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Prenatal Haloperidol Reduces the Number of Active Midbrain Dopamine Neurons in Rat Offspring

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ZHANG, J., L. WANG AND D. K. PITTS. *Prenatal haloperidol reduces the number of active midbrain dopamine neurons in rat offspring.* NEUROTOXICOL TERATOL **18**(1) 49–57, 1996.—The dopamine (DA) receptor antagonist, haloperidol (HAL, 1.25 or 5 mg/kg), or vehicle, dimethyl sulfoxide (DMSO), was administered (SC) daily to pregnant Sprague-Dawley dams from gestational day (GD) 8 to GD 20. The average body weight of 2-week-old male offspring was significantly lower in all of the HAL-treated groups relative to controls. In extracellular electrophysiological studies, the male 2-week-old offspring from all HAL treatment groups were found to have significantly reduced average numbers of spontaneously active midbrain dopamine (DA)-containing neurons in both the substantia nigra (A9) and the ventral tegmental area (A10) relative to controls. In DA neurons classified as bursting neurons, HAL exposure (5 mg/kg) caused a significantly increased level of burst activity in A10 but not A9 DA neurons relative to controls. For both the A9 and A10 regions, the proportion of DA neurons classified as bursting or nonbursting was unaffected by HAL treatment. These results suggest that prenatal HAL exposure influences the development of midbrain DA neurons.

Development Substantia nigra Ventral tegmental area Dopamine Electrophysiology Ontogeny Antipsychotic drugs Neuroleptics

HALOPERIDOL (HAL) is an antipsychotic agent, which is a potent, but relatively nonselective dopamine (DA) receptor antagonist (60). The therapeutic effects of HAL are generally believed to be due to its blockade of DA D_2-like receptors (60). The effects of pre- or postnatal exposure to HAL on nervous system development have been reported by many investigators [e.g., (1,2,8,15,16,36,37,56,58,63,73)]. The value of such studies are severalfold: (a) to gain further understanding of the possible role that dopamine receptors play in the development of the nervous system, (b) to provide a comparison of the responses of the immature and mature nervous systems to the repeated exposure to DA antagonists, and (c) to assess the nature of potential clinical risk factors involving alterations in dopaminergic neurotransmission during critical phases of development.

Midbrain dopamine neurons undergo differentiation over the 11th to 15th day of gestation in the rat (31). Dopamine receptors appear in the embryonic rat brain (3,16,17,54), and have been demonstrated in the substantia nigra by gestational day (GD) 17 and in the caudate putamen by GD 14 (54). The projection pathways of the midbrain dopamine neurons are well developed, but still immature at birth (33,44,59,69). Postnatal synaptogenesis occurs in the striatum to give rise to adult-like innervation patterns by the fourth postnatal week (25,33,42,44). Prenatal HAL treatment does not appear to alter the DA neurotransmitter or metabolite levels in the striatum of 1- to 58-day-old offspring (51,73). Prenatal HAL treatment has been reported to decrease the number of postsynaptic DA receptors (37,50,51,56). However, there has been at least two negative reports (36,58), and one report suggesting an increase in DA receptor binding (41). Prenatal or perinatal exposure to HAL in the rat has been shown to alter the responses of offspring to drug challenges that affect dopaminergic systems (15,50,55,63).

Prenatal HAL exposure decreases cell proliferation in the forebrain (2,46) and alters the expression of DNA polymerases

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in the forebrain and mesencephalon of neonatal offspring (8). It has been reported that prenatal HAL reduces both the expression of nerve growth factor receptor mRNA and receptor density in the neonatal rat forebrain (1). Nerve growth factor has been reported to regulate the differentiation of the cholinergic neurons in the striatum (39). The studies suggesting that prenatal HAL exposure has a significant impact on forebrain development are important when considering possible specific effects of prenatal HAL exposure on midbrain DA neurons. Midbrain DA neurons appear to influence (29,32,42,45,67), and in turn, be influenced by (6,34,35) the development of forebrain target areas.

At least two functional aspects of midbrain DA neuron activity have been reported during normal development within the late embryonic period, depolarization-induced DA release that is calcium dependent (43) and DA reuptake (76). Because electrophysiological studies have shown that the mesencephalic DA-containing neurons are spontaneously active in vivo during the first postnatal week (65,70,72) and other catecholamine-containing neurons, such as those in the locus coeruleus (53), have been shown to be capable of responding to sensory stimuli during late gestation, it is possible that the mesencephalic DA neurons exhibit activity before birth. Pharmacological studies have shown that DA D_1- and D_2-like receptor stimulation can affect fetal behavior (40). The early appearance of functional capabilities in mesencephalic DA neurons would be consistent with their suggested participation in the development of the forebrain (29,32,42,45,67).

Although a number of studies suggest that prenatal HAL exposure affects the development of the DA system, the effect of prenatal HAL on impulse flow from single midbrain DA neurons has not been examined. An initial examination of the electrophysiological activity of midbrain DA neurons from neonatal offspring exposed prenatally to HAL is reported.

METHOD

In Utero Haloperidol Exposure

Fifteen timed-pregnant Sprague–Dawley rats (Hilltop, Scottdale, PA) were housed in standard animal facilities for a minimum of 2 days prior to haloperidol (HAL) exposure. HAL was dissolved in a small volume of dimethyl sulfoxide (DMSO). Both HAL (1.25 or 5 mg/kg) and the same volume of vehicle (DMSO, 200 μl/kg) were administered daily (between 1600–1900 h) by SC injection from GD 8 to GD 20. Five dams were used for the vehicle treatment, seven were used for the high dose (5 mg/kg SC) HAL treatment, and three were used for the low dose (1.25 mg/kg SC) HAL treatment. During the course of the study, two vehicle-treated and one low dose HAL-treated dam were not found to be pregnant. Therefore, only the remaining two low-dose HAL-treated and three vehicle-treated dams and their respective offspring were used in the present study. All experimental groups with the exception of three dams used in the high dose HAL treatment group were crossfostered with untreated lactating dams beginning on postnatal day (PD) 1. Control animals receiving DMSO were matched to the high-dose HAL-treated dams of similar weight and pair fed a standard diet of laboratory rat chow. Litters were culled to males only on PD 3. Only correctly identified 2-week-old male offspring were used in electrophysiological studies.

Electrophysiology

Male 2-week-old rat pups (PD 14 and 15) were anesthetized with chloral hydrate (400 mg/kg, IP) and placed in a stereotaxic apparatus (ASI Instruments, Warren, MI). The animals were intubated following a tracheotomy to facilitate spontaneous respiration. Body temperature was maintained at 37 ± 1°C with a thermostatically controlled heating pad. Single-unit extracellular recordings were made from spontaneously active DA neurons with glass micropipettes (1.5 mm diameter, World Precision Instruments, Inc., Sarasota, FL) broken to achieve a tip diameter of approximately 1–2 μm. The pipettes were filled with a 2 M NaCl solution and typically had impedances that measured 1.8 to 2.5 megohms at 15 Hz in physiological saline. Details regarding the electrophysiological technique for identifying and recording from midbrain dopamine-containing neurons in neonates have been previously reported (48,65,70–72).

In order to sample midbrain DA neuron activity, stereotaxic coordinates were used to define a block of tissue. This consisted of 12 different electrode tracks divided equally between the A9 and A10 cell body regions. The coordinates were as follows: A9: 1.8, 2.0, or 2.2 mm anterior to lambda and 1.4 or 1.6 mm lateral to the sagittal suture; A10: 1.8, 2.0, or 2.2 mm anterior to lambda and 0.4 or 0.6 mm lateral to the sagittal suture. Two mean values (A9/A10) were calculated for the number of cells per track, and another two mean values (A9/A10) for the average firing rate for a total of four mean values calculated per rat. The cells-per-track data represents the average number of cells recorded from the six different electrode tracks within either the A9 or A10 cell body regions. The average firing rate represents the mean of the firing rate of all A9 or A10 DA neurons from each rat. For specific experimental groups, interspike interval (ISI) histograms (500 consecutive active potentials per histogram) were collected from DA neurons as previously described (14,48,70). These ISI histograms were later used for discharge pattern analysis using software developed by Dr. L. A. Chiodo (Department of Pharmacology, Texas Tech University). The criteria for the designation of a cell as a bursting cell were as follows: (a) The occurrence of bursts defined to begin with an interspike interval less than 80 ms and terminate with the first interspike interval greater than 160 ms; (b) a minimum of two three-spike bursts were encountered within 500 active potentials (11,22, 48,70).

Data Analysis

Changes in dam body weights (g) were evaluated using an analysis of covariance with repeated measures (gestation day). The covariate, pretreatment weight (g), was used to decrease error variance and increase power (47). Dunnett's test was used for post hoc comparisons of treatment values to pretreatment values. Offspring weights (g) were evaluated using a one-way analysis of variance (ANOVA). The average number of cells per electrode track and average discharge rate were analyzed for treatment effects using a two-way ANOVA (factors: treatment group and cell type) with repeated measures on cell type (A9 or A10). A Newman–Keuls multiple comparison test was used for posthoc comparisons.

The discharge pattern was evaluated using the ISI files collected for each cell. For discharge pattern analysis, DA neurons were divided into two categories: bursting DA neurons and nonbursting DA neurons. The distribution of bursting and nonbursting DA neurons among treatment groups was evaluated using a three-way frequency (i.e., log-linear) analysis. The three factors were treatment group (HAL vs. vehicle), cell type (A9 vs. A10), and discharge pattern category (bursting vs. nonbursting). Seven different burst parameters were

examined within the cells categorized as bursting neurons (11,22,48,70). Due to the intercorrelation between burst parameters, factor analysis was used to divide the parameters into subgroups that were found to be the same as those previously reported (48,70). A two-way MANOVA was used to examine the effect of treatment among the subgroups of burst parameters. Independent variables were treatment group and cell type. The dependent variables were divided between the two different factors as follows: the first factor included discharge rate (spikes/s), average within-burst ISI (ms), post-burst inhibitory period (ms), burst length (in number of action potentials), and the second factor included percentage of spikes in bursts, duration between burst events (s), and number of bursts (per 500 action potentials). All variables except discharge rate and within-burst ISI were log transformed prior to statistical analysis (48,70). Contrast analysis and the Newman-Keuls multiple comparison test was also used for further evaluation of treatment effects among the discharge pattern variables. The coefficient of variation, CV, was calculated for all cell interspike interval files. A three-way ANOVA was used for analysis of the CV according to treatment group, cell type, and discharge pattern category.

All data are expressed as arithmetic mean ± standard error of the mean unless otherwise indicated. With the exception of discharge pattern analysis, all sample sizes reported refer to the number of animals. The sample sizes used in discharge pattern analysis included within Tables 1 and 2 and Fig. 3 refer to the number of cells. The application of many of the above statistical procedures has been previously described in detail (47,48,70).

RESULTS

Growth and Survival of the Dams and Neonatal Offspring

The initial pretreatment body weight (g) of the dams was not significantly different ($p > 0.10$), with an overall average of 243 ± 10 g. The body weight gain for the two different groups of dams treated with 5 mg/kg HAL and used for the non-crossfostered ($n = 3$) and crossfostered experiments ($n = 4$), was not significantly different (ANCOVA, $p > 0.40$). The weight data for these two groups was, therefore, pooled (Fig. 1A). The maternal weight gain (from GD 9 to GD 21) of the pair-fed vehicle controls was not significantly different from that of the 5 mg/kg HAL-treated dams (ANCOVA, $p > 0.05$). However, when GD 9 to GD 15 were examined, the average weight of the dams was found to be significantly lower (Dunnett's test, $p < 0.05$) than their respective pretreatment values for both the 5 mg/kg HAL-treated group (GD 9 to GD 13 significantly lower) and the vehicle-treated group (GD 10 to GD 12 significantly lower). The pattern of weight gain in the vehicle-treated animals likely reflects the effect of paired feeding.

All rat pups were born after a 20 to 22 day period of gestation. The transfer of offspring to untreated lactating dams for crossfostering occurred on the first postnatal day (PD 1). No significant differences in the litter size or number of male and female offspring were found among the various treatment groups. A certain number of the offspring in the present study died within the first 2 days following birth. Animals surviving past the second postnatal day lived until they were used in electrophysiological experiments at the age of 2 weeks old. The survival rate of the offspring observed at 2 days following birth was 86.5% (83 out of 96) for the 5 mg/kg Hal treatment, 78% (28 out of 36) for the 1.25 mg/kg treatment, and 100% ($n = 44$) for vehicle-treated controls.

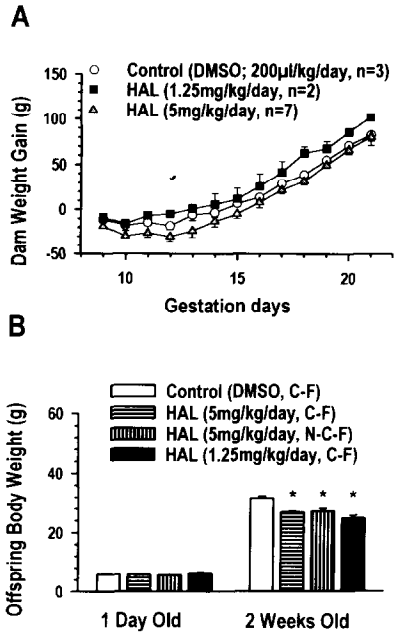

FIG. 1. Effect of prenatal HAL treatment on the maternal weight gain and postnatal body weight. (A) The maternal weight gain (from GD 9 to GD 21) of the pair-fed vehicle controls ($n = 3$) was not significantly different (ANCOVA, $p > 0.05$; pretreatment weight as the covariate) from that of the pooled data (crossfostered and non-crossfostered) from 5 mg/kg HAL treatment groups ($n = 7$). The average weight of the dams was found to be significantly lower (Dunnett's test, $p < 0.05$) than their respective pretreatment values for both the 5 mg/kg HAL-treated group (GD 9 to GD 13 significantly lower) and the pair-fed vehicle controls (GD 10 to GD 12 significantly lower). The small sample representing the 1.25 mg/kg HAL group ($n = 2$) was not significantly different from pair-fed controls (ANCOVA, $p > 0.05$). (B) The high dose (5 mg/kg) HAL-treated offspring are divided between two groups: crossfostered (C-F) and non-crossfostered (N-C-F). The average body weight of the HAL-treated offspring at PD 1 (including both male and female offspring) was not significantly different from the vehicle controls (ANOVA, $p > 0.20$). The average body weight of 2-week-old offspring (males only) from all three HAL-treated groups was found to be significantly lower than that of the vehicle-treated controls (ANOVA, $p < 0.01$; *Newman-Keuls, $p < 0.05$ for each comparison of HAL treatment with control; $p > 0.10$ for comparisons among HAL treatment means).

The average body weight of the HAL-treated pups at PD 1 (including both male and female offspring) was not significantly different from the vehicle controls (ANOVA, $p > 0.20$; Fig. 1B). However, the average body weight of 2-week-old pups (males only) in the three HAL-treated groups was found to be significantly lower than that of the vehicle-treated controls (ANOVA, $p < 0.01$; Newman-Keuls $p < 0.05$ for each comparison of HAL treatment with control; $p > 0.10$ for comparisons among HAL treatment means, Fig. 1B).

Number of Spontaneously Active Midbrain DA Neurons in 2-Week-Old Offspring

Figure 2A shows that there was a significant reduction in the mean number of spontaneously active DA cells per track for the HAL-treated groups (ANOVA, treatment group effect, $p < 0.001$; Newman-Keuls, $p < 0.001$ for each compar-

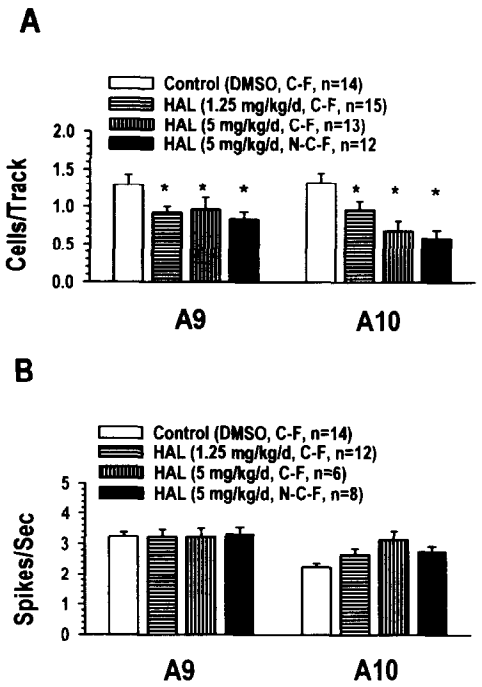

FIG. 2. Effects of prenatal HAL treatment on the number of spontaneously active A9 and A10 midbrain DA neurons and their discharge rate in 2-week-old offspring. (A) The mean number of spontaneously active DA cells per electrode track was significantly reduced relative to controls for for male offspring from the HAL-treated groups (ANOVA, treatment group effect, $p < 0.001$; Newman-Keuls, $*p < 0.001$ for each comparison of HAL treatment to respective control). The extent of the decrease in the number of cells per track was similar for both A9 and A10 cell groups (ANOVA, cell type × treatment interaction, $p > 0.50$) and across HAL treatment groups for a given cell type (Newman-Keuls, $p > 0.05$ for each comparison). (B) Prenatal HAL treatment did not significantly change the average discharge rate of A9 and A10 DA neurons (ANOVA, treatment group effect, $p > 0.20$). The number of subjects in the HAL treatment groups is fewer in B relative to A. For each animal, the criterion of a minimal sample size of three cells per A9 and per A10 cell group area was used to determine inclusion in the analysis.

ison of HAL treatment to control). The extent of the decrease in the number of cells per track was similar for both A9 and A10 cell groups (ANOVA, cell type × treatment interaction, $p > 0.50$) and across HAL treatment groups for a given cell type (Newman-Keuls, $p > 0.05$ for each comparison).

Figure 2B shows that prenatal HAL treatment did not significantly change the average discharge rate of A9 and A10 DA neurons (ANOVA, treatment group effect, $p > 0.20$). Because fewer cells were found in the HAL-treated offspring, the animals with less than a minimum of three A9 and three A10 DA neurons were not included in the statistical analysis or Fig. 2B. For each animal, the discharge rate analysis included two mean values (representing the A9 and A10 cell groups), each calculated from a minimal sample size of three. Therefore, the number of offspring (n) indicated in Fig. 2B is lower than that used in Fig. 2A. It is possible that significant group-dependent changes in discharge rate may not have been detected due to the reduction in sample size (i.e., number of animals included in the analysis) and loss of statistical power. In contrast to the treatment group effects, the average discharge rate of A9 DA neurons was found to be significantly higher than that of the A10 DA neurons (ANOVA cell type effect, $p < 0.01$).

Discharge Pattern of Midbrain DA Neurons from 2-Week-Old Offspring

A total of 282 interspike interval (ISI) histograms (163 from A9 DA neurons and 119 from A10 DA neurons) were collected from the two 5 mg/kg HAL-treated groups and the control group. The DA neurons used in the discharge pattern analysis were divided into two subgroups, bursting and nonbursting DA neurons, based on the criteria described in the methods. The distribution of bursting and nonbursting DA neurons among the drug treated and control groups are shown in Tables 1A and 1B. In the HAL-treated groups, the distribution of bursting and nonbursting DA neurons among the A9 (Table 1A) and A10 (Table 1B) regions was not found to be different (three-way log-linear analysis: treatment × cell type × discharge pattern) from that of the vehicle-treated controls ($p > 0.20$, for treatment group and all treatment interactions). The cell type by discharge pattern interaction was found to be significant ($p < 0.001$). The significance of the interaction can be attributed to the relative proportion of bursting to nonbursting DA neurons in the A9 and A10 cell regions. The ratio of bursting to nonbursting neurons is smaller for the A9 region relative to the A10 region.

Figure 3 illustrates another parameter used in the analysis of discharge pattern, the coefficient of variation (CV). The effect of treatment on CV was examined for all cells by a three-way ANOVA (treatment group × cell type × discharge pattern). No significant differences among the different treatment groups was found for the average CV ($p > 0.20$, for the treatment group effect, and $p > 0.05$ for all treatment interactions). For all treatment groups, however, the average CV for bursting DA neurons was significantly higher than that of the nonbursting DA neurons ($p < 0.001$, for the discharge pattern effect; and $p > 0.20$ for the discharge pattern interactions). The average CV of A10 DA neurons was also found to be significantly higher than those of A9 DA neurons ($p < 0.01$, for the cell type effects, and $p > 0.05$ for all cell type interactions).

A more detailed analysis of the bursting DA neurons from the different treatment groups was accomplished by examining seven burst-parameters (see Table 2A and 2B). A multivariate analysis of variance (MANOVA) was used on each of two subgroups of interrelated variables (see the Method section). No significant differences among treatment groups were found in the first set of variables, which included discharge rate, within burst ISI, postburst inhibitory period, and burst length (MANOVA, treatment group effect, $p > 0.20$). The A10 bursting neurons had a significantly lower discharge rate

TABLE 1A

DISTRIBUTION OF BURSTING AND NONBURSTING A9 DA NEURONS

Treatments	Bursting	Nonbursting	Total (n)
Vehicle	14 (25.9%)	40 (74.1%)	54
HAL (5 mg/kg) crossfostered	18 (30.0%)	42 (70.0%)	60
HAL (5 mg/kg) noncross-fostered	13 (26.5%)	36 (73.5%)	49
Total (n)	45 (27.6%)	118 (72.4%)	163

TABLE 1B

DISTRIBUTION OF BURSTING AND NONBURSTING A10 DA NEURONS

Treatments	Bursting	Nonbursting	Total (*n*)
Vehicle	14 (36.8%)	24 (63.2%)	38
HAL (5 mg/kg) crossfostered	25 (59.5%)	17 (40.5%)	42
HAL (5 mg/kg) noncross-fostered	17 (43.6%)	22 (56.4%)	39
Total (*n*)	56 (47.1%)	63 (52.9%)	119

and significantly longer postburst inhibitory period than the A9 bursting DA neurons (cell type effect, $p < 0.01$; Newman-Keuls test, $p < 0.05$ comparing A9 and A10 for both variables).

A significant treatment group effect (MANOVA, treatment group effect, $p < 0.05$) was detected among the second set of variables, which included the percentage of burst-related spikes, the duration between bursts and the overall number of bursts per 500 spikes (Table 2A and 2B). A simultaneous evaluation of all three variables using contrast analysis indicated that there were significant treatment group effects only within the A10 cell group. The two HAL treatment groups were both significantly different from vehicle controls ($p < 0.01$, in each case; Table 2B). A significantly greater average percentage of spikes in bursts (Newman-Keuls, $p < 0.05$ for each comparison) and significantly shorter mean duration between bursts (Newman-Keuls, $p < 0.01$ for each comparison) was found for the HAL-treated groups relative to the vehicle-treated controls. In addition, a trend for a greater number of bursts per 500 spikes for the HAL treatment groups vs. vehicle controls was noted (Newman-Keuls, $p < 0.05$ for the crossfostered group and $0.05 < p < 0.10$ for the non-crossfostered group). This last result is consistent with the shorter duration between bursts for the HAL-treated groups. The mean duration between bursts was also found to be longer for A10 bursting DA neurons than for A9 bursting DA neurons (cell type effect, $p < 0.01$; Newman-Keuls, $p < 0.001$).

DISCUSSION

Prenatal HAL exposure was found to significantly affect the electrophysiological characteristics of midbrain DA neurons from offspring in the present study. Both the 1.25 and the 5.0 mg/kg prenatal treatment with HAL significantly reduced the number of spontaneously active midbrain DA neurons in neonates relative to pair-fed vehicle controls. The effect of HAL exposure on the number of spontaneously active midbrain dopaminergic neurons did not appear to be dose dependent, suggesting that the two doses studied may be exerting maximal or near maximal effects. This effect on the number of spontaneously active DA neurons was also found to be independent of the cell group studied, being the same for both the A9 and A10 regions. The doses used in this study are similar to those used in previous studies of the developmental effects of HAL [e.g., (2,15,36,56,63)], and the low HAL dose, in particular, is within the range of doses typically used to study the effects of repeated neuroleptic treatment on brain function in the rat [e.g., (4,10,52,74)].

Midbrain DA neurons can be divided among at least three categories according to their activity: (a) spontaneously active neurons discharging in the single spike mode, (b) spontaneously active neurons discharging in the burst mode, and (c) electrophysiologically inactive cells with little or no basal activity (5,14,21,22). A significant portion of the midbrain DA neurons are apparently inactive at any given time, and these neurons can be activated by the application of iontophoretic or systemic drugs (4,5,10,12,23,74,75). A reduction in the physical number of DA neurons present or a reduction in the number of spontaneously active DA neurons (e.g., a decrease in the ratio of active to silent cells) will result in a decrease in the number of spontaneously active DA neurons detected by the electrophysiological method used in this study. The present study cannot determine if the decrease in the number of spontaneously active DA neurons per electrode track is due to a physical loss of cells or an increase in the proportion of silent cells. Histological studies will be necessary to determine if there is a physical reduction in the midbrain DA neuron population of offspring exposed prenatally to HAL.

In addition to its effect on the number of spontaneously active DA neurons, prenatal HAL exposure was also found to affect the discharge pattern of DA neurons. DA neurons discharging in a burst mode in vivo are believed to release more DA in the forebrain than DA neurons discharging at a similar average discharge rate in the single spike mode (18,19). Although the functional role of the DA neuron burst discharges is still not completely understood, a number of hypotheses have been discussed (5,14). Afferent activity plays an important role in the generation of burst discharges in the DA neurons. Burst activity is absent in vitro, when afferents are severed in the brain slice preparation (24,28). The relative importance of specific excitatory influences (e.g., disinhibition, neuropeptides, excitatory amino acids) for eliciting burst discharges in vivo is still a matter under active investigation (5,9,20,22,28,61,62,64,78). In the present study, prenatal HAL treatment did not appear to affect the proportion of DA neurons classified as bursting neurons relative to the pair-fed vehicle controls. However, the A10 neurons classified as bursting cells within both the crossfostered and non-crossfostered 5 mg/kg HAL treatment groups had significantly more burst activity (i.e., higher percentage of spikes in bursts and lower duration between bursts) relative to similarly classified pair-fed vehicle controls. This result, although statistically signifi-

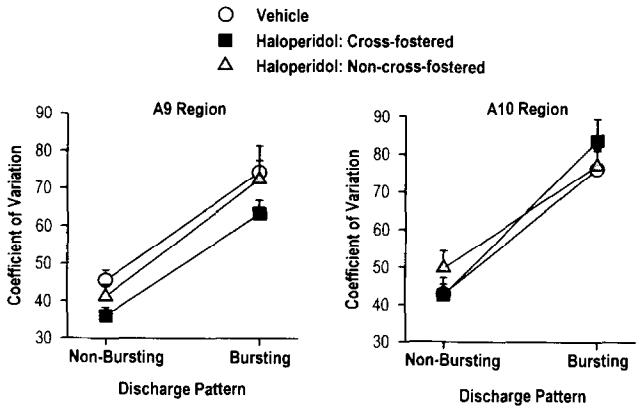

FIG. 3. Effects of prenatal HAL treatment on the coefficient of variation (CV) for interspike intervals from DA neurons. No significant differences among the different treatment groups was found for the average CV ($p > 0.20$, for the treatment group effect, and $p > 0.05$ for all treatment interactions). The number of cells (*n*) included for each group can be found in Table 1A and 1B.

TABLE 2A

ANALYSIS OF BURSTING A9 DA NEURONS

Burst Parameters	Vehicle	HAL (5 mg/kg) Crossfostered	HAL (5 mg/kg) Noncrossfostered
(*n*)	14	18	13
Discharge rate (spikes/s)	4.4 ± 0.4	3.9 ± 0.3	4.5 ± 0.3
Within-burst ISI (ms)	73 ± 3	72 ± 3	75 ± 3
Post burst inhibition (ms)	357 ± 23 [348]	467 ± 44 [436]	323 ± 9 [322]
Burst length (no. spikes/burst)	2.6 ± 0.2 [2.5]	2.6 ± 0.2 [2.5]	2.6 ± 0.1 [2.5]
Percentage of spikes in bursts	30 ± 4 [27]	38 ± 5 [31]	35 ± 7 [24]
Duration between bursts (s)	2.5 ± 0.4 [2.1]	1.9 ± 0.3 [1.6]	3.6 ± 2.0 [1.6]
No. of Bursts (per 500 spikes)	64 ± 10 [54]	80 ± 12 [62]	69 ± 13 [49]

[Geometric mean].

cant, must be interpreted with caution, because the sample sizes used within the context of a multivariate design are somewhat small. The burst parameter averages reported for the pair-fed vehicle controls, however, are quite similar to those previously reported for A9/A10 DA neurons from untreated 2-week-old animals (48,70). The fact that bursting DA neurons from both 5 mg/kg HAL treatment groups (i.e., crossfostered and noncrossfostered) had significantly more burst activity than those from pair-fed vehicle controls adds strength to this finding.

An additional variable, the coefficient of variation (CV; standard deviation/mean × 100%) was used to describe the relative dispersion within interspike interval histograms for the cells of each treatment group. Although this variable is generally used to describe normal distributions, and DA neuron interspike interval histograms are often not normally distributed, the CV has been reported in the past to reflect the degree to which adult DA neurons discharge in a regular (low average CV) or irregular (high average CV) pattern (26,78). Bursting neurons tend to have a higher average CV, while cells discharging in a predominantly single-spike mode have a lower average CV (26,78). In the present study, no significant treatment effects on CV were found. One possible explanation for the lack of significant treatment-induced changes in CV observed in this study is that there were no changes in the proportion of DA neurons classified as bursting cells, resulting in a lower power for an analytical method that does not categorize the discharge patterns.

In some previous studies, prenatal HAL exposure was reported to cause a small but significant decrease in the brain weight and body weight of rat offspring (68,73). Although the mechanism responsible for the HAL-induced changes in the midbrain DA neurons of offspring is unknown, it appears to be unlikely that the changes in the characteristics of midbrain DA neurons are due to an effect on maternal weight or nutrition. Similar effects on the rate of weight gain for both the 5 mg/kg HAL-treated and the pair-fed vehicle-treated pregnant dams were observed. The significant decrease in the rate of weight gain relative to pretreatment values caused by HAL or paired-feeding has been reported previously and differs from the normal steady increase seen during gestation (73). A significant reduction in body weight was observed in the 2-week-old (males only), but not in the 1-day-old (both males and females) HAL-treated offspring relative to controls. This suggests that there was a delayed effect on the growth of the HAL-treated offspring. However, a sex-dependent difference in the effect of HAL on PD 1 body weight cannot be ruled out because the offspring weights for the two sexes were not measured separately on this day. A previous report (73) suggests that a similar prenatal HAL treatment regimen caused a significant reduction in PD 1 body weight. A significant reduction in the offspring body weight following prenatal HAL exposure in the rat has been reported for both adult offspring (68,73) and PN day 30 offspring (73). The results of the present study and that of a previous study using pair-fed controls (73) indicate that the stunting of offspring growth cannot likely be attributed to maternal undernutrition. A significant and long-lasting decrease of approximately 10% in the brain weight of offspring exposed prenatally to HAL has also been reported (73).

Repeated daily HAL administration in adult rats has also been shown to cause a significant decrease in the number of spontaneously active midbrain DA neurons per electrode track in the A9 and A10 cell groups (4,10,12,74,75). This phenomenon has been reported to be due to depolarization inactivation of a significant portion of the DA neurons (4,10,12,23,74,75). Although the results from the present study and the studies conducted in adults appear to be similar, there are several factors suggesting that different mechanisms may be involved. D_2 receptor density has been reported to be increased in the adult rat forebrain following exposure to HAL or other neuroleptics (60). However, D_2 receptor binding sites may be reduced (37,50,51,56) or unaffected (36,58) in the forebrain following prenatal neuroleptic treatment. The reduction in the number of spontaneously active DA neurons in the present study was determined in 2-week-old offspring from mothers

TABLE 2B

ANALYSIS OF BURSTING A10 DA NEURONS

Burst Parameters	Vehicle	HAL (5 mg/kg) Crossfostered	HAL (5 mg/kg) Noncrossfostered
(*n*)	14	25	17
Discharge rate (spikes/s)	3.0 ± 0.3	3.7 ± 0.3	3.8 ± 0.2
Within-burst ISI (ms)	69 ± 2	66 ± 3	70 ± 3
Post burst inhibition (ms)	621 ± 150 [513]	571 ± 68 [506]	486 ± 35 [467]
Burst length (no. spikes/burst)	2.3 ± 0.1 [2.3]	2.7 ± 0.2 [2.6]	2.8 ± 0.2 [2.7]
Percentage of spikes in bursts	15 ± 2 [12]	36 ± 4 [28]*	37 ± 6 [29]*
Duration between bursts (s)	11.8 ± 5.4 [6.6]	3.5 ± 0.7 [2.4]†	3.2 ± 0.7 [2.3]†
No. of Bursts (per 500 spikes)	32 ± 5 [25]	66 ± 8 [53]	68 ± 10 [55]*

[Geometric mean].

*Newman-Keuls, $p < 0.05$ compared to vehicle controls.

†Newman-Keuls, $p < 0.01$ compared to vehicle controls.

that had HAL administration terminated approximately 1 to 2 days before birth (approximately 15 to 17 days before electrophysiological recordings). When radiolabeled HAL is administered to pregnant dams on GD 21, the half-life of the drug in the brain of exposed neonates has been reported to be approximately 2 days (36). In the present study, the possibility of continued exposure to HAL through the treated dam's milk did not affect the outcome for the offspring. There was no significant difference between the results obtained from crossfostered or non-crossfostered pups. In adult studies the reduction in the number of spontaneously active DA neurons exhibited partial recovery within 1 week (4) and complete recovery within 2 weeks (13) following HAL withdrawal. However, in the present study, the possibility that there is a significant residual HAL concentration in the neonatal brain cannot be ruled out. There apparently is a greater accumulation of HAL in the neonatal vs. the maternal brain following gestational administration (36). Continued presence of significant amounts of HAL in the neonatal brain would suggest the possibility that the midbrain DA neurons might be inactivated by prolonged depolarization in a manner similar to adults. Furthermore, this might suggest that HAL-induced depolarization inactivation could occur at an early developmental age. However, some of the electrophysiological properties attributed to depolarization inactivation in adult animals, such as reduced action potential amplitude, prolonged burst discharges, and longer postburst inhibition (10,23,74,75), were not observed in the present study. Our results suggest that prenatal HAL exposure might have induced a physiological alteration in the midbrain DA neurons by an unknown mechanism and as yet undetermined duration for postnatal life.

An alteration in the physical number of midbrain DA neurons is another possible mechanism by which prenatal HAL might decrease the number of spontaneously active DA neurons in the present study. Unlike adults, the midbrain DA neurons of neonatal rats appear to be undergoing apoptosis during the early postnatal period (27,66). This is consistent with the observation that the number of spontaneously active DA neurons per electrode track appears to be somewhat higher in the 2-week-old rat midbrain [(71) and the present study] than in the adult midbrain (10,12,74,75,77). The electrophysiological and pharmacological characteristics of neonatal midbrain DA neurons are also different from those of DA neurons in adults (48,65,70,72). Perinatal or prenatal exposure to the monoamine reuptake inhibitor, cocaine, has been similarly reported to reduce the number of spontaneously active DA neurons in neonatal (71) and adult (38) rat offspring. Although speculative, it is possible that prenatal treatments that affect catecholaminergic transmission may affect apoptotic events within the midbrain DA neuron population. Quinolinic lesions of the striatum, have been shown to cause reductions in the number of surviving DA neurons in the ipsilateral substantia nigra (6,34). This loss of DA neurons has been attributed to apoptosis and a possible loss of neurotrophic factors (6,34,35). Dopamine receptors have also been shown to affect growth cone motility in cultured retinal neurons (30). Alterations in the motility of growth cones may affect the ability of DA neurons to reach or interact with their target sites appropriately. Finally, the influence of a neurotoxic pyridinium metabolite, 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]pyridinium (HPP^+), that is derived from HAL cannot be ruled out in the present study. HPP^+ has been shown to have MPP^+ (1-methyl-4-phenylpyridinium)-like neurotoxic effects on adult midbrain DA neurons (49). It is possible that such a metabolite might reach the fetal CNS during prenatal HAL treatment.

In summary, examination of 2-week-old offspring revealed that prenatal HAL exposure caused a significant decrease in the number of spontaneously active midbrain DA neurons in the A9 and the A10 cell groups. In addition, this prenatal HAL treatment appeared to cause an increase in the level of burst activity associated with A10 neurons classified as bursting cells, but not an increase in the proportion of A10 cells classified as bursting cells. Further studies will need to be conducted to determine if the decrease in the number of active DA neurons results from a physical loss of cells or a physiological alteration in the level of spontaneous activity.

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