National Institute on Drug Abuse

RESEARCH

MONOGRAPH SERIES

Behavioral Studies of Drug-Exposed Offspring: Methodological Issues in Human and Animal Research

164

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

Behavioral Studies of Drug-Exposed Offspring: Methodological Issues in Human and Animal Research

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NIDA Research Monograph 164
1996

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

National Institute on Drug Abuse
5600 Fishers Lane
Rockville, MD 20857

ACKNOWLEDGMENT

This monograph is based on the papers from a technical review on "Behavioral Studies of Drug-Exposed Offspring: Methodological Issues in Human and Animal Research" held on July 12-13, 1993. The review meeting was sponsored by the National Institute on Drug Abuse.

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National Institute on Drug Abuse
NIH Publication No. 96-4105
Printed 1996

NIDA Research Monographs are indexed in the *Index Medicus*. They are selectively included in the coverage of *American Statistics Index, BioSciences Information Service, Chemical Abstracts, Current Contents, Psychological Abstracts,* and *Psychopharmacology Abstracts.*

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Introduction

Cora Lee Wetherington, Vincent L. Smeriglio, and Loretta P. Finnegan

For several years the use of drugs during pregnancy, particularly cocaine, has been a major public health issue because of the concern about possible adverse behavioral effects on the neonate and the developing child. While many popular press publications have warned of the severe adverse effects of prenatal drug exposure, the scientific literature has been less clear on this issue, in part because of complex methodological issues that confront research in this field.

On July 12 and 13, 1993, the National Institute on Drug Abuse conducted a technical review at which researchers reviewed the state of the art regarding behavioral assessments of offspring prenatally exposed to abused drugs. Presenters identified and addressed the complex methodological issues that abound in both human and animal studies designed to assess behavioral effects of prenatal drug exposure, and they stressed the caveats involved in drawing causal conclusions from associations between maternal drug abuse and adverse behavioral outcomes in the offspring. This research monograph is based upon revisions of presentations made at that technical review. The fundamental aim of this research monograph is to clarify the methodological issues for future research in this field, to provide caution in the interpretation of research findings, and to suggest future research directions.

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Long-Term Effects of Developmental Exposure to Cocaine on Learned and Unlearned Behaviors

Charles V. Vorhees

INTRODUCTION

The purpose of this chapter is to review the effects of exposure to central nervous system (CNS) stimulants on the neurobehavioral development of experimental animals. The focus is on the effects of cocaine.

A search was made of the experimental literature on the effects of prenatal and/or early postnatal exposure to cocaine. The search encompassed the years 1982 to mid-1993. Only one selection criterion was imposed on the search: only articles reporting original experimental results were included. The search generated 57 relevant articles. A tabular summary of these, presented in chronological order, is provided in table 1. The table is structured with authors and date in the first column, with subsequent columns for dose (expressed as the hydrochloride unless specified as the free base), species and strain, dose rate expressed on a per diem basis, exposure period (embryonic or postnatal age given in days), route of drug administration, the concentration of the drug in solution, the major types of control groups used, principal variables investigated, and finally the major effects obtained (including negative findings).

DESIGN CONSIDERATIONS

Several points regarding design of developmental studies appropriate for cocaine are discussed in this chapter. Some of these arise from developmental considerations, some from the nature of cocaine's pharmacological effects, and some from considerations of experimental design logic.

The control groups used in studies of cocaine exposure are particularly important for several reasons. First, cocaine is a potent anorectic agent and therefore suppresses food consumption and weight gain. This is obviously important during pregnancy in developmental studies, when

weight gain is a normal process that accompanies embryonic development. While all experiments reviewed below included an ad libitum fed and saline-injected (or water-gavaged) control group (AL), only a subset of the experiments reviewed included nutritionally matched pair-fed (PF) controls (i.e., groups given diet in the amount equal to that consumed by yoked cocaine-treated animals on the same gestational day) (Vorhees 1986). Pair-fed controls in cocaine-exposure experiments, especially in prenatal studies, are one of the most important controls needed; their absence raises questions about the interpretation of the results obtained. An exception to this rule is when the dose given does not induce an alteration in food consumption or weight gain. As shown in table 1, several authors have reported data in which the dose and dosing regimen used did not significantly reduce maternal weight in either absolute terms or in terms of weight gain during gestation.

A second relevant control in prenatal studies arises from the potential for maternal carryover effects. In this situation, the use of surrogate or other fostering procedure is indicated. The purpose of these procedures is to remove the treated neonates from the influence of their treated (biological) dam. One approach is for treated and control neonates to be reared by untreated dams prepared separately and timed to deliver shortly before the experimental groups. Such offspring are said to be surrogate fostered. Another approach is when neonates are reared by dams of the opposite treatment group. Litters reared in this fashion are known as cross-fostered.

Finally, the converse of cross-fostering occurs when neonates are reared by dams from within the same treatment group but not by their biological dam. These offspring are known as fostered (Vorhees 1986). In table 1, surrogate fostering has been designated S-FOS, while cross-fostering or fostering has been designated FOS. Only a subset of the experiments reviewed in table 1 included either type of fostered rearing control.

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993.*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Fantel and MacPhail 1982	50,60, 75	Rat:SD	1/d	E8-12	IP	n.g.	AL PF	Visceral malformations	50:-- 60:↑ resorp. ↓ fetal wt. 75:↑ mat. leth. ↑ fetal edema m60: ↓ fetal wt.
	60	M:SW	1/d	E7-16	IP	n.g.			
Church et al. 1988*a*	20,25, 30,35, 40,45	Rat:LE	2/d	E7-19	SC	20	AL	Preg. outcome	↑ mat. leth.: 60-90 ↓ mat. wt. gain all grps. ↑ fetal. mort.: 70-90 ↓ fetal wt.: 90

KEY: --=no change; ↑=significant increase; ↓=significant decrease; E=embryonic day; PN=postnatal day; AL=ad lib fed control; PF=pair fed control; S-FOS=surrogate-fostered control; FOS=cross-fostered control; SD=Sprague-Dawley rat; LE=Long-Evans rat; W=Wistar rat; SC=subcutaneous; IP=intraperitoneal; PO=per oral (gavage); M=mouse with specific strain noted; n.g.=concentration not given in article; SW=Swiss-Webster; m=male; 2-DG=2-deoxyglucose; F=female; T ½=half-life; mat=maternal; neg geo=negative geotaxis; surf. rt.=surface righting; spont. alt.=spontaneous alternation; act. av.=active avoidance; det.=detachment; ret. lat.=retention latency; BAEP=brainstem auditory evoked potential; NTD=neural tube defect; d-A=d-amphetamine; n. accumb.=nucleus accumbens; cx.=cortex; BS=brainstem; Str.=striatum; TH-IR=tyrosine hydroxylase-immunoreactivity; lc=lab control; Trph=tryptophan hydroxylase; ½ FOS=½ the animals were cross-fostered.

NOTE: Doses are expressed in mg/kg. Except where noted, dose was expressed as the hydrochloride. Drug concentration is expressed as mg/ml of solution.

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Church et al. 1988*b*	20,25, 30,35, 40,45	Rat:LE	2/d	E7-19	SC	20	AL	Preg. outcome	same as above
Dow-Edwards et al. 1988	25 50	Rat:SD	2/d 1/d	P1-2 P3-10	SC	n.g.	AL	2-DG	↑ metab. act. in F in multiple cerebral areas
DeVane et al. 1989	30 (base)	Rat:SD	1/d	E18 or 19	IP	30	None	T½	mat. 45 min fetal 55 min
Dow-Edwards et al. 1989	40,80 20,40	Rat:SD	1/d	E0-16	PO SC	26-32 20,40	AL	mat. wt. gain mat. peak	↓ ~500ng/mL ¼ hr. ~1,000 ¼ hr.

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Fung et al. 1989	30 (base)	Rat:SD	Con-tinuous	E2-birth	mini-pump	NA	AL S-FOS	loco. ontogeny activity spiperone bind. DA turnover	-- -- -- -- -- --
Hutchings et al. 1989	30,60	Rat:W	1/d	E7-21*	PO	6,12	AL PF S-FOS	Mat. wt. gain activity	↓ ↑ at 60 mg/kg on P20 and 23
Smith et al. 1989	10	Rat:LE	1/d	E3-17*	SC	10	AL	mat. wt. offspring wt. neg. geo. surf. rt. spont. alt.	-- -- -- -- ↓ in m

* = Adjusted for evidence of conception as embryonic day EO.

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Smith et al. (cont.)								open-field	↓ in m
								DRL-20	↑ response rate
								act. av.	--
								tail-flick	↓ latencies
								T-water maze	↑ lat. m, early trials only
								sleep time	--
								shock sensit.	↓
Spear et al. 1989*a*	40	Rat:SD	1/d	E7-19*	SC	13.3	AL PF	mat. wt.	--
								gest. length	--
								litter size	--
								offspring wt.	--
								eye opening	--
								lower incisors	--
								surf. rt.	--
								cliff avoid.	--
								horiz. screen	--
								vert. screen	--
								neg. geo.	--

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Spear et al. 1989*a* (cont.)								odor cond. shock-induced wall climbing and act. shock sensit.	↓ ↓ ↑ --
Spear et al. 1989*b*	10,20, 40	Rat:SD	1/d	E7-19*	SC	13.3	AL	coc. 2 hr. at 40 mg/kg plasma mat. fetal brain mat. fetal	 ~2200 ng/mL ~800 ~2900 ~2200
Wiggins et al. 1989	10,60	Rat:LE	1/d	E19 or 20	PO	n.g.	None	fetal: mat. brain fetal: mat. plasma	~.7 at 1.5 h at 60 mg/kg ~1.5 at 1.5 h at 60 mg/kg

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Church et al. 1990	20,30, 40,50	Rat:LE	2/d	E7-20	SC	20	AL PF	mat. wt. gain mat. mort. offspring wt. litter size offspring mort. pinna det. incisors fur dev. eye opening vag. patency	dose-dep. ↓ dose-dep. ↑ ≥40 dose-dep. ↓ ≥30 ↓ at 50 ↑ at 40 and 50 ↓ at 20, 40, 50 -- ↓ at 40 and 50 ↓ at 30, 40, 50 ↓ at 40 and 50
Church and Overbeck 1990*a*	20,30, 40,50	Rat:LE	2/d	E7-20	SC	20	AL PF	neg. geo. spont. alt. activity passive av. act. av.	-- -- (freq.) ↑ left bias -- vs. PF ↓ ret. lat. at 50 -- vs. PF

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Church and Overbeck 1990*b*	20,30, 40,50	Rat:LE	2/d	E7-20	SC	20	AL PF	BAEP	↑ interpeak lat. at 50 mg/kg ↓ amplitude at 50 mg/kg
Dow-Edwards 1990	30,60	Rat:W	1/d	E7-21*	PO	n.g.	--	¼ h. at 60: mat. plasma fetal plasma mat. brain fetal brain	~5400 ng/mL ~3000 ~3500
Dow-Edwards et al. 1990	60	Rat:W	1/d	E7-20*	PO	12	PF S-FOS	2-DG	↓↓ hypothal. ↓ nigrostriat. ↓ MFB ↓ hippo. ↓ septum ↓ amygdala

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Finnell et al. 1990	20,40, 60	DBA/2J SWV	1/d	E6-8 E8-10	IP	n.g.	AL	stage effects	--
								implantations	--
								resorptions	--
								mat. wt. gain	--
								fetal wt.	--
								malform. (NTD and urinary)	↑ 40,60,DBA ↑ all doses SWV
Giordano et al. 1990[1]	30	Rat:SD	1/d	E12-21	SC	30	AL	activity w/	
								d-A chall.	--
								coc. chall.	--
Henderson and McMillen 1990	15 (base)	Rat:SD	2/d	E1-birth	SC	n.g.	AL S-FOS	birth wt.	↓
								litter size	--
								surf. rt.	↓
								eye opening	--
								act. P30	↑
								act. P60	--

1 = This study contained only 2 litters per group.

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Heyser et al. 1990	40	Rat:SD	1/d	E7-19*	SC	13.3	AL	mat. wt. gain	↑
								litter size	--
								birth wt.	--
								sens. precond.	↓ at P8
									↓ at P12
									-- at P21
								1st order cond.	↑↓ at P8
									-- at P12
									-- at P21
Raum et al. 1990	10	Rat:SD	single dose	PO	SC	n.g.	AL	0.5 h.estradiol	↓
								0.5 h. testost.	↓
	10,30	Rat:SD	2/d	E15-20	SC	n.g.	AL	mat. wt. gain	↓ at 30
								litter size	--
								offspring wt.	--
								anogenit. dist.	--
								scent marking	↓ in males
								intromiss. lat.	↓
								plasma LH	↑
								plasma FSH	--

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Raum et al. (cont.)	3		2/d	E15-20 and P1-5			AL and S-FOS	testosterone organ wts. birth wt. P50 wt. scent marking	-- -- ↓ -- ↓ in males
Scalzo et al. 1990	40	Rat:SD	1/d	E7-19*	SC	13.3	AL	recept. bind. P2 striatum D_1 striatum D_2 n. accumb. D_1 n. accumb. D_2	 -- ↑ -- --
Sobrien et al. 1990	40	Rat:SD	1/d	E14-20*	SC	n.g.	AL	mat. wt. gain litter size birth wt. offspring wt. pinna detach. incisor eruption eye opening	-- -- -- -- -- -- --

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Sobrien et al. 1990 (cont.)								neg. geo.	--
								surface rt.	↑
								olfact. behav.	--
								cliff avoidance	↑
								startle dev.	↑
								air righting	--
								act. w/ d-A	attenuated ↑
								act. w/ coc.	attenuated ↑
Barron et al. 1991	60	Rat:LE	1/d	E13-20*	PO	13.3	AL	mat. wt. gain	↓
								mat. plasma coc.	430 ng/mL 5-50
								fetal wt.	--
								litter size	--
								umbilical lgth.	↓
Church et al. 1991	30	Rat:LE	2/d	E7-20	SC	20	AL PF S-FOS	mat. wt. gain	↓
								birth wt.	--
								offspring mort.	--

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Church et al. (cont.)								pinna detach.	--
								fur dev.	--
								eye opening	--
								vag. patency	--
								neg. geo.	--
								loco. act.	↓
								pass. av.	--
								act. av.	--
Church and Overbeck 1991	30,40	Rat:LE	2/d	E7-20	SC	20	AL PF S-FOS	BAEP threshold latency	 ↑ at 40 ↑ at 40
Clow et al. 1991	10,20, 40	Rat:SD	1/d	E7-19*	SC	3.33 6.66 13.3	AL PF S-FOS	mat. wt. gain ^{3}H-naloxone autoradiog.	↑ at 40 in med prefrontal rost. olf. tuber cingulate cx. hippo. CA1 DG mol layer motor cx. sensory cx. entorhinal cx.

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
El-Bizri et al. 1991	2.1, 4.2, 8.5,17, 34	Rat:SD	1/d	E0-19	IP SC	n.g.	AL	mat. wt. gain IP peak (5") T½ SC peak offspring wt. brain DA brain NE act. embryo culture	↓ 510 ng/mL 21" 147 ng/mL -- -- -- -- ↓ development
Hughes et al. 1991	50	Rat:SD	1/d	P1-10 P11-20	SC		AL	act. w/ d-A 0.1 mg/kg 0.25 mg/kg	 ↑ early grp. ↓ late grp.
Rodriguez-Sanchez et al. 1991	40	Rat:W	1/d	E7-19 E7-P15 P0-15	SC	n.g.	AL some were S-FOS	Somatostatin-IR front. cortex Hippocampus	 ↓ prenatal grps. ↑ prenatal grps. -- P0-15 grp.

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Rodriguez-Sanchez et al. (cont.)								SS receptors	↑ no. in cortex ↓ affinity in cx.
Rodriguez-Sanchez and Arilla 1991	40	Rat:W	1/d	E7-19 E7-P15 P0-15	SC	n.g.	AL	Somatostatin-IR ^{125}I-Tyr Somat. in striatum	-- all grps ↑ receptors in E7-19 and E7-P1 ↓ affinity E7-19
Seifert and Church 1991	40,50	Rat:LE	2/d	E7-20	SC	n.g.	AL PF S-FOS	Offspring wt. femur dry wt. femur ash wt. femur organ. wt	↓ at 50 mg/kg ↓ at 40 mg/kg ↓ at 40 mg/kg ↓ at 40 mg/kg

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Webster et al. 1991 (based on Webster and Brown-Woodman 1990)	70 60 50	Rat:SD	1/d 1/d 2/d	E16 only	IP	20	AL	Cx necrosis Cx cavitation BS cavitation Str. hemorrhage	↑ ↑ ↑ ↑
Foss and Riley 1991*a*	60 40	Rat:LE	1/d	E13-20* E7-20*	PO SC	13.3 13.3	AL PF	mat. wt. gain litter size offspring wt. acoustic startle startle habit. prepulse inhib.	↓ -- both grps. -- both grps. -- both grps. -- both grps. -- both grps.
Foss and Riley 1991*b*	40	Rat:LE	1/d	E13-20*	SC	13.3	AL PF	mat. wt. gain startle w/ coc. open-field w/ coc. chall.	↓ -- no pattern

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Riley and Foss 1991*a*	60 40	Rat:LE	1/d	E13-20* E7-20*	PO SC	13.3 13.3	AL PF	holeboard	-- both grps.
Riley and Foss 1991*b*	60	Rat:LE	1/d	E13-20*	PO	13.3	AL PF	pass. av. act. av. Morris maze	-- -- --
Akbari et al. 1992	40 40,10	Rat:SD	1/d	E13-22 E13-P5	SC	13.3	AL	^{3}H-paroxetine 5HT-IR ^{3}H-paroxetine	↓ P1,7 pre grp. ↓ hipp., pre grp. -- or ↑ early in pre/post grp. P28 all ▵ gone
Akbari and Azmitia 1992	40	Rat:SD	1/d	E13-22	SC	13.3	AL S-FOS	TH-IR	↑ hippocampus ↑ ant. cingulate ↑ parietal cortex
Bilitzke and Church 1992	40	Rat:LE	2/d	E7-20	SC	20	AL PF S-FOS	mat. wt. gain resorptions offspring wt. pinna detach.	↓ ↑ (after birth) -- up to P120↓ --

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Bilitzke and Church (cont.)								fur dev.	--
								ear opening	--
								eye opening	--
								offspring mort.	--
								Porsolt test	↓ immob. time
Church and Rauch 1992	50	Mouse: BALB/c xSJL	1/d	E7-18	SC	20	AL PF	mat. wt. gain	--
								water consump.	↑
								food consump.	--
								resorptions	↑
								birth wt.	↓
								offspring wt.	↓
								pinna detach.	--
								fur dev.	--
								ear opening	↓
								eye opening	↓

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Goodwin et al. (II) 1992	40	Rat:SD	1/d	E7-19*	SC	13.3	AL FOS	P7 cond. odor avoid.	↓ C40-C40 on train. trls. 2-4 ↓ av FOS/C40 on train. trls. 2-3
								P17:	
								cond. aud. av.	--
								cond. odor av.	--
								shock aggr.	↓ lat C40-C40 & C40-FOS grps.
								intruder aggr.	--
Heyser et al. (I) 1992*c*	40	Rat:SD	1/d	E7-19*	SC	13.3	AL FOS	mat. wt. gain	↓
								litter size	--
								birth wt.	--
								pup retrieval	--
								mat. aggr.	
								FOS/lc	--
								FOS/C40	
								lc/FOS	--
								lc-lc	↑ lat. to attack

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
									↑ intruder freez
Heyser et al. (I) 1992*c* (cont.)								C40/FOS	--
								C40-C40	↑ attacks
									↑ lat. to attack
									↑ intruder submission
Heyser et al. 1992*b*	40	Rat:SD	1/d	E7-19*	SC	13.3	AL PF S-FOS	mat. wt. gain	↓
								litter size	--
								offspring mort.	--
								offspring wt.	--
								cond. place. pref.	↓
								cham. entries	↑
Heyser et al. 1992*a*	40	Rat:SD	1/d	E7-19*	SC	13.3	AL cellulose control	Coc.-induced odor preference	↓ Coc.-induced odor pref. at low dose only in prenatal coc. grp.

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Johns et al. (I) 1992*a*	15	Rat:SD	2/d	E1-20 E2-3,8-9, 14-15,19-20	SC	n.g.	PF S-FOS	mat. wt. gain litter size offspring wt. surface rt. eye opening act. P30, 15" Diurnal nocturnal act. P60, 15" diurnal nocturnal	↓ coc-D grp. ↑ coc-I grp. -- both grps. -- both grps. -- both grps. ↑ coc-D grp. -- both grps. ↓ coc-I grp. -- both grps. -- both grps. -- both grps.
Johns et al. (II) 1992*b*	15	Rat:SD	2/d	E1-20 E2-3,8-9, 14-15,19-20	SC	n.g.	PF S-FOS	spont. alt. open-field win-stay maze P30 and 60	-- both grps. ↑ non-entries coc-D ↑ entry coc-I -- (both ages)

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Meyer et al. 1992	20	Rat:SD	2/d	E10-19*	SC	6.6	AL PF S-FOS	P 11 Coc. chall.	
								vocalizations	--
								digging time	--
								grooming time	--
								stationary time	--
								loco. time	--
								wall clim. time	no ↑ in coc. offspring at mid and low coc. chall. doses
Minabe et al. 1992	40	Rat:SD	1/d	E7-19*	SC	13.3	AL PF S-FOS	active cells in A9 and A10 nuclei	↓ in A10 and A9 -- after apomorphine challenge

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Rodriguez-Sanchez and Arilla 1992	40	Rat:W	1/d	E7-19 E7-P15 P0-15	SC	n.g.	AL	somatostatin-IR	↓ E7-19 grp. ↑ E7-P15 grp. -- P0-15 grp.
								^{125}I-Tyr Somat. in olfactory bul	↑ receptors and ↓ affinity E7-19 and P0-15 grps.
Tyrala et al. 1992	10	M:IcR	1/d	E6-14	IP	n.g.	AL	fetal brain cell culture	↓ specific act.
								AchE protein	↓
Weaver et al. 1992	10,20	Rat:SD	1/d	E20	IP	n.g.	AL	c-FOS in SCN	↑
								pre-trt. D_1 ant. SCH-23390	blocks ↑
Dow-Edwards et al. 1993	50	Rat:SD	1/d	P11-20	SC	10	AL	2-DG males	↓ n. accumbens ↓ piriform cx ↑ med. genicul. ↑ auditory cx.

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Dow-Edwards et al. 1993 (cont.)								females	↑ 5/6 mot. area ↑ 7/17 limbic ↑ 2/8 hypothal. ↑ 3/11 sensory ↑ 1/3 assoc.
Factor et al. 1993	10,40	Rat:SD	contin-uous	E8-22	mini-pump	NA	AL some S-FOS	PPT[2] mRNA PPT TrpH act. subst. P in med. raphe n	-- all ages -- all ages -- all ages -- all ages
Seidler and Slotkin 1993	30,100	Rat:SD	3/d	E8-20 E18-20	SC	3.3 11.1	AL	ODC activity ^{3}H-thymidine DNA content protein/DNA	↑ ODC on P2, no change thereafter -- all other parameters

2 = Preprotachykinin

TABLE 1. *Effects of prenatal and/or early postnatal exposure to cocaine on experimental animals, 1982-1993 (continued).*

Authors	Dose	Species	Rate	Exposure	Route	Conc.	Controls	Variable	Effect
Vathy et al. 1993	10	Rat:SD	2/d	E11-18	SC	n.g.	AL ½ FOS	surf. righting vaginal patency open field act. F lordoses after ovariectomy m No. mounts intromissions post-ejac. time preoptic n.	-- -- -- ↓ ↑ ↑ ↓ ↑ NE ↑ DA

Overview of Design Features of the Current Literature on Cocaine

In order to provide a sense of the areas of investigation among the 57 reports reviewed here, table 2 provides a summary by focus area. Slightly more than 42 percent of the studies focused on behavioral effects, while 27 percent concentrated on neurochemical effects, nearly 16 percent on teratogenesis or early postnatal physical outcome (termed

TABLE 2. *Summary of experiments on developmental exposure to cocaine, 1982 to 1993.*

Area	No. of Articles*	Percent
Behavioral teratologic	24	42.1
Pharmacokinetic	5.5	9.6
Developmental toxicity/teratologic	9	15.8
Neurochemical	15.5	27.2
Neurophysiologic	3	5.3
Total	57	100.0

KEY: * = Some experiments were difficult to classify and were therefore divided and half placed in two separate categories.

"developmental toxicity" in table 2), nearly 10 percent on pharmacokinetics (although these studies are all very limited in scope), and about 5 percent on neurophysiological measures.

Table 3 reveals the distribution of test species in the literature. No studies in monkeys were found. All the studies identified were conducted in rodents, and rat studies constituted better than 93 percent of the published literature. Of the 54 articles reporting results in rats, nearly 60 percent were conducted using the Sprague-Dawley (SD) strain, nearly 30 percent in Long-Evans (LE), and about 10 percent in Wistar (W) rats.

A further examination of table 1 reveals that about 50 percent of the published articles on developmental cocaine exposure have included

TABLE 3. *Summary of experiments on developmental exposure to cocaine, 1982 to 1993.*

		No.*	Percent
Species	Rat	54	93.1
	Mouse	4	6.9
Strain (rat)	SD	32	59.3
	LE	16	29.6
	W	6	11.1

KEY: SD = Sprague-Dawley; LE = Long-Evans; W = Wistar; * = total sums to more than 57 because some articles reported results from multiple experiments in two species.

pair-fed or similar nutritional controls. Of those studies reporting postnatal outcome measures, about 50 percent included some type of fostering controls, most being of the surrogate fostering type. The confluence of nutritional and fostering controls occurred in roughly 25 percent of the prenatal exposure experiments that included postnatal outcome. Experiments without postnatal evaluation obviously do not need fostered controls; this category includes teratologic and fetal neurochemical experiments.

Table 4 shows the distribution of exposure periods. Interestingly, better than 83 percent of studies used only prenatal exposure, just under 8 percent a combination of prenatal and postnatal exposure, and about 9 percent early postnatal exposure only (prior to weaning). In light of the fact that early postnatal exposure in rodents is roughly analogous to late second and third trimester exposure in humans in terms of CNS development (Bayer et al. 1993), the relative neglect of this latter exposure period is unfortunate as it suggests that most of the experiments to date have modeled less than half of the early developmental stages that are critical to human intrauterine brain development.

Table 5 summarizes another aspect of the developmental cocaine literature. The top half of the table shows the dose rate schedules of cocaine administration. Although cocaine has a short biological half-life (0.3 hour after intravenous (IV) administration, 0.75 hour after intraperitoneal (IP) administration, and 2 hours after subcutaneous (SC)

TABLE 4. *Summary of experiments on developmental exposure to cocaine, 1982 to 1993.*

Exposure Period	No.*	Percent
Prenatal	54	83.1
Pre- and postnatal	5	7.7
Postnatal	6	9.2
Total	65	

KEY: * = Total number exceeds 57 because several experiments were reported in which there were multiple treatment regimens used (e.g., the articles by Rodriguez-Sanchez and colleagues included a prenatal only group, a pre- and postnatal group, and a postnatal only treated group). Each group was tallied separately.

TABLE 5. *Summary of experiments on developmental exposure to cocaine, 1982 to 1993.*

Dose Rate/Route	No.	Percent
Dose rate		
Once/day	39	68.4
Twice/day	15	26.3
Over twice/day	1	1.8
Continuous	2	3.5
Total	57	
Route of administration		
SC	42	70.0
IP	7	11.7
PO	9	15.0
IV	0	0
Infusion	2	3.3
Total	60	

NOTE: Total exceeds 57 because these articles report experiments using more than one route of administration.

KEY: SC = subcutaneous; IP = intraperitoneal; PO = per oral (gavage); IV = intravenous.

administration in rats (DeVane et al. 1989; Nayak et al. 1976)), over 68 percent of the studies relied upon single daily dose administration regimens. About 26 percent used twice per day schedules, while only one experiment (1.8 percent) used more than twice per day administration. That experiment, by Seidler and Slotkin (1993), used thrice per day dosing. This chapter also summarizes preliminary data from an experiment conducted by the author using 5-times-a-day dosing of cocaine during gestation.

The bottom half of table 5 shows the routes of drug administration that have been employed. By a large margin (70 percent), experimenters have used SC administration. Slightly less than 12 percent used the IP route, and not one of these examined behavioral outcomes. The IP administration experiments are predominately teratologic, with a small number being pharmacokinetic or neurochemical. Interestingly, all of these studies, save the one that was exclusively pharmacokinetic, report finding cocaine-induced effects on the progeny. A similar minority (15 percent) of the studies used an oral (PO) route of administration. None of the published studies used an IV route. Finally, 3.3 percent of the studies used infusion via subcutaneously implanted osmotic minipumps for cocaine delivery.

Deficiencies in the Current Literature on Cocaine

Having summarized the approaches reported in the developmental cocaine literature, it is germane to point out the gaps that exist. First, the rat, and primarily the SD rat, has been the dominant species and strain used. Since no single species provides an entirely adequate model of human disorders, there is a clear need to expand the range of models to other species. Among rats, the heavy reliance upon the SD rat, although understandable in terms of its broad scientific acceptance and use by regulatory agencies as a standard in investigations of developmental toxicity, may overly weight the findings towards this one strain. If the literature were replete with striking cocaine-related developmental effects, one would be less concerned about the possible limitations of the SD rat; but given that this is not the case (see below), exploration of other strains may prove fruitful.

Another shortcoming is the heavy focus on exposure during most of pregnancy. Two obvious gaps exist here: the first is the value of rodent postnatal exposure as a model of human late second and all of third trimester, and second is the value of investigating discrete exposure

intervals. Of the published prenatal studies, almost all have used exposure throughout most of pregnancy. The opportunity to find critical period effects is lost with this strategy, and thus important effects may exist that have yet to be uncovered.

Another area where gaps exist is in the dose-rate schedules that have been used. Heavy reliance upon once-a-day dosing may be insufficient. Again, if the existing literature supported the view that cocaine given once per day was inducing clear neurodevelopmental effects, there would be no compelling reason to question this approach. However, the contrary is the case, with few effects having been established with certainty. This situation, taken together with the fact that cocaine has a biological half-life in rodents of 0.3 to 2 hours depending on the route of administration, raises questions about what may be being missed by current models that administer the drug only once a day.

Finally, these studies have relied upon the SC route of administration to a great extent. Using the same logic as expressed above, no concern would exist had striking effects been reported using this route. As this is not the case, further exploration of other routes seems worthwhile. Among the other routes needing further attention are the IP and IV routes. Further work with PO administration may also prove useful and consideration should be given to inhalation exposure. Given the degree of sophistication possible in facilities with equipment and expertise in inhalation toxicology, the technical barriers to this approach should not prevent this route from being investigated.

In toto, the current developmental cocaine experimental literature appears heavily weighted towards a limited range of experimental models that have produced mixed results. However, if one ignores for the moment the possible limitations of the models, the next pertinent question becomes, what effects related to cocaine exposure have been reported?

BEHAVIORAL EFFECTS OF DEVELOPMENTAL COCAINE EXPOSURE

Of the 57 experiments reviewed, 24 have been behavioral teratologic studies. Of these 24, 15 have reported finding cocaine-related effects (table 6). These 15 studies are individually reviewed below. However, it should be noted that describing the results as positive in 15 of 24, or 62.5 percent, of the studies overstates the apparent strength of the

TABLE 6. *Summary of positive behavioral teratologic experiments on prenatal cocaine, 1982 to 1993.*

Article	Route	Dose	Exp.	Effect
Hutchings et al. 1989	PO	30,60	7-21*	↑ Loco. act. P20 and 23 only at 60 mg/kg
Smith et al. 1989	SC	10	3-17*	↓ Spont. alt. freq. (males only)
				↓ Open-field act. (males only)
				↑ DRL-20 res. rate
				↓ Tail-flick lat.
				↑ T-water mz. lat. (M, early trls. only)
				↓ Shock sensitivity
Spear et al. 1989*a*	SC	40	7-19*	↓ Odor conditioning
				↓ Shock-induced wall climbing
				↑ Act. during shock-ind. wall climb.
Church/Overbeck 1990*a*	SC	20,30, 40,50	7-20	↑ Left bias in spont. alt. (non-D-dep.)
				↓ Pass. av. ret. at 50 mg/kg
Henderson/McMillen 1990	SC	15	1-birth	↓ Surface righting dev.
				↑ Loco. act. at P30; - at P60
Heyser et al. 1990	SC	40	7-19*	↓ Sens. precond., P8, 12; not at P21
				↓ 1st order cond., P8; not at P12, 21
Raum et al. 1990	SC	10,30	15-20	↓ Scent marking in males
				↓ Lat. to intromission
Sobrien et al. 1990	SC	20	14-20*	↑ Surface righting and cliff avoid. dev.
				↑ Startle dev.
				↓ Res. on loco. act. to d-A and coc.
Church et al. 1991	SC	30	7-20	↓ Loco. act.
Heyser et al. 1992*b*	SC	40	7-19*	↓ Cond. place preference
				↑ No. chamber entries

TABLE 6. *Summary of positive behavioral teratologic experiments on prenatal cocaine, 1982 to 1993 (continued).*

Article	Route	Dose	Exp.	Effect
Johns et al. 1992*a*	SC	15	1-20 2-3, 8-9, 14-15, 19-20	↑ Loco. act. P30 1st 15' (Coc-D grp.) ↓ Loco. act. P30. dark cycle (Coc-I grp.)
Johns et al. 1992*b*	SC	15	Ibid	↑ Open-field non-entries (Coc-D grp.) ↑ Open-field act. (Coc-I grp.)
Bilitzke and Church 1992	SC	40	7-20	↓ Immobil. time on Porsolt swim test
Goodwin et al. 1992	SC	40	7-19*	↓ Odor cond. P7; coc-coc, 2-4 train. trls. FOS/coc 2 and 3 train. trials only - aud. cond. P17; - Odor cond. P17 ↓ Lat. to 1st attack shock-induc. aggr. in coc-coc and fos-coc grps.
Meyer et al. 1992	SC	20	10-19*	Effects tested using coc. challenge: ↑ wall climb. all ch. doses in AL and PF ↑ wall climb. high dose only in coc. grp.

NOTE: Papers are listed in chronological order. Two papers (Raum et al. 1990 and Vathy et al. 1993) reporting positive findings on sexual behaviors are not included in this table, but are shown in table 1.

KEY: * = Adjusted for evidence of conception as embryonic day E0.

findings since every report contained multiple behavioral and other measures. In most of the experiments with positive findings, there were as many (or in some cases, more) negative than positive findings.

Therefore, one should bear this in mind as the positive effects are discussed in greater detail.

Among the behavioral teratogenic studies, Hutchings and colleagues' (1989) is the only study with positive findings that used the oral route of administration. These authors reported that offspring exposed in utero to 60 milligrams per kilograms (mg/kg) of cocaine administered by gavage on embryonic days (E) 7 to 20 showed increased locomotor activity on days 20 and 23, but on surrounding test days cocaine-exposed offspring did not show similar effects. Progeny exposed by the same regimen to a lower dose of 30 mg/kg showed no changes in activity. While significant cocaine-related effects were found in this experiment, the small number of test days showing a change and their sporadic occurrence within a larger context of more numerous test days when no effects were obtained raise doubt as to the strength and reproducibility of these findings.

Among the studies administering cocaine by injection, Smith and colleagues (1989) reported decreased spontaneous alternation frequency and open-field activity only among males, increased responding on a differential reinforcement of low rate (DRL)-20 operant schedule, decreased tail-flick latencies, increased latencies to reach a goal in a water T-maze, and decreased footshock sensitivity in rats exposed in utero to 10 mg/kg of cocaine administered once per day on E3 to 17. Superficially these results appear to support a large number of cocaine-related effects, but the findings are difficult to interpret. The effects reported stem from analysis of variance (ANOVA) interaction terms. The significant F-ratios obtained were small and significant only because of the large degrees of freedom. A concern is that the effects account for only a small percentage of the variance and may not prove to be meaningful or replicable. If the authors or others cannot replicate these effects, then they are not reliable indices of cocaine-induced developmental neurotoxicity. As discussed below, there is little indication in the literature that the findings reported in this article are seen by other investigators.

Spear and colleagues (1989*a*) administered SC cocaine to rats on days E7 to 19 and found that the offspring showed a decrease in odor conditioning, a decrease in shock-induced wall climbing, and increased activity during the wall-climbing test. These findings may represent a promising lead because the authors have replicated at least portions of these findings (decreased odor conditioning). Moreover, they have extended the findings to other effects of prenatal cocaine exposure. Some of these other effects are similar to those reported in this first experiment (Spear et al. 1989*a*) and

some are quite different, but together they suggest that cocaine is capable of inducing developmental effects in progeny exposed in utero.

Among the positive behavioral effect studies is that published by Church and Overbeck (1990*a*). This report is part of a series published by the Church laboratory on prenatal cocaine exposure using a wide range of dependent variables. The authors reported finding two behavioral effects: an increase in left turning bias in animals tested on a spontaneous alternation procedure with no cocaine-related effects on the primary response measured on this task (viz., alternation frequency) and a decrease in passive-avoidance retention times. Church and Overbeck (1990*a*) obtained these results in rats administered doses of cocaine ranging from 20 to 50 mg/kg twice a day given to the dams on days E7 to 20. The principal difficulties in interpreting these findings are that the left turning bias is of unknown significance, the increase was not dose-dependent, and the behavior was not central to what the task was intended to measure. The passive-avoidance effect was found in the high-dose group, but no other cocaine-exposed group was affected on this measure. The high-dose group in this study received a dose that produced significant maternal and offspring toxicity. Specifically, the 50 mg/kg dose increased maternal death and reduced maternal weight gain. In the offspring, this dose reduced litter sizes and increased neonatal mortality. Obviously, these types of toxicity are not trivial. In such a context, the passive-avoidance effect, while interesting, stands in pale contrast with death. This view might be altered if it could be shown that cocaine-exposed offspring have persistent and substantial memory impairments at lower doses, but converging evidence employing multiples tests of memory have yet to appear in the literature. At present, the passive-avoidance effect does not appear commensurate to the other effects of cocaine at very high doses.

Henderson and McMillen (1990) administered 15 mg/kg cocaine twice a day on E1 to 22 and found decreased surface righting development and increased locomotor activity at 30 days but not 60 days of age. A number of investigators have tested cocaine-exposed offspring for reflex ontogeny, including using tests of surface righting, but no such effects were obtained by others even at higher doses (Johns et al. 1992*a*; Spear et al. 1989*a*; Vathy et al. 1993). Thus, it is difficult to reconcile this finding with those of other investigators who have not found such effects No published replication of the effect has yet appeared. The increase in day 30 activity is interesting and may be significant, but other laboratories have reported opposite effects (Church et al. 1991; Smith et al. 1989).

One similar effect on activity has been reported and is discussed below (Johns et al. 1992*a*).

Heyser and colleagues (1990) administered 40 mg/kg of SC cocaine on E7 to 19 and found decreased sensory preconditioning in offspring on postnatal days 8 and 12, but the effect was no longer present by day 21. Odor conditioning was also reduced in the cocaine-exposed progeny on day 8, but not on days 12 or 21. These data, although different in their details from previous work (Spear et al. 1989*a*), nevertheless represent apparently consistent findings. To the extent that these data converge, they signify the first instance in the experimental literature on prenatal cocaine of consistent evidence of behavioral effects. Note, however, that these are neonatal effects. These early effects tell little about whether cocaine is capable of inducing long-term effects, which is the principal concern about this drug when taken by women during pregnancy.

In another experiment, Raum and colleagues (1990) administered cocaine at doses of 10 or 30 mg/kg twice a day on E15 to 20 or 3 mg/kg twice a day on E15 to postnatal day (PN) 5 and found decreased scent marking in males at the two lower doses, but not at the higher dose. They also found decreased intromission latencies, increased plasma leutinizing hormone (LH) levels, and short-term decreases in estradiol and testosterone levels. These data may be important as they suggest that cocaine may have hormonal effects during critical stages of sexually dimorphic CNS organization. Such influences could lead to long-term alterations in sex-related behaviors such as scent marking. The observation of an apparent biphasic effect of cocaine dose, with effects seen at 3 and 10 mg/kg but not at 30 mg/kg, is intriguing, but must be interpreted with caution until it can be replicated.

Sobrien and colleagues (1990) administered a dose of 20 mg/kg of SC cocaine on E14 to 20 and found increased surface righting, cliff avoidance, acoustic startle development, and an attenuated increase in locomotor activity after acute challenge doses of either d-amphetamine or cocaine. The surface righting findings are at variance with the other data for cocaine's effects on surface righting and are the opposite of Henderson and McMillen's (1990) findings mentioned above, who found decreased development of this reflex. It is not possible to resolve these discrepancies until more laboratories have examined the righting reflex of in utero cocaine-exposed offspring. However, with three studies reporting no effects, one an increase, and one a decrease, it appears that cocaine has no major effect on the development of this reflex. As noted, Sobrien and

colleagues also found accelerated cliff avoidance and startle development. Similar effects have not yet been reported by other laboratories. Diminished stimulant-induced locomotor activity could be a significant finding and should be further investigated.

Church and colleagues (1991) reported that 30 mg/kg SC cocaine twice a day on E7 to 20 induced reductions in locomotor activity in the exposed progeny at 20 days of age. Although direct comparisons are difficult because of methodological differences, this observation is certainly not consistent with that of Henderson and McMillen (1990), who reported increased cocaine-related activity at 30 days of age. Although the activity measuring devices were similar, length of testing (15 minutes versus 6 hours) and age at testing (20 versus 30 days of age) differed between the two studies. How such discrepancies can be resolved is unclear given the general reluctance in this field for investigators to replicate each others' findings. Heyser and colleagues (1992*b*) have reported that progeny exposed to 40 mg/kg of SC cocaine administered to the dam on E7 to 19 exhibit decreased conditioned place preferences (CPP) measured as time spent in one of three chambers, and concomitantly display an increase in the number of chamber entries during testing. In other words, the cocaine-exposed progeny did not remain in the chamber previously associated with acute cocaine exposure as long as controls and moved between chambers more often, resulting in higher chamber entry counts. The place preference effect was complex; however, inasmuch as the effect occurred most clearly in comparison with ad libitum fed controls, a comparison may not be informative. The more important comparison, with pair-fed controls, was only significant under very specific circumstances. The cocaine-exposed group differed from pair-fed controls only at a conditioning dose of 5 mg/kg of cocaine when a black test chamber was used, but a similar reduction occurred only at a conditioning dose of 2 mg/kg of cocaine when a white chamber was used. The other groups, the 2 mg/kg black test chamber group and the 5 mg/kg white test chamber group, performed no differently from controls. This unusual conditioning-dose dependency by test chamber interaction is difficult to explain and raises questions about the robustness of the effect. Should others find the same effect, it may eventually prove to be important, but until then this effect should be viewed as preliminary.

Johns and colleagues (1992*a,* 1992*b*) reported that rats administered SC cocaine at a dose of 15 mg/kg twice a day on E1 to 20 (daily group) or E2 to 3, 8 to 9, 14 to 15, and 19 to 20 (intermittent group) exhibited increased initial activity at 30 days of age (daily group), decreased

nocturnal activity (intermittent group) during 24-hour testing, increased nonentries (daily group) (i.e., instances of not leaving the start chamber in a separate short-term open-field test), and increased section crossings (intermittent group) in the open field. The increased day 30 initial activity in the daily exposure group is consistent with the data of Henderson and McMillen (1990) and should be confirmed by further experiments. The change in nocturnal activity could have implications for a cocaine effect on the circadian clock. Weaver and colleagues (1992) have reported that cocaine can alter c-fos expression in the supra-chiasmatic nucleus after prenatal exposure. Moreover, this effect can be blocked by pretreatment with the dopamine (D) type 1 (D_1) antagonist SCH-23390. If cocaine is capable of interfering with the biological clock by acting on dopamine receptors critical for early circadian entrainment, this could represent an important finding.

Bilitzke and Church (1992) reported that 40 mg/kg SC cocaine twice a day on E7 to 20 reduced swimming immobility time on a Porsolt swim test. The Porsolt swim test was developed as a screening procedure for drugs with potential antidepressant activity. Why prenatal cocaine might induce an effect consistent with the acute effects of antidepressants is not clear, but the effect appeared clear cut and represents a promising lead worthy of further investigation. If the underlying effect of prenatal cocaine exposure is to increase reactivity, then reduced immobility might be seen as a reflection of exaggerated responsiveness to stress.

Goodwin and colleagues (1992) reported that 40 mg/kg of cocaine administered SC to dams on E7 to 19 produced offspring showing decreased odor conditioning at 7 days of age, no changes in auditory or odor conditioning at 17 days, and decreased shock-induced aggression as adults. This experiment included a fostering/cross-fostering design intended to help factor out the direct effects of cocaine on the offspring from those occurring indirectly through changes in maternal rearing. One might suspect that maternal rearing could be altered by maternal exposure to a drug such as cocaine. When the maternal rearing variable was factored in, the findings on day 7 odor conditioning showed that cocaine-exposed offspring raised by cocaine-exposed dams showed no evidence of odor conditioning when given 2, 3, or 4 training trials. This finding was in contrast with controls that showed good conditioning under all three training trial conditions. Cocaine-exposed offspring raised by untreated foster dams, however, did not show evidence of odor conditioning when given 2 or 3 training trials but did when given 4 trials, indicating that being raised by a control dam ameliorated the severity of the odor

conditioning impairment. This finding raises the important point that studies which fail to control for maternal carryover effects from cocaine exposure may overestimate the drug's effects on the progeny.

However, it would be imprudent to extrapolate these data too far. First, this (Goodwin et al. 1992) is the only study reporting such a maternal modifying effect (and the only one that has systematically looked for one as well). The effect on odor conditioning was the only behavior on which a maternal-base modifying influence was found, and no other measured responses showed such modification. The magnitude of the maternal influence on odor conditioning was not great, and does not suggest a major interpretational problem exists due to maternal effects. While some effects, such as this one, may be exacerbated by allowing cocaine-exposed dams to rear their own offspring, other effects may go in the opposite direction. No conclusions concerning over- or underestimation are possible based on the limited data currently available. For shock-induced fighting, maternal rearing did not alter the outcome for in utero cocaine-exposed offspring; under both rearing conditions, cocaine-exposed offspring exhibited reduced aggression latency. Therefore, the effect on offspring aggression appeared to be a direct (maternally unmodified) effect. However, this effect was also not very large, being insignificant by standard ANOVA methods and only significant by reliance upon preplanned comparison tests. While the design of the experiment may imply a rationale for an a priori comparison test such as the one performed on these data, it is not clear whether this comparison was explicitly preplanned or merely deemed logical; the report does not discuss this point.

Meyer and colleagues (1992) reported that 20 mg/kg SC cocaine twice a day on E10 to 19 to dams induced a failure of the normal increase in wall climbing by the offspring caused by an acute dose of cocaine when given at 11 days of age. This effect occurred at mid and low cocaine challenge doses, but was not apparent at the higher dose tested. These results may bear some resemblance to Spear and colleagues' (1989*a*) finding of reduced shock-induced wall climbing in cocaine-exposed progeny, but with only two such studies published, comparisons are difficult. An idea raised by these data, however, is whether cocaine exposure leads to postnatal changes in the offspring's response to stimulation. Altered responsiveness may occur regardless of whether the signal administered to the organism is a stimulant such as a cocaine challenge or an environmental stimulation such as shock. These two studies' data provide a hint

that this may be the case, but the findings thus far are too limited to reach any conclusions.

Overview of Behavioral Findings

The positive behavioral teratologic experiments reviewed here provide little convergence of findings, but they provide leads worthy of further investigation. Areas that appear promising are cocaine's possible effects on critical organizing events of sexually dimorphic differentiation (Raum et al. 1990; Vathy et al. 1993), cocaine's possible effects on circadian clock entrainment (Weaver et al. 1992), cocaine's effects on early classically conditioned odor learning (Goodwin et al. 1992), and cocaine's possible long-term effects on adult cognitive capacities (see below). These areas are especially relevant to the long-term effects of cocaine on offspring behavior and could have direct relevance to understanding the risks this drug poses to children exposed prenatally.

Another point revealed by the data summarized in tables 1 and 6 and the study reported below is the number of sex-specific effects reported in cocaine-exposed offspring. Future research in this area should evaluate and separately report findings in terms of male-female differences whenever differential effects are encountered, as this information may prove to be a crucial distinction for understanding cocaine's developmental neurotoxicity.

Clear deficiencies are evident in the existing literature. These gaps in knowledge need to be rectified by inclusion of better control groups, replication of findings within and across laboratories, greater scrutiny and diversity in the parameters of the models being testing (including dose, dose rate, route of administration, and pattern of internal dose), and extension of the models to other species. Greater attention to these areas should permit the field to discern the correct relationship between prenatal cocaine exposure and possible long-term effects on the brain and behavioral development.

An Alternate Model

In order to illustrate one alternate approach to modeling the effects of prenatal cocaine exposure, the results of a preliminary experiment that has not been previously published are described (Vorhees et al., in press). Because of the short half-life of cocaine discussed earlier, the author decided to administer cocaine 5 times per day at 2-hour intervals instead

of once or twice a day as all of the models reviewed herein have done. SD rats were mated in the laboratory (plug day was considered E0) and assigned on a weight-matched basis to one of four treatment groups. Cocaine (20 mg/kg) was administered SC five times a day on days E7 to 12 or days E13 to 18. Controls were injected with vehicle on days E7 to 12 or days E13 to 18 and pair fed to one of the matched cocaine-exposed dams. The dose of cocaine was expressed as the free base and was dissolved in a citrate buffer in a dosing volume of 6.67 mg per milliliter (mL) at pH 5.0 A stability test performed on this solution by gas chromatography mass spectrometry confirmed what the literature reported: cocaine is stable for more than 1 week in solution at a pH of 5.0. On the day of birth (P0), litters were randomly culled within sexes to 4 males and 4 females per litter when possible. Litters were weaned on P28.

On days P9, 11, and 13 all offspring were tested for olfactory orientation to their home cage bedding. On P10, 12, and 14 they were tested for early crawling and pivoting in a small photocell activity monitor designed for mice (1 minute a day). After weaning, 2 males and 2 females were retained. On P50 to 51, all remaining offspring per litter were tested for acoustic and tactile startle in a prepulse inhibition paradigm. The interstimulus interval (ISI) was 50 milliseconds (ms), the signal was 115 decibels (A scaled) (dB(A)), and the signal duration was 20 ms. On P53 each rat received a single 30-minute test in a photocell activity monitor. On day 58 each rat was tested in a 150 centimeter (cm) straight swimming channel for swimming ability and motivation to escape from water. This swim test was followed by testing in a Morris hidden platform maze. Rats received 2 days of acquisition (8 trials/day) to find the hidden platform followed by 4 test trials on the third day to assess memory in the absence of the platform.

After the test trials were completed, the platform was replaced and 4 additional reinstatement trials were given. On the fourth and fifth test days rats were given 8 trials per day with the platform shifted to the opposite quadrant. Finally, 1 week after the completion of the Morris maze, rats received testing in the Cincinnati multiple-T water maze, 2 trials per day in what is referred to as path B (the elective-choice path of the maze).

All the data in this experiment were analyzed using litter, not individual, offspring as the unit of analysis (litter mean stratified by sex, with sex used as a within-subjects factor) by analysis of variance. There were 12 litters in each of the 2 cocaine-exposed groups and 8 each in the PF

groups for a total of 40 litters in the experiment or approximately 320 offspring for the preweaning tests and 160 for the postweaning tests.

Two sets of results are discussed to illustrate those tests that showed cocaine exposure-related findings. First, regardless of exposure period, cocaine-exposed offspring exhibited delayed olfactory orientation as neonates compared to controls. The effect was not strictly significant, occurring at $p < 0.07$ (see figure 1). In the Morris maze, a significant effect of cocaine was found for the early treated group among females, with the cocaine-exposed animals having longer search latencies on the first 8 (day 1) of the 16 acquisition trials compared with PF controls. Why this effect was only seen among females is not yet known. The effect is illustrated in figure 2. A related effect was found on another maze test (not shown).

The effect of cocaine exposure on performance in the Morris maze, while not large, is nevertheless in contrast with the findings reported by Riley and Foss (1991*b*) who found no effect of prenatal cocaine exposure on Morris maze acquisition. What could account for this difference? Two possibilities worth considering are differences in dose rate and exposure period. Riley and Foss administered 60 mg/kg of cocaine once a day by gavage on days E13 to 20, whereas the author administered 20 mg/kg of cocaine 5 times per day on days E7 to 12 in the affected group. In the Riley and Foss study, cocaine exposure was during late embryogenesis and fetogenesis while exposure in the author's study was during organogenesis. In Riley and Foss' study, the once a day exposure undoubtedly produced a single short-lived daily peak whereas the author's experiment produced 5 smaller peaks spread throughout the diurnal phase. The difference in outcome may be due to differences in peak concentration, duration of exposure per day, or to the stage of development cocaine exposure occurred. Each of these factors is sufficiently important pharmacologically and embryologically to easily account for differences in outcome. This kind of alternative modeling that may prove beneficial to understanding cocaine's potential for inducing developmental neurotoxicity.

FUTURE DIRECTIONS

Although this chapter does not review postnatal cocaine exposure or teratological or neurochemical studies beyond what is shown in table 1, it should be stressed that many of these experiments have generated

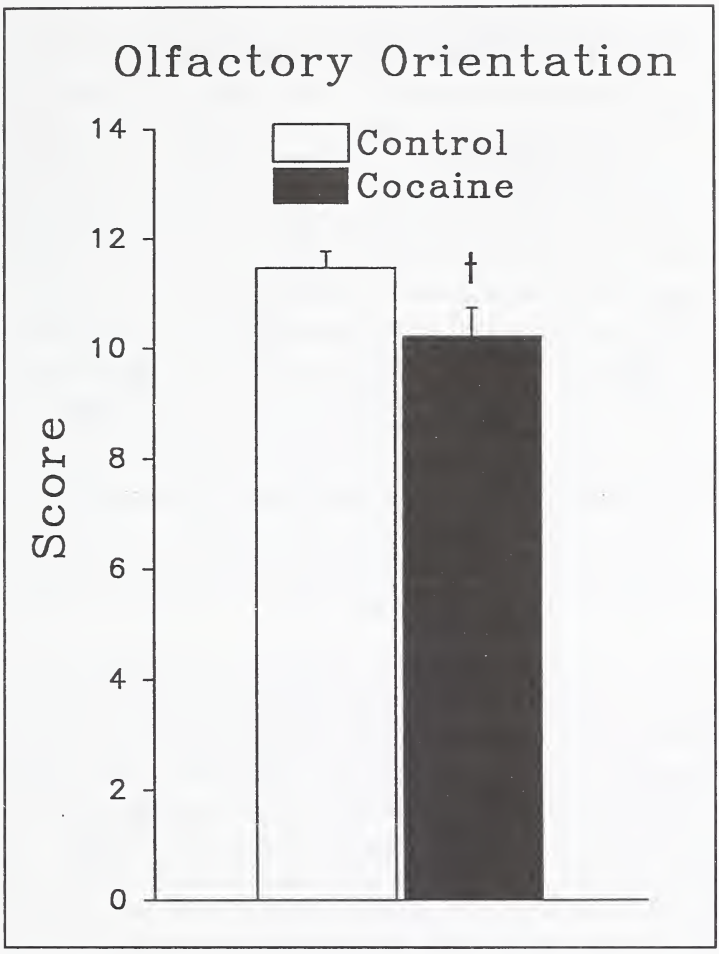

FIGURE 1. *Effect of prenatal cocaine exposure (20 mg/kg five times a day) on olfactory orientation scores of offspring on days P9, 11, and 13 in response to the scent of home cage versus clean bedding in a two-choice task. Analysis of variance showed a main effect of treatment with a probability of $p < 0.07$. Exposure period (E7-12 or E13-18), sex, and day of testing were not significant nor were the interactions between these terms. $p < 0.07$ compared to control.*

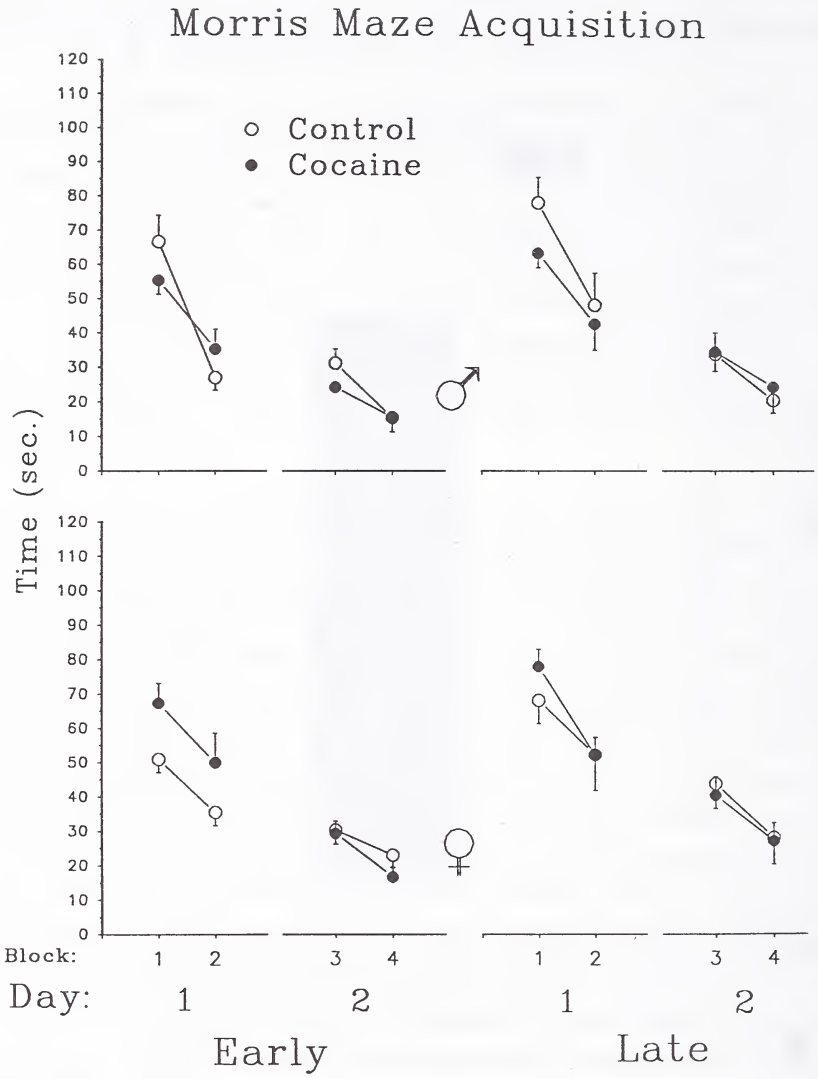

FIGURE 2. *Effect of prenatal cocaine exposure compared to pair-fed controls on acquisition (latency by seconds) on the Morris hidden platform maze. A 2-between (treatment, exposure period), 3-within (day, trial, sex) ANOVA on latency data showed a significant treatment - trial - sex interaction ($p < 0.02$) and a significant main effect of exposure period ($p < 0.02$). As shown, the female cocaine-exposed group had longer latencies on day 1 (particularly trials 2-8) than their pair-fed controls. Data are plotted in blocks of 4 trials each.*

interesting evidence of cocaine-related developmental effects. Of these studies, those producing the least evidence of major effects have been the teratological experiments. In general, the teratological experiments indicate that cocaine does not induce major dysmorphic effects in rodents. By contrast, some of the most promising data have been those (Dow-Edwards et al. 1989, 1993; Hughes et al. 1991) (table 1) showing that early postnatal exposure induces autoradiographic evidence of long-term changes in 2-deoxyglucose activity and concomitant changes in startle and locomotor activity. Perhaps more research should be directed towards late gestational events in the case of cocaine, since dopaminergic neurotransmitter release and postsynaptic receptor function are relatively late events in brain development. Given cocaine's known effects on dopaminergic systems, it is logical to believe that cocaine may have more pronounced effects on these systems during synaptogenesis (dendritic arborization and pruning) than during earlier stages when proliferation and migration are the dominant events. The early stages of ontogeny should not be neglected, but both logic and data now suggest that greater attention to later stages of development may be warranted.

In sum, although the experimental evidence of cocaine's effects on early CNS development remains unclear, there is gradually mounting evidence suggesting that some specific effects may exist. It appears that early investigations looking for gross dysmorphogenesis, increased rates of embryonic death, or severe behavioral impairments have not been borne out. However, as recent investigations have focused on specific systems that cocaine might be expected to affect such as dopamine receptors important in setting circadian rhythms or interactions with hormonal priming agents such as testosterone, it has become apparent that subtle but potentially important effects may in fact be present. These newer lines of research, along with better exposure models, should produce new insights into the developmental effects of cocaine.

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ACKNOWLEDGMENT

The author's development of this manuscript was made possible by support from Public Health Service grant DA06733 from the National Institute on Drug Abuse.

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The Effects of Prenatal Cocaine Exposure on Subsequent Learning in the Rat

Edward P. Riley and Michael H. LaFiette

INTRODUCTION

The purpose of this chapter is to examine data obtained with various animal models of prenatal cocaine exposure on subsequent learning abilities. There are two caveats that need to be mentioned, however. The first is that the term "learning" is rather loosely applied and interpreted, and in fact some of the effects mentioned may be due to performance-related deficits, memory impairments, or some other dysfunction. The authors hope that the reader will not be too critical of this loose interpretation at this stage of the inquiry into the effects of prenatal cocaine exposure. The second caveat is that some studies (e.g., those involving conditioned place preference) are not included in this chapter because they are covered elsewhere in this monograph (Spear, this volume). Experimental animal studies of the effects of prenatal cocaine exposure on subsequent behavior are relatively recent, and there are few published studies assessing the effects of prenatal cocaine exposure on learning. Therefore, this chapter first presents the findings of each study individually and then provides an overall summary and conclusions. A summary of these studies and their findings is given in table 1.

THE DATA

In one of the first studies on the effects of prenatal cocaine on subsequent behavior, Spear and colleagues (1989*b*) examined the acquisition of first-order appetitive odor conditioning. In this study, pregnant Sprague-Dawley rats were given daily subcutaneous (SC) injections of 40 milligrams per kilogram (mg/kg) of cocaine hydrochloride (C40) or an equal volume of saline (C0) on gestational days (GDs) 8 to 20. Animals in these two groups also received liquid diets as their sole source of nutrition from GD-6 until birth so that pair-feeding could be easily done. In this case, on each day of pregnancy a C0-treated animal was fed the

TABLE 1. *Studies that have examined the effects of prenatal cocaine in learning tasks. Types of learning tasks are presented in alphabetical order. Studies are presented by year of publication.*

Type of learning task study	Strain	Dosage (mg/kg/day)	Route of administration	Postnatal age (days)	Deficit
Appetitive classical conditioning					
Odor-milk					
Spear et al. 1989*b*	SD	40	SC	7	Yes
Odor-cocaine					
Heyser et al. 1992*a*	SD	40 (dam)	SC		
		2 (pup)	IP	7	Yes
		5 (pup)	IP	7	No
		10 (pup)	IP	7	No
Aversive classical conditioning					
Odor-shock					
Spear et al. 1989*a*	SD	40	SC	7	No
				17	Yes
				18	Yes
Heyser et al. 1990	SD	40	SC	8	Yes
				12	No
				21	No
Goodwin et al. 1992	SD	40	SC	7	Yes
				18	No
Sound-shock					
Goodwin et al. 1992	SD	40	SC	17	No

KEY: SD = Sprague-Dawley rats; LE = Long-Evans rats;
SC = subcutaneous injection; GV = gavage; IP = intraperitoneal.

TABLE 1. *Studies that have examined the effects of prenatal cocaine in learning tasks. Types of learning tasks are presented in alphabetical order. Studies are presented by year of publication (continued).*

Type of learning task study	Strain	Dosage (mg/kg/day)	Route of administration	Postnatal age (days)	Deficit
Avoidance, passive					
Church and Overbeck 1990	LE	40	SC	19	No
				80	No
		60	SC	19	No
				80	No
		80	SC	19	No
				80	No
		100	SC	19	No
				80	No
Church et al. 1991	LE	60	SC	17	No
				63	No
Riley and Foss 1991	LE	60	GV	21	No
Retention					
Church and Overbeck 1990	LE	40	SC	19	No
				80	No
		60	SC	19	No
				80	No
		80	SC	19	No
				80	No
		100	SC	19	Yes
				80	No
Avoidance, shuttle					
Smith et al. 1989	LE	10	SC	92	No
Church and Overbeck 1990	LE	40	SC	80	No
		60	SC	80	No
		80	SC	80	No
		100	SC	80	Yes

TABLE 1. *Studies that have examined the effects of prenatal cocaine in learning tasks. Types of learning tasks are presented in alphabetical order. Studies are presented by year of publication (continued).*

Type of learning task study	Strain	Dosage (mg/kg/ day)	Route of administration	Postnatal age (days)	Deficit
Conditional discrimination					
Heyser et al. 1992*b*	SD	40	SC	67	No
Reversal					
Heyser et al. 1992*b*	SD	40	SC	80	Yes
Schedules of reinforcement DRL-20					
Smith et al. 1989	LE	10	SC	94	Yes*
Schedules of reinforcement FR-10					
Heyser et al. 1992*b*	SD	40	SC	60	No
Sensory preconditioning (odor-shock)					
Heyser et al. 1990	SD	40	SC	8 12 21	Yes No No
Spontaneous alternation					
Smith et al. 1989	LE	10	SC	25	Yes

KEY: * = Although a significant drug x days interaction was found by Smith and colleagues, there was no cocaine-related deficit in acquisition of DRL behavior nor in asymptotic DRL performance. Early in training all groups obtained a comparable number of rewards; late in training cocaine-exposed subjects obtained significantly more rewards than control animals.

TABLE 1. *Studies that have examined the effects of prenatal cocaine in learning tasks. Types of learning tasks are presented in alphabetical order. Studies are presented by year of publication (continued).*

Type of learning task study	Strain	Dosage (mg/kg/day)	Route of administration	Postnatal age (days)	Deficit
Spontaneous alternation (continued)					
Church and Overbeck 1990	LE	40	SC	21	No
				80	No
		60	SC	21	No
				80	No
		80	SC	21	No
				80	No
		100	SC	21	No
				80	No
Johns et al. 1992	SD	30	SC	32	No
				35	No
				40	No
				45	No
Visual discrimination					
Smith et al. 1989	LE	10	SC	90	No
Water maze					
Smith et al 1989	LE	10	SC	134	Yes
Riley and Foss 1991	LE	60	GV	70	No
Johns et al. 1992	SD	30	SC	30	No
				60	No

amount of liquid diet consumed ad libitum by a paired C40-treated animal. This pair-feeding procedure allows for control of cocaine's effect on food intake, and thus any difference between these two groups could not be attributed to differential nutritional intake. As a further control, a nontreated (NT) group that had free access to standard lab chow and

water throughout pregnancy and was not injected was also included in the study design. At 7 days of age, 3 pups per litter (mixed sexes) had intraoral tongue cannulas inserted so that milk could be infused directly into the oral cavity. Each pup was then assigned to one of the three experimental groups.

Pups in the first-order conditioning groups (paired groups) received three consecutive trials in which they were first exposed to banana scent conditioned stimulus (CS-) for 3 minutes and then exposed to lemon scent (CS+) for 3 minutes. During the CS+ exposure, pups were infused for 5 seconds with 0.3 milliliters (mL) of half-and-half dairy cream every 30 seconds. Two control groups for first-order conditioning were used; pups in one group received milk infusions 20 minutes prior to exposure to the two odors (backward conditioning or unpaired group), and pups in the other control group received milk infusions without any exposure to the odors (unconditioned stimulus [US] only group). All groups were matched with respect to the duration of exposure to the stimuli employed.

Following the third trial, animals were given a preference test of the CS- and CS+ odors. The preference test consisted of placing the animal on a screen on the midline of two adjacent containers holding the CS- and CS+ scents, respectively. The main measure of learning was the amount of time spent over an odor for a total test time of 2 minutes. Preference tests were conducted 3 minutes after the last conditioning trial, and a test of retention 24 hours later. This paradigm has been used previously and was developed to establish Pavlovian learning in neonatal animals (Spear et al. 1982).

From an analysis of the data, the pair-fed (PF) and NT control animals that were in the paired condition spent significantly more time over the lemon odor (CS+) than the banana odor (CS-) during both the immediate and 24-hour retention test. This was the predicted outcome, since in the paired condition the CS+ was associated with milk infusion. In contrast, PF and NT animals in the nonpaired condition spent more time over the banana scent in the immediate test, although in the 24-hour retention test they spent slightly more time over the lemon odor. Cocaine-exposed animals in the paired condition also spent more time over the banana odor, the stimulus not paired with milk, than over the lemon odor in the immediate test and about an equal amount of time over both odors in the 24-hour retention test. In essence, the animals prenatally exposed to cocaine in the paired condition acted like animals from the PF and NT

groups in the nonpaired condition, demonstrating no learning of the odor-milk association.

In terms of statistical significance, an analysis of variance (ANOVA) of difference scores (time over CS+ minus time over CS-) indicated significant main effects of prenatal treatment (C40, C0, NT), condition (paired, unpaired), and retention interval (immediate, 24 hour). However, in order to show a differential effect of prenatal treatment on learning, the interaction of prenatal treatment by condition should have been significant. In the ANOVA this interaction did not reach statistical significance ($p < 0.088$), and thus the authors used planned comparisons to statistically verify that the cocaine-exposed animals were deficient in learning the odor association. These planned comparisons were conducted on paired and unpaired difference scores within each treatment group at each retention interval. These tests revealed no significant differences between paired and unpaired C40 animals at either retention interval. Data from paired and unpaired NT animals were significantly different on the immediate and 24-hour retention tests, while the PF paired and unpaired groups were different only on the immediate test. This difference between the PF and NT animals at the 24-hour retention interval may reflect some effect of the feeding regimen.

In the study by Spear and colleagues (1989*b*), cocaine-exposed animals showed a significant lack of association between milk reward and odor only when planned comparisons were conducted. The use of planned comparisons and in particular the number of such comparisons that should be made in breaking down a nonsignificant interaction has been debated statistically, since there is no control for within-experiment error rates. However, these data suggest an effect of prenatal cocaine on subsequent odor association learning in young rat pups.

It should also be mentioned that in discussing their data, the authors (Spear et al. 1989*b*) argue convincingly that this lack of associative learning is the result of a disruption in learning and not due to some nonassociative factor. For example, the animals exposed to cocaine did not experience any meaningful alteration in physical development nor did these cocaine-exposed animals appear to have any difficulty detecting or discriminating the CS+ and CS- odors.

Spear and colleagues (1989*a*) reported similar preliminary data using an aversive conditioning, rather than an appetitive conditioning, paradigm. Although the details are sketchy, animals were tested on postnatal days

17 and 18 and these animals were littermates of those used in the Spear (1989*b*) study. Animals exposed to footshock in the presence of a particular odor evidenced an aversion to that odor in subsequent preference tests either immediately or 1 hour after conditioning. Animals exposed to cocaine prenatally showed an attenuated association relative to pair-fed and untreated controls. In that review, however, the authors do mention that in another experiment there was no evidence of an odor-shock conditioning deficit in 7-day-old animals. As stated by the authors, animals from all prenatal treatment conditions demonstrated excellent conditioning and memory. The reason that 7-day-old animals did not evidence the cocaine-related attenuation of odor conditioning found in 17- to 18-day-old animals is not addressed by the authors, and it is surprising in light of the findings of Heyser and colleagues (1990).

The Spear laboratory has further studied early odor association learning following prenatal cocaine exposure, hypothesizing that one possibility for the deficit in the cocaine-exposed animals might be due to a delay in maturation. This possibility was addressed in a study by Heyser and colleagues (1990) in which they examined sensory preconditioning in young rats prenatally exposed to cocaine. In sensory preconditioning, one element of a compound stimulus acquires strength as a conditioned stimulus (CS) only after the other element of the compound stimulus is explicitly paired with a US. Interestingly, young animals between 8 and 17 days of age readily exhibit sensory preconditioning while older animals do not. Thus, if there is a maturational delay following cocaine exposure, these cocaine-exposed animals should demonstrate sensory preconditioning at older ages than nontreated control animals. If the previously noted deficits were due to a cognitive dysfunction rather than a general developmental delay, then this would not be the case.

In the standard sensory preconditioning paradigm, two neutral stimuli (CS1 and CS2) are paired together, then one element of the compound (e.g., CS2) is paired with a US. In the test of sensory preconditioning, the other element of the compound (e.g., CS1) is presented alone. If sensory preconditioning has taken place, CS1 will elicit a conditioned response (CR) comparable to the one established during CS2 and US pairings.

Offspring from Sprague-Dawley rats given SC injections of 40 mg/kg cocaine on GDs 8 to 20 and control rats given injections of saline began testing at 8, 12, and 21 days of age. Both groups were allowed free access to lab chow and water. As in all of the studies from this lab, only

one subject per litter was assigned to any given experimental group at each age. Furthermore, subjects were fostered to untreated dams to preclude any effects of being raised by a dam treated with cocaine during pregnancy.

In addition to examining sensory preconditioning, this study (Heyser et al. 1990) included an experiment assessing first-order conditioning in which a lemon odor was paired with footshock (almond CS-, lemon CS+). The preference test consisted of a test between the lemon odor and a novel orange odor. There were paired and unpaired groups, similar in design to the control groups in the sensory preconditioning experiment. An ANOVA on the first-order conditioning groups indicated that at 8 days of age there were significant effects of prenatal treatment, condition (paired and unpaired), and a treatment x condition interaction. Post-hoc tests revealed that there was no difference between cocaine-exposed paired and unpaired groups, indicating that cocaine exposure disrupted the odor aversion association. There were significant differences between paired and unpaired saline-injected control animals; the pairing of lemon odor with shock caused the paired group to spend a greater amount of time over the novel orange odor relative to unpaired subjects. When animals were trained and tested at 12 and 21 days of age, there were significant conditioning effects, but these did not interact with prenatal treatment. This finding indicates that the disruption in odor-aversion learning seen in 8-day-old cocaine-exposed animals was not apparent by 12 days of age.

In the sensory preconditioning experiment of this study, the sensory preconditioning (SP) group had a banana odor (CS1) simultaneously paired with a lemon odor (CS2) for 3 minutes. The lemon odor was then paired with footshock (0.5 milliampere [mA] for 3 seconds) in a procedure in which animals were placed in a chamber containing the lemon odor and then received two shocks. Following a 1-minute intertrial interval (ITI), this shock session was repeated. The preference test was then given in which the animal could spend time over the banana odor (CS1) or a novel orange odor. Two controls were included to assess conditioning effects. If sensory preconditioning occurred, then it would be expected that the animals would avoid the banana odor given its previous association with the lemon odor, subsequently paired with shock.

At 8 days of age in the sensory preconditioning experiment there were significant effects of prenatal treatment, condition, and a treatment x

condition interaction. Post-hoc tests showed that there were no differences in the amount of time spent over the CS1 odor (banana) during the test between paired cocaine-exposed animals and cocaine-exposed animals from the two unpaired conditions, indicating a lack of sensory preconditioning. Paired lab chow (LC) control animals spent significantly less time over the banana odor than did LC animals from the unpaired groups, a standard demonstration of sensory preconditioning.

At 12 days of age there was a significant main effect of condition and a significant treatment x condition interaction. As with the 8-day-old animals, paired and unpaired cocaine-exposed animals spent most of the test time over the banana odor with little difference between conditioning groups. Paired LC animals spent less time over the CS1 banana odor than unpaired counterparts, again illustrating the sensory preconditioning phenomenon. Finally, at 21 days of age there were no significant main effects or interactions on time spent over the CS1, with all groups spending most of the test time over the banana scent. Clearly, young (8 and 12 days of age) cocaine-exposed animals demonstrated a deficit relative to age-matched controls on sensory preconditioning. The cocaine-exposed animals spent more time over the CS1 stimulus than LC animals after pairings of CS2 and footshock.

Older cocaine-exposed animals (21 days of age) spent the same amount of time over the CS1 odor as paired and unpaired LC animals of the same age, indicating that there was no sensory preconditioning in older animals. Heyser and colleagues (1990) concluded that these results suggest that deficits in young animals induced by prenatal cocaine exposure in forming classically conditioned associations are not due to a maturational delay; if they were, one would have expected to detect sensory preconditioning in the 21-day-old animals. Rather, the deficits are due to some general impairment in the mechanism responsible for associating classes of stimuli.

However, the results of this study also indicate that the odor-aversion association was not learned by animals prenatally exposed to cocaine at 8 days of age, although by 12 days of age the animals appear to behave similarly to controls. This might support the role of a developmental dysfunction in these first-order conditioning effects. Furthermore, at 8 and 12 days of age, cocaine-exposed animals do not demonstrate the normal sensory preconditioning expected at these ages. Preconditioning was readily demonstrated in the control animals, thus making the use of

the sensory preconditioning paradigm for assessing developmental delays in cocaine-exposed animals problematic.

In yet another followup investigation of early associative learning in cocaine-exposed rats from the same lab, Goodwin and colleagues (1992) examined odor-aversion learning and auditory-aversion learning. Using similar prenatal treatments to those described above, young (7 days of age) rats received either 2, 3, or 4 pairings of an odor-footshock association. Animals were also tested at 17 days of age for an auditory-footshock association and at 18 days of age for an odor-footshock association.

This study also sought to determine the role of fostering in experiments involving prenatal cocaine exposure. In all behavioral teratology studies, one concern is that any effects noted in the offspring might be the result of being raised by a mother who was treated with the drug during pregnancy. This drug treatment might alter maternal behavior either directly or indirectly and, given the maternal-pup interaction, can have a significant effect on behavioral outcomes. Thus, these fostering studies are essential to determine whether behavioral alterations were the effect of direct in utero exposure to the drug. In order to accomplish this, following parturition, pups were fostered in the following manner: Offspring exposed to cocaine in utero and unexposed offspring were fostered (FOS) to untreated dams (FOS/C40 and FOS/LC, respectively) or were raised by their biological mothers (C40/C40 and LC/LC). In addition, untreated pups were fostered to dams treated with cocaine during pregnancy (C40/FOS) or to untreated dams (LC/FOS). These combinations address whether being raised by a cocaine-treated dam influences offspring behavior.

In the first experiment, olfactory conditioning commenced at postnatal age (PN) 7 using a paradigm similar to that described with footshock. Within each prenatal treatment condition there were two groups, a paired and an unpaired group. The paired group received exposure to a CS- (banana odor) for 20 seconds, immediately followed by a 20-second exposure to CS+ (lemon odor) plus two 3-second, 0.5 mA shocks. Subjects received either 2, 3, or 4 such trials with an ITI of 1 minute. Animals in the unpaired condition received 4 trials of footshocks (8 total shocks) 20 minutes prior to exposure to both the CS- and CS+.

Following these exposures, subjects were returned to a holding cage for 3 minutes prior to the test of conditioning. Preference testing for paired

and unpaired groups consisted of placing the animal on the midline between the CS+ odor and a novel odor (orange) for 3 minutes. The main dependent measure was time over each odor.

For the younger animals, the results revealed significant effects of group, condition (paired versus unpaired), and a prenatal treatment x condition interaction. Subsequent tests indicated that animals prenatally exposed to cocaine did not evidence any conditioning, spending as much time over the CS+ as unpaired animals. Fostering among animals prenatally exposed to cocaine also had an effect. Animals prenatally exposed to cocaine and fostered to nontreated dams (FOS/C40) learned the association when given 4 training trials, but not when given only 2 or 3 trials. Animals not exposed to cocaine evidenced good conditioning regardless of the number of trials and regardless of their rearing conditioning. Thus, it appears as if prenatal cocaine exposure had an effect on subsequent conditioning, and this effect could be exacerbated by rearing the animal with a mother treated with cocaine during pregnancy.

Olfactory conditioning was also conducted at 18 days of age. Aversive odor conditioning for the 18-day-old animals consisted of 30 seconds exposure to the CS- odor (almond) and 30 seconds exposure to the CS+ odor (methyl salicylate) plus two footshocks (1.6 mA, 2 seconds in duration). In the unpaired condition, footshock was administered 20 minutes prior to odor exposure. Conditioning was assessed in the traditional preference test between the CS+ and a novel odor (lemon) and in a freezing test. In this freezing test, subjects were exposed to the CS+ alone and the tendency to freeze in the presence of the CS+ recorded. Both tests were given immediately after conditioning or 3 hours later (test order was counterbalanced and used as a factor in the analysis). Because the order of testing (freezing versus preference) had an effect on the demonstration of conditioning, only data from subjects that received the preference test first are germane to this discussion.

The analysis of the 18-day-old animals data in the normal preference test indicated that there were significant main effects of condition (paired versus unpaired) and time (immediately versus 3 hours) and the condition x time interaction approached significance ($p < 0.07$). Basically, animals in the paired groups tested immediately after conditioning spent less time over the CS+ than unpaired animals. Importantly, there was no effect of prenatal cocaine exposure or of fostering, nor did these factors interact with any other factors. In the freezing test, there was a significant effect of condition, with subjects who had received the odor-footshock pairings

freezing more than unpaired controls. In neither the preference test nor the freezing test did prenatal cocaine exposure or rearing history have any significant influence.

In another experiment within the Goodwin and colleagues (1992) study, auditory conditioning at 17 days of age was examined. Conditioning consisted of placing the animals in a distinct chamber and pairing shock (1 mA for 0.5 seconds) with a tone CS+ (15 seconds of a pulsing tone). There were two conditioning groups, one of which received shock beginning at the offset of the CS+ (0 interstimulus interval [ISI]) and another in which shock onset occurred 20 seconds after the CS+ terminated (20-ISI). Various control groups were also included and testing for conditioning consisted of measuring the suppression of activity induced by the CS+. Testing occurred 24 hours after the last conditioning trial. An analysis of the test data indicated a significant effect of conditioning, with animals in the 0-ISI group demonstrating a greater suppression of activity than the 20-ISI group or the control groups. Again, there was no significant effect of prenatal cocaine exposure or of fostering condition, nor did these factors interact with any other factor.

In summary, the Goodwin and colleagues (1992) study demonstrated that very young rats gestationally exposed to cocaine required more trials to learn an odor-shock association than controls. This effect was exacerbated by being reared by a dam who had been treated with cocaine during pregnancy. In the 17- and 18-day-old animals, the tests were sufficiently sensitive to demonstrate conditioning, but in both the odor-shock and auditory-shock associations, the learning ability of the animal was not compromised by gestational cocaine exposure.

Another group has also examined first-order Pavlovian learning in animals prenatally exposed to cocaine using a less traditional unconditioned stimulus. Heyser and colleagues (1992*a*) examined preference for lemon versus orange scent following pairings of lemon odor and acute administration of cocaine hydrochloride. Subjects were offspring of Sprague-Dawley rats given injections of 40 mg/kg cocaine on GDs 8 to 20 (C40), a nutritional control group that was given free access to a diet composed of cellulose and lab chow and injected with saline on GDs 8 to 20 (NC), and an untreated lab chow control group (LC). At 6 days of age, male and female subjects were exposed to an orange scent for 5 minutes, given an SC injection of saline, and returned to the orange odor for an additional 25 minutes. The next day, subjects were exposed to lemon scent for 5 minutes and then given an SC

injection of either 0, 2, 5, or 10 mg/kg of cocaine. Following injections of either drug or saline, subjects were exposed to the lemon scent for an additional 25 minutes. Twenty-four hours after conditioning, subjects were given a 6-minute preference test between lemon scent (CS+) and orange scent (CS-).

Initial analyses of the study data indicated that there were no sex-related differences, thus, data were collapsed across sex. An ANOVA indicated significant effects of prenatal treatment, dose, and a prenatal treatment by dose interaction. In this study a cocaine-induced odor preference was defined as a significant increase in time spent over the lemon odor relative to the time spent over the lemon odor by saline-injected animals in the same prenatal treatment group. C40-treated subjects injected with 2 mg/kg cocaine spent about as much time over the lemon odor as saline-injected control C40 animals. However, C40 animals injected with 5 or 10 mg/kg of cocaine spent significantly more time over the lemon odor than saline-injected C40 subjects. NC and LC subjects injected with cocaine spent more time over the lemon odor after all doses than did NC and LC saline-injected control animals, indicating that prenatal control animals did not have a deficit in learning. One possible explanation for the deficit noted in C40-treated offspring is that prenatal cocaine exposure adversely affected brain reward systems, rendering cocaine less effective as a reinforcer for C40-treated animals than for NC or LC subjects.

In another assessment of cocaine's behavioral teratogenicity, Heyser and colleagues (1992*b*) examined adult rats prenatally exposed to cocaine for the acquisition and reversal of a conditional discrimination using odor cues. Pregnant Sprague-Dawley rats were given SC injections of 40 mg/kg cocaine on GDs 8 to 20. The control groups consisted of pair-fed saline-injected animals, a saline-injected nutritional control group that received a cellulose/lab chow diet, and a nontreated lab chow group. Pups were cross-fostered to surrogate dams until weaned. Only male offspring were tested beginning at 60 days of age.

The learning task employed was a conditional discrimination task. Animals were first trained to lever press on two levers in a conditioning chamber, one on the right side and the other on the left side, for food reward. A fixed-ratio 10 (FR-10) schedule was employed so that 10 responses were required prior to reward. Once stable responding on the two levers occurred, conditional discrimination training commenced.

In the initial acquisition phase, either banana odor or almond odor was present in the conditioning chamber on a given training day, although neither odor was presented for more than 2 consecutive days. On those days when the odor was present, only one of the two levers was active; the lever that produced food depended on which odor was present. For half of the subjects, banana scent was present when the right lever was active and almond odor was present when the left lever was active; these contingencies were reversed for the other half of the subjects. Sessions were 20 minutes in duration.

After acquiring the discrimination, a reversal phase was begun in which the animal had to learn the opposite discrimination from that learned during acquisition. The criterion for the initial odor discrimination was 80 percent or more correct responses in the first 10 responses of a session and 90 percent or more correct responses over the entire session for 5 consecutive days.

There were no differences between any of the groups in the number of sessions required to learn the FR-10 lever-pressing response. Similarly, there were no group differences in the number of sessions required to learn the original conditional discrimination. However, during the reversal phase, the cocaine-exposed offspring required significantly more sessions to learn the discrimination than the control groups (approximately 33 versus 42 sessions for controls and C40-treated animals, respectively) and importantly there were no differences between groups in response rates.

Two discrimination indices (DI1 and DI2) were used to assess the discrimination and determine when the criterion had been reached. DI1 was a percentage of the number of correct responses among the first 10 responses of a session. DI2 was the percentage of correct responses among all responses during a session. In assessing the responses during the 5 days prior to reaching the criterion during acquisition and reversal, there was a significant prenatal treatment x phase interaction. Again, during reversal but not during the original acquisition of the discrimination, C40-treated animals differed from controls.

From the data provided (Heyser et al. 1992*b*), it appears that the controls made about 90 percent of their first 10 responses on the correct lever during the reversal phase compared with about 85 percent correct responses by the C40-treated animals. There were no differences in DI2 between the prenatally treated groups during the initial discrimination or

during the reversal phase. From the error data provided (Heyser et al. 1992*b*, figure 4, p. 842), it appears as if the C40-treated animals did indeed make about 1.5 more responses on the incorrect lever compared with about 1 response by the controls prior to the first reward, which required 10 correct responses (FR-10).

In another paper frequently cited as showing the behavioral teratogenicity of cocaine, Smith and colleagues (1989) reported effects of prenatal cocaine exposure on differential reinforcement of low rate (DRL) performance and on performance in a water maze. This was a rather large study that examined numerous learning tasks. In this study, Long-Evans rats were treated with daily SC injections of 10 mg/kg cocaine on GDs 4 to 18. Control animals received injections of saline, and all animals had ad libitum access to lab chow and water. In this study, spontaneous alternation was examined in a T-maze on postnatal days 25 to 45. Bar pressing for food reward was tested on a DRL schedule of reinforcement. Food delivery was dependent on an inter-response time greater than or equal to 20 seconds (DRL-20 seconds). Subjects were 94 days of age at the start of training. A visual discrimination (light/dark) task was included, in which bar-presses during the presence of illuminated cue lights was rewarded. Subjects were 90 days of age at the start of training. Two-way shuttle avoidance was tested, in which an auditory cue signaled, and was paired with, footshock. The animal could avoid the shock by moving from one compartment of the shuttlebox to the other during the CS-US interval. Subjects were 92 days old at the start of testing, which consisted of 30 trials per day for 5 days. The study included a water-maze task in which the latency to find a platform submerged in opaque water was measured. Subjects had been exposed to the DRL-20 task and were between 134 and 137 days old when testing in the water maze began.

Smith and colleagues (1989) found no effects of prenatal cocaine exposure on shuttle avoidance or on the visual discrimination problem. However, significant effects (which Smith and colleagues state support the behavioral teratogenicity of cocaine) were found in the spontaneous alternation task, the water maze, and the DRL test. In the test of spontaneous alternation, the cocaine-exposed males alternated less on the second trial than controls, although there were no differences among females. In the DRL experiment an ANOVA indicated a significant prenatal treatment x day interaction, which appears to be due to the cocaine-exposed animals obtaining more reinforcers than control animals with increasing numbers of test days (33.6 + 3.52 versus 26.0 + 3.41 for

the last 5 days of testing) making more responses late in testing. However, it is extremely important to point out that no followup tests to the overall ANOVA are presented and that the significant interaction of prenatal treatment with day involved an F equal to 1.50 on 29 and 906 degrees of freedom, $p < 0.05$. It also does not appear that the authors made any statistical correction for the repeated measure design (e.g., Geiser-Greenhouse correction). Given the lack of appropriate statistical correction, the small F, and the large number of degrees of freedom, it is unlikely that this interaction accounts for much of the total variance.

The interpretation of the water-maze data presents similar problems. In this case, there is a significant three-way interaction ($F(18,396) = 2.71$, $p < 0.0005$) involving prenatal treatment x day x trial for latencies to escape in the maze. According to the authors, the interaction is due to the cocaine-exposed offspring taking longer on the early trials during day 1 of testing, although no supporting tests are presented. Although sex does not appear to have been a significant factor in the study, there was a trend ($p < 0.1$) for sex to interact with prenatal treatment and trial. When separate ANOVA were done for each sex (no justification is provided), no significant effects were seen in females, while males showed the three-way interaction of prenatal treatment, day, and trial. Again, no tests subsequent to the ANOVA are presented to confirm these findings, nor is there any correction for the repeated measures design.

Overall, the results of study by Smith and colleagues (1989) do not provide much evidence of the adverse effects of cocaine on learning. A significant cocaine-related deficit was found only in males in the spontaneous alternation task and in the latency measure of the water maze. Males were less likely to alternate during spontaneous alternation assessment, and in the water maze the males were slower on only the first few trials on the first day of acquisition. This latter effect was considered marginal and there were no differences between groups on the error measure. On other complex cognitive tasks, visual discrimination and two-way shuttle avoidance, there were no significant effects of gestational cocaine exposure. On the DRL task, cocaine-exposed animals performed better than their untreated counterparts; this effect was marginal at best.

Other studies also do not provide much substantial evidence for the effects of prenatal cocaine on subsequent learning using traditional tasks. For example, Church and Overbeck (1990) tested offspring exposed to cocaine gestationally for spontaneous alternation, passive avoidance, and

shuttle avoidance. Pregnant Long-Evans rats were administered 40, 60, 80, or 100 mg/kg/day of cocaine, with half of the dose administered in the morning and half in the afternoon by SC injection between GDs 7 to 20. Control animals received saline injections or were untreated, and a pair-feeding procedure was employed to help control for possible nutritional effects.

One male and one female from each litter were tested for spontaneous alternation at 21 days of age and again at 80 to 90 days of age. Animals were tested in a T-maze for 5 trials or until the animal alternated to the side opposite that entered on the original trial. Other littermates were tested for passive avoidance learning at 19 days of age and again at 80 to 90 days of age. Animals were placed in an illuminated chamber and allowed to move into a darkened chamber where they received a shock (0.5 mA or 1.2 mA for the younger and older animals, respectively). This procedure continued until the animal remained in the illuminated chamber for 180 seconds on two consecutive trials. Animals were also tested for retention 48 hours after the acquisition phase. Other littermates were tested for shuttle avoidance in an automated shuttlebox at 80 to 90 days of age. Similar to the Smith and colleagues (1989) study, the animals could avoid shock (1.2 mA) by moving from one side of the shuttlebox to the other during the CS/US interval. A total of 50 trials were administered.

In the spontaneous alternation test, there were no differences between the prenatal treatment groups in the latency to enter one of the T-maze arms on the initial or second trial. Nor were there differences between groups on the number of trials prior to alternation. Thus, in contrast to the findings of Smith and colleagues (1989), there was no evidence of any differences between the groups on spontaneous alternation despite much higher doses used by Church and Overbeck (1990). A lack of group differences was also found in the acquisition of the passive avoidance response at 19 days of age. These investigators found no significant effects of prenatal cocaine treatment on the latency to enter the dark chamber or on the number of trials to reach criterion in preweanling animals. The highest dose cocaine-treated group (100 mg/kg), however, did exhibit a significant retention deficit, moving into the dark compartment somewhat faster than all other groups. It must be stressed, however, that this 100 mg/kg dose is extremely high and there was no indication of any dose-response relationship. There were no significant effects of prenatal treatment at 80 to 90 days of age on either acquisition or retention of the passive avoidance response. On the shuttle avoidance

task there was a significant treatment effect on the number of avoidances, with males exposed to the 100 mg/kg dose and their pair-fed controls having fewer avoidances than the ad libitum group. The 100 mg/kg cocaine-treated group also made fewer escapes than the ad libitum group. Females showed the same trend but these effects did not reach statistical significance.

In another investigation, Church and colleagues (1991) examined the neurobehavioral teratogenicity of gestational alcohol plus cocaine exposure with the results of the cocaine-treated only groups germane to this review. Dosing in this study consisted of daily SC injections of 60 mg/kg/day cocaine twice per day with half of the dose administered in the morning and half in the afternoon, from GDs 7 through 20. The control groups consisted of a pair-fed saline-injected group and an untreated group. Animals were assessed for passive-avoidance learning at 17 days and again at 63 to 67 days of age, and for shuttle avoidance at 63 to 67 days of age. These investigators found no significant effects of gestational cocaine exposure on any measure of passive avoidance, retention of passive avoidance, or the acquisition of shuttle avoidance.

Overall, the results of Church and Overbeck (1990) and Church and colleagues (1991) suggest that prenatal cocaine exposure had no influence on preweanlings' (17, 19 days of age) ability to learn a passive-avoidance response, nor did it inhibit the natural tendency to move from light to dark. There also does not appear to be any major effect of prenatal cocaine exposure on shuttle avoidance.

Riley and Foss (1991) also examined animals prenatally exposed to cocaine on a number of traditional tasks. They examined the acquisition of both passive and shuttle avoidance using procedures previously used to validate the behavioral effects of prenatal alcohol exposure. Besides using tasks previously shown to be sensitive to another behavioral teratogen, both of these tasks are known to be altered by prenatal hypoxia, one of the fetal insults that may result from cocaine use during pregnancy (Woods et al. 1987). Riley and Foss (1991) also examined learning and memory on a spatial task, the Morris water maze. The prenatal manipulation involved giving pregnant Long-Evans rats daily doses of 60 mg/kg of cocaine by intubation on GDs 14 to 21. The control groups either received an equal volume of saline or were untreated. For the passive avoidance task, animals were 21 days old; for shuttle avoidance they were 81 days of age; and in the Morris maze, subjects were tested between 70 and 78 days. Passive-avoidance tests

consisted of placing the animal in the white compartment of a two-compartment chamber where a lamp in the white compartment was illuminated when the guillotine door separating the two compartments was raised. If an animal crossed over to the dark compartment, the light was extinguished, the door was shut, and a 0.5-second, 0.5 mA footshock was delivered to the floor of the chamber. The animal was then returned to a holding cage for 30 seconds.

If an animal remained in the lit compartment for 180 seconds, the trial was terminated, the animal removed from the chamber, and the ITI initiated. Trials were continued until the subject remained in the white compartment for 180 seconds on two consecutive trials. Twenty-four hours after initial training, subjects were tested for retention of passive-avoidance learning. Dependent measures were the latency to cross when the door was open and the number of trials needed to reach the criterion.

In the shuttle avoidance experiment, a compound CS+ (light + tone) preceded a 0.6 mA footshock (US) by 5 seconds. Crossing from one compartment of the shuttlebox to the other during the compound stimulus terminated the stimulus and ended the trial. Crossovers (escapes) during shock terminated the shock and ended the trial. The ITI was 50 seconds. Training was conducted for 4 consecutive days with 50 trials per day. The dependent measures included the number of avoidance trials, escapes, and crossings during the ITI.

A spatial navigation task, the Morris water maze, was also used to detect the behavioral teratogenicity of prenatal cocaine exposure. The apparatus consisted of a circular tank filled with opaque water. A platform could be placed at different locations within the tank, and the top of the platform when placed in the tank was 2.5 centimeters (cm) below the surface of the water. The opacity of the water prevented subjects from visually locating the platform. Training was conducted on 3 consecutive days; the first 10 trials on each day were training trials. If the subject did not find the platform within 90 seconds of placement in the tank, the subject was placed on the platform by the experimenter. Animals remained on the platform for 15 seconds. On the 11th trial of each day and on the sole trial of day 4, the platform was removed and the animal was simply allowed to swim for 30 seconds while being videotaped for later analysis. The behavioral measure was the percentage of time spent in the area of the tank formerly occupied by the platform over a 30-second period. The other measure involved was the latency to find the platform during training.

The results of these behavioral assessments provided no indication that prenatal cocaine exposure compromised later behavior. Prenatal treatment was not a significant main effect in any of the analyses, nor did it interact with any other experimental factor. All subjects tended to decrease the number of entries into the dark chamber over trials, as well as their entry speed into that chamber, during the passive avoidance test. In shuttle avoidance, the number of avoidance responses increased over days, escapes decreased, and ITI crossovers were not systematically affected by days of training. In the Morris water maze, there was a significant prenatal effect. However, post-hoc tests revealed that there was a significant difference between the two control groups, with the cocaine-exposed subjects not significantly different from either control group.

Riley and Foss (1991) found no statistical evidence that rat offspring were adversely affected by cocaine exposure. All three tasks involved components of learning and memory, and in all tasks cocaine-exposed animals performed no differently than control groups, showing normal learning and retention of these three different tasks.

Johns and colleagues (1992) have also examined the effects of prenatal cocaine exposure on learning. In this study, pregnant Sprague-Dawley animals were exposed to 15 mg/kg cocaine SC twice daily for a total dose of 30 mg/kg/day on GDs 1 to 20 or were given the same dose on 2 consecutive days every 5 days beginning on GD 6. This latter intermittently cocaine-exposed group was meant to model "weekend users" of cocaine. Animals were assessed on both spontaneous alternation and water maze performance. Spontaneous alternation was tested in 5 massed trials on postnatal days 32, 35, 40, and 45 in a standard T-maze.

A water-maze task was conducted at 30 or 60 days of age. Basically, this water-maze test involved working memory, in that the animals were given a reference trial indicating which response (left or right) would be rewarded with escape from the maze. Five minutes after the reference trial, animals were given a test trial in which both choices (right or left) were possible and the animal had to recall the reference trial in order to make a correct response and escape the maze. Animals received four of these two trial sequences per day for 19 days.

There were no differences between the groups on any measure of spontaneous alternation, similar to the findings from Church's lab (Church and Overbeck 1990; Church et al. 1991). In the water-maze escape task there was a significant effect of block indicating that the

animals improved over trials, but there were no effects due to prenatal cocaine exposure.

Barron (personal communication, May 1993) has also been examining the effects of perinatal cocaine exposure on subsequent behavior. What is interesting about this research is that cocaine is administered neonatally via an indwelling stomach cannula. Because of differences in timing in brain development between rodents and humans, this postnatal administration has been proposed as a model of human third trimester exposure. Additionally, since in this procedure pups are artificially reared away from the dam, the confounding effects of drug-induced alterations in maternal behavior are eliminated.

In this procedure pups are implanted with intragastric cannulas on postnatal day 4 and fed an artificial milk diet every 2 hours via a pump connected to the cannula. On postnatal days 4 to 10 the pups are reared away from the dam and exposed to either a 20 or 60 mg/kg dose of cocaine. The pups are then returned to the dam and subsequently tested. Barron assessed both spontaneous alternation and passive avoidance using procedures similar to those described in the aforementioned studies. Passive avoidance was tested at 23 and 24 days of age and there were no differences between cocaine-exposed animals and controls on any measures. Similarly, there were no effects on spontaneous alternation.

SUMMARY

This chapter has reviewed the animal studies related to prenatal cocaine administration and subsequent learning. A summary of these studies and their findings is given in table 1. It is important to note that there is a relative scarcity of reports in this area. Given the potential number of infants that might be exposed to cocaine prenatally, it is certain that more work needs to be done. Second, from this table and the preceding review, it is difficult to conclude that prenatal cocaine exposure has wide-ranging effects. There certainly appears to be ample evidence from Spear's lab that prenatal cocaine exposure disrupts early olfactory learning.

These findings are in need of independent replication. In fact, the authors have partially replicated a deficit in an odor-aversion task following gestational cocaine administration. The evidence provided by Spear and colleagues (1989*a,* 1989*b*), however, indicates that this deficit is relatively transitory and is not found in animals after perhaps 15 days of

Furthermore, it does not appear that these cocaine-exposed animals are incapable of learning. In the Goodwin and colleagues (1992) study, where animals were given either 2, 3, or 4 acquisition trials, animals exposed to cocaine prenatally and reared by surrogate untreated mothers did learn the association when given 4 trials. It must be stressed, however, that early deficits that diminish as the animals mature or deficits which can be overcome by repetition can still have long-lasting consequences. Requiring more experience with an association prior to learning that association or having a transitory learning deficit in no way diminishes its potential importance for the organism.

On more common tasks of learning, such as passive and shuttle avoidance and maze learning, there is really very little evidence to support the notion that cocaine is acting as a behavioral teratogen. The available evidence would appear to indicate that significant, biologically meaningful deficits on these tasks are not found over a wide range of doses.

There are a number of reasons for these failures to find effects. It may be that these tasks are too simple to detect underlying behavioral anomalies. Passive avoidance is a simple task that is easily learned by animals by the time of weaning. Active avoidance is much more difficult, but it too may not place enough challenges on the organism. The work by Heyser and colleagues (1992*b*) may signal that more complicated tasks, such as reversal of a conditional discrimination, might be necessary to show cocaine's behavioral teratogenic action. However, even in this case the effect was not very substantial, consisting of less than one extra incorrect response relative to controls prior to the first reward. More challenging situations such as successive discriminations (e.g., learning to learn) might lead to bigger differences in performance between cocaine-exposed animals and controls. These types of tasks need to be assessed. Similarly, perhaps cocaine-exposed animals need to be challenged physiologically, either by placing them in extremely stressful situations or assessing their response to other drugs that disrupt normal physiological functioning (see Spear, this volume).

Another reason for a failure to find substantial effects on a wide range of behavioral assessments is that cocaine may act as a behavioral teratogen through a number of different mechanisms. It might be a direct toxin to certain developing neurotransmitter systems, it may function indirectly by inducing hypoxia, or both. If the mechanism of action varies in different animals and the effects are not large in any animal, then it would be extremely difficult to detect group differences using the sample sizes

normally assessed in these studies. It may be that only a small subset of a group of animals is affected and that group variability must be assessed in addition to alterations in group means.

Finally, it may be that cocaine is not a behavioral teratogen that has wide-ranging consequences. Whatever the answer, additional research is obviously necessary before any firm conclusions can be reached.

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ACKNOWLEDGMENT

This work was supported by National Institute on Drug Abuse grant DA04275 awarded to Edward P. Riley, Department of Psychology, San Diego State University. Reprints may be obtained from Edward Riley.

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Prenatal Exposure to Drugs of Abuse: Methodological Considerations and Effects on Sexual Differentiation

Robert F. McGivern and Robert J. Handa

INTRODUCTION

Studies conducted over the past 25 years have revealed a biological pervasiveness to the neurobehavioral sexual differentiation process that extends far beyond behaviors associated with reproduction. This pervasiveness is now recognized to include sex differences in cognition and affect (Halpern 1992) as well as structural and biochemical differences in the central mechanisms involved in their expression (Arnold and Gorski 1984). In humans, sex differences have been observed in communication style, verbal and spatial skills, mathematical reasoning ability, and even play behavior in children (Beatty 1984; Benbow 1988; Nyborg 1984). Many of the nonlinguistic sex differences in humans have been observed in other mammals, attesting to the primary role of biology over cultural factors in the determination of such differences (Beatty 1979). These differences include juvenile play behavior (Meaney and Stewart 1981), pain sensitivity (Mogil et al. 1993; Pare 1969), social interaction (Kellogg et al. 1991), taste preferences (Valenstein et al. 1967), and spatial skills (Stewart et al. 1975). The reader is referred to a series of reviews by Beatty (1979, 1984, 1992) for references and discussion of nonreproductive sex differences in animals. Several reviews of nonreproductive sex differences in humans have addressed cognitive, emotional, and neuropsychological function (Beatty 1984; Halpern 1992; Levy and Heller 1992; Maccoby and Jacklin 1974).

While biology provides a template for the neurobehavioral development of sex-related behaviors across the phylogenetic scale, the flexibility of this template has increased with evolutionary pressure. Such flexibility has allowed adult mammalian behavior to reflect environmental influences during development to a degree not observed in the fixed action patterns seen in insects, fish, or reptiles. However, an untoward effect has been the increased susceptibility of the mammalian neurobehavioral sexual differentiation process to disruption by extrinsic factors such as drugs.

Animal models of prenatal or perinatal exposure to drugs of abuse have demonstrated a variety of subtle behavioral alterations in offspring in the absence of any clear teratological consequences related to physical development. These have included alterations in attention, learning, emotional reactivity, drug sensitivity, and sexual behavior (Gorski 1974; Meyer and Riley 1986*b*; Vorhees 1986). The most recent class of behaviors to come under scrutiny following prenatal drug exposure is that of nonreproductive sexually dimorphic behaviors.

Investigations into the mechanisms mediating these behavioral alterations have identified drug-induced disruptions of gonadal function or neuronal or glial development in sexually dimorphic brain regions. This finding has provided a basis to interpret the enduring alterations in the expression of these behaviors (Arnold and Gorski 1984; McGivern and Riley 1993; Ward 1992; Weinberg et al. 1991). This chapter provides a brief overview of basic mechanisms involved in sexual differentiation of the mammalian brain, followed by a consideration of selected methodological issues associated with studies of neurobehavioral sexual differentiation. The inclusion of sexually dimorphic behaviors as an area of study in the field of developmental neuroteratology has introduced methodological considerations specific to sex-related behaviors. Therefore, the authors have attempted to address some specific methodological considerations relevant to the area of animal models of sexual differentiation. Finally, a brief synopsis of findings concerning the effects of several major drugs of abuse on the sexual differentiation process is presented.

MECHANISMS OF SEXUAL DIFFERENTIATION

Normal sexual differentiation is determined by genetic and phenotypic factors acting during different stages of development (George and Wilson 1988). Genetic or chromosomal sex is determined at the time of fertilization by (as yet) unidentified factors associated with the X and Y chromosomes. Genetic activity related to the sex chromosomes subsequently differentiates the embryonic gonad into a testis or an ovary. Phenotypical sex is under the direct control of gonadal hormone secretion and other biochemical factors. Phenotypical expression relates to development of sex organs such as the penis or vagina as well as sex-specific regional differentiation of the central nervous system (CNS). Current evidence indicates that factors associated with phenotypical differentiation are also involved in the expression of affective and cognitive sex differences in humans (Halpern 1992; Reinisch and Sanders 1992). Drugs

generally influence the phenotypical aspects of the sexual differentiation process. The drugs that influence biochemical factors critical to the sexual differentiation process, such as sex steroid hormones (McEwen 1988) or catecholamines (Mirmiran et al. 1988), are of most concern.

Broadly speaking, gonadal hormones serve to organize the brain during critical periods of development to respond with a masculine or feminine pattern to later activational effects of hormonal stimulation (Phoenix et al. 1959). Organizational effects of hormones are long-lasting and occur in both the brain and periphery through direct effects on genomic activity in the developing organism (McEwen 1992). By definition, organizational actions of hormones are restricted to critical periods of development to induce their long-lasting consequences. Regardless of the developmental timeframe of a given species to reach maturity, there is a good correlation between the timing of the critical period for sexual differentiation and the stage of biological development (Phoenix et al. 1959). Thus, prenatal exposure of either males or females to adequate levels of testosterone at the proper point in fetal development leads to later masculine sex behavior patterns (i.e., mounting or intromission). Such behavior is observed only in the presence of a receptive female and adequate circulating testosterone levels. Conversely, a lack of adequate hormonal exposure during a critical organizational period produces an adult who fails to respond to the same amount of testosterone with normal sex behavior.

Activational effects of a hormone occur rapidly in response to circulating levels of the hormone. The sensitivity of such effects to hormone stimulation is often determined by the prior exposure to testosterone or estrogen during critical organizational periods. However, the activational effects of gonadal hormones can also be influenced by environmentally induced alterations in the biochemical milieu at the time the hormone is present, such as those induced by stress or the estrual cycle (Becker and Cha 1989; Levine et al. 1989).

Some aspects of masculine sex behavior are organized by genomic actions of androgens such as testosterone or dihydrotestosterone acting directly through the androgen receptor. Other aspects of male sex behavior are organized by estrogens such as estradiol acting in specific brain regions such as the hypothalamus, preoptic area, or amygdala (McEwen 1992). Part of testosterone's role in the masculinization process is to serve as a substrate for estrogen. Estrogen in the male brain is derived primarily from the intracellular aromatization of testosterone by aromatase. Aromatase is a P450 enzyme which has a limited regional

distribution in the brain to areas such as hypothalamus, amygdala, and cerebral cortex. Its activity in adulthood is sexually dimorphic, a phenomenon determined by hormonal exposure during early development as well as genetics (Roselli and Resko 1993).

Females are protected from the masculinizing effects of ovarian estrogen by a plasma-binding protein that prevents estrogen from crossing the cell membrane. In the rat, this binding protein is α-fetoprotein; in the human, it is sex hormone binding globulin (SHBG). Both SHBG and α-fetoprotein remain elevated during the infantile period and decline significantly prior to puberty, allowing the onset of adult patterns of negative feedback control of gonadotropin secretion in females (Ojeda and Urbanski 1988). Masculinized behavior patterns have been observed in women prenatally exposed to high levels of adrenal androgens such androstenedione (Galatzer and Laron 1989) or to estrogens such as diethylstilbestrol (Hines and Shipley 1984) which are not bound by SHBG. Limited evidence also exists indicating that ovarian steroids may be important for complete feminization of the brain (Dohler et al. 1984; Fitch et al. 1991; Gerall and Dunlap 1971). In the rat model of sexual differentiation, it has generally been assumed that behavioral masculinization of females following exposure to drugs that activate the adrenal gland is due to the increased release of androstenedione (McGivern et al. 1984; Meyer and Riley 1986*a*), similar to that which takes place in humans (Casey et al. 1992). However, recent evidence indicates that androstenedione is not made in the adrenal gland of the rat (Fitch et al. 1992; van Weerden et al. 1992), indicating that other mechanisms are mediating such masculinization.

Sex steroids influence genomic activity through binding to the intracellular receptor. This is accomplished by the binding of the steroid-receptor complex to hormone response elements in deoxyribonucleic acid (DNA). Such genomic actions appear to be intimately connected with neuronal survival and growth (McEwen 1992). This process is also thought to permanently define a neuron's subsequent responsiveness to stimulation (Hasegawa and Sakuma 1993), as suggested by studies showing that sex steroids can produce long-term changes in estrogen stimulation in synapses and postsynaptic membranes in brain areas such as the hypothalamus (Hasegawa and Sakuma 1993; Naftolin et al. 1990).

The timing of any disruption in the steroid milieu during development is of particular importance in determining the long-term consequences on brain and behavior. The normal masculinization process in males is dependent in part upon surges of testosterone secretion during critical

periods of development. In the rat, the critical period for sexual differentiation is considered to extend from approximately the last week of gestation through the first few days postnatally. During this period, there is a normal surge of testosterone on days 18 and 19 of gestation (McGivern et al. 1988*a*; Weisz and Ward 1980), as well as a second surge at birth (Corbier et al. 1978; McGivern et al. 1993). If the amplitude or time course of either surge is altered, sexual differentiation of the male will be incomplete.

Corresponding surges of testosterone have been identified in the human male fetus and neonate. A prenatal surge of testosterone occurs around 16 to 20 weeks of gestation (Parker 1993). A postnatal testosterone surge has also been observed during the first few hours after birth (Corbier et al. 1990; Stahl et al. 1978). It is presumed that interference with either surge will lead to incomplete masculinization as occurs in the rat (Corbier et al. 1992; Roffi et al. 1987). No prenatal surge of estrogen has been observed in females, and the capacity to produce estrogen from exogenous precursors primarily evolves during the infantile period (Ojeda and Urbanski 1988).

Results from studies of the effects of prenatal stress or alcohol exposure provide good examples of the impact environmental stimuli can have on the sexual differentiation process. Exposing a pregnant rat to stress accelerates the testosterone surge in the male fetus to day 17, resulting in incomplete masculinization of sex behavior in the adult male offspring (Ward 1972). Alcohol exposure attenuates the prenatal testosterone surge (McGivern et al. 1988*a*) rather than shifting it, but the behavioral effects (McGivern and Riley 1993) on reproductive behaviors appear to be broadly similar to those of prenatal stress (Ward 1972, 1980).

Recent studies have also revealed notable effects of neurotransmitters on the process of sexual differentiation in the CNS. In addition to their neurotrophic actions early in brain development (Lauder and Krebs 1986), serotonin and norepinephrine (NE) have also been identified as playing a significant modulatory role on sex steroid actions during development (Beyer and Feder 1987; Handa et al. 1986; Jarzab et al. 1986; Raum and Swerdloff 1981; Raum et al. 1984). A novel interaction of the gamma aminobutyric acid (GABA) system with neurosteroids to influence social behavior has been observed by Kellogg and coworkers (1991) in the course of their studies of the effects of prenatal diazepam exposure. Specifically, they have identified an important developmental role for the GABA receptor in the adult modulation of social behavior by

steroids. Such findings have led to an increased appreciation of alternative mechanisms to explain alterations in sexual differentiation in the absence of any apparent change in steroid secretion or action (Beyer et al. 1992; Reisert and Pilgrim 1991).

METHODOLOGICAL CONSIDERATIONS

Stress

Stress interacts both behaviorally and physiologically with sex-related behaviors (Levine et al. 1989). Stress reactivity in the adult animal can be influenced by developmental exposure to stress (Levine et al. 1989) or to drugs such as ethanol (Taylor et al. 1982; Weinberg 1988). For these reasons, it is imperative to limit the influence of stress as much as possible when studying sex-related behaviors *per se* in prenatally drug-exposed animals. The subtlety of potential stress effects in development studies is highlighted by results from Meyer and colleagues (1992), who observed an effect of saline treatment of dams during pregnancy on the responsiveness of offspring to cocaine. These authors observed no effect of increasing doses of cocaine (1.25, 2.5, or 5.0 milligrams per kilogram (mg/kg) subcutaneously (SC)) on locomotor activity of 11-day-old males from dams injected twice daily with saline from days 11 to 20 of gestation, compared with a linear response in males from untreated dams. Females from saline-injected dams exhibited less locomotor activity following saline injections than untreated controls. Their locomotor response to cocaine was similar to all three doses and an increase over saline injection. In contrast, females from untreated dams were unresponsive to the lowest doses of cocaine and exhibited a decrease to the highest dose. These results point to the importance of including untreated controls in studies of drug effects on development.

One of the basic organizational actions of androgens is to desensitize the adult hypothalamus to the positive feedback effects of estrogen on luteinizing hormone (LH) secretion. Using this information, LH responsiveness to estrogen can be used to study masculinization of females or incomplete defeminization of males following prenatal exposure to a drug such as ethanol (Handa et al. 1985). In castrated female rats, a positive feedback response to estrogen occurred approximately 54 hours after a 2 microgram (μg) injection of estrogen benzoate at 10 a.m. (Handa et al. 1985). To study this phenomenon, the rat is often implanted with an

indwelling jugular catheter 48 to 72 hours prior to the LH surge, so that hourly blood samples can be obtained before and during the LH surge.

A major consideration for this type of study is the experimental conditions under which a blood sample is obtained from the rat. A large stress-induced rise in glucocorticoids several hours before the surge can attenuate or eliminate the positive feedback response to LH. Therefore stress must minimized both during the time the catheter is hooked up, as well as the during the blood sampling period, to obtain accurate reflections of hypothalamic-pituitary-gonadal (HPG) function in these animals.

The ability of glucocorticoids to suppress LH (Baldwin and Sawyer 1974; Olster and Ferin 1987; Ringstrom and Schwartz 1985) is also a major consideration. A reduction in LH or change in its pulsatile characteristics can result in significant decreases in sexual behavior. For the rat, a novel situation such as a test arena is a stressor and leads to a notable rise in glucocorticoids (Fitch et al. 1992). For this reason, male rats often exhibit little sex behavior in the presence of a receptive female during the first test session. Asymptotic levels of behavior are generally not reached until the second or third test session. Consequently, assessment of an animal's sex behavior potentials should be made on the basis of performance over multiple test sessions.

Even under optimized test conditions, alterations in sex behavior by prenatally drug-exposed animals may still reflect an increased responsiveness to environmental stress such as that reported consistently in animals prenatally exposed to alcohol (Taylor et al. 1982; Weinberg 1988, 1992). Thus, reductions in sex behavior of drug-exposed animals could reflect a secondary effect of increased stress reactivity rather than a basic dysregulation of the HPG. Optimally, all testing for sex behavior potentials in drug-exposed animals would include followup studies of stress hormone responsiveness as well as extensive habituation to the testing situation. Such baseline data obtained under minimal stress conditions can be used to assess the independent effect of the prenatal exposure regimen on stress-responsive systems that might be secondarily influencing sex-related behaviors.

Housing

Housing is also a major consideration in tests of sex behavior. The rodent is a social animal and normal behavior is best assessed in animals raised under group-housed conditions. Individual housing is a potent

stressor (Valzelli 1981) that can result in significant changes in monoaminergic systems (Segal et al. 1973) and opioid systems (Adler et al. 1975*a*, 1975*b*) after 3 to 4 weeks. Marked alterations in circadian patterns of hormone release and hypothalamic NE content are also apparent (Greco et al. 1992). These changes are presumed to underlie the alterations in normal sex behavior potentials observed in individually housed animals (Valzelli 1981). Therefore, animals used for assessment of alterations in sex-related behaviors following prenatal drug exposure should be group housed from weaning.

Gestational Period of Drug Exposure

While exposure to a drug throughout gestation can provide important preclinical information regarding its teratogenic potential for sex-related behaviors, such broad exposure periods are very restricted with respect to understanding potential mechanisms involved. The extended presence of ethanol prior to the differentiation of the HPG axis raises the possibility that effects of ethanol on sex-related behaviors result from an indirect effect on progenitor cells.

In the rat, the development of the pituitary gland begins about day 12 of gestation (Hebel and Stromberg 1986). The hypothalamus and gonads differentiate over the course of the following 3 days, and by day 15.5 the testes are capable of secreting testosterone in response to LH (Nemeskeri et al. 1984; Warren et al. 1984). Subsequently, as noted above, two surges of testosterone occur in the male that are important for complete neurobehavioral masculinization and defeminization. This series of events in the male is important to consider when designing experimental protocols to study the effects of a drug on sex-related behaviors or physiology. Generally, the timing of the drug exposure should be correlated with the gestational period of differentiation or secretion related to the structures considered to mediate potential alterations in behavior or biology. This period of exposure can then be contrasted with effects observed in animals exposed to ethanol before or after this period in development. For interpretive reasons, care should be taken to entirely include or exclude drug exposure during a testosterone surge. Thus, a commonly used regimen of exposure from days 12 through 18 should be avoided for the study of sex-related effects in males since the drug exposure period would end in the middle of the prenatal testosterone surge.

Hormone Levels and Blood Sampling

The pulsatile or circadian patterns of hormone secretion in plasma make single time point estimations of hormone levels of limited value, especially when small sample sizes are employed (e.g., N = 6 to 10), as is the norm in animal studies. This limitation can be easily illustrated with testosterone, which has a broad normal range for serum values as well as a marked circadian rhythm. Normal testosterone values in rat plasma can vary between 2 and 10 nanograms per milliliter (ng/mL). A significant difference ($p < 0.05$) in testosterone levels between two groups of males, although still within the normal range (e.g., 4.2 versus 3.5), can be difficult to interpret unless it can be associated with a clear alteration in sex behavior in the same animals. Since stress can also depress testosterone levels by inhibiting LH secretion, counterbalanced procedures between groups for obtaining blood samples are also important to consider when assessing data from single blood sample determinations.

Serial blood samples obtained from freely moving catheterized animals provide much more information than single point determinations with respect to hormonal secretory profiles and their relationship to function. When using this method is not possible, such as in the measurement of the fetal testosterone surge, the litter or a litter representative should be considered the unit of analysis rather than treating data from several individuals within the litter as statistically independent points.

Maturational and Physiological Status of the Animal

Several sex-related behavioral tests are influenced significantly by the age or physiological state of the animal. These include open-field behavior, wheel-running activity, taste preferences, and neuroendocrine stress responsiveness. Both open-field behavior and wheel-running activity of female rodents change during the estrous cycle (Beatty 1979), primarily through estrogen's actions on the nigrostriatal dopamine system (Castner et al. 1993). When comparing male and female postpubertal behavior on these type of tests, the stage of estrus should be determined by vaginal cytology. Alternatively, endogenous hormone levels can be removed and circulating levels clamped by implanting a hormone capsule or by hormone injection.

Age and sex differences in neuroendocrine responsiveness are also well documented (Brett et al. 1983; Critchlow et al. 1963). Estrogen (Burgess and Handa 1992; Phillips and Poolsanguan 1978) or the stage of estrus

(Viau and Meany 1992) has been shown to influence the adrenocorticotropic hormone (ACTH) and adrenal steroid response to environmental stress. In most cases, estrogen appears to enhance the response of the hypothalamic-pituitary-adrenal (HPA) axis (Burgess and Handa 1992; Phillips and Poolsanguan 1978; Viau and Meany 1992). In contrast, androgen appears to inhibit the HPA response to stress (Handa et al. 1994). Thus, response differences in neuroendocrine activity due to age and sex should be considered in any study of stress responsiveness of drug-exposed offspring.

Water consumption is also sexually dimorphic in the rat, a fact that is not generally recognized in psychobiological studies of taste preferences. Adult female rats consume approximately 15 percent more water per day than males when consumption is calculated on the basis of body weight (McGivern and Henschel 1990). This fact appears to relate to the sex difference in circulating levels of vasopressin (Crofton et al. 1985). The authors have conducted a series of studies (McGivern et al., in press-*a*) to further characterize this sex difference in the Sprague-Dawley rat. It was found that the sex difference is present at weaning, but tends to be masked by the marked drop in water consumption that occurs in both sexes from weaning through adulthood. At weaning, the water consumption range for male and female rats is approximately 24 to 30 mL/day per kg -0.1 of body weight. A clear sex difference is always present by 60 days of age, when values drop to 16 to 20 mL/day for females and 12 to 16 mL/day for males. Daily consumption continues to decline until about 120 days of age when values are approximately 10 to 12 mL/day/kg^{-1} for females and 8 to 10 mL/day/kg^{-1} for males. This decrease in water intake appears to reflect the decrease with age in the percentage of body weight made up by water (Hays 1980).

Generally, studies looking at taste preferences in adult males and females, including the authors' studies (McGivern et al. 1984, 1987), have not examined daily water consumption. Therefore it is unknown how sex differences in consumption patterns relate to sex differences in taste preferences. However, the authors' data indicate that animals should be tested at ages that are within a few days of each other to determine sex-equivalent water consumption.

Anogenital (AG) distance at birth is a commonly used marker of masculinization, since it reflects the amount of androgenic stimulation during the prenatal period. Specifically, this region is stimulated by testosterone or dihydrotestosterone acting through the androgen receptor.

However, AG distance, like internal organ size, is correlated with body size. This is exemplified by studies of AG distance in males prenatally exposed to alcohol. At birth, AG distance in alcohol-exposed males has been consistently found to be smaller compared with controls (Chen and Smith 1979; McGivern 1987; McGivern et al. 1992; Rudeen et al. 1986; Udani et al. 1985). However, the difference is insignificant when corrected for body weight (McGivern 1987; McGivern et al. 1992). Therefore, in prenatal drug studies where birthweight is reduced, a correction for body weight or crown-to-rump length is essential for an accurate assessment of androgenization. Graham and Gandelman (1986) have found that body weight appears to be a somewhat better correction factor than body length, although either can be used.

Reproductive Versus Nonreproductive Sex Differences

The study of nonreproductive sex-related behaviors has a relatively recent history compared with the study of reproductive behaviors, with the result that much less is known regarding the biological substrates of nonreproductive compared with reproductive behaviors. However, it is becoming increasingly clear that while the neurobehavioral organization of the two classes of behavior are biologically related, their organization and expression can differ substantially. Knowing the performance of an animal with respect to a nonreproductive behavior does not necessarily predict its behavior potentials for reproductive behaviors. For instance, in male rats with normal testosterone levels prenatally exposed to cocaine, the authors observed significant reductions in adult scent marking, but found no changes in mounting, intromission, or ejaculation behavior of the same animals (Raum et al. 1990). Overall, little data have been collected regarding a systematic consideration of the relationship within animals between the expression of reproductive and nonreproductive behaviors in the rat. Until such data become available, interpretation of one class of behaviors appears to have very limited value with respect to other behavioral classes.

DRUGS OF ABUSE: EFFECTS ON SEXUAL DIFFERENTIATION

For the purposes of this chapter, the authors have provided only a brief consideration of the effects of drugs of abuse on the sexual differentiation process. A more extended consideration of the perinatal influence of drugs of abuse on neurobehavioral sexual differentiation is provided in

any of several recent reviews (McGivern and Riley 1993; Segarra and McEwen 1992; Ward 1992; Weinberg et al. 1991).

Cocaine

A very limited amount of information is currently available regarding the influence of cocaine on the sexual differentiation process. Pharmacologically, the drug is well known to block reuptake of monoamines in adult animals and to release dopamine (Heikkila et al. 1975; Komiskey et al. 1977; Ritz et al. 1987; Ross and Renyi 1969). Prenatal exposure might be expected to have significant effects on the sexual differentiation process, since monoamines have been demonstrated to modulate steroid actions as well as to have neurotrophic actions early in development (Lauder and Krebs 1986). Fetal brain tyrosine hydroxylase activity is significantly increased by cocaine exposure (Akbari and Azmitia 1992; Meyer and Dupont 1993), consistent with the marked hyperactivity of the noradrenergic system observed in neonatal brains of males and females exposed to cocaine from days 8 to 20 of gestation (Seidler and Slotkin 1992). Little or no effect of prenatal cocaine exposure was observed on dopamine activity, although it altered dopamine and serotonin receptor number (Byrnes et al. 1993; Henderson et al. 1991; Scalzo et al. 1990).

Raum and colleagues (1984) observed that adrenergic stimulation of the neonatal brain inhibited hypothalamic nuclear incorporation of estrogen. The authors subsequently demonstrated that cocaine administered intracerebroventricularly to 4-day-old female rats significantly inhibited the incorporation of estradiol in the hypothalamus. Since estradiol is a critical factor in the masculinization of the brain, these results suggest that prenatal cocaine exposure might interfere with neurobehavioral masculinization in males.

To date, two published studies have directly addressed this question with conflicting behavioral results. The authors treated pregnant Sprague-Dawley dams with cocaine (10 mg/kg SC) twice a day during the last week of gestation and studied only male offspring (Raum et al. 1990). No effects of cocaine were observed on AG distance at birth. In adulthood, males prenatally exposed to cocaine were found to have increased latencies to initiate sexual behavior, but other aspects of masculine sex behavior were similar to controls. However, another testosterone-dependent behavior, territorial scent marking, was significantly reduced in these animals.

Some evidence for alterations of adult endocrine function was also detected. Plasma LH in cocaine-exposed males was significantly higher than controls, whereas testosterone levels were the same as controls, suggesting some measure of insensitivity to negative feedback by testosterone in these animals. Other endocrine measures were normal, as were sex organ weights, but sperm counts were significantly reduced. The reduction in scent marking in the face of normal circulating levels of testosterone and elevated LH suggests a relative CNS insensitivity to androgens in these animals compared with controls.

Vathy and colleagues (1993) treated dams of the same strain with the same dose regimen of cocaine from days 11 through 18 of pregnancy and observed a different pattern of results. Adult cocaine-exposed males were observed to have facilitated sexual activity patterns, as well as markedly reduced postejaculatory intervals. Conversely, the sexual behavior of cocaine-exposed females was significantly inhibited. Catecholamine levels in the preoptic area of cocaine-exposed males, but not females, were significantly higher than controls. The reasons for the different findings of the two studies are not immediately apparent, but could reflect differences in timing of gestational drug administration or differences in housing of the offspring. Animals in the Raum and colleagues (1990) study were group housed from weaning and during the several weeks of sex behavior testing, whereas the animals in the study by Vathy and colleagues (1993) were singly housed throughout from weaning. Thus housing conditions may have influenced the pattern of results observed. If so, the results imply that prenatal exposure to cocaine may significantly influence the way males and females respond to long-term environmental stress in adulthood. Clearly, additional studies are needed to assess the long-term effects of cocaine on sexual differentiation.

In a study examining locomotor and stereotypy responses to cocaine in animals prenatally exposed to cocaine, Peris and colleagues (1992) reported that prenatal cocaine exposure influences dopamine release from nigrostriatal terminals in a sex-dependent manner. Both males and females exhibited an increased sensitivity to cocaine compared with controls, but females prenatally exposed to cocaine also exhibited increased locomotor activity following saline injection. Sex differences were also detected in amphetamine-induced release of ^{3}[H]-dopamine from striatal slices. In utero cocaine exposure increased amphetamine-stimulated release in females, but decreased release in males. However, since the stage of the estrual cycle was not reported, it not clear whether these effects might also reflect changes in hormonal status during the estrual cycle.

The effect of prenatal cocaine exposure on locomotor activity and acoustic startle response of the offspring appears to be sex related. Hughes and colleagues (1990) found that baseline locomotor activity was reduced in 21- to 22-day-old female rats exposed to cocaine (60 mg/kg) from days 8 to 22 of gestation. Cocaine-exposed females were also observed to respond significantly less to an injection of amphetamine (Hughes and Dow-Edwards 1991). No effect was observed in the baseline activity or drug-related activity of cocaine-exposed males at this age compared with controls. Females exposed to this same dose regimen exhibited a decreased acoustic startle response at 60 to 65 days of age compared with controls (Hughes and Dow-Edwards 1992), but no effect of the prenatal drug exposure was observed on the startle response of males. Jackson and colleagues (1992) reported that prenatal exposure to 15 mg/kg cocaine injected twice daily during the last week of gestation reduced striatal tyrosine hydroxylase immunoreactivity in 20-day-old females but not males. These results appear to be consistent with the effects on locomotor activity observed by Hughes and colleagues (1990, 1991).

Several other studies have noted sex-related differences in cocaine's effects on development which suggest that females may be more affected than males. Sex-related differences in regional brain glucose utilization were noted in adult animals injected with 50 mg/kg cocaine from days 1 to 10 (Dow-Edwards et al. 1988) or 11 to 20 (Dow-Edwards et al. 1993) postnatally. Cocaine-exposed females exhibited increased glucose utilization in several cortical and limbic regions, whereas little change, or a decrease, was observed in males. In another study (Kunko et al. 1993), prepubertal females, but not males, were observed to exhibit greater sensitization to cocaine-induced stereotypy following prenatal cocaine exposure. Levin and Seidler (1993) recently reported that exposure to 30 mg/kg cocaine from days 8 to 20 of gestation resulted in impaired radial arm performance in females, but not males.

Sex-related differences in cocaine toxicity have been reported in adult rats with respect to cardiovascular function (Morishima et al. 1993). The effect appears to be sex steroid-mediated since it is dependent upon the presence of ovaries (Morishima et al. 1993). However, it is not known at this time whether sex differences in toxicity extend to fetal development. The relatively larger number of toxic effects reported to date in females prenatally exposed to cocaine compared with males suggests that a sex difference in fetal toxicity could be present.

Another important issue to the overall toxicity of cocaine may be the dose of cocaine to which the animals has been exposed prenatally. Evidence from the authors' studies in which pregnant dams have been injected with one of three doses of cocaine during the last week of gestation suggests that the neurobehavioral effects of the drug on the offspring are not always linearly related to the dose of cocaine to which the animals was exposed prenatally. In a recent study involving adult males only, the authors observed a significant lack of sensitization to cocaine-induced stereotypy in adult males exposed prenatally to 3.0, 10.0, or 30.0 mg/kg twice daily for the last week of gestation (McGivern and Hutcheson 1993). In adulthood, the animals were administered 8 daily injections of cocaine (10 mg/kg SC) and their behavior was monitored in the open field for 60 minutes after injection. One week later the animals were again injected with cocaine to examine sensitization to the drug. Sensitization to cocaine was observed in controls, but not in animals prenatally exposed to cocaine. The lack of sensitization to cocaine-induced stereotypy extended to all animals exposed to cocaine, regardless of prenatal dose. A similar lack of sensitization to cocaine has been recently reported in mice prenatally exposed to cocaine (Byrnes et al. 1993).

However, differential effects of prenatal dose were noted for the behavioral responsiveness to cocaine with respect to locomotor activity and rearing behavior (McGivern and Hutcheson 1993). In this study, the authors noted an overall inverted U-shaped function with respect to cocaine responsiveness which was related to the prenatal dose of cocaine exposure. This response pattern is similar to the behavioral results for scent marking which the authors observed in a previous study in adult males prenatally exposed to the same three doses (Raum et al. 1990). A similar inverted U-shaped response to cocaine has been observed by Meyer and colleagues (1992) in 11-day-old males and females exposed to cocaine from gestational days 11 to 20. Since cocaine has pharmacologically relevant anesthetic properties at higher doses (Gifford and Johnson 1992), it may be producing developmental effects that differ from its classic action of reuptake blockade at lower doses.

Opiates

Data concerning the long-term effects of opiates on sexual differentiation are also limited. Prenatal exposure to morphine from day 5 to 14 (Vathy et al. 1983) or from days 11 to 18 (Vathy et al. 1985) reduced lordosis frequency in females. The effect was more pronounced in animals exposed from days 11 to 18 of gestation (40 to 57 percent) compared

with those exposed from days 5 to 14 (20 percent). This difference suggests the importance of morphine exposure during the entire period of hypothalamic differentiation in producing this effect. Sexual behavior of males was unaltered, with the exception that morphine-exposed males had significantly shorter postejaculatory intervals (Vathy and Katay 1992; Vathy et al. 1985). Lordosis behavior was not examined in males.

Hypothalamic NE content was dramatically altered in these animals. The NE content in morphine-exposed females was elevated by 95 percent, while the content in males was reduced by 57 percent relative to controls (Vathy and Katay 1992). It should be noted that animals used in these studies were single housed from weaning, which may have contributed to eliciting the differences in drug-exposed animals. No differences were observed in estrogen receptor regulation in the hypothalamic-preoptic region of morphine-exposed animals (Vathy et al. 1985).

Perinatal morphine exposure had no effect in golden hamsters on the ability of the female to display hormone-induced male or female sexual behavior patterns (Johnston et al. 1992). Morphine-exposed males exhibited normal masculine sex behavior patterns but significantly more lordosis behavior than controls, indicating incomplete defeminization.

Other evidence for a masculinizing effect of morphine on females has been obtained in the rat, in addition to the reduced lordotic potential observed by Vathy and coworkers (1983, 1985, 1992). Lapointe and Nosal (1982) reported increased AG distance in females at weaning after exposure to morphine from conception through postnatal day 16. Delayed vaginal opening was also reported in females exposed to morphine from days 5 to 12 of gestation (Litto et al. 1983). This latter effect contrasts with the effect of postnatal administration of morphine, which has been found to induce precocious puberty in females (Sonderegger et al. 1977).

Plasma levels of the androgens testosterone and androstenedione on day 20 of gestation were significantly reduced in males, but not females, exposed to methadone from days 14 through 19 of gestation (Singh et al. 1980). However, no evidence for a direct effect of the drug at the site of the testis, nor any effect of the drug on aromatization of testosterone to estrogen, was observed. The results of this study, as well as many of the above-cited studies on the prenatal effects of morphine, should be interpreted with caution since cessation of morphine treatment causes severe withdrawal in rodents, and is known to induce long-term effects on growth and differentiation. Sparber has provided evidence that many

of the effects attributed to prenatal morphine exposure, both biochemical and behavioral, are a result of withdrawal rather than the direct effects of the drug (Lichtblau and Sparber 1984; Sparber 1986).

While the limited evidence to date is not conclusive with respect to the effects of opiates on the sexual differentiation process, circumstantial evidence strongly justifies further study. Perinatal opiate treatment decreases dendritic arborization in cortical neurons (Ricalde and Hammer 1990). Mu opioid receptors are present in the brain as early as day 14 of gestation (Bayon et al. 1979; Clendeninn et al. 1976). This class of opioid receptors binds opiates such as morphine, heroin, and methadone, and is distributed throughout brain regions integral to reward and the expression of sex behavior including the medial preoptic area (MPOA) and the ventral tegmental area (VTA). Stimulation of mu receptors in adult animals inhibits luteinizing hormone-releasing hormone (LH-RH) release through an inhibitory influence on excitatory NE projection to the MPOA (Kalra and Kalra 1984) and inhibits female sexual behavior through an inhibition of NE release in the ventromedial hypothalamus of females (Vathy et al. 1991). Injection of beta-endorphin directly into the MPOA produces a cessation of copulation in male rats (Hughes et al. 1987).

Chronic morphine treatment during the last 2 weeks of gestation did not alter the number or functional efficacy of the mu receptors in striatal and cortical slices from fetal brain at 21 days of gestation (DeVries et al. 1991). However, electrically stimulated, calcium-dependent release of both dopamine and NE in morphine-exposed tissue was dramatically enhanced, indicating an excessive activation of signal transduction mechanisms regulating catecholamine release. These opioid-related actions on catecholamines, combined with evidence that opiate receptor systems modulate development of catecholaminergic systems (Seidler et al. 1982), suggest that morphine has a strong potential to alter the normal development of neurobehavioral sexual differentiation. Studies of the susceptibility of nonreproductive sex-related behaviors to disruption by prenatal opiate exposure will be important in assessing the overall impact of the drug on the sexual differentiation process.

Nicotine

Male offspring prenatally exposed to nicotine have been found to exhibit demasculinized behavior patterns in adulthood. Decreased mounting and intromission behavior in adult nicotine-exposed males has been reported

(Segarra and Strand 1989), indicating incomplete masculinization of the brain in these animals. Bernardi and colleagues (1981) reported a decrease in the postejaculatory interval of males prenatally exposed to cigarette smoke, suggesting an increase in sexual drive. Evidence for feminization of the brain in males prenatally exposed to nicotine is provided by data indicating an increase in saccharin preference in these animals (Lichtensteiger and Schlumpf 1985).

Female sex behavior has not been found to be altered, but an increase in ovarian weight in females prenatally exposed to nicotine was observed (Segarra and McEwen 1992). Other sex-related nonreproductive behaviors have generally been found to be unaffected in females following perinatal nicotine exposure. These behaviors include saccharin preference (Lichtensteiger and Schlumpf 1985), salt preference (Segarra and McEwen 1992), radial arm-maze performance (Levin et al. 1993), and open-field behavior (Peters and Tang 1982). An exception is active avoidance behavior, which was found to be improved in adult females exposed to nicotine throughout gestation (Genedani et al. 1983). Meyer and Carr (1987) found a consistent delay in vaginal opening in females exposed to a low or high dose of nicotine either prenatally or postnatally. In addition, elevations in peripubertal LH values were observed in nicotine-exposed males and females, suggestive of a relative insensitivity to steroid negative feedback.

In males exposed prenatally to nicotine, plasma testosterone levels have been reported to be significantly reduced in adulthood (Segarra and Strand 1989) and when measured on day 18 of gestation in male fetuses (Lichtensteiger et al. 1988), which appear to be consistent with the effects of the drug on masculine sex behavior. However, since the prenatal surge peaks on days 18 to 19 (McGivern et al. 1988*a*; Weisz and Ward 1980), additional time points during this period of gestation need to be measured to reach a more definitive assessment of nicotine's effect on fetal testosterone levels. AG distance at birth was also reported to be significantly smaller in nicotine-exposed males, but birthweight was significantly lower in these animals compared with controls, which likely accounted for the decrease in AG distance. Peters and Tang (1982) observed decreased birthweight in male, but not female, offspring from dams treated with 6 mg/kg of nicotine prior to and during gestation, which is in the same range as the animals from the study of Lichtensteiger and Schlumpf (1985). Segarra and Strand (1989) failed to find a difference in birthweights of either sex from dams treated with 0.25 mg/kg of nicotine twice daily from days 3 to 21 of gestation.

The mechanisms whereby nicotine might alter the sexual differentiation process are multiple. Nicotine receptors in the brain are most dense in the hypothalamus and preoptic area (Clarke et al. 1988) and cholinergic regulation in the preoptic area is modulated by gonadal steroids (Commins and Yahr 1984). Prenatal nicotine exposure has been reported to induce transient increases in nicotinic receptors in fetal and postnatal brains of rats (Slotkin et al. 1987); however, long-lasting effects of prenatal nicotine exposure on the cholinergic system have not been studied. Nicotine treatment of the fetus is known to have long-term effects on catecholaminergic function. Deficiencies in postnatal catecholamine activity have been observed (Navarro et al. 1988, 1990; Ribary and Lichtensteiger 1989; Seidler et al. 1992), in contrast with significant increases in catecholamine turnover observed in fetal brain (Lichtensteiger et al. 1988). Such increases could hypothetically result in a decrease in hypothalamic nuclear binding of estradiol and subsequent demasculinization of the male (Raum et al. 1984, 1990).

Nicotine administration elevates several pituitary hormones, including ACTH, LH, vasopressin, endorphins, and prolactin (Fuxe et al. 1989). Fetal adrenal function, as well as aromatase activity in fetal forebrain, is affected by prenatal nicotine exposure (von Zigler et al. 1991). Activation of the HPA axis in pregnant animals by stress or ACTH injection is known to demasculinize or feminize reproductive behavior of male offspring (Segarra and McEwen 1992); this mechanism may account for a significant portion of the long-term effects of prenatal nicotine exposure on sex-related behaviors.

Marijuana

The animal literature regarding the effects of perinatal exposure to marijuana is quite small, but demonstrates consistent demasculinizing effects of the drug on sexual differentiation of males. In male mice, exposure to Δ^9-tetrahydrocannabinol (THC) or cannabinol during the last week of gestation reduced masculine sex behaviors in adulthood (Dalterio 1980; Dalterio and Bartke 1979). No other behavioral studies of sex-related behaviors have been conducted to the authors' knowledge. In light of the recent identification of the cannabinoid receptor and its widespread distribution in the CNS (Howlett et al. 1990), more studies of the effects of this drug appear warranted.

Walters and Carr (1986) observed long-term decreases in striatal tyrosine hydroxylase activity as well as decreases in dopaminergic autoreceptor

binding in the cortex of rats exposed prenatally to crude marijuana extract. These results suggest a potential role for involvement of catecholamines in alterations of sex-related behaviors of cocaine-exposed offspring. Stronger evidence indicates a role for reduced action of androgens. Marijuana has been consistently observed to decrease testosterone in adults rats and humans (see Ward 1992 for review). Current evidence indicates that it has a similar effect in the fetus and neonate. Dalterio and Bartke (1981) found a decrease in testosterone and dihydrotestosterone in male mice on day 16 of gestation when exposed to THC or cannabinol (50 mg/kg) from days 12 to 16 of gestation. AG distance was significantly increased in these animals, in spite of the fact that body weights were significantly smaller than controls and AG distance was not corrected. Given the fact that AG distance is dependent upon circulating androgen levels, these results appear inconsistent with an effect of the drug on AG distance which is mediated through a reduction in androgens.

However, the reduced androgen level in drug-exposed males is consistent with a significant reduction in testosterone and LH in male rats at birth following exposure to 6 mg/kg THC from days 14 to 19 of gestation (Ahluwalia et al. 1985). Abnormal prostate morphology and long-term deficits were observed in fertility of these rats, which was accompanied by lower prepubertal, but not postpubertal, plasma levels of LH and testosterone. Reductions in fertility have also been observed in mice exposed to THC postnatally (Dalterio 1980; Dalterio and Bartke 1979) and rat offspring of dams exposed to marijuana smoke during pregnancy (Freid and Charlebois 1979).

Alcohol

The literature regarding the effect of alcohol on neurobehavioral sexual differentiation is significantly greater than that for other drugs of abuse and has recently been extensively reviewed (McGivern and Riley 1993). Ethanol is well known to suppress HPG function in adults and this action provided the original basis to hypothesize effects of the drug on the sexual differentiation process (Chen and Smith 1979).

Reproductive and Maternal Behaviors. Several aspects of sexual behavior have been found to be altered in adult rodents prenatally exposed to alcohol. Chen and Smith (1979) reported the first study of sexual behavior in the fetal alcohol-exposed (FAE) male rat in which they observed poorer penile reflexes in FAE males compared with controls. Udani and colleagues (1985) reported a decrease in intromission behavior

in FAE males in the presence of receptive females, suggesting incomplete masculinization. Hard and colleagues (1984) reported increased lordosis in FAE males primed with estrogen and progesterone, indicating incomplete defeminization in FAE males. However, others have not observed differences in FAE males with respect to masculinization (Dahlgren et al. 1989; Hard et al. 1984; McGivern and Handa, unpublished observations) or feminization (Dahlgren et al. 1989; McGivern and Handa, unpublished observations) of sexual behavior.

This inconsistency between studies may reflect the effect of prenatal alcohol exposure on the sensitivity of the HPA axis. Prenatal ethanol exposure has also been found to have long-term effects on the developing HPA axis. FAE female rats exhibit a greater response to stress in adulthood as measured by the release of corticosterone from the adrenal gland. This effect has been found with both prenatal (Taylor et al. 1982; Weinberg 1988, 1992) and postnatal alcohol exposure (Kelly et al. 1991). Stress responsiveness of FAE males was not found to be affected in these studies, but a recent study indicates that increased HPA activation in response to stress can also be observed in adult FAE males (Weinberg 1992). Stress-induced suppression of reproductive behavior is well known (Sachs and Meisel 1988), an effect that is mediated by glucocorticoids (Baldwin and Sawyer 1974; Brann et al. 1990; Kononen et al. 1993; McGivern and Redei 1994). Thus it is possible that the decreases in sexual behavior observed by some investigators reflect a lack of habituation to the open-field testing situation, a situation well known to increase HPA activation in the rat (Fitch et al. 1992). Alternatively, olfactory cues important to initiation of sexual behavior in the rat may be compromised due to the loss of mitral cells in the olfactory bulb following early alcohol exposure (Bonthius et al. 1992).

Fewer sex-related effects of prenatal alcohol exposure have been observed in FAE female offspring. However, a delay in the onset of sexual maturation in mice and rats, as measured by date of vaginal opening, has been consistently reported in FAE females (Boggan et al. 1979; Esquifino et al. 1986; Farry and Tittmar 1975; McGivern and Yellon 1992; McGivern et al. 1992). In a recent study, the date of vaginal opening in females exposed to ethanol during days 7 to 21 of gestation was compared with that of females exposed from days 14 to 21 of gestation (McGivern et al. 1992). An equal period of delay was observed in both groups. These results suggest that exposing the developing hypothalamus to ethanol during the last week of gestation is a more important factor in causing this delay than alcohol exposure to the developing ovary, which

differentiates around day 12 of gestation. This suggestion is supported by the findings of Sonderegger and colleagues (1986), who observed no effect of prenatal ethanol exposure on days 1 to 7 or 8 to 14 of gestation on female reproductive function. However, females exposed to a much higher level of ethanol only on day 8 of gestation have been reported to be more sexually responsive to estrogen in adulthood (Minetti and Fulginiti 1991). Neither prenatal nor postnatal alcohol exposure has been found to alter fertility (Hard et al. 1985; Mitchell 1994; Sonderegger et al. 1986). However, the authors have found that prenatal exposure to alcohol during the last week of gestation accelerates the age-related loss in estrous cyclicity in females (McGivern et al. 1995). These results suggest that prenatal alcohol exposure can shorten the window of reproductive competence in the life of the female.

FAE females display deficits in maternal behavior as evidenced by taking longer to retrieve their pups and poorer nest building (Hard et al. 1985). Retrieval deficits can also be observed in virgin FAE rats presented repeatedly with pups from another mother (Barron and Riley 1985), indicating that alcohol has a disruptive effect on the organization of this behavior. However, the role played by increased stress responsiveness of FAE females needs to be determined to better assess whether prenatal ethanol exposure directly influences the organization of maternal behavior.

Nonreproductive Behaviors. Several sex-related behaviors unrelated to reproduction are feminized in adult FAE males. Such behaviors include saccharin preference, maze performance, and juvenile play behavior, all of which are organizationally dependent upon testosterone for their sexually dimorphic expression. Normally adult female rats consume greater quantities of sweetened solutions such as saccharin than males (Valenstein et al. 1967). This behavior, like most sexually dimorphic behaviors, can be altered by changes in the steroid hormonal environment during either late prenatal or early postnatal life (Beatty 1979). Following both prenatal (McGivern et al. 1984) and neonatal (Barron et al., in press) alcohol exposure in rats, the normal sex difference in saccharin preference has been reported to be eliminated due to an increase in FAE males and a decrease in FAE females. However, in another study (McGivern et al. 1987), preference was increased in FAE males, but unchanged in FAE females. In mice, fetal alcohol exposure has been found to increase saccharin consumption by both sexes (Middaugh et al. 1993).

The literature on the effects of prenatal alcohol exposure on learning documents consistent decrements in learning abilities of alcohol-exposed animals (Meyer and Riley 1986*b*). However, in studies involving spatial mapping when both sexes have been tested, sex-dependent effects have also been observed in complex learning paradigms that involve spatial learning or spatial mapping. Males' learning ability in spatial tasks is generally found to be significantly better than females' (Beatty 1979, 1984, 1992). Prenatal alcohol exposure appears to influence adult performance of a spatial task in a sex-dependent manner. In the Lashley III maze, FAE males required more trials to learn the maze than controls, while the performance of FAE females improved to the level of control males (McGivern et al. 1984). Performance of FAE males in other complex mazes is also impaired (Blanchard et al. 1987; Zimmerberg et al. 1991).

Sex-dependent effects of prenatal alcohol exposure have also been observed in play behavior. Juvenile rats engage in a type of rough and tumble play that resembles wrestling. The degree to which this behavior is expressed is dependent upon perinatal testosterone levels; males typically engage in more of this play behavior than females (Meaney and Stewart 1981). FAE males have been found to display less of this aggressive behavior than controls, while FAE females display more (Meyer and Riley 1986*a*), again suggesting a partial masculinization of females and a demasculinization of males.

Social behavior in the rat is sexually dimorphic (Kellogg et al. 1991) when conspecific animals are paired in a familiar versus an unfamiliar environment. Males exhibit much less interaction with another male in an unfamiliar environment compared with one that is familiar. This difference is not observed in females. Kelly and Dillingham (1994) recently observed a loss of this sexual dimorphism in adult animals exposed to alcohol from postnatal days 4 to 12. This is a period in rat brain development that roughly corresponds to the third trimester in humans. Males exposed to alcohol during this period exhibited significant decreases in social behavior compared with controls, whereas a significant increase was observed in alcohol-exposed females. This feminization of male behavior and masculinization of female behavior by postnatal alcohol exposure was accompanied by reduced cell number in the amygdala region of males, but not females. However, alcohol-exposed females exhibited significant increases in dopamine metabolism in this region. The amygdala has been shown to play a critical role in the expression of social behavior (Meaney and McEwen 1986).

The authors originally proposed that masculinization of FAE females might be due to ethanol-induced activation of the HPA axis, resulting in excessive release of adrenal androgens such as androstenedione (McGivern et al. 1984). However, recent evidence indicates that androstenedione is not released in response to stress in the rat (Fitch et al. 1992), unlike the human, because it is not synthesized in significant quantities in the adrenal gland (van Weerden et al. 1992). Thus it seems unlikely that the masculinization observed in FAE females is related to excessive androgen production.

Other learning paradigms also reveal sex differences in sensitivity to alcohol's effects, but these differences appear to depend upon the period of exposure. While males appear more affected in some paradigms following prenatal alcohol exposure, the reverse appears to be true following postnatal alcohol exposure. When mice prenatally exposed to alcohol were trained to press a bar for reward in an operant learning task, the normal sex differences were eliminated and males appeared to be more affected than females (Gentry and Middaugh 1988). Control males responded at a significantly higher rate than control females under a fixed-ratio 5 (FR-5) schedule. This sex difference was absent in ethanol-exposed mice, primarily due to lower responding by the males. When the response rate by the same animals on the FR-5 schedule was compared with the rate of a differential reinforcement of other behavior (DRO) schedule designed to produce low rates of responding, elevated responses were observed in ethanol-exposed animals of both sexes. The authors argue convincingly that these results may reflect a diminished efficacy of the reinforcer in ethanol-exposed animals (Gentry and Middaugh 1988).

In a passive avoidance paradigm, a simple learning task in which the subject must learn to inhibit its normally preferred response to avoid punishment, both sexes had difficulty with learning following prenatal alcohol exposure (Riley et al. 1979). However, following neonatal alcohol exposure, only females were impaired (Barron and Riley 1990). When spatial navigation was examined in a Morris water maze, the performance of adult females was impaired while adult male performance was unaffected following postnatal alcohol exposure (Kelly et al. 1988).

Fadem (1993) recently examined the behavioral and anatomical effects of postnatal alcohol exposure in the opossum, a species that emerges from the womb at a very immature stage of development. No effect of ethanol was observed in the reproductive behavior, anatomy, or physiology of either sex. However, some evidence was obtained for decreased

fecundity in alcohol-exposed female opossums. In addition, ethanol exposure masculinized threat behavior and scent-marking behavior in females, while feminizing the expression of these behaviors in males. These findings indicate some generalization across species in the effects of ethanol on the sexual differentiation process.

Alterations in Neuroendocrine Function. Ethanol is well known to depress HPG function in both males and females (Purohit 1993) resulting in reduced testosterone levels in males. Ethanol also has been found to produce a marked depression in fetal and neonatal production of testosterone in males, an effect that is quite consistent with its behavioral effects. The prenatal testosterone surge on days 18 and 19 of gestation are greatly attenuated in FAE male fetuses (McGivern et al. 1988*a*). A less marked but significant attenuation has also been observed in the postnatal surge of males from dams consuming approximately 14 g/kg/day of ethanol during the last week of gestation (McGivern et al. 1993). A decrease in testosterone levels of FAE males around the time of birth has been reported by others (Kelce et al. 1989; Rudeen et al. 1986), consistent with an attenuation of the postnatal testosterone surge. This depression of testosterone production appears to relate to a depression in 17-alpha-hydroxylase activity in neonatal testes from FAE males (Kelce et al. 1989, 1990), although changes in LH secretion or sensitivity to LH cannot be ruled out (McGivern et al. 1988*a*). Exposure to lower amounts of ethanol during this period does not appear to influence the postnatal rise in testosterone levels (Dahlgren et al. 1989). Aromatase activity in fetal and neonatal brain is elevated by prenatal alcohol exposure in males, but not females (McGivern et al. 1988*b*). To some degree, this increase in enzymatic activity might be expected to limit the effect of a reduction in testosterone on the defeminization process by increasing the conversion of the available substrate.

Present evidence indicates that LH secretion is decreased in older adult FAE animals of both sexes. Basal LH secretion in adult castrated FAE males and females at 5 to 6 months of age was found to be reduced to nearly half the level of pair-fed controls (Handa et al. 1985). In addition, alterations in the amplitude and duration of pulsatile LH release were observed. This may reflect an accelerated rate of aging in FAE animals. Studies of reproductive function in females have established an age-related decline in circulating plasma LH levels (see Gerall and Givon 1992 for review). Plasma LH is 2 to 4 times less in old rats than young rats, with middle-aged rats exhibiting intermediate levels between the two. The authors have recently observed that FAE females enter

anestraul sterility at a significantly earlier age than pair-fed (PF) or chow-fed (CF) females (McGivern et al. 1995, in press-*b*). Such results indicate that fetal alcohol exposure in females reduces the window of reproductive competency in the lifespan of the animal.

Both sexes have been reported to exhibit reduced sensitivity to sex steroid feedback in the brain (Handa et al. 1985; Jungkuntz-Burgett et al. 1990). Such effects may contribute significantly to the delay in puberty onset in FAE females as well as the demasculinized sex behavior of adult FAE males. However, corticosteroid levels were not measured in the studies cited above, leaving open the possibility that decreases in basal LH or in response to estrogen-induced positive feedback result in part from the increased stress responsiveness of FAE females.

Data concerning the effects of prenatal ethanol exposure on the adult male HPG axis are inconsistent. Reduced sex organ weights in FAE adult males, including testes, prostate, and seminal vesicles have been reported by Udani and colleagues (1985) in animals exposed to ethanol from day 12 through parturition. However, similar reductions were not observed in FAE males of the same strain exposed to ethanol during either the last 2 weeks of gestation or the last week alone (McGivern et al. 1992). In addition, males in this study were observed to have normal testosterone levels and normal sperm counts. Other studies have reported reduced plasma testosterone levels in adult FAE males (Dahlgren et al. 1989; Udani et al. 1985), although the measured plasma values were still in the normal male range. Given this fact, as well as the inconsistency between studies with respect to male sex behavior, the significance of these reductions is not clear at this time.

A number of studies have reported that uncorrected AG distance in FAE males is reduced at birth (Chen and Smith 1979; Rudeen et al. 1986; Udani et al. 1985), which has been interpreted to indicate either reduced levels of testosterone or a decrease in 5-alpha reductase activity to convert testosterone to dihydrotestosterone. Significant reductions in AG distance of FAE males at birth have been observed (McGivern 1987; McGivern et al. 1992), but the results were no longer significant when AG distance was indexed to body weight. Thus, the authors believe that the reduction in AG distance primarily reflects an effect of prenatal ethanol exposure on somatic growth rather than a specific effect of the drug on peripheral androgen metabolism or sensitivity.

Neurotransmitter Function. Animal studies suggest that monoamine neurotransmitters such as NE and serotonin can act as modulators of neuroanatomical and behavioral sexual differentiation during prenatal development (Handa et al. 1986; Jarzab et al. 1986; Raum et al. 1984). Excessive NE activity is known to inhibit the actions of sex steroid hormones in areas of the brain such as the hypothalamus in the neonatal rat (Raum and Swerdloff 1981). Data from other studies indicate that both NE and serotonin play an important role in the structural development of the brain (Lauder and Krebs 1986; Mirmiran et al. 1988). Prenatal alcohol exposure has been shown to have long-term effects on neurotransmitters in the developing brain (Cooper and Rudeen 1988; Detering et al. 1980; Druse and Paul 1989; Druse et al. 1990; Rathbun and Druse 1985). Taken together, these data indicate that an interaction between monoamines and sex steroid hormones on brain development may be an important variable when considering the effects of prenatal alcohol exposure on sexual differentiation.

Functional consequences of catecholaminergic alterations in FAE animals that may be sex-related are suggested by findings from two recent studies. Becker and colleagues (1994) found that mice prenatally exposed to ethanol were more sensitive to the stimulation of locomotor activity by ethanol. This effect was more marked in FAE females than FAE males compared with controls. Both sexes were relatively more sensitive as adults to the antagonistic action of amphetamine on ethanol-induced stimulation of locomotor activity, a finding consistent with reduced monoaminergic function in the brain of FAE animals. Following ethanol administration (0.5 or 1.0 g/kg), Blanchard and colleagues (1993) measured dopamine release by microdialysis in the striatum and nucleus accumbens of FAE adult male and female rats. Dopamine release was absent in FAE females at both doses, while release was evident in FAE males at the higher dose. In controls, release was observed at both doses. The decreased responsiveness in dopamine release in the accumbens appears consistent with the hypothesis of Gentry and Middaugh (1988) of reduced efficacy of reinforcers in FAE animals.

Neuroanatomical Changes. The preoptic area of the hypothalamus is known to play an important role in sex and maternal behaviors in rats (Numan 1988). Within this area is the sexually dimorphic nucleus of the preoptic area of the hypothalmus (SDN-POA), which is several fold larger in males than in females (Gorski et al. 1978). Following either prenatal or perinatal alcohol exposure, this nucleus has been found to be

smaller in adult males, indicating a demasculinizing effect of alcohol during perinatal development (Barron et al. 1988; Rudeen et al. 1986).

The corpus callosum in rats has also been shown to be influenced by prenatal alcohol exposure in a sexually dimorphic manner. Typically, the corpus callosum is larger in males than females. However, data from a recent study suggest that prenatal alcohol exposure reduces or eliminates this sex difference (Zimmerberg and Scalzi 1989). A similar effect of prenatal alcohol exposure on cortical asymmetry has also been reported (Zimmerberg and Reuter 1989). In normal males, the right hemisphere of the cerebral cortex is thicker than the left cortex, whereas females show no such asymmetry. Following prenatal alcohol exposure, this cortical asymmetry appears reduced in males, again suggesting a demasculinizing influence of ethanol.

In the mouse, ethanol reduced the number of immunoreactive gonadotropin-releasing hormone (Gn-RH) neurons detectable at 18 days of gestation following exposure to a high dose of ethanol administered on day 8 of pregnancy (Scott et al. 1992). However, no effect of prenatal ethanol exposure was observed in the number of immunoreactive Gn-RH neurons in 44-day-old female FAE rats with delayed onset of puberty (McGivern and Yellon 1992). Subtle alterations were detected in the morphological characteristics of the neuronal processes of Gn-RH cells in these animals, but the significance is unclear at present. Differences in species, as well as prenatal timing and amount of exposure to alcohol, make comparisons to the results found in the mouse difficult. It remains to be determined whether the decreases in LH secretion of FAE animals reflect a functional deficit related to the Gn-RH neuron.

SUMMARY

The pattern of results from the studies reviewed above indicates that alcohol, morphine, nicotine, marijuana, and possibly cocaine can influence reproductive aspects of the neurobehavioral sexual differentiation process to varying degrees. However, with the exception of alcohol, little is currently known regarding the effects of these drugs on nonreproductive sex-related behaviors. Future studies are needed to define the extent of perinatal disruption induced by each drug on the nonreproductive aspect of the sexual differentiation process.

It is increasingly clear that the neurobehavioral development of reproductive and nonreproductive behaviors is not influenced to the same degree by alterations in the perinatal hormonal or monoaminergic environment, probably reflecting a fundamental underlying difference in the relative contributions of different brain areas to each behavior (Meaney and McEwen 1986). This fact points to the necessity of greater inclusion of sex-related behaviors in animal models used to assess the teratogenic potential of a given drug on the sexual differentiation process.

In light of recent demonstrations of regional structural sex differences in the human CNS (Allen et al. 1989, 1991; deLacoste-Utamsing and Holloway 1982; Hofman et al. 1988; Swaab and Hofman 1988) as well as reports of structural differences in male homosexuals (LeVay 1992; Swaab and Hofman 1990), there is an increasing interest in the contribution of prenatal drug exposure to homosexuality in humans. These findings appear to have led some investigators to interpret behavioral results from animal studies of prenatal drug exposure as being relevant to understanding the causes of homosexuality in humans (Dahlgren et al. 1991; Hard et al. 1984). However, while data from the animal models reviewed above can provide invaluable preclinical evidence to help understand the effects of perinatal drug exposure on brain development and the process of sexual differentiation, the authors believe that the results of these studies provide minimal useful information with respect the prenatal influence of these drugs on homosexual behavior in humans.

Animal models of homosexuality are inherently inadequate for several reasons. No adequate model exists for homosexual behavior in the rodent in the absence of pharmacological administration of steroids. Normal male rats that show low levels of masculine sex behavior in the presence of estrous females do not exhibit increased tendencies to mount other males nor to lordosis when mounted by another male. In fact, male preference behavior for an estrous female rat does not appear to be influenced by perinatal androgen exposure (Merkx 1984).

A second issue that cannot be addressed in an animal model is the fact that sexual orientation in humans is determined by an interaction between hormonal, environmental, and cultural factors (Money 1987). This problem, and others, with a developmental animal model of human homosexuality have been considered by Sachs and Meisel (1988), to which the reader is referred for a more extensive discussion.

Finally, in humans there is also the issue of gender identity, which refers to traits or conditions of maleness or femaleness. The degree to which gender identity in humans is causally linked to cultural or biological influences is an area of current debate (Gentile 1993; Unger and Crawford 1993), but such identity is clearly beyond the scope of animal modeling. Therefore, issues related to sexual orientation of humans and prenatal drug exposure likely await data from future human studies for further resolution.

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ACKNOWLEDGMENTS

Dr. McGivern's work was supported in part by National Institute on Drug Abuse grant no. DA-04490 and National Institute on Alcohol Abuse and Alcoholism grant no. AA-06478. Dr. Handa's work was supported in part by NIAAA grant no. AA-08696 and NSF, BNS grant no. 910-9226.

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Assessment of the Effects of Developmental Toxicants: Pharmacological and Stress Vulnerability of Offspring

Linda Patia Spear

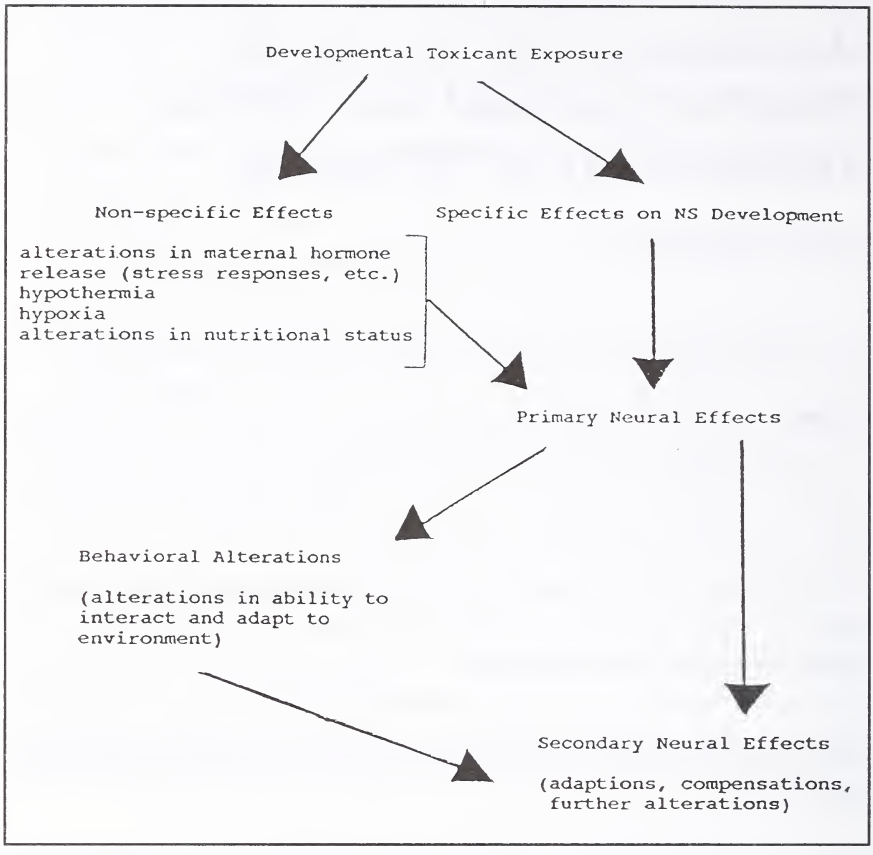
In developmental toxicology studies, behavioral testing of offspring typically is conducted in carefully controlled situations characterized by minimal environmental stressors and distractors. However, increasing the demands of the test situation through the use of environmental or pharmacological challenges may reveal or unmask deficits that may not be evident under basal testing conditions. The purpose of this chapter is to discuss how assessment of pharmacological and stress vulnerability has proved to be useful in revealing alterations in offspring exposed to developmental toxicants. Studies of prenatal exposure to ethanol and cocaine in the laboratory rat are used as examples.

NERVOUS SYSTEM ADAPTATION AFTER DEVELOPMENTAL INSULTS

Table 1 presents a simplistic outline of how developmental toxicants may influence neural development. Exposure to a developmental toxicant may have a number of specific effects and various nonspecific effects that ultimately can affect nervous system development. Together these specific and nonspecific effects lead to a pattern of primary neural alterations that may emerge during or shortly after exposure to the neurotoxicant. These primary neural effects may lead to alterations in the ability of the organism to interact and adapt to the environment, which may lead to further neural alterations. But, particularly important for the purposes of this chapter, the primary neural alterations themselves may result in secondary neural adaptations as the nervous system attempts to adjust and compensate for these initial neural alterations.

The nervous system is a highly interactive and intrinsically self-regulating system that is often homeostatically driven. Manipulating nervous system activity at any stage of life may lead to compensatory adaptations

TABLE 1. *Developmental toxicant exposure.*



in other components of the nervous system, although the nature of those compensatory processes appears to vary not only with the nature of the insult but also with age at the time of the insult. As an example of the latter, chronic blockade of dopamine (DA) receptors in adulthood leads to an upregulation in DA receptor binding (Burt et al. 1977), whereas chronic blockade of these receptors early in development results in a decrease in DA receptor binding (Rosengarten and Friedhoff 1979). Simplistically, it is as if chronic receptor blockade induces receptor upregulation in adulthood in an apparent attempt to maintain homeostatic equilibrium in the DA system; conversely, during development fewer receptors may be formed because fewer appear to be needed due to the chronic presence of the DA antagonist. (See Spear and Scalzo 1986 for further review and discussion.)

The remarkable capacity of the nervous system to adapt to insults has been recognized for decades as an important principle in the field of developmental toxicology. For instance, as stated by Hughes and Sparber (1978, p. 366) 25 years ago: "Mammalian organisms frequently retain apparently normal function after extensive lesions of the CNS with functional reorganization and subsequent recovery of function occurring after destruction of as much as 98 percent of some brain regions." Yet, there may be a cost to such reorganization, with this cost being reflected by a decrease in adaptability. Certain behavioral and physiological functions may appear normal under basal testing conditions, but underlying deficits may be unmasked when subjected to challenges of various kinds. (See Hughes and Sparber 1978 for further discussion.)

TYPES OF CHALLENGES THAT MAY REVEAL UNDERLYING DEFICITS

If the nervous system is often capable of showing at least partial functional reorganization and recovery after exposure to a developmental insult, then exposing the drug-exposed offspring to challenges may unmask deficits that may not be evident under baseline testing conditions. A variety of types of challenges potentially can be used to reveal or unmask underlying deficits. Among the types of challenges that may be useful include assessment of responsiveness to pharmacological challenges, adaptability and responsivity to stressors, age-related alterations, and the ability to recover from subsequent brain damage. The first two of these challenges have been most frequently examined and are the focus of this chapter, although the other approaches listed are also promising. For instance, neural alterations that normally occur with aging may be accelerated or exacerbated after exposure to developmental toxicants. Moreover, exposure to a developmental toxicant may constrain subsequent adaptability in response to later brain damage. For instance, Gottesfeld and colleagues (1989) observed that prenatal ethanol exposure led to a suppression of the normal plasticity seen in dopaminergic terminals in the olfactory tubercle following olfactory bulbectomy. This approach of examining how early neurotoxicant exposure alters later nervous system recovery from brain damage is an interesting one that has been little investigated to date.

In focusing on the first two types of challenges, findings derived from research examining the effects of prenatal exposure to ethanol and cocaine exemplify these approaches. Ethanol was chosen because there is a fairly

large database of animal studies collected over the past 2 decades examining this substance. Cocaine was chosen as the second example because this substance is a particular focus of much current research, although it should be recognized that there is a more limited database with this compound, with most of the animal work in this area published only in the past 5 years. Findings are illustrated using a few examples of work from the author's laboratory examining the developmental toxicology of cocaine in rats.

Pharmacological Challenges

There are two basic approaches that have been used with regard to pharmacological challenges. The first approach has been to use neuro- and psychopharmacological challenges to assess functional alterations in specific neurotransmitter systems induced by the developmental toxicant. This approach has been used extensively in alcohol research and has been useful, in conjunction with neurochemical studies, in documenting alterations in a variety of neurotransmitter systems including the dopaminergic, cholinergic, and serotonergic systems following prenatal ethanol exposure (Bond 1985, 1986*a*, 1986*b*). This approach is beginning to be used in cocaine research as well. For instance, researchers have found that prenatal cocaine exposure results not only in increases in opiate binding in many brain regions (Clow et al. 1991), but also an increased responsiveness to a variety of opiate receptor agonists, particularly mu opiate agonists (Goodwin et al. 1993). Gestational cocaine exposure also increases dopamine type 2 (D_2) receptor binding (Scalzo et al. 1990) and results in an increased psychopharmacological sensitivity to the D_2 receptor agonist quinpirole (Moody et al. 1992).

The second pharmacological approach is to assess postnatal responsiveness to the same drug that was administered prenatally. Basically, the question is whether offspring exhibit a decreased sensitivity (i.e., tolerance) or an increased sensitivity (i.e., sensitization) to the drug to which they were exposed early in life. This approach is of special interest with regard to subsequent drug self-administration: Does early exposure to a drug increase or decrease later self-administration of that substance?

Sensitivity to Later Ethanol Challenge After Prenatal Ethanol Exposure

Table 2 presents a summary of representative findings regarding how prenatal ethanol exposure influences later sensitivity to ethanol challenges. As shown, the effects of early ethanol exposure on later

TABLE 2. *Sensitivity to later EtOH challenge after prenatal EtOH exposure.*

Response	Sensitivity	Reference
Hypothermia	Decreased	Anandam et al. 1980 Abel et al. 1981 Molina et al. 1987
	Increased	Taylor et al. 1983
Hypnotic	No change	Abel 1979 Randall and Bogan 1980 Perez et al. 1983 Randall et al. 1983
Later EtOH intake	Increased	Bond and DiGiusto 1976 Phillips and Stainbrook 1976 Randall et al. 1983 Molina et al. 1987
	No change	Abel and York 1979

ethanol sensitivity appear to depend upon the response measure used. For instance, although there appears to be general consensus that prenatal ethanol exposure does not alter the later hypnotic effects of ethanol as indexed by ethanol-induced sleep times (Abel 1979; Perez et al. 1983; Randall and Boggan 1980; Randall et al. 1983), such exposure has been observed to alter ethanol-induced hypothermia. There is not perfect concordance across laboratories, however, in terms of the nature of the alterations in ethanol-induced hypothermia that are seen in offspring exposed gestationally to ethanol. Whereas most studies have found that prenatal ethanol exposure decreases sensitivity to the later hypothermic effects of ethanol (Abel et al. 1981; Anandam et al. 1980; Molina et al. 1987), Taylor and colleagues (1983) reported an increased hypothermic effect to a later challenge dose of ethanol in these offspring. Differences among laboratories that might have led to these discrepant results are not readily apparent.

As can be seen in table 2, the majority of studies of later ethanol intake have reported that prenatal alcohol exposure increases alcohol self-administration in adulthood (Bond and DiGiusto 1976; Molina et al.

1987; Phillips and Stainbrook 1976; Randall et al. 1983), although this finding is not ubiquitous (Abel and York 1979). It should be recognized that most of these studies examining ethanol intake have used two bottle intake tests where the amount of ethanol consumed is generally without pronounced pharmacological consequences, and where such intake could be influenced by flavor factors and taste neophobia rather than the pharmacological consequences of ethanol per se. Thus, although the data to date are consistent with the suggestion that early ethanol exposure may generally increase later ethanol self-administration, these findings need to be confirmed using other procedures for the initiation of ethanol intake and for the control of taste sensitivity/neophobia.

Sensitivity to Later Stimulant Challenge After Prenatal Cocaine Exposure

With regard to sensitivity to stimulants (cocaine and amphetamine) after prenatal cocaine exposure, less consensus has been reached, probably due in part to the more limited database available. As shown in table 3, in terms of stimulant-induced activity, decreases in sensitivity to stimulants have been reported in testing early in life (Meyer et al. 1992; Sobrian et al. 1990), whereas increases (Foss and Riley 1991; Peris et al. 1992) or no effect (Giordano et al. 1990; Heyser et al., unpublished) on stimulant-induced activity have been observed in adulthood. In terms of stimulant-induced startle effects, both decreased responsiveness in females (Hughes and Dow-Edwards 1992) and no effect (Foss and Riley 1991) have been reported. In general, in those instances where altered sensitivity to stimulants has been observed on activity or startle tests in adulthood, it appears that these effects are relatively modest. With regard to other response measures, the author and coworkers have observed that cocaine-exposed offspring are somewhat less sensitive to the discriminative stimulus effects of cocaine (Heyser et al., unpublished) and are less sensitive to the reinforcing properties of cocaine as indexed by cocaine-induced conditioned odor preferences in infancy (Heyser et al. 1992*a*) and cocaine-induced conditioned place preferences (CPP) in adulthood (Heyser et al. 1992*b*). Such deficits in cocaine-conditioned preferences presumably reflect an apparent reduction in the reinforcing efficacy of cocaine that may be related to a possible alteration in drug abuse liability.

The basic principle behind the CPP procedure is that when drug administration is paired with a particular place on a number of occasions, animals develop a preference for that location to the extent that they find the drug reinforcing. In the study by Heyser and colleagues (1992*b*),

TABLE 3. *Sensitivity to later stimulant challenge after prenatal cocaine exposure.*

Response	Sensitivity	Reference
Stimulant-induced activity		
In infancy	Decreased	Sobrian et al. 1990 Meyer et al. 1992
In adulthood	Increased	Foss and Riley 1991 Peris et al. 1992
	No effect	Giordano et al. 1990 Heyser et al. 1994
Stimulant effects on startle	Decreased (females)	Hughes and Dow-Edwards 1992
	No effect	Foss and Riley 1991
Cocaine discriminability	Decreased	Heyser et al. 1994
Cocaine-induced preferences		
In infancy (odor pref.)	Decreased	Heyser et al. 1992*a*
In adulthood (CPP)	Decreased	Heyser et al. 1992*b*

offspring from three groups of dams were studied: dams subcutaneously injected with 40 milligrams per kilogram per 3 milliliters (mg/kg/ 3mL) daily on gestational days 8 through 20 (C40); dams injected daily with saline and whose daily food and water intake was paired with that of cocaine-exposed dams (PF—this pair-fed nutritional control group was used to control for the transient anorexia seen in cocaine-exposed dams at the onset of treatment); and untreated control dams given ad libitum access to lab chow (LC). Adult offspring from each prenatal treatment group were exposed 30 minutes a day to a white chamber and to a black chamber. For half of the animals, the black chamber was always paired

with a saline injection, whereas the white chamber was paired with an injection of either saline or 2 or 5 mg/kg cocaine. The other half of the animals received these injections in the opposite chambers. On the test day, animals were not injected prior to being given a 15-minute preference test where the amount of time spent in each of these chambers or a novel gray chamber was recorded.

Normal adult animals exhibit a CPP for cocaine—that is, on the test day animals that received cocaine in a particular chamber spend more time in that chamber than animals that received saline injections in that chamber. Indeed, as shown in figure 1, LC control animals that received 2 or 5 mg/kg cocaine during conditioning exhibited significant place preferences in both the black and white chambers. Similarly, PF control animals that received 5 mg/kg in the black chamber and 2 mg/kg in the white chamber during training also exhibited significant place preferences. In contrast, no evidence of a cocaine-induced place preference was seen in adult offspring prenatally exposed to cocaine when trained with either dose of cocaine in either chamber. A similar deficit was seen in cocaine-exposed offspring when tested in infancy for the development of cocaine-induced conditioned odor preferences (Heyser et al. 1992*a*).

There are several possible explanations of these findings. The lack of significant CPP in the cocaine-exposed offspring could reflect a learning deficit. This possibility, however, is rather unlikely; it was previously shown that adult cocaine-exposed offspring do not differ from controls in their ability to learn a rather complex conditional discrimination task (Heyser et al. 1992*c*). It also does not appear that this deficit in the formation of cocaine-induced CPP is related to any alteration in cocaine pharmacokinetics in these animals; no differences were found among C40, PF, and LC offspring in brain levels of cocaine at any time examined (5 to 60 minutes postinjection) following intraperitoneal administration of a challenge dose of 10 mg/kg cocaine in adulthood (Heyser et al. 1994). A final possibility is that this lack of cocaine-induced CPP may reflect an attenuation in the reinforcing consequences of cocaine in these animals.

These data may reflect an alteration in drug abuse liability in the cocaine-exposed offspring. Although there are some exceptions, manipulations that decrease CPP generally increase self-administration and vice versa. (See Le Moal and Simon 1991 for references and discussion.) The typical interpretation of these findings is that manipulations which decrease the

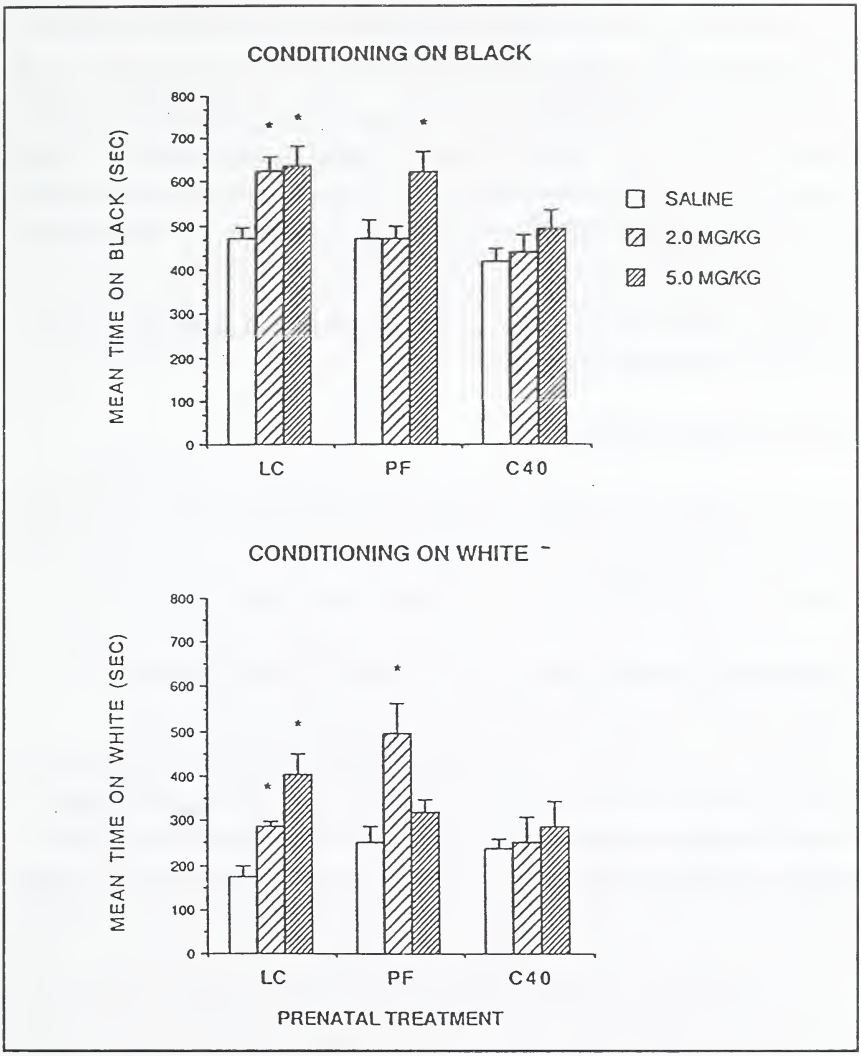


FIGURE 1. *Mean time (seconds) spent on the test day in the previously drug-paired compartment when animals from each prenatal treatment group (C40 = cocaine; PF = pair-fed; LC = non-treated control) were conditioned in black (top) and white (bottom). Place conditioning was defined to occur if the cocaine-conditioned animals spent significantly more time in the drug-paired chamber than animals receiving saline (* = $p < 0.05$ for these comparisons).*

SOURCE: Reprinted from Heyser et al. 1992*b*. Copyright 1992 with kind permission from Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 OBW, UK.

reinforcing properties of a drug result in increased drug self-administration because higher doses of the drug are necessary to compensate for the decreased rewarding efficacy of the drug. That is, because the animals find the drug less reinforcing, they self-administer more of the drug to obtain its psychoactive consequences (Le Moal and Simon 1991). Taken together, these findings raise the possibility that the decrease in cocaine-induced odor and place preferences seen in offspring exposed gestationally to cocaine (Heyser et al. 1992*a*, 1992*b*) may be associated with an increase in the later self-administration of cocaine. This possibility should be considered speculative until directly tested using intravenous (IV) self-administration protocols.

Stress Responsivity

A second challenge that may help unmask underlying neural alterations induced by developmental toxicants is assessment of stress responsivity. This type of challenge has been shown to be a sensitive and robust indicator of the effects of prenatal ethanol and cocaine exposure.

Responsivity to Stressors after Prenatal Ethanol Exposure. Prenatal exposure to ethanol has been shown to alter later stress responsivity. As shown in table 4, these findings are remarkably consistent across studies. In infancy, offspring prenatally exposed to ethanol exhibit increased plasma and brain corticosterone levels and a blunted pituitary-adrenocortical response to stressors such as injection, ether, and ethanol challenge that lasts for at least the first postnatal week (Taylor et al. 1982, 1986; Weinberg 1989). By contrast, when tested in adulthood, these ethanol-exposed offspring showed no alterations in basal corticosterone levels but exhibited a hyperresponsive pituitary-adrenal response to stressors such as footshock, ether, and ethanol challenge (Nelson et al. 1986; Taylor et al. 1982; Weinberg 1988; Weinberg and Gallo 1982; Weinberg et al. 1986). These hormonal effects are robust, particularly in female offspring, and have been replicated across laboratories. Offspring exposed prenatally to ethanol have not been assessed for their behavioral responsiveness to stressors as frequently as they have been assessed hormonally. Nevertheless, the available evidence suggests that gestational ethanol exposure also alters behavioral adaptability to stressors in adulthood as indexed by decreased immobility in swim tests (Bilitzke and Church 1992; Nelson et al. 1984).

TABLE 4. *Responsivity to stressors after prenatal EtOH exposure.*

Response measure	Reference
Hormonal alterations to stress	
In infancy	
Increased corticosterone levels - birth	Taylor et al. 1982, 1986 Weinberg 1989
Blunted pituitary-adrenal response to stressors	Taylor et al. 1986 Weinberg 1989
In adulthood	
No alteration in basal corticosterone	Taylor et al. 1982 Weinberg et al. 1986 Weinberg 1988
Hyperresponsive pitituary-adrenal response to stressors	Taylor et al. 1982 Weinberg and Gallo 1982 Nelson et al. 1986
Altered behavioral adaptability to stress	
In adulthood	
Decreased immobility in swim tests	Nelson et al. 1984 Bilitzke and Church 1992

Responsivity to Stressors after Prenatal Cocaine Exposure. Altered responsivity to stress also appears to be a robust and reliable finding in studies of prenatal cocaine exposure (table 5), although the focus to date has been on behavioral rather than hormonal assessments. These findings are particularly notable in that they may represent the clearest example of replicable findings at this early stage of animal research in the developmental toxicology of cocaine. Like ethanol-exposed offspring, adult offspring prenatally exposed to cocaine do not differ in basal corticosterone or adrenocorticotropic hormone (ACTH) levels (Cabrera et al. 1993; Kuhn and Spear, unpublished observations); to the author's knowledge, there are no publications to date regarding pituitary-adrenal stress responsivity in these animals. However, in terms of behavioral responsivity to stress, there are a number of reported alterations in cocaine-exposed offspring in their acute and long-term

TABLE 5. *Responsivity to stressors after prenatal cocaine exposure.*

Response measure	Reference
Hormonal	
No alterations - basal ACTH or corticosterone level	Cabrera et al. 1993 Kuhn and Spear (unpubl. observ.)
Behavioral	
Decreased immobility	
Swim tests	Bilitzke and Church 1992 Molina et al. 1994
During intermittent shock exposure	Molina et al. 1994
Altered behavioral responsivity following prior footshock	
Decreased open field immobility	Molina et al. 1994
Increased reactivity to later footshock	Smith et al. 1989
"Frantic swimming" - water maze tasks	McMillen et al. 1991 Johns et al. 1992 Smith (personal commun., 1992)

responses to a variety of stressors. Among the notable behavioral alterations are decreases in immobility (Bilitzke and Church 1992; Molina et al. 1994) in response to acute stressors, as well as several longer lasting behavioral alterations following prior exposure to footshock (Molina et al. 1994; Smith et al. 1989). Increases in "frantic" behavior (Johns et al. 1992; McMillen et al. 1991; Smith, personal communication, 1992) also have been reported.

The results of a recent study illustrate these findings (Molina et al. 1994). In this study, adult male C40, PF, and LC offspring were assigned to one of three groups. Each animal in one group (FS) was given a 5-minute

forced swim in room-temperature water on the first day; animals in the second group (SHOCK) were individually given 20 brief footshocks over a 10-minute period on this day; animals in the third group (CTRL) were not manipulated on the first day. All animals were then given a 5-minute open-field test on the second day. Normal adult animals, when exposed to a stressful situation such as a forced swim or intermittent footshock, exhibit an increase in immobility not only during the stressor, but also frequently after the stressor when confronted with a novel situation such as an open field (Armario et al. 1991; De Pablo et al. 1989; Van Dijken et al. 1992). According to Bolles (1970) and others (De Pablo et al. 1989; Fanselow 1986), this immobility is thought to be an adaptive response to stress.

As shown in figure 2*a*, cocaine-exposed offspring exhibited less immobility than control offspring during the forced swim test, thereby replicating findings reported previously by Bilitzke and Church (1992). Similarly, cocaine-exposed offspring also exhibited less immobility during the intermittent footshock exposure (see figure 2*b*). Moreover, when animals were tested 24 hours later in the open field, LC and PF control offspring that received prior exposure to footshock exhibited more immobility than their previously unstressed littermates (figure 2*c*). This increase in immobility induced by prior footshock was not seen in the cocaine-exposed offspring. Thus, both during and following exposure to an acute stressor, offspring subjected to cocaine prenatally exhibited less immobility than both groups of control offspring. To the extent that immobility is an adaptive species-specific defense response to stressors (see Bolles 1970), these data suggest that prenatal cocaine exposure may disrupt later stress adaptability. As noted in table 5, a number of studies report similar findings. This alteration in stress responsivity appears to be a robust and reliable finding, even given the limited number of investigations to date examining the behavioral toxicology of cocaine in animal models.

SUMMARY AND CONCLUSIONS

From this brief summary of alterations in pharmacological and stress responsivity following gestational ethanol and cocaine exposure, a number of conclusions can be reached, although some of these conclusions are more speculative than others.

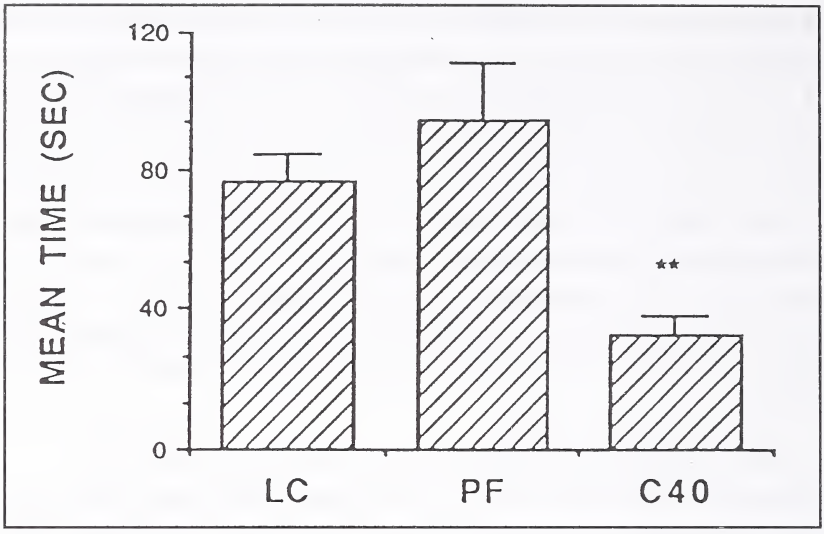


FIGURE 2a. *Mean time (seconds) spent in immobility during A) the forced swim test, B) the footshock session, and C) the subsequent open-field test by adult male offspring prenatally exposed to cocaine (C40), pair-fed control offspring (PF), and nontreated control offspring (LC) (N=8-11 per test condition and treatment group). In C) animals were previously submitted to one of three conditions 24 hours prior to the open field test: 5 minutes of forced swim (FS), 10 minutes of intermittent footshock (SHOCK); or nonmanipulated (CTRL). Error bars indicate SEMs.*

KEY: A): ** = $p < 0.001$ when compared with LC and PF groups; B): * = $p < 0.05$ when compared with LC and PF groups; C): * = $p < 0.05$ when compared with corresponding CTRL groups).

SOURCE: Data derived from Molina et al. 1994.

Both pharmacological challenges and stress responsivity have been shown to be sensitive to the effects of prenatal ethanol as well as prenatal cocaine exposure.

In terms of pharmacological sensitivity, there is some inconsistency in the findings obtained. Nevertheless, there is limited evidence to suggest that prenatal exposure to ethanol (and potentially cocaine) may increase later

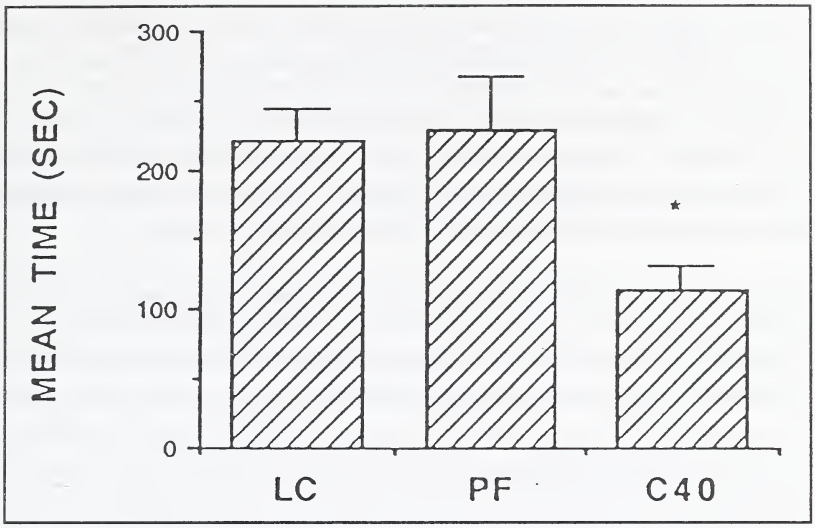


FIGURE 2b.

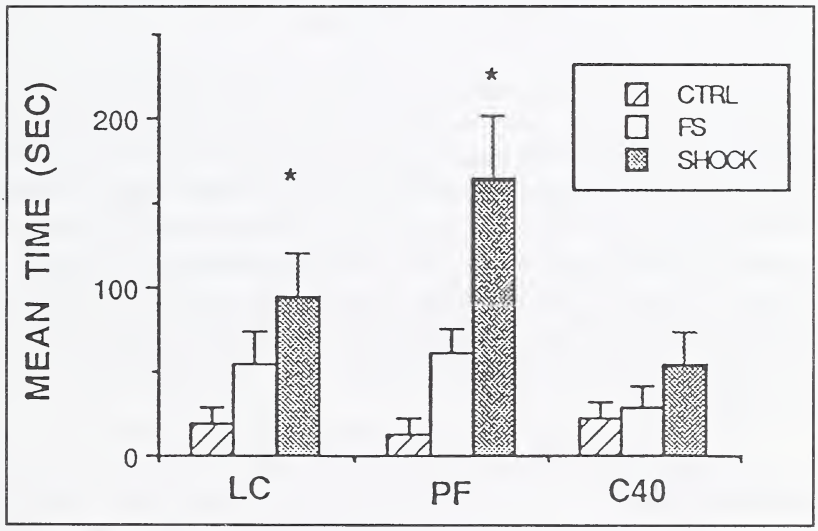


FIGURE 2c.

self-administration of the exposed drug. It should be recognized, however, that this possibility remains to be directly tested with cocaine, and needs further verification with ethanol using additional test procedures. It is interesting that a similar finding has been reported in the opiate literature: adult offspring exposed in utero to methadone exhibit an increase in subsequent morphine self-administration (Peters and Hovious

1983). Taken together, these data support the intriguing but still speculative suggestion that early chronic exposure to a drug of abuse may increase the propensity for later self-administration of that or related substances. More systematic research is needed to test this possibility and to determine whether alterations may be seen in later self-administration of other classes of abused drugs—that is, whether early drug exposure may increase the later propensity for general drug abuse.

In terms of stress vulnerability, offspring exposed prenatally to ethanol predominantly have been assessed in terms of hormonal response measures, whereas the focus to date for offspring exposed prenatally to cocaine has been on alterations in behavioral responsivity to stressors. Yet prenatal exposure to either substance has been shown to result in consistent and long-lasting alterations in later responsivity to stressors in the absence of alterations in basal hormone levels in adulthood. For ethanol, these effects are particularly robust in female offspring; sex differences in stress responsivity in cocaine-exposed offspring have not been reported although few studies to date have been designed specifically to assess potential sex differences.

As previously noted, the nervous system has a remarkable capacity to reorganize following insults at any age. The cost of neural reorganization following developmental insults may be associated with a decrease in adaptability that may not necessarily be evident under basal, nondrug, minimal stress, low distractibility testing conditions. Yet it is perhaps worth noting that these are the very conditions under which subjects are tested. In future work in developmental toxicity, it may prove useful to increase the demands of testing by assessing offspring under challenge conditions to reveal or unmask deficits that are not evident under basal test situations.

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ACKNOWLEDGMENTS

The preparation of this article and the research presented therein were supported by National Institute on Drug Abuse grants R01 DA14478 and K02 DA00140.

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Comparability of Human and Animal Studies of Developmental Cocaine Exposure

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Human and animal studies of cocaine exposure during development can be compared on many levels and from many different perspectives. The most logical place to begin is to pose the question: What is the best animal model for cocaine abuse during pregnancy, and does the information obtained in a given animal model apply to humans? Of course, due to the complex nature of cocaine's pharmacology, there is no perfect animal model. It is virtually impossible to perfectly model human development in anything other than a human being. Since invasive research is not done in humans, researchers are forced to study an approximation of human development. Each approximation of the human condition, or model, has specific strengths and weaknesses. The data obtained from each model must be evaluated in terms of the strengths and weaknesses of the model.

The major pharmacological actions of cocaine in the maternal-fetal unit include cardiovascular and hemodynamic effects as well as effects on fetal physiology with major targets in the central and peripheral nervous systems. What then are the characteristics that can be ascribed to individuals developmentally exposed to cocaine? Cocaine can affect the individual on many levels: behavioral, cognitive, developmental, and structural. The central nervous system (CNS) receives particular attention in this chapter since cocaine has potent effects on the CNS of the adult.

The biggest hurdle for the animal model is the problem of the polydrug microculture. The influence of lifestyle and, in particular, alcohol and cigarette use, on development including postnatal development is significant. While the effects of alcohol, other drugs, and nicotine can be addressed in animal models, the multidimensional confounds such as socioeconomic status, poor prenatal care, and sexually transmitted disease are difficult to adequately address in animal models. The value of the animal model, or preclinical research, is the ability to determine the biological effects of a substance independent of cultural and sociologic

influences. It is a pure biological system in which the pharmacological and physiological effects of a drug can be studied in detail and quantified.

Historically, specific hypotheses generated through clinical experience and anecdotal evidence are tested in animal models. In the case of cocaine, however, animal studies have, on the whole, been run concurrently with clinical studies. For example, the first animal studies reported teratologic effects of cocaine in the early 1980s (Fantel and MacPhail 1982; Mahalik et al. 1980) and the earliest clinical reports describing structural defects in cocaine-exposed infants were in the mid-1980s (Bingol et al. 1987; Chasnoff et al. 1988; Dixon and Bejar 1989). However, the more recent and carefully controlled animal studies indicate that cocaine is not teratogenic unless it is administered intraperitoneally (IP).

Webster and colleagues (1991) and Webster and Brown-Woodman (1990) produced digit and CNS malformations when cocaine was administered to the rat IP on gestation day 16. Finnell and colleagues (1990) found that in the mouse, cocaine produced congenital malformations, including cardiovascular defects, limb abnormalities, and genitourinary malformations when administered IP either during gestation days 6 to 8 or 8 to 10. (The IP route of administration increases the teratogenic potency of drugs due to extraplacental diffusion of the drug to the fetus, resulting in higher drug levels in the fetus than in maternal plasma or tissues (DeVane et al. 1989).)

More recent clinical studies, which include some large retrospective studies such as reviews of birth certificates, also did not find an association between cocaine and structural defects with the possible exception of hydronephrosis (Chavez et al. 1989; Hutchings 1993; Martin et al. 1992; Mehanny et al. 1991). Therefore, as far as structural teratogenesis is concerned, both animal research and clinical studies have found that cocaine is not teratogenic.

In addition to structural alterations, growth of the organism is often used as a measure to determine whether a compound is developmentally toxic. Growth, in general, is the result of multiple influences, including maternal nutrition; uteroplacental blood flow and function; a variety of peripheral receptors in the developing organism; and the function of the maternal and fetal hypothalamic-pituitary axes (HPA) which regulate growth, including growth hormone releasing hormone (GHRH), somatostatin, growth hormone (GH), and thyroid hormone. Recently, prenatal cocaine exposure has been shown to increase somatostatin levels in forebrain and olfactory bulb following prenatal and/or postnatal

exposure (Rodriguez-Sanchez et al. 1991). Whether somatostatin levels in various brain regions correlate with hypothalamic levels was not addressed in the report. However, if they are, in fact, related, one could speculate that since somatostatin inhibits somatic growth, the increased somatostatin levels in fetal brain may be responsible for the reduced body size.

Clinically, a decrease in birthweight is often found in cocaine-exposed neonates. Some authors have described altered patterns of growth, with the head being most affected, while others have found no alteration in body proportionality (Frank et al. 1990; Little and Snell 1991; Mitchell et al. 1988). Generally, in the rat, prenatal cocaine does not reduce birthweight or alter postnatal growth patterns unless it is administered at toxic levels (Church et al. 1990). However, to the author's knowledge, the proportionality of growth in exposed pups has not been determined. Since animal studies do not show altered birthweights following all but the most toxic doses of cocaine, the decreases in body weights often cited in human studies are most likely caused by factors other than cocaine, such as polydrug, alcohol, and cigarette use.

Both the teratologic effects and the growth effects are generally attributed to cocaine's effects on the cardiovascular system. Cocaine has potent cardiovascular effects, particularly during pregnancy when levels of cholinesterase (the enzyme which breaks down cocaine) are reduced (Shnider 1965) and cardiac tissue is primed with progesterone (increasing sensitivity to catecholamines) (Sharma et al. 1992). According to Wilkerson's work in the dog, low doses of cocaine increase blood pressure and heart rate while high doses take on the typical local anesthetic effects of slowing the heart and decreasing blood pressure (Wilkerson 1988).

Several groups have examined the cardiovascular effects of cocaine in the pregnant sheep model, which is considered the best model to study cardiovascular changes in pregnancy since both the mother and the fetus can be monitored (Burchfield et al. 1991*b*; Moore et al. 1986; Woods et al. 1987). Cocaine administration to the mother results in dose-dependent increases in maternal blood pressure and heart rate and decreases in uteroplacental blood flow and fetal oxygenation (Woods et al. 1987). Once plasma cocaine levels return to nonpharmacologic levels, the cardiovascular parameters return to normal.

Although there have been no studies published of human pregnancies under the conditions of cocaine abuse, scientists generally accept the sheep as a good model for drug effects in humans. The situation is

somewhat different in the rat. That is, cocaine administered to the awake and freely moving mother increases blood pressure only transiently and actually decreases heart rate (Dow-Edwards et al. 1993; Morishima et al. 1992). Nevertheless, both the Morishima study and the Dow-Edwards study found that cocaine reduced blood flow to the placenta. The author's study (Dow-Edwards et al. 1993) also found that cocaine reduced oxygenation in the near-term fetus. Since hypoxia has, in and of itself, been demonstrated to produce long-term neurobehavioral alterations in the rat (Longo and Hermans 1992), the actions of cocaine on the cardiovascular system and the reduction in uteroplacental blood flow could be responsible for a portion of the neurobehavioral effects currently attributed to cocaine's actions on neurotransmitters (see below). Some interesting data published by Koegler and colleagues (1991) demonstrate that blocking the vasoactive effects of cocaine blocks the decrease in ornithine decarboxylase, a key regulatory enzyme in the control of neural cell differentiation. However, until the timecourse and degree of hypoxia produced by cocaine are documented and appropriately modeled in the pregnant rodent, the contribution of hypoxia to the overall effect of cocaine on structural as well as neurobehavioral development in the rodent cannot be estimated. Therefore, although cardiovascular responses to cocaine are dampened in the rodent compared with humans, it is clear that cocaine's effects on the cardiovascular system interact with its effects on neurotransmitters and development.

In terms of neurobehavioral effects, scientists have relied on research in the adult animal to formulate hypotheses about the pharmacology and physiology of cocaine in development. Researchers utilizing animal models, most often the rat, have exerted an intense effort to determine the effects of cocaine on brain function and neurochemistry. This effort has greatly facilitated the understanding of cocaine's actions in the adult brain and provided direction for research into cocaine's developmental effects

In the adult, cocaine has multiple and interactive effects. In addition to its local anesthetic effects, cocaine acts by inhibiting reuptake and therefore metabolism of the three major neurotransmitters—dopamine (DA), serotonin (5-HT), and norepinephrine (NE)—thus increasing their concentration in the synapse and potentiating the effects of all three neurotransmitters. Effects of cocaine on the 5-HT system are even more complex due to uptake inhibition of the 5-HT precursor tryptophan and inhibition of the enzyme that synthesizes 5-HT, tryptophan hydroxylase. These effects would be expected to decrease serotonergic function over the long run.

At this time, the behavioral and neurochemical responses to cocaine are believed to be similar in rodents, nonhuman primates, and humans. For example, sensitization occurs in all three species; repeated exposure to cocaine at doses that initially produce simple behavioral activation eventually produce increasingly bizarre behavior that is quantitatively and qualitatively different from that initially observed (Post and Rose 1976). Sensitization is associated with specific neurochemical changes (i.e., a decrease in striatal DA concentration and alterations in the concentrations of DA receptors). The receptors can be increased or decreased depending on the brain region, duration of drug exposure, and the time since the last drug dose (Farfel et al. 1992; Goeders and Kuhar 1987; Kalivas et al. 1988; Kleven et al. 1988, 1990; Yeh and De Souza 1991). Generally, however, the concentration of DA and the numbers of DA type 1 (D_1) receptors are decreased.

While these neurochemical changes have been most thoroughly investigated in the rodent brain, parallel changes in DA receptors have been found in chronic cocaine abusers using positron emission tomography (PET) (Volkow et al. 1992) and in human postmortem samples (Hurd and Herkenham 1993). Therefore, there is empirical evidence that changes in the DA system following chronic cocaine exposure are parallel in human and rodent brain.

While DA is known to be important in the reinforcing effects of cocaine, all three neurotransmitters have been implicated as being important in the development of the CNS. In addition, the developing brain is quite different from the adult brain and some phenomena, such as sensitization, do not occur in developing organisms at all (Meyer and Yacht 1993). Therefore, while data collected in adult animals can serve as a guide for developmental studies, they clearly cannot replace developmental studies. The details of the neurobehavioral effects of cocaine exposure during development are found elsewhere in this monograph and the mechanisms of action have also recently been reviewed (Dow-Edwards 1995). Due to the multiplicity of cocaine's effects, it is truly unique as a behavioral teratogen.

EXAMPLES OF CROSS-SPECIES COMPARABILITY

Historically, structural teratogens have been more thoroughly studied than functional teratogens. A wide range of potentially teratogenic substances has been examined for cross-species comparability and several

excellent reviews have been published (Brown and Fabro 1983; Hemminki and Vineis 1985; Schardein et al. 1985). The authors generally conclude that although the specific structural damage produced by a given substance may be different in animals compared with man, compounds that are found to be teratogenic in man are also teratogenic in animals and vice versa. For example, Brown and Fabro (1983) state that of the agents known to be teratogenic in humans, 97 percent of the tests in another single species also showed the agents to be teratogenic in that species. However, of 165 compounds believed to be nonteratogenic in humans, only 28 percent of the compounds were nonteratogenic in animals. Therefore, animal studies found relatively greater teratogenesis than human studies. This result is expected due to the greater numbers of animals that can be examined for events such as terata which have a low natural rate of occurrence. In addition, the compounds showing no teratogenesis in human may actually be teratogenic to a degree that is too low to be detected in the populations sampled. On the other hand, some substances may be teratogenic only in animals due to species-specific metabolism, pharmacokinetics, or developmental characteristics (see below).

A review of cross-species comparability for neurobehavioral endpoints was the subject of a meeting held in Williamsburg in 1989. This workshop, "Qualitative and Quantitative Comparability of Human and Animal Developmental Neurotoxicity," was cosponsored by the National Institute on Drug Abuse (NIDA) and the U.S. Environmental Protection Agency (EPA). The participants concluded that given the limitations of incomplete information available, particularly with regard to human dosing and exposure periods, the degree of across-species comparability for a wide range of compounds was considered remarkable. Several categories of behavior were evaluated, including motor development and function, cognitive function, sensory function, motivational/arousal behavior, and social function (Stanton and Spear 1990) (table 1). Across a wide range of developmental toxicants, similar effects were found for each agent. In addition, certain measures tap processes that are closely comparable across several species; examples of these are given in table 1.

That is, for each neurobehavioral endpoint listed (e.g., acoustic and tactile startle), motor activity, sleep-wake cycles, habituation, short-term and long-term memory), good cross-species comparability was found for a range of compounds. A given toxicant may or may not have altered a given endpoint, but the same pattern of alterations was found in humans, nonhuman primates, and rodents for the endpoints listed (see Stanton and

TABLE 1. *Comparability of endpoints in developmental neurotoxicology.*

Functional Category	Species		
	Rodents	Nonhuman Primates	Humans
Sensory	--	--	Sensory psychophysics
	PI-ASR	PI-ASR	PI-ASR
	Sensory-evoked potential	Sensory-evoked potential	Sensory-evoked potential
Motivation/ arousal	Activity	Activity	Activity
	Sleep-wake	Sleep-wake	Sleep-wake
	--	--	Impulsivity
	Seizures	Seizures	Seizures
Cognitive	--	--	Bayley MDI
	--	--	IQ
	--	Visual recognition memory	Visual recognition memory
	--	--	Language development
	Habituation	Habituation	Habituation
	Short-term memory	Short-term memory	Short-term memory
	Long-term memory	Long-term memory	Long-term memory
	Pavlovian conditioning	Pavlovian conditioning	Pavlovian conditioning
	SCOB	SCOB	SCOB
Motor	--	--	Bayley PDI
	Reflex development	Reflex development	Reflex development
	Locomotor development	Locomotor development	Locomotor development
	Motor control	Motor control	Motor control
	EMG	EMG	EMG
Social	Suckling	Suckling	Suckling
	Mother-infant contact	Mother-infant contact	Mother-infant contact
	Communication	Communication	Language
	Aggression	Aggression	Aggression
	Play	Play	Play
	Reproductive behavior	Reproductive behavior	Reproductive behavior

KEY: DEV = development; EMG = electromyograph; MDI = Mental Development Index; PDI = Physical Developmental Index; PI-ASR = prepulse inhibition of acoustic startle response; SCOB = schedule-controlled operant behavior.

SOURCE: Reprinted from *Neurotoxicol Teratol* 12:261-268, Stanton, M.E., and Spear, L.P., 1990, with kind permission from Elsevier Science Ltd., The Boulevard, Langford Lane, Kidlington OX5 1GB, UK.

Spear 1990 for a comprehensive review). In theory, examination of these specific endpoints would produce closely comparable results for a given developmental toxicant. This also is the case for cocaine.

THE IMPORTANT ISSUES

Once the concept that certain behavioral measures tap similar processes across species is accepted, the next step is to determine the best model for cocaine administration during pregnancy. Three important issues allow one to model human development in animals and draw comparisons: pharmacokinetics, dose, and timing. Each outcome measure (dependent variable) may be sensitive to a unique set of circumstances. As seen in the adult, cocaine is a very complex drug with at least four major actions: inhibition of reuptake of DA and NE, effects on the 5-HT system, and local anesthetic actions. Data collected from studies in which cocaine is administered to adult rats indicate that the dosing schedule (continuous versus intermittent), the dose, the animal's gender, the conditions under which the dependent measures are collected, and particularly the time since the last administration are important in determining the magnitude and direction of the cocaine response. Even the route of administration is important in determining which systems respond to cocaine as well as the direction of the response.

PHARMACOKINETICS

Studying cocaine's effects in adult and developing animals is quite challenging due to the complex interactions of pharmacokinetics and pharmacologic responses. Within the last 2 years, rodent studies have appeared which indicate that cocaine effects in the adult depend to a great extent on the route of administration, presumably due to the importance of the rate at which cocaine occupies its receptors in the brain and the periphery. Whereas intravenous (IV) cocaine produces increased metabolic rates in components of the motor, sensory, and limbic systems, IP cocaine stimulates only the motor and sensory areas (Porrino 1993). Broderick (1992) has shown that IV and subcutaneous (SC) administration of cocaine produce the opposite effects on extracellular DA (ECDA) levels in accumbens. That is, IV cocaine increased ECDA as expected, while SC administration actually decreased ECDA. When a drug's route of administration can determine not only the magnitude of the

neurochemical alteration but also the direction, pharmacokinetic considerations take on additional significance.

What then is the best route of administration for use in cocaine studies in animals to maximize data comparability to the human condition? This question would be answerable if researchers knew precisely what plasma and brain cocaine levels were associated with the various routes of administration in humans. Of course, brain cocaine levels would be almost impossible to determine in humans were it not for PET, which can determine the uptake of labeled cocaine in human volunteers. Fowler and colleagues (1989) determined that uptake of cocaine was highly correlated with subjective ratings. Human brain cocaine levels can also be inferred by evaluating the high produced following various routes of administration. Jones (1990) has examined human volunteers for differences in plasma (venous) cocaine levels following smoking and IV cocaine administration, and found that the maximum subjective high does not correlate with the maximum venous blood levels. Although smoking cocaine is widely acknowledged as the most reinforcing way to administer cocaine, the venous blood levels following this route (100 milligrams (mg) in pipe) were not as great as those following IV administration (0.6 mg/kg) and neither were the subjective ratings (Jones 1990; see table 2).

Recent data presented by Evans and colleagues (1995) also compared smoked and IV cocaine. They found the two routes produced roughly equivalent arterial and venous cocaine levels as well as similar cardiovascular responses. The timecourses of the cardiovascular and subjective effects were highly correlated with the arterial plasma curve following both routes of administration. However, smoked cocaine actually produced somewhat lower subjective ratings of liking and feeling stimulated compared with IV cocaine. Although cocaine is probably most frequently abused by smoking, the available data on plasma cocaine levels do not provide physiologic support for the popularity of this route. Even though the human physiological data do not clarify the basic mechanism involved in producing cocaine's subjective effects, smoking is the best route to model in animal studies of development since smoking is generally considered the most popular route of administration.

There has been some progress in modeling crack smoking in animals, but this method of administration remains problematic. Work in sheep by Burchfield and colleagues (1991*a*) found that while a model of inhalation could be demonstrated, the peak plasma cocaine levels were one

TABLE 2. *Pharmacokinetics of cocaine: Comparison of species and routes of administration.*

Species	Route	Dose (mg/kg)	Peak Value (ng/ml)	Peak time	T1/2 (min)	Mat-fet ratio	Source
Human	IV	0.23	221	<5 min			Javid et al. 78
	IV	0.44	250	5 min			Evans et a. 95
	IV	0.46	308	<5 min			Javid et al. 78
	IV	0.6	550	10 min			Jones 90
	IV	1.47	1,000	<5 min	38		Barnett et al. 81
	IV	2.95	6,000	<5 min	87		Barnett et al. 81
	smoked	0.4	225	10 min			Jones 90
	smoked	0.67	160	5 min			Evans et al. 95
	IN	0.23	53	60 min			Javid et al. 78
	IN	0.91	115	30 min			Javid et al. 78
	IN	1.37	206	30 min			Javid et al. 78
	IN	2	350	70 min			Jones 90
	IN	2	170	90 min			Wilkinson et al. 80
	IN	2	160	60 min			Van Dyke et al. 78
	PO	2	290	80 min			Jones 90
	PO	2	242	65 min			Wilkinson et al. 80
	PO	2	209	60 min			Van Dyke et al. 78
Macaque-preg	IM	1.0	288	15 min	1.2 hr	7	Binienda et al. 93
Sheep	IV	2	20,000	1 min	3.4		Burchfield et al. 91*a*
	smoked	1.3	350	1 min	1.6		Burchfield et al. 91*a*
	smoked	1.5	902	1 min			Burchfield et al. 91*a*
Sheep-preg	IV	2	7,900	30 sec	1		Woods et al. 87
	IV	2	11,432	<1 min	5	26	DeVane et al. 91
Rat	IV	6	1,000		1.46 hr		Boni et al. 91
	IV	7.5	1,757	7.5 min			Pan et al. 91
	IV	8	610	15 min	0.3 hr		Nayak et al. 76
	IV	10	2,000		1.32 hr		Boni et al. 91
	IV chronic	7.5	1,970				Pan et al. 91
	smoked	0.26	95	45 sec	1.9 hr		Boni et al. 91
	smoked	1.54	205	45 sec	1.54		Boni et al. 91
	IP	7.5	130	15 min	108		Lau et al. 91
	IP acute	7.5	2,242	10 min			Pan et al. 91
	IP	15	230	15 min	72		Lau et al. 91
	IP	30	610	60 min	54		Lau et al. 91

TABLE 2. *Pharmacokinetics of cocaine: Comparison of species and routes of administration (continued).*

Species	Route	Dose (mg/kg)	Peak Value (ng/ml)	Peak time	T1/2 (min)	Mat-fet ratio	Source
	IP chronic	7.5	4,242	30 min			Pan et al. 91
	PO	7.5	130	45 min	90		Lau et al. 91
	PO	15	150	45 min	54		Lau et al. 91
	PO	30	250	30	96		Lau et al. 91
	SC	15	240	180 min	120		Lau et al. 91
	SC	20	490	4 hr	1 hr		Nayak et al. 76
	SC	20	494	4 hr			Mule & Misra 77
	SC-chronic	20	500	1 hr	2 hr		Nayak et al. 76
	SC-chronic	20	502	1 hr			Mule & Misra 77
Rat-preg	IV	.33/min	1,660	na	4.5		Morishima et al. 92
	IV	3	3,725	30 sec			Mactutus et al. 94*b*
	IP	30	2,000	<30 min	46	<1	DeVane et al. 89
	IG	60	5,400	15 min	23	1.8	Dow-Edwards 90
	IG	30	1,000	15 min	44	1.4	Dow-Edwards 90
	SC	40	3,000	2 hr	2.8		Spear et al. 89
Mouse	IP	10	380	15 min			Shah et al. 80
	IP	10	2,000	5 min	16		Benuck et al. 87
	IP	25	7,000	5 min	16		Benuck et al. 87
Mouse-preg	IP	10	300	15 min			Shah et al. 80

KEY: IV = intravenous; IP = intraperitoneal; IM = intramuscular; SC = subcutaneous; IG = intragastric; IN = intranasal; PO = oral; preg = pregnant.

hundredfold lower than following IV administration. This difference was presumably due to the fact that the sheep do not intentionally inhale the smoke and do not hold their breath to establish high blood cocaine levels like humans do. Boni and colleagues (1991) compared IV administration and inhalation of cocaine in the rat and found that heart rate and blood pressure changes were generally dose dependent and temporally correlated with peak arterial cocaine concentrations. However, inhalation of cocaine by rats produced significantly lower plasma cocaine levels than IV administration, presumably because the rat also does not hold its

breath. Therefore, at least in the rat and sheep, smoking does not mimic cocaine pharmacokinetics produced by smoking in humans.

The next best route would be IV administration. Until recently, IV administration during pregnancy has been used only in studies of the acute effects of cocaine in sheep. Quite recently, however, a few groups have presented promising data from IV administration in the pregnant rat (Kunko et al. 1993; Mactutus et al. 1994*b*; Peris et al. 1992). The venous port system that Mactutus and colleagues (1994*b*) devised seems quite reliable, imparts minimal stress to the animal, and appears to be suitable for multiple daily injections without tethering the animals. Therefore, although smoked cocaine (crack) remains the most frequently used route of administration in humans, IV administration in animals most closely mimics the rapid rise and fall of plasma cocaine levels seen following crack smoking in humans (see table 2 for additional studies).

If a rapid delivery of cocaine to the brain is the administration pattern most desirable to model in adult animals, is it also the best pattern to model in developmental studies? At this time, it is unknown whether the rate of change in maternal plasma cocaine levels, the peak blood cocaine levels, or perhaps area under the time-concentration curve (AUC) is the most important factor for the production of a given developmental endpoint. While maternal drug-taking patterns may produce several rapid peaks in the plasma cocaine levels, the exposure of the fetus may be quite different.

The fetus does not metabolize cocaine as readily as the mother and thus would be expected to be exposed to the drug for a longer period of time than the mother. Nau (1986) has discussed the salient issues in this area and cites examples where peak plasma drug levels produce a given response (because continuous low levels of drug do not) or AUC correlates with the production of a given developmental endpoint. While IV cocaine in animals may model the situation in the adult human crack user, it is still too early to say whether this rapid rise in arterial and brain cocaine levels produces the greatest developmental toxicity. The effects of cocaine in the fetus are more difficult to measure and may be entirely unrelated to the effects in the adult. However, if peak plasma level is the major factor in determining developmental toxicity, then IV cocaine should be the most effective. If AUC is more critical, then SC cocaine would provide the greatest AUC for the plasma concentration versus time curve; SC administration results in a slow release of cocaine and peak plasma levels in about 3 hours (see table 2).

Intragastric (IG) administration, which is used in the author's lab, results in relatively rapid peak plasma cocaine levels (within 15 min) and pharmacologically effective plasma levels for about 90 minutes without the need for surgical intervention, which undoubtedly is somewhat of a stressor. In addition, IV administration produces a very narrow plasma concentration curve (a rapid rise and fall) but may not produce developmental toxicity simply because the plasma drug levels might be above threshold for only a very short time.

It will be several years before researchers know which route of administration reliably produces a given developmental insult since few laboratories are examining more than one route of administration. In addition, with the increasing awareness of animal rights issues, it is difficult, if not impossible, to obtain approval for within-laboratory replication.

DOSE

A quick glance at table 2 would show even the most naive investigator that there are many inconsistencies in peak value, peak time, and half-life (T½) even when cocaine is administered in equivalent doses via the same route of administration in the same species. For example, in three studies, cocaine was administered at 2 milligrams per kilogram (mg/kg) via oral and intranasal routes to human volunteers. In two studies, oral administration produced higher blood levels of drug while in the third, the Jones study, intranasal administration produced the greater blood cocaine levels.

In the rat, administration of greater amounts of cocaine resulted in relatively lower peak plasma values compared to humans. For example, Jones (1990) administered 0.6 mg/kg to human volunteers and obtained peak plasma levels of 550 nanograms per milliliter (ng/mL). Nayak and colleagues (1976) administered 8 mg/kg to rodents and obtained plasma values of 610 ng/mL. Thus a thirteenfold increase in dose in the rat results in an equivalent plasma level although identical routes of administration are used. Rees and colleagues (1990*b*) make the point that although there may be a great dichotomy in administered dose of a compound when comparing animals to humans, the internal dose produced is often quite different from the administered dose (see table 3). This is, of course, due to differential metabolism of the drug in animals and man. Differences in enzymes that metabolize cocaine, differences in blood flow to the organs that metabolize cocaine, and differences in cardiac output all contribute to the production of differences in metabolism of cocaine.

Another issue is that of sampling. Evans and colleagues (1995) found tenfold higher cocaine levels in arterial blood than venous blood following both smoking and IV administration. Most rodent studies utilize trunk blood, which is a mixture of the two. One study (Lau et al. 1991) compared trunk blood to blood collected by snipping the tip of the tail and found that while the two were highly correlated, trunk blood contained 2.5 times more cocaine than tail tip blood across a wide range of administered doses. In other cases, investigators undoubtedly missed the peak of plasma cocaine by sampling too late or infrequently. For example, Nayak and colleagues (1976) found that the peak plasma level following 8 mg/kg cocaine was 610 at 15 min. Pan and colleagues (1991), on the other hand, administered 10 mg/kg cocaine IV and got levels of 1752 at 7.5 min. Mactutus and colleagues (1994*a*) found peak plasma levels at 30 seconds postinjection using the IV port for administration.

TABLE 3. *Administered versus internal dose comparison for phenytoin.*

	Human	Rodent
Administered daily dose	6-12 mg/kg	50-200 mg/kg
Measured blood levels	8-25 μg/mL	13-23 μg/mL

SOURCE: Data from Adams et al. 1990.

Another issue is that of chronic dosing. While virtually all human volunteers in the studies listed were acknowledged cocaine users, few rodent studies examined chronic exposure. However, these animal studies have shown that under some circumstances, chronic exposure facilitates the absorption process and produces either higher blood levels or a more rapid peak in plasma cocaine concentration. Pan and colleagues (1991) reported that previous exposure to IP cocaine almost doubled the peak plasma cocaine levels. Nayak and colleagues (1976) found that chronic SC cocaine had no significant effect on peak plasma level but did decrease the time of the peak blood level from 4 hours to 1 hour. Therefore, the question of what dose to select to establish a relevant plasma cocaine level in an animal model relies on first establishing the human situation and then designing a dosing protocol in the animal model that mimics the human situation. However, even if this could be accomplished, differences in the timing of developmental events

between animals and people must be considered before a given model is accepted as the best model.

TIMING

In development, the time during which the drug exposure occurs is obviously an important factor in trying to resolve issues of vulnerability. Although the general processes unique to nervous tissue during development are relatively similar for all mammalian species, the absolute time at which each event occurs differs by days or weeks across species. There is an orderly progression of events such as cell division, migration, differentiation, and cell death that is roughly similar for humans and rodents but the rate of occurrence of each process is different for each species.

Using gross anatomical features, links can be made across the species at specific developmental time points. Bayer and colleagues (1993) have matched the gross anatomical features of human embryo brains with rat embryo brains and established a comparative time table across the entire period of development. They have found that the state of maturation of the rat brain at birth, for example, is equivalent to the human brain at about 19 weeks (Bayer et al. 1993). It is during the postnatal period in the rat that the brain develops, as does the human brain during the last half of gestation. Therefore, although the basic sequence of development is similar in rat and human, the absolute time following fertilization at which a given event occurs and the time of birth in relation to the maturational state of the brain are quite different. On the other hand, the basic organization of the nervous system, the roles of the various cell groups that comprise nuclei, and the functions of these cell groups in behavior and physiology are similar across species. For example, the extrapyramidal motor system performs similar functions in humans and rodents. The neurochemical composition in each brain system is similar across species, and with the limited information available in the human, the pharmacologic and neurochemical effects of stimulants are also similar (see above). Due to the similarities in the overall developmental processes and the similarities in pharmacology and function of the brains, rodent models have yielded a wealth of information about regulation of specific developmental events and how interference with one event can alter the course of development of many subsequent events.

As Dobbing (1968) demonstrated in the 1960s, the timing of the brain growth spurt or maximal expansion of the brain with respect to the day of

birth is quite different for rodents and human beings. In humans, the brain growth spurt occurs just prior to the time of birth (completely within the month preceding birth) and in the rodent it occurs between 5 and 15 days postnatal. The human fetus opens its eyes at 25 weeks gestation (2/3 of prenatal development), and the rodent at 15 days postnatal. Late postnatal events such as the attainment of 50 percent of the adult weight in the cerebellum or 50 percent myelination of the corpus callosum are easier to compare since these occur at a time when tissue is available in both species. For example, the cerebellum attains 50 percent adult weight in humans at about 12 months of age and in the rat at about 15 days of age (Howard 1973). Myelination of 50 percent of the corpus callosum occurs at about 18 months in humans but not until 45 days in rats (Wiggins 1982). Both human babies and rodents undergo a postnatal maturation of the peripheral nervous system, the cardiovascular system, and the neuroendocrine system, all of which facilitate their adaptation to the environment. Thermoregulation, for example, takes 18 months to fully develop in humans and 18 days in rodents (Kleitman et al. 1937; Verlag 1962). Rodier (1994) has recently reviewed the postnatal development of a variety of reflexes across several species and concludes that the development of a specific reflex depends to a large degree upon the necessity of that reflex for the survival of the particular animal. Some species may be relatively precocious in one reflex and relatively delayed in another. Therefore, the appearance of reflexes should not be used to compare species in terms of timing of development of the brain.

The appropriateness of the model then depends upon the question being asked. For example, if the question pertains to the effects of cocaine on a specific brain region or nucleus, the model should examine cocaine administration during the period of cell division of the precursor cells of that region, the period of differentiation within that region, and the period during which connections with that region mature. Each region undergoes these processes at specific yet unique times and drug administration should be timed to encompass each event. If, on the other hand, one wants to determine general effects on brain development produced by chronic, low-level exposure or even binge crack smoking, different models are appropriate.

Efforts to design a model must include consideration of the pharmacokinetics within the context of the differences in timing. For example, one may have decided to administer cocaine to a pregnant rat at a dose and via a route that result in pharmacologically relevant cocaine levels in

the fetal plasma for 3 hours. Using Bayer and colleagues' (1993) comparison, events occurring between E11 and E17 in the rat that may take one day take two days in the human. Therefore, if the dose were administered between 11 and 17 days gestation in the rat, this 3-hour exposure would be equivalent to a person taking cocaine such that the fetus would be exposed for 6 hours. If, however, this dose producing a 3-hour exposure in the rat were administered during gestation days 18 to 22, it would be equivalent to human exposure lasting 1.75 days, since the time ratio changes to 1 rat day equaling 14 days of human development. The events in brain development which occur in the rat during a 3-hour period at this stage of development occur over a span of 1.75 days in the human. During postnatal life of the rat, a 3-hour exposure in the rat approximates a 1.1-day exposure in the human. Therefore, one can see that the use of once or twice daily dosing regimen during pre- and postnatal life of the rat are equivalent to exposure periods of different lengths in human brain development depending on when in development the dosing occurs. Certainly, every effort must be made to produce an animal model that mimics human exposure patterns. However, it will be impossible to produce the perfect model that would encompass the typical cocaine use pattern because of the differences in timing of developmental events, the fact that human use patterns in pregnancy are not accurately known, and the fact that single or multiple daily dosing approach translates into different exposure patterns depending upon the stage of development during which the drug is given.

EFFECTS OF GESTATIONAL COCAINE EXPOSURE

A summary of the reported effects of gestational cocaine exposure appears in table 4. The asterisks indicate similar effects in animal and human studies while the italics denotes differences. Although it is not possible to discuss each effect in this chapter, one can see that in most cases, similar effects have been attributed to cocaine in both animal and human studies. A more detailed comparison of two aspects of development, the teratogenic effects and the effects on growth, was presented at the beginning of this chapter. Briefly, human and animal studies agree that cocaine is not teratogenic while they disagree with regard to its effects on growth. Animal studies find no effect on growth except at high doses, and clinical studies often find a positive association. In animals, reproductive effects are also identified only at toxic doses of cocaine, while in humans reproductive effects are frequently reported.

TABLE 4. *Summary of reported effects of gestational exposure to cocaine in humans and laboratory animals.*

	Human	Animal
Reproductive effects	*placental abruptions *IUGR *prematurity* *spontaneous abortion	*placental abruptions-HD *IUGR-HD *normal gestation length* *fetal resorption/death-HD
Early infant outcome	*low birthweight *few/no congenital malformations *respir. abnormal/SIDS *abnormal cardiac function*	*low birthweight-HD *few/no congenital malformations *respir/ abnormal *normal cardiac α & β receptors*
Neural effects	*dec. head circumference* seizures/abnormal EEG *cranial infarct/hemor. *alt. brain metabolism *altered plasma catechols.	*little effect on brain weight* not reported *cranial hemorrhage *alt. brain metabolism *inc. NE turnover *complex DA changes alt. 5-HT, opiate, cholinergic, and somatostainergic systems disrupted gliogenesis
Behavioral effects		
Sensory	*abnorm. audit. evok. pot.	*abnorm. audit. evok. pot.-HD
Motivational/ arousal	orientation/state reg. *inc. reactivity* *alt. stress response	*inconsis alt. reactivity* *alt. stress response
Cognitive	*IQ dec.	*dec. classical condit *dec. sensory precondit. alt. DRL 20 & water maze impair. reverse condit. discrim.
Motor	*persist. primitive reflex* inc. extensor tone deficit in volit. movement	*inconsis. alt. reflex* inconsis. alt. activity
Social	dec interact. behav. *less play insecure attach.	*dec. play inc. submissive play demasculinization inc. aggressive behav.
Other		dec. reinforc. efficacy of cocaine lower threshold brain stimulation alt. drug responsivity

SOURCE: Adapted from Spear 1994.

KEY: DRL 20 = differential reinforcement of low rates of responding, 20-second interval.
* = similar effect in human and animal studies; HD = occurs at high or toxic doses only. *Italic* indicates effects that are not similar in human and animal studies.

Early infant outcome measures appear to be similar across species with the exception of cardiac function. While cocaine-exposed infants have smaller heads and animals do not appear to be affected, both species show a variety of neurobehavioral effects that are remarkably similar (see table 4). The most speculative area is that of intelligent quotient (IQ). The reader is referred to Stanton and Spear (1990) for a complete discussion of the comparison of assessments of human IQ and measurements used in animal behavior. Experts generally agree that in rodents, certain behavioral tests, including classical conditioning and maze performance, tap some of the same processes as those used in human IQ measures. Findings that cocaine alters these processes in rodents and IQ is reduced in offspring of cocaine and polydrug abusers (Griffith et al. 1994) indicate that the development of these complex processes in both species may be sensitive to the effects of cocaine.

Another interesting area is that of altered stress responsivity. Although the Spear chapter documents that prenatal ethanol exposure alters pituitary-adrenal responsiveness to stress, it also reveals that more work needs to be done in this area. Two groups have examined stress responsivity in the rodent following cocaine exposure. Both studies show that prenatal cocaine exposure decreases immobility in a forced swim test, a change consistent with an enhanced catecholamine or 5-HT function (Bilitzke and Church 1992; Molina et al. 1994). The author has noticed that intermediate doses of cocaine (30 mg/kg) appear to increase adrenal size while high doses (60 mg/kg/day) reduced adrenal size (Dow-Edwards, unpublished data). Owiny and colleagues (1991) demonstrated that maternal cocaine administration in sheep produced an increase in fetal adrenocorticotropin and an increase in maternal and fetal cortisol levels. Clinically, a pilot study by Mirochnick and colleagues (1991) showed that cocaine-exposed babies have increased levels of the NE precursor dihydroxyphenylalanine which the authors state may be related to an increased level of stress in the infants. Davidson Ward and colleagues (1991) have identified increased NE levels in cocaine-exposed infants, while Magnano and colleagues (1992) found no difference in basal cortisol levels in saliva of cocaine-exposed infants and a decreased response to a stressor such as a neuroexam or heel stick. Since there are conflicting reports of alterations in stress responses in the clinical literature (Eisen et al. 1991) and the handling of stress is a necessary skill for successful adaptation to life, there is clearly the need for additional work in this area, particularly in the basic sciences.

As with any study of drug-exposed newborns, health care providers have been concerned about neonatal abstinence since the pioneering work of

Finnegan (1984) and the development of the Neonatal Abstinence Scale. To date, the majority of studies agree that prenatal cocaine exposure without opiate exposure does not produce significant neonatal abstinence. Only one animal study has addressed this point and it also found that prenatal cocaine did not alter ultradian rhythms at a time when prenatal methadone exposure did (Zmitrovich et al. 1992).

One final point can be used to compare animal and human data on prenatal cocaine exposure. Early clinical reports described abnormal ventilatory patterns and a significant increase in the occurrence of sudden infant death syndrome (SIDS) in populations prenatally exposed to cocaine (Chasnoff et al. 1989; Davidson Ward et al. 1986). However, reviews of large samples have shown that an increased incidence of SIDS is not associated with cocaine use during pregnancy (Bauchner et al. 1988; Kandel and Gaines 1991). Olsen and Weil (1992), however, have shown that prenatal cocaine exposure in guinea pigs does alter breathing patterns in a manner consistent with an increased tendency for SIDS. While cocaine may have some direct effect on the development of brainstem respiratory centers, the concomitant use of alcohol and cigarettes in cocaine-using pregnant women may account for many of the adverse effects of the drug. An interesting and new area of research is the use of PET in populations exposed to cocaine prenatally. Tyler and colleagues (1993) have found reductions in glucose utilization in about half of the children prenatally exposed to cocaine and other drugs. The author and colleagues' studies in rats show a very similar pattern of altered metabolism following prenatal cocaine exposure (Dow-Edwards et al. 1990). Use of techniques such as in vivo imaging that are identical in animals and humans (Blin et al. 1991) will certainly facilitate understanding of the developmental toxicity of cocaine, particularly once the confounding variables associated with clinical research on drug abusers can be controlled.

CONCLUSIONS

Certainly both clinical and preclinical research on prenatal cocaine exposure have a wide range of findings in common. Animal studies have been able to reproduce most clinical reports, particularly when toxic doses of cocaine are used; certainly there are individuals who use cocaine in the toxic dose range.

Several additional points can be made. First, as Vorhees (this volume) points out, the focus has been on the early postnatal period in both human and animal studies. Greater attention should be paid to long-term changes. Both animal and clinical investigators should improve control procedures (e.g., blinding of observers) and the reporting of important variables.

Within the animal literature, all in all, there is a low level of demonstrated reproducibility, perhaps due to the fact that no two authors examine the same set of endpoints. Although several authors have found significant effects that often parallel clinical findings, no two studies have found the same effects. The most robust effects of cocaine are seen when the organism is challenged, either pharmacologically or by using a more difficult task. Animal researchers have focused on one strain of rat and single daily doses. Consideration must be given to studies utilizing different routes of administration and dosing paradigms. Manipulation of environmental variables and polydrug models must also be developed to improve the relevance of the animal studies.

Lester and coauthors (this monograph) have elegantly described the clinical literature and illustrated that the mother and infant mutually regulate behavior in the context of the greater environment. The role of cocaine in this relationship is very complex. There is a biological effect in the mother during pregnancy and in the infant following exposure. This effect depends upon the dose and frequency of cocaine and other drugs during pregnancy as well as the drug-associated microculture. This microculture includes not only polysubstance abuse, poor prenatal care, and poor nutrition, but also sexually transmitted disease (STD).

Animal studies can address the biological and neurobiological effects of cocaine in the mother and infant, but the role of the drug abuse microculture on neurobehavioral outcome measures is much more difficult to quantify and model experimentally. Cocaine seems to make the child more vulnerable to the effects of a poor caretaking environment. Therefore, although the major focus of this chapter is on the effects of cocaine on the development of human and animal brain function, the reader must remember that the biological and neurobiological effects of cocaine are only a part of the overall picture and the magnitude of effects may be different for each child examined.

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ACKNOWLEDGMENT

Supported by NIDA grant DA04118.

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Studies of Cocaine-Exposed Human Infants

Barry M. Lester, Lyn LaGasse, Kiti Freier, and Susan Brunner

INTRODUCTION

No reminder is necessary that the so-called cocaine problem took society by surprise. It struck the academic, health, political, government, and legal communities and quickly became known as an epidemic. Based on little scientific evidence, early reports of the effects of cocaine were exaggerated (Mayes et al. 1992) and people were soon ready to write off an entire generation of children.

On the positive side of this rush to judgment is the valid concern for the health and development of drug-exposed children. This concern has led to a substantial effort on the part of the scientific community to understand the effects of prenatal drug exposure on the developing child. In a relatively brief amount of time there has been an explosion of research in this area including an infusion of cross-fertilization and interdisciplinary collaboration. No doubt because of the attention that this particular area of scientific inquiry has drawn, the process of scientific inquiry has been accelerated. This process has, however, resulted in some misinformation, compelling society to understand what is known and what is unknown as the second generation begins.

Investigators have begun the second wave of research on in utero cocaine exposure and child outcome. The purpose of this chapter is to review what was learned from the first wave of work and to consider how this knowledge can be applied in the second wave of research. This chapter focuses on neurobehavioral studies and presents a quasi-meta-analysis. The analysis is quasi in the sense that it is more descriptive than statistical. One goal of this chapter is to address the question: Is there enough information in the database of neurobehavioral studies to even attempt a formal meta-analysis?

META-ANALYSIS

The Meaning of Neurobehavioral. The starting point for this analysis is to define "neurobehavioral" so that the appropriate corpus of literature can be identified. In actuality, the term "neurobehavior" was developed to refer to older children but is now applied to infants as well. In older children the term refers to an expanded neurological examination that involves sophisticated observation of higher cortical function and motor output, often combined with an assessment of the maturation of the central nervous system (CNS) or a search for minor neurological indicators. The authors use the term broadly to reflect the notion that all human experiences have psychosocial as well as biological or organic contexts.

The term "neurobehavioral" recognizes bidirectionality—that biological and behavioral systems dynamically influence each other and that the quality of behavioral and physiological processes is dependent on neural feedback. Neurobehavior becomes the interface of behavior and physiology and includes neurophysiological mechanisms that mediate specific behaviors or psychological processes. Thus, the authors include the study of specific physiological systems that reflect these neurophysiological mechanisms. For example, some aspects of cardiorespiratory function such as vagal tone (Porges 1991), a measure of respiratory sinus arrhythmia, are thought to mediate behavior by facilitating attention. Therefore, this kind of measure would be included as a neurobehavioral measure. On the other hand, studies that are interested in structural defects of the heart would not be included because such studies do not involve a psychological process. Neurobehavioral measures provide an estimate of biobehavioral function and integrate the influences of neurobiology, thought, affect, and experience.

Inclusion Criteria. In order to be included in this chapter, studies had to include a neurobehavioral measure or study a neurobehavioral process in human subjects using cocaine during pregnancy (other drugs could also be present). Based on the criteria used in the Lutiger and colleagues' (1991) meta-analysis on prenatal cocaine exposure and pregnancy outcome, the authors were able to identify a total of 60 neurobehavioral studies. Ten studies that did not include original empirical data (N = 2), a control or comparison group (N = 7), inferential statistical analysis (N = 2), and publication in a refereed or peer-reviewed journal (N = 1) were excluded (two studies were excluded for more than one methodological limitation). The appendix lists the 50 studies that met the criteria for inclusion in the review.

Subject Characteristics. Inspection of the publication dates of the 50 studies included in the appendix shows the recency of this area of investigation. The first studies of cocaine use during pregnancy and child outcome were published in 1985. However, of the 50 studies, 45 (90 percent) were published since 1989. Not surprisingly, with most of the work being recent, there are very few studies of older children. The majority of subjects were less than 1 month old when tested. Some studies included preterm and term infants. Twenty studies included preterm infants tested before they reached term and 41 studies included term infants. Only seven studies (14 percent) included infants up to 4 months of age. In addition, there are only two longitudinal studies (4 percent) in which infants have been followed from birth into the second or third year of life. There is considerable variation in the sample size of these studies. The typical sample size for the exposed infants across all 50 studies ranges from 21 to 50; 7 studies were conducted with fewer than 10 exposed infants.

WHAT IS NOT KNOWN

Drug Information. Table 1 shows the drug information from the 50 studies. The table shows the number of studies and the percentage in which each drug was reported; the means by which the drug was identified; and the amount, frequency, timing of use, and route of administration. The data clearly show that the cocaine problem is really a polydrug problem; "cocaine only" use was described in only two studies (4 percent). Marijuana (23 percent), alcohol (21 percent), and nicotine (26 percent) are the substances most often used with cocaine. It is surprising that in six studies (12 percent) no information about other drug use was even reported or addressed.

A few studies attempted to address the polydrug problem by controlling for the use of drugs other than cocaine. Four methods were used to control for polydrug effects: stratification, matching, exclusion, and statistical. Depending on the drug, stratification was used in 1 study, matching was used between 1 and 9 times, exclusion of other drugs was used between 3 and 15 times, and statistical control was used between 2 and 4 times. In other words, the majority of studies failed to use any method of control for polydrug use. No method of control was reported for phencyclidine (PCP) in 38 studies (76 percent), heroin or barbiturates in 37 studies (74 percent), methadone in 36 studies (72 percent),

TABLE 1. *Number and percentage of studies reporting drug information (N = 50).*

	Number of Studies	Percentage
Drug type		
Cocaine alone	2	4
Alcohol	21	42
Tobacco	26	52
Marijuana	23	46
Heroin	7	14
Methadone	7	14
Opiates	6	12
PCP	3	6
Amphetamines/methamphetamine	6	12
Methaqualone	1	2
Unspecified narcotics	4	8
Unspecified polydrug use	6	12
Unspecified use of legal drugs (e.g., alcohol and tobacco)	7	14
Not reported	6	12
Method of detection		
Urine only	23	46
Self-report only	2	4
Meconium only	1	2
Urine and self-report	11	22
Meconium and urine	1	2
Hair and urine	1	2
Urine and/or self-report	10	20
Not reported	1	2
Pattern of use		
Frequency of use	3	6
Trimester of use	8	16
Amount used	4	8
Not reported	35	70
Route of administration		
Intranasal	4	8
Intravenous	4	8
Freebase	4	8
Not reported	35	70

marijuana or alcohol in 35 studies (70 percent), tobacco in 33 studies (66 percent), and opiates in 32 studies (64 percent).

The term "polydrug use" is also confusing and studies do not explain how they are using the term. For example, polydrug use can mean the simultaneous use of more than one drug such as when cocaine and alcohol are ingested together. The term can also mean that multiple substances are used but not necessarily together. In the first case, the neurobehavioral outcome may be affected by cross-reactivity or the interaction between two drugs. It has been suggested, for example, that alcohol can potentiate the effects of cocaine. In the second case, different neurobehavioral consequences may result from multiple exposures to different drugs.

Table 1 also shows that the single index of a urine screen was used in almost half of the studies. Urine analysis and self-report account for almost all of the studies. The limitations of these methods are that for women who used drugs during pregnancy but did not use within 72 hours prior to delivery (the range of the urine screen), a urine test for cocaine will be negative and these women could be included in the control group. Similarly, with self-report, a mother who used drugs but denies use may also be included as a control. In the two studies in table 1 mentioned earlier that claimed cocaine as the only drug used, a single urine screen at birth with no history was used as the method of drug detection. Thus, there is a reasonable likelihood that other substances may have been involved.

Meconium assay, which is fast becoming the scientific standard, was used in only two studies. Information on the amount, frequency, timing, and route of administration was reported in only a few studies.

Demographic and Medical Information. Table 2 shows the number and percentage of studies reporting demographic information. It is unfortunate that so little demographic information has been reported in these studies because it makes it virtually impossible to understand the populations on which the neurobehavioral data are based. There appears to be an implicit assumption that studies are conducted on lower socioeconomic status (SES) families but there is little supporting documentation. In addition, there is substantial variability within social class stratum, parenting, childrearing, caretaking, the quality of the physical environment, and factors such as stress and violence—all of which can affect the neurodevelopmental outcome of the child. Determining that samples are from lower SES families does not provide an environmental control.

TABLE 2. *Number and percentage of included studies reporting demographic information (N = 50).*

		Number of Studies	Percentage
Race/ethnicity		50	100
Gender		35	70
Maternal:	Age	22	44
	SES	10	20
	Education	14	28
	Welfare status	6	12
	Work status	5	10
	Prenatal care	32	64

KEY: SES = socioeconomic status.

Table 3 shows the number of studies that have attempted to control for demographic and medical (obstetrical and perinatal) variables. Most studies have not controlled for these factors. Of the methods used to control for confounding variables (demographic or medical), matching was used in 25 studies (50 percent), exclusion in 18 (36 percent), stratification in 4 (8 percent), and statistical control in 7 (14 percent) studies. Confounding variables were reported as controlled but the methods were not specified in 7 studies (14 percent).

As with demographic factors, medical factors can also have an effect on child neurobehavioral outcome. The argument is sometimes raised that factors such as prematurity should not be controlled because cocaine may cause prematurity, so that controlling for prematurity would blur the effects of cocaine. The problem with this argument from the neurobehavioral perspective is that prematurity is known to potentially affect neurodevelopmental outcome. If prematurity is not controlled, it becomes impossible to separate the effects of cocaine from the effects of prematurity on neurobehavior. For example, is the cocaine-exposed preterm infant different from the unexposed premature infant given comparable medical insult and illness history?

Two other interesting if not disturbing findings emerged from this survey. First, of the 50 studies reviewed, only 20 (40 percent) reported that the

TABLE 3. *Number and percentage of studies attempting to control for demographic and medical variables (N = 50).*

	Number of Studies	Percentage
Demographic variables		
SES	7	14
Race	24	48
Gender	10	20
Maternal age	22	44
Maternal education	5	10
Maternal welfare status	4	8
Maternal work status	1	2
Maternal marital status	2	4
Medical variables		
Prenatal care	14	28
Parity	14	28
Gravidity	8	16
Medical complications	23	46
Prematurity	16	32
Gestational age	20	40
Birthweight	10	20

neurodevelopmental examiners were masked or unaware of the exposure status of the child. Information on masking was not even reported in 24 (48 percent) of the studies. The second issue has to do with intervention. Intervention services for the mother (e.g., drug treatment), the child (e.g., early intervention), or both are common in this population and can affect neurodevelopmental outcome. Yet, information about such services was not reported in 36 (72 percent) of the studies.

Little is known about the actual environments in which these children are raised or about measures of who the caregivers are. The kind of information that is necessary includes the number and duration of caretakers; the age of the child with each caretaker; whether relatives or other foster parents are involved; how many other children are being cared for at the same time; continuity of care; and intervention by the protective service system, the healthcare system, the legal system, and the caregiving

system. Are these children actually afforded the time to develop adequate interpersonal relationships?

To summarize, the knowledge base of neurodevelopmental studies comes mostly from studies of young infants; the problem is one of polydrug use, not cocaine alone; the methods used to identify exposure status are questionable; and there is a serious confounding of demographic and medical factors. In addition, there appear to be problems in how and what information is reported in peer-reviewed journals. Basic information such as the route of administration, timing and amount of drug used, social class, masking of examiners, and role of social services is underreported.

WHAT IS KNOWN

Neurobehavioral Effects. The authors divided the neurodevelopmental measures reported in these 50 studies into three domains: behavior, medical, and psychophysiology/neurochemistry. Table 4 shows the neurodevelopmental measures that were used in each of the three domains and the number of studies that showed statistically significant effects related to prenatal drug exposure. Of the 16 measures in the behavior domain, most were used in only one or two studies. Two measures of abstinence were each used in four studies; one measure showed three significant effects, the others showed three nonsignificant effects. The Brazelton Neonatal Behavioral Assessment Scale (BNBAS) was used in eight studies, and showed significant effects in seven. However, only one finding from the BNBAS was reported in more than one study. Two studies found poorer habituation in exposed infants.

The medical domain includes 1 study that showed no seizures in exposed infants and 22 studies using the Apgar score, 12 of which showed no effects. In the psychophysiology/neurochemistry domain, most measures were used once or twice.

In a traditional meta-analysis, one goal is to estimate effect sizes and determine whether findings replicate across different studies. Table 5 shows what such an analysis could look like if focused on the behavioral measures. In order to calculate effect size, the number of subjects and mean and standard deviation (SD) per group need to be reported. This information was not available in some of the behavioral studies, hence some are not represented in table 5. Effect size is determined in SD units

TABLE 4. *Summary of results of neurodevelopmental studies.*

	Number of Studies	
	Significant	Not Significant
Behavior		
BNBAS	7	1
Neonatal Abstinence Score	3	1
Stress/abstinence/withdrawal	1	3
Neurobehavioral status and state organization	1	0
Sucking	1	0
Neonatal perception inventory	0	1
Nursing child assessment of feeding	1	1
Cry	2	0
Glabella reflex	2	0
Movement assessment of infants	1	0
Bayley Scales	0	1
Fagan Test of Infant Intelligence	1	0
Developmental quotient	1	0
Attachment	1	0
Play	1	0
Behavior/development problems	1	0
Neurological		
Seizures	0	1
Apgar scores	10	12
Psychophysiology/neurochemistry		
EEG	2	0
Auditory brainstem response	1	1
Blood pressure	1	0
Respiration	3	0
Heart rate	1	1
Vagal tone	1	0
MRI	0	1
Catecholamines	2	0

and effect type is based on Cohen's criteria, with small, medium, and large effect sizes corresponding to < 0.5 SD, 0.5 to 0.75 SD, and > 0.75 SD, respectively. For example, five effects were reported using the BNBAS. The differences between exposed infants and controls

TABLE 5. *Summary of effect sizes of neurodevelopmental studies.*

Measure	Effect Size	r	r^2	Effect Type
BNBAS				
State organization	1.14	0.48	0.23	Large
Autonomic	0.45	0.19	0.04	Small
Reflexes	0.70	0.33	0.11	Medium
Habituation	0.57	0.26	0.07	Medium
Habituation	0.81	0.37	0.14	Large
MAI				
Muscle tone	1.19	0.51	0.26	Large
Primitive reflexes	0.93	0.41	0.17	Large
Volitional movement	0.64	0.30	0.09	Medium
Fagan Test	0.70	0.33	0.11	Medium
Sucking	0.36	0.15	0.02	Small
Developmental quotient	0.87	0.39	0.15	Large
Play	2.16	0.71	0.50	Large

KEY: BNBAS = Brazelton Neonatal Behavioral Assessment Scale;
MAI = Motor Assessment Inventory.

ranged from 0.45 to 1.14 SD, including one small effect, two medium effects, and two large effects. The r value in the table is the correlation between exposure status and the outcome variable. The percentage of variance in the outcome variable explained by exposure status is r^2. On the BNBAS, between 7 and 23 percent of the variance was explained by drug exposure. Habituation appears twice because, as mentioned above, it is the only finding that was reported more than once.

This analysis is meant only to illustrate what could be done, and should not be considered a legitimate meta-analysis for several reasons. First, the analysis assumes that the exposed and control groups have equal sample sizes. Second, the analysis was done on a subset of measures. Third, only one effect (habituation on the BNBAS) was found in more than one study. An adequate meta-analysis requires a consistent set of findings that appear across studies so that effect sizes can be estimated. In short, the authors have concluded that a meta-analysis of behavioral effects is not possible at this time.

As can be seen from the data in tables 1 to 5, knowledge about the effects of in utero cocaine exposure is fairly limited. Most studies have been conducted with young infants using a wide array of instruments, making it difficult to compare findings across studies. Few findings have been replicated and longitudinal data are sorely lacking. There are several issues embedded here. One is the stability and reliability of a finding, which cannot be determined because most studies use an assessment at a single point in time. A repeated-measures design of the same measure within a short period of time would shed light on the stability of a single finding and help determine whether reported effects are transitory or more long lasting. Longitudinal studies are affected by attrition; thus the cohort available for analysis at one point in time is usually different from the cohort available later. If these cohorts represent different populations, the generalizability of the findings is different at one age from another. A related issue is that even if comparable effects are reported across age, the same children may not be affected.

Differences in group mean scores do not reflect individual differences. For example, to show that exposed children differ on the Bayley Scales at 12 and 36 months does not necessarily mean that the children with low scores at 12 months were the same children with low scores at 36 months. Yet this is exactly the information needed from a clinical as well as scientific point of view. Are the same children consistently affected? If they are not, intervention programs would not know which children to target. One would have to conclude that individual differences with regard to effects of drug exposure are not stable.

At this time only one cohort of children has been followed to 3 years of age (Azuma and Chasnoff 1993). At age 3 the drug-exposed children are performing within normal limits on standard intelligence quotient (IQ) tests. There are more drug-exposed children than controls who fall outside the normal range, and drug-exposed children show lower scores on some subscales of function (e.g., language). However, the average IQ of the drug-exposed and control groups does not differ. This study is complicated by the fact that the mothers were in and out of drug treatment and followup. Thus, intervention effects may have mitigated the effects of prenatal drug exposure.

HOW KNOWLEDGE IS MOVING THE SECOND WAVE

The Four A's of Infancy. The subtlety of the effects reported in the Chasnoff study (Chasnoff et al. 1990) is consistent with other reports of short-term and long-term effects. There is a consensus in the literature that when drug effects are observed they tend to be found in more subtle domains of function rather than along gross developmental measures such as general mental or motor developmental scores or IQ.

There has been some attempt to understand the effects of cocaine on development through the study of neurotransmitters and behavior. Monoaminergic neurotransmitters (norepinephrine, dopamine, and serotonin) play an important role in the central control of basic processes, including autonomic function, state regulation, and responses to sensory stimuli. The effects of cocaine on autonomic system activity mediated by monoaminergic transmitters are suggested by findings that elevated circulating norepinephrine levels and heart rates were found in prenatally exposed infants at 2 months of age. A preliminary study provided some evidence that higher levels of norepinephrine were related to poorer responsivity on the BNBAS (Mirochnick et al. 1991).

Cocaine use during pregnancy may very well affect neuroregulatory mechanisms that result in disorders in behavioral regulation. Effects on the monoaminergic system would lead to activity associated with limbic, hypothalamic, and extrapyramidal function (Volpe 1992). Lester and Tronick (1994) (figure 1) suggested that the associated disorders in behavioral regulation are manifest as the "four As of infancy": attention, arousal, affect, and action. These four areas seem to be particularly affected by prenatal drug exposure.

- *Attention* refers to perceptual abilities that relate to the intake and processing of information from the environment.

- *Arousal* includes control and modulation of behavioral states from sleep to waking to crying, ability to display the entire range of states, excitation, and inhibition to incoming stimuli.

- *Affect* relates to the development of sociality and emotion, the mutual regulatory processes of social interaction and social relationships.

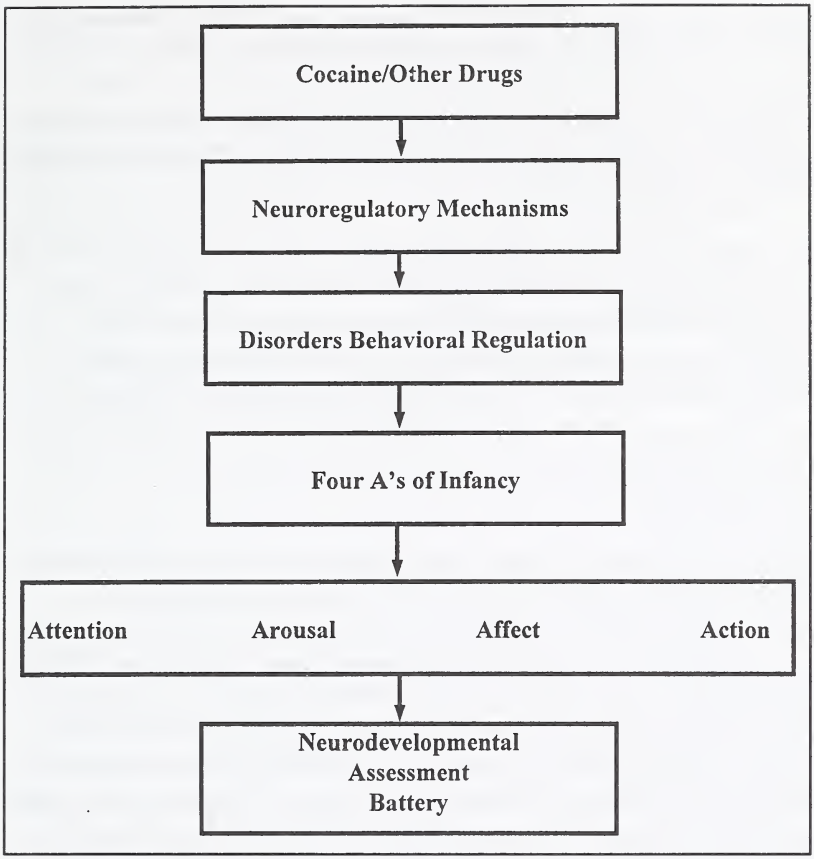

FIGURE 1. *Theoretical model of the effects of prenatal cocaine exposure on child behavior.*

- *Action* indicates motor function, the development of fine and gross motor skills, and the acquisition of knowledge and social exchange through motor patterns.

Direct and Indirect Effects. It is possible that different neurobehavioral effects may result from direct and indirect effects of cocaine on the fetus and infant (Jones and Lopez 1988). Preclinical studies have shown that the teratogenic effects of a drug can be produced by an action on the maternal animal, directly on the fetus, or by alteration of normal maternal-fetal metabolic pathways (Inglass et al. 1952). Direct effects include the action of cocaine on the fetus consequent to transfer of the drug through the placenta. These systemic effects of cocaine on the nervous system are

probably mediated by the changes in synaptic transmission resulting in an excess of neurotransmitter at the receptor sites (Richie and Greene 1985). This mechanism affects the sympathetic nervous system and produces vasoconstriction, an acute rise in arterial blood pressure, tachycardia, and a predisposition to ventricular arrhythmia and seizures (Cregler and Mark 1986; Tarr and Macklin 1987).

Indirect effects can be attributable to changes in the fetal environment and effects on the mother's CNS that place the infant at risk. During pregnancy, uterine blood vessels supplying oxygen and nutrients to the developing fetus are maximally dilated, but they vasoconstrict in the presence of catecholamines. Cocaine blocks the reuptake of catecholamines (Richie and Greene 1985), thereby increasing their concentration, resulting in vasoconstriction of the uterine arteries and impaired oxygen delivery to the fetus.

In pregnant cocaine-using women, vasoconstriction, sudden hypertension, or cardiac arrhythmias may interrupt blood supply to the placenta and reduce perfusion to various fetal tissues in early gestation, causing deformation or disruption of morphogenesis in late gestation (Bingol et al. 1987). Vasoconstriction, tachycardia, and increased blood pressure caused by cocaine all increase the chance for intermittent intrauterine hypoxia, preterm labor, precipitous labor, and abruptio placentae followed by hemorrhage, shock, and anemia (Tarr and Macklin 1987). Vasoconstriction at the uterocomplex coupled with anorexic effects of cocaine might explain the growth retardation that occurs in some of the offspring of cocaine-using mothers (Fulroth et al. 1989; Hadeed and Siegel 1989; Yoon et al. 1989). Hypoxia resulting from vasoconstriction has been shown to reduce fetal weight in animal studies (Mahalik et al. 1984).

In summary, cocaine has a specific direct effect on brain function and an indirect effect through the influence of fetal nutritional status. It is possible that these direct and indirect effects have different influences on neurobehavioral functioning. Support for this hypothesis comes from a study of the direct and indirect effects of cocaine using acoustic cry analysis as the neurobehavioral outcome (Lester et al. 1994). Two neurobehavioral syndromes were identified as related to direct versus indirect effects of cocaine. Excitable cry characteristics (e.g., higher pitch, more variability, and longer cries) could result from the direct effects of cocaine. The action of cocaine on mesolimbic systems (Wise 1984) triggers the cry, which is activated by the hypothalamic-limbic system and controlled by the midbrain and brainstem regions (Lester and

Boukydis 1992). The effects of cocaine on the tegmentum and raphe nuclei (Wise 1984) could directly affect midbrain and brainstem control.

Depressed cry characteristics (longer latency to cry onset, fewer cries, and lower amplitude cries) could result from the indirect effects; cocaine resulted in lower birthweight or intrauterine growth retardation (IUGR) in infants, which in turn affected cry. The cocaine effect on placental vasoconstriction can result in decreased nutrient supply to the fetus, hypoxia, and IUGR. Depressed catecholamine responses have been found in IUGR rat pups (Shaul et al. 1989), and depressed behavior in IUGR human infants has been reported in other studies of cry (Lester and Zeskind 1978), feeding behavior (Mullen et al. 1988), and infants assessed using the BNBAS (Lester et al. 1986).

The notion of excitable and depressed neurobehavioral syndromes in cocaine-exposed infants is supported by studies using other assessments of similar behaviors. For example, in studies using a narcotic withdrawal index, some findings suggest heightened responsivity, increased motor tone, and irritability consistent with excitability, whereas other studies describe the infants as underaroused and lethargic. In the authors' clinical experience with the BNBAS, these patterns have been observed. In addition, there appears to be a third or mixed pattern in which cocaine-exposed infants initially appear underaroused, hard to wake up, and difficult to bring to a quiet alert state. They then become highly excitable, irritable, and hypertonic, and remain in an insulated cry state. These infants appear to be unable to modulate their level of arousal once awake. They are mostly in lower (sleep) states or higher (cry) states and are unable to maintain a state of quiet alertness. In some infants, massive consolability maneuvers by the examiner can achieve brief periods of quiet alertness.

Table 6 shows a system for scoring the BNBAS on the excitable and depressed dimensions. This system is currently being used in several studies. In a study by Tronick and colleagues (1994), a dose-response relationship was reported between prenatal cocaine use and the excitability score.

Neurodevelopmental Assessment. Traditional tests of developmental outcome such as the Bayley Scales provide global estimates of neurobehavioral function and have the advantages of being standardized, widely known and accepted, and relatively easy to administer and score.

TABLE 6. *Proposed drug scoring system for the Brazelton Neonatal Behavioral Assessment Scale.*

Recent research has used the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) to study the effects of prenatal substance abuse, primarily cocaine, on newborn behavior. The effects of cocaine are sometimes difficult to detect due, in part, to methodological problems including determining patterns of cocaine use and confounding with other drug and nondrug effects, but also because some of the effects of cocaine may be relatively subtle.

The traditional seven-cluster scoring system for the BNBAS may not be adequate to capture effects due to prenatal cocaine exposure. Specifically, reading of the cocaine literature suggests that at least two patterns or neurobehavioral syndromes can be described in these infants, an excitable pattern and a depressed pattern. The seven-cluster scoring system does not readily lend itself to describing these patterns of behavior. Therefore, the data reduction system described below was developed.

To use this system, the infant is assigned 1 point for each item that he or she meets the criteria for excitable or depressed behavior. There are 13 items on both the excitable and depressed scales. Therefore, each infant may have a range of 0-13 for the excitable and depressed scores. Please note that each infant will have two scores. If missing data is a problem (some infants do not have all scores) it might be useful to compute the mean (i.e., divide the total excitable or depressed score by the number of excitable or depressed items that are available for the infant).

EXCITABLE	DEPRESSED
Tone > 6	Ball < 4
Motor maturity < 4	Rattle < 4
Cuddliness < 3	Face < 4
Consolability < 4	Voice < 4
Peak excitement > 7	Face and voice < 4
Rapidity buildup > 6	Alertness < 4
Irritability > 5	Tone < 4
Activity > 6	Pull to Sit < 4
Tremulousness > 5	Defensive < 4
Startles > 4	Peak excitement < 4
Lability skin > 7	Rapidity buildup < 4
Lability state > 3	Irritability < 3
Self-quieting < 3	Activity < 4

However, this measure may not be suitable for detecting the specific areas affected by cocaine. Thus, it is possible that findings reported to date that show no significant differences or that show findings that are difficult to interpret or contradictory may be due to the type of tests being used. Lester and Tronick (1994) developed a neurodevelopmental battery for drug-exposed infants based on the four A's of infancy as part of a large, multisite longitudinal study of prenatal drug exposure and child outcome for the National Institutes of Health (NIH), National Institute of Child Health and Human Development (NICHD), and the National Institute on Drug Abuse (NIDA). It includes state-of-the-art assessments that should be sensitive to even subtle effects of cocaine. The battery should also help identify neurobehavioral patterns such as the excitable and depressed syndromes described earlier. The ability to describe these patterns of individual differences will enable researchers to study specific mechanisms by which cocaine affects behavior as well as to develop clinical programs that deal with the specific behavioral domains affected.

LESSONS FROM THE PAST

There is a certain deja vu associated with the study of prenatal cocaine exposure. Prenatal influences and insults on child development are much studied areas and it might be useful to consider cocaine exposure as a special case of this larger problem. In doing so, researchers need to understand what can be learned from the past as well as what is unique about this particular problem. Arguably, the study of preterm infants provides a good model.

Starting in the 1950s with the Collaborative Perinatal Study of some 20,000 pregnancy and delivery outcomes, substantial effort was devoted to the effects of prematurity (Niswander and Gordon 1972). The prevailing zeitgeist was that being born prematurely was a form of biological insult likely to affect CNS development and the long-term outcome of the child. As supporting evidence, studies showed that premature infants were overrepresented in many populations of abnormal outcomes, including cerebral palsy and mental retardation (Lilienfeld and Parkhurst 1951; Pasamanick and Knoblock 1966). A related movement called for the development of early stimulation programs to help these infants make up for their biological deficits and perhaps prevent poor developmental outcome.

The second wave of studies of the effects of preterm birth told a different story. Research showed that the evidence that preterm infants were overrepresented among the handicapped population, even if true, was based largely on retrospective data. Prospective longitudinal studies showed that when preterm infants were followed from birth, most developed normally. Studies such as the Kauai study showed that in fact it was the environments of these children that were predictive of their developmental outcome rather than their medical status at birth (Werner et al. 1971). The seminal paper by Sameroff and Chandler (1975) brought these issues to the forefront in the form of the transactional model, in which the dynamic response of the caretaking environment to the characteristics of the child is seen as the primary determinant of child outcome. Parallel work in the biological domain showed substantial plasticity and mechanisms for recovery of function from insult and injury to the developing nervous system (Waddington 1966). Doom was replaced by optimism for the developing preterm infant.

It was learned that preterm infants are not a homogeneous group. As babies began to survive at lower and lower birthweights, the medical community distinguished between low birthweight (1500 to 2500 grams) and very low birthweight (< 1500 grams). Today reference is made to the "micropreemie," an infant weighing less than 900 grams. Smaller babies are at higher biological risk not only because they are smaller, but also because they are more prone to insult, injury, and illness. Brain injury such as intraventricular hemorrhage and respiratory illness such as bronchopulmonary dysplasia mostly occur in smaller babies and often occur together.

There has been a longstanding bias in the research community, influenced in part by funding and public policy issues, that cognitive and intellectual outcomes are of primary importance. Most studies of preterm infants looked only at cognitive and intellectual outcomes so that differences were viewed only in terms of intelligence. However, more recent work with preterm infants has reflected an appreciation of the importance of noncognitive outcomes, including social and emotional development, parent-child relationships, temperament, and peer interaction. These noncognitive outcomes are important in their own right. Researchers have learned that it is somewhat simplistic to separate cognitive from noncognitive outcomes, because factors such as social and emotional behavior, temperament, and motivation influence intellectual achievement and school performance. A child with emotional or behavioral problems may not do well in school even if he or she is intellectually competent.

Preterm infants also are not homogeneous with respect to their behavior and development. They show a wide range of behavioral and developmental trajectories that are multidetermined. The dynamic response of the caregiving environment to the changing behavioral organization of the infant is the best window into the long-term developmental outcome of the preterm infant.

Application to Drug-Exposed Infants. Like prematurity, drug exposure can be viewed as another potential insult or injury to the developing fetus. Researchers do not know whether and how drugs affect the fetus; the effects of polydrug use; or the effects of timing, dosage, and frequency of use. In some infants there may be true injury, in others there may be any degree of insult, and many infants may escape unscathed. It is also possible that there are effects that simply cannot be measured or effects that are not manifest until the child is older. Drug effects also interact with other prenatal factors such as poor nutrition or illness, which also potentially compromise the infant.

There is a relationship between drug exposure and early delivery, probably because of the effects of cocaine on labor, although possibly related to lack of prenatal care. Thus, drug-exposed infants constitute an increasingly large percentage of the infants in the special care nursery. It is not known if the drug-exposed preterm infant is any different from the unexposed preterm infant with a comparable medical history. That is, does drug exposure have an additional or synergistic effect when factors such as birthweight, other sickness, and insults are taken into account?

The vast majority of drug-exposed infants are not born prematurely. Many are born at term and are otherwise normal and healthy, while others are born at term but are growth retarded (IUGR or small for gestational age (SGA)). As with preterm infants, drug-exposed infants are not a homogeneous group with respect to how they present medically or behaviorally. Researchers have just begun to describe some of the different behavioral patterns that drug-exposed infants manifest, and it is likely that these different beginnings may result in different developmental trajectories as the demands of the caregiving environment come into play.

Study of preterm and other high-risk infants revealed that many standard developmental tools are not sensitive to the behavioral variations of these infants. Not surprisingly, this is also turning out to be true for the drug-exposed infant. In preterm infants, measures that are more sensitive to

behavioral processes such as the four A's of infancy are better able to describe the behavioral organization of these infants than tests of gross developmental outcome or milestones.

Although the database is very small, studies have shown differences in symbolic play and attachment relationships in drug-exposed infants who score within normal limits on developmental tests (Beckwith et al. 1994). In a 3-year followup (Azuma and Chasnoff 1993), although drug-exposed infants fell within normal IQ range, they showed differences on some subtests such as language.

From the study of preterms and other at-risk populations, multiple risk models have been developed that should be useful in the study of drug-exposed infants. Cumulative risk models suggest that it is the number (rather than the nature) of specific risk factors that determines developmental outcome (Sameroff et al. 1987). Other models study the resilient or invulnerable children, those who do well despite the presence of multiple risk factors (Garmezy et al. 1984; Lester et al. 1994). Despite exposure to similar adverse factors, some substance-exposed infants are able to survive and develop well, whereas others are not. There is a need to understand the individual differences in reactions to similar adverse factors and identify characteristics of resilience (Johnson et al. 1990). This had lead to the study of protective factors that may serve as regulators or re-regulators of development and help buffer the effects of high-risk factors. These models need to be applied to the study of drug-exposed infants.

The study of drug-exposed infants is probably best viewed as a special case of the infant at risk. This suggests that study of drug-exposed infants would benefit from the knowledge gained in the study of high-risk infants. This includes the abandonment of preconceived biases that these infants are damaged and doomed to fail and that they are all alike. The long-term developmental outcome of these children is likely to be a function of how the caregiving environment responds to the behavioral constellation of the infant, with the understanding that both the behavior of the infant and the caregiving environment make dynamic adjustments to each other and are influenced by other forces. The study of the exposed infant should be approached from a holistic perspective in which the full range of child behavior (i.e., cognitive as well as noncognitive) is examined.

Unique Aspects of Drug-Exposed Infants. It is important to address issues that may be unique to the study of the drug-exposed child. One issue is whether there is a unique pharmacological effect of drugs and

how this effect interacts with other pre-, peri-, and postnatal biological and environmental factors. A second issue is SES. Although many high-risk infants grow up in impoverished environments, drug-exposed infants (at least those the authors study) are almost exclusively from the poorest segment of society. The developmental consequences of poverty have only recently been acknowledged and require far more investigation. Beyond the obvious problems of nutrition and health, children raised in poverty are likely to face homelessness, violence, and crime. Families ranked as low SES are not, however, a homogeneous group. The variation in parenting and other environmental caretaking factors within social strata that can affect child outcome requires study.

Poverty is also associated with minority status, race, and ethnicity. The complexities of these issues affect the ability to communicate and establish rapport, to understand cultural factors that affect use of drugs, and childrearing practices. There are psychometric concerns regarding the appropriateness of tests that have been developed and standardized based on middle-class American values. How does one determine what behavioral processes to study and how to interpret the findings without knowing the meaning of these processes in the local culture? For example, there is a belief in much of the United States that eye contact between mother and infant is important in the development of the mother-child relationship. Some cultures, however, discourage this practice and the relationship is based on other behaviors. Clearly, one would not want to penalize a mother from a different culture if she did not look at her baby the way many American mothers do. This illustrates the need to incorporate cultural issues in instrument development when studying families from different cultures.

There are also other subpopulations that need to be studied separately, such as teenage mothers. There is already a parenting risk associated with teenage mothers. There is the belief that the teenage mother using drugs puts her child in double jeopardy, but this is probably too simplistic. Like their infants, teenage mothers are not a homogeneous group. For example, depending on their level of emotional development, some are better parents than others. The effects of drug exposure need to be understood in the context of the teenage parenting phenomenon.

In the case of the exposed infant there is the potential involvement of the social service and legal community because drug use is illegal and has implications for child abuse and neglect. There is also the issue of multiple caretakers and multiple placements. Some children experience

as many as eight foster care placements in the first year of life (Beckwith et al. 1994). When studying the attachment relationship, it is not always obvious who the primary caretaker is. In fact, researchers may even be asking the wrong question about attachment in these situations. Rather than identifying the attachment classification in children who undergo multiple placements, perhaps the question should be, "How do children form attachments in the face of multiple placements? What is the role of the biological mother in these cases?"

The unique problem of maternal drug use and possible addiction needs to be treated somewhat independently of the child. On the other hand, maternal preoccupation with drugs, associated personality disturbances, possible psychopathology, and a chaotic lifestyle clearly impact on the mother-child interaction (mutual regulatory system) and on the ability of the child to thrive in this environment.

Finally, there is the issue of identification of exposure status. The 1992 NIDA Household Survey showed that although the prevalence of crack cocaine use has declined overall, in certain groups the drug continues to be used at high or increasing rates (NIDA 1992). Not surprisingly, it is inner-city minority groups that are most affected. Also, women of childbearing age seem to be particularly susceptible. Prevalence rates range from 3 percent to almost 50 percent, with the highest rates reported by centers that serve poor inner-city mothers. However, there are two problems with this survey data. First, it is based on self-report, and self-report is known to be especially unreliable when illegal activities are involved. Second, the report is based on individuals living in households. That is, respondents had to live in a household to be in the survey. These criteria do not identify a group representative of the drug-using population.

Epidemiological statistics will improve as better toxicology assays become available. Moreover, currently available techniques (discussed below) only provide reliable qualitative information on the presence or absence of drugs. They do not provide quantitative information about the frequency, timing, or amount of drug use necessary to establish dose-response relationships.

Epidemiological information is also affected by the populations that are screened. Depending on hospital policy, pregnant women can be screened if they have a prior history of drug use or when there are clinical reasons to suspect drug use. There are no official criteria for clinical suspicion but, in general, criteria include obstetrical events such as no prenatal care,

premature labor, and placental abruption. Since these conditions are more often associated with poverty, poor people and minorities are more often screened. Therefore, most of the data about drug use comes from pregnant women living in poverty. It is possible to do anonymous screens in which the patient is not identified. One such study was done of pregnant women in Florida and the surprising finding was that the incidence of illegal drug use was comparable between lower-class and middle-class patients (Chasnoff et al. 1990). This study, if replicated, would change the way society thinks about illegal drug use during pregnancy. Further, a middle-class study sample would provide the methodological opportunity to study drug-exposed children growing up in more enriched environmental conditions.

Toxicology Assays. There are many issues unresolved in the use and development of toxicological assays. Urine screens have been the standard but reflect only use over the preceding 72 hours. The meconium assay is a more recent development and has the advantage of recording drug use through the second half of pregnancy. Hair analysis is a third technique that has the potential to provide an even longer record of drug use. However, there are methodological problems with hair assay and the need for informed consent that have so far limited the use of this technique.

Toxicological assays involve a two-step process. There is an initial screen that can yield a presumptive positive. The screen is presumptive until it is confirmed by a second assay. Many presumptive positive screens are not confirmed, resulting in a high false-positive rate. Therefore, it is important to verify presumptive positive results with a confirmation analysis. Some methods for screening and confirmation are more reliable than others. For example, in forensic work, gas chromatography/mass spectrometry (GC/MS) is used for confirmation. However, this method is usually considered too expensive for clinical use. Also, some metabolites are more difficult to confirm than others. For example, tetrahydrocannabinol (THC), the metab-olite of marijuana, is much more difficult to confirm than drugs such as cocaine and opiates.

As previously mentioned, all of the toxicology assays in current use provide limited qualitative data. Quantitative methods have not been established. One cannot determine how much of the drug was ingested, how many times it was ingested, or at what stage during gestation it was ingested. There is no biochemical marker for alcohol, so toxicology cannot be used to determine alcohol use during pregnancy. This information has to be determined from maternal report.

Cocaethyline is a metabolite of cocaine that is present when cocaine and alcohol are used together. The presence of this metabolite indicates only that cocaine and alcohol were used together some time during pregnancy. Cotanine can be used to determine cigarette smoking, although this variable has not yet been used in a study of prenatal substance abuse.

Another problem that has not been solved is how to separate drugs used licitly from drugs of abuse. Licit drugs may include prescription medication taken during pregnancy or medication used during labor and delivery. Opiates used for pain relief can result in a positive toxicology screen but may not indicate illegal drug use. On the other hand, some mothers abuse prescription medication such as codeine. Even if the validity of a positive toxicology screen is questioned because of prescription medication, the mother may have abused the prescription medication or used illegal drugs as well as prescription medication. These questions cannot be answered by a toxicology analysis alone and in some cases the drug use history may never be known.

There has been some recent investigation of passive exposure, including the absorption of cocaine by a child through environmental exposure such as inhaling smoke or powder. In a study of 460 children between 1 and 60 months of age seen in an emergency department for pediatric problems unrelated to drugs or child abuse (e.g., crying, fever, diarrhea), cocaine was found in 5.4 percent of the urine specimens (Rosenberg et al. 1991). The environment may have pharmacological as well as social effects. There are no studies of other environmental hazards of toxins such as lead or polychlorinated biphenyls (PCBs) and how exposure to these substances may interact with drugs. Inner-city children in some areas of the country are likely to be exposed to lead as well as drugs. There are poor fishing communities where drugs and PCBs likely co-occur.

RESTATEMENT OF THE PROBLEM

Arguably the most important contribution of the first generation of cocaine research is a better understanding of the problem itself (figure 2). Researchers learned that the problem was far more complicated than had been originally described for two reasons. First, the drug issue is one of polydrug use, not of cocaine alone. Most women who use cocaine also use other drugs; alcohol, marijuana, and cigarettes are most common, but other drugs such as heroin are also used. There may be women who use

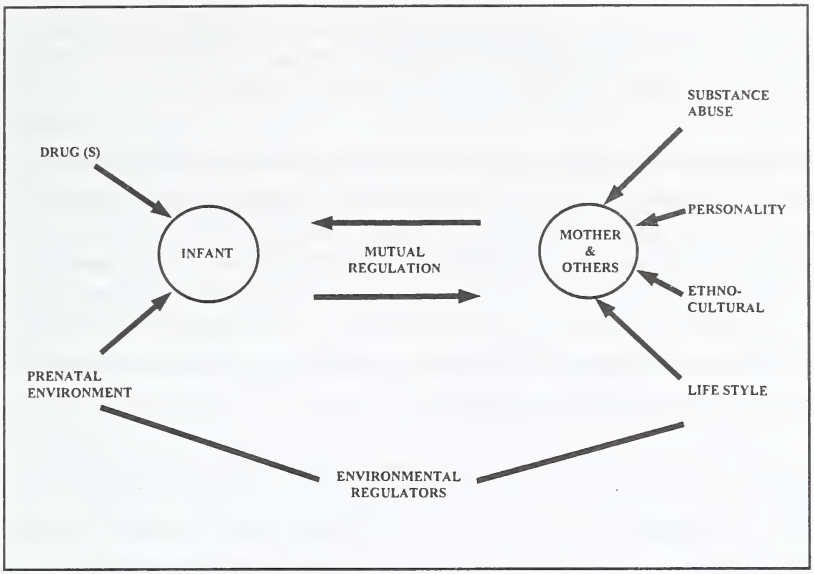

FIGURE 2. *Systems approach to study of cocaine.*

only cocaine but they seem to be more the exception than the rule. Thus one must assume polydrug use from the outset.

The second complicating factor is what has been termed "environmental" or "lifestyle" issues. Environment is used to describe a complex set of interrelated factors including psychological and social factors that lead a mother to use drugs, as well as the neighborhood and general conditions in which drug-exposed children are often raised. These conditions may involve inadequate and even more disruptive forms of parenting; poverty; high stress; exposure to violence; and a chaotic, disorganized lifestyle, factors that could lead to poor developmental outcome independent of prenatal drug exposure. Therefore, drug effects (pharmacological effects) are confounded by environmental effects. If developmental outcome is compromised, is this due to drug exposure or the environment?

Cocaine seems to be a variable marker for polydrug use and a lifestyle associated with poverty that may jeopardize normal developmental outcome. Figure 2 shows a systems approach to the problem as currently understood. It is reasonable to expect that the combination of prenatal drug exposure and other factors such as poor prenatal care and a poor reproductive history combine to produce, in some cases, an acute neurobehavioral vulnerability or fragility. Many of these infants are

probably not damaged. In fact, many appear quite normal. However, there is a significant proportion of these infants who display many stress behaviors and show disorders of behavioral regulation.

In a reasonably supportive environment, these infants would probably recover and have every chance for a normal developmental outcome. However, environmental factors can be regulators or disregulators, buffers and stabilizers or destabilizers of child behavior. Unfortunately, all too often these infants do not grow up in environments that have a positive effect on even average child behavior. An infant who is already stressed has that much more to overcome and may recover poorly in an unsupportive caregiving environment.

As shown in figure 2, the immediate caretaking of the infant may be compromised by a mother who has a drug problem, by personality disorders undoubtedly related to her use of drugs, and historically based psychopathology. These factors impinge on the mutual regulatory process of the mother-infant interaction that could re-regulate infant regulatory disorders. More distal factors may be added to these proximal factors, including a lack of social support and the larger environmental stressors associated with poverty.

In this model, drugs have a direct acute effect and an indirect long-term effect. Drugs have the potential to predispose the infant to a short-term neurobehavioral vulnerability as a direct pharmacological effect on the four A's of infancy. The interaction between the neurodevelopmental vulnerability and the response of the caregiving environment determines the long-term developmental outcome of the child. The longer-term drug effect is indirect and is mediated by environmental factors.

This model enables study of the effects of cocaine in the context of multiple risk factors that may affect the regulatory capacities of the child. With this framework one can generate a discussion of the issues that need to be addressed based upon this current understanding of the problem of prenatal cocaine/polydrug exposure and child outcome.

THE SECOND WAVE

Researchers learned from the first wave of research in cocaine abuse that drug effects had been exaggerated and had caused a widespread misperception that a generation of children was doomed. It would be

equally dangerous to assume that the maternal lifestyle or larger environment is to blame. At this time very little is known about the range of developmental outcomes to expect in drug-exposed children or the etiology of such outcomes. It is probably fair to say that these children are at increased biological and social risk, that their outcome is undetermined, that the full range of intellectual and social-emotional outcomes are possible, and that neither biological nor environmental factors have been proven or disproven to determine the developmental outcome in these infants.

The authors believe that a wide range of individual differences in patterns of development will be found in these children. These patterns will be lawfully but differentially related to the interplay between biological (including drug exposure) and social forces. Thus, biological vulnerability makes a child more vulnerable to the effects of a poor caretaking environment. By understanding these patterns of individual differences and their biosocial etiologies, researchers will be able to understand the developmental outcome of drug-exposed children. This understanding will enable development of effective preventive and ongoing treatment programs to facilitate child development.

SUMMARY AND RECOMMENDATIONS

The authors consider the results of this attempt at a meta-analysis of neurobehavioral studies and cocaine exposure informative and to some extent shocking. Important data are not routinely reported in peer-reviewed publications. When basic information such as the masking of examiners to exposure status in neurodevelopmental studies is not reported in almost 50 percent of the articles, it becomes virtually impossible to draw conclusions about neurobehavioral effects. Even when adequate information is reported, studies have such severe methodological limitations that any attempt to draw conclusions about neurobehavioral effects of prenatal drug exposure is impeded. The authors strongly recommend that journal editors be more stringent and require that minimum information be reported in all studies of drug-exposed infants. Perhaps NIDA could develop a list of recommended or required reporting information and circulate such a list to journal editors.

The good news is that identification of these methodological problems will allow definition of more sophisticated future studies and the methodological issues that need to be addressed. Clearly there is a need for

longitudinal followup studies that pay adequate attention to repeated-measures analysis of the same factors and individual differences in the stability and reliability of findings.

Some issues, such as polydrug use and the confounding of medical and demographic factors, are issues for which there are methodological strategies. Due to their complexity, these issues require additional conceptual thought. For example, although there are methodological techniques to deal with the problem of polydrug use, it has also been argued that if polydrug use is the norm it should be regarded as the variable under investigation rather than trying to isolate a pure cocaine effect that may actually be a rare event. There are arguments to be made on either side of this issue and scientists need to be clear about the strengths and limitations of each approach.

Other issues such as toxicology analysis await further methodological advances. These include improved ability to detect prenatal drug use, the development of quantitative assays to determine dose-response relationships, and how drug interactions may affect behavior.

Finally, this attempt at an analysis was probably premature. Not all studies report the information necessary and sufficient studies using the same outcome measures have not been reported. The neurobehavioral database is small, fragmented, and lacks consistency in measures used as well as in study design. However, by relating the study of prenatal drug exposure to the study of other high-risk populations such as premature infants, researchers can build on the existing knowledge base and also appreciate the uniqueness of the present situation.

This new wave promises to be exciting. With the present knowledge base researchers have a much better understanding of the problem than when the studies reviewed here began. Perhaps the most important contribution of the first wave of research was a solid handle on the problem itself. Answers seem within reach, but there is still a great deal to learn while a sizable and very precious part of part of society, children and mothers, remains in jeopardy.

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APPENDIX 1. List of included articles (N = 50).

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ACKNOWLEDGMENTS

This work was supported in part by National Institute of Child Health and Human Development (NICHD) SRC grant no. 1U10-HD27904 and NICHD grant no. N01-HD-2-3159.

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Exposure to Cocaine: Behavioral Outcomes in Preschool and School-Age Children

Linda C. Mayes

Within the last 5 years, increasing numbers of children exposed prenatally to cocaine and crack have been evaluated in the preschool and school-age years. Although there are few published reports to date about the findings of longitudinal followup efforts beyond the first 12 to 24 months of age (Chasnoff et al. 1992; Griffith et al. 1994), sufficient data are beginning to emerge from animal and human studies to allow some refinement of hypotheses and more informed choices of measures for studying such children.

In early reports, cocaine exposure was predictively linked to moderate to severe developmental delays across all domains. Subsequent studies have reported mild to no impairments in overall developmental functioning in cocaine-exposed children compared with noncocaine-exposed groups. Most recently, the developmental profiles of a group of 106 cocaine/alcohol-exposed 24-month-olds followed from birth were compared with the performance of 45 toddlers exposed to marijuana and/or alcohol but not cocaine and 77 nondrug-exposed children (Chasnoff et al. 1992). Mothers of infants in the two comparison groups were similar to the cocaine-using mothers in socioeconomic status (SES), age, marital status, and tobacco use during pregnancy. On repeated developmental assessments using the Bayley Scales (Bayley 1969) at 3, 6, 12, 18, and 24 months, albeit with a high rate of attrition from the original cohort, there were no mean differences in either the mental or motor domains, although the investigators cautioned that a higher percentage of cocaine-exposed infants scored two standard deviations (SD) below the mean (Chasnoff et al. 1992). Cocaine-exposed children from this cohort followed through age 3 years continued to show no differences on overall performance on the Stanford-Binet Intelligence Scale from the noncocaine-exposed controls (Griffith et al. 1994), although the cocaine-exposed group scored significantly lower on verbal reasoning.

Three other investigative groups have reported similar failures to find differences among cocaine-exposed groups on general measures of developmental competency in the first, second, and third years of life

(Anisfeld et al. 1991; Arendt et al. 1993; Billman et al. 1991). Findings such as these have required a reevaluation of earlier concerns about global developmental delay in cocaine-exposed children. Conversely, as more children who were prenatally exposed have been evaluated in a variety of research and clinical contexts, more evidence has accumulated about the insensitivity of measures such as the Bayley or the Stanford-Binet to the types of clinical problems displayed by many cocaine-exposed children.

When specific developmental domains or neurodevelopmental functions have been studied, mild to moderate impairments have been reported in the following areas (albeit based on one or two studies for each area): recognition memory, visual habituation, language development, and capacity for symbolic play. In addition, concern has been raised about impaired parent-child interactions and distorted or impaired attachment. More generally, studies dealing with the parenting, home, and community environments of cocaine-exposed children suggest increased incidence of physical abuse, neglect, abandonment, and foster placement, events that also carry implications for later psychological and developmental dysfunctions.

The findings related to attention and reactivity functions among cocaine-exposed children reflect, at least in part, links with central nervous system (CNS) monoaminergic systems and contribute to the emergence of language, play, and capacities for social interaction (Mayes 1992). The central question for outcomes such as language, symbolic play, attachment, and parent-child interactions that are more multidetermined and socially embedded is whether or not the possible increased incidence of impairments in preschool cocaine-exposed children is uniquely different from that seen in children from dysfunctional or multirisk families not affected by substance abuse. Additionally, continued parental cocaine use postnatally provides another level of cocaine exposure for the young child and may or may not have unique effects on parenting capacities that in turn affect such domains as language development.

ATTENTION AND HABITUATION

When an infant is presented with a novel stimulus, the infant will orient and attend. If that stimulus is presented repeatedly or continuously, the infant's attention will wane and the point of decrement is called habituation. If a second novel stimulus is presented, the infant will

reorient and attend again. Conversely, such an increase in attention or visual fixation will not occur with subsequent presentation of the first, familiar stimulus (Bornstein 1985, 1989). The habituation task provides information about the organization of looking behavior and attention in the first 1 to 2 years, and the habituation process represents an early form of some type of information processing and encoding by the infant and child (Bornstein 1985; Cohen et al. 1979; Colombo and Mitchell 1990). The habituation procedure has been used to study a host of questions about early memory, concept formation, and infants, and young children's capacities to detect, categorize, and discriminate incoming information.

Habituation in infancy measured quantitatively or qualitatively shows adequate test-retest reliability and, for certain measures such as accumulated looking time at the stimulus, is moderately stable month to month (Bornstein and Mayes 1992). Additionally, as would be hypothesized for a function related to information processing, habituation measures show improved efficiency with maturation (Bornstein et al. 1988; Mayes and Kessen 1989). Habituation measured between 3 and 6 months of age predicts Bayley performance, language production and comprehension, and full-scale intelligence quotient (IQ) test performance up to 12 years (Bornstein 1989; Bornstein and Mayes 1992). The median of predictive correlations in these studies reaches the 0.50 region (Bornstein and Sigman 1986). Additionally, the habituation response has been used in a number of studies of infants at risk for impairments in attention or early information processing, such as very preterm infants or those preterm infants with intraventricular hemorrhage, and has been shown to discriminate among such groups (Landry et al. 1985; McDonough and Cohen 1982; Millar et al. 1991).

Links between the dopaminergic system and attentional mechanisms that are likely to involve the habituation process (Coles and Robbins 1989) make it plausible to hypothesize that prenatal cocaine exposure could affect subsequent habituation performance. To support this hypothesis, evidence from animal studies reveals a relation between prenatal cocaine exposure and impaired postnatal associative learning (Dow-Edwards 1988, 1989; Spear et al. 1989). Two studies have examined the habituation response or similar measures in cocaine-exposed children. Struthers and Hansen (1992) reported on recognition memory in a group of 36 cocaine- and/or amphetamine-exposed infants and 26 nondrug-exposed infants who were evaluated between 27 and 52 weeks of age. The drug-exposed infants showed significantly lower scores on serial measures of visual

recognition memory. Tests of recognition memory, which are moderately predictive of later cognitive performance (Fagan and Montie 1988; Fagan et al. 1986), rely on the infant's capacity to habituate to familiar stimuli and to orient preferentially to novel information; thus, though procedurally different from standard habituation paradigms, recognition memory relies on habituation processes.

A recent series from the author's own laboratories (Mayes et al. 1995) examined 108 infants—61 cocaine-exposed and 47 controls—at 3 months of age in an infant-control habituation and novelty responsiveness procedure. Habituation studies were performed by investigators blind to drug exposure status. In the standard habituation paradigm, a visual stimulus appears on the screen when the infant looks directly ahead and is removed from the screen when the infant looks away. The habituation stimulus is presented repeatedly until the looking times decline to the so-called habituation criterion, which is defined as a percentage of a baseline that is the mean of the duration of the first two looks at the stimulus. Following the habituation phase, a novel stimulus is presented to measure recovery of attention to novel information. Changes in the child's level of arousal and state of alertness are monitored and coded throughout the session.

The basic unit of measurement in the habituation sequence is the duration of a look. From the duration of looks, the following measures describe habituation performance: the duration of the first and second look, duration of the longest look or peak looking time, duration of the criterion look, number of looks to criterion, and the cumulative looking time before criterion is reached. Recovery of visual attention to novel stimuli is calculated as a proportion of the duration of the look to the novel stimulus compared with the last look at the habituation stimulus. Accumulated looking, peak look, and recovery to novelty are predictive of later information-processing capacities measured by full-scale IQ with the median in the 0.50 range (see also above; Bornstein and Mayes 1992; Bornstein and Sigman 1986).

In this series, 37 of the 108 infants fussed or cried on the presentation of the habituation stimulus and were unable to begin the habituation phase. A greater proportion of these infants were from the cocaine-exposed group (N = 27 of 61, or 44 percent) than from the nondrug-exposed group (N = 10 of 47, or 21 percent), chi square = 6.23, $p = 0.01$. Also, at the beginning of the novel stimulus presentation, an additional seven infants became sufficiently irritable that they could not complete the novel stimulus test phase. Four of these infants had been cocaine exposed.

Thus, in the overall habituation procedure, 44 infants became fussy and irritable, and a greater proportion of these were from the cocaine-exposed group, $\chi^2 = 5.89$, $p = 0.02$. Infants who were unable to begin the habituation phase were not significantly different in terms of gestational age, birthweight, length, and head circumference or maternal alcohol, tobacco, and marijuana use, age, and education.

For the 71 infants (34 cocaine-exposed and 37 noncocaine-exposed) who completed the habituation phase and reached criterion, variables describing habituation performance are shown in table 1. Because time data are often positively skewed, log transformations of looking times were performed, and relations between drug exposure status and habituation performance were examined. Table 1 shows no differences between the two groups in habituation performance. With the criterion for significance set to 0.05, the sample size of infants reaching habituation criterion was sufficient to detect moderate group differences (equal to 0.25 population SD) more than half the time (power = 0.56) and large group differences (equal to 0.4 population SD) more than 90 percent of the time (power = 0.92). Also, because lower birthweight has been related to differences in measures of visual attention (Gotlieb et al. 1988) and measures of habituation change significantly with age (Bornstein et al. 1988; Mayes and Kessen 1989), comparisons of habituation variables were covaried for birthweight and age in days. There were no significant differences for drug exposure status on habituation performance. These means on measures of habituation and recovery to novel information are comparable to those obtained in the author's laboratory from a large group of nondrug-exposed children from families of middle to upper SES (Mayes and Kessen 1989).

Thus, cocaine-exposed infants appeared more labile and reactive to novel stimuli, but if the infant was able to attend there was no difference in measures of habituation performance. That is, if the infant was able to maintain an alert, oriented state, measures of early information processing were no different between drug-exposed and nondrug-exposed groups. Before expanding on the possible implications of differences in reactivity to novelty, it is important to underscore that other aspects of the study sample may have contributed to the difference in reactivity between the cocaine- and noncocaine-exposed groups. As others have found (Zuckerman et al. 1989), cocaine-exposed infants were at increased perinatal risk as indicated by differences in prenatal care, number of obstetric complications, and indices of fetal growth. They were more often from families in which mothers had more limited education and

TABLE 1. *Habituation performance and cocaine exposure.*

	MEAN (SD)	
Habituation Measures	Cocaine Exposed N = 34	Noncocaine Exposed N = 37
First look (sec)	5.8 (3.5)	8.5 (8.1)
Baseline looking (sec)	5.2 (2.4)	7.6 (7.6)
Peak look (sec)	11.9 (10.2)	16.5 (25.2)
Criterion look (sec)	1.5 (0.8)	1.8 (1.8)
Looks to criterion	9.2 (6.7)	7.9 (4.6)
Cumulative looking (sec)	39.7 (35.2)	34.3 (22.7)
	N = 30	N = 34
Recovery to novelty	0.64 (0.18)	0.62 (0.17)

were more likely to use alcohol, marijuana, and tobacco. Thus, for a number of reasons in addition to prenatal cocaine exposure, the cocaine-exposed group was at potentially increased risk for neurodevelopmental impairments. Rather than being an effect specific to cocaine or cocaine plus other drugs, it may be that the infants' overall irritability reflected their more general compromised and complicated perinatal course related to a state of relative fetal hypoxia due to cocaine's effect on placental blood flow and measured by reduced birthweight. Small for gestational age and low birthweight infants have been described well into the first and second years as more irritable and less adaptable to novelty on measures of sleep-wake patterns, temperament, and reactivity (Watt 1987; Watt and Strongman 1985). In addition, low-birthweight infants may show compromised attention to visual discrimination tasks, with more attention difficulties apparent among these infants with compromised medical courses (Gotlieb et al. 1988; Landry et al. 1985; Sigman et al. 1977).

With this caveat about overall perinatal compromise in mind, these findings regarding habituation suggest that reaction to novelty and the capacity to regulate attentional states may be important lines of investigation to follow with children prenatally exposed to cocaine. For one, closer examination of the patterns of reactivity in those infants who fail to enter

the habituation process (including level of motor activity, gaze aversion, change in facial affect, and heart rate patterns prior to their actual change to an irritable, crying state) will provide information on the latency to changes in arousal following the presentation of a novel stimulus. Second, examining items commonly used in developmental assessments that employ novel materials or tasks provides similar opportunities to study reactivity to novelty or to the demand to focus and attend. For example, during the administration of the Bayley Scales, the child is faced with a number of novel and increasingly difficult tasks that place demands on arousal-regulating systems. Systematic study of shifts in states of arousal and attentiveness during standardly administered developmental assessments may provide additional information about the question of reactivity. Suggestive data support this possibility (Hawley and Disney 1992); cocaine-exposed 24-month-olds had more difficulty attending to several objects at the same time and in structuring an approach to an unfamiliar task on their own in the context of the developmental assessment.

It will also be important to continue to use tasks through the preschool years that rely upon habituation and novel discrimination processes and provide assessments of reactivity, frustration tolerance, and task persistence. Tasks such as those used by Ruff (1986), in which the child is sequentially presented with several novel toys to explore for a fixed period of time, assess not only the capacity to sustain exploration but also how the child reacts to multiple shifts in tasks or toys. Impairments in these types of domains have important implications for later school performance, where there are many more novel situations in any given day and increasing demands on a capacity to focus and move smoothly between tasks and new information.

The other domains for which some results are available from studies of preschool cocaine-exposed children are language, play, and parenting and attachment. In these areas, the available literature becomes far more scant and methodologically problematic. The few available studies to date have used small samples with mixed prenatal as well as postnatal drug exposure and combinations of preterm infants as well as SES-matched comparison groups. The measures of the particular domains have been quite variable and not conceptually linked to earlier hypotheses about how cocaine might affect functions such as reactivity and attentional regulation that in turn underlie capacities for language, play, and sustained social interaction. Additionally, only the most general measures of parenting, home environment, and parent-child interaction have been used. However, there are conceptually salient reasons for concern about the

domains of language, play, and parent-child interaction among cocaine-exposed children and their families.

LANGUAGE AND SYMBOLIC PLAY

Language development and the capacity for symbolic play are closely related since both involve maturing capacities for representation and for communication. Whereas play in the first year of life is largely exploratory or nonsymbolic, play in the second and third years involves the capacity for substituting function (e.g., using a cup to stand for something other than a cup) and for pretending. The capacity for verbal language emerges in parallel and likely facilitates increasingly sophisticated symbolic, pretend play. The progression from nonsymbolic to symbolic play is not only gradual, with its own rate of maturation; there are also marked individual differences in the amount of symbolic play shown by children in their second year of life. At 13 months, some toddlers never exhibit symbolic play, whereas for others as much as half of their play is symbolic (Tamis-LeMonda and Bornstein 1990, 1991). Further, capacity for symbolic play at 13 months predicts development at 20 months and thereafter; again, there is a close predictive and correlative tie between language and play sophistication.

There are many sources of such individual variation in the development of capacities for language and play, including differences in overall cognitive competency, but at least two relate to areas of concern for children prenatally exposed to cocaine. First, language development is partially dependent upon a capacity for sustained attention and exploration. Basic problems in the regulation of arousal and alert states will indirectly affect the emergence of language. Second, maternal stimulation increases children's use of language and level of play, both nonsymbolic and symbolic (Tamis-LeMonda and Bornstein 1990, 1991; Vibbert and Bornstein 1989). A combination of maternal social (e.g., physical, affectionate contact) and attention directing (or didactic) activities best explains the level of sophistication of the toddler's play. As addressed below, cocaine-abusing mothers may be more likely to have difficulty with the kinds of interactive tasks that support both language and play development.

To date very few published studies have specifically addressed the language development of cocaine-exposed children. In one study, 30 preschool children from The Netherlands who were prenatally exposed to cocaine in addition to methadone and heroin (van Baar 1990) were found,

at age 30 months, to perform less well on the Bayley Mental Index than comparison children. However, when all Bayley items involving language (receptive or expressive) were removed and a nonverbal development index created, there were no differences between the drug-exposed and nondrug-exposed groups.

While it may be problematic to examine group differences in individual items from standardized tests, such an approach is useful for generating hypotheses. However, findings of specific receptive or expressive delays do not directly address language or communicative functions but only suggest that tasks involving verbal demands or requiring verbal response may be more difficult for drug-exposed children. No study has yet examined communicative functions such as joint attention (Bruner 1975) or other early communicative strategies that underlie verbal language and communication and which, given concerns about attention and reactivity in cocaine-exposed infants, may be impaired.

Further studies of language development in cocaine-exposed preschool children will need to rely not only on specific measures of communication and receptive/expressive language, but will also need to examine communication precursors such as joint attention. Additionally, it will be essential for studies of language to have parallel studies of mother-child interaction.

The two available studies of the symbolic play capacities of drug-exposed toddlers, like the examination of language, provide only the most preliminary outlines for further investigation but do suggest areas of concern. In a study of symbolic play at 13 months of age, 18 children prenatally exposed to cocaine, phencyclidine (PCP), heroin, and/or methadone were compared with 41 SES-matched preterm children. The drug-exposed children were significantly less likely to engage in representational play or nonrepresentational exploration, but rather exhibited disorganized, poorly modulated play such as scattering and throwing toys (Rodning et al. 1989). These findings were replicated in a second cohort of 31 children and the differences were not statistically related to differences in home environment (Beckwith et al. 1994).

In addition to progression to different levels of symbolic activity, subsequent studies of play also need to focus on such measures as how long the child is able to sustain play with a given object and how often and under what types of conditions play is disrupted. Similarly, following on the earlier discussion of reactivity and lability, measures of the child's approach, and use of novel toys or situations are conceptually analogous

to the simpler novel stimulus tasks of infancy and tap the same potentially problematic domains as assessed by the habituation tasks.

PARENT-CHILD INTERACTION AND ATTACHMENT

Perhaps the most methodologically problematic area in the study of prenatal cocaine exposure in the preschool child has been the evaluation of the parenting environment (Mayes, in press). The domains of attention, reactivity, language, and play are each influenced by parental interaction and structuring activities (Tamis-LeMonda and Bernstein 1989). While attention and reactivity more directly reflect neuropsychological functions that are biologically based, over time these functions appear quite sensitive to the effects of environmental disorganization and neglect. The degree of sensitivity is individually variable for any given infant, but models of cumulative risks are particularly relevant for drug-exposed infants and children living in drug-using households.

Parents who are actively abusing cocaine and other substances have problems caring for their children, as indicated in part by the increased incidence of physical abuse and neglect in such families and by the proportionately higher numbers of children from substance-abusing families who are in foster care or other types of placements (Lawton 1992). Additionally, evidence is accumulating from substance abuse treatment programs that a high proportion of children and their mothers are witnesses to and victims of physical and verbal violence on a nearly daily basis (Lawton 1992). How such environmental events influence both a parent's ability to care for and protect a child and the child's ability to modulate aggression and develop basic capacities for empathy and relatedness are areas essentially unstudied in substance-abusing families; both are pressing issues in understanding the specific nature of parent-child interactions in these families.

Indirect though conceptually related measures of parenting include studies of attachment. Rodning and colleagues (1989, 1991), studying the same cohort described earlier, showed that drug-exposed toddlers are more likely to be insecurely attached to their mothers, while most of the comparison group of nondrug-exposed premature infants were securely attached. In addition, the drug-exposed children showed higher rates of disorganized attachment behaviors (group D; Main and Solomon 1986). The first study from Rodning's group (1989) suggested that the high rate of insecure attachment was related more to postnatal environmental

conditions than to prenatal drug exposure, since drug-exposed children reared in foster care or by a relative were less likely to be insecurely attached than those living with their biological mothers. However, similar differences in the frequency of insecure attachments among biological, relative, or foster care parents were not found in a second study of 39 infants (Rodning et al. 1991).

In addition to the difficulties already cited of mixed drug exposure and small sample sizes, the attachment studies raise two methodological concerns. First, attachment is a broad construct and the method traditionally used to assess attachment behaviors, the Strange Situation Paradigm (Ainsworth et al. 1978), can be particularly stressful for a child left alone with a stranger, usually in a strange place. How the child responds to the mother on her return is, in part, a measure of their relationship and the child's experience with seeking her comfort. However, the situation may impose an additional level of stress on children who have potential problems with reactivity, lability, and state control in novel or strange situations. Such additional stress may add to the appearance of particularly impaired attachment behaviors in the drug-exposed group.

The second methodological concern involves the question of whether or not these differences in insecure or disordered attachment are unique to prenatal drug exposure or more reflective of the overall increased disorganization, stress, abuse, and exposure to violence among drug-using families. An increased incidence of disordered attachment behaviors has been described for severely dysfunctional families in a number of studies (Carlson et al. 1989; O'Connor et al. 1987). Moreover, failure to find a difference between prenatally exposed infants in foster care and those in the care of their biological mothers may not reflect a direct relationship between prenatal cocaine exposure and overall attachment, since children in foster care have usually been in their biological parents' care for months to years and have experienced more than one foster placement. Their caregiving situation at the time of the attachment assessment does not necessarily reflect the situation even a month earlier. Careful study of this issue will require far larger sample sizes and efforts to quantify the amount of exposure to the various caregiving situations.

An additional problem with measures of parenting as well as attachment rests with the issue of characterizing the actual caregiving situation. Most of the studies of parenting among substance-abusing families have relied on parent report on instruments such as the Parental Attitudes Research Instrument (Wellisch and Steinberg 1980) that describe factors such as

the degree of parental control, use of supports, or reliance on authoritarian techniques. On measures such as these, substance-abusing mothers exhibit a range of parenting difficulties, including reliance on a more disciplinarian, threatening style of parenting and negative reinforcement (Bauman and Dougherty 1983).

In addition to the often cited problems of using self-report instruments with actively substance-abusing adults, reliance on such measures for descriptions of parenting styles does not address the questions of whether and how active cocaine abuse limits or distorts a mother's actions with her children. Infant attention, exploration, and use of language are influenced by maternal behaviors such as directing the infant's attention to a new toy, naming and pointing, or elaborating on the child's play. Because of the acute and chronic effects of cocaine on an adult's responsiveness, in addition to the more general effects of chronic stress and poverty, it is likely that cocaine-abusing mothers are able to do fewer of the types of activities most central to influencing the infant's attentional regulatory capacities, use of language, exploration, and play. Additionally, cocaine use is associated with a higher incidence of depressive symptomatology that may be both a premorbid state as well as a result of chronic cocaine use (Woods et al. 1991). In either case, such symptoms also impair an adult's ability to adequately care for a child.

There are very few direct observational studies of the interactive behaviors of cocaine-using women with their children. In one study of five polydrug-using mothers without a comparison group, drug-using mothers showed a reduction in reciprocal behaviors with their infant and infrequently structured and mediated the environment (Burns et al. 1991). These findings suggest problems with attention directing and structuring activity, but far more work is needed in this area.

METHODOLOGIC DILEMMAS

Throughout this chapter, several points have been made about methodological problems inherent in the available studies to date. In summary, these are, first, the fact that samples are characterized by mixed drug exposure. No study has dealt with cocaine exposure alone, including the most recent ones dealing with habituation and recognition memory in which maternal cocaine use during pregnancy was more carefully defined. At the very least, any findings that appear specific to the cocaine-exposed group reflect cocaine/alcohol exposure. It will take time to accumulate

comparison groups exposed to alcohol only. The important developmental question may not be "What are the effects of cocaine exposure alone?" but, "Are there effects apparently related to a combination of cocaine/alcohol exposure?" In any case, the caution about attributing any findings in the present literature solely to cocaine use needs repeated highlighting. Additionally, all outcome studies in cocaine-exposed children are plagued by uncertain data about the timing, amount, and duration of the exposure to cocaine as well as other drugs.

The second problem is the considerable attrition in study samples and the likelihood that those families who remain in a longitudinal cohort do so for a variety of reasons including their own wish for help or concerns about the child. The most frequently voiced concern is that families who remain in treatment and in study cohorts are the most motivated and concerned, not the most impaired or dysfunctional. Thus, findings of only mild or no differences on various child assessments may reflect a sample biased toward children at lesser risk and thus are not generalizable to the larger population of prenatally exposed children. This concern has important policy implications, and underscores the need for some types of assessments of families who drop out of studies. Although such assessments cannot be as detailed as those already described, which provide a global measure of risk and need, the data are necessary to inform future research.

As has been stated by many reviewers, blinded assessments of the children are critical. Besides instituting a number of techniques such as coding from videotapes and using different examiners to see families, the author has begun asking examiners at the end of their session to indicate whether or not they feel they know the infant or child's exposure status and if so, why. The ability to guess correctly has been only slightly greater than chance, and the reasons given for choices indicate a number of deep-seated biases about the relation between drug abuse, parenting, and level of general disorganization.

The third methodological issue involves the point implicit throughout this chapter: how prenatal exposure interacts with the postnatal environment to affect those behavioral/developmental outcomes of most concern. Even basic neuropsychological functions such as reactivity and attention are, as cited, quite sensitive to environmental effects. The three levels of most active effects are as follows.

(1) Overall increased perinatal risks due to the effects of cocaine on maternal health during pregnancy and the general effects of cocaine on

fetal growth (apart from the specific effects on fetal brain development) contribute to an increased risk status for the infant and potentially result in an infant who is more difficult to care for.

(2) Continued maternal crack use after delivery puts the infant and child at risk for passive exposure. No studies of early development and behavior in prenatally cocaine-exposed infants have considered the possibility of acute effects of cocaine due to recent postnatal exposure and the effects of chronic passive exposure on postnatal brain development, particularly during the period of synaptogenesis and synaptic remodeling occurring in the first postnatal months (Goldman-Rakic 1987).

(3) As noted above, continued postnatal use potentially affects the child's caregiving environment at three levels. Adults who are under the influence of cocaine are less able to respond adequately to their children at any given time. The effects of cocaine on an adult's attentiveness, as well as the effects of alcohol or other drugs, impair (at least during acute intoxication) the adult's ability to care for the child. More generally, because of the lifestyle associated with cocaine use (e.g., prostitution, crime, exposure to violence, and the overwhelming power of the addiction), the overall environment for these children is often chaotic, violent, and neglectful. The psychological/personality factors that lead an adult to substance abuse (e.g., chronic affective disorder) (Rounsaville et al. 1982) may impinge on the adult's capacity to care adequately for the child. Additionally, some psychiatric disorders may also be associated with genetic risks for similar disorders in the child.

Importantly, none of these levels of prenatal or postnatal cocaine effects is more operative than another. If there is a specific effect of cocaine on fetal brain development, the exposed child may be more vulnerable to the effects of postnatal exposure and environmental discord and chaos. These types of interactive models emphasize why it is important to examine multiple aspects of the cocaine-exposed child's functioning and environment and to think in terms of cumulative effects. Some outcomes in part related to the prenatal exposure may only become behaviorally apparent months to years after birth as a result of cumulative effects of multiple environmental failures and stressors.

CONCLUSION

In summary, the following areas are either potentially fruitful or much needed lines of investigation as part of ongoing studies of prenatally cocaine-exposed preschool children:

(1) studies of reactivity, persistence, attentional regulation, and the stability of such capacities from infancy into the second and third year of life;

(2) studies of language and communication with attention to early communicative precursors;

(3) direct observations of parent-child interaction with emphasis on parental attention-directing and structuring activities; and

(4) studies of the effects of chronic exposure to violence on such functions as a capacity for empathy or for mediating aggression.

Closer studies of basic functions that mature over time but nevertheless underlie broader developmental competencies and the interaction of such functions with the parental environment will provide a more adequate profile of potential specific and nonspecific problem areas of cocaine-exposed children as they reach school age.

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ACKNOWLEDGMENT

This chapter was prepared with support from the National Institute on Drug Abuse grant no. DA-06025.

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Exposure to Opiates: Behavioral Outcomes in Preschool and School-Age Children

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HISTORICAL OVERVIEW

The purpose of this chapter is to review current literature on the developmental outcomes of preschool and school-aged children prenatally exposed to opiates, and in so doing to provide a sense of where the field has been and directions for the future. Clearly, this is a challenging task; the literature is relatively sparse and was primarily generated in the seventies and early eighties. The majority of data for children between 3 and 6 years of age are from the work of a few groups of investigators: Kaltenbach and Finnegan of Philadelphia, Rosen and Johnson of New York, Strauss and colleagues of Detroit, Hans and colleagues of Chicago, and Wilson and colleagues of Houston. This work consists primarily of longitudinal studies that began in the mid to late 1970s and culminated in the mid-1980s. At present there are no prospective data available for school-age children. While it may appear reasonable to assume that the paucity of recent literature reflects a decline in opiate use among pregnant women, the reality is that the incidence of prenatal opiate exposure continues to be significant. In recent years, however, the focus has been on investigating the effects of cocaine exposure, often to the exclusion of differentiating whether a woman uses opiates in addition to cocaine. A review of the literature on prenatal cocaine exposure yielded fewer than five studies that identified whether opiates were used in addition to cocaine. Such an approach to the delineation of drug exposure is not unique to current investigations, but reflects a typical lack of specificity that is evident throughout the literature on prenatal exposure to illicit drugs.

Although the occurrence of newborn drug withdrawal associated with maternal opiate dependency has been known for over a century, the reemergence of heroin use in the late 1950s and early 1960s among women of childbearing age led to concern regarding the effects of opiates on the developing fetus. The advent of methadone maintenance for pregnant opiate-dependent women in the early 1970s provided the

impetus for investigations to determine both short- and long-term neurobehavioral effects of prenatal opiate exposure. The impetus was twofold: there was concern regarding potential risks to the progeny of methadone-maintained women, and women in methadone treatment programs provided accessible study populations. It is important to note, however, that methadone exposure does not preclude exposure to other psychoactive agents. Research investigating prenatal opiate exposure includes exposure to heroin, methadone, or both, and may also include exposure to amphetamines, barbiturates, benzodiazepines, cocaine, alcohol, nicotine, and propoxyphenes. For example, a study by Rosen and Johnson (1988) included three groups of pregnant women: women on methadone maintenance, nonmethadone-maintained polysubstance abusers, and those who used no drugs. However, within the methadone maintenance group, 12 percent used only methadone.

Prenatal Opiate Exposure and Perinatal Outcome

In a review of outcome data for preschool and school-age children exposed to opiates in utero, it is necessary to consider relevant neonatal and infant outcomes. Studies that have compared infants born to heroin-dependent women not maintained on methadone with infants born to heroin-dependent women receiving methadone have found higher birthweights in infants born to methadone-maintained women (Connaughton et al. 1975, 1977; Kandall et al. 1976, 1977; Zelson 1973). Kandall and colleagues (1976) reported a significant relationship between the first trimester maternal methadone dose and birthweight. This study suggests that methadone may promote fetal growth in a dose-related fashion even after maternal heroin use, whereas heroin itself is associated with fetal growth retardation. Stimmel and colleagues (1982) analyzed the birth records of 239 infants born to narcotic-dependent women on supervised methadone maintenance, women on unsupervised methadone maintenance, women on street heroin, and women who were polydrug users. They found that perinatal outcome was significantly improved in those infants born to women on supervised methadone maintenance as compared with all other groups.

In general, studies that have compared methadone-exposed infants and nondrug-exposed infants have yielded consistent findings. Studies that compared methadone-exposed infants with nondrug-exposed infants found that methadone-exposed infants had lower birthweights than comparison infants. Remarkably, the mean birthweights for methadone-exposed infants across these studies were relatively the same, with a range of 2,830 to 2,882 grams (gms) (Chasnoff et al. 1982; Hans 1989;

Kaltenbach and Finnegan 1987; Lifshitz et al. 1983), although two studies reported somewhat higher birthweights. Strauss and colleagues (1975) found no difference in birthweight between methadone-exposed (X = 3,005 gm) and nondrug-exposed (X = 3,203 gm) infants. In a study by Rosen and Johnson (1982) in which methadone-exposed infants and a drug-free comparison group were matched for weight (±250 gm), the mean birthweight for methadone-exposed infants was over 3,100 gms.

Neonatal Abstinence

Infants born to heroin- or methadone-dependent mothers have a high incidence of neonatal abstinence. Neonatal abstinence is described as a generalized disorder characterized by signs and symptoms of central nervous system (CNS) hyperirritability; gastrointestinal dysfunction; respiratory distress; and vague autonomic symptoms that include yawning, sneezing, mottling, and fever. Neonates undergoing abstinence often suck frantically on their fists or thumbs, yet they may have extreme difficulty feeding because they have an uncoordinated and ineffectual sucking reflex. Infants who undergo abstinence generally develop tremors that are initially mild and occur only when the infant is disturbed, but which progress to the point where they occur spontaneously without stimulation. High-pitched crying, increased muscle tone, and irritability develop (Finnegan and Kaltenbach 1992).

With appropriate pharmacotherapy, neonatal abstinence can be satisfactorily treated without any untoward neonatal effects. It has been recommended that an abstinence scoring system be used to monitor the passively addicted neonate in a comprehensive and objective way to assess the onset, progression, and diminution of symptoms of abstinence (Finnegan 1986; Finnegan and Ehrlich 1990; Finnegan and Kaltenbach 1992). However, the initiation, type, and duration of pharmacotherapy for the treatment of neonatal abstinence varies across studies. In addition, the relationship between maternal methadone dose and severity of withdrawal symptoms has not been clearly established. Wilson and colleagues (1981) reported that although the incidence of severity of neonatal abstinence was similar for heroin- and methadone-exposed infants, neonatal abstinence was of longer duration in the methadone-exposed infants. Ostrea and colleagues (1976) and Madden and colleagues (1977) reported a significant relationship between severity of neonatal withdrawal and maternal methadone dose. Kaltenbach and colleagues (1990) examined maternal methadone dose during pregnancy and neonatal outcome and found no relationship between methadone dose and severity of withdrawal.

Few studies have examined the relationship between severity of withdrawal and developmental outcome. Kaltenbach and Finnegan (1986*a*) found no relationship between severity of withdrawal and developmental scores on the Bayley Scale of Infant Development. Similarly, Lifschitz and colleagues (1983) reported no relationship between severity of withdrawal and cognitive outcome at 3 years of age. It is important to note that in both these studies infants were objectively assessed for abstinence and received pharmacotherapy when indicated.

Infant Studies

There have been a number of studies investigating the neurobehavioral and developmental outcome of infants exposed to opiates in utero. Procedures used in infant followup studies are quite similar. Children are evaluated throughout infancy, typically at 6-month intervals, with the Bayley Scales of Infant Development (Bayley 1969). Children born to nondrug-dependent women from comparable socioeconomic and racial backgrounds are used as comparison groups.

Overall, most studies suggest that infants through 2 years of age function within the normal range of development. Strauss and colleagues (1976) found both methadone-exposed infants and comparison infants scored well within the normal range on the Bayley Mental Development Index (MDI) and the Motor Development Index (PDI) at 3, 6, and 12 months of age. PDI scores for the methadone-exposed infants, however, declined with age and differed from comparison infants at 12 months of age. Wilson and colleagues (1981) also found no difference in MDI scores at 9 months of age, but found lower PDI scores for the methadone-exposed infants. Although Rosen and Johnson (1982) found no difference between groups on MDI and PDI scores at 6 months of age, they found methadone-exposed infants to have lower MDI and PDI scores at 12 and 18 months of age. In a sample of 2-year-olds, Hans (1989) found no difference in MDI scores, but methadone-exposed infants had lower PDI scores and poorer gross and fine motor coordination as measured by the Bayley Infant Behavior Record (IBR). In comparison, Hans and Marcus (1983) reported no differences between groups in MDI or PDI scores at 4 and 12 months of age; Chasnoff and colleagues (1984) reported no difference in MDI or PDI scores at 3, 6, 12, and 24 months of age; and Kaltenbach and Finnegan (1986*b*) found no difference in MDI scores at 6, 12, and 24 months of age.

Such diverse findings often reflect the numerous confounding variables that exist within these studies. Mothers differed on amounts of daily methadone dose, length of methadone maintenance during pregnancy, type and quantity of polysubstance abuse, amount of prenatal care, and obstetrical complications. The use of pharmacological intervention for neonatal abstinence was not consistent with variations in initiation, type, and duration of treatment. The effect of the caretaking environment on children in substance-abusing families has also been identified as a critical factor that must be taken into account.

Additionally, some of the diverse findings may be explained by differences in the sample populations within studies. Although the infant studies are presented as longitudinal in design, the difficulties inherent in subject retention for this population often result in different sample compositions at different age points. The Rosen and Johnson (1982) study reported data gathered at 6, 12 and 18 months of age. Although all of the subjects were enrolled in the study at birth, data for the age points did not necessarily include repeated assessments of the same infants. Conversely, the length of time required to obtain longitudinal data may result in initial publications having smaller samples. The outcome data for 2-year-old infants reported in the Hans (1989) study was obtained from a larger sample (N = 74) that included infants (N = 39) from the Hans and Marcus study (1983) that reported on outcome at 4 and 12 months of age.

Studies of Preschool Children

The preceding discussion presents the context in which the review of the available preschool data should be considered. Wilson and colleagues (1979) reported differences between opiate-exposed children and three different comparison groups that included drug-naive subjects in drug-using households, a high-risk group, and a socioeconomic comparison group. This cross-sectional study assessed subjects between approximately 3 to 6 ½ years of age during a 5-month period. Maternal drug use was based on self-report, and hospital records were abstracted to obtain perinatal data.

The drug-exposed group (N = 22) was comprised primarily of infants born to women who used heroin continuously during their pregnancy, with one-third of the women reporting concomitant abuse of other drugs. The drug environment comparison group (N = 20) consisted of infants born to women involved in the drug culture, either through their partner or their own heroin use subsequent to the birth of the child, but who

reportedly used no drugs during their pregnancy. The high-risk comparison group (N = 15) consisted of nondrug-exposed infants who had medical risk factors similar to the heroin-exposed infants such as dysmaturity, intrauterine growth retardation, and fetal distress. The socioeconomic comparison group (N = 20) lived in the geographic area near the city hospital where they were born. Pregnancy and delivery were uneventful and mothers reported no history of drug abuse.

This study (Wilson et al. 1979) used an extensive battery of assessments, including the Illinois Test of Psycholinguistic Abilities (ITPA) (Kirk et al. 1968), the Columbia Mental Maturity Scale (Bergemeister et al. 1972), the McCarthy Scales of Children's Abilities (McCarthey 1972), and the Minnesota Child Development Inventory (Ireton and Thuirg 1974). Differences on the ITPA were found between the three risk groups and the socioeconomic comparison group but were within normal range. No difference between groups was found on the Columbia Mental Maturity Scale or Minnesota Child Development Inventory. However, the heroin-exposed children performed more poorly than the comparison groups on the general cognitive index (GCI) and on the perceptual, quantitative, and memory subscales. This study also included a videotaped 5-minute free play and structured doll-play situation in which no difference among groups was found in activity level or speech and language function.

Strauss and colleagues (1979) evaluated children from the original (Strauss et al. 1976) sample studied when the children were 5 years of age. These children were part of the original sample of newborns but were not necessarily in the sample assessed at 3, 6, and 12 months of age. Moreover, there had been no contact with the subjects for 4 years. Children in the drug-exposed group were born to methadone-treated heroin-addicted women participating in the Methadone Maintenance-Obstetric Care program; no other maternal drug use information was provided. Children were assessed with the McCarthy Scales of Children's Ability and a 15-minute videotape of behavior in the waiting room. No differences were found between methadone-exposed children (N = 33) and nondrug-exposed comparison children (N = 30) on the McCarthy GCI or any of the subscales. Scores for both groups were well below the standardization means (methadone-exposed children X = 87; comparison children X = 26). Videotapes of waiting room behavior were coded for children's playing and talking; interaction with caregiver and other children; wandering; and for mother's playing with child, positive/negative responses, initiation of interaction, and reprimands. No difference was found between the two groups of children or their mothers during the waiting room observation.

However, the drug-exposed children were found to be more active, energetic, and immature, and displayed more task-irrelevant activity during the structured testing situation, as measured by a modified version of the IBR.

Other studies include longitudinal investigations in which the sample was enrolled at birth and assessed repeatedly throughout development. All of the studies reflect significant subject attrition from their original samples. Lifschitz and colleagues (1985) evaluated 92 children between the ages of 3 to 6 drawn from a previous (Wilson et al. 1981) sample. The study sample was comprised of a methadone-exposed group (N = 26), a heroin-exposed group (N = 25), and a drug-free comparison group (N = 41). The methadone-exposed infants were born to women enrolled in a methadone maintenance treatment program, and the heroin-exposed infants were born to heroin-addicted women who were not in a treatment program. Maternal drug use history included program records of daily methadone dose and results of qualitative urine screenings. Cigarette smoking and alcohol consumption were also assessed. Almost all (95 percent) of the women receiving methadone continued to use illicit and/or prescription psychoactive drugs. Cognitive development during the preschool years was assessed with the McCarthy Scale of Children's Abilities. Physical and psychosocial characteristics of the child's home were measured by the Home Observation for Measurement of the Environment (HOME) inventory (Caldwell 1972). Performance on the McCarthy GCI was comparable for all three groups (methadone-exposed children X = 90; heroin-exposed children X = 85; comparison children X = 89). Variables identified as predictive for cognitive performance were amount of prenatal care, prenatal risk, and the HOME inventory; the degree of maternal opiate use was not a factor.

Kaltenbach and Finnegan (1989) evaluated 47 children (27 methadone-exposed and 17 nondrug-exposed comparison children) from their longitudinal sample at 4 ½ years of age. The mean daily maternal methadone dose during pregnancy was 38.42 milligrams (mg), and 72 percent of the children required pharmacotherapy for neonatal abstinence. No difference was found between groups on the McCarthy GCI or any of the subscales (methadone-exposed children X = 107, comparison children X = 106). These scores were higher than those reported in other studies, and are higher than one would expect for both groups considering that children from low socioeconomic backgrounds usually score lower than average on cognitive tests (Ramey et al. 1985). It may have been that mothers who continued to participate throughout the 5-year study were a self-selected sample of motivated mothers

especially interested in their children's development and/or their ongoing participation provided an informal intervention.

Rosen and Johnson (1985) evaluated their longitudinal sample at 3 years of age. They were able to assess 62 children from their original cohort of 94. The methadone-exposed children (N = 39) and comparison children (N = 23) were assessed at 36 months with a 30-minute videotaped free play and structured-task situation and with the Merrill-Palmer Scale of Mental Tests (Stutsman 1931). They found no difference between groups on the Merrill-Palmer scores and percentiles. The videotapes were used to assess spontaneous language production. No differences were found between groups but the mean length of utterances (MLU) for both groups were lower than those reported for middle-class samples.

In general, by the late 1970's to mid-1980's, investigators began to move from a bivariate approach to a recognition that research needed to address the multiple factors that may have a direct or indirect effect on the outcome of children born to substance-abusing mothers.

A review article by Kaltenbach and Finnegan (1984) recommended that outcome studies investigating the effects of prenatal drug exposure should also take into account maternal characteristics, maternal psychiatric morbidity, degrees of life stress, patterns and stability of child care, and mother-child interaction. The later work of Lifschitz and colleagues (1985) was able to show that the outcomes of drug-exposed children do not differ from a high-risk comparison group when sociodemographic, biological, and health factors that are frequently altered by the narcotic user's lifestyle are taken into account. In a study by Johnson and colleagues (1987), path analysis was used to determine the impact of multiple variables such as maternal medical history, drug abuse, neonatal outcome, and family functioning characteristics on developmental outcome. Neonatal complications and social disorganization were found to have direct effects on outcomes at 36 months. This study suggested that in assessing outcomes of children born to substance abusers, maternal drug use is not necessarily the most important factor; family characteristics and functioning play a significant role.

Future Directions

Before the potential of a multifactorial approach could be fully realized, research investigating the effects of prenatal opiate exposure basically ceased as concern shifted to maternal cocaine use. However, the data on

the effects of prenatal opiate exposure indicate, overall, that opiate-exposed infants through 2 years of age function well within the normal range of development and that children between 2 and 5 years of age do not differ in cognitive function from a high-risk population. Moreover, the data consistently suggest that psychosocial demographic factors may have as much, or more, effect on development as maternal opiate use. Clearly this finding has provided a foundation for future research. Although the drug of choice (and hence the focus of investigations) may change, the effects of maternal substance abuse must be examined within an interactive context of biological, psychosocial, and environmental factors in order to fully understand the etiology of developmental effects.

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Behavioral Outcomes in Preschool and School-Age Children Exposed Prenatally to Marijuana: A Review and Speculative Interpretation

Peter A. Fried

INTRODUCTION

In considering the relationship between marijuana use during pregnancy and the impact of such use upon the behavioral outcome of the young children of these pregnancies, the paucity of objective information is striking and, from one point of view, quite surprising. Marijuana is far from being the "new kid on the block," with references to its use in civilizations thousands of years ago (Abel 1980), and, in fact, has had a role in pregnancy folklore for many centuries.

The very limited number of contemporary scientific studies that focus upon marijuana's potential long-term effect on the developing fetus becomes a major concern when one considers the number of women of reproductive age who use this drug. In some cases, marijuana may be the only potentially teratogenic substance used; in other cases, it may be used with other legal, potentially teratogenic agents (e.g., alcohol and tobacco); while in further instances marijuana may be combined with other illegal substances that are under extensive investigation for their possible role in affecting the unborn child. As one example, the majority of the studies using samples in which the long-term consequences of in utero exposure to cocaine are being determined report that the use of that substance is highly correlated with marijuana use (Chasnoff et al. 1992; Frank et al. 1988). Although it is sometimes possible to control, to a certain extent, marijuana's impact by statistical means, knowing the role of cannabis upon the dependent variable in question is clearly of great importance in interpreting the nature of the contribution of other substances.

Although overshadowed in both the public media and scientific publications by the current concern with crack cocaine, marijuana remains the most commonly used illicit drug among women of childbearing age. In

the National Institute on Drug Abuse's (NIDA's) recently completed National Pregnancy and Health Survey (NIDA 1994), which provides national estimates of prevalence and patterns of substance use among women delivering live-born infants in the United States between October 1992 and August 1993, self-reported marijuana use during pregnancy was 2.9 percent compared with 1.1 percent cocaine (0.9 percent crack). In the past few years, marijuana use appeared to be increasing among women in their reproductive years. In NIDA's Monitoring the Future Study (Johnston et al. 1994*a*) 1993 data, 10.4 percent of 19- to 32-year-old women reported using marijuana in the past month. Further, among U.S. high school seniors, the annual use of marijuana increased between 1992 and 1993 from 21.9 percent to 26.0 percent (sexes not differentiated), reversing a previous declining trend seen since the early 1980s (Johnston et al. 1994*b*).

A number of studies have examined the extent of use of marijuana during pregnancy, but in many instances the prevalence rates may not be representative of marijuana use in the general population, as sampling procedures involved populations selectively biased towards drug use. On the basis of either interviews or urine screens conducted prenatally or postpartum, a rate of 27 percent was reported among a high-risk, predominantly nonwhite, Boston inner-city sample (Zuckerman et al. 1989). In another high-risk sample in Pittsburgh (Day and Richardson 1991), a random sampling of women from an outpatient prenatal clinic found a 30 percent rate. In a relatively low-risk sample at the Yale New Haven Hospital, the rate at any time during pregnancy was found to be 10 percent (Hatch and Bracken 1986) and among another low-risk population in the Seattle area, the rate was 17 percent (Streissguth et al. 1989). In a comparison between Florida public health clinics and private obstetrical offices, the rate based on urine screens was quite similar, with 12.4 percent in the former and 11.3 percent in the latter group (Chasnoff et al. 1990). In contrast, in Chicago (MacGregor et al. 1990), based on urine screens at the time of admission into the labor-and-delivery unit, a marked difference for marijuana rates was noted between clinic patients (32 percent) and private patients (7.5 percent).

In the author's work in Ottawa, Canada (described below), among predominantly middle-class volunteers (Fried et al. 1984, 1985) in the year before pregnancy, 80 percent did not use any marijuana, 12 percent used the drug irregularly, 3 percent smoked two to five joints per week, and 5 percent smoked more than that amount. After the recognition of pregnancy, usage declined significantly, although during each of the three

trimesters the percentages remained relatively constant. Approximately 6 percent reported irregular use, 1 percent reported smoking two to five joints per week, and 3 percent continued to smoke a greater amount. The heaviest users were the most likely to reestablish prepregnancy levels of consumption in the year following the birth of the baby.

In spite of the fact that marijuana is the illicit drug most used by pregnant women (see above), there is a notable lack of information about its long-term consequences. The major reasons for this state of affairs lie in the ethical and practical difficulties surrounding quasi-experimental research (Kilbey and Asghar 1992). Obviously, drugs cannot be administered to gravid women and so exact doses or amounts utilized and the timing of such use are not quantifiable. Further, potentially confounding factors (such as other drug use or socioeconomic factors) cannot be controlled by random assignment to groups. Human studies, particularly those investigating the long-term effects of in utero exposure, have to be based on volunteer samples and reports of drug use gathered either before (prospectively) or after (retrospectively) birth. These limitations are severe. Although a degree of control can be attained with statistical procedures, the interpretation and conclusions drawn from the research must be placed in the proper context.

Aside from one or two studies, all of the information pertaining to the behavioral effect of prenatal exposure to marijuana in children beyond the toddler stage is limited to the reports coming from the Ottawa Prenatal Prospective Study (OPPS) (Fried et al. 1980). The protocol and the limitations of this Canadian work are described below in some detail. Additional information can be found elsewhere (Fried et al. 1980).

THE OTTAWA PRENATAL PROSPECTIVE STUDY

As recently as 1980, the only information pertaining to the effect marijuana may have upon the pregnant user and her offspring was limited to two polydrug case reports. This lack of information, the results of animal work (reviewed in Dalterio and Fried 1992; Fried 1984), the extent of usage among women of reproductive age, and the cooperation of the teaching hospitals in the Ottawa area combined to set the climate and the opportunity for the inception of the OPPS in 1978.

Data have been and continue to be collected in a prospective fashion from approximately 700 women residing in the Ottawa, Canada, region.

Pregnant women volunteered after being informed of the study by a variety of means including via their physicians, by notices located in the waiting rooms of obstetricians, or by notices located in the reception rooms of prenatal clinics in the major Ottawa hospitals. The information that was disseminated at this juncture did not mention marijuana but rather discussed, in general terms, how lifestyle habits during pregnancy may influence the developing fetus. Upon contacting the research facility, the potential subject was given further details about the particular habits of interest—use of marijuana, alcohol, and cigarettes. It was emphasized that, for purposes of comparison, the researchers wished to recruit women who used any of these substances to a very small degree or not at all. After volunteering and signing an informed consent, the mother-to-be was interviewed once during each of the trimesters remaining in her pregnancy by a trained female interviewer.

This procedure of recruiting volunteers has both strengths and weaknesses that pervade the entire OPPS. The self-selection procedure limits the extent to which generalizations can be made in terms of epidemiological information collected, the possibility of selection bias being obvious. However, as noted elsewhere (Fried et al. 1980, 1984), on several key demographic variables including parity, age, and family income, the OPPS volunteer sample is quite similar to nonparticipating women living in the Ottawa area who give birth in the hospitals taking part in the study.

The recruitment procedure used has the advantage of increasing the likelihood of the reliability of self-report (elaborated below) and of increasing the probability of a long-term commitment to the study. Aside from subjects who have moved from the Ottawa area (about a third), a retention rate of over 95 percent has been maintained over the past decade.

During each of the prenatal interviews information was collected on such variables as socioeconomic status, mother's health (both current and before pregnancy), the health history of the father, obstetrical history of previous pregnancies, a 24-hour dietary recall (including an assessment of caffeine intake), as well as past and present drug use patterns. Detailed information is gathered with respect to marijuana, cigarettes, and alcohol use. To establish the use patterns of these three drugs, information was gathered both for the year preceding the pregnancy and for each trimester of the pregnancy. Further details of the interview and the categorization of the various drugs have been described previously (Fried et al. 1980).

There was an extensive range of marijuana use in the sample and the drug was not used by a similar proportion of subjects. As a result of these factors, for descriptive and statistical purposes, the marijuana use data were treated categorically. Volunteers were classified as nonusers, irregular users (one joint or less per week), moderate users (two to five joints per week), and heavy users (more than five joints per week).

The women who smoked marijuana regularly during their pregnancy differed from the nonusers and irregular users on a number of factors that have the potential of influencing offspring development. These factors were dealt with by various statistical procedures. These possible confounding factors included lower socioeconomic level, less formal education, and increased cigarette smoking. Although no difference in parity was noted, the heavy users were 3.2 years younger than the nonusers. There were no differences among the four groups in terms of nutritional adequacy and weight gain during pregnancy.

The self-report procedure used in the OPPS to assess drug habits raises the critical issues of validity and reliability. Despite the obvious shortcomings of this mode of assessing drug use, at the time of the collection of data (primarily between 1979 and 1983) no practical alternative was available. Today, laboratory tests can measure the presence of metabolites of marijuana up to 1 to 2 weeks after the time of use. The uses of both the interview and biological assessment approaches are critically discussed in a well-reasoned paper by Day and Richardson (1991).

In the OPPS, procedures were undertaken to enhance the likelihood of accurate data collection. A congenial relationship between the interviewer and the individual being interviewed in a comfortable environment (typically the mother's home) had been part of the protocol of the OPPS, and the same female interviewer followed the mother-to-be during her entire pregnancy. A second procedure designed to enhance the accuracy of the self-reports involved the number of times the same drug-related questions are asked. The questionnaire was administered once during each trimester; during each of these interviews, the questions pertaining to drug use during the preceding trimester and the 12 months before the pregnancy were repeated, permitting a test-retest reliability measure.

Neurobehavioral Observations

Although the focus of this chapter is on preschool children and beyond, it is relevant to highlight some of the observations (and lack of observations)

noted at earlier ages. The literature pertaining to the behavioral effects of prenatal marijuana exposure is relatively sparse and, although provocative, is far from definitive. The first report in 1980 examined 4-day-old babies born to 12 regular users in the OPPS (Fried 1980), and the findings were replicated in a subsequent, much larger study using the Ottawa sample (Fried and Makin 1987). Prenatal exposure to marijuana was associated with decreased rates of visual habituation and increased tremors, frequently accompanied by exaggerated startle responses that were both spontaneous and in response to minimal, external stimulation. Similar observations were noted at 9 and 30 days of age using the Prechtl neurologic assessment (Fried et al. 1987). Further, at 9 days, increased hand-to-mouth behavior was found among the babies born to the marijuana users.

These possible indicants of impairments in nervous system state regulation and/or mild withdrawal were noted by some others (Chasnoff 1990) but not by all (Richardson et al. 1989; Tennes et al. 1985). Other signs of alterations in nervous system integrity have also been associated with in utero marijuana exposure. Sleep cycling and motility in newborns differed between marijuana-exposed and nonexposed babies (Scher et al. 1988) and disturbed sleep patterns were still associated with prenatal exposure when the offspring were 3 years of age (Dahl et al. 1988). The observations of the OPPS sample in the newborn period are briefly described above as they were the only significant associations noted with prenatal marijuana exposure for a number of years as the children were followed.

When the children in the OPPS were examined at 1 year of age (Fried and Watkinson 1988) using the Bayley Scales (Bayley 1969), no adverse effects of prenatal marijuana exposure were noted. The Bayley Scales consist of three components. The Mental Developmental Index (MDI) assesses sensory perceptual abilities, early acquisition of object constancy, memory, problemsolving, vocalization, and the onset of words. The Psychomotor Developmental Index (PDI) assesses gross and fine motor movement. The Infant Behavior Record (IBR) evaluates the infant's attitudes, interests, and temperament. The failure to find a relationship between the infant's behavior and maternal marijuana use is consistent with other reports assessing the children at the same age (Astley and Little 1990; Tennes et al. 1985).

At 24 months, prenatal marijuana exposure was not negatively correlated with overall scores on the Bayley Scales (Fried and Watkinson 1988). Using the Reynell Developmental Language Scale (Reynell 1977), a

negative association with a measure of language comprehension, but not language expression (Fried and Watkinson 1988), was observed. This association did not persist after statistically adjusting for other variables, especially ratings of the home environment.

At 3 years of age, children in the Ottawa sample (Fried and Watkinson 1990) were administered the Reynell test of language expression and comprehension as well as the McCarthy Scales of Children's Abilities (McCarthy 1972). This latter instrument is based upon six scales: verbal, perceptual, quantitative, general cognitive (a composite of the three previous scales), memory, and motor. As found when the children were a year younger, after controlling for potentially confounding variables, prenatal marijuana exposure was not significantly associated with any of the outcome variables.

At 4 years of age the same sample was given the test battery that was administered a year earlier plus the Peabody Test of receptive vocabulary and a series of motor tests (Fried and Watkinson 1990). General, global intellectual measures were not related to prenatal cannabis exposure, congruent with the findings of another study in which marijuana was not the primary drug of interest (Streissguth et al. 1989). However, on tests of verbal ability (both the McCarthy subscale and the Peabody) and memory, the children of regular marijuana users were significantly inferior to other children. This relationship persisted after statistically controlling for a host of potentially confounding factors including the home environment. This negative relationship was the first reported association beyond the neonatal stage. The observation of a significant neurobehavioral effect at this age (and not earlier) may indicate that the degree and type of deficits noted can be identified only when normal neurological development has proceeded to a certain level of maturity and when complex behavior can be examined at a more specific, rather than global, level. This maturation hypothesis reflects the notion that the effects of prenatal exposure to marijuana are subtle and that their consequences on complex behavior are not manifested and/or cannot be tested before 4 years. This line of thinking is elaborated below.

The difficulty in unraveling the long-term consequences of in utero marijuana exposure becomes very apparent when one examines the data gleaned from the cognitive and language assessment of the 5- and 6-year-old OPPS participants (Fried et al. 1992). These children were given the same battery as when they were 4 but, unlike the findings at 48 months, statistical analysis found no relationship at either 5 or 6 years of age

between any of the subscales of the McCarthy or the Peabody tests and maternal marijuana use.

The reason for the disparity of observations is not at all clear. One possibility may be the increasing effect of environmental variables. As the children get older, they are exposed to an increasing similarity of postnatal influences that bear on cognitive development. For example, by 5 years of age, 89 percent of the nonexposed children and 87 percent of the exposed children had a year of formal schooling. Could it be that this common feature would tend to overwhelm some of the quite subtle differences in memory and verbal abilities noted at an earlier age?

Possible indirect evidence of the influence of ubiquitous, relevant environmental factors may be seen in the catching up scores of the marijuana-exposed children. The McCarthy verbal and memory scores at 4 and 5 years of age were essentially unchanged for the nonexposed children, being 1 to 1.5 standard deviations (SD) above age norms at both 4 and 5 years of age. On the same subscales, the marijuana-exposed children improved their scores by approximately half an SD between the ages of 4 and 5, to 1 SD above the age norm at 60 months. Thus, the postnatal influence of school may have served to overcome the marijuana-associated observations noted at 4 years.

Instruments that provide a general description of cognitive abilities may not be capable of identifying nuances in neurobehavior that may discriminate between the marijuana-exposed and nonexposed children. However, tests that examine specific characteristics that may underlie cognitive performance may be more appropriate and successful. This approach to assessing the consequences of prenatal marijuana exposure was examined in a recent study (Fried et al. 1992) in which impulse control and sustained attention were examined in 6 year olds.

The children were assessed using two forms of a computerized vigilance task with a one-button solid-state console (McClure and Gordon 1983). In order to examine the child's ability to withhold responding, a 6-second differential reinforcement of low rate responding (DRL) schedule was employed. Under this regimen, reinforcement (points displayed on a screen) would be obtained when a button press occurred 6 seconds after the emission of a previous response. Responses that occurred prior to the end of this 6-second period were not reinforced and served to reset the timer so that 6 seconds of no button pressing would have to elapse before the next button press would result in a reinforcement. Thus, on this DRL

6-second schedule, a child would receive reinforcement for every button press emitted after an interval of 6 seconds.

Three sets of data were obtained: the absolute number of responses, the total number of rewarded responses, and an efficiency ratio (ER) that was obtained by dividing the number of rewarded responses by the total number of responses.

The same apparatus was used to examine sustained attention. A series of single-digit numbers was shown on the screen at a rate of one per second. They were displayed for 200 milliseconds (ms) with an 800-ms interval between each signal. Each subject was asked to press a button whenever the target stimulus appeared on the display screen among a series of randomly presented numbers. The scores were the number of correct responses, the number of omissions (missed target stimuli), and the number of commissions (button press to nontarget stimuli). The scores were computed for each of three 3-minute blocks and then totaled for the overall 9-minute trial.

As an additional facet of this work, parents assessed their child's impulsivity/inattention at home by using portions of the Conners' Parent Rating Scale-48 (Conners 1989). This 48-item behavioral symptom checklist was completed by the child's mother at the time of testing using a four-point rating system. The scale yields six behavioral clusters, one of which—the Impulsive-Hyperactive Scale—was used for this assessment. The four items that enter into this scale include excitable/impulsive; restless or "squirmy"; wants to run things; and restless, always on the go.

The results suggested that prenatal marijuana exposure was not associated with poorer impulse control, as the children of the heavy marijuana users were not deficient in the delay task in either the number of rewards or the efficiency ratio. In the vigilance task, the commission errors were very similar among all three marijuana-exposed groups (again suggesting no impairment in impulse control), but the omission errors and the number correct were differentiated, in a dose-related fashion, among the children of the various marijuana-exposure groups. Further, across temporal epochs within the vigilance task, only the children in the heavy marijuana exposure category increased their omission errors. The overall increase in omission errors and the greater number towards the end of the vigilance task may reflect a deficit in sustained attention.

There was a significant tendency for the women who used marijuana heavily during pregnancy to rate their children as being more impulsive/ hyperactive. The nature of the scale emphasizes overall activity rather than attention behavior. Although consistent with the more objective measurements, there is a difficulty in interpreting these results. The fact that women in the heavy marijuana use group tended to identify their children as more problematic in this domain may be an accurate reflection of the child's behavior, or it may represent the mother's perception and attitude toward this behavior. Do the present observations indicate a true behavioral difference in the attention-related domain or is there a lowered parental tolerance? Ratings by other observers such as teachers and additional assessments of maternal parenting attitudes and expectations might help to clarify this issue.

In a recent preliminary report, O'Connell and Fried (1991) examined the school-aged (6 to 9 years of age) offspring of regular marijuana users and matched (in terms of alcohol and cigarette use during pregnancy) controls participating in the OPPS on a battery of neurobehavioral tests. These included assessment of intellectual abilities, visual perceptual skills, distractibility, memory, language comprehension, academic achievement, visual motor skills, and parental rating of behavior.

Measures that discriminated between the study groups and on which the children of the marijuana users scored more poorly included parental behavior ratings (particularly conduct problems), visual perceptual and visual memory tasks, language comprehension, and distractibility. It is striking that these are behaviors that have cropped up in work with these children at earlier ages. On the other hand, the data from this work are not without interpretative complications. For the measures of visual memory and language comprehension, the mother's age at the child's birth potentiated the effect of cannabis use to produce lowered scores for children of young, cannabis-using mothers relative to children of young, nonusing mothers. Further, when controlling for the influence of the mother's age at delivery, mother's self-rated personality (the marijuana-using cohort being higher on neuroticism and lower on agreeableness and conscientiousness), and the home environment (greater aggression and less supervision were present in the marijuana-using homes), the discriminating variables were no longer statistically significant.

Whether the inclusion of the personality and home environment variables as statistical controls is appropriate is a difficult issue that has been discussed elsewhere (Fried and Watkinson 1988; O'Connell and Fried

1991), and also is considered below. Briefly, the important question is whether this inclusion results in a conservative approach to the data analysis. The finding of differing personality and home environment ratings between the users and nonusers of marijuana may well be viewed in a transactional framework (Sameroff and Chandler 1975). This model states that the developmental outcomes are the product of both maternal and child characteristics and the relationship between the mother and child characteristics is a reciprocal one. Thus home environment measures and personality characteristics may be outcomes in themselves, arising from interactions with a behaviorally altered child.

Interpretative Issues

It is quite apparent that the data available to date make it very difficult to come to any definitive conclusion about the long-term implications of marijuana use during pregnancy. This difficulty arises for a number of reasons—some of which are generic to virtually all longitudinal, prospective teratogenic studies and others that are particular issues with marijuana. It is appropriate to include a brief discussion of these interpretative caveats in this chapter.

At a general level, separating the in utero effects from postnatal effects becomes more and more problematic as the child gets older. As discussed in detail elsewhere (Fried 1993; Kilbey and Asghar 1992) consequences of drug exposure noted in the offspring may be caused not only by the drug in question, but also by the lifestyle and parent-child interaction that often are related to a particular drug habit. Attempting to parcel out the statistically unique contribution of a drug, after controlling for so-called confounding factors, may obscure the reality of the drug effect(s). If there are effects of marijuana use, clearly they are very subtle. As discussed above, there is the real potential for a transactional state of affairs; thus this possible over-control becomes even more of an interpretative issue.

In other publications (e.g., Fried and Watkinson 1988) arising from the OPPS, it has been argued that it is more likely that the drug's real association with the behavioral outcomes in question may lie between the drug's unique contribution (after potential confounds are considered) and its zero-order correlation (with no potential confounds considered). In the latter approach, variance attributable to drugs may be as high as 12 percent, whereas, as stated earlier, the unique contribution is often in the region of 1 or 2 percent. The likely contribution or influence of the drug may well fall between these two figures.

Related to the above discussion is the fact that the amount of outcome variability in question that may be attributed to almost any prenatally used drug is relatively small compared with other factors and diminishes as the child gets older. In the author's work, spanning more than a decade, nondrug lifestyle habits account for up to 35 percent of the cognitive outcome variability (Fried and Watkinson 1988), but the behavioral effects uniquely associated with maternal drug use (tobacco or alcohol or marijuana) range only from 1.5 to 8 percent after the variance due to other potentially confounding factors is parceled out. In other laboratories with higher risk samples, the figure is frequently less. This low proportion of unique, explained variance should not be interpreted as indicating that maternal drug use is of little significance. Not only are there real, measurable effects as described above, drug use is also one of the few variables that can realistically be modified—more so than other lifestyle factors such as socioeconomic status that impinge on the mother and child. Furthermore, rarely does a drug act in isolation or in a statistically unique fashion. It interacts with a host of factors including other drugs and other environmental and genetic risk factors.

However, the small proportion of unique variance attributable to maternal drug use does lead to a variety of interpretative problems and emphasizes the importance of longitudinal investigations in which suspected drug effects from maternal drug usage can be examined across many ages. If one notes effects in the very young infant along particular dimensions of behavior and continues to see effects in related spheres as the offspring gets older, more confidence can exist in attributing some of the findings to the in utero exposure.

Two additional points have to be kept in mind in interpreting the findings with respect to prenatal marijuana exposure described in this chapter. The women in the Ottawa work represent a very low-risk sample. There is a considerable body of literature (animal and human) to suggest that the drug's effect is potentiated in a higher risk environment (reviewed in Fried 1993) and thus one must be very cautious in extrapolating the present observations to other marijuana-using populations. There is also the concern that the potency of marijuana preparations, in terms of tetrahydrocannabinol content, has increased several fold (Elsohly and Elsohly 1989) since the entrance of pregnant women into the Ottawa study in the late 1970s and early 1980s. This increase in drug potency heightens the importance of interpreting the present results as representing conservative observations.

ATTEMPT AT A SYNTHESIS

What can one conclude from the material described up to this point, bearing in mind the interpretative issues just raised? On the surface it appears that the only definitive statement would be that, if there are long-term consequences of prenatal exposure to marijuana, such effects are very subtle. However, the data may allow conclusions that go somewhat beyond this level.

The marijuana findings may be summarized in the following manner. In the newborn and neonate, although far from definitive, there appears to be an association between nervous system state regulation and prenatal exposure to marijuana. However, between 6 months and 3 years of age no neurobehavioral consequences of marijuana have been reported in the OPPS children, although, at 2 years of age, language comprehension was lower among the children of cannabis users prior to statistical control for the home environment. At 4 years, tests of verbal ability and memory statistically discriminated between the offspring of regular marijuana users and the remainder of the children in the OPPS sample. At 5 and 6 years of age, prenatal marijuana exposure was not associated with global tests of cognition and language after statistically controlling for potentially confounding data. However, at approximately these ages and slightly older, tests that examined more specific aspects of behavior did appear to suggest a relationship between performance and in utero exposure to marijuana. In school-aged children, a deficit in sustained attention was noted on a task that differentiated between impulsivity and vigilance. Further, parental ratings of behavior indicated greater problems (particularly in the area of inattention and conduct) among the children of cannabis users. Finally, visual perception, visual memory, language comprehension, and distractibility discriminated between the 6- to 9-year-old offspring of marijuana users and nonusers. The latter findings did not remain statistically significant upon the inclusion of maternal personality and home environment conditions as potential confounds, although this statistical control (as discussed above) may be inappropriate.

Two issues that arise from these data are the seeming absence of prenatal cognitive effects of marijuana at 4 years of age and the question of whether there is any common theme among the effects and trends noted at 4 years and beyond.

Dealing with the latter issue first, the areas of vulnerability that have emerged over the course of the OPPS are quite consistent with the cognitive construct that several authors have termed "executive function"

(Duncan 1986; Luria 1966; Welsh and Pennington 1988). This function is defined as the ability to maintain an appropriate problemsolving set for attainment of a future goal and involves the integration of cognitive processes. The executive function behaviors noted to be negatively associated with prenatal marijuana exposure include those that involve self-regulatory abilities (the dysfunction possibly manifesting itself in the form of behavioral problems), the ability to maintain attention (noted as impairments in vigilance and distractibility), and the ability to act on accumulated knowledge (poorer performance on facets of language and memory).

Executive function is thought to serve as a marker of prefrontal lobe function, and thus this part of the central nervous system (CNS) may be particularly vulnerable to prenatal marijuana exposure. Frontal lobe development is not an all-or-none phenomenon but appears to be a multistage process, as is executive functioning (Welsh and Pennington 1988). Although aspects of executive functioning are present in infants and toddlers (e.g., object permanence behavior), certain aspects of prefrontal functioning are not apparent or are difficult to test (e.g., self-control, strategies to enhance problemsolving such as the generation and maintenance of goal-oriented sets involving memory and self-monitoring) until children approach or reach school age. This would certainly be very congruent with the results reported in this chapter.

An important further property of executive functioning is that it is disassociated from measures of global intelligence. This is consistent with the observation of the sparing of intelligence quotient (IQ) after frontal lobe damage (Damasio 1979) and may reflect the fact that traditional, global intelligence tests evaluate overlearned information and established cognitive sets. One of the consistent findings noted among the children in the OPPS was that prenatal marijuana exposure was not associated with a lowering of general IQ.

Recent observations from diverse fields within the marijuana research literature also implicate the frontal lobes in that drug's effects. The discovery of receptors for cannabinoid substances in the mammalian brain (including humans) provides very convincing evidence for the possibility of direct action of marijuana on mental processes (e.g., Herkenham et al. 1990, 1991; Matsuda et al. 1990). In long-term, chronic adult users, that action includes fragmentation of thought; difficulty in short-term memory tasks; and disturbances in attention, concentration, and judgment—tasks that are associated with frontal lobe functioning. In the rat, within different regions of the cortex, the frontal area has been reported to contain the

highest density of binding sites (Herkenham et al. 1991). In nine chronic adult marijuana users, Tunving and colleagues (1986) reported reduced blood flow throughout the cerebral cortex. Intriguingly, only two of the users were not polydrug users and in these cases the prefrontal area was the most affected (Lundqvist, personal communication, July 1993). Finally, Struve and coworkers (1989, 1993) have recently reported that chronic, daily use of marijuana results in a marked alteration in alpha activity, primarily in the frontal region, even after prolonged cessation of use.

Together, then, a suggestive (although at this stage, highly speculative) picture is beginning to emerge. The behavioral evidence gathered primarily from the children participating in the OPPS over the past years, the temporal sequence of the observed effects, and the recent findings linking altered frontal lobe functioning with chronic marijuana exposure are certainly compatible with the notion that prenatal marijuana exposure may result in altered frontal lobe functioning in the offspring. One of the next steps in this research is to examine the children in the OPPS in tasks that are thought to be particularly sensitive to frontal lobe dysfunction. These include tests of problemsolving that require cognitive flexibility, route finding tasks, measures of distractibility and attention, and working memory. These assessments are presently underway.

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ACKNOWLEDGMENTS

The OPPS has been supported in the past by grants from the National Research Council of Canada and National Health and Welfare, Canada. In recent years and presently the work is supported by National Institute on Drug Abuse grant DA-04874. The thoughful, insightful, and stimulating comments pertaining to frontal lobe functioning and clinical observations of chronic marijuana users by Drs. Dan Ericsson and Thomas Lundqvist of Lund, Sweden, at the International Cannabis Research Society meeting in June, 1993 in Toronto, Canada, are gratefully acknowledged. The work described could not have been carried out without the continuing and ongoing assistance of long-time associates over the years, including B. Watkinson, H. Linttell, and R. Gray, and the enduring patience of the mothers and their children during the past decade.

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Prenatal Drug Exposure: Behavioral Functioning in Late Childhood and Adolescence

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Following widespread publicity about cocaine babies (Kantrowitz 1990; Toufexis 1991), the American public has shown considerable concern about the potential long-term consequences to children's development from prenatal exposure to drugs of abuse. Many communities have gone so far as to consider or implement special school-based programs for children with histories of prenatal drug exposure. Yet very little is known about long-term development in children of drug-using parents and even less about what specific aspects of development, if any, are linked to prenatal exposure. Longitudinal followup of children with documented histories of prenatal drug exposure requires tremendous commitment from investigators and funders (Hans 1991), and few research studies have followed children past infancy and early childhood.

This chapter reviews the current state of research knowledge about the long-term development of children prenatally exposed to drugs of abuse. Because so little is yet known about this topic, the author discusses some general themes in the study of human development that need to be integrated into future research on older drug-exposed children.

REVIEW OF LITERATURE ON PRENATALLY EXPOSED CHILDREN IN LATE CHILDHOOD AND ADOLESCENCE

Most of what is known about the late childhood and adolescent offspring of drug-using parents comes from several older studies with cross-sectional research designs. The largest such study is the work of Sowder and Burt (1980) with children of heroin addicts. Through east coast drug treatment programs, the investigators recruited a sample of children and adolescents whose mothers or fathers were former or current heroin addicts. Altogether, the sample included 126 offspring of heroin addicts between the ages of 8 and 17 years. Two-thirds of the group were African American, while the remaining subjects were of varying ethnicity. The investigators also recruited a comparison group of children from the same neighborhoods whose parents had no history of heroin use or abuse.

This was done through contacting families randomly selected from telephone directories and inquiring if the household had a child in the appropriate age range. Data for both index and comparison groups were collected from a variety of sources including interviews with the parents, interviews with the young people, school reports, and reports from a variety of community social service and law enforcement agencies.

The investigators compared children of drug-addicted and nondrug-using parents on dozens of variables, and concluded that the offspring of heroin addicts were at greater risk for a variety of problems. The index children were more likely to have missed school for reasons other than illness, and index families were more likely to have been contacted by their children's schools for absences. Eighty-five percent of index children had missed six or more days of school during the previous year, compared with 55 percent of comparison children. Teachers were more likely to have reported that index children were disobedient, did not work well in groups, were destructive of school property, and repeated grades. Index children were more likely to report having received counseling for fighting and receiving tutoring or special education. Eighteen percent of children in both groups reported having been in trouble with police, but children in the index group had a greater number of contacts with the police and more serious offenses.

Sowder and Burt did ask parents about whether the children had been exposed to drugs in utero—asking separately about heroin and other drugs, although not about alcohol. Parents reported that 44 percent of the children had been prenatally drug exposed. Comparing exposed and unexposed children on a set of outcome variable factors, the investigators found little relationship between prenatal exposure and outcome. Altogether, the Sowder and Burt data suggest that children and adolescents with drug-addicted parents are young people at risk. However, their retrospective and probably unreliable (Day and Robles 1989) measures of prenatal exposure provided no support for the hypothesis that such risk is related to the teratological effects of heroin.

That nonteratological factors might be major contributors to the behavior problems of children with drug-using parents is made even more plausible by other reports. Herjanic and colleagues (1979) studied 32 school-age and adolescent children whose fathers, but not mothers, were opiate addicts. These children, who were raised in a home with a heroin user but who were not prenatally exposed to a drug, showed outcomes very similar to those of the Sowder and Burt study, including both academic

problems and antisocial behavior. Problems were more pronounced in the adolescents than the school-age children.

Wilson (1989) was the first to report a long-term followup of a sample of children with documented prenatal drug-exposure histories. Wilson conducted a partial followup of an earlier Houston sample of infants who had been prenatally exposed to opiates. This sample included fairly equal representations of African-American, Hispanic, and English-speaking white children. The followup study was conducted with limited resources, and the sample included only those families who could be reached by mail or telephone at previously identified residences. The followup sample included 68 percent of the original heroin-exposed children, 36 percent of the methadone-exposed children, and 36 percent of the unexposed control children. The total sample contained 32 prenatally exposed children and 12 controls between the ages of 6 and 11 years. Parent interviews and school reports formed the basis of the data. Wilson found a high prevalence of academic problems in both groups; one-quarter of the drug-exposed and control groups had repeated one or more grades and had required special educational services. Maternal opiate use was related to behavioral problems: 75 percent of the index cases and only 50 percent of the comparison cases were identified by parents or school as having behavior problems. Psychiatric referrals had been made for 7 index children (22 percent) and only 1 control child (8 percent).

The author recently completed a 10-year followup of a longitudinal sample of methadone-exposed children and a comparison group of unexposed children identified prenatally (for reports on this sample during infancy, see Hans 1989; Jeremy and Hans 1985). All children were African Americans living in very low income families. Over 90 percent of the original methadone-exposed sample were found and assessed (excluding children who died during the first year of life), as well as over 75 percent of the original comparison sample. Altogether, this school-age sample included 36 drug-exposed children and a slightly larger number of comparison children.

Extensive information on children's behavior problems was collected using multiple instruments and multiple informants. The parents completed the Achenbach Child Behavior Checklist (Achenbach 1991*a*), and teachers completed the Achenbach Teacher Report Form (Achenbach 1991*b*). On this instrument, teachers rated approximately half the children in both groups as having "poor" academic performance. Nationally, teachers use the "poor" rating for only 7 percent of children. The groups were also

comparable in terms of the rates of retention in grade and placement in special education. Based on data from the Teacher Report Form, there was a trend for the drug-exposed children to have more externalizing problems; fully half of the drug-exposed children had externalizing scores more than one standard deviation above scale norms, although their scores were not statistically different from those of comparison children. There were no parent-reported differences on any of the major Achenbach subscales.

The author administered a semistructured psychiatric interview that involved reports from children and their caregivers—the Diagnostic Interview for Children and Adolescents (DICA) (Welner et al. 1987). Guidelines in the "Diagnostic and Statistical Manual of Mental Disorders," 3d. ed. rev. (DSM-III-R) (American Psychiatric Association 1987) were used for diagnoses of the children based on DICA interviews data supplemented by information from the Achenbach checklists. These diagnoses were made by a psychiatric social worker who was blind to information about parents' history of drug abuse. High proportions of the children from both groups met the criteria for at least one diagnosis: 47 percent of the drug-exposed children and 37 percent of the comparison group. Diagnoses of the drug-exposed children included (in order of prevalence): attention deficit-hyperactivity disorder (ADHD), disruptive disorders (conduct and oppositional), functional enuresis, disorders of the affective spectrum, and separation anxiety. Compared with the control group, drug-exposed children were somewhat more likely to receive ADHD and disruptive behavior diagnoses but were not at higher risk for affective disorders.

Altogether these new data replicate other cross-sectional and longitudinal reports by indicating a trend for more conduct and attentional problems in children with drug-using parents. A high incidence of academic problems was observed in these children, but as in Wilson's prospective study, no differences were found between prenatally exposed and comparison children.

Ratings of symptoms are not ideal outcome measures for exploring the potential effects of prenatal exposure on child development. Behavior problems are complex and multiply determined patterns of behaviors. To detect prenatal exposure effects, one needs to look at simpler, more basic behaviors—preferably those with known links to brain damage. The author's longitudinal study included a set of neurobehavioral measures in the assessment battery. These measures included a continuous performance test (CPT) (Rosvold et al. 1956)—a computer-administered measure of

sustained attention. In this task children are required to watch numbers flash tachistoscopically on a computer screen for prolonged periods, pushing a button whenever a target number appears. Outcome indices for this test have a known relationship with anoxic events at birth, even in samples of children from low-risk environments (O'Dougherty et al. 1984).

On the CPT, opiate-exposed children in the author's sample had significantly lower hit rates and higher false alarm rates than unexposed children. Low hit rates reflect deficits in basic attentional processes, while high false alarm rates reflect impulsivity. Although all children performing poorly on the CPT also received ratings of poor attention from their teachers, many other children received such teacher ratings. The CPT task seemed to be more specific to exposure history than clinical ratings of attentional problems. Further analyses are being conducted to determine whether opiate effects on attention might be mediated by environmental variables, history of perinatal problems, parental neuropsychological deficits, or other types of child behavior problems.

To summarize the knowledge of the effects of prenatal drug exposure during late childhood and adolescence, it seems that prenatally drug-exposed children are definitely at high risk for behavioral and academic problems, although possibly not any more so than sociodemographically matched controls. Since behavioral and academic problems are present even in children with drug-using parents who were not prenatally exposed, it seems unlikely that the primary source of these problems is directly related to the prenatal exposure. Nevertheless, early evidence suggests that there could be information-processing deficits observable at school age in some prenatally exposed children that might contribute to difficulties in school.

As researchers work to expand this base of knowledge on the associations between prenatal drug exposure and behavior in later childhood and adolescence, they face enormous methodological and technical challenges. These challenges are related to two of the major conceptual themes that have occupied all thinkers about human development: the nature of change and continuity across the lifespan, and the interplay between biology and experience (Kagan 1984; Lerner 1986). The remainder of this chapter elaborates on how these themes are played out in research on the long-term development of children prenatally exposed to drugs of abuse.

CHANGE AND CONTINUITY ACROSS THE LIFESPAN

Most work in human behavioral teratology has focused on behavior during the first months after birth. This age period the most convenient from a logistical point of view; furthermore, scientists feel more comfortable interpreting connections between prenatal events and later behavior as having a causal basis when less time has elapsed. Yet present knowledge of early problems or the lack thereof in prenatally exposed children is insufficient to permit informed statements relevant to older children. Scientists studying potential behavioral teratogens are forced to face the fact that individuals grow and develop throughout the lifespan. Different competencies are demanded of individuals at different ages. Individuals are placed in new social environments at different ages, behavior and thought are organized in qualitatively different patterns at different ages, and one would expect the effects of a teratogen to be different at different ages. The brain with its tremendous plasticity can show remarkable recovery from early insults, but new problems can emerge with development, and old problems can take different forms throughout the lifespan.

As an example of how behavioral disorders can change over the lifespan, consider the literature on individuals with ADHD. Research in behavioral teratology suggests this syndrome may be relatively more common in children prenatally exposed to drugs (Hans 1992). ADHD often goes undetected during infancy, may be first noticed during early childhood, but is most readily identified during the school years. Classic ADHD symptoms are inappropriate degrees of inattention, impulsivity, and early activity. Yet the symptoms of this disorder may change as children enter adolescence and adulthood. While inattention, impulsivity, and restlessness are likely to continue, in many cases the most salient problems of ADHD children as adolescents become antisocial behavior—defiance, aggression, temper outbursts—and sometimes substance abuse (Barkley et al. 1991; Mannuzza et al. 1991). It is assumed that much of the change in symptom expression is related to the increasing ability of individuals to control their own environment—to remove themselves from situations such as classrooms in which prolonged quiet attention are required—and to the increasing self-awareness some ADHD children may develop at adolescence of the ways they differ from other individuals and may be limited by their behavioral characteristics.

Closer to the issue of prenatal drug exposure is recent work on the development over time of children with fetal alcohol syndrome (FAS) (Streissguth et al. 1991). These researchers reported that the predominant

physical and behavioral symptoms of FAS may change at puberty. After puberty, the faces of patients with FAS or fetal alcohol effects (FAE) are not as distinctive. Patients remain short and microcephalic, although their weight becomes somewhat closer to average. Streissguth also suggests that FAS patients have increasing behavior problems with age. Young FAS children, although intellectually handicapped, are often extremely good natured. In adolescence, attention and judgment problems remain serious, but conduct problems (e.g., lying and defiance) that had not been present at younger ages begin to emerge. Streissguth suggests that it is impossible to determine the primary origins of the behavioral problems: they could be direct results of poor environments, reflect a potentially greater vulnerability of biologically impaired children to such poor environments, or they could be the result of difficult-to-rear children eliciting nonoptimal responses from their caretakers.

Not only is it important to understand that a teratogen might alter behavior in a different manner at different ages, it is important to understand that behavioral problems can emerge at older ages that were undetectable during infancy and early childhood. The opiate drugs studied by the author for over a decade are an example of a category of drugs that appear from the literature to have little effect on child development after neonatal withdrawal. Several studies have shown small or no differences between the behavior of exposed and unexposed infants and preschool children (Hans 1992; Kaltenbach, this volume). The author's own findings of attention deficits in 10-year-old opiate-exposed children provide evidence of how effects can be discovered at a later age in a sample of children whose early development appeared normal when assessed on standardized tests. Fried's work on prenatal exposure to marijuana (this volume) is a further example of a study in which prenatal exposure effects during infancy were minimal, but became increasingly clear with age.

It is not unheard of for behavioral abnormalities whose roots may be present early in life to be undetected until later in human development. So-called sleeper effects are implicit in a number of types of brain-based developmental disorders. For example, schizophrenia is a disorder whose symptoms often emerge suddenly and dramatically in adolescence or adulthood. Yet schizophrenia is now generally believed to be a neurodevelopmental disorder involving abnormalities in brain development that are present early in life, but that are not reflected in symptoms of psychopathology until much later (Fish et al. 1992; Marcus et al. 1993). Dyslexia is another disorder that cannot be identified early in life—it is

not detected until children learn to read—yet it is believed to have roots in early brain development (Duane and Gray 1991; Galaburda 1993).

What are the reasons that behavioral disorders whose roots can be traced back to genetics or prenatal events are only first observable at later ages? Are there any reasons one might expect to see drug-related findings only at later ages?

Late-to-emerge effects might involve normally late-maturing competencies or aspects of behavior linked to late-maturing parts of the brain. Considerable neurocognitive development occurs during human adolescence (Flavell 1985). Human thought processes increase dramatically in complexity and abstractness, and the capacity for metacognitive processing (i.e., the capacity to think about one's own thought processes and to use that awareness to enhance thought), emerges. These changes, although not well documented, certainly involve underlying changes in brain organization. The example of schizophrenia may be relevant here also. One explanation for the emergence of the disorder later in life is that abnormalities in the prefrontal cortex are core to schizophrenia (Weinberger et al. 1991). The prefrontal cortex, involved in the highest order cognitive processes, is very late to develop pathways to other parts of the brain (Spreen et al. 1984). Thus a late-to-emerge disorder such as schizophrenia may involve late-to-mature regions of the brain. Similarly, prenatal substance exposure effects that impact normally late maturing parts of the brain might not be observable early in life. Fried (this volume) argues that deficits in executive functions related to prefrontal lobe maturation similarly might not be observable in drug-exposed children until relatively late in development.

Late-to-emerge effects might also involve behavior whose emergence is triggered by hormonal changes at puberty. The most obvious example of this would be sexual behavior. Numerous animal studies suggest alterations in sexual behavior related to exposure to drugs and alcohol (McGivern, this volume; Segarra and McEwen 1992). Additionally, two studies of methadone-exposed children (Sandberg et al. 1990; Ward et al. 1989) suggest alterations in normal sexual identity related to drug exposure, particularly in male children.

Late-to-emerge effects might also involve vulnerabilities that cannot be observed until the child enters the social milieu of adolescence. Perhaps the strongest candidate for such effects would be drug-use behavior. Spear (this volume) reports animal studies suggesting that prenatal

exposure may alter the adult animal's sensitivity to the effects of a drug. While it is possible that prenatal substance exposure creates a biological vulnerability to addiction in humans, such a vulnerability would normally be impossible to detect until the child matures to an age when the social environment provides opportunities for the use of drugs. Such opportunities rarely occur until adolescence or sometimes even adulthood.

To date, the published literature on the familial transmission of substance abuse has been impressively blind to the possibility that prenatal exposure might alter vulnerability to later drug use. Family studies investigating the origins of drug abuse are not as common as those studying familial patterns of alcoholism. Those that exist, such as the work of Rounsaville and colleagues (1991) with opiate addicts, have suggested that, compared with relatives of normal subjects, the relatives of opiate addicts have considerably higher rates of substance abuse. Cadoret and colleagues (1986) conducted the only adoption research focusing specifically on transmission of drug abuse. They found that substance abuse (primarily alcohol abuse) by biological parents was associated with drug abuse in offspring. These family studies, however, have not investigated the role of prenatal exposure in familial transmission.

Numerous studies of drug use in adolescents—studies sampling general populations of teenagers and studies sampling high-risk youth—have suggested that young people who initiate drug use in high school are more likely to have substance-abusing parents. With a few exceptions, the issue of prenatal exposure to substances has largely been overlooked in these studies as well. For example, Brook and colleagues (1989), who have produced much interesting information on the antecedents of adolescent drug involvement, examined the role of prenatal and perinatal factors in subsequent drug use, but failed to separate out the potential effects of prenatal substance exposure other than exposure to prescribed medications. Others (Gilchrist et al. 1990; Lohr et al. 1992) studying the drug-use patterns of pregnant adolescents examined the impact mothers' drug use had on the drug use of the adolescent, but did not try to take into account whether mothers of pregnant adolescents had been using drugs when they themselves were pregnant.

The only published human studies relevant to the issue of whether prenatal exposure creates a vulnerability to addiction in later life do not concern maternal drug abuse, but rather use of analgesic drugs during labor and delivery. In their research, Swedish investigators (Jacobson et al. 1988, 1990) recruited a sample of several hundred drug-addicted

adults and their nonaddicted siblings. These individuals were identified through drug treatment programs, criminal records, and autopsy records. The researchers used the extensive Scandinavian medical databases to retrieve hospital records from the time of the subjects' births. After controlling for a variety of potentially confounding variables, they found a dose-response relationship between nitrous oxide administered during labor and the offspring's later amphetamine addiction. They also found a dose-response relationship between morphine administered during labor and later opiate addiction in offspring. These investigators argue that these effects may be related to some kind of pharmacological imprinting mechanism involving a specific drug, but acknowledge that other factors such as prolonged perinatal hypoxia could also be involved. These study data are provocative, but remain to be replicated.

A final issue regarding change and continuity across the lifespan is understanding the stability or lack thereof of the behavior of individuals across different developmental periods. In the field of human behavioral teratology, it is also important not only to document the correlates of prenatal substance exposure at a variety of different ages across the lifespan, but also to take advantage of the strengths of the longitudinal method to document whether the same individuals are experiencing problems at different ages. Future research could pose questions such as whether the infants who have difficulties with state regulation are the same children who have attention difficulties at school age and conduct problems as adolescents. It is actually quite remarkable that, given the large number of ongoing longitudinal studies of drug-exposed children, virtually none has taken advantage of the longitudinal design to analyze patterns of behavior continuity or discontinuity over time (an exception is the work of Johnson et al. 1987).

BIOLOGY AND EXPERIENCE

Part of researchers' discomfort with long-term followup of prenatally exposed children lies with the legitimate concern that the effects of environment on child development are important and become increasingly greater over time.

In the study of prenatally exposed children, researchers have historically gone through transformations in attitudes toward children's environments. The earliest studies of drug-exposed children tried to easily dispense with the issue of environment. They sampled unexposed

comparison children of comparable socioeconomic background to the drug-exposed children and then gave no further attention to the issue of environment. As researchers became more sophisticated, they measured environmental factors and looked for exposure effects that remained significant after statistically controlling for environmental variables confounded with exposure.

Perhaps the field is on the verge of another transition: no longer viewing the environment as a nuisance variable to be ignored or controlled for. It is time that studies of prenatal exposure began to examine issues such as under what environmental conditions exposure variables are related to behavioral problems. Vorhees (1986), in stating principles of behavioral teratology, identified this as the principle of environmental determination: "The type and magnitude of a behavioral teratogenic effect depend on the environmental influences on the organism, including both prenatal and postnatal environmental factors" (Vorhees 1986, p. 36).

At present, researchers know little about how this principle operates within human populations. Some have argued that exposure to substances can only be correlated with behavior in samples of children drawn from relatively low-risk environments; the baseline rate of problems in children from high-risk environments is simply so high that it obscures exposure effects. Fried's work (this volume) in middle-class children with prenatal marijuana exposure may be an example of how effects are more clearly identified in samples drawn from relatively low-risk environments. An opposite argument has also been made that drug exposure effects will be most clearly observable in high-risk environments. Spear (this volume) has suggested that drug exposure effects might act by creating a vulnerability to stress in offspring and therefore be particularly apparent in high-stress situations. In work on opiate-exposed children (Hans 1989), the author observed that the relationship between prenatal exposure and neurobehavioral outcome at age 2 was strongest within children from the most disadvantaged circumstances. Investigators working with human populations need to explore the moderating effects of different environmental conditions, particularly stressful conditions or potentially ameliorating conditions such as intervention and foster care. This approach requires as much care be taken with measures of environment as with measures of exposure and behavior.

A closely related research design strategy that embraces the role of environment in development explores sources of variability within groups

of drug-exposed children that are related to environmental experiences. While this approach does not address basic teratology research questions, studies of individual differences within drug-exposed children may provide the most useful types of information from a clinical viewpoint. Researchers need to answer questions such as, "Under what environmental conditions do early effects of drugs disappear?" "Under what conditions do they develop into serious disorders?" "Are there conditions that foster the development of resilient children?" "Can some of these conditions be modified through intervention?" Answering such questions requires intensive longitudinal study of children's behavior and their experiences.

Although it is important to understand the basic scientific questions of whether and how prenatal drug exposure affects children's behavior, it is also important not to forget that researchers' work can answer questions that are important to clinicians, program planners, and policymakers. Within these audiences, it is critically important to understand which children exposed to drugs in utero "make it" and which do not. Since many of the markers of "making it" or "not making it" most valued by society are only measurable at adolescence—school dropout, early parenthood, criminal behavior, drug abuse—it is obviously important to follow children until the age when these important markers can be assessed.

SUMMARY

The knowledge base on the long-term implications of prenatal drug exposure in human development is extremely limited, but suggests that children with drug-using parents are at high risk for nonoptimal development. The literature, however, provides some evidence that this risk is related to environmental factors. To date, there is no compelling evidence that history of prenatal drug exposure affects long-term development either directly or through transactions with experiential factors.

This chapter has discussed how study of the long-term development of drug-exposed children requires careful conceptualization of issues of change and continuity in development as well as the role of biology and experience in development. Consideration of these issues will lead to fuller scientific understanding of development and also help the scientific literature better address questions of relevance to clinicians, program planners, and policymakers who are concerned with the welfare of drug-exposed children.

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ACKNOWLEDGMENTS

This chapter was prepared with support from National Institute on Drug Abuse grant R01DA05396. The author acknowledges the role of her colleagues Victor Bernstein, Ph.D., and Linda Henson, M.A., in the design and execution of the longitudinal study summarized herein.

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Drug Effects: A Search for Outcomes

Barry Zuckerman

INTRODUCTION

The National Institute on Drug Abuse (NIDA) Technical Review that generated this monograph represents ongoing support to advance investigations regarding prenatal drug exposure. Previous research conferences that focused on defining the independent variable led to studies establishing the importance of the use of biological markers to more accurately identify prenatal drug use. Other issues, such as measuring and controlling appropriate confounding variables and identifying nonbiased samples, continue to be refined in the present studies and further increase the validity of results. Identification of dose and timing remain underinvestigated in part because of the difficulty in conducting such studies in humans. The chapters presented in this monograph summarize the current status of findings, identify methodologic problems, and recommend fruitful avenues of future research while emphasizing the importance of selecting specific behavioral outcome measures so as not to miss adverse consequences of prenatal cocaine exposure.

SUMMARY OF PRESENT FINDINGS

Animal Studies

The chapters by Riley, Spear, and Vorhees provide important information and perspective on the present status of findings from animal studies on developmental, behavioral, and learning outcomes. Vorhees makes an important contribution by systematically reviewing the experimental animal literature on the effects of prenatal and/or early postnatal exposure to cocaine, covering studies published from 1982 to mid-1993 (Vorhees, this volume). Of the 24 behavioral teratologic studies, 15 reported finding cocaine-related effects. However, Vorhees cautions that this rate of 62 percent positive studies may overstate the apparent strength of the finding, since for every report of positive findings, there were many (or in some cases, more) negative findings. Vorhees concludes that findings of

an adverse effect of prenatal cocaine exposure are inconsistent and prevent firm conclusions from being drawn at this time.

Vorhees (this volume) also provides an important critique of the study methodologies. Methodological problems that may have obscured more consistent adverse effects of prenatal cocaine exposure are as follows:

(1) Lack of consistent use of nutritionally matched pair-fed controls. This is especially important since cocaine induces anorexia and therefore suppresses food consumption and weight gain.

(2) Lack of control for the potential of maternal carryover effects since drug use may affect mothering.

(3) Limitation of studies to one species: rats.

(4) Exposure consisting only of a single daily dose of cocaine. This is theoretically important since cocaine has a short biological half-life, and preliminary data suggest it is an important consideration.

(5) Limitation to subcutaneous route of administration.

(6) Limitation of exposure to the whole pregnancy instead of discrete exposure intervals.

Riley (this volume) came to a similar conclusion on the research findings, stating that prenatal cocaine exposure does not appear to have "wide-ranging effects." However, this conclusion was qualified by Riley's citing the theoretical perspective and preliminary data by Spear that is described in more detail elsewhere (Spear, this volume). Spear hypothesizes that neural reorganization due to prenatal cocaine exposure may result in a decreased adaptability that may not be evident under basal, nondrug, minimal stress, low-distraction testing conditions. Spear's preliminary data support adverse effects in response to pharmacological and social stresses. Thus, studies that do not use a stress or challenge paradigm may miss important consequences of prenatal cocaine exposure. Interestingly, Hans' discussion of human studies offers a similar perspective in stating that the field of human behavioral teratology is moving beyond questions of independent effects of prenatal cocaine exposure to examining the conditions under which the adverse effects of prenatal cocaine exposure might be identified (Hans, this volume). Thus, two important and prominent

researchers, one in animal investigations and the other in human investigations, come to a similar perspective.

Human Studies

Although the earliest reports suggested the existence of dramatically disturbed newborn behavior associated with prenatal cocaine exposure, an overview of studies that used the Brazelton Neonatal Behavior Assessment Scale (BNBAS) fails to show any consistent pattern of effects (table 1). Where Chasnoff's studies showed deficits in orientation, motor and state control, and reflexes (Chasnoff et al. 1985, 1989), Eisen found only deficient habituation (Eisen et al. 1991), Neuspiel found abnormal motor development (Neuspiel et al. 1990), and Coles found differences in autonomic control and reflexes (Coles et al. 1992). Most recently, Mayes and colleagues (1993) have replicated Eisen's finding of decreased habituation as the sole correlate in the newborn period.

The inconsistency of these data makes conclusions regarding cocaine's behavioral teratogenicity difficult. If there is any agreement in these data, it might be in the early impairment of habituation. Decreased habituation in cocaine-exposed newborns most likely reflects an inability to dampen sensory input and control arousal. A related phenomenon might be the finding of augmented reactivity in cocaine-exposed infants to a controlled eye-blink stimulus (a glabellar tap), with or without a 90 decibel (dB) tone (Anday et al. 1989).

Mayes' preliminary data show infants beyond the newborn period may continue to have difficulty regulating arousal (Mayes, this volume). Cocaine-exposed infants became fussy and irritable early in the habituation procedure when presented with the first novel stimuli. When the infants were focused or able to attend, there were no differences in measures of habituation. Habituation in newborns is qualitatively different from habituation in later infancy, which, as Mayes points out, is thought to be associated with information processing. Thus, if the infant is able to maintain an alert, oriented state, there do not appear to be any differences in early information processing between drug-exposed and nondrug-exposed groups. However, difficulty in regulating arousal prevents cocaine-exposed infants' opportunities to take in and process information or function adaptively in selected situations. The author's clinical experience supports this observation. Cocaine-exposed infants appear to function normally in low-stress situations but show increased arousal and disorganization related

TABLE 1. *Cocaine effects on Brazelton Neonatal Behavioral Assessment Scale (BNBAS) scores in term infants not exposed to opiates.*

	Habituation	Orientation	Motor	State Range	State Regulation	Autonomic Regulation	Abnormal Reflexes
Chasnoff 1989 N = 79	0	+	+	0	+	0	+
Eisen 1991 N = 52	+	0	0	0	0	0	0
Neuspiel 1990 N = 111	0	0	+*	0	0	0	0
Coles 1992 N = 107	0	0	0	0	+**	+**	0
Mayes 1993 N = 86	+	0	0	0	0	0	0

KEY: + = less optimal scores in cocaine exposed; 0 = no difference between exposed and unexposed; * = only at 2 weeks of age; ** = only at 14 and 28 days.

to transitions or other stimuli. In addition, when they get upset, they appear to have difficulty self-regulating and continue to spiral out of control. This observation is consistent with Spears and Hans' perspective that adverse behaviors due to prenatal cocaine exposure are contextually related.

Little is known about the development of cocaine-exposed infants beyond the neonatal period. A single study performed by nonblind examiners found that cocaine/polydrug-exposed infants scored more poorly on an assessment of motor functioning at 4 months of age than unexposed infants (Schneider and Chasnoff 1992). Subsequently, this same research group reported that cocaine-exposed infants were similar to unexposed infants on the Bayley Scales of Infant Mental Development at 24 months of age (Chasnoff et al. 1992) and on the Stanford-Binet intelligence quotient (IQ) test at 36 months of age (Azuma and Chasnoff 1993).

However, using path analysis with the data collected at 36 months, drug exposure (defined as cigarettes, alcohol, marijuana, with or without cocaine) was directly and indirectly associated with measurements on the Stanford-Binet IQ test. The indirect effects were mediated through head circumference at 3 years of age, the home environment, and perseverance at tasks. The only other longitudinal investigation of cocaine-exposed infants also included exposure to phencyclidine (PCP). Infants in this study showed deficits in unstructured play at 18 months and high rates of insecure, disorganized attachment (Rodning et al. 1991, 1993).

BIOLOGIC BASIS FOR CHOOSING OUTCOME MEASURES

Clinical impressions and preliminary data such as those by Mayes (this volume) suggest that cocaine-exposed infants and children are different. The findings from studies of prenatal exposure to methadone and marijuana are summarized by Hans (this volume) and Fried (this volume), who emphasize that assessments limited to global developmental functioning potentially underestimate the effects of prenatal drug exposure on specific neurodevelopmental or neurobehavioral functions. Traditional clinical developmental outcome measures—the Bayley Scales and even the BNBAS—have not shown robust effects of prenatal exposure. The neurophysiologic correlates of prenatal cocaine exposure provide a theoretical basis for choosing more selective outcome measures. Autonomic nervous system (ANS) dysfunction is thought to be associated with regulatory disorders of attention, arousal, and the ability to deal with complex environmental inputs necessary for learning and adaptive social interactions. Neurophysiological concepts can contribute to clinical research by selecting specific outcomes to measure. For example, deficient control of autonomic regulation and arousal may underlie observed hyper- or hyposensitivity to stimuli and the resulting learning and behavior problems.

Cocaine blocks the reuptake of norepinephrine, epinephrine, and dopamine at the presynaptic membrane. This results in a magnification of activity of these agents at the postsynaptic membrane, leading to behaviors such as increased motor activity, increased vigilance, euphoria, and physiologic responses such as increased heart rate and increased blood pressure. Three studies in humans have been published so far that have directly measured neurochemical changes associated with prenatal cocaine exposure. One study reported levels of venous norepinephrine 1.8-fold higher in 22 infants exposed to cocaine compared with 15 age-matched controls (Ward et al. 1991). The samples were obtained at

approximately 2 months of age. Venous epinephrine and dopamine did not differ between groups, nor did measures of alpha and beta receptor binding on peripheral blood components. No attempt was made to separate out the effects of cocaine from that of other drugs of abuse. Birthweight was not controlled, although this differed significantly, with a high predominance (27 percent) of low birthweight (< 2500 grams (g)) among exposed infants. Elevated plasma norepinephrine was interpreted as possibly reflecting increased sympathetic tone.

Circulating catecholamines were measured in a small pilot study by Mirochnick and colleagues (1991). In 12 infants known to be cocaine exposed, with negative histories and toxicology screens for opiates or other illicit drugs, the mean concentration of dihydroxyphenylalanine was increased nearly two-fold (10.3 versus 5.9, $p = 0.055$). Dopamine and norepinephrine were not different between groups. Norepinephrine, however, was negatively correlated with the orientation cluster on the BNBAS. Other chemicals measured were not significantly related to behavior. As with the previous study, the potential confounding effect of gestational age and intrauterine growth were not controlled, due to small sample size. Samples were obtained at 24 to 48 hours postpartum, when acute effects of recent cocaine exposure might still be operative in some children.

Studies of peripheral catecholamine levels provide data that are several steps removed from the area of greatest interest, the central nervous system (CNS). In order to obtain more proximal information about CNS functioning, monoamine precursors and metabolites in the cerebrospinal fluid of infants exposed to cocaine were assessed (Needlman et al. 1993). The major finding was lower homovanillic acid (HVA) among cocaine-exposed infants. Other substances—tyrosine, tryptophan, 3-methoxy-4-hydroxyphenyl-glycol (MHPG), and 5-hydroxyindole acetic acid (5-HIAA)—did not differ between groups. The association between cocaine and lower HVA remained significant after removing from the analysis mothers who used other substances including cigarettes and other potentially confounding factors. Interpretation of this finding is difficult because of the small sample size and because of the uncertain relationship between spinal fluid levels of neurotransmitters or metabolites and actual alterations in structure or function in the brain. For example, decreased levels of HVA could be due to decreased global production or decreased production only in specific brain regions. Despite these limitations, these findings provide the most direct look at neurochemical changes associated with prenatal cocaine exposure in humans.

In the fetal brain, neurotransmitters contribute to brain development by influencing neuronal migration and differentiation, synaptic proliferation (Lauder 1988), and receptor number (Miller and Friedhoff 1988). Cocaine readily crosses the placenta as well as the blood-brain barrier. Brain concentrations of cocaine have been reported as high as four times that of plasma levels (Farrar and Kearns 1989). Thus, cocaine may affect the development of brain structure, especially in areas of the brain that have a higher concentration of dopamine. When cocaine was injected into pregnant rats or directly into rat fetuses on day 20 of gestation, the dopamine receptors in the suprachiasmatic nuclei (SCN) were most affected, with little effect elsewhere in the fetal brain and in the maternal SCN (Weaver et al. 1992). Since the SCN contributes to circadian functioning, prenatal cocaine exposure at a specific time might lead to selected behavioral and/or endocrine changes associated with perturbations of circadian rhythm. Cocaine has also been shown to deplete dopamine in the corpus striatum (Weese-Mayer et al. 1993). Since the corpus striatum is linked to the prefrontal lobe, executive functions such as behavioral flexibility, planning, and self-monitoring may be affected and therefore need to be assessed.

Two studies looking at physiological functions in human newborns that may be related to CNS dopaminergic or ANS functioning support this approach. Evaluation of cry data suggests the existence of two distinct types of infant behavioral response (Lester et al. 1991). Another study has found an elevated sensitivity to sugar: Cocaine-exposed infants sucked less on the pacifier and more on a sweetened pacifier than did controls, suggesting a possible difference in CNS reward circuitry (Maone et al. 1992)

Outcome Measures

Outcome measures need to include tasks that assess regulation of arousal and attention as well as frontal lobe executive functions such as planning, behavioral flexibility, and self-monitoring. Preliminary data support the need for such assessments. Performance on the continuous performance test (CPT) (a computer-administered measure of sustained attention) has been shown to be more sensitive than IQ scores or caretaker reports to the effects of prenatal exposure to cigarettes and polychlorinate biphenyl (PCB) on sustained attention, speech processing, and impulsivity (Jacobson et al. 1992; Streissguth et al. 1984, 1986). In the face of finding no impact of prenatal marijuana exposure on cognitive and language scores, Fried (this volume) found that an increase in omission

errors, especially at the end of a vigilance task, may reflect a deficit in sustained attention in early school-aged children. Children prenatally exposed to marijuana were also rated as more impulsive and hyperactive by their mothers. Hans (this volume) also identified attention deficits in children prenatally exposed to methadone.

Importance of Longitudinal Followup

Failure to find strong cocaine effects on infant developmental tests after the neonatal period does not obviate the need for evaluation of cocaine-exposed children at later ages (Hans, this volume). Findings from followup of other perinatal insults support this recommendation. Effects of prenatal marijuana exposure were noted at birth and again at age 48 months, with no effects on interim developmental test scores at 12 and 24 months (Fried and Watkinson 1990). Low birthweight children found to have learning disabilities at school-age frequently perform in the normal range during infancy (Hunt et al. 1982). Furthermore, the manner in which an early biological insult is expressed may change over time. Among low birthweight infants, delayed motor function at 1 year of age significantly predicted lower IQ, expressive language delay, and articulation deficits at age 3 years (Ross et al. 1985).

Some domains are difficult to assess during infancy due to limitations in an infant's response capacities, the lack of suitable assessment tools, or the immaturity (i.e., developmental unavailability) of higher order skills. Children's performance on tasks purported to assess prefrontal functions (e.g., selective attention, organization, sensory-motor integration) undergoes substantial changes with maturation. Potential developmental deficits in areas such as social competence with peers, complex language, and sustained attention may not be evident until the social/cognitive demands of school entry.

Hans (this volume) further emphasizes the potential importance to follow children through adolescence and even young adulthood because some CNS-related disorders usually do not appear until this time. Other problems such as drug use and abuse need a specific social context to occur. Whether children exposed to drugs prenatally have a greater susceptibility to later drug abuse or addiction has important clinical and public policy implications.

SUMMARY

In order to best understand the developmental and behavioral effects of prenatal cocaine exposure, two important activities must occur. The first is the continuing development and refinement of research methodologies. Information-sharing activities such as conferences support this goal. Second, agencies such as NIDA need to support longitudinal followup of prenatally drug-exposed child cohorts and controls to identify outcomes beyond infancy and toddler years. Without support for information exchange and longitudinal followup, researchers will still be in the dark regarding the effects of prenatal drug exposure on school functioning when the next drug epidemic occurs. This message was not heeded in the 1970s and early 1980s, leaving researchers in the 1990s in the uncomfortable position of saying they do not know the longer term effects of prenatal drug exposure.

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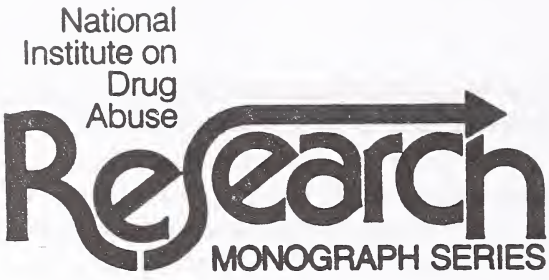

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NIH Publication No. 96-4105
Printed 1996