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Relationships Between Midembryonic 5-HT_2 Agonist and/or Antagonist Exposure and Detour Learning by Chickens

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BOLLWEG, G. AND S. B. SPARBER. *Relationships between midembryonic 5-HT_2 agonist and/or antagonist exposure and detour learning by chickens.* PHARMACOL BIOCHEM BEHAV **60**(1) 47–53, 1998.—The importance of serotonin (5-HT) as both a transmitter and a regulatory signal during development of many species is well established. The availability of 5-HT receptor subtype agonists and antagonists will enable pharmacological dissection of the importance of one or more of the 5-HT receptors for their involvement in the mediation of developmental insults by drugs that are less selective but include actions upon serotonergic function. Such insults include exposure to cocaine or opiate withdrawal, both of which are blocked or attenuated by 5-HT_2 antagonists. The 5-HT_2 receptor agonist dimethoxyiodophenylaminopropane (DOI), like cocaine, causes vasoconstriction during embryogenesis, herniated umbilici in hatchlings, and altered detour learning by young chickens after injection into eggs at late stages of embryogenesis. The 5-HT_2 antagonist ritanserin (RIT) blocks or significantly attenuates these effects. This study describes an effect of DOI on posthatch detour learning when injected earlier during embryogenesis (i.e., on embryonic day 12, E12) which is opposite its effect when injected later (i.e., on E15). Both effects are blocked by an inactive dose of RIT (0.3 mg/kg egg) and by a higher dose of RIT (0.9 mg/kg egg), which itself retards posthatch detour learning following E12 injection. Thus, excessive stimulation or blockade of 5-HT_2 receptors around midembryogenesis can cause a similar behavioral teratogenic outcome. The data are discussed in relation to the likelihood that potential use of 5-HT_2 antagonists for treating pregnant women and their fetuses who are not at risk is nil. © 1998 Elsevier Science Inc.

5-HT_2 receptor Cocaine Developmental toxicity Behavior

ONE of cocaine's pharmacologic actions is to block synaptic reuptake of monoamine neurotransmitters including serotonin (5-hydroxytryptamine, 5-HT). Our laboratory (2,28–30) and others (1,3–5,8) have been studying the relationship between perturbed 5-HT function during development and the potential developmental toxicity of cocaine. The central nervous system (CNS) may be especially susceptible to such insults because of its lengthy development and intricate anatomy/physiology (24,27,34).

Because of similarities between the acute effects of cocaine and the expression of opiate withdrawal, including signs indicative of autonomic nervous system and cardiovascular activation, our work has focused on the potential developmental consequences of stimulation and blockade of the 5-HT_2 receptor. 5-HT_2 receptors are involved in vasoconstriction mediated by 5-HT (25), the 5-HT_2 agonist dimethoxyiodophenylaminopropane, DOI (36), and cocaine (29,36). Drugs that block 5-HT_2 receptors have also proven effective in blocking the expression of true and quasi-opiate withdrawal (10,18–20). More recently, we have observed that the 5-HT_2 antagonist ritanserin (RIT) antagonized some developmental toxicity of cocaine (28,29,36).

To examine direct effects of altered 5-HT_2 receptor stimulation or blockade during development and avoid potential confounding factors inherent in mammalian studies (e.g., from maternal–fetal interactions), we have used the developing chicken as an experimental subject, injecting DOI or RIT into eggs with embryos at various stages of development. Because fertilized chicken eggs develop independently in incubators, use of this species avoids another complication of mammalian studies, incorrect attribution of litter effects (between-litter variance) to experimental treatment (9).

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We have carried out preliminary experiments in which the more selective, direct acting 5-HT_2 agonist DOI was used to determine if its effects upon embryonic motility, vasoconstriction of extraembryonic blood vessels, capacity to cause herniated umbilici, and altered behavior of hatchlings when injected into chicken eggs with embryos later during embryonic development (e.g., embryonic day 15, E15, or E18) are similar to effects caused by injection of cocaine at these stages of development (2,29,30,36). The combined outcomes of these experiments strongly suggest that many of cocaine's acute and short-term (i.e., early perinatal) toxic effects in this species are mediated in great part by excessive indirect stimulation of 5-HT_2 receptors.

Another reason for interest in the possible effects of excessive developmental stimulation (or blockade) of 5-HT receptors is that in addition to its transmitter role, 5-HT functions as a regulatory signal or neurotrophic factor during development (11,12). Thus, agents that substantially modify 5-HT–related processes during development could also modify structural organization and function. Administration of the 5-HT–depleting drug parachlorophenylalanine (which inhibits tryptophan hydroxylase, the rate-limiting enzyme for 5-HT synthesis) in the chicken embryo indirectly affects the enzyme necessary for synthesis of the transmitter acetylcholine (choline acetyltransferase) after, but not before, E12. This suggests that 5-HT stimulates cholinergic neurotransmission necessary for maturation of motoneurons that allow more coordinated motor control in preparation for hatching (31). 5-HT injected into egg during E7–E14 resulted in lethality that depended on the day of administration, suggesting a transient increase in 5-HT receptors in middevelopment (35). 5-HT immunopositive neurons in chick spinal cord increased from E5–9, plateaued at E9–E12, then decreased from E12–E17 before rising again from E17 until E21, the day of hatching (32). Taken together, these results suggest that 5-HT's role changes rapidly and transiently in mid-development of the chicken embryo, and that agents modifying 5-HT function at that time could modify 5-HT–dependent processes, with potential short and long-term consequences.

We recently reported that acute motility suppression and altered [enhanced; see Discussion in (2)] posthatch detour learning followed E15 injection of 1.0 mg DOI/kg egg into eggs with embryos, an effect blocked by treatment 1 h later with RIT (0.3 or 0.9 mg/kg egg) (2). To further characterize mid-development, we hypothesized that the same treatment earlier, at E12, may result in a different outcome due to a smaller and/or less functional 5-HT_2 receptor population on E12 compared to that on E15.

The purpose of the present experiment was to determine whether E12 injection of DOI into eggs with embryos would affect motility recorded 2.5 h later, hatchability, or posthatch detour learning, and if so, whether either of the RIT doses could block one or more of DOI's effects on these variables. Another experimental question was whether these RIT doses themselves might affect any of the dependent variables. Although we have observed few effects on such variables after injecting a range of RIT doses (0.1–2.7 mg/kg egg; unpublished observations) on E14, we have not investigated consequences of earlier RIT administration.

METHOD

Pre- and posthatch test methods and treatment of subjects were similar to those used previously and described in detail elsewhere (2). A shortened description is given below.

Subjects and Their Treatment

Eggs with fertilized chicken embryos (Babcock B300 strain) were obtained from ASP Hatchery, Silver Lake, MN, on embryonic day 8 (E8) and incubation was continued in our laboratory in a rotating forced air incubator. Eggs were candled for viability (~95%) and nonviable eggs were discarded. One to 2 days before drug administration holes were drilled in eggshells for injections, using care to avoid puncturing the membranes beneath the shell. Holes were then covered with a small piece of transparent tape. Average egg weight on E10–E11 was ~56 g. Eggs were numbered and randomly assigned to six treatment groups for drug administration and motility recording (n = 8/group): 1) saline-Tartrate 0.05 M; 2) saline-RIT 0.3 mg/kg egg; 3) saline-RIT 0.9 mg/kg egg; 4) DOI 1.0 mg/kg egg-Tartrate; 5) DOI 1.0 mg/kg egg-RIT 0.3 mg/kg egg; 6) DOI 1.0 mg/kg egg-RIT 0.9 mg/kg egg.

In addition to subjects for which motility was recorded (six groups, n = 8/group), another identical set (n = 8/group) was injected at the same time for later hatchability observations (n = 16/group) and detour learning. Thus, of all subjects used for hatchability and detour learning, half in all groups were used for recording their motility after drug injections on E12, 12 h. Subjects from both sets were used to determine if treatments substantially affected hatchability and detour learning. Except for brief transfer and handling periods (e.g., injections), subjects were maintained in heated, humidified incubators throughout the experiment.

Drug solutions were prepared the day of the motility experiment and kept chilled on ice before administration. Avian saline (0.85% NaCl) or DOI HCl (RBI Inc., Natick, MA) were administered in 20 μl injection volumes with 50 μl Hamilton syringes (Reno, NV). RIT (RBI Inc., Natick, MA) or its tartrate vehicle (0.5 M (+)-tartartic acid, Calbiochem, Los Angeles, CA) were injected in 40 μl injection volumes.

Motility Recordings

Motility, measured as changes in amplified voltages derived from embryonic movement, was recorded as previously described (2) with the addition of another recording channel that allowed two subjects to be recorded simultaneously. Channel function and amplification were confirmed each day before recordings with a reference device emitting a fixed 3 Hz, 1 mV signal connected to the recording electrodes and visualized on the display grid of a Philips PM3335 oscilloscope (Philips, Enschede, The Netherlands). Although amplification was verified in both channels prior to use, the possibility that small sensitivity differences might systematically affect the outcome was controlled by recording four subjects from each group on both channels (4 subjects × 2 channels × 6 groups = 48 subjects recorded). To control for possible effects of age differences at recording time, recordings were scheduled such that half were made before E12,12 h and half after E12,12 h.

During recordings eggs were placed on a triangular configuration of phonograph cartridges to minimize transmission of ambient room vibration to eggs. Two 28-gauge platinum wire electrodes were inserted 2–3 mm into the holes drilled earlier to detect electric potential produced by embryonic movement. Voltage detected by the electrodes was amplified and filtered, then sent to an analog-to-digital converter (MacADIOS 8AIN, GW Instruments, Somerville, MA). The digital signal was analyzed with a commercial wave analysis application (Superscope, GW Instruments, Somerville MA) run on a Macintosh IIci computer (Apple Computer, Cupertino, CA). Electrical signals were recorded as minimum (Min), maximum

(Max), range (Max-Min), and standard deviation (SD; all measures in volts) data in spreadsheet format for later statistical analysis. Recordings were comprised of 20 15-s "waves" at an 80 Hz sampling rate (5 min total recording time). Thus, for each 15-s "wave," there was one each of Min, Max, Range, and SD. The SD value was based upon $80 \times 15 = 1200$ voltage samples and used as a measure of variability about a value of zero volts.

Drug injections and motility recordings were done as follows: eggs were removed from the incubator, DOI or saline were injected into eggs, and eggs were replaced. One hour later the same eggs were removed from the incubator and injected with RIT or tartrate and again replaced. Two and a half hours after DOI or saline (1.5 h after RIT or tartrate) eggs were placed on the recording cradles and electrodes were inserted with micromanipulators. After a 5-min acclimation period, motility was recorded (20 15-s "waves," 5 min total recording time). After recordings were completed, eggs were returned to the rotating incubator until E18, when they were placed in a hatcher and observed for hatchability.

Motility Statistics

Data were transferred to a statistical analysis application (Statview II, Abacus Concepts, Berkeley, CA) and analyzed by analysis of variance (ANOVA). Planned group mean comparisons (saline-tartrate vs. each of saline-RIT 0.3, saline-RIT 0.9, DOI 1.0-Tartrate, DOI 1.0-RIT 0.3, DOI 1.0-RIT 0.9) were made with Dunnett's test and nonparametric (Kruskal–Wallis, Mann–Whitney *U*) tests.

Hatchability and Statistical Analysis: Posthatch Weighing, Banding, and Housing

Eggs were checked twice per day for hatchlings on E19–E21. Potential treatment effects on hatchability were assessed by chi-square analysis. After hatching, chicks were weighed and fitted with numbered leg bands for identification, then placed in a heated, five-level community brooder furnished with ad lib food and water. Hatchling body weight was analyzed by one-factor ANOVA. Chicks were separated into two sets for alternate day detour learning sessions (there were too many to test on one day) the day before detour learning sessions began.

Detour Learning

The night before posthatch day 6 (set 1) or 7 (set 2), chicks were deprived of food in preparation for detour learning testing the next day. Set 1 was tested on posthatch days 6, 8, and 10; set 2 was tested on posthatch days 7, 9, and 11. The detour learning apparatus is a fluorescently illuminated metal enclosure with a lid, separated into two compartments (social and isolation sides) by a Plexiglas wall (2). To return to the social side, isolated subjects must turn away from the transparent wall and detour through the open tunnel. Under these conditions the opportunity for access to food and broodmates are appropriate stimuli for reinforcing the detour response, resulting in shorter latencies as subjects learn to detour.

Six chicks (one from each treatment group) were randomly selected from the community brooder and placed on the social side of the detour apparatus, which contained a Petri plate with a small amount of moistened chick food. Subjects were allowed access to the food and social reinforcement for 30 s, after which one was selected and placed in the center of the isolation side of the apparatus. This subject was allowed 180 s to face away from the reinforcing complex and detour through the open tunnel. If no detour response was made during this time, its latency was recorded as the maximum 180 s and the subject was gently guided through the tunnel with a wooden ruler, terminating the trial. This sequence was repeated with a subject from the next group until each of the six chicks had received four trials. The colored, numbered leg bands allowed the experimenter to control for order effects by systematically rotating the sequence of testing for each clutch of six chicks. They were then returned to another brooder level furnished with water and ad lib food, and another group of six food-deprived chicks was selected. The procedure was repeated until all 36 chicks in the group had completed four trials. After 6 days of testing each chick in the six treatment groups ($n = 12$/group) received 12 trials.

Detour Learning Statistics

Response latency (s) was recorded with a stopwatch. Latency data were analyzed by two-factor (treatment, trial) repeated-measures ANOVA. Overall detour performance was evaluated by averaging each chick's latency across all 12 trials and comparing group means for this measure by one-factor (treatment) ANOVA, followed by Dunnett's test.

RESULTS

Motility

To detect possible effects of recording channel that may have confounded treatment effects, voltage (motility) data were analyzed with two-factor (treatment, channel) ANOVA. There was no effect of treatment or channel, or any treatment by channel interaction (data not shown). Channel data were thus combined and analyzed by one-factor (treatment) ANOVA. There were no overall effects of treatment by one-factor ANOVA for any of the motility measures: minimum voltage, $F(5, 42) = 0.37$, $p = 0.87$; maximum voltage, $F(5, 42) = 0.80$, $p = 0.56$; range voltage, $F(5, 42) = 0.57$, $p = 0.73$; SD voltage $F(5, 42) = 0.52$, $p = 0.77$. None of the planned comparisons (saline-Tartrate vs. each of the other five groups) approached statistical reliability. Because the experimental design precluded using motility data prior to drug injections as a baseline, the data were highly variable, with coefficients of variation ranging from 23–56%, indicative of large individual differences (Table 1). Data were thus analyzed with nonparametric tests (Kruskal–Wallis, Mann–Whitney *U*-tests). None of these tests approached statistical reliability for any of the motility measures (Min, Max, Range, and SD voltage). Therefore, there were no apparent effects on motility monitored shortly after treatment at this age.

Hatchability and Herniated Umbilici

Although there was a small reduction in hatchability due to drug treatment (Table 2), chi-square analysis showed no overall effect of treatment on hatchability (chi-square = 6.85, $df = 5$, $p = 0.23$). Hatchability data are shown in Table 2.

Two herniated umbilici were observed in the DOI 1.0-Tartrate group (none in other groups), resulting in a nearly significant overall effect on this variable (overall chi-square = 10.21, $df = 5$, $p = 0.07$).

Hatchling Body Weight

Chicks were weighed shortly after hatching. Analysis by one-factor ANOVA showed no effects of any treatment. Av-

TABLE 1

NEITHER DOI (1.0 mg/kg EGG) NOR RITANSERIN (RIT, 0.3, 0.9 mg/kg EGG) AFFECT E12 EMBRYONIC MOTILITY (2.5 H AFTER DOI, 1.5 H AFTER RIT)

	Motility Measure (Mean ± SD, Volts)			
Treatment	Minimum	Maximum	Range	Standard Deviation
Saline-tartrate	−0.386 ± 0.177	0.368 ± 0.198	0.754 ± 0.373	0.165 ± 0.087
Saline-RIT 0.3	−0.441 ± 0.247	0.418 ± 0.162	0.860 ± 0.407	0.182 ± 0.091
Saline-RIT 0.9	−0.377 ± 0.183	0.351 ± 0.198	0.728 ± 0.378	0.145 ± 0.071
DOI 1.0-tartrate	−0.317 ± 0.098	0.256 ± 0.060	0.573 ± 0.154	0.126 ± 0.037
DOI 1.0-RIT 0.3	−0.384 ± 0.194	0.357 ± 0.185	0.741 ± 0.377	0.167 ± 0.084
DOI 1.0-RIT 0.9	−0.403 ± 0.194	0.376 ± 0.179	0.778 ± 0.366	0.155 ± 0.072

Values represent mean ± SD Voltage for each group of eight subjects.

erage hatchling weight in the control group was 41.8 ± 3.4 g (mean ± SD, $n = 16$).

Detour Learning

Detour learning results are depicted in Fig. 1. Detour response latency data (s) were analyzed by two-factor repeated-measures ANOVA, with treatment as the fixed factor. There were significant effects of treatment, $F(5, 66) = 4.39$, $p < 0.01$, and repeated latency reduction over trials 1–12 as the detour response was acquired. There was no treatment by repeated measures interaction, $F(55, 726) = 1.12$, $p = 0.26$. Because the ANOVA showed an effect of treatment, it was of interest to determine which treatments differed. Mean latencies for each group averaged across all 12 trials were compared with saline-Tartrate controls via Dunnett's test. Latencies were increased in the DOI 1.0-Tartrate group compared to controls (121.9 s vs. 75.7 s, respectively). This increase was blocked by both RIT doses (DOI 1.0-RIT 0.3, 78.0 s; DOI 1.0-RIT 0.9, 93.4 s). Mean latency in the saline-RIT 0.3 group (86.1 s) did not differ from controls, but it was elevated in the saline-RIT 0.9 group (134.1 s) compared to controls (75.7 s). Thus, large latency reductions (learning) were observed across trials for all groups, with increased latency (retarded learning) in the DOI 1.0-Tartrate and saline-RIT 0.9 groups, compared to controls.

DISCUSSION

The behavioral consequences of relatively selective developmental stimulation or blockade of 5-HT_2 receptors were investigated following injection of a 5-HT_2 agonist and 5-HT_2 antagonist into eggs with chicken embryos on E12. As in a prior study carried out with 15-day-old embryos, we administered DOI (1.0 mg/kg egg) and 1 h later, the 5-HT_2 antagonist RIT (0.3 or 0.9 mg/kg egg) or its vehicle. We found no evidence of short-term (within 2.5 h) effects on embryonic motility, or substantially altered overall hatchability 8–9 days later, though samples were small and the latter conclusion should be tempered accordingly. However, significant functional effects of the agonist or the higher dose of the antagonist were detectable 1–2 weeks after hatching. Detour response latencies of DOI-exposed subjects were significantly increased, an indicator of impaired performance of a simple learning task. RIT blocked the DOI effect and had no effect when administered at the lower dose, but the higher RIT dose, which also blocked DOI's effect, impaired acquisition of the detour response.

TABLE 2

HATCHABILITY AFTER E12 EXPOSURE TO DOI 1.0 mg/kg EGG, FOLLOWED 1 H LATER BY EITHER 0.05 M TARTRATE OR RIT 0.3, 0.9 mg/kg EGG

Treatment	Hatched	% Hatched
Saline-tartrate	16/16	100
Saline-RIT 0.3	15/16	94
Saline-RIT 0.9	14/16	88
DOI 1.0-tartrate	14/16	88
DOI 1.0-RIT 0.3	12/16	75
DOI 1.0-RIT 0.9	12/16	75

The results both resemble and differ from our earlier work (30) in which DOI 2.5–25.0 mg/kg egg was administered as single doses at various stages of embryogenesis. Observations following those treatments included frank lethality at higher doses, interference with the hatching process at lower doses injected late during embryogenesis, or after high doses injected on E3, and induction of herniated umbilici. RIT attenuated these gross measures of DOI toxicity. Unlike higher doses used in that experiment, the effect of DOI 1.0 mg/kg egg injected on E12 in the present experiment was equivocal with regard to induction of herniated umbilici and hatchability. However, effects of this dose injected on E12 were robust 2–3 weeks later when detour learning was assessed. Like our earlier report (30) in which RIT attenuated DOI hatch suppression and induction of herniated umbilici, both RIT doses blocked DOI's effects on detour learning. However, the RIT 0.9 mg/kg egg dose appeared to be unnecessarily high and also suppressed learning.

The present results also illustrate the dependence of developmental toxicity on timing of exposure to DOI and/or RIT: 1) DOI 1.0 mg/kg egg injected on E15 significantly reduced embryonic motility recorded 2.5 h later (2), while the same dose injected on E12 had no effect (Table 1); and 2) DOI 1.0 mg/kg egg injected on E12 increased detour learning latency relative to saline-Tartrate treated controls (Fig. 1), while an opposite effect upon performance was observed following E15 injection. A simple explanation for the differences is that the 5-HT_2 receptor population is immature at E12 (e.g., fewer receptors or weaker transduction capabilities), but more mature at E15. If a functional 5-HT_2 receptor population mediates motility suppression, its absence on E2 and presence on E15 might account for DOI 1.0 mg/kg egg suppressing motility, while the same dose injected on E12 had no effect. How-

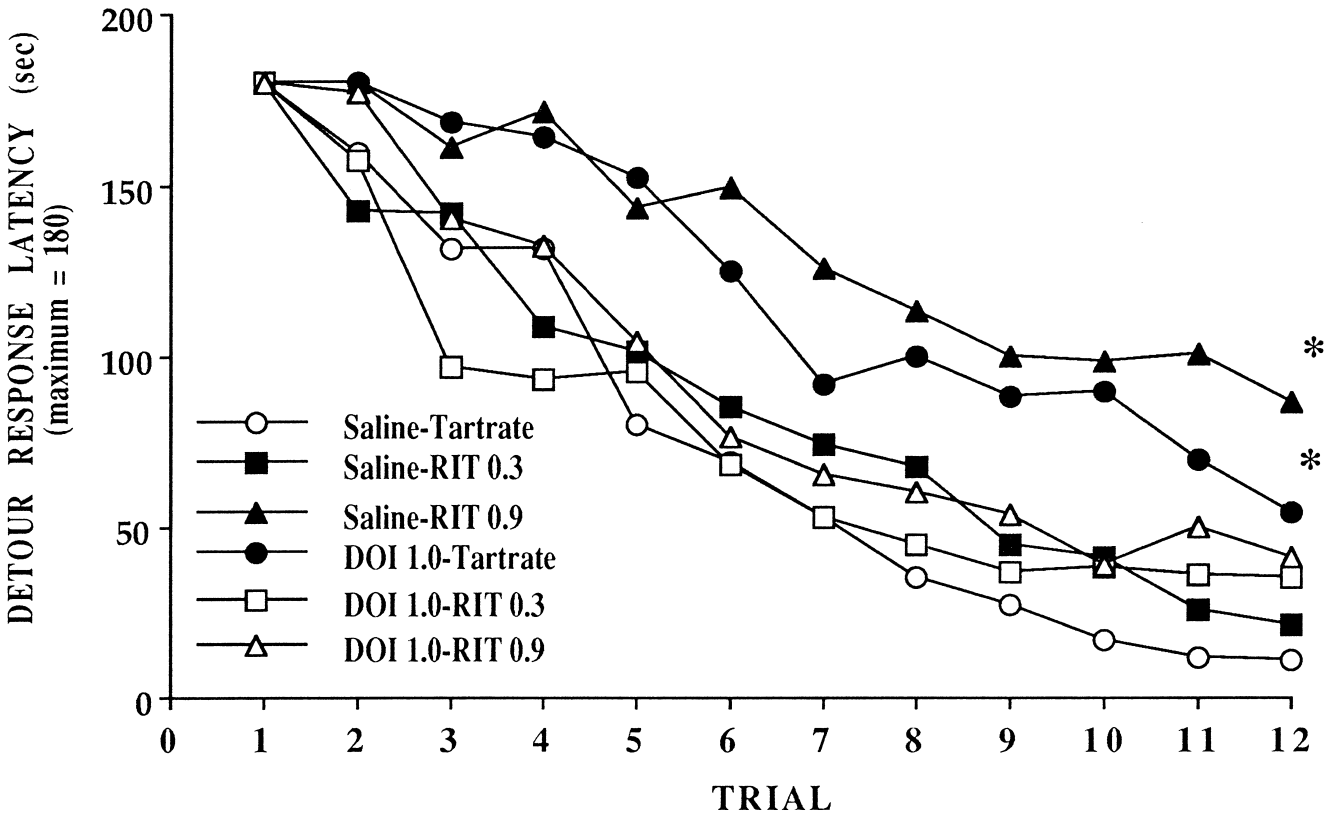

FIG. 1. DOI modifies posthatch days 6–11 detour learning after injection into eggs with developing chicken embryos on day 12 of embryogenesis (E12), an effect blocked by both doses of RIT; however, the high RIT dose also retards detour learning. Each point depicts the mean latency (s) for a group of 12 chicks. *$p < 0.01$ for effect of treatment vs. saline-Tartrate by two-factor repeated measures ANOVA.

ever, interpretation of the present results is complicated with regard to when the biologic insult that resulted in altered detour learning actually occurred. Because there was no embryonic motility suppression 2.5 h after DOI was injected on E12 (1.5 h after RIT), it is not known whether distribution and embryonic absorption of the drug had occurred at that time. Thus, the biologic effect resulting in altered detour learning could have occurred after E12, confounding the comparison of equal injected doses at different developmental stages that may result in very different absorbed or target organ concentrations. Metabolism of drugs also depends on developmental stage. Other workers (14) have reported that chick cytochrome P-450 metabolism is only weakly induceable by phenobarbital until ~E18, making it unlikely that DOI or RIT were rapidly metabolized after E12 administration, and supporting the notion of a delayed effect. Alternatively, the drug may be distributed 2.5 h after E12 injection, but the 5-HT_2 receptor population may not be functional. Our earlier finding that injection of 1.0 mg DOI/kg egg on E15 results in motility suppression 2.5 h later suggests that the drug effect(s) that altered detour learning in the present experiment occurred between E12 and E15. This ambiguity might have been reduced had motility been assessed in these subjects at both E15 and E12.

The present finding that RIT 0.3 and 0.9 mg/kg egg injected on E12 blocked DOI's effects on altered detour learning, but that the 0.9 RIT dose itself affected learning also allows more than one interpretation. As occurred after E15 injection of DOI at a dose of 1.0 mg/kg egg (2), treatment 1 h later with both RIT doses blocked DOI-mediated changes in posthatch detour learning no matter how DOI's effect upon this variable was manifest. However, the present observation of a posthatch detour learning effect of 0.9 mg RIT/kg egg injected on E12 does not indicate when that effect occurred, or even that it was mediated by 5-HT_2 receptors. Although relatively selective, RIT can interact with histamine H1, alpha-2 adrenergic and dopamine D_2 receptors, as well as the 5-HT_2 receptors (13). Thus, in addition to binding transient or developing 5-HT_2 receptors, RIT could interact with other transmitter systems and alter long-term function. In other words, it may be that altered detour learning in the saline-RIT 0.9 group is a nonspecific effect of too high a dose (i.e., a dose at least three times that necessary for blocking the 5-HT_2 agonist's effect). From a therapeutic perspective, it should be remembered that RIT would not be used unless there were real or threatened pathophysiological outcomes of pregnancy (e.g., risks modeled by DOI).

Also of interest are the very large detour learning latency differences between control subjects in the present experiment [e.g., trial 8, ~40 s) and controls in our earlier work (trial 8, ~125 s; (2)]. This occurred despite very similar conditions in both experiments (e.g., age of subjects during detour learning, administered drug dose, experimenter, laboratory, apparatus). Several factors may account for this difference. First, there were six chicks per detour learning "clutch" in the present experiment, while there were four chicks per "clutch" in the other experiment, resulting in variation in the amount

of time between trials during detour learning testing. This allowed more time for establishment of stronger working memory or memory consolidation in subjects in the present experiment, which may have resulted in acquisition of the detour response after fewer trials. Second, there were slight differences in chick strains, e.g., White Leghorn vs. Babcock B300 in the present experiment. Last, there were seasonal differences, i.e., late summer (2) vs. late winter in the present experiment. The large response variation may have resulted, in part, from such factors or their interaction, reinforcing the need for contemporaneous controls in behavioral teratology experiments.

The utility of more than one behavioral outcome measure and testing at more than one time during development is apparent from the present results. Had the detour learning assessment not been made, one might have concluded that there was no treatment effect of agonist or antagonist, at the doses used, following injection on E12. The present findings support other work suggesting that selectively perturbing 5-HT_2 receptors during development may result in "silent damage" (23) that may manifest at any time during the lifespan. For example, neither 5-$HT_{2A/2C}$ receptor density or adrenocorticotropin (ACTH) response to DOI was affected at postnatal day 28 in male rat progeny of pregnant rats exposed to fluoxetine during mid–late gestation, but at postnatal day 70, both receptor density (−35%) and ACTH response (−58%) were decreased (4). Developmental exposure to less selective drugs, such as cocaine, which initially affect many transmitter systems, may also lead to potentially detrimental, although probably more complex consequences, in part due to interaction with other neurotransmitter systems in addition to 5-HT. This may have also been the case after the apparently unnecessarily high RIT dose (0.9 mg/kg egg) in the present study.

If any of the treatments described in the present results led to 5-HT release from affected neurons or other cells, such cells, if migrating, could be arrested, because 5-HT added to cultures of actively growing neurons causes cessation of neurite elongation in certain 5-HT subtypes (7,33). Because nonserotonergic receptors and transmitters may have also been perturbed by the treatments in the present experiment, a multitude of other cellular functions could also have been affected (15), potentially modifying developing structures and their subsequent function.

In summary, we found posthatch functional consequences following administration of a 5-HT_2 agonist (DOI) and antagonist (RIT) during midembryogenesis in the chicken. Such effects vary with dose and developmental stage, and may be delayed. Because cocaine is an indirect 5-HT agonist, developmental cocaine exposure may initiate consequences that are detectable with tests of function (e.g., behavior), but that are not yet observable with other methods. In addition, RIT has been proposed as supportive pharmacotherapy for drug abuse (16), and tested for efficacy in treatment of alcoholism (17), anxiety (22), negative symptoms of schizophrenia (6), and sleep disturbances (26). The present results suggest that functional or behavioral teratogenicity should be considered during preclinical and clinical testing of RIT and other drugs (e.g., risperidone, fluoxetine) that modify 5-HT_2 receptor-mediated processes. At least one recent study (21) is encouraging in this regard, both for its investigation of the issue and the negative findings reported.

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