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Behavioral Effects of Prenatal Haloperidol Exposure

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SCALZO, F. M., S. F. ALI AND R. R. HOLSON. *Behavioral effects of prenatal haloperidol exposure*. PHARMACOL BIOCHEM BEHAV **34**(4) 727–731, 1989.—Pregnant albino rats were exposed to vehicle (CON), 2.5 mg/kg (LOW) or 5.0 mg/kg (HIGH) haloperidol (HAL) from the sixth through the twentieth day of gestation. The effect of prenatal HAL exposure on offspring was assessed with the following five behavioral measures: 1) milk-induced behavioral activation on the sixth postnatal day (PND 6), 2) shock-precipitated wall climbing (PNDs 9, 11, 13, 15 and 17), 3) amphetamine-induced stereotypies (PND 30), 4) apomorphine-induced stereotypies (PND 30) and 5) duration of barbiturate anesthesia (PNDs 34 and 62). Measures taken very early in life indicated that prenatal HAL reduced arousal. Inactivity scores were elevated in HAL-exposed pups on PND 6 during milk-induced behavioral activation. Shock-precipitated wall climbing was reduced in the HAL animals on PNDs 9 and 11, but not thereafter. At PND 30, no prenatal treatment effect was detectable on stimulant-induced stereotypies or on duration of barbiturate anesthesia. On PND 62, barbiturate anesthesia duration was significantly reduced in both sexes of HIGH HAL animals. These findings suggest that prenatal HAL effects follow a dynamic, changing course as the exposed rat pup matures. Early reductions in arousal (milk-induced behavior and shock-precipitated wall climbing) wane with age, perhaps to be replaced by an actual increase in arousal as HAL pups approach adulthood.

Prenatal drug effects Dopamine Development Haloperidol Stereotypy Barbiturate anesthesia

IN a companion paper we report that prenatal haloperidol (HAL) exposure reduces dopamine (DA) D1 and D2 receptor binding sites in caudate nucleus and nucleus accumbens (8). It is important to determine whether such reductions are accompanied by functional alterations in behaviors thought to be in part DA-dependent.

The literature on this subject suggests that perinatal HAL exposure produces a number of behavioral abnormalities suggestive of lowered arousal, and perhaps linked directly to reduced functional status of the forebrain DA system. Thus, such exposure has been reported to reduce both amphetamine (7,10) and apomorphine-induced stereotypies (6,10) in rodent offspring. This lowered responsivity to stimulants is evidently accompanied by an increased sensitivity to CNS depressants, including HAL, arecoline (10) and barbiturates (12).

The only published failure to obtain such results was that of Madsen *et al.* (5). These investigators obtained evidence for a lowering of apomorphine sensitivity on PND 10, but not thereafter, in offspring of dams exposed to 5 mg/kg HAL on gestational days (GD) 4–20. They also demonstrated that clearance of HAL from the neonatal brain is quite slow; their behavioral findings were accordingly attributed to a lingering presence of levels of HAL in the CNS of very young rat pups.

While it very much remains to be seen whether residual drug presence can account for behavioral abnormalities reported 30 (6) or even 60 days (12) following birth and cessation of treatment, the above findings definitely suggest the possibility of a direct residual HAL effect upon behavior shortly after birth. The present experiments were designed with the above possibility in mind. We deliberately included two behavioral assessments of the neonate (milk-induced behavior at PND 6 and shock-precipitated wall climbing over PNDs 9–17), together with an evaluation of amphetamine or apomorphine-elicited behaviors on PND 30, and barbiturate anesthesia duration measurements on PNDs 34 and 62.

There is reason to believe that all four of these behavioral measures are sensitive to the effects of prenatal HAL exposure. Three of the tests (milk-induced behaviors, wall climbing behavior and drug-induced stereotypies) are known to be sensitive to manipulations of catecholamine systems (1, 2, 6, 11). The fourth test, duration of barbiturate anesthesia, has been previously reported to be reduced in animals prenatally exposed to HAL (12).

Inclusion of four different behavioral measures, at various ages from early neonatal to near-adult would, it was hoped, provide a clearer description of the nature and time course of functional deficits elicited by prenatal HAL exposure.

METHOD

Subjects and Dosing

Sprague-Dawley derived albino nulliparous females were placed

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overnight with experienced male breeders. The next morning (gestational day 0) plug-positive females were housed individually in transparent acrylic cages with wood shavings for bedding in a temperature- (23°C) and humidity- (50%) controlled environment with a 12-hr light/12-hr dark cycle (light on at 0700 hr). Food and water were available ad lib, and cage bedding was changed twice a week, throughout gestation and weaning. At birth (postnatal day 1), litters were randomly culled to 4 ± 1 pups of each sex, or 8 pups per litter. Pups stayed with the mother until weaning at postnatal day 21, at which time they were housed as above in same-sex and prenatal dose condition groups.

Prenatal HAL exposure was done in three groups: vehicle control (CON), low-dose HAL at 2.5 mg/kg (LOW) and high-dose HAL at 5 mg/kg (HIGH). Dosing was by daily subcutaneous injection, over gestational days 6 through 20. Further details are given in the companion paper (8).

Experiment 1—Milk-Induced Behaviors

Apparatus. A milk delivery system consisting of two 5 cc syringes in a Sage Instruments Syringe pump (341) controlled by a Syrelec Timer was programmed to supply a 0.04 ml infusion of a 50% Carnation Evaporated milk/water solution over a 10-sec period every minute through PE-50 (Clay Adams 7411) tubing. The tubing was attached to a posteriorally placed tongue cannula made of PE-10 (Clay Adams 7400) tubing.

Procedure. Pups were deprived of food and the dam in a warm (33–34°C), humidity-controlled isolette (Air-Shields Inc. C-77) for 18–20 hr prior to testing on PND 6. At the onset of the deprivation period and immediately prior to testing body weights were recorded. Cannulations were performed at least 1 hr prior to testing using the procedures described by Hall (4) for posterior tongue cannula placements. Cannulas were implanted using a stainless steel wire bent in a semi-circle at one end. The curved end of the wire was inserted on the midline of the ventral surface of the jaw and directed up through the digastric muscle and tongue and out the pup's mouth. The cannula was then fitted over the wire and the wire pulled back through the lower jaw positioning the cannula in the back of the tongue.

Two pups at a time were placed individually in clear plastic cups in the isolette. Each tongue cannula was attached to the milk delivery system and baseline activity of each pup was recorded for 5 min in the absence of milk infusions (Premilk). Mouthing, probing, forward locomotion, forelimb paddling, hindlimb treading, wall climbing, rolling and curling, lie still and twitching behaviors were recorded using a time sampling procedure for 5 sec every 20 sec during the 5-min observation period. Immediately following this 5-min Premilk period, milk infusions commenced and continued for two 5-min observation periods (Milk 1 and Milk 2 respectively). Postinfusion period activity was then observed for 5 additional minutes (Postmilk).

Experiment 2—Shock-Precipitated Wall Climbing

Apparatus. The test apparatus consisted of two clear, bottomless, Plexiglas boxes ($17 \times 17 \times 20$ cm) which rested on a suspended grid floor (0.2 cm diameter parallel bars separated by 0.4 cm) connected to a shock generator (Lafayette Instr. 820404/5-SS). The boxes were divided into four quadrants by a center hash mark on each side.

Procedure

Behavioral testing was conducted on PNDs 9, 11, 13, 15 and 17. Two pups were tested during each session. Each pup was placed in the center of the test chamber and given a 5-min orientation period during which baseline behaviors were recorded (No-Shock testing). During the next 5 min, 3-sec footshocks (0.5 MA) were delivered (Shock testing) on a variable interval (30 sec maximum) schedule. Test sessions were videotaped for later scoring. Wall climbing bouts and their durations and matrix crossings were scored from the video tape during the intershock intervals of the Shock testing period.

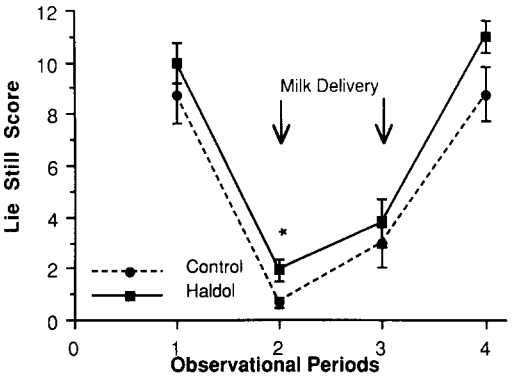

FIG. 1. Milk-induced behavior (PND 6). Twelve CON and 12 HIGH (5.0 mg/kg) prenatal HAL subjects, 6 of each sex, were assessed for milk-induced behavioral activation. Shown is the mean "lie still" score over 5 minutes prior to milk delivery, for two consecutive 5-min observtional periods following milk delivery, and for a final 5-min postmilk period. Means ± the standard error of the mean. *Significantly different from control, Duncan's Multiple Range Test. Drug, $F(1,20) = 5.1$, $p < 0.05$; Period, $F(3,60) = 67.7$, $p < 0.0001$.

Experiment 3—Stimulant-Induced Behavioral Stereotypies

Stereotypic behavior and locomotor activity were assessed on PND 30. Behaviors were observed with subjects, placed individually in $45 \times 45 \times 45$ cm chambers, with one-way Plexiglas forming the front wall. The floor of each chamber was ruled into 9 (5×5 cm) squares. Following injection with either 3.5 mg/kg d-amphetamine (IP) or 0.6 mg/kg apomorphine (IP), stereotypic behavior was monitored for 15 sec every 3 min, for a total of 90 min for amphetamine and 60 min for apomorphine, using the following scoring scale:

0 = normal daytime activity (asleep or stationary);

1 = periodic repetitive stereotypic behavior, periodic exploratory activity;

2 = continuous repetitive stereotypic behavior, periodic exploratory activity;

3 = continuous repetitive stereotypic behavior, very brief or no locomotor activity;

4 = continuous repetitive stereotypic behavior interrupted by periodic licking with very brief or no locomotor activity.

In addition to level of stereotypic behavior, type of behavior was also recorded by entering a code for any of the following behaviors which occurred during each 15-sec observational period: sleep, inactivity, forward locomotion, rearing, grooming, stereotypic wall climbing, paw treading, licking, gnawing, stereotypic repetitive head movements, sniffing and rotating. Motor activity was also assessed by counting square entries in each 15-sec period.

Experiment 4—Duration of Barbiturate Anesthesia

Animals were taken from the home cage on PND 34 or 62,

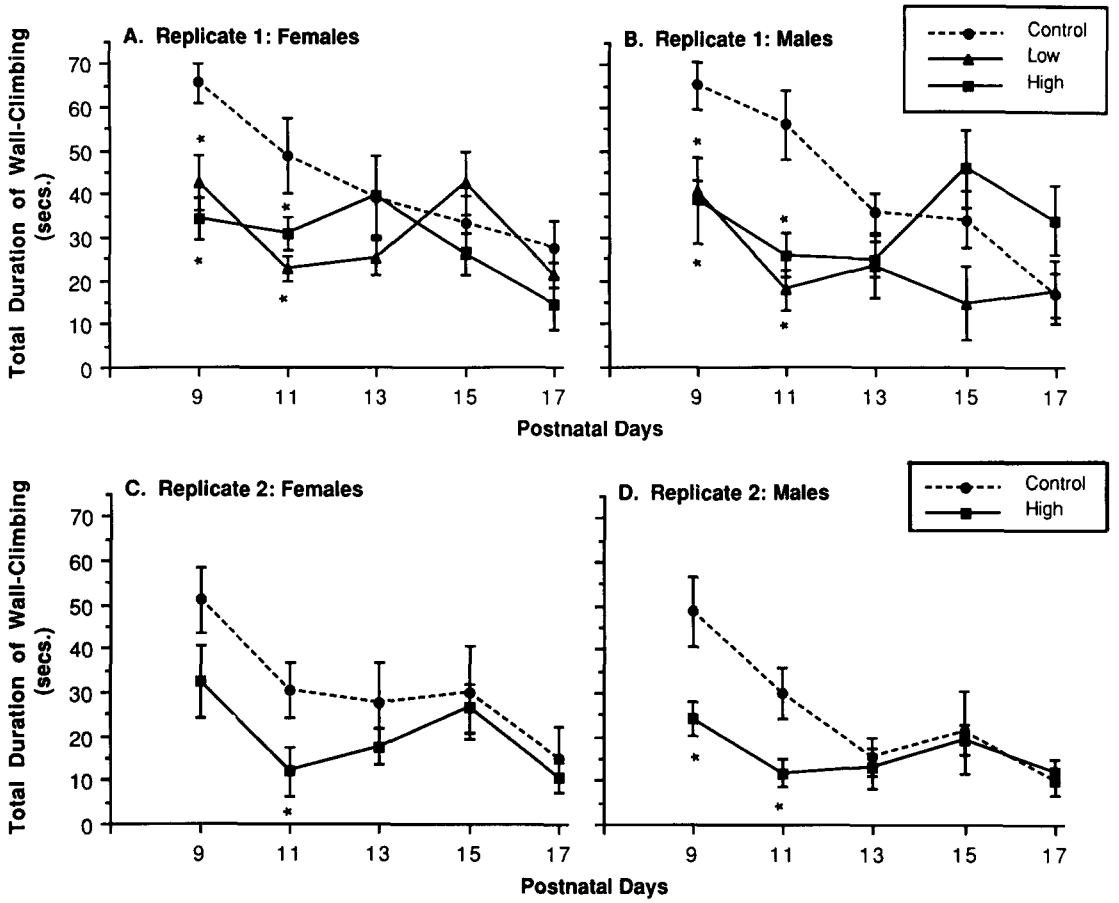

FIG. 2. Shock-precipitated wall climbing. Shown is total duration of wall climbing over 5 consecutive test days on PNDs 9, 11, 13, 15 and 17. Data are given as means ± standard error for CON (prenatal vehicle) LOW (2.5 mg/kg) and HIGH (5.0 mg/kg) prenatal HAL exposure. Females (A) and males (B) for the first replicate. Females (C) and males (D) for the second replicate. *Significantly different from controls, Duncan's Multiple Range Test.

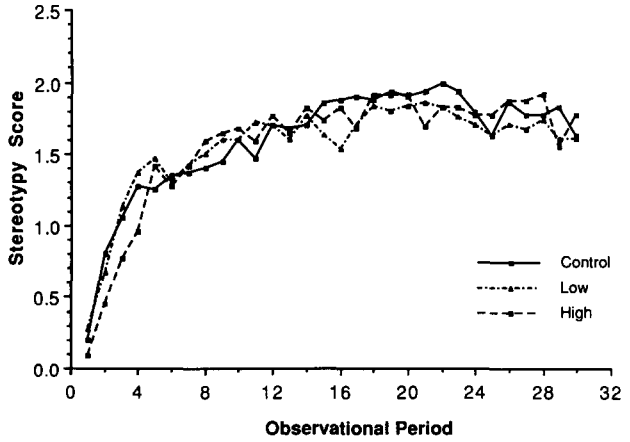

FIG. 3. Stimulant-induced stereotypy. Mean amphetamine (3.5 mg/kg) stereotypy scores for CON, LOW and HIGH subjects. Mean scores are shown for each of 30 consecutive 15-sec observational periods, over a total of 1½ hours.

weighed and given an injection of 1% sodium Nembutal (0.45 ml/kg, IP). Following injection, the rat was placed on a table top until loss of the righting reflex occurred. The time at which this reflex was lost was recorded. The animal was then placed on its back and left until it spontaneously rolled over, with all four feet in contact with the table top. This time was recorded and anesthesia duration was calculated as the difference between the two times. Time was measured to the nearest second on a digital timer.

Experimental Design and Statistical Analysis

For all four experiments, both males and females were tested. However, the source of males and females (within or between litters) and the level of prenatal HAL exposure differed both within and between experiments. These experiments were conducted on subjects drawn from two distinct replicates. In the first replicate, shock-induced wall climbing, amphetamine-induced stereotypies and PND 35 barbiturate anesthesia duration were evaluated on different pairs of littermates for each of the three tests. One male and one female were tested per litter, so sex was a correlated, within-litter factor in this replicate. Three levels of the prenatal HAL factor were included (CON, LOW and HIGH). In the second replicate, milk-induced behavioral activation, shock-precipitated wall climbing, apomorphine-induced stereotypies and PND 64 barbiturate anesthesia duration were all evaluated. This replicate

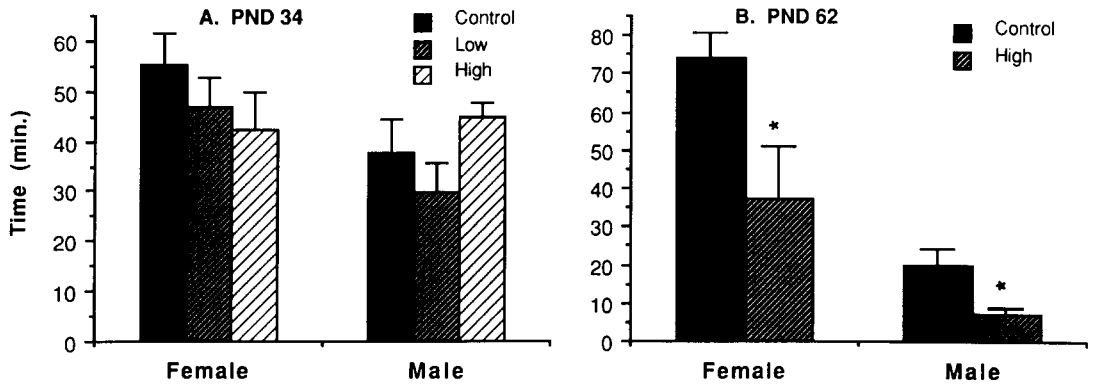

FIG. 4. Duration of barbiturate anesthesia. Mean duration (min) of barbiturate anesthesia duration ± standard errors of the mean. (A) PND 34, CON, LOW and HIGH values for males and females. (B) PND 62 CON and HIGH values for males and females. *Significantly different from controls.

differed from the first in that males and females were drawn from separate litters (hence, sex was a between-litters factor) and only CON and HIGH levels of prenatal HAL exposure were studied. Finally, three of the four experiments involved repeated measures over time (milk-induced behavior, wall climbing and stereotypy).

As a result of the above considerations, details of statistical analysis varied across tests. For milk-induced activation, sex was included in a repeated-measures subject (sex × prenatal treatment) × time ANOVA. Since source of subjects by sex as well as prenatal treatments differed by replicate for wall climbing, data were analyzed separately by replicate. In replicate 1, a subject (dose) by days ANOVA was conducted separately for males and females; in replicate 2, a subject × sex × prenatal treatment × days ANOVA was conducted. Results for both stereotypy experiments were analyzed by replicate as was wall climbing. Finally, barbiturate anesthesia duration was analyzed separately by sex at PND 34, using a one-way ANOVA design to detect prenatal HAL effects. PND 62 barbiturate anesthesia duration, in contrast, was analyzed with a two-way, sex × prenatal HAL ANOVA design.

RESULTS

There was no apparent difference between prenatal HAL groups in response to milk infusions on PND 6. Eight behavioral categories of this response showed no such effect (data not shown). However, the HIGH prenatal HAL subjects were less active throughout this single 20-min test [Fig. 1; lie still score prenatal HAL effect, $F(1,20)=5.1$, $p<0.05$. Effect of milk delivery, $F(3,60)=67.7$, $p<0.0001$].

The total duration of shock-precipitated wall climbing was reduced early in life. This effect was seen clearly in both sexes, and in two independent replicates of the experiment (Fig. 2). In all instances, prenatal HAL exposure resulted in a decrease in total duration of wall climbing on PNDs 9 and/or 11, the first two days of testing, but not thereafter. [*Replicate 1:* Females, Prenatal HAL effect, $F(2,19)=5.2$, $p<0.02$; Prenatal HAL by days interaction, $F(8,76)=2.3$, $p<0.03$. Males, prenatal HAL effect, $F(2,19)=2.9$, $p<0.08$; Days, $F(4,26)=10.5$, $p<0.0001$, Prenatal HAL by days interaction, $F(8,76)=5.3$, $p<0.0001$. *Replicate 2:* Sex of subject did not interact with prenatal HAL exposure. Main effect of prenatal HAL, $F(1,20)=6.2$, $p<0.03$; Days effect, $F(4,75)=13.3$, $p<0.0001$; Prenatal HAL by days interaction, $F(4,75)=2.8$, $p<0.05$].

A single IP injection of either amphetamine (Fig. 3) or apomorphine (data not shown) on PND 30, in contrast, produced no prenatal HAL exposure differences in either behavioral stereotypies or in open-field activity (data not shown).

Similarly, there was no prenatal HAL exposure effect on barbiturate sleep duration on PND 34 (Fig. 4A). In contrast, by PND 62, prenatal exposure to 5.0 mg/kg HAL clearly reduced total anesthesia duration in both sexes (Fig. 4B). By this age the well-documented sex difference in barbiturate anesthesia duration was also evident [Prenatal HAL effect, $F(1,28)=9.5$, $p<0.005$; Sex effect, $F(1,28)=27.4$, $p<0.0001$].

In none of the above experiments was there a sex difference in the response to prenatal HAL.

DISCUSSION

These findings are suggestive of a dynamic, changing course of prenatal HAL effects over postnatal development. Thus, an early reduction in activity during the PND 6 milk-induced activation was coupled with a reduction in the catecholamine-mediated (1,3) duration of shock-elicited wall climbing on PNDs 9 and 11. Thereafter, wall climbing was not affected, nor were stimulant-induced stereotypies on PND 30 or barbiturate anesthesia duration on PND 34. By PND 62, in contrast, barbiturate sleep times were actually reduced in males and females exposed prenatally to HAL. Thus, exposure effects seemed to move from an early hypoarousal (decreased wall climbing behavior) through apparent normality at PND 30 to something resembling enhanced arousal by PND 62.

The early behavioral effects of prenatal HAL reported here are consonant with the suggestion of Madsen *et al.* (5) that functionally active traces of HAL can linger in the neonatal brain for some days following cessation of exposure late in gestation. The lack of any effect on wall climbing after PND 11, and normal levels of stimulant-induced stereotypy by PND 30, would indicate that any such effect did not extend beyond PND 12.

There is, however, a major problem in understanding the failure of prenatal HAL exposure in this experiment to create any alteration in apomorphine- or amphetamine-elicited stereotypies on PND 30. In a companion paper (8), we report that D1 and D2 binding was decreased at PND 30 in the caudate nucleus of HAL-exposed littermates. In the experiments of Rosengarten and Friedhoff (6) such a reduction in receptors was accompanied by a clear reduction in apormorphine stereotypies. It is unclear why our animals were not similarly hyporesponsive to stimulation of the dopamine system. Possible explanations for the differences between the two findings include the fact that D2 binding in our animals was substantially less reduced by prenatal HAL than in the Rosegarten and Friedhoff study, and possibly the use of a higher dose of apomorphine in our experiment (0.6 mg/kg vs. 0.3

mg/kg). Whatever the explanation, one is still left with the problem of determining the functional effect (if any) of a 12% reduction in D2, and a 20% reduction of D1 binding in the caudate in PND 30 rats.

Another substantial problem is the contradiction between our finding of lowered barbiturate sleep time in PND 62 prenatally HAL-exposed rats, and the report by Yanai and Fishman (12) of an increase in anesthesia duration at PND 60 in rats subject to a very similar prenatal HAL regime. One can only speculate on the source of this discrepancy, especially without PND 60 DA binding data from either laboratory. In this regard, it could be important that Yanai and Fishman (12) used a large barbiturate dose, obtaining sleep durations almost six times those reported here.

In conclusion, our data indicate that the effect of prenatal HAL may reverse during development, moving from early hypo- to later hyperarousal. Before these indications can be accepted as fact, much data will be needed on the time course of prenatal HAL effects on relevant behavioral and neurochemical variables. In light of the present findings, it will be especially important to track developmental changes in response to both CNS depressants and stimulants, from the early neonatal period through adulthood. It is also important to expose subjects to not one but a range of doses of selected pharmacological challenges at each stage of development. Finally, such measurements will need to be accompanied by parallel determinations of neurotransmitter and receptor levels in the forebrain DA system. Only after collecting such data will we know whether the tantalizing hints provided by the findings reported here reflect the true state of affairs.

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