Ann. Ist. Super. Sanità, vol. 29, n. 1 (1993), pp. 139-146

Haloperidol effects on the developing dopamine system: conflicting results and implications for neurobehavioral teratology research

Frank M. SCALZO *(a, b)*, Syed F. ALI *(b)*, R. Robert HOLSON *(b)* and Robert L. WILLIAMS *(c)*

(a) Department of Pediatrics, Arkansas Children's Hospital Research Center and The University of Arkansas for Medical Sciences, Little Rock, Arkansas,USA
(b) National Center for Toxicological Research, Jefferson, Arkansas,USA
(c) Agency for Toxic Substance and Disease Registry, Atlanta, Georgia, USA

Summary. - In an attempt to further develop basic principles to guide research in neurobehavioral teratology, six experiments were conducted to examine the effects of prenatal haloperidol (a D2 dopamine antagonist) exposure on striatal D1 and D2 binding sites. Another laboratory has repeatedly reported that prenatal exposure to this dopamine antagonist reduces striatal dopamine binding sites in exposed offspring. Our initial studies were successful in replicating and extending these previously reported reductions in D2 dopamine binding sites in caudate of rats exposed prenatally to haloperidol. However, additional experiments in our laboratory, in which pups were exposed to a range of haloperidol doses over gestational periods when the dopamine system has been reported to be most vulnerable to prenatal haloperidol exposure effects, have repeatedly failed to replicate our initial findings. Three other laboratories have also failed to duplicate this effect. The results of these studies suggest that beyond "standard" confounding variables, neurobehavioral teratologists are faced with as yet poorly understood factors that influence replication of findings within and between laboratories. These findings also emphasize the importance of within- and between-laboratory replication of experimental findings.

Key words: haloperidol, dopamine, prenatal drug effects, dopamine receptors.

Riassunto *(Effetti dell'aloperidolo sullo sviluppo del sistema dopaminergico: replicabilità dei risultati e implicazioni per la teratologia neurocomportamentale)*. - Alcuni esperimenti sono stati condotti nel nostro laboratorio al fine di valutare nel ratto l'effetto di esposizioni prenatali ad aloperidolo (un antagonista dopaminergico D2) sulla densità dei siti di legame dopaminergici D1 e D2 nello striato. Numerosi studi condotti in un altro laboratorio hanno riportato in seguito all'esposizione prenatale allo stesso agente una riduzione nei piccoli trattati dei siti di legame dopaminergici a livello striatale. Studi condotti nel nostro laboratorio hanno inizialmente replicato ed esteso tali effetti anche al nucleo caudato. Successive ricerche, tuttavia, condotte sia nel nostro che in altri laboratori hanno evidenziato, usando un più ampio spettro di dosi di aloperidolo e somministrandolo in un periodo prenatale in cui il sistema è particolarmente vulnerabile a tale agente, risultati contrastanti. In generale tali sudi suggeriscono come, al di là delle consuete variabili confondenti, la teratologia neurocomportamentale debba far fronte ad altri fattori ancora scarsamente compresi che influenzano la replicazione dei dati in uno stesso laboratorio così come in laboratori differenti. Si vuole enfatizzare l'importanza della replicazione del dato sperimentale entro e tra laboratori tra i principi guida della ricerca in teratologia neurocomportamentale.

Parole chiave: aloperidolo, dopamina, esposizione prenatale, recettori dopaminergici.

Introduction

Alterations in the dopamine system after prenatal exposure to haloperidol, a compound which blocks dopamine D2 receptor sites, were first reported by Rosengarten and Friedhoff [1]. In this report, a decrease in striatal [^{3}H]spiroperidol binding was observed in offspring whose dams were treated with 2.5 mg/kg haloperidol on gestational days (GD) 5-21. This reduction persisted until postnatal day (PND) 60, which was the last day of neurochemical analysis. Treatment with alpha-methyl-p-tyrosine (AMPT), a compound which blocks dopamine synthesis, also reduced striatal [^{3}H]spiroperidol binding, but to a lesser degree. This reduction did not persist past PND 21. In this same study, apomorphine produced significantly less stereotypy in rats exposed prenatally to haloperidol or AMPT, indicating that the observed decrease in dopamine binding sites had functional consequences. Further studies from the same laboratory demonstrated that administration of l-dopa for a short period toward the end of the second and beginning of the third gestational week greatly increased striatal [^{3}H]spiroperidol binding [2]. A sensitive period for the effects of prenatal haloperidol and l-dopa exposure

was also described [3]. Administration of haloperidol or l-dopa during GD 15-18 produced decreases and increases, respectively, in striatal [^{3}H]spiroperidol binding. Administration of haloperidol prior to GD 15 or after GD 18 had no effect on [^{3}H]spiroperidol binding. Similarly, administration of l-dopa after GD 18 had no effect on [^{3}H]spiroperidol binding [3]. An increase in striatal muscarinic ([^{3}H]QNB) sites has also been reported after prenatal exposure to haloperidol on GD 5-20 [4] or GD 14.5 - 17.5 [5].

Since the initial report by Rosengarten and Friedhoff [1] that prenatal haloperidol exposure produced enduring changes in the dopamine system, there have been few studies that have replicated these effects. Madsen *et al.* [6] did not obtain a decrease in [^{3}H]spiroperidol binding on PND 15 or 30 following prenatal haloperidol administration and attributed a reduction in [^{3}H]spiroperidol binding affinity on PND 7 to the lingering presence of residual haloperidol in the brain. Moon [7], using autoradiographic techniques, observed an increase in [^{3}H]spiroperidol binding after administration of haloperidol to dams via osmotic minipump throughout gestation. Schmidt and Lee [8] failed to observe any alteration in striatal D2 receptor affinity or density after prenatal exposure to either haloperidol, the thioxanthene thiothixene, or the phenothiazine trifluoperazine, during GDs 15-18 or 5-20. On the other hand, prenatal reserpine exposure has been reported to decrease B_{max} for [^{3}H]spiroperidol binding in the caudate, but only in female not male offspring [9].

In an attempt to clarify inconsistencies in the literature and to further develop basic principles to guide research in neurobehavioral teratology, we have conducted several experiments examining the neurobehavioral effects of prenatal haloperidol exposure. Our initial studies were successful in replicating and extending the previously reported effects of prenatal haloperidol exposure on D2 dopamine binding sites in caudate [10]. However, five additional experiments in our laboratory, in which pups were exposed to haloperidol over periods when the dopamine system has been reported to be most vulnerable to prenatal haloperidol exposure effects [3, 11], have failed to replicate these previous findings. In this paper we present the results of all six experiments. We then discuss the implications of these failures to replicate for experimental design and technique in the larger area of neurobehavioral teratology.

Materials and methods

Subjects

Sprague-Dawley derived albino nulliparous female rats were placed overnight with experienced male breeders. The next morning (GD 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 h light/12 h dark cycle (light on at 0700 h). Maternal weights were collected daily from the onset of dosing to parturition. Food and water were available ad libitum, and cage bedding was changed twice a week throughout gestation and weaning. At birth (PND 1), litters were weighed and randomly culled to 4±1 pups of each sex, or 8 pups per litter. Pups remained with the dam until weaning into same-sex group caging on PND 21.

Haloperidol preparation

Haloperidol (Sigma Chemical, St. Louis, MO) was dissolved in a drop of lactic acid, then brought up to a concentration of 2.5 or 5.0 mg/ml with distilled water. The pH of the final solution was maintained at 4.2 with lactic acid. Solutions were made fresh weekly and kept in light-tight containers. Dams were injected s.c. every morning with vehicle or haloperidol at a standard volume of 1 ml/kg.

Experimental design

The designs of each of the six experiments are summarized in Table 1 and are described in more detail below.

Experiment 1. Dams were given single daily s.c. injections of vehicle (control), 2.5 (low) or 5.0 (high) mg/kg haloperidol over GD 6-20. Offspring were sacrificed on PNDs 29-31, and D1 and D2 receptor densities were assayed in several brain regions. Behavioral measures were conducted on littermates of those sacrificed to obtain brain tissue for neurochemical analyses (see [12] for the behavioral results of this experiment and [10] for further details of the neurochemical results).

Experiment 2. Dams were given single daily injections of vehicle (control), 2.5 (low) or 5.0 (high) mg/kg haloperidol over GD 6-20. Offspring were sacrificed on PND 1, 15, 30 or 58. Caudates were assayed for D1, D2 and muscarinic binding, and for monoamine content [13].

Experiment 3. Dams were injected with either vehicle or 5.0 mg/kg haloperidol on GD 6-20, and the offspring were sacrificed at PND 29. Again caudates were assayed for D1, D2 and muscarinic binding. Caudates of littermates were also assayed for content of acetylcholine and choline acetyltransferase [13].

Experiment 4. Dams were injected with either vehicle or 5.0 mg/kg haloperidol on GD 6-20. To control for possible effects of maternal care-giving, offspring were cross-fostered on the day of birth, and to control for the aphagic effects of haloperidol, a group of dams was pair-fed (see [13] for details of pair-feeding procedure). Offspring were sacrificed at PND 30, and D1, D2 and muscarinic binding assays were conducted on caudate membrane.

Table 1. - Experimental design summaries for each experiment and location of results from each experiment

Experiment	Sacrifice date	Number of treatments	Litters	Measures	Results
1	8/88	I. vehicle II. 2.5 mg/kg haloperidol III. 5.0 mg/kg haloperidol	72	D1 and D2 binding	Table 2
2	12/88	I. vehicle II. 2.5 mg/kg haloperidol III. 5.0 mg/kg haloperidol	112	D1, D2 and muscarinic binding	Fig.1
3	5/89	I. vehicle II. 5.0 mg/kg haloperidol	39	D1, D2 and muscarinic binding	Table 3
4	6/89	I. vehicle II. 5.0 mg/kg haloperidol III. pair fed controls IV. 5.0 mg/kg haloperidol reared by control mothers	43	D1 and D2 muscarinic binding	Fig. 2
5	1/90	I. vehicle II. 5.0 mg/kg haloperidol	12	Scatchard analysis of D1 and D2 binding	Table 4
6	6/90	I. vehicle II. 2 x 5 mg/kg haloperidol daily (GD 12-16 or GD 16-20)	18	D1 and D2 binding	Fig. 3

Experiment 5. Dams were injected with vehicle or 5.0 mg/kg haloperidol on GD 6-20. These offspring and a separate group of pups, whose dams were pair-fed to the haloperidol dams, were sacrificed on PND 33. Scatchard analyses for D1 and D2 binding were conducted using methods previously described [14].

Experiment 6. Dams were injected with vehicle or 10.0 mg/kg (two daily 5.0 mg/kg injections) haloperidol on GD 12-16 or 16-20 and sacrificed on PND 30. Once again striatal D1 and D2 binding sites were quantified.

Neurochemical analysis

Offspring were sacrificed at the various ages indicated in each experiment. Brains were removed, weighed, then immediately dissected over ice into major subregions which were weighed and then quickly frozen over dry ice and stored at - 80 °C until neurochemical analysis. All neurochemical analyses were conducted using methods previously described [10, 14, 15].

Data analysis

In all analyses, litter was the basic unit of statistical evaluation. At a given age, one male and one female were sacrificed per litter. Data were then evaluated independently by sex (yielding one subject per litter), or male and female values were averaged within litter, and these litter means then formed the unit of analysis. In some cases, a litter contributed only one male or female to an experiment.

Results

D1, D2 and muscarinic binding

Experiment 1. Prenatal haloperidol exposure decreased both D1 and D2 dopamine receptor binding in the caudate nuclei of offspring exposed to 2.5 or 5.0 mg/kg haloperidol on GD 6-20 compared to vehicle injected controls (for D1: $F(2,46) = 3.5$, $p < 0.05$; for D2: ($F(2,46) = 4.7$, $p < 0.01$) (Table 2).

Experiment 2. No differences were found in caudate D1 or D2 dopamine or muscarinic binding in pups exposed to vehicle, 2.5 or 5.0 mg/kg haloperidol on GD 6-20 and sacrificed on PND 1, 15, 30 or 58 (Fig. 1).

Experiment 3. No differences were observed in caudate D1 or D2 dopamine or muscarinic binding in pups exposed prenatally to vehicle or 5.0 mg/kg haloperidol on GD 6- 20 and sacrificed on PND 30 (Table 3).

Experiment 4. No differences were found in caudate D1 or D2 dopamine or muscarinic binding in pups from

Table 2. - [^{3}H]SCH-23390 (D1) and [^{3}H]spiroperidol (D2) binding (mean fmol/mg protein ± SEM) in various regions of the forebrain dopamine system in offspring of females exposed to vehicle (control), 2.5 mg/kg haloperidol (low) or 5.0 mg/kg haloperidol (high) on gestational days 6- 20. Number of subjects or litter means in parentheses

	D1			D2		
Brain Region	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Caudate nucleus	791.8 ± 23.7 (21)	695.9 ± 36.2 (*) (13)	648.9 ± 27.2 (*) (15)	434.1 ± 9.3 (21)	399.4 ± 9.2 (*) (13)	383.7±14.4 (*) (15)
Nucleus accumbens	34.6 ± 2.9 (16)	20.6 ± 2.7 (*) (10)	28.2 ± 2.2 (22)	180.0 ± 9.9 (26)	140.4 ± 9.4 (*) (16)	148.8 ± 7.9 (*) (30)
Frontal cortex	4.7 ± 1.1 (7)	8.4 ± 1.7 (8)	9.1 ± 1.9 (9)	144.0 ± 6.3 (7)	149.6 ± 5.2 (8)	148.1 ± 5.9 (9)
Amygdala	36.4 ± 2.5 (7)	34.7 ± 2.1 (8)	34.6 ± 2.2 (9)	70.3 ± 15.4 (7)	51.5 ± 3.7 (8)	57.8 ± 3.6 (9)

(*) Significantly different from control values, $p < 0.05$.

Table 3. - Experiment 3. Caudate D1, D2 and muscarinic binding on postnatal day 30 (fmol/mg protein)

	Receptor type		
	D1	**D2**	**Muscarinic**
Control (n=19)	752.4 ± 30.1	299.9 ± 11.9	1689.2 ± 50.7
Haloperidol (n=20)	693.3 ± 27.7	289.7 ± 9.9	1742.4 ± 52.2

any of the following treatment conditions: vehicle (C), 5.0 mg/kg haloperidol on GD 6-20 (H), 5.0 mg/kg haloperidol on GD 6-20 and cross-fostered to untreated dams (HCF) or dams pair-fed (PFC) to the H dams (Fig. 2).

Experiment 5. No differences were observed in caudate D1 or D2 dopamine receptor binding (K_d or B_{max}) in pups exposed to vehicle or 5.0 mg/kg haloperidol. Caudate D1 and D2 binding in pups whose dam was pair-fed to the haloperidol dams did not differ from either vehicle or haloperidol treated groups (Table 4.)

Experiment 6. No differences were observed in caudate D1 or D2 dopamine receptor binding in pups exposed to vehicle or 10.0 mg/kg haloperidol (Table 5).

Brain Weights

Unlike binding, the weight of whole brain and most brain regions was reduced by GD 6-20 haloperidol exposure in a highly replicable fashion (Table 6, data from experiments 2-5). The 10% to 15% weight reduction in brain was matched by a very similar decrease in whole body weight. These haloperidol effects seemed to be permanent, in that they were seen with undiminished effect in exposed offspring as old as 5 months [13].

Discussion

A series of six experiments, conducted in the same laboratory with highly consistent techniques over a two-year period, has produced very puzzling results. In the first of these six experiments we replicated the Rosengarten and Friedhoff finding that prenatal haloperidol exposure in rats can reduce the B_{max} of dopamine binding sites in the caudate of offspring. Subsequently, we have been unable to replicate our own findings in five consecutive experiments.

We are unable to explain this failure to replicate. We believe that over the course of these experiments we have excluded the more obvious possibilities. Thus, strain of rat and all housing and animal care techniques have been constant over the two years of this project. Drug exposure was also consistent over the first five experiments, being restricted to s.c. injection of 2.5 or 5 mg/kg haloperidol over GD 6-20. In the sixth experiment we doubled the previous dose and administered this higher dose over the gestational age shown to be most sensitive by Rosengarten and Friedhoff. Assay technique was held constant, and all single point assays were conducted by the first author. Assay results were consistent over assays, agreed closely with published values from other laboratories, and showed the predictable increase with age. Nor can we attribute

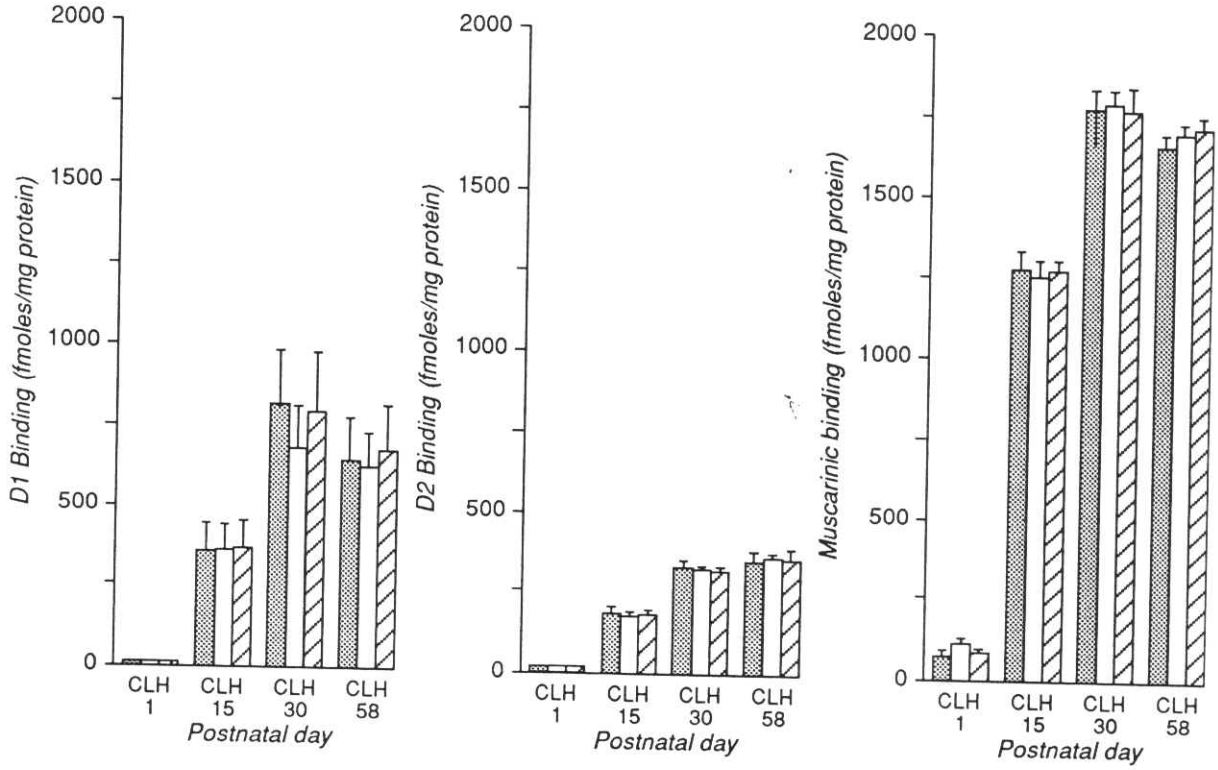

Fig. 1. - Experiment 2. Caudate D1, D2 and muscarinic binding on postnatal days 1, 15, 30 and 58 (mean fmol/mg protein ± SEM).

the failure to replicate our first results to problems with the compound. In all experiments the drug had closely similar effects on dams, causing severe catalepsy and a consequent failure to eat or drink [13]. As discussed above, the drug also consistently reduced brain and body weight of offspring, so it was certainly not without effect.

Problems with replication are often attributed to "seasonal effects" or small sample sizes. We do not believe that seasonal effects account for these results. As shown in Table 1, two of these six experiments were conducted in winter, while the other four (including the first, positive experiment) were conducted in summer. Further, our sample sizes were adequate to detect a 10% to 20% alteration in binding. While we could speculate endlessly about other factors which might account for these puzzling results, we feel that such speculation would be largely idle at this point. The important conclusion that must be drawn from these experiments is that any effect of prenatal haloperidol on dopamine or muscarinic binding sites in the brain cannot be consistently reproduced in this laboratory.

This intra-laboratory problem with replication is certainly matched by problems in replicating the Rosengarten and Friedhoff effects between laboratories. Thus, even in our first, positive experiment the size of the effect on D2 sites was lower in our laboratory, and we did not replicate the reduction in stereotypy in response to a drug challenge also reported by Rosengarten and Friedhoff [1]. Three other laboratories have had even greater problems in duplicating these effects [6-8]. In two of these cases, investigators took great pains in attempts to obtain these effects. Thus Madsen and colleagues carefully followed the exposure protocol of Rosengarten and Friedhoff. They then used the same neurochemical and behavioral techniques (apomorphine challenge) to assess the effects of haloperidol exposure. These experimenters failed to obtain any effects on any measure after PND 10. A reduction in the activity of haloperidol-exposed rats elicited by an apomorphine challenge on PND 10 but not thereafter was attributed to the possible presence of residual amounts of haloperidol in pup brain. This finding is reminiscent of our finding that shock-induced wall climbing, a catecholaminergic neonatal behavior, was reduced before but not after PND 12 in haloperidol- exposed pups [12].

Schmidt *et al.* attempted an independent replication of the Rosengarten and Friedhoff results [8]. Pregnant rats were exposed to a range of doses of haloperidol or to one of two other potent neuroleptics, either during the late-pregnancy sensitive period reported by Friedhoff, or over the full GD 5-20 period originally used by Rosengarten and Friedhoff. Fetal brain levels of all three

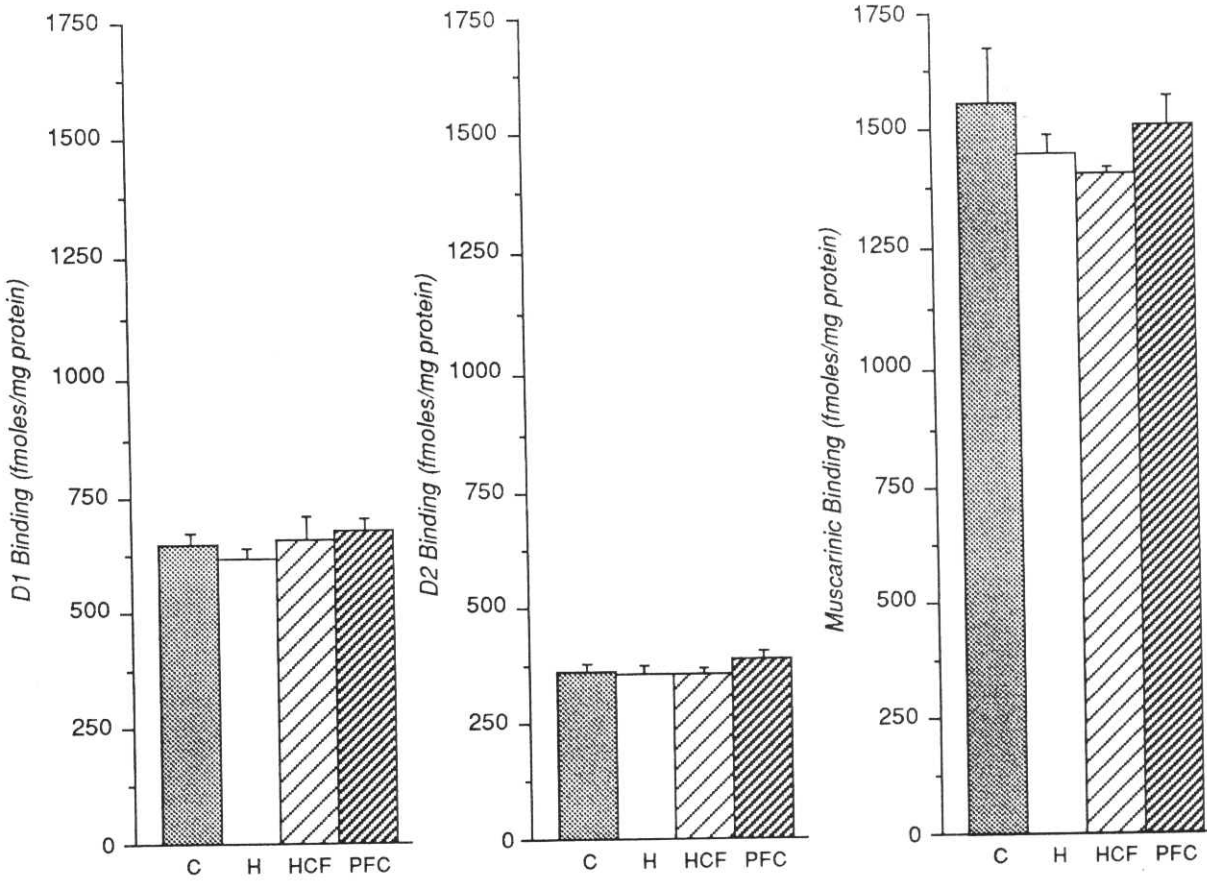

Fig. 2. - Experiment 4. Caudate D1, D2 and muscarinic binding on postnatal day 30 (mean fmol/mg protein ± SEM).

Table 4. - Experiment 5. K_d (nM) and B_{max} (fmol/mg protein) for caudate D1 and D2 binding on postnatal day 30 (4 litters were used for each prenatal treatment). All values represent the mean ± SEM of 3 independent experiments with duplicates of total and non-specific binding

Receptor type		Prenatal treatment		
		Control	Haloperidol	Pair-fed
D1	K_d	.597 ± .06	.687 ± .07	.660 ± .09
	B_{max}	867.7 ± 19.6	837.0 ± 13.9	887.7 ± 52.6
D2	K_d	.592 ± .04	756 ± .03	.779 ± .12
	B_{max}	245.8 ± 21.0	269.7 ± 18.1	263.1 ± 2.2

compounds were assayed, and all binding assays were conducted with a full Scatchard analysis. Again, no effect of any compound, at any dose level, was found on PND 14 caudate D2 K_d or B_{max} values.

We conclude that the effect of prenatal haloperidol exposure on striatal dopamine binding sites is inconsistent, both within and between laboratories. We certainly do not question Rosengarten and Friedhoff's findings, since we have obtained and published similar results. We do, however, believe that the decrease in D1 and D2 binding is not a robust effect. For reasons not currently understood, this effect is not found in all laboratories, or even (in our case) in all experiments within a single laboratory.

This evident inconsistency has implications well beyond the understanding of prenatal neuroleptic exposure effects on brain and behavior. These results show that at least some biologically important effects of early drug exposure have intrinsically high variability. By implication, they suggest that the developing mammalian nervous system may respond like other

Table 5. - Experiment 6. Caudate D1 and D2 binding. Mean (fmol/mg protein) ± SEM (n)

Exposure period	Prenatal treatment	D1	D2
GD 12-16	Control	607.1 ± 27.1 (11)	305.1 ± 10.3 (11)
	5.0 mg/kg haloperidol	581.7 ± 35.9 (12)	325.0 ± 12.4 (12)
GD 16-20	Control	566.2 ± 29.8 (10)	317.9 ± 11.6 (10)
	5.0 mg/kg haloperidol	644.6 ± 33.9 (8)	347.2 ± 23.4 (8)

Table 6. - Gestational haloperidol exposure (5 mg/kg, GD 6-20) had highly replicable effects on dams and offspring. Mean ± SEM (number of litters in brackets)

		Experiment 2	Experiment 3	Experiment 4	Experiment 5
Litter size (n of pups per litter)	Vehicle	12.6 ± 0.5 (14)	13.7 ± 1.0 (12)	12.0 ± 0.8 (12)	13.9 ± 0.8 (12)
	Haloperidol	13.2 ± 0.7 (11)	12.2 ± 0.8 (12)	11.9 ± 1.0 (18)	11.8 ± 0.7 (12)
Maternal GD 20 Wt. (gm)	Vehicle	399.4 ± 5.9 (14)	409.3 ± 13.3 (12)	409.9 ± 9.4 (14)	404.3 ± 6.5 (12)
	Haloperidol	345.6 ± 5.3 *(*)* (11)	353.3 ± 6.0 *(*)* (12)	359.6 ± 7.5 *(*)* (18)	363.0 ± 8.7 *(*)* (12)
PND 30 Male Body Wt. (gm)	Vehicle	86.4 ± 2.5 (14)	77.7 ± 3.2 (12)	109.9 ± 4.4 (14)	106.0 ± 4.8 (12)
	Haloperidol	76.3 ± 1.9 *(*)* (11)	74.6 ± 1.4 (12)	93.6 ± 2.9 *(*)* (18)	93.1 ± 2.6 *(*)* (12)
PND 30 Male Brain Wt. (gm)	Vehicle	1.56 ± 0.01 (14)	1.53 ± 0.03 (12)	1.63 ± 0.02 (14)	1.61 ± 0.02 (12)
	Haloperidol	1.39 ± 0.02 *(*)* (10)	1.45 ± 0.01 *(*)* (12)	1.44 ± 0.02 *(*)* (18)	1.44 ± 0.02 *(*)* (12)
PND 30 Male Brain/Body Wt. Ratio	Vehicle	1.83% ± 0.05 (14)	2.00% ± 0.07 (12)	1.51% ± 0.05 (14)	1.54% ± 0.06 (12)
	Haloperidol	1.81% ± 0.04 (10)	1.95% ± 0.05 (12)	1.56% ± 0.04 (18)	1.55% ± 0.03 (12)

(*) significantly different from vehicle controls, $p < 0.05$.

highly complex systems to perturbation. That is, as in other areas of chaos theory, the developing brain may respond very differently to treatment depending upon subtle, small and poorly understood differences in initial conditions.

This disquieting possibility has major implications for research in the field of neurobehavioral teratology. If developmental effects can be this variable, then replication becomes an even more essential part of good experimental technique. While the importance of between-laboratory replication is grasped by most experimenters in this field, these results underline the critical importance of replication of findings within laboratories. We suspect that most experimenters still feel that use of statistical significance tests at or below the 0.05 level provides adequate protection against such problems. However, this protection only exists if our biological results follow the statistical model - that is, only if the variability of replicate results is distributed as the classical statistical sampling distribution, with a standard deviation equal to the obtained within-group standard deviation divided by the square root of the sample size.

In conclusion, the results reported here suggest that in important instances this model may not be biologically correct. In other words, variability of effects over replication may be biologically greater than that posited by the statistical model. If this inherently high variability is a reality, then replication of experimental results within a laboratory, especially for important new findings, must become as important as replication of such findings across laboratories.

Acknowledgement

This research was supported in part by an appointment to the Oak Ridge Associated Universities (ORAU) Postgraduate Research Program at the National Center for Toxicological Research administered by ORAU through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration. The acetylcholine and choline acetyltransferase assays were conducted by Dr. Joanne Sweeny. The authors thank Pat Sullivan-Jones, Peggy Webb, Bobby Gough and Lora J. Burge for technical assistance and Marjorie McMinn for editorial assistance.

Submitted on invitation.
Accepted on 25 September 1992.

REFERENCES

1. ROSENGARTEN, H. & FRIEDHOFF, A.J. 1979. Enduring changes in dopamine receptor cells of pups from drug administration to pregnant and nursing rats. *Science* **203**: 1113-1135.
2. FRIEDHOFF, A.J. & MILLER, J.C. 1983. Clinical implications of receptor sensitivity modification. *Ann. Rev. Neurosci.* **6**: 121-148.
3. ROSENGARTEN, H., FRIEDMAN, E. & FRIEDHOFF, A.J. 1983. Sensitive periods for the effect of haloperidol on development of striatal dopamine receptors. *Birth Defects* **19**: 511-513.
4. FRIEDHOFF, A.J. & MILLER, J.C. 1983. Prenatal psychotropic drug exposure and the development of central dopaminergic and cholinergic neurotransmitter systems. *Monogr. Neural. Sci.* **9**: 91-98.
5. MILLER, J.C. & FRIEDHOFF, A.J. 1986. Prenatal neuroleptic exposure alters postnatal striatal cholinergic activity in the rat. *Dev. Neurosci.* **8**: 111-116.
6. MADSEN, J.R., CAMPBELL, A. & BALDESSARINI, R.J. 1981. Effects of prenatal treatment of rats with haloperidol due to altered drug distribution in neonatal brain. *Neuropharmacology* **20**: 931-939.
7. MOON, S.L. 1984. Prenatal haloperidol alters striatal dopamine and opiate receptors. *Brain Res.* **323**: 109-113.
8. SCHMIDT, M.H. & LEE, T. 1991. Investigation of striatal dopamine D2 receptor acquisition following prenatal neuroleptic exposure. *Psychiatr. Res.* **36**: 319-328.
9. ALI, S.F., BUELKE-SAM, J. & SLIKKER, W. 1986. Prenatal reserpine exposure in rats decreases caudate nucleus dopamine receptor binding in female offspring. *Toxicol. Lett.* **31**: 195-201.
10. SCALZO, F.M., HOLSON, R.R., GOUGH, B.J. & ALI, S.F. 1989. Neurochemical effects of prenatal haloperidol exposure. *Pharmacol. Biochem. Behav.* **34**: 721-725.
11. MILLER, J.C. & FRIEDHOFF, A.J. 1985. The window of vulnerability in the ontogeny of the dopamine receptor. *Psychopharmacol. Bull.* **21**: 683-685.
12. SCALZO, F.M., ALI, S.F. & HOLSON, R.R. 1989. Behavioral effects of prenatal haloperidol exposure. *Pharmacol. Biochem. Behav.* **34**: 727-731.
13. WILLIAMS, R.L., ALI, S.F., SCALZO, F.M., SOLIMAN, K. & HOLSON, R.R. 1992. Prenatal haloperidol exposure: effects on brain weights and caudate neurotransmitter levels in rats. *Brain Res. Bull.* **29**: 449-458.
14. SCALZO, F.M., ALI, S.F., FRAMBES, N.A. & SPEAR, L.P. 1990. Weanling rats exposed prenatally to cocaine exhibit an increase in striatal D2 dopamine binding associated with an increase in ligand affinity. *Pharmacol. Biochem. Behav.* **37**: 371-373.
15. ALI, S.F., SLIKKER JR., W., NEWPORT, G.D. & GOAD, P.T. 1986. Cholinergic and dopaminergic alterations in the mouse central nervous system following acute trimethyltin exposure. *Acta Pharmacol. Toxicol.* **59**: 179-188.