Research report

Comparative behavioral changes between male and female postpubertal rats following neonatal excitotoxic lesions of the ventral hippocampus

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Abstract

Neonatal ventral hippocampal (nVH) lesioned male rat has been used as a model to test the hypothesis that early neurodevelopmental abnormalities lead to behavioral changes putatively linked to schizophrenia. There are significant gender differences in schizophrenia with male and female individuals differing in the age of onset, course and outcome of the disorder. In order to assess whether the behavioral effects of nVH lesions extend to or are different in female rats, we investigated spontaneous locomotion, grooming, social interactions and spatial memory in male and female rats post-pubertally at postnatal day (P) 56 following bilateral ibotenic acid of the ventral hippocampus at P7. The spontaneous locomotor activity in a novel environment of both male and female nVH lesioned rats was significantly enhanced compared to their respective sham-operated controls. In tests of social interactions, the number of encounters was significantly decreased in female lesioned rats, whereas the male nVH lesioned rats showed a significantly reduced duration of active social interactions. Furthermore, Morris water maze test showed a deficit of spatial learning/memory in only male lesioned rats with significant decrease in the latency to find hidden platform. These results suggest that while nVH lesions affect post-pubertal behavior in both sexes of rats, the males appear to be affected to a greater extent than the females underscoring the influence of sex differences in the development of behaviors in the nVH lesioned animals.

1. Introduction

Neonatal ventral hippocampus (nVH) lesions in rats lead, after puberty, to behaviors that are believed to reflect an increased mesolimbic dopamine (DA) transmission. These include increased locomotor activity in response to a novel environment, stress oramphetamine, decrease in social interactions, deficits in spatial learning and memory and changes in the dopamine receptor levels and dopamine release in the nucleus accumbens [6,12,21,38,54]. These studies have suggested that neonatal lesions of the VH may be considered as a model to test the hypothesis that early neurodevelopmental abnormalities may lead to behavioral changes putatively linked to schizophrenia. These studies, however, have been mainly carried out in male rats. While schizophrenia affects both genders with similar prevalence rates, there are gender differences in the age of onset, clinical features, course and prognosis of the disorder [13,20,57]. Schizophrenic males exhibit more severe clini-
cal profile and poorer prognosis [51] with earlier age of onset [25,35], greater negative symptomatology [1] and cognitive deficits [24,27], whereas females have slightly later onset compared to the males as well as better social outcome [25,60]. In addition, some reports have demonstrated that schizophrenic males have a greater number of significant abnormalities in the brain than the females such as the ventricular enlargement [45].

Animal neurochemical data shows that there are sex differences in central DA levels [14] with male and female rats having differential sensitivities to amphetamine, cocaine and apomorphine [4,5,11,48,57]. Sexual dimorphism of the DA system is further revealed by the responsiveness of DA neurons to gonadal hormones as ventral tegmental area (VTA) neurons appear to be more responsive to the hormone milieu compared to the substantia nigra compacta (SNc) neurons [7,18]. Gonadectomized male rats show an increase in the density of tyrosine hydroxylase immunoreactivity in the prefrontal cortex [2]. In addition, numerous reports have shown estrogen regulation of DA receptor and transporter expression [29,34,41,42].

The role of the gonadal hormones in the nVH lesion has been studied [8,37], however, the data did not clarify the relation between the gonadal hormones and this animal model in rats. The absence of gonadal hormones by castration of male rats not only does not prevent the appearance of the behavioral changes in the nVH lesion animals but also even exacerbates them [37]. In addition, the effect of nVH lesion on spontaneous locomotor activity in female compared with male rats suggests that both groups of animals present increased the locomotion after puberty, however, the female rats delayed the effect after postnatal day (P) 100 [8].

The present investigation was designed to assess whether nVH lesions affect the post-pubertal development of select behaviors in female rats and if any significant difference existed in the behavioral profile between male and female lesioned animals. We compared the spontaneous behavioral consequences at postnatal day (P) 56 of ibotenic acid-induced bilateral nVH lesions in male and female Sprague–Dawley pups at P7.

2. Material and methods

2.1. Neonatal lesions

The procedure followed was essentially as described previously [21,38]. Pregnant Sprague–Dawley rats were obtained at gestational day 14–17 from our facilities (University of Puebla). Animals were individually housed in a temperature and humidity controlled environment on a 12-h–12-h light–dark cycle with free access to food and water. The day following birth, litters of three to four male and three to four female pups were formed and on postnatal day 7 (P7), each pup (weighing 15–17 g) was assigned to either a sham (males n=12, female n=12) or lesion (male n=12, female n=12) group. All surgical procedures described in this study were in accordance with the guidelines of the Laws and Codes of Mexico in The Seventh Title of the Regulations of the General Law of Health Regarding Health Research and the guidelines of the Canadian Council for Animal Care as approved by the McGill University Animal Care Committee. Anesthesia was induced by placing the pups on wet ice for 12–15 min. The pups were then positioned on a modified platform fixed to a stereotaxic Kopf instrument, 0.3 mm from the midline, 2.5 mm lateral to the midline, and 3.0 mm ventral to the cortical surface. A 30-gauge stainless steel cannula (0.1 M phosphate-buffered saline, PBS, pH 7.4) was injected in the ventral hippocampus over a 2-min period through a 30-gauge stainless steel cannula positioned at the following coordinates: AP 3.0 mm, ML±3.5 mm to bregma and VD 4.9 mm from dura. After the procedure, the pups were placed on a heat-pad for recovery and then returned to their dams. On P21, animals were weaned and grouped two to three animals per cage. Since the hormonal milieu of female rats can influence their behavioral performance, all behavioral tests in these animals were carried out during the estrous phase (characterized by a predominant population of cornified cells at the vaginal smear). Vaginal smears were taken daily from female rats to determine the stage of estrous cycle and locomotor behavior assessments were done when the female rats were in the estrous stage (P56–59).

2.2. Behavioral testing

Seven weeks (P56) after neonatal lesions, the behavior of each animal was assessed under four testing paradigms namely:

1. Spontaneous locomotor activity in a novel environment: Locomotion of sham and ibotenic acid-lesioned rats was assessed in the eight-photocell activity boxes connected to a counter (Tecnologia Digital, Mexico). Rats were removed from their home cages and placed in the activity boxes to which they were unacclimatized previously. Horizontal beam crosses were recorded in 10-min bins for a total of 60 min.
2. Grooming: Two days after locomotor activity, nVH and sham-lesioned rats were placed in testing boxes (60×60×20 cm) individually for a 15-min period and their behavior was videotaped. Total time spent grooming and total number of episodes (face and head washing, body licking, scratching) was scored by an investigator blind to the conditions using a computer program (Grooming, developed by the University of Puebla).
3. Social interaction: This paradigm has been broadly validated as a useful test for studying anxiety in rats.
In this test, one rat is faced with another one on the basis of gender and weight, so that the member of a pair do not differ more than 20 g. All animals are tested only once under high light and unfamiliar conditions. In this study, a modified version of the original model [19] was used for evaluating the social abilities of the lesioned animals. Briefly, 2 days after grooming behavior assessments each pair of rats were randomly selected within the same test group (sham–sham, lesion–lesion) and placed into an acrylic cage (50×76×38 cm). Their activity behavior was recorded for 10 min. Only the following behaviors were considered as active social interaction: sniffing, following, grooming, mounting, wrestling, jumping on and crawling under or over the partner [26]. The experiment was videotaped and scored by an investigator blind to the surgical status of the rat through a PC software developed by the University of Puebla. Data are expressed as total number of interactions as well as the time spent in the active behaviors.

4. Morris swim maze: The water maze test was performed in a circular water tank located in the center of an experimental room (450×300 cm). Lighting was provided by four floor-mounted (200 W) lamps, one in each corner of the room. The quiet room had different visual cues on the walls. Four equidistant start locations (North, South, East, and West) were allocated, thus delineating four quadrants (NE, SE, NW, and SW) of the pool. The circular tank consisted of a white fiberglass pool, measuring 150 cm in diameter and 60 cm in height. The water (26 °C) was made opaque by the addition of milk powder. On each trial, a rat was placed in the pool facing the wall at one of the four start locations. The circular escape platform (12 cm in diameter) was made from white plastic and it was submerged 2 cm below water level. Four trials were given daily (i.e. from the four quadrants) for 5 consecutive days and the swim behavior was videotaped [43]. Each acquisition trial was terminated either when the rat located the hidden escape platform or after 120 s had elapsed. If the animal located the platform, it was allowed to remain there for 10 s. If the rat was unable to find the platform after 120 s, it was removed by hand and put on the platform for 10 s. The videotape was analyzed for the time to find the platform (latency) by an investigator blind to the experimental status of the animals.

2.3. Statistical analyses

Behavioral results were analyzed by two-way ANOVA, followed by Newman–Keuls tests for post-hoc comparisons, with lesion and sex as independent factors (P<0.05 was considered significant). Morris water maze test results were analyzed with a repeated measure ANOVA.

2.4. Brain processing

All sham and lesioned rats per group were anesthetized with sodium pentobarbital (75 mg/kg, i.p.) 48 h after the last testing day. Brains were rapidly removed, frozen in isopentane maintained at −40 °C and stored at −80 °C until use. For assessment of lesion size, 30-μm-thick coronal sections at the level of ventral hippocampus [47] were stained with 0.5% cresyl violet and examined under a light microscope.

3. Results

3.1. Verification of the lesion

Cresyl violet-stained sections obtained from neonatal VH-lesioned of male and female animals at adult age reveal a significant bilateral damage of the ventral hippocampus with neuronal loss, atrophy and apparent retraction of the hippocampal formation (Fig. 1) as reported previously [12,21]. Cavity resulting from the lesion was also frequently seen.

3.2. Behavior

The effect of neonatal lesion on spontaneous locomotor activity in a novel environment is illustrated in Fig. 2. Two-way ANOVA revealed that the locomotion was significantly affected by the lesion ($F_{1,48}=13.69$, $P=0.0006$), by sex ($F_{1,48}=35$, $P<0.0001$), but not by lesion×sex interactions ($F_{1,48}=0.45$, $P=0.50$). Post-hoc tests showed that the spontaneous locomotor activity of all female rats were significantly higher than males, and that the lesion caused a significant increase in the locomotor behavior in both male and female VH-lesioned animals ($P<0.01$, 0.05, respectively) compared with their respective sham-lesioned controls.

The effect of neonatal VH lesion on grooming in male and female rats is shown in Fig. 3. ANOVA reveals that the total time spent grooming was significantly affected by sex ($F_{1,43}=4.21$, $P=0.046$) but not by lesion ($F_{1,43}=2.06$, $P=0.15$) or lesion×sex interaction ($F_{1,43}=0.71$, $P=0.40$). However, the number of grooming episodes did not show significant effects of sex, lesion or lesion×sex interaction.

Fig. 4 shows the effect of neonatal VH lesion on social behavior in the male and female rats. The total time spent in social interactions was significantly affected by lesion ($F_{1,24}=10.27$, $P=0.003$), sex ($F_{1,24}=14.37$, $P<0.001$) and lesion×sex interactions ($F_{1,24}=6.21$, $P=0.02$). The number of episodes of social encounters was not affected by lesion ($F_{1,24}=0.04$, $P=0.83$), however, there were significant effects of sex ($F_{1,24}=4.89$, $P=0.03$) and lesion×sex interactions ($F_{1,24}=8.16$, $P=0.008$). Post-hoc tests show that male VH-lesioned animals spent significantly less time in the social encounters ($P<0.001$) without changes in the
number of social encounter episodes. Female lesioned rats, however, showed a significantly decreased number of social encounter episodes ($P<0.05$).

In the Morris swim maze test for spatial memory (Fig. 5), both male and female lesioned and sham animals show learning of the task as reflected in the reduced latency to

![Fig. 1. Areas of least and greatest hippocampal lesion as determined by Nissl stain in rats receiving neonatal ibotenic acid. Female lesion animals (A) and male lesion animals (B). Gray, reconstruction of the neuronal loss and gliosis in the hippocampus of the rat with the most widespread lesion. Black, extent of the lesion in the rat with the minimal lesion considered significant. Numbers indicate distance posterior from bregma according to Paxinos and Watson [47].](image)

![Fig. 2. Locomotor activity (mean number of beam interruptions per 60 min±S.E.M.; n=12 per group) in a novel environment of female and male sham-operated or neonatal VH-lesioned animals tested at PD56. *$P<0.05$, significantly different from the same sex sham-operated group. **$P<0.01$, significantly different from the same sex sham-operated group.](image)

![Fig. 3. Grooming behavior in female and male rats with neonatal lesioned of the VH. Age at testing was 58 days old. Upper panel: time (s) spent in grooming (mean±S.E.M.), bottom panel: number of grooming (mean±S.E.M.).](image)
find the hidden platform across 20 trials. However, repeated measures one-way ANOVA ($F_{3,57}=26.55, P<0.0001, n=12$ per group) revealed that the latencies to find a hidden platform were significantly higher in male VH-lesioned animals compared with their corresponding controls ($P<0.05$). No significant difference in the latency to find hidden platforms was observed between female sham and VH-lesioned animals.

4. Discussion

The aim of the present study was to compare the post-pubertal behavioral consequences of the nVH lesion in male and female rats. We report here that this procedure in male animals produces significant reductions in the time spent grooming and social interaction with a deficit in memory and learning, whereas female lesioned rats do not show significant changes in this behavior. Spontaneous locomotor activity, however, was similarly increased in both male and female neonatal VH-lesioned animals. The findings in male rats reported here are in agreement with previous studies in which increased locomotor response to novel environment, stress, amphetamine or MK-801 [3,21] as well as a decrease in social interaction were reported [6,54]. Black et al. [8] recently compared spontaneous and amphetamine-induced locomotion in male and female nVH lesioned animals at two time points post-pubertally and reported an enhanced spontaneous locomotion in female lesioned rats only at P100, whereas amphetamine-induced hyperlocomotion was evident at P56. However, our results suggest an appearance of spontaneous hyperlocomotion in the female lesioned rats at P56–59 similar to that observed in male animals. While the reasons for these differences in locomotor results are not clear because of general similarities in the lesion procedure, one variable, i.e. the stage of estrous cycle may be important. Whereas our locomotor tests in females were done during the estrous phase, no
assessment of estrous cycle was made in the study of Black et al. [8]. The spontaneous locomotor activity of female rats is generally reported to be higher than the male [10,59] and the stage of estrous cycle in females has been reported to influence spontaneous locomotion in female rats [17]. Thus it is probable that the lesion may interact with female sex hormones to produce effects on spontaneous locomotion in a novel environment. Likewise, the environmental variables in the development are important on the outcome of the lesion [61].

Consistent with other studies in male nVH lesioned rats, our results also show deficits of social interactions in the male lesioned rats [6,54]. This deficit is attributable to the time the rats spend during the social contacts but not to the number of contacts that are made, i.e. each social encounter of lesioned male rats is, on average, of shorter duration than the control males. Female lesioned rats, however, show an attenuation of the number of social interaction episodes but not the total time spent during contacts. Our results of the reduced time of social contact by female sham lesioned animals is generally consistent with reports that female rats show less social behavior and emotionality in open field testing than males [2,9,17,31,49]. In so far as the duration of social contacts is a more critical determinant of social behavior, it can be argued that nVH lesion produces greater deficits in the behavior in male rats. Deficits in social interactions may not be due to increased spontaneous locomotion caused by neonatal lesions as both male and female lesioned rats show similar hypolocomotion. The social behavior of animals is sensitive to dopaminergic and glutamatergic manipulations as D1, D2 agonists or PCP administration are reported to reduce social behavior [53]. In this context it is noteworthy that male neonatal VH lesioned animals show hypersensitivity to both dopamine agonists as well as PCP in paradigms of locomotor activity [21,28,38]. The data on PCP sensitivity of female neonatal VH lesioned rats are not available, however, female lesioned rats are shown to be more sensitive to the locomotor effects of amphetamine [8]. Furthermore, there is evidence that the gonadal androgens are critical in the organization of the social interaction [32,52] and the nVH lesion in the male rats may in part be blocked by the effect of the androgen in the organization of the social interaction.

The result from total time spent grooming only suggests that there are differences between male and female animals with no significant differences in the lesion condition, however, male nVH lesion animals exhibited a non-significant attenuation of the total time spent grooming. The mechanism by which a neonatal VH lesion produces differential effects in the males and females in social behavior and grooming, is not clear. Although a role of gonadal hormones in male rats has been ruled out in the locomotor or stereotyped behaviors following nVH lesions [37], it is possible that they may have an influence on the social behavior and grooming following lesions. Several reports have demonstrated that gonadal androgens appear to organize environment-related social interaction in male rats during adolescent development [32,50]. Furthermore, castrated males prior to the onset of puberty, but not in adulthood, tested at adult age, displayed a pattern of social interaction similar to the intact adult female rats [50] and this effect of juvenile castration may be prevented by chronic exposure to testosterone propionate [50]. Another possibility is that the differential effects of neonatal lesions may arise from a differential functional alteration of the activity of the medial prefrontal cortical neurons. Neonatal VH lesions in male rats are reported to lead to deficits in prefrontal tasks such as delayed alternation [40] and changes in the dendritic morphology of prefrontal pyramidal neurons (unpublished observations).

Monkeys with neonatal temporal lobe lesions, as adults, show altered prefrontal regulation of subcortical dopamine release [55]. Prefrontal cortices in rats seem to participate in a range of cognitive, emotional and locomotor functions including locomotor activity in novel environment, social behaviors, spatial orientation, habituation, temporal ordering and working memory [2,21,33]. Sex differences identified in many of these behaviors suggest that the prefrontal cortex of the rat, like that of monkeys and man [16,23], is strongly influenced by gonadal hormones.

Our results suggest that male, but not female, neonatally VH lesioned animals show deficits in spatial learning and memory in the Morris maze test. These data are consistent with a previous report that male neonatally VH lesioned rats show pronounced deficits in radial arm maze choice accuracy both pre as well as post-pubertally [15]. Thus neonatal VH lesion in the male animals demonstrates interesting parallels with schizophrenia in which a variety of cognitive abnormalities have been reported, including attention and working memory impairments. However, the cognitive impairments in schizophrenia correlate with functional abnormalities of the prefrontal cortex [22]. While a role of prefrontal cortex in the behavior of neonatal VH lesioned animals is possible, animal research has shown that Morris maze deficits are more linked to the hippocampal functioning [43]. Lesions of the dorsal hippocampus or fimbria-fornix consistently produce spatial learning abilities of animals, however, lesions of the ventral hippocampus are reported to spare learning disabilities unless nearly all of the ventral hippocampus is removed [44]. Given the size of lesion in our nVH lesioned animals where nearly all of the ventral hippocampus is affected, we believe that spatial learning deficits in this model may derive primarily from the damage to the hippocampus. The question why female neonatal VH lesion animals were spared a deficit in learning/memory in this task may be related to the influence of ovarian hormones in the females. Animal reports have suggested that estrogens enhance learning/memory on a task utilizing spatial memory [39,46]. In addition, some data in postmenopausal women suggested that estrogen replacement
therapy enhances immediate and delayed verbal recall in both naturally and surgically menopausal woman [30,58]. Taken together, these observations suggest that estrogen in the female rats may in part protect memory and learning from the neonatal VH lesion. However, this assertion cannot be overemphasized as we pooled the data from the female animals that may have been at different stages of estrous cycle on different days of water maze trials.

In summary, the nVH lesion model of schizophrenia shows that there are essential gender differences in the behavioral outcome of the lesion at early adulthood with male rats showing more pronounced deficits in social behavior, grooming and water maze learning than the females. There is ample evidence supporting sex differences in the clinical features of schizophrenia [13,20,57]. Schizophrenic males exhibit more severe clinical profile and poorer prognosis [51] with early-onset of first episode (before 25 years old) [25,35], and more cognitive and negative symptoms [1,24,27], whereas schizophrenic women have better social course with later-onset of first episode [25,60]. These gender differences in schizophrenia support the hypothesis of a mild protective effect of estrogens [25,36,56]. Our data are also consistent with the idea that sex differences may have a significant influence over the behavioral changes produced by nVH lesions.

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