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

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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 (1989b) examined the acquisition of first-order appetitive odor conditioning. In this study, pregnant Sprague-Dawley rats were given daily subcutaneous (SC) injections of 40milligrams 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. 1989b	SD		SC		Yes
Odor-cocaine					
Heyser et al. 1992a	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. 1989a	SD		SC	7	No
				17	Yes
				18	Yes
Heyser et al. 1990	SD		SC	8	Yes
				12	No
				21	No
Goodwin et al. 1992	SD		SC	7	Yes
				18	No
Sound-shock					
Goodwin et al. 1992	SD		SC		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		SC	17	No
				63	No
Riley and Foss 1991	LE		GV		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		SC		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. 1992b	SD		SC		No
Reversal					
Heyser et al. 1992b	SD		SC		Yes
Schedules of reinforcement		DRL-20			
Smith et al. 1989	LE		SC		Yes*
Schedules of reinforcement		FR-10			
Heyser et al. 1992b	SD		SC		No
Sensory preconditioning (odor-shock)					
Heyser et al. 1990	SD		SC	8	Yes
				12	No
				21	No
Spontaneous alternation					
Smith et al. 1989	LE		SC		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		SC	32	No
				35	No
				40	No
				45	No
Visual discrimination					
Smith et al. 1989	LE		SC		No
Water maze					
Smith et al 1989	LE		SC		Yes
Riley and Foss 1991	LE		GV		No
Johns et al. 1992	SD		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 5seconds with 0.3 milliliters (mL) of half-and-half dairy cream every 30seconds. 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 signifi-cant. In the ANOVA this interaction did not reach statistical significance ($p < 0.088$), and thus the authors used planned comparisons to statis-tically 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 (1989b), 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. 1989b) 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 (1989a) 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 (1989b) 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 8days 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 (8and 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 8days 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 8and 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) 20minutes prior to exposure to both the CS- and CS+.

Following these exposures, subjects were returned to a holding cage for 3minutes 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 3trials. 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 20minutes 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 3hours 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 (1mA 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 (1992a) 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 6days 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 2mg/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 (1992b) 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 40mg/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 80percent or more correct responses in the first 10 responses of a session and 90 percent or more correct responses over the entire session for 5consecutive 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. 1992b), 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. 1992b, 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 4to18. 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 discrimi-nation (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 spon-taneous 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 tothe overall ANOVA are presented and that the significant interaction of prenatal treatment with day involved an F equal to 1.50 on 29 and 906degrees of freedom, $p < 0.05$. It also does not appear that the authorsmade 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 alterna-tion 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 90days 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 compart-ment 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 60mg/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 10trials 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 2con-secutive 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 adminis-tration 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 (1989a, 1989b), however, indicates that this deficit is relatively transitory and is not found in animals after perhaps 15 days of age. 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 (1992b) 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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Click here to go to page 78