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Postnatal haloperidol eliminates the deficit in circling behavior produced by prenatal exposure to the same drug

Marcelo Javier Wolansky[1,2], Mariano Soiza-Reilly, Mariana Fossati, Julio Marcos Azcurra*

Interdisciplinary Project on Neuroteratology (IPON), Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Piso 4, Pab II, Ciudad Universitaria, Universidad de Buenos Aires, C1428EHA Buenos Aires, Argentina

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Abstract

Up to 35% of pregnant women take psychotropic drugs at least once during gestation [Austin and Mitchell, 1998]. From concurrent animal and human evidence, it has been proposed that exposure to several psychoactive medications in utero or during lactation increases the risk for permanent brain disorders. Present preventive or therapy practices applied on humans for this type of long-lasting behavioral alterations are mainly based on empirical results. Here, we test an experimental approach designed to counteract a circling performance deficit that appears in Sprague–Dawley rats at puberty on exposure to the dopaminergic blocker haloperidol (HAL) during gestation [J.L. Brusés, J.M. Azcurra, The circling training: A behavioral paradigm for functional teratology testing, in: P.M. Conn (Ed.), Paradigms for the study of behavior, Acad. Press, New York, 1993, pp. 166–179. *Method Neurosci.* 14]. Gestational exposure to HAL (GD 5–18, 2.5 mg/kg/day ip) induced the expected circling activity decrease in the offspring at the fifth week of life. When prenatal exposure to HAL was continued through lactation (PD5–21, 1.5 mg/kg/day ip), rats otherwise showed a control-like circling performance. No difference was yet found between lactation-only, HAL-exposed pups and saline (SAL)-treated controls ($n = 8$ each group). We further performed saturating (^{3}H)-spiroperidol (SPI) binding assays on striatal P2 membrane fractions 2 months later. The dopamine-type D2-specific binding results suggested that above circling behavior findings could be partially explained by enduring HAL-induced neurochemical changes. The role of critical periods of sensitivity as transient windows for opportunistic therapies for behavioral teratology is discussed.

Keywords: Haloperidol; Behavioral Teratogen; Therapeutic Agent

1. Introduction

Most psychotropic drugs pass through the placenta and/or accumulate in breast milk [36,51,62]. Children exposed in utero or during lactation to neuroactive drugs are often at neurodevelopmental peril and at a higher risk for permanent behavioral damage. This is a direct result of the cause–effect relationship between exposure to drugs during the critical periods of early development and perturbation of brain maturation [54,61]. This neurological susceptibility is supported by an increasing body of evidence from experimental animal models. In several cases, these findings have been consistent with those derived from human studies [39,58,60]. For pharmaceuticals, longitudinal studies of human behavioral teratology have more recently started to appear [17,53]. Animal models to study cellular, pharmacological, and environmental factors influencing developmental neurotoxicology should be investigated further, as they might offer important perspectives for treatment in humans. We have proposed the circling training (CT) test as a rat model for the study of enduring alterations in motor function and learning induced by psychoactive drugs when administered during early development [5,6]. Among the prototypic drugs we have selected for our experiments is the antipsychotic haloperidol (HAL), a dopamine antagonist selective for D2 receptors (D2Rs) [3,14]. Accumulated evidence shows HAL to be considered a relatively safe neuroleptic treatment for childbearing women in terms of structural teratology [2,24], but a suspected behavioral teratogen, when compared with

* Corresponding author. C. F. Melo 4127 (C.P. 1602), Buenos Aires, Argentina. Tel./fax: +54-11-47093444.

E-mail addresses: wolansky.marcelo@epa.gov (M.J. Wolansky), azcuipon@bg.fcen.uba.ar (J.M. Azcurra).

[1] Present address: Neurotoxicology Division, National Health and Environmental Effects Research Laboratory (B105-04), US EPA, RESEARCH TRIANGLE PARK, NC 27711, USA.

[2] In memory of my parents.

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other neuroleptics [39,45]. In rats, chronic exposure to HAL during gestation or early postnatal life induces persistent changes in motor and associative-spatial systems and affects the expression of central neurotransmitters and receptors [9,15,16,26,35,37,46,50,63]. During postnatal development in rats, the potency and efficacy of dopamine antagonists decrease with age [1]. In addition, a couple of weeks after HAL exposure during lactation, a persistent increase in D2R binding in the striatum (STR) is found [42]. This change in D2R density is accompanied by altered D2R gene activity, although the evidence is equivocal [21,41,65]. Thus, these and other findings place lactation as a target period to test experimental interventions for treating dopamine system disturbances originating during gestation. Interestingly, several behavioral, neurochemical, and molecular alterations induced by HAL are dependent on the stage of development during which it is administered. HAL induces long-lasting down- or up-regulation of striatal D2Rs after prenatal or early postnatal exposure, respectively [42], and other brain plasticity-associated markers display similar stage-dependent effects of HAL exposure during perinatal development [11,19]. Furthermore, motor system function and its response to pharmacological challenges are strikingly dependent on the time of HAL exposure during early development [9,16,46,50]. Likewise, by testing adolescent rats exposed to HAL during gestation or lactation, we found a lower [6] or higher (unpublished data) circling speed, respectively. In light of this stage-specific sensitivity of the dopamine system, here, we tested whether the long-term effects of gestation-only exposure to HAL on pubertal circling behavior could be attenuated by administering HAL during lactation.

2. Materials and methods

2.1. Animals

Primiparous Sprague–Dawley female rats (local facilities, originally purchased from Holtzman Institute) were used for breeding. Cages containing three female rats and one male each were placed in a controlled environment (22–24 °C) under a 12-h dark/light cycle. The appearance of sperm after vaginal flushing with saline (SAL) was considered an index for gestational day (GD) 1.

2.2. Doses

Dosages were selected taking into account that man is generally more vulnerable than are experimental animals, by a factor of about 10, to toxic drug effect [20], and our previous experience on enduring circling alterations after early exposure to HAL [5,6]. The HAL dose, on the basis of body weight (BW), was roughly 10-fold higher than a medium one used in antipsychotic therapy [3].

2.3. Prenatal treatments

Pregnant rats were randomly divided into two groups. The treatments were administered intraperitoneally, once daily, from GD 5 to 18 as follows: SAL vehicle ($n = 11$); 2 ml NaCl (0.9% w/v) sterile solution per kilogram BW; or HAL ($n = 12$), 2.5 mg/kg BW, from 20% v/v final solution obtained by diluting HAL in SAL (HAL ampoules, 5 mg/ml, from Janssen Laboratories, BA).

2.4. Postnatal treatments

Stainless steel cages ($22 \times 18 \times 45$ cm^3) were used to foster litters. The litters were reduced to eight pups by maximizing the presence of males. Pups were randomly relocated at postnatal day (PD) 2 to follow up the postnatal step of the experiment with an attenuated litter effect. The offsprings were then injected intraperitoneally once daily, from PD5 to 21, between 3 and 6 p.m. with SAL solution, 10 μl/g BW, or HAL, 1.5 mg/kg BW, from 5% v/v solution obtained by diluting HAL in SAL. Four experimental groups (each group consisting of four cages, $n = 8$ each cage) were thus designated, considering prenatal/postnatal corresponding treatments, as follows: control C (SAL/SAL), SAL/HAL, HAL/SAL, and HAL/HAL. After weaning (PD22), rats from the same pharmacological schedule were regrouped as four individuals per cage. Four cages from each treatment group were moved to the behavioral testing room at PD29 and stayed there, under the same controlled environmental conditions and light cycle as mentioned above, until the functional assessment was completed (PD37). Before testing, two rats from each cage were designated at random for behavioral assessment. The other eight nontested littermates from each pharmacological treatment served to evaluate the expected weight gain pattern alterations induced by CT test associated experimental conditions [6]. Nontrained animals from the control group C (denoted below as C_{NT} group) further served later as a fifth group in D2R selective binding assays. This group controlled for activity-dependent plastic changes that appear in striatal neuroreceptors by the effect of physiological motor exercise executed during the rat striatal critical period [27,47]. The animals were fed with high-protein food and water ad libitum, except when indicated. The rats were taken care of in compliance with the NIH Guide for the Care and Use of Laboratory Animals.

2.5. Behavioral evaluation

Trained animals (water deprived by 24 h) were submitted to the CT test starting at PD30. The procedures used for behavioral testing have already been described [5,6]. Briefly, the rats learn the conditioning in session zero (S_0) and then train daily, for seven days (S_1 up to S_7, one session a day), according to a continuous reinforcement schedule. A drop of 50 μl 10% (w/v) sucrose per clockwise turn was the reward.

Each session is finished when the rat runs for 30 min or after 100 (in $S_{0\text{-}1\text{-}2\text{-}3}$) or 150 turns (in $S_{4\text{-}5\text{-}6\text{-}7}$). The total turns (one turn = one reinforcement) in each session divided by the required time (in minutes) represents the circling velocity (V). The accumulated reinforcement (R_a) after each actual training session, S_1 to S_7, is equal to the total sum of rewarded turns that the rat runs session by session. Turn detection, reinforcement delivery, and time counting are automatically executed. Before training sessions $S_{0\text{-}1\text{-}3\text{-}7}$, the BWs of rats from all treatment groups were recorded. After training, the rats were returned to the general housing facilities, and BWs were recorded twice a week until sacrifice.

2.6. Striata homogenization

At P90, five randomly selected rats from each group were sacrificed by decapitation. Brains were quickly removed, and the cerebellum (CBL) and rostral parts of the STR (e.g., left side = L-STR, right side = R-STR) were then dissected over ice and were weighed. Accordingly with the asymmetric exercise executed by rats during the CT test sessions, all procedures of homogenization and radioligand binding assays were performed, maintaining striatal hemiparts separated. Ice-cold 20 mM Tris–ClH buffer, pH 7.4, containing 0.32 M sucrose, 1 mM EDTA, and 0.5 mM PMSF, was added to the tissues (10% w/v). Homogenization (1300 rpm, 3×20 strokes) was performed in a Potter–Elvehjem glass homogenizer with a Teflon pestle (clearance = 0.25 mm). After centrifugation to $1090 \times g$, 10 min at 4 °C, supernatants were saved, and the pellets were washed twice with ice-cold homogenization buffer and then recentrifuged. Pooled supernatants were centrifuged again to $13\,000 \times g$, 30 min at 4 °C, and the pellets were resuspended in buffer containing 0.02% NaN_3 and were stored at − 70 °C.

2.7. (^{3}H)-spiroperidol binding assay

Membrane samples from each rat were analyzed separately. D2-specific sites were measured on 100 μg membrane protein [33] by saturating 1.6 nM (^{3}H)-spiroperidol [(^{3}H)-SPI; from NEN, 20.5 Ci/mmol] binding assay, as described before [27]. Each determination was made in triplicate, and nonspecific bound (^{3}H)-SPI was determined in a duplicate assay in the presence of 10 μM (−)-sulpiride (Armstrong Laboratories, BA). After 2 h incubation with (^{3}H)-SPI in 1 ml buffer 50 mM Tris–HCl, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2 mM $CaCl_2$, at 30 °C, the samples were filtered through Whatman GF/B discs under vacuum. Radioactivity from washed and dried filters was determined by liquid scintillation spectrometry (LKB Wallac).

2.8. Statistical analysis

To analyze the motor response in the CT test, a two-way ANOVA test was applied, with session (S, repeated measure) and treatment (T) as the main factors. In satisfying the corresponding validation requirements (e.g., Mauchley's Sphericity test), we analyzed records from four representative sessions ($S_{1\text{-}3\text{-}5\text{-}7}$). Once ANOVA results showed significance in the main effects or the interaction [$S \times T$], we analyzed data by Tukey test as multiple comparison method. On the purpose of examining the differences between sessions when circling velocities reach near-maximal performance, we took the last four sessions (e.g., S_4 to S_7). We also applied a derivation of Hernstein's matching law equation described by Heyman and Beer [25]. In our case, we use accumulated reinforcers instead of reinforcement frequency [6]. It consisted in computing the linear regression between V and V/R_a from sessions S_1 to S_7. The maximal theoretical velocity (V_m) was thus obtained for each group by extrapolating the corresponding function to the abscissa (i.e., where $y = 0$). Pairwise group comparison by applying analysis of covariance (ANCOVA) on the above regression results was precluded because of nonfulfillment on ANCOVA prevalidation requirements. We therefore calculated V_m for each rat by applying a regression model. V_m values, grouped by treatment, were statistically examined by ANOVA and then paired off by Tukey test. BWs, recorded before or after CT assessment, and the patterns of BW loss during testing were analyzed with two-way ANOVA, with representative days or sessions ($S_{0\text{-}1\text{-}3\text{-}7}$) as repeated-measure levels, respectively. BWs were likewise examined by comparing trained versus nontrained rats. Tissue weights and radioligand binding results were subjected to ANOVA analysis, with T as the main effect. These latter nonbehavioral results were a posteriori examined with the Scheffé test. In the case of STR, ANOVA analysis was further applied to examine the main effect, striatal-side.

3. Results

3.1. Gross physical development

We did not observe differences in the basic fostering or breast-feeding behaviors demonstrated by pregnant rats after the delivery of litters. Likewise, no change with respect to SAL-exposed rats was detected in HAL-treated rats for any of the following perinatal records: gestation time, sex ratio, malformation rate, neonatal mortality, and mother–pup cannibalism. HAL-treated groups, though, showed slight trends for BW delay during postnatal follow-up (for all pairwise comparisons by Scheffé test for treated vs. control groups, $P > 0.05$; data not shown). At PD90, we evaluated if the dosages of HAL, used in this study, during early development had resulted in some enduring weight alteration in the STR and CBL. The weights (in mg, as means ± S.D.) for L-STR, R-STR, and CBL were, respectively, C_{NT}: 51.9 ± 9.2, 50.9 ± 7.3, and 270 ± 36.2; C: 53.9 ± 8.9, 52.3 ± 6.9, and 263 ± 33.3;

SAL/HAL: 55.9 ± 8.1, 54.0 ± 6.1, and 250 ± 38.7; HAL/SAL: 46.0 ± 8.0, 46.6 ± 8.3, and 239 ± 34.0; HAL/HAL: 50.9 ± 7.7, 49.6 ± 6.7, and 246 ± 28.2. No difference between striatal hemiparts [$F(1,70) = 0.35$, $P = 0.56$] was found. In addition, a slight statistical significance resulted for the main effect T [$F(4,70) = 2.80$, $P < 0.05$]. Yet, the only nearly significant difference was found for a higher STR weight in group SAL/HAL with respect to that from HAL/SAL (i.e., for 21% increase in L-STR, $P < 0.053$). No effect of treatment on CBL weight data was detected [$F(4,35) = 1.05$, $P = 0.40$].

3.2. CT test assessment at 30 days of age

Regardless of treatment, the weight loss patterns during testing were similar with those historically associated with CT test conditioning [6,27,47,64]. Furthermore, we did not find any treatment-related difference in BW ratio evolution by comparing trained versus nontrained/nondeprived rats from each treatment (data not shown). The weight pattern differences between trained and nontrained animals appeared totally recovered about one month after CT testing, as expected from previous work [6].

3.2.1. Behavioral findings

Fig. 1 shows the functional consequences of early-life exposure to HAL on circling behavior as assessed in the CT test at puberty. The circling velocities (V) are presented in Panel A. ANOVA results were significant for both T [$F(3,28) = 8.30$, $P < 0.0005$] and S [$F(3,84) = 549$, $P < 10^{-5}$] and for the interaction $T \times S$ [$F(9,84) = 2.90$, $P < 0.005$]. Group SAL/HAL did not show any circling speed difference compared with control C group. A decrease in motor response in rats exposed to HAL during gestation appeared otherwise by pairing off circling results for groups HAL/SAL versus C from session S_3 onwards ($P < 0.05$). Remarkably, pups treated with HAL during lactation, after exposure to the same drug during gestation (e.g., group HAL/HAL), later had 35% (average) faster CT circling speeds with respect to those from group HAL/SAL ($P < 0.01$). In addition, for most CT assessment, the performance of group HAL/HAL did not show discrimination from the C control. A further examination of circling patterns showed that the session from which an asymptotic response originated was dependent on treatment. In control group C, no statistical difference was found after pairwise comparison between the circling speed means between sessions S_6 and S_7. The

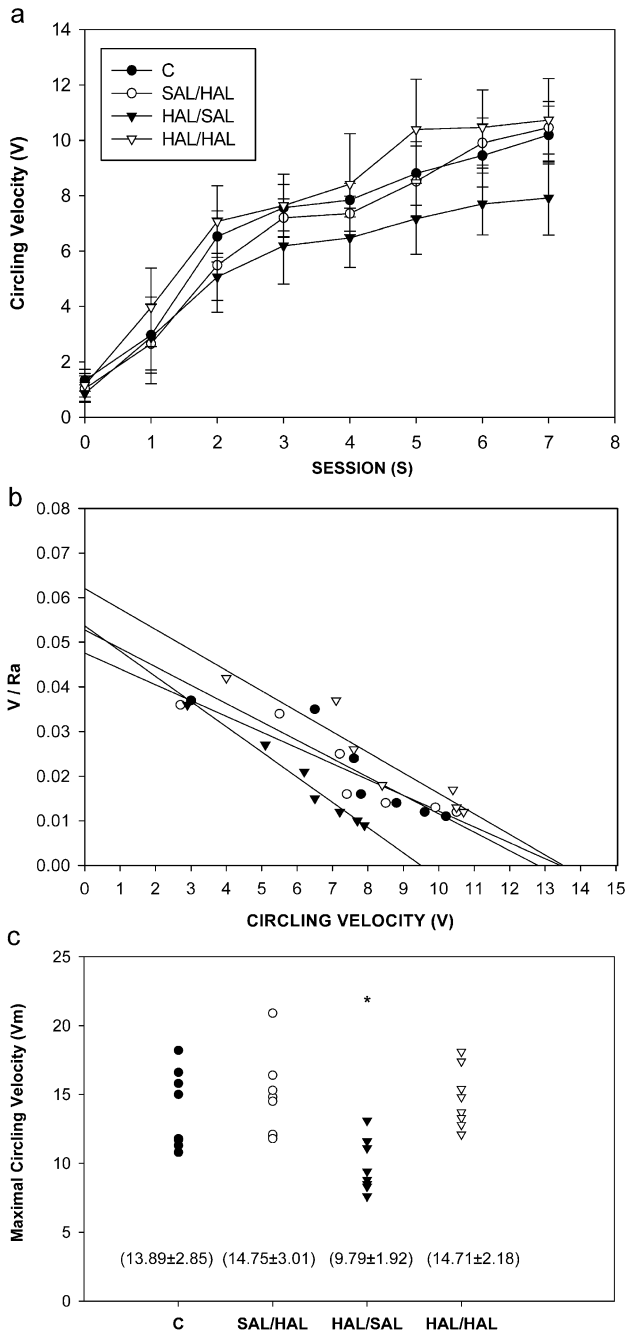

Fig. 1. Performance in the CT test at puberty. Groups: C (control), administered SAL during gestation and lactation; HAL/SAL and SAL/HAL, gestation- or lactation-only exposure to HAL, respectively; and HAL/HAL, exposure to HAL during gestation and lactation. At PD30, these groups were introduced to the CT test, a 7-day training to turn in a circular maze from sessions S_1 to S_7. S_0: session for teaching motor–spatial conditioning. (a) Circling velocities (V) are expressed in turns per minute, as means ± S.D. ($n = 8$ each group). Only sessions $S_{1,3,5,7}$ were taken into account for ANOVA analysis computing. Both the main effects and the interaction [$T \times S$] resulted significant. Comparing the HAL-treated groups to group C, significant differences among the means did emerge for the circling pattern of group HAL/SAL, and just for session S_5 in the case of group HAL/HAL ($P < 0.05$). (b) Scatchard-like transformation of data shown in Panel A. A graphical approximation for the maximal theoretical velocities (V_m) can be appreciated at the intersection of the corresponding regression with the x axis (at $V/R_a = 0.0$). R_a = accumulated reinforcement. See Panel A for group identification. (c) V_m was obtained by fitting each individual data plot to a linear regression model (each rat is thus represented by a dot). These V_m values were then examined by ANOVA. The V_m means (turns/min ± S.D.) are shown at the bottom of the corresponding plot (for main effect T, $P < 0.002$). Only group HAL/SAL presented statistical difference with respect to group C (marked with an asterisk; $P < 0.05$).

circling activity obtained during these last sessions represents that expected for 1-month-old rats, as has been reported elsewhere [6,27]. Group SAL/HAL reached this plateau at S_6, too. In groups treated with HAL during gestation, the maximal motor performance commenced, otherwise, at earlier sessions: in S_4 and S_5 for groups HAL/S and HAL/HAL, respectively. In Panel B, data adjustments to linearity are shown. This mathematical processing has been previously detailed [5,6]. The estimated maximal circling velocities (V_m) are presented in Panel C. The effect of T on V_m values resulted significant [$F(3,28)=6.95$, $P<0.002$]. Similar fitting parameters were found for most groups (e.g., no statistical difference was found by pairing off groups SAL/HAL or HAL/HAL vs. C). Consistent with V patterns shown in Panel A, group HAL/SAL presented a significant 30% lower V_m ($P<0.05$).

3.3. Striatal (^{3}H)-SPI specific binding assay at 90 days of age

The effect of training per se upon nontreated animals, as well as HAL-induced changes on the dopaminergic marker D2R, can be appreciated in the saturating (^{3}H)-SPI binding assay results shown in Fig. 2. The differences between striatal hemiparts in specific radioligand binding to P2 membrane samples were not significant [$F(1,40)=2.67$, $P=0.12$]. Highly significant changes were specifically obtained for analyzing the main effect T [$F(4,40)=31.8$, $P<10^{-9}$]. Furthermore, as expected from previous work [27], a decrease in striatal (^{3}H)-SPI binding was found in trained controls (group C) with respect to nontrained rats from group C_{NT} (-26.4% and -30% for L-STR and R-STR, respectively; $P<10^{-6}$). In addition, pairwise comparisons between treated groups with respect to control C detected a significant 18% drop in striatal (^{3}H)-SPI specific binding from gestation-only exposed rats (group HAL/SAL, $P<0.05$). This radioligand density drop appeared still deeper when the HAL/SAL group was alternatively compared with both groups treated with HAL, including lactation ($P<0.005$). The SAL/HAL and HAL/HAL groups did not differ from group C. Significant decreases in (^{3}H)-SPI specific binding were yet observed in these groups with respect to the nontrained control C_{NT} (group SAL/HAL, -17% and -25%; group HAL/HAL, -24% and -25%; for L-STR and R-STR, respectively; $P<0.0001$). Again, comparing treated groups to the nontrained control group C_{NT}, the largest binding drop in the STR was detected in the case of gestation-only exposure to HAL (for group HAL/SAL, -39% and -43%, on L-STR and R-STR, respectively; $P<10^{-9}$). Furthermore, a nearly significant effect of T on CBL binding data resulted by ANOVA test [$F(4,20)=2.83$, $P<0.052$]. However, a 44% decrease in (^{3}H)-SPI specific binding from group HAL/SAL did not reach statistical significance ($P=0.14$).

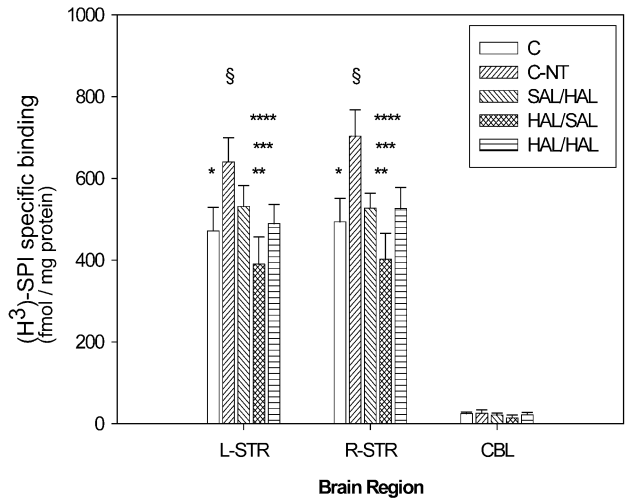

Fig. 2. (^{3}H)-SPI binding assay at adulthood. At PD90, brain regions from the same experimental groups shown in Fig. 1 were dissected, homogenized, and processed to perform (^{3}H)-SPI specific binding assays on P2 membrane fractions. A group of SAL-treated, nontrained rats (group C_{NT}) served to control for persistent activity-dependent D2R changes. The examined regions were the STR, L-STR and R-STR hemiparts, studied separately, and CBL. The data are expressed in fmol (^{3}H)-SPI sites per mg protein as means ± S.D. See details for ANOVA test statistics in the text. A highly significant effect of T was detected on the STR ($P<10^{-9}$). The symbols at the top of the corresponding bars mark significant results emerged on applying the Scheffé test for multiple comparisons ($n=5$ per group). $*P<10^{-6}$, C vs. C_{NT}. $**P<0.05$, HAL/SAL vs. C. $***P<10^{-9}$, HAL/SAL vs. C_{NT}. $****P<0.005$, HAL/SAL vs. SAL/HAL, HAL/SAL vs. HAL/HAL. $^{§}P<0.005$, SAL/HAL vs. C_{NT}, HAL/HAL vs. C_{NT}.

4. Discussion

In the present study, we tested the hypothesis that long-term behavioral and neurochemical changes that psychotropic drugs induce, which are age-specific, could be attenuated by appropriate drug interventions at the proper stages. We used a behavioral test paradigm with demonstrated sensitivity for detecting circling behavior alterations after early exposure to HAL [5,64], Vitamin A [6], phenytoin (manuscript in preparation), and diazepam (unpublished results). Most of our experience has been derived from pubertal rats. By 1 month of age, most nigrostriatal cholinergic and dopaminergic phenotypic differentiation has already occurred [13], including maximal expression of striatal D2R mRNA and protein [52,65]. In animals, including humans, puberty would represent the last preadult ontogenetic period in which a greater likelihood of detecting primary deficits induced by behavioral teratogens exists because further compensatory adaptations may have raised later in life by effect of parental care, activity-dependent plasticity, and social and self-teaching experiences [7,48].

We induced behavioral disturbance in group HAL/SAL in the absence of mass physical damage, although a trend to a

decrease in striatal weight was present at PD90. Nevertheless, only when comparing striatal weights in groups SAL/HAL and HAL/SAL that a close by statistical significance was found. These findings agree with a series of reports showing slight but enduring changes in striatal weight after early exposure to HAL [12,42,46,63]. Because drug-free periods (from last HAL dose to CT testing) were 33 and 9 days for the HAL/SAL and SAL/HAL–HAL/HAL groups, respectively, and the time elapsed between HAL exposure and D2R binding assays was longer than 2 months, the direct pharmacological actions of HAL (i.e., sedative) could not have been responsible for the results of this work.

4.1. Gestational exposure to HAL

In rats, during prenatal development, the brain is vulnerable to the inhibitory actions of HAL, particularly, the nigrostriatal system during the last week of gestation, when the STR begins to receive afferent dopaminergic neuron branching from substantia nigra [13,35,37]. Besides, striatal neurogenesis takes place from the second week of gestation to the first few days after birth [34,40,59]. Although several cellular targets have been proposed [11,23,26], the mechanisms by which early exposure to HAL affects behavioral ontogenesis have not been elucidated yet [37,50].

4.2. Lactational exposure to HAL

It is well known that young and adult rats subjected to repeated administration of HAL present later supersensitivity to pharmacological challenges selective for D2R agonists [3,37]. Besides, it has already been demonstrated that early postnatal exposure to HAL induces later increased locomotor activity [50]. We did not find differences by pairing off groups SAL/HAL versus C (see Fig. 1). Yet, we have previously observed the occurrence of higher circling speed in the CT test after a similar scheme of postnatal treatment if the eight-day CT testing is performed from age PD40 (unpublished data) instead of PD30. In the field of developmental neurotoxicology, there are several examples of chemical-induced behavioral changes that have a striking dependency on the age selected for functional testing [49,50].

4.3. Density of (^{3}H)-SPI specific binding sites 2 months after behavioral assessment

In rats and mice, the ability of the nigrostriatal pathways to modulate motor function would be particularly sensitive to dopamine during postnatal development [55]. In rat STR, chronic treatment with HAL induces the presynaptic inhibition of glutamate-mediated excitatotory synaptic transmission elicited by dopamine and dopamine agonists, probably acting on an altered D2R system [10]. Furthermore, motor activity per se may modify dopaminergic markers in STR, like tyrosine–hydroxylase (TH) activity [22], and rats submitted to CT test at age PD30 present later activity-dependent decreases in D2 and acetylcholine muscarinic receptor expression in the STR at adulthood [27,47]. Therefore, we attempted to search long-term dopamine system consequences on early exposure to HAL- and CT-associated motor exercise. This was done with saturating D2-specific binding assays in the same rats submitted to CT test at puberty and in nontrained (SAL-treated) controls (group C_{NT}). A D2R decreasing effect of CT was found in the STR at PD90 (compare groups C and C_{NT} in Fig. 2). The magnitude of this binding drop should be theoretically included in binding values resulting from HAL-treated (and trained) animals. By pairing off HAL-treated groups to trained control C, the only statistical difference was in the (^{3}H)-SPI binding drop after gestation-only exposure to HAL. This finding agrees with a reduction of D2R-specific binding sites obtained on striatal samples from 1-month-old rats treated with HAL during gestation reported by Rosengarten and Friedhoff [42]. The apparent effect-addition between the D2R changes induced by HAL and by circling activity deserves more work to be confirmed. On the other hand, the detection of enduring D2R effects in rats following early exposure to HAL could be partially masked by the natural process of dopamine receptor pruning, which occurs in the STR from puberty onwards [56]. Remarkably, we found the abovementioned HAL-induced changes in circling behavior and D2R system at 1 and 3 months of age, respectively, suggesting the presence of sequels that the program of brain maturation could not counterbalance. Yet, we do not know if the long-term D2R change detected in group HAL/SAL under our experimental conditions remained associated to any class of motor behavior deficit in adulthood. The study of the potential correlation between the enduring effects in dopamine receptor system and circling behavior, in adult rats exposed to HAL during critical periods of early development, would be of particular interest. The absence of training-dependent (^{3}H)-SPI binding changes in CBL agrees with preliminary work from our laboratory (unpublished data). Either high S.D./mean ratios or the relatively low *n* per experimental group included in the neurochemical assays did not allow an unequivocal conclusion about HAL effects on cerebellar D2R system. Nevertheless, a 44% drop in D2-specific radioligand binding in group HAL/SAL was unexpected due to the relatively low dopamine D2R level in this region. In the cerebellum, the density of dopamine receptors is far lower than that in the caudate/putamen, and mostly marked by D3R-selective radioligands [32]. The precise mechanism by which HAL could alter motor activity by acting at cerebellar levels has not been elucidated [4].

4.4. Recovery of control-like performance in group HAL/HAL

Long-term blockade of D2R with HAL may increase the activity of the striatal cholinergic system in a concerted action involving striatal dopamine D1 receptors [28,43]. In addition,

a compensatory decrease in the binding of the muscarinic antagonist [*N*-^{11}C-methyl] benztropine is observed following treatment with the D2R antagonist *N*-methyl-spiperone [18]. During postnatal exposure, HAL could have further affected development in the nigrostriatal system, for instance, apoptotic cell death [8,38], synaptogenesis [29,55], and/or phenotypic maturation, which is only 15–30% complete during the first week of life [13]. It was not the focus of the present work to reveal the relative participation of each stage of development in long-term HAL-induced effects. However, in group HAL/HAL, the absence of differences in circling performance and D2R binding with respect to control group C seems to be explained by neutralization between the changes that HAL induced during gestation or lactation.

A deep awareness of age-dependent responses seems to be a cornerstone in designing rational strategies for behavioral teratology prevention or attenuation. The first experimental models were based on surgical procedures to correct the functions altered during pregnancy due to trauma. This approach used embryonic neural grafts implanted into the affected brain regions as appropriate. It was then postulated that the partial or complete recovery of functionality occurred due to the interaction between the implanted (healthy) neural tracks and the damaged brain [31]. Other strategy was found by placing the graft to specifically interfere the activity of the impaired neural pathways that are suspected to regulate the examined behavior [30]. It has been also reported the correction of phenobarbital-induced (e.g., a nontraumatic case) behavioral teratology by implanting fetal neurons as postnatal therapy [66]. More recently, pharmacological interventions have been reported for alcohol-induced behavioral teratology [57]. More related to our work, Saleh and Kostrzewa [44] showed that postnatal administration of a particular tripeptide attenuated the alterations observed in striatal D2R system development on early postnatal exposure to SPI. Ultimately, we obtained the above-presented (circling behavior and dopamine system) results by taking advantage of the existence of critical periods of sensitivity [40,54,61], as well as looking at them as restricted periods for therapeutic opportunities.

A final point that merits more study is a risk/benefit analysis for psychotropic use in the case of child-bearing age women under treatment with antipsychotic drugs because HAL has been detected in breast milk, and suckling infants may present plasma concentrations from noneffective to nearly therapeutic levels [51,67]. In light of our results, it would seem that the interruption (withdrawal or substitution) of HAL therapy after delivery could be more risky for the development of the dopamine system in breast-fed infants than continuing the treatment during lactation.

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