Pharmacology Biochemistry and Behavior, Vol. 61, No. 1, pp. 9–17, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00013-6

Open-Field and LPS-Induced Sickness Behavior in Young Chickens: Effects of Embryonic Cocaine and/or Ritanserin

LISA M. SCHROTT, MARY E. GETTY, PAUL W. WACNIK AND SHELDON B. SPARBER

Department of Pharmacology, University of Minnesota Medical School, 3-249 Millard Hall, 435 Delaware St. S.E., Minneapolis, MN 55455

Received 21 September 1997; Revised 3 December 1997; Accepted 30 December 1997

SCHROTT, L. M., M. E. GETTY, P. W. WACNIK AND S. B. SPARBER. *Open-field and LPS-induced sickness behavior in young chickens: Effects of embryonic cocaine and/or ritanserin.* PHARMACOL BIOCHEM BEHAV **61**(1) 9–17, 1998.—Exposure to drugs of abuse during embryogenesis may adversely affect nervous, immune, and endocrine systems development. We compared exposure on embryonic day 18 (E18) by single or multiple cocaine (COC) injections (56.25 mg/kg total dose for both) or saline on hatching and activity measures. In saline-exposed controls, repeated testing, age, and gender affected activity levels. A single or multiple COC injections increased the median latency to explore and multiple COC injections decreased the median number of lines crossed by female chicks in the open field. We also determined if pretreatment with the serotonin$_2$ (5-HT$_2$) receptor antagonist ritanserin could attenuate COC's effects on open-field behavior as well as behaviors sensitive to immune system stimulation (lipopolysaccharide (LPS)-induced sickness behavior). Eggs containing embryos were pretreated on E17 with 0.4 mg ritanserin/kg or its vehicle followed by multiple COC injections or saline on E18. E18 COC treatment decreased the median number of lines crossed and distress vocalizations in females. Ritanserin pretreatment mitigated the COC induced effects. E18 COC exposure also suppressed LPS-induced sickness behaviors in both males and females, increasing food consumption and the time spent awake and active, as well as decreasing the time spent sleeping. Ritanserin alone had no effect on the food consumed or time spent active, nor did this dose affect COC-induced alterations in sickness behavior. Ritanserin alone decreased time spent sleeping and also failed to affect the COC-induced suppression. Thus, embryonic COC exposure can suppress open field and LPS-induced sickness behavior in the young chick, and ritanserin pretreatment can block the former, but not the latter effects at the dose chosen for these experiments. © 1998 Elsevier Science Inc.

Open field Ritanserin Sickness behavior Lipopolysaccharide Cocaine *Gallus domesticus*

PRENATAL exposure to drugs of abuse such as cocaine (COC) has the potential to alter the physiology and behavior of the developing organism. Although there has been extensive research in humans on pregnancy complications (4,10), perinatal health (12,23), and infant and child development (11,25,44,47,48), there is much controversy as to the degree and existence of any deleterious effects attributable solely to COC (38–40,50). Parallel research with animal models may provide more insight into possible effects and mechanisms for COC-induced physiological and behavioral manifestations. Although animal research has clarified many of the physiological effects of prenatal COC, including its cardiovascular and occasional dysmorphic properties [i.e. (46,60)], both short-term and long-term behavioral effects of COC exposure remain controversial (15,57). In his critical review of the developmental COC literature from 1982–1993, Vorhees [(57); p. 34] states "In toto, the current developmental cocaine experimental literature appears heavily weighted towards a limited range of experimental models that produced mixed results." These studies have primarily been conducted in the rat after COC administration during the equivalent of the human first and second trimesters of pregnancy. Often these studies lacked adequate controls, relied on invasive procedures to administer COC, and could not discriminate between maternal response to COC or the means of its administration and direct action of COC on the offspring. The domestic chick has a

Requests for reprints should be addressed to Lisa M. Schrott, Department of Pharmacology, University of Minnesota Medical School, 3–249 Millard Hall, 435 Delaware St. S.E., Minneapolis, MN 55455.

number of features that makes it useful for developmental studies of abused drugs. *In ovo* drug administration eliminates maternal undernutrition. Because the chick is precocial, early postnatal manipulations can be conducted without maternal influence, a potential source of confounding early experience (51). This laboratory has used the chick model extensively to demonstrate effects of opiate exposure and withdrawal (35,36) and COC (31,52,54) on cardiovascular responsiveness, brain biochemistry, hatchability, and behavior. In addition, Hughes et al. (24) has found alterations in immune responsiveness and behavior in the chick exposed to COC during mid-embryogenesis, suggesting its usefulness for studying neural-immune interactions following exposure to drugs of abuse.

Unconditioned behaviors, such as open-field activity, have been well studied in rodents exposed prenatally to COC. However, results are equivocal, with modest activity alterations at best. Experimental design has been inconsistent with respect to route of administration, duration of exposure, and test age, making it difficult to compare results. Studies examining juvenile open-field behavior have found decreased activity in male rats in the periweaning period (13,27,32,49), whereas other studies have found no or inconsistent effects in this age range (19,45). Young swine exposed to COC during the last third of gestation showed reduced locomotion and rooting and spent more time immobile (37) and young chickens (7–12 days of age) exposed to COC during mid-embryogenesis showed decreased activity and decreased distress vocalizations (24). There is a general failure to find effects of prenatal COC exposure on activity measures in older animals (13,18,37,45), although Johns et al. (26) reported increased latency to explore by adult rats exposed prenatally to COC.

In addition to effects on nervous and cardiovascular systems, COC can affect immune function. Immunosuppressive effects of COC have been widely documented in humans and rodents under *in vitro* and *in vivo* conditions [e.g., (59)]. However, little is known about COC's effect on the development of interactions among immune, nervous, and endocrine systems. One method of assessing these interactions is to examine sickness behaviors induced following immune system stimulation. These highly conserved and survival-promoting behaviors include decreased food intake, activity, and grooming; withdrawal from social interactions; appropriate thermoregulatory behaviors; and changes in sleep–wake cycles [i.e. (14,21,22)]. Sickness behaviors are induced by infectious agents, immunostimulatory substances such as the endotoxin lipopolysaccharide (LPS) which stimulates the production and/or release of endogenous cytokines [i.e., (20,30)], and exogenous cytokines themselves (14,16,43). Like other species, chickens display sickness behavior following LPS exposure characterized by reduced food intake and plasma iron concentrations, and increased body temperature, sleep time, and corticosterone concentrations (28,29). Although much is known about factors affecting sickness behavior in mature rodents, it has been less intensively studied in developing organisms and nonrodent species.

In parallel with our study of COC's developmental effects, we are investigating potential therapies that could ameliorate detrimental COC effects. In addition to blocking synaptic reuptake of dopamine and norepinephrine, COC's blockade of the serotonin (5-HT) transporter increases activity at 5-HT receptors. Prior studies in our laboratory found that embryonic COC suppressed chick embryonic motility (31,52), reduced hatchability (31), enhanced postnatal detour learning, and decreased distress vocalizations in a social pairing (52). 5-HT_2 agonists such as dimethoxyiodophenylaminopropane (DOI) can mimic many of these effects (7,53) and 5-HT_2 antagonists, such as ritanserin, can attenuate or block them (6,31). Interestingly, a single injection of the 5-HT_2 agonist DOI, administered early or late in embryonic development, can induce herniated umbilici, while a single injection of COC consistently fails to induce them [(53); unpublished observations]. Herniations are indicative of excessive smooth muscle spasm caused by 5-HT_2 receptor activation. The difference in the inducibility of herniations between DOI and COC may be due to their potency and/or durations of action. COC has a very short plasma half life (61) and to obtain high peak concentrations and a long duration of action, multiple injections are necessary (58). In fact, we found that when eggs containing embryos received multiple injections of COC, the incidence and severity of herniated umbilici was significantly greater in these chicks than those receiving the same absolute dose as a bolus (63).

In the chick, the behavioral effects of multiple COC injections compared to a bolus have not been examined. Moreover, because humans rarely self-administer a single bolus or smoke COC a single time, instead having "binge" patterns of COC use (17), experiments with nonhuman species should attempt to mimic more closely these human use patterns. In Experiment 1 we examined this issue by comparing open-field behavior in chicks receiving a saline, a bolus, or multiple injections of COC during late development, on embryonic day 18 (E18). We also characterized chick open-field behavior by examining sex and developmental differences. In Experiment 2 we extended the behavior analyses to include LPS-induced sickness behavior. We also examined the effects of E17 exposure to the 5-HT_2 antagonist ritanserin on both open field and sickness behavior, as well as its potential to block any of effects of E18 COC exposure on these behaviors.

METHOD

Experiment 1

Treatment. Cocaine hydrochloride (COC, generously provided by the National Institute on Drug Abuse) or avian saline (0.85% NaCl) were administered on E18. All drug and vehicle solutions were filtered with a 0.2 μm filter (Millpore, Bedford, MA) and were kept at 4°C prior to injections. There were three treatment groups: bolus COC, multiple COC, and controls. The bolus COC group received one injection of 56.25 mg COC/kg egg and four injections of saline. The multiple injection group received five injections of 11.25 mg COC/kg egg (total dose = 56.25 mg/kg). Controls received five saline injections. In several prior studies we have found no effect of a bolus vehicle injection on similar behaviors [e.g. (6)] and thus, we used the more conservative control of multiple saline injections for comparison. Injection volume was 21 μl per injection, and the injection interval was 1.5 h.

Experiment 2

Treatment. Treatments were administered on E17 and E18. E17 treatment was either 0.4 mg ritanserin/kg egg (Rit; Research Biochemical International, Natick, MA) or its vehicle, 0.1 M tartaric acid (TA). This dose was chosen because of its efficacy in blocking herniated umbilical formation following DOI exposure (53) and because it is within the range that has been found to be behaviorally inactive for detour learning when injected in the chick at late stages of embryogenesis (7). The following day (E18), five injections of COC or avian sa-

line (Sal) were administered to half of each E17 treatment group. Each COC injection was 11.25 mg/kg egg, for a total dose across the five injections of 56.25 mg/kg egg. Thus, the treatment groups were TA-Sal, Rit-Sal, TA-Coc, and Rit-Coc. Drug preparation and injection schedule were the same as for Experiment 1.

General Method

Subjects. Eggs from a Rhode Island Red × White Leghorn cross were obtained from the Poultry Nutrition Research Center (University of Minnesota, St. Paul, MN). The eggs were stored overnight at 14–16°C to synchronize embryo development. The eggs were then set in a rotating forced air incubator/hatcher (Humidairre, New Madison, OH) with the temperature at 37–38°C and the relative humidity at 58–60%. The set day was designated as embryonic day 0 (E0). The eggs were candled on E14 and treatment was assigned randomly to eggs with viable embryos. An injection site for drug administration was marked about 2.0 cm below the air cell, avoiding membrane-bound blood vessels that could be observed during the candling procedure. The shell surface at the injection site was sterilized with a small drop of 2% iodine tincture and wiped off with a gauze pad moistened with 70% ethanol. A 1.2-mm diameter dental burr and a small drill were used to drill injection holes without puncturing the underlying membrane. Holes were covered with a small piece of transparent plastic tape (3M, St. Paul, MN). Drugs were administered on E18 in Experiment 1 and on E17 and E18 in Experiment 2. On E18 or E19 the eggs were transferred to the hatcher and the hatcher was checked twice a day from E20–E22 for new hatchlings. Hatchlings were removed from the hatcher and banded for identification (day of hatching designated as posthatch day 0). Weight, presence of a herniated umbilicus, and umbilical size were recorded. A herniated umbilicus was recorded if there was a discolored umbilical protrusion and herniated umbilical size was measured with a caliper to the nearest 0.5 mm. Chicks with physical abnormalities that could interfere with behavioral testing were euthanized and those to be behaviorally tested were placed in a heated brooder. Water was provided ad lib upon placement in the brooder and ad lib food (Country Choice Medicated Chick Starter, Land-O-Lakes, Inver Grove Heights, MN) was provided from day 2 forward, except during the food deprivation phase of the sickness behavior assessment in Experiment 2. The chicks were placed on a 12 L:12 D cycle with lights on at 0700 h in Experiment 1 and 0800 h in Experiment 2. It is not possible to distinguish young males from females in the hybrid chicks used in these studies. Therefore, at the conclusion of testing, the chicks were euthanized, the abdominopelvic cavity was opened, and the reproductive organs identified.

Behavioral testing. In Experiment 1 chicks were tested in the open field on posthatch days 3, 5, and 7 (repeated exposure group) or day 7 only (single exposure group). For the saline group $n = 14$ and for the bolus COC and multiple COC injection groups $n = 12$ for both repeated and single exposure groups. In Experiment 2 chicks were tested in the open field on day 3 and for LPS-induced sickness behavior on day 10. There were 14 subjects per treatment group. A block randomization design was used to determine test order.

Open-Field Behavior

The testing paradigm was modified from Kuwahara and Sparber (34). Open-field testing was conducted in a 90.75 × 90.75 × 35.50 cm arena with a gray floor and black walls. Yellow tape divided the field into 25 squares (18.15 × 18.15 cm). The room was dark except for a light with a 60 W bulb, which was placed 30.5 cm directly above the center of the open field floor, yielding an illumination level of 1.25 foot candles. A wide-angle, low-light–sensitive 8-mm video camera was situated above the open field and was used to record the test sessions. Distress vocalizations were recorded throughout testing via the audio input on the video camera. Chicks were tested individually. Each chick was placed into the center square of the open field and allowed to roam freely for 3 min. Excreta were removed from the test arena, which was then wiped with 70% ethanol prior to testing the next chick. The videotaped data were scored for the latency to begin exploration (latency to leave the center start square and begin open field exploration), number of lines crossed, number of escape attempts (attempts to jump or fly out of the test arena), and number of distress vocalizations (loud, high pitched chirps emitted when chicks are isolated). For Experiment 1, the videotapes were scored blindly by two independent observers. Coefficients of variation (CV) were calculated between the scorers' values for each subject and measure. Any measure that had a %CV greater than 10% was rescored by a third person. The % CVs across all measures averaged 1.5% (range 0–3.6%). For Experiment 2, the videotapes were scored by one person, and 20% of the subjects were randomly selected and their videotape was scored by a second observer. CVs for these subjects were in the same range as in Experiment 1.

Sickness Behavior

The testing procedure was modified from Johnson et al. (28,29). Chicks were food deprived (water available) 24 h prior to testing. One hour prior to testing they received an intraperitoneal (IP) injection of 5 mg LPS/kg body weight (Sigma, St. Louis, MO; serotype 0128:B12) in a volume of 2 μl/g body weight. Testing occurred in a 51 × 61 × 37 cm chamber that was designed for chick detour learning (6). The chamber's front and back walls were covered with mirrors to decrease isolation-induced distress vocalizations and to facilitate accurate assessment of eye closure. The test room was dark, but the arena was illuminated by fluorescent lights. A Petri dish containing moistened chick food (same as ad lib food described above) was weighed and placed in the center of the arena. The food was moistened to make it easier to eat in the absence of drinking water and to prevent spread during pecking. The chick was placed in the front right corner of the arena facing the dish and the 5-min test session begun. A timer was used to record the following behaviors: eating (pecking at food in the dish); activity (noneating horizontal or vertical movement); and sleep (inactivity accompanied by closure of one or both eyes, a change in breathing, and cessation of vocalizations). The number of bouts of each of these activities was recorded. In addition, the latency to begin eating was recorded and the food dish was weighed before and after testing to determine the apparent amount of food consumed.

RESULTS

Experiment 1

Hatching data. A χ^2 analysis found no significant difference in the hatching rates among the groups, although there was a 10.5% decrease in hatching in the Bolus COC injected group and a 6.4% decrease in hatching in the multiple injection COC group compared to the controls. Only subjects that hatched on E21 were used for behavioral testing. Neither bo-

lus or multiple injections COC affected body weight in comparison to the saline controls. We replicated our prior study (63), finding that multiple COC injections induced more herniated umbilici than the bolus COC or saline injections, $\chi^2(1) = 13.96$, $p < 0.0002$, for multiple COC vs. saline injections, and $\chi^2(1) = 3.92$, $p < 0.05$ for multiple COC vs. bolus COC injection (multiple COC = 48.78%; bolus COC = 25.81%; saline = 8.82%). The herniations were also larger in the Multiple COC injected subjects than in the other two groups [Treatment main effect, $F(2, 103) = 8.64$, $p < 0.0003$; multiple COC vs. controls, $p < 0.0001$; and multiple COC vs. bolus COC $p < 0.03$, Fisher's protected LSD; multiple COC = 2.93 mm ± 0.50; bolus COC = 1.48 mm ± 0.48; controls = 0.44 mm ± 0.25].

Behavioral data. Experiment 1 examined two questions for which the data were analyzed separately: 1) are there developmental or sex differences in open-field behavior in saline-treated young chicks?; and 2) Does embryonic exposure to COC, either as a bolus injection or via multiple injections, affect open-field behavior? Because we were not able to confirm the nature of the distributions of data and there were apparent deviations from normality, the data were analyzed by nonparametric statistics. Thus, median values and individual data or ranges are reported in figures and tables.

Developmental and Sex Differences in Open-Field Behavior. Only subjects receiving saline injections on E18 were included in these analyses. The data were collapsed across minutes of the test, and a Freidman test was used to examine differences across testing days. Males and females were examined separately because of suggestions in the literature that there are sex differences in open-field behavior in the chick (56). For males, the nonparametric Friedman ANOVA test was significant for latency to begin exploring, $\chi^2(2) = 6.25$, $p < 0.05$, escape attempts, $\chi^2 = 6.52$, $p < 0.04$, and distress vocalizations, $\chi^2 = 7.00$, $p < 0.04$. There was a marginal effect for the number of lines crossed ($p < 0.08$). The latency to begin exploring decreased across days, while the other measures increased across days. For females, significant effects were found for escape attempts, $\chi^2 = 12.25$, $p < 0.003$, and a marginal effect was found for the number of lines crossed ($p < 0.07$). In females, escape attempts increased across test days.

To determine if the age effects were a consequence of repeated testing or nervous system maturation, 7-day-old males that had received prior testing were compared to naive 7-day-old males (repeated testing effect) and 3-day-old naive males were compared to naive 7-day-old males (developmental effect) using Mann–Whitney *U*-tests. Escape latency in males decreased as a function of repeated testing, $z = 2.84$, $p < 0.005$ (day 7 repeat testing: median = 21.5 s; range 1–50 s, and day 7 naive testing: median = 50.5 s; range 24–92 s). For escape attempts in males there was a marginal effect for repeated testing, $p < 0.10$ and a significant developmental effect, $z = 3.21$, $p < 0.002$, with 7-day-old naive males having more escape attempts (median = 8.0; range 4–13) than 3-day-old naive males (median = 0; range 0–6). The change in escape attempts in females across days was also a developmental effect, $z = 2.02$, $p < 0.05$, with a similar pattern to that seen in males.

Embryonic COC Exposure and Open-Field Behavior

Because significant sex and age interactions were found in saline-treated subjects, COC effects were examined in each sex separately, at each age. The data were analyzed by a Kruskal–Wallis test for Treatment effects (controls, bolus COC, and multiple injection COC) followed by Mann–Whitney *U*-tests to determine specific effects. There were approximately equal numbers of males and females across the treatment groups.

Males. No significant effects of E18 COC exposure were found.

Females. On day 3 there was a significant treatment effect for latency to begin exploring, $H(2) = 8.14$, $p < 0.02$ (Fig. 1) and the number of lines crossed, $H = 6.14$, $p < 0.05$ (Fig. 2). Compared to controls, both a single injection of COC, $z = 2.42$, $p < 0.02$, or five injections of COC, $z = 2.34$, $p < 0.02$, increased the latency to explore. Multiple COC injections also decreased the number of lines crossed compared to saline controls, $z = 2.25$, $p < 0.03$. By days 5 and 7, there was no longer evidence of a COC-induced effect on these measures.

Experiment 2

Hatching. The hatching and herniated umbilici data from subjects in Experiment 2 have been previously reported (63). Briefly, Treatment did not affect hatching rate or weight. TA-COC subjects had more and larger herniated umbilici than TA-Sal controls. Ritanserin pretreatment on E17 blocked this effect, decreasing both herniation incidence and size.

Behavior. This study was designed to address six specific issues and the data were analyzed to address these questions: 1) can we replicate the COC-induced changes in female chicks open-field behavior found in Experiment 1?; 2) Does E17 ritanserin exposure affect open-field behavior?; 3) Can E17 ritanserin exposure prevent the COC-induced alterations in open-field behavior measures?; 4) Does E18 COC exposure alter LPS-induced sickness behavior?; 5) Does E17 ritanserin exposure affect LPS-induced sickness behavior?; and 6) If E18 COC exposure affects LPS-induced sickness behavior, can pretreatment with ritanserin on E17 mitigate the COC-induced changes? The behavioral data were summed across minutes and analyzed as described in Experiment 1.

Open-Field Behavior

Effects of E18 COC exposure alone. Because significant sex effects were found in Experiment 1, data for males and females were analyzed separately. There were equal number of males and females in each of the treatment groups. Analyses were conducted to determine if we could replicate the suppression of activity in E18 COC-treated subjects by comparing the TA-Sal controls (E17 and E18 controls) to the TA-COC group (E17 vehicle and E18 COC treatment). Again, there were no significant effects for males. In females, E18 COC exposure decreased the median number of lines crossed, $z = 1.98$, $p < 0.05$ (Fig. 3) and the median number of distress vocalizations, $z = 2.11$, $p < 0.04$ (Fig. 4), compared to the TA-Sal females. Unlike Experiment 1, no effect was found for the escape latency measure.

Effects of E17 ritanserin exposure alone. Rit-Sal–treated females (E17 ritanserin and E18 control) did not differ from the combined E17/E18 control TA-Sal subjects on any of the measures.

Effects of E17 ritanserin combined with E18 COC exposure. To determine if blockade of 5-HT_2 receptors prior to COC administration could prevent the COC-induced open-field effects, the Rit-COC females (Rit pretreatment on E17 followed by COC treatment on E18) were compared to the TA-Sal female controls. Ritanserin pretreatment negated the significant COC effects found in the first comparison. Rit-COC females did not differ significantly from TA-Sal females on the measures affected by COC (Figs. 3 and 4).

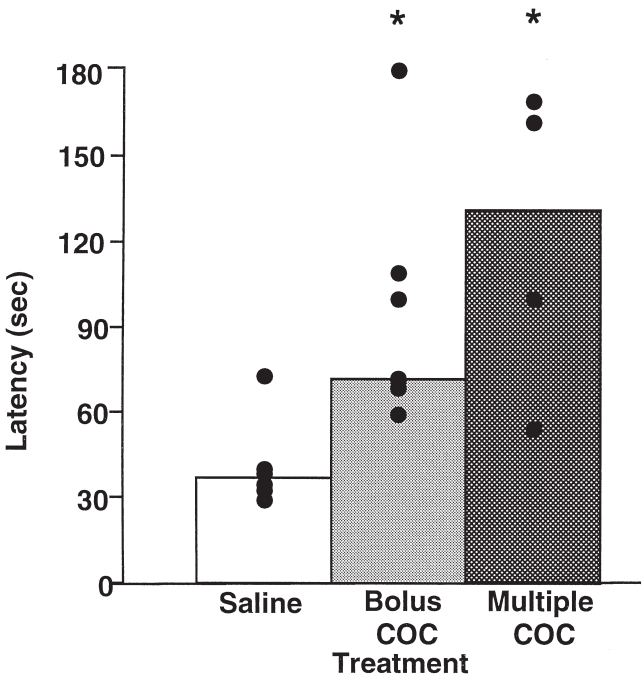

FIG. 1. Median latency in seconds for female chicks to begin exploring the open field following E18 exposure to saline ($n = 6$), a single COC injection (bolus COC; $n = 7$) or 5 COC injections (multiple COC; $n = 4$). Solid circles represent the individual values. Chicks were tested at 3 days of age and the data were summed across the 3-min test. *$p < 0.05$ compared to saline controls (Mann–Whitney *U*-test).

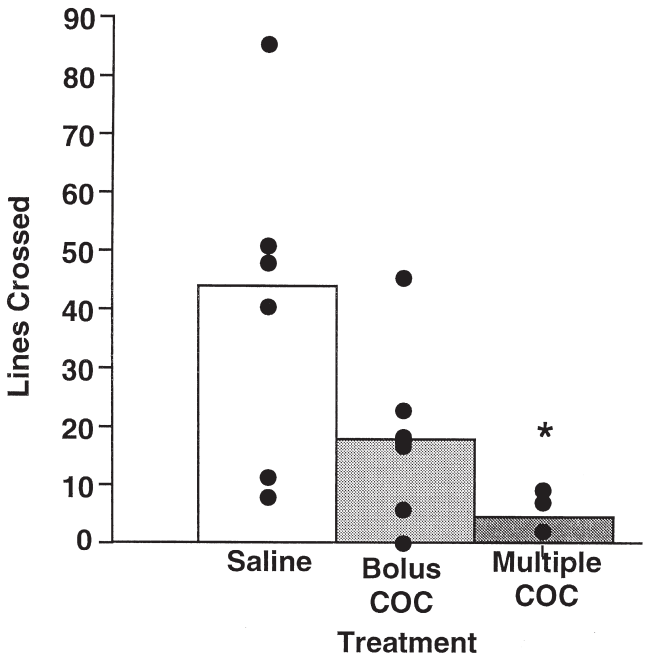

FIG. 2. Median number of lines crossed in the open field for female chicks following E18 exposure to saline, a single COC injection (bolus COC) or 5 COC injections (multiple COC). See Fig. 1 legend for analysis detail.

LPS-Induced Sickness Behavior

Effects of E18 COC exposure alone. There were no sex effects for any of the sickness behavior measures so the data were collapsed across sex. Because of the orthogonal nature of the data, the measures were highly intercorrelated and a subset were chosen for analyses. Table 1 displays the data by treatment groups. In controls (TA-Sal), LPS injection induced a behavioral pattern consisting of low food consumption, little time spent in eating or activity, and substantial time spent sleeping. Exposure to COC on E18 (TA-COC group) attenuated this pattern, increasing food consumption, $z = 2.95$, $p < 0.004$, and the time spent awake and active, including time spent eating, $z = 2.70$, $p < 0.007$, as well as decreasing the time spent sleeping, $z = 3.11$, $p < 0.002$. Time spent in quiet inactivity was not affected.

Effects of E17 ritanserin exposure alone. Exposure to ritanserin on E17 (TA-Rit) had no effect on the food consumed or time spent awake and active compared to the TA-Sal controls. A different pattern was found for time spent sleeping, where E17 ritanserin exposure affected E18 Sal-treated subjects; the Rit-Sal chicks slept less than the TA-Sal controls, $z = 2.36$, $p < 0.02$.

Effects of E17 ritanserin combined with E18 COC exposure. To determine if ritanserin pretreatment could mitigate the COC-induced attenuation of LPS-induced sickness behavior, chicks treated with ritanserin on E17 followed by COC on E18 (Rit-COC) were compared to the TA-Sal controls. Unlike the open-field measures, the dose of ritanserin chosen for this experiment did not eliminate the E18 COC effects for these measures (Table 1). RIT-COC subjects ate more food, $z = 2.05$, $p < 0.05$, spent more time active, $z = 2.49$, $p < 0.02$; and less time asleep than TA-Sal controls, $z = 2.49$, $p < 0.02$.

DISCUSSION

In the present set of studies we examined open-field and LPS-induced sickness behavior following embryonic exposure

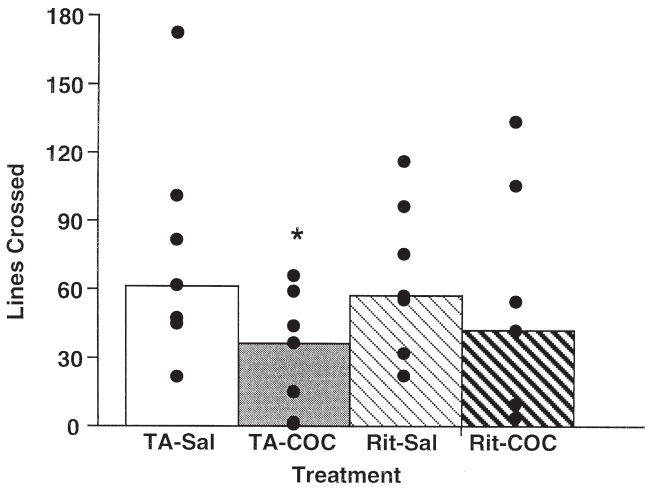

FIG. 3. Median number of lines crossed in the open field for female chicks exposed to tartaric acid vehicle (TA) or ritanserin (Rit) on E17 followed by saline (Sal) or 5 cocaine (COC) injections on E18 ($n = 7$ for all groups). See Fig. 1 legend for analysis detail. *$p < 0.05$ compared to TA-Sal controls (Mann–Whitney *U*-test).

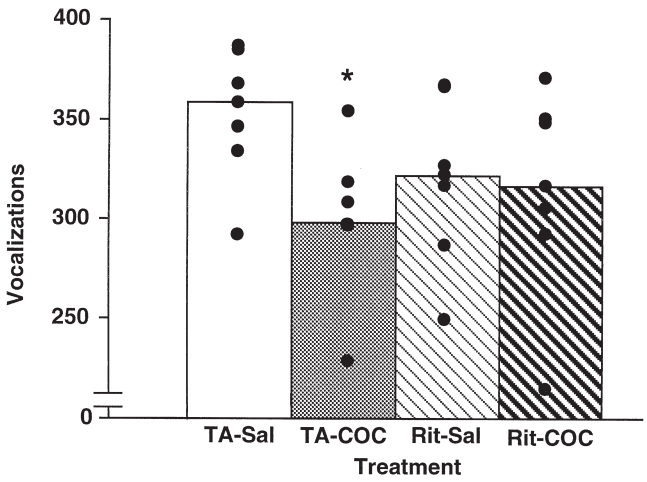

FIG. 4. Median number of distress vocalizations in the open field for female chicks exposed to tartaric acid vehicle (TA) or ritanserin (Rit) on E17 followed by saline (Sal) or 5 cocaine (COC) injections on E18 ($n = 7$ for all groups). Median value for TA-COC group represents three data points. See Fig. 1 and 3 legends for analysis detail.

to COC and/or ritanserin. The dose of cocaine used (56.25 mg/kg egg, divided into five equal injections) was not lethal to the embryos, as there was no reduction in hatching in either study, although it did increase the incidence and size of the minor structural malformation, herniated umbilici. COC suppressed open-field activity in female chicks when they were tested at 3 days of age and 0.4 mg ritanserin/kg egg blocked this effect, suggesting a role for 5-HT_2 receptors in modulating COC's effect on these behaviors. Embryonic COC also attenuated LPS-induced reductions in food consumption and activity; however, the dose of ritanserin chosen was unable to block these effects. Exposure to either COC or ritanserin alone decreased the time spent sleeping in the LPS-induced sickness behavior assessment, and ritanserin did not block the COC effect. In addition, we describe sex differences and effects of repeated testing in open-field behavior for saline-treated chicks. These effects and their implications will be discussed separately.

Chick Open-Field Behavior: Effects of Sex and Repeated Testing

Like the rodent, patterns of open-field behavior in the chick appear to differ by sex and as a function of test experience. In males, changes in open-field behavior were seen across test days. The decrease in the latency to begin exploring was a consequence of repeated testing, while the increase in the number of escape attempts was primarily a consequence of age, most likely because the birds were larger (weighed approximately 30% more on day 7 than day 3), although habituation to the novel environment with repeated testing may have influenced both of these observations. For the number of lines crossed and distress vocalizations neither repeated testing nor developmental effects were significant, suggesting that it was a combination of these factors involved in producing the changes from day 3 to day 7. Females, on the other hand, were not as affected by the repeated testing as the males. The increase in escape attempts in females was a developmental effect, like that seen in males. These findings are consistent with those Valloritgara and Zanforlin (56), who found sex differences in open-field behavior, as well as other unconditioned behaviors, in young chickens. These data also suggest that gender should be analyzed when examining treatment effects for such unconditioned behaviors in chicks.

E18 COC Exposure: Suppression of Activity and LPS-Induced Sickness Behavior

In both Experiments 1 and 2 E18 COC exposure decreased the number of lines crossed by 3-day-old female chicks in a 3-min open-field test. In Experiment 1, the latency to begin exploring was increased in the females, while in Experiment 2 the number of distress vocalizations was decreased. In Experiment 1 multiple injections of COC gave a greater suppression than a single bolus injection, although both total doses were the same. These data are similar to the herniated umbilici findings (63), where lengthening the duration of COC action increased the induction of herniations. No effects of embryonic COC on open-field measures were seen for males in either experiment, or for females on days 5 and 7 in Experiment 1. Thus, a transient and gender-specific suppression of activity was found, much like that previously reported in the literature. For example Smith et al. (49) found that in rats males were more sensitive to activity suppressant effects of prenatal

TABLE 1

LPS-INDUCED SICKNESS BEHAVIOR BY EMBRYONIC TREATMENT

Measure	TA-Sal	TA-COC	Rit-Sal	Rit-COC
Food consumed (g)				
Median	0.0	0.36*	0.0	0.12*
Range	0–0.76	0–1.89	0–0.62	0–2.17
Eating + Active Time (s)				
Median	0.0	113.5*	37.0	77.5*
Range	0–211	0–220	0–169	0–223
Sleep Time (s)				
Median	58.5	0.0*	0.0†	0.0†
Range	0–248	0–101	0–179	0–64

Data were summed across the 5-min test.
$n = 14$ for all groups.
Significantly different than TA-Sal, *$p < 0.01$; †$p < 0.05$.

COC exposure than females, and the subject's age interacted with the treatment effect. Church et al. found that prenatal COC suppressed activity in 20-day-old male rats, but not 63–70-day-old males (13). In swine exposed to cocaine prenatally, Laferriére et al. (37) found that young (2–9 days of age), but not older swine (22–29 days of age) showed suppressed locomotion and rooting behavior. Juvenile activity suppression after prenatal COC exposure may be a developmental consequence, with a sensitive period for detection right after hatching in the chick and around the time of weaning in the rat. In addition or alternatively, it may be a consequence of the recency of the drug exposure and lack of time for the advent of maturational or compensatory processes that may obviate or mask them. Regardless of the mechanism, if prenatal COC exposure alters the behavior of the neonate, even transiently and in one sex, the developmental trajectory of these affected subjects may likely be altered. In animal models, survivability may be affected, while in exposed humans, caregiver–infant relationships may be disrupted for the reasons discussed below.

The suppression of activity by embryonic COC may result from alterations in the behavioral state regulation of the chick and/or its response to novel situations. The fact that embryonic COC effects were found only on the first day of testing supports this latter interpretation. There has been a suggestion from the literature that prenatal COC exposure may alter the stress response of rats and this could interact with performance in novel environments, such as an open field. For example, adult rats prenatally exposed to COC had decreased immobility time in the Porsolt swim test (5,41) and open-field immobility following inescapable foot shock (41). These latter data suggest that exposure to a stressor may help to unmask differences in neuroendocrine function resulting from early COC exposure. There have been reported significant alterations in the neuroendocrine axis following prenatal COC exposure [i.e., (8,9)]. Thus, prenatal COC could act as a generalized maternal stressor, and, in the case of the chicken embryo, a more direct stressor on the developing nervous system, influencing postnatal behavior, especially when the organism is challenged. In addition to the number of lines crossed in the open field, distress vocalizations were also suppressed by embryonic COC treatment. In a prior study, Sparber et al. (52) found a dose-dependent decrease in distress vocalizations by chicks treated during late embryogenesis with COC when they were paired with like-treated broodmates, although there was no effect on isolation-induced vocalizations. However, Hughes et al. (24) also found a decrease in isolation-induced vocalizations in chicks exposed to COC during mid-embryogenesis. Distress vocalizations have traditionally been considered an index of isolation induced stress in a chick [i.e. (42,55)], and therefore, might be expected to increase if the COC-exposed chick is challenged by a novel environment. On the contrary, data from the present study, along with that of Sparber et al. (52) and Hughes et al. (24), suggest that the decreased vocalizations following embryonic COC exposure may have adverse consequences. By decreasing vocalizations, and thus the attention and potential protection from predators by a hen or conspecifics, embryonic COC exposure may compromise survival of a young chicken in novel surroundings "in the real world."

In addition to its effects on activity, E18 COC exposure also affected LPS-induced sickness behavior. Following LPS injection, a reduction in food consumption, decreased activity and increased sleep are seen in both rodents [i.e. (14)] and chickens (28,29). However, E18 COC exposure interfered with the expression of LPS-induced sickness behavior, allowing greater food consumption and activity, and decreasing sleep relative to control subjects injected with LPS. Sickness behaviors result from stimulation of the immune system via the direct actions of cytokines on the nervous system or via indirect actions on the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system. Manipulations, such as embryonic COC exposure, that may affect the production of cytokines, HPA axis hormones, and/or sympathetic neurotransmission, may, in turn, affect the expression of sickness behaviors. For example, administration of LPS elevates plasma corticosterone in chickens (28) and in rodents (20). If HPA axis development/function is compromised by prenatal COC treatment, as discussed above, these LPS-induced neuroendocrine changes may be abnormal. Similar arguments can be made for actions of embryonic COC on the developing sympathetic nervous and immune systems. Acting in concert, such attenuation of activity, distress vocalizations, and sickness behavior caused by embryonic COC exposure can be thought of as nonadaptive, decreasing chances of survival under conditions of predation by macro (e.g., predatory birds)- or microorganisms.

E17 RIT Exposure: Effect on COC-Suppressed Activity and LPS-Induced Sickness Behavior

E17 ritanserin exposure, at a dose of 0.4 mg/kg egg, did not affect open-field activity, or LPS-induced lowered food consumption and activity. For the open-field activity measures, this dose of ritanserin was efficacious in mitigating COC's activity suppressant effects, such that Rit-Coc treated–females did not differ from TA-Sal controls. These data suggest that 5-HT_2 receptor stimulation is involved in the etiology of the activity suppression, and the results are similar to that seen for chick detour learning, where E14 exposure to 0.3 mg ritanserin/kg egg was behaviorally inactive, but blocked the effects of the 5-HT_2 agonist DOI (7). However, the 0.4 mg/kg dose of ritanserin was not able to alter COC-related modulation of LPS-induced sickness behavior. It may be that these behaviors are not influenced by the indirect action at 5-HT_2 receptors following E18 COC exposure or that different doses of ritanserin are needed to block this effect. Because examination of a dose–response relationship was not incorporated in the present study, it is not possible to distinguish between these possibilities at present.

One finding that was particularly intriguing was the effect of E17 ritanserin exposure on LPS-induced sleep in the sickness behavior assessment. E17 ritanserin treatment, like E18 COC treatment, decreased the amount of time spent sleeping. This paradoxical effect may be a consequence of the biphasic nature of 5-HT on the immune response, whereby 5-HT receptor blockade/antagonism (3,62) and excessive stimulation (1,2,33) both lead to immunosuppression, suggesting that ritanserin may attenuate LPS effects, as it did for the sleep time measure in this study. Future studies will determine the extent of ritanserin's ability to either enhance or attenuate immunological and behavioral effects of LPS, because in the present study only a single dose was examined and the ritanserin suppression was limited to sleep time. The induction of sleep by LPS may be controlled by different mechanisms than suppression of food intake and activity which may be more sensitive to alterations in the immune system induced by both 5-HT_2 receptor blockade and excessive stimulation. Moreover, additional, perhaps even more selective, 5-HT_2 agents should be tested to determine if ritanserin's actions or lack thereof are mediated at 5-HT_2 receptors and its subtypes.

CONCLUSIONS

E18 COC exposure suppressed open-field activity in 3-day-old female and LPS-induced sickness behavior in 10-day-old male and female chicks. E17 ritanserin treatment, at a dose of 0.4 mg/kg, mitigated COC's suppression of open-field activity, but did not mitigate COC effects on LPS-induced sickness behavior. Ritanserin itself affected the amount of time spent asleep in this assessment. Because the dose of ritanserin used in the present study was only partially efficacious and had some effects of its own, future studies will examine dose–response relationships for ritanserin efficacy. Embryonic COC exposure may compromise an organism's ability to effectively respond to infectious agents by interfering with communication between the nervous, immune, and endocrine systems and thus, this model may be useful for studying a source of such health ramifications of prenatal COC exposure.

ACKNOWLEDGEMENTS

This work was supported in part by USPHS grants DA04979, DA08131, and T32 DA07097.

REFERENCES

1. Arzt, E.; Costas, M.; Finkielman, S.; Nahmod, V. E.: Serotonin inhibition of tumor necrosis factor-α synthesis by human monocytes. Life Sci. 48:2557–2562; 1991.
2. Arzt, E.; Fernández-Castelo, S.; Finocchiaro, L. M. E.; Criscuolo, M. E.; Díaz, A.; Finkielman, S.; Nahmod, V. E.: Immunomodulation by indoleamines: Serotonin and melatonin action on DNA and interferon-γ synthesis by human peripheral blood mononuclear cells. J. Clin. Immunol. 8:513–520; 1988.
3. Aune, T. M.; Golden, H. W.; McGrath, K. M.: Inhibitors of serotonin synthesis and antagonists of serotonin 1A receptors inhibit T lymphocyte function *in vitro* and cell-mediated immunity *in vivo*. J. Immunol. 153:489–498; 1994.
4. Bell, G. L.; Lau, K.: Perinatal and neonatal issues of substance abuse. Ped. Clinics North Amer. 42:261–281; 1995.
5. Bilitzke, P. J.; Church, M. W.: Prenatal cocaine and alcohol exposures affect rat behavior in a stress test (the Porsolt swim test). Neurotoxicol. Teratol. 14:359–364; 1992.
6. Bollweg, G.; Sparber, S. B.: Ritanserin blocks DOI altered embryonic motility and posthatch learning in the developing chicken. Pharmacol. Biochem. Behav. 55:397–403; 1996.
7. Bollweg, G.; Sparber, S. B.: Relationships between mid-embryonic 5-HT_2 agonist and/or antagonist exposure and detour learning by chickens. (submitted). Pharmacol. Biochem. Behav. 60:47–53; 1998.
8. Cabrera, T. M.; Levy, A. D.; Li, Q.; van de Kar, L. D.; Battaglia, G.: Cocaine-induced deficits in ACTH and corticosterone responses in female rat progeny. Brain Res. Bull. 34:93–97; 1994.
9. Cabrera, T. M.; Yracheta, J. M.; Li, Q.; Levy, A. D.; van de Kar, L. D.; Battaglia, G.: Prenatal cocaine produces deficits in serotonin mediated neuroendocrine responses in adult rat progeny: Evidence for long term functional alterations in brain serotonin pathways. Synapse 15:158–168; 1993.
10. Chasnoff, I. J.; Burns, W. J.; Schnoll, S. H.; Burns, K. A.: Cocaine use in pregnancy. N. Engl. J. Med. 313:666–669; 1985.
11. Chasnoff, I. J.; Griffith, D. R.; Freier, C.; Murray, J.: Cocaine/polydrug use in pregnancy: Two year follow-up. Pediatrics 89: 284–289; 1992.
12. Chasnoff, I. J.; Griffith, D. R.; MacGregor, S.; Dirkes, K.; Burns, K. A.: Temporal patterns of cocaine use in pregnancy. Perinatal outcome. JAMA 261:1741–1744; 1989.
13. Church, M. W.; Holmes, P. A.; Overbeck, G. W.; Tilak, J. P.; Zajac, C. S.: Interactive effects of prenatal alcohol and cocaine exposures on postnatal mortality, development, and behavior in the Long–Evans rat. Neurotoxicol. Teratol. 13:377–386; 1991.
14. Dantzer, R.; Bluthé, R. M.; Kent, S.; Goodall, G.: Behavioral effects of cytokines: An insight into mechanisms of sickness behavior. In: De Souza, E., ed. Neurobiology of Cytokines. San Diego: Academic Press; 1993:130–150.
15. Dow-Edwards, D.: Comparability of human and animal studies of developmental cocaine exposure. NIDA Res. Monogr. 164: 146–174; 1996.
16. Dunn, A. J.: Role of cytokines in infection-induced stress. Ann. NY Acad. Sci. 697:189–202; 1993.
17. Foltin, R. W.; Fischman, M. W.; Levin, F. R.: Cardiovascular effects of cocaine in humans: Laboratory studies. Drug Alcohol. Depend. 37:193–210; 1995.
18. Foss, J. A.; Riley, E. P.: Failure of acute cocaine administration to differentially affect acoustic startle and activity in rats prenatally exposed to cocaine. Neurotoxicol. Teratol. 13:547–551; 1991.
19. Fung, Y. K.; Reed, J. A.; Lau, Y.-S.: Prenatal cocaine exposure fails to modify neurobehavioral responses and the striatal dopaminergic system in newborn rats. Gen. Pharmacol. 20:689–693; 1989.
20. Goujon, E.; Parnet, P.; Aubert, A.; Goodall, G.; Dantzer, R.: Corticosterone regulates behavioral effects of lipopolysaccharide and interleukin-1β in mice. Am. J. Physiol. 269:R154–159; 1995.
21. Hart, B. L.: Biological basis of the behavior of sick animals. Neurosci. Biobehav. Rev. 12:123–137; 1988.
22. Hart, B. L.: Behavioural defense against parasites: Interaction with parasite invasiveness. Parasitology 109:S139–151; 1994.
23. Hoyme, H. E.; Jones, K. L.; Dixon, S. D.; Jewett, T.; Hanson, J. W.; Robinson, L. K.; Msall, M. E.; Allanson, J. E.: Prenatal cocaine exposure and fetal vascular disruption. Pediatrics 85:743–747; 1990.
24. Hughes, R. A.; Cunnick, J. E.; Kojic, L. D.: Cocaine during embryogenesis alters social, defensive, and immunologic responses in a precocial avian model. Exp. Clin. Psychopharmacol. 2:396–404; 1994.
25. Jacobson, S. W.; Jacobson, J. L.; Sokol, R. J.; Martier, S. S.; Chiodo, L. M.: New evidence for neurobehavioral effects of *in utero* cocaine exposure. J. Pediatr. 129:581–90; 1996.
26. Johns, J. M.; Means, M. J.; Anderson, D. R.; Means, L. W.; McMillen, B. A.: Prenatal exposure to cocaine II: Effects on open-field activity and cognitive behavior in Sprague–Dawley rats. Neurotoxicol. Teratol. 14:343–349; 1992.
27. Johns, J. M.; Means, L. W.; Means, M. J.; McMillen, B. A.: Prenatal exposure to cocaine I: Effects on gestation, development, and activity in Sprague–Dawley rats. Neurotoxicol. Teratol. 14:337–342;1992.
28. Johnson, R. W.; Curtis, S. E.; Dantzer, R.; Bahr, J. M.; Kelley, K. W.: Sickness behavior in birds caused by peripheral or central injection of endotoxin. Physiol. Behav. 53:343–348; 1993.
29. Johnson, R. W.; Curtis, S. E.; Dantzer, R.; Kelley, K. W.: Central and peripheral prostaglandins are involved in sickness behavior in birds. Physiol. Behav. 53:127–131;1993.
30. Kent, S.; Kelley, K. W.; Dantzer, R.: Effects of lipopolysaccharide on food-motivated behavior in the rat are not blocked by an interleukin-1 receptor antagonist. Neurosci. Lett. 145:83–86; 1992.
31. Kim, D. G.; Sparber, S. B.: Ritanserin blocks cocaine's motility depressant and lethal effects upon chicken embryos. (submitted). In preparation.
32. Kunko, P. M.; Wallace, M. J.; Robinson, S. E.: Gestational cocaine and ethanol exposure alter spontaneous and cocaine-induced behavior in weanling rats. Pharmacol. Biochem. Behav. 55:559–564; 1996.
33. Kut, J. L.; Young, M. R. I.; Crayton, J. W.; Wright, M. A.; Young, M. E.: Regulation of murine T-lymphocyte function by spleen cell derived and exogenous serotonin. Immunopharmacol. Immuntoxicol. 14:783–796;1992.
34. Kuwahara, M. D.; Sparber, S. B.: Behavioral consequences of embryonic or early postnatal exposure to *l*-α-noracetylmethadol

(NLAAM) in the domestic chicken. Neurobehav. Toxicol. Teratol. 4:323–329; 1982.
35. Kuwahara, M. D.; Sparber, S. B.: Prenatal withdrawal from opiates interferes with hatching of otherwise viable chick fetuses. Science 212:945–947; 1981.
36. Kuwahara, M. D.; Sparber, S. B.: Continuous exposure of the chick embryo to *l*-α-noracetylmethadol does not alter brain protein or nucleic acid content. Dev. Pharmacol. Ther. 3:12–24; 1981.
37. Laferriére, A.; Ertug, F.; Moss, I. R.: Prenatal cocaine alters openfield behavior in young swine. Neurotoxicol. Teratol. 17:81–87; 1995.
38. Lester, B. M.; LaGasse, L.; Freier, K.; Brunner, S.: Studies of cocaine-exposed infants. NIDA Res. Monogr. 164:175–210; 1996.
39. Lindenberg, C. S.; Alexander, E. M.; Gendrop, S. C.; Nencioli, M.; Williams, D. G.: A review of the literature on cocaine abuse in pregnancy. Nurs. Res. 40:69–75; 1991.
40. Lutiger, B.; Graham, K.; Einarson, T. R.; Koren, G.: Relationship between gestational cocaine use and pregnancy outcome: A meta-analysis. Teratology 44:405–414; 1991.
41. Molina, V. A.; Wagner, J. M.; Spear, L. P.: The behavioral response to stress is altered in adult rats exposed prenatally to cocaine. Physiol. Behav. 55:941–945; 1994.
42. Nelson, E.; Panksepp, J.; Ikemoto, S.: The effects of melatonin on isolation distress in chickens. Pharmacol. Biochem. Behav. 49:327–333; 1994.
43. Plata-Salaman, C. R.: Interferons and central regulation of feeding. Am. J. Physiol. 32:R1222–R1227; 1992.
44. Richardson, G. A.; Hamel, S. C.; Goldschmidt, L.; Day, N. L.: The effects of prenatal cocaine use on neonatal behavioral status. Neurotoxicol. Teratol. 18:519–528; 1996.
45. Riley, E. P.; Foss, J. A.: Exploratory behavior and locomotor activity: A failure to find effects in animals prenatally exposed to cocaine. Neurotoxicol. Teratol. 13:553–558; 1991.
46. Sandstrom, L. P.; Pennington, S. N.: Embryonic growth inhibition induced by cocaine is associated with suppression of ornithine decarboxylase activity. Proc. Soc. Exp. Biol. Med. 202:491–498; 1993.
47. Singer, L.; Arendt, R.; Minnes, S.: Neurodevelopmental effects of cocaine. Clin. Perinatol. Care 20:245–262; 1993.
48. Singer, L. T.; Yamashita, T.; Hawkins, S.; Cairns, D.; Baley, J.; Kliegman, R.: Increased incidence of intraventricular hemorrhage and developmental delay in cocaine-exposed, very low birth weight infants. J. Pediatr. 124:765–71; 1994.
49. Smith, R. F.; Mattran, K. M.; Kurkjian, M. F.; Kurtz, S. L.: Alterations in offspring behavior induced by chronic prenatal cocaine dosing. Neurotoxicol. Teratol. 11:35–38; 1989.
50. Snodgrass, S. R.: Cocaine babies: A result of multiple teratogenic influences. J. Child Neurol. 9:227–233; 1994.
51. Sparber, S. B.; Lichtblau, L.; Kuwahara, M. D.: Experimental separation of direct and indirect effects of drugs upon neurobehavioral development. In: Krasnegor, N. A.; et al., eds. Advances in Behavioral Pharmacology, vol V: Developmental Behavioral Pharmacology. Hillsdale, NJ: Lawrence Erlbaum Associates; 1986:225–263.
52. Sparber, G. R.; Lauerman, M.; Kim, D. G.; Sparber, S. B.: Injection of cocaine into eggs on day 19 of development suppresses chicken embryonic motility and alters detour learning and distress vocalization 1–2 weeks postnatally. FASEB J. 5:A1588; 1988.
53. Sparber, S. B.; Rizzo, A.; Berra, B.: Excessive stimulation of serotonin$_2$ (5-HT$_2$) receptors during late development of chicken embryos causes decreased embryonic motility, interferes with hatching, and induces herniated umbilici. Pharmacol. Biochem. Behav. 53:603–611; 1996.
54. Sparber, S. B.; Wasserman, A.; Bollweg, G.: Ritanserin (RIT) blocks the vasoconstriction caused by injection of cocaine (COC) into chicken eggs with 15 day old embryos. NIDA Res. Monogr. 153:169; 1995.
55. Sufka, K. J.; Hughes, R. A.; McCormick, T. M.; Borland, J. L.: Opiate effects on isolation stress in domestic fowl. Pharmacol. Biochem. Behav. 49:1011–1015; 1994.
56. Vallortigara, G.; Zanforlin, M.: Open-field behavior of young chicks (*Gallus gallus*)*:* Antipredatory responses, social reinstatement motivation, and gender effects. Animal Learn. Behav. 16: 359–362; 1988.
57. Vorhees, C. V.: Long-term effects of developmental exposure to cocaine on learned and unlearned behaviors. NIDA Res. Monogr. 164:3–52; 1996.
58. Vorhees, C. V.; Reed, T. M.; Acuff-Smith, K. D.; Schilling, M. A.; Cappon, G. D.; Fisher, J. E.; Pu, C.: Long-term learning deficits and changes in unlearned behaviors following *in utero* exposure to multiple daily doses of cocaine during different exposure periods and maternal plasma concentrations. Neurotoxicol. Teratol. 17:253–264; 1995.
59. Watzl, B.; Watson, R. R.: Immunomodulation by cocaine—A neuroendocrine mediated response. Life Sci. 46:1319–1329; 1990.
60. Webster, W. S.; Brown-Woodman, P. D. C.; Lipson, A. H.; Ritchie, H. E.: Fetal brain damage in the rat following prenatal exposure to cocaine. Neurotoxicol. Teratol. 13:621–626; 1991.
61. Wiggins, R. C.; Rolsten, C.; Ruiz, B.; Davis, C. M.: Pharmacokinetics of cocaine: Basic studies of route, dosage, pregnancy and lactation. Neurotoxicology 10:367–381; 1989.
62. Young, M. R. I.; Kut, J. L.; Coogan, M. P.; Wright, M. A.; Young, M. E.; Matthews, J.: Stimulation of splenic T-lymphocyte function by endogenous serotonin and by low-dose exogenous serotonin. Immunology 80:395–400; 1993.
63. Zhang, X.; Schrott, L. M.; Sparber, S. B.: Evidence for a serotonin-mediated effect of cocaine causing vasoconstriction and herniated umbilici in chicken embryos. (in press). Pharmacol. Biochem. Behav. 59:585–593; 1998.