Behavioral assessment of the "schizophrenia-like" phenotype in an animal model of neonatal lesion in the ventral hippocampus (NLVH) of young and adult rats

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Abstract

Schizophrenia is a serious mental disorder that affects one in 100 people in the world and it is characterized by distorted thoughts and perceptions. Several animal models have been developed for the study of schizophrenia, primarily based on the study of the mechanism of action of antipsychotic drugs. In this paper, we present a number of classic behavioral tests (memory, social interaction [SI] and pre-pulse inhibition) related with the disease, using the neonatal ventral hippocampal lesion (NVHL) model in juvenile and adult rats. The NVHL animal model is a heuristic model that discriminates, by means of behavioral tests, the "schizophrenia-like" phenotype from other behavioral paradigms, such as depression and anxiety, specifically in adult animals. The study of the genomics of this model holds promise as an important generator of candidate genes for schizophrenia in human beings. (Gac Med Mex. 2014;150:419-29)
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Introduction

Schizophrenia is a serious mental disorder that affects one in 100 people in the world; it is characterized by distorted thoughts and perceptions, and it impacts on the patient all life long[1]. Although its etiology remains unknown, a multifactorial etiology is accepted. For example, the neurodevelopmental theory proposes that genetic predisposition, together with early environmental agents (trauma, stressor or virus), would produce disturbances in the brain's connectivity during its development, predominantly in the control of dopaminergic system-mediated subcortical areas[2,3]. It has also been suggested that schizophrenia can be induced by "accidents" or lesions during the individual's brain early development, which entail a reduction of neural connections of different regions[4].

Schizophrenia is mainly characterized for having positive, negative and cognitive symptoms, such as memory loss[5]. Positive symptoms are associated with psychotic symptoms such as loss of touch with reality, delusions and hallucinations, whereas negative symptoms correspond to a state of deficit in behavioral processes, which are decreased or absent and appear as impoverishment or decline of thought, emotion, behavior and language [6].

The participation of different neurotransmission systems is also proposed in schizophrenia[1]. For example, the dopaminergic hypothesis of schizophrenia has its origin in the observation that drugs of abuse, which increase dopamine release (for example, cocaine and amphetamines), can induce psychotic symptoms, and in the discovery that anti-psychotic drugs preferentially block the D2 and D4 dopaminergic receptors[7]. Other system associated with schizophrenia is the GABA-ergic; recently, the *GABRB1* gene T-allele, which encodes for the gamma-aminobutyric acid type A (GABA-A) receptor subunit β-1, has been found to be strongly associated with the disease, and thus, it has been proposed as a molecular marker[8]. The role of serotonin in schizophrenia was originally proposed based on the hallucinogenic effects of lysergic acid (LSD), a compound

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that activates the 5-HT2A serotoninergic receptors; for example, atypical antipsychotics, such as clozapine, olanzapine and risperidone are antagonists to different serotonin receptors[9].

Study of schizophrenia in animal models

The use of animal models in psychiatry has clear limitations, since complex human behaviors are tried to be reproduced in an animal[10-13]. However, animal models of psychiatric disorders have allowed for the therapeutic potential of specific medications in the treatment of these disorders to be explored, as well as for relevant data on the mechanisms of action of these drugs to be obtained, and they are also valuable tools for the determination of neurobiological substrates of psychiatric disorders[13].

The contribution of animal models to the neurobiology of psychiatric disorders depends on their validity and therefore, it has been proposed that models should meet the following criteria[14].

- Predictive validity criterion: implying that drugs that modify pathological states in humans must do it in the animal model as well, covering the sensitivity, selectivity and relative potency requirements.
- Face validity criterion: which refers to the phenomenological similarity between the model and the studied disorder; it is proposed that the model has to show the most representative symptoms of the disorder.
- Construct validity or hypothetical validity criterion: which establishes that the hypothesis that explains the psychiatric disorder must also serve as the rationale of the model[14].

For the study of schizophrenia, several animal models have been proposed: pharmacological, genetic (induced by gene mutations or deletions) and neurodevelopmental (induced by a physical or neurotoxic lesion or by environmental factors during neurodevelopment)[15].

Neurodevelopmental models

It has been suggested that schizophrenia can be induced by events or "lesions" during the individual's early development, which result in a reduction of neural connections in different regions of the brain, such as the prefrontal cortex, as well as a decrease of synapses in projection sites such as the cingulum, the cortex and the ventral striatum[16]. Based on this idea, models have been developed where the development of the brain is affected, either during prenatal or neonatal stage, such as the NVHL validated model[17].

This model is induced with a small excitotoxic injury in the immature brain hippocampus of neonate rats, allowing for their later maturation. In the adult stage, the lesioned animals display behaviors associated with positive and negative symptoms of the disease, such as hyperlocomotion, pre-pulse inhibition reduction, memory deficit and decreased Social Interaction, among others[18]. This model is based on the neurodevelopmental hypothesis, since it proposes that in schizophrenia, neuronal migration is altered on the second trimester of gestation (first neonatal days in rodents), and affects several of the neurotransmission systems associated with schizophrenia[19]. According to the above review, we think that the animal model mostly supporting the characteristics of schizophrenia is the NVHL model, since it can be useful to study the disease from a pathophysiological perspective[20].

The purpose of the present work was to analyze age-dependent behavioral differences (at 45 and 90 days) with schizophrenia-related behavioral tests in the NVHL model.

Methodology

Biologic material

The study started with 48 male rats of the Wistar strain, stemming from litters of laboratory-impregnated rats on individual isolation, and that were put on a 12-h light-dark inverted cycle. The total sample was divided into 6 groups: 8 juvenile-NVHL rats, 8 falsely lesioned juvenile rats (sham), 8 juvenile rats without manipulation (naïve), 8 adult-NVHL rats, 8 falsely lesioned adult rats (sham) and 8 juvenile rats without manipulation (naïve).

All experiments were conducted following the regulations established by the Mexican official standard on the use and care of laboratory animals (NOM-062-ZOO-1999), as well as the International Association for the Study of Pain ethics committee regulations[21].

Neonatal ventral hippocampal lesion: the 5-7 days-aged baby rats were anesthetized by hypothermia by placing them on ice for 12-15 min; then, they were placed in a stereotaxic instrument with special adaptor for neonate animals. On each baby rat, an incision was performed into the skin of the skull and 0.3 ul of ibotenic acid (dissolved in phosphate buffer 0.1 M, pH 7.4) were administered directly in the ventral hippocampus for 2 min with a 30-gauge stainless-steel cannula. The coordinates

were: anteroposterior: 2.0 mm, midlateral: ± 2.5 mm relative to bregma, and ventrodorsal: –3.3 mm from the dura mater[20]. Subsequently, the animals were stitched and were left to recover under a 40-W lamp-indirect heat until they recovered their temperature. The operated animals were returned to their respective mothers until the moment of weaning at 21 days of age[20,22].

Behavioral tests

Tests were conducted on 45 and 90 days-aged animals; prior to these tests, the rats were isolated for 5 days in individual boxes (34 x 16 x 24 cm) and on the sixth day, the tests below-described were conducted.

Motor coordination test

Motor coordination was measured using a piece of equipment (rotarod) consisting of a 7 cm-diameter cylinder that rotated at a speed of 11 rpm. The animals were put to walk on the cylinders for 5 min, after two training sessions. In the test, the number of falls of the animal was recorded[23]. Usually, this test is useful to observe the animal's motor skills; in this case, we used it to determine if the neonatal operation did not interfere with the motor skills and locomotion of the animal to be assessed, since if the animal falls more than once or twice during the test, it is regarded as having motor skills abnormalities and it is discarded from the test.

Open field test

To analyze locomotor activity, the animals were subjected to the open field test. The rats were placed individually in an acrylic box (30 x 60 x 15 cm) with the floor divided into 24 squares. The number of times that the animal entered into each square with all four legs was recorded over a 5-min period. Measurements were conducted in a dark environment, illuminated by a red lamp placed above the open field. The test was videotaped to be recorded later[23,24]. With the open field test, hyperlocomotion is expected to be observed in animals with NVHL. It is worth mentioning that this test is one of the parameters that have been most evaluated in schizophrenia models, either pharmacological, genetic or due to some lesion[25-27].

Social Interaction (SI) test

The animals were individually isolated for five days before starting the test. Two animals were placed in a 70 cm-diameter x 38 cm-height transparent cylinder, in a dark room and under bright light. The interaction accumulated time was recorded for 10 min using a video camera, and the following behaviors were measured: mounting, sniffing, jumping and crawling over the partner's body[28,29]. Reduced SI is one of the most representative negative symptoms of schizophrenia, which has been observed in several schizophrenia pharmacological models as well[28,29].

Object recognition test

The test was divided in three different sessions (habituation, familiarization and retention sessions) and was carried out during two consecutive days. The habituation session is necessary in order for all the animals to adapt to the experimental scenario without any specific stimulus for 15 min, 24 h before the test. On the second day, the animals had a familiarization session, 10 min with the presence of two identical wooden or metal objects. The objects were placed at the center of the floor of the arena and the animals explored the objects freely during 10 min. Immediately after the familiarization session, the rats were removed from the arena. The test was repeated 60 min later (retention session) in order to test the short-term memory. In this case, one of the objects was replaced by a new one (new object), and the rat was introduced to the arena. The objects' positions were randomly permuted for each animal. Exploration time was recorded (time the animals spend sniffing or touching the objects with the nose and/or front paws). The time spent on exploring each object was recorded with a digital video camera. The discrimination index was calculated to determine the rats' preference to explore the new object, as related to the known object[25,30-32]. To measure the cognitive function, the recognition index (RI) was used, which is obtained by dividing exploration time of the new object by total exploration time. A RI close to 0.5 indicates that the animals spend a similar amount of time to explore the familiar object and the new object, whereas values higher than 0.5 indicate preference to explore the new object[32].

Pre-pulse inhibition test

The acoustic chambers consisted of an acrylic tube or immobilization device (8.2 cm-diameter and 22 cm-length) with an accelerometer mounted below the tube. The animal was placed in the immobilization device in a sound-attenuating dark box; the acoustic stimuli were

promoted by the computer's software, through a speaker placed 10 cm above the tube. The software converts the accelerometer measurements into a digital signal. The background noise was 70 dB. Each session started with a 5 min-acclimation, followed by six assay blocks that included a 120 dB-pulse stimulus (baseline amplitude) and a pre-pulse 15 dB above the background noise. The pre-pulses always preceded the 120 dB stimulus after 100 ms; the interval between each test was 10/37 s. After the tests, the immobilization devices were cleaned with a soft soap solution before the next animal was tested. The startle amplitude was calculated as the mean of the six assays. The pre-pulse inhibition percentage (PIP%) was calculated as follows: 100 – (startle amplitude/baseline startle amplitude) x 100[33]. This test evaluates the individual's responsiveness to an auditory stimulus, as well as its processing; in animals, abnormalities in the processing of information and attention, which are characteristics that are present in schizophrenia, can be measured[34,35].

Forced swimming test

The swimming sessions were conducted in individual glass cylinders (46 x 20 cm) with warm water (23-25 °C) and 30 cm depth, without the animals touching the floor with the legs. The test was performed in two sessions: a 15-min initial training, and 24 h later, a 5-min session. The rats were removed from the cylinder, excess of water was removed with towels, and were placed in warm cages for 30 min, and later they were returned to their cages. Each session was video-recorded for later analysis[33]. The forced swimming model measures the animal's behavioral despair; depression is known to be a feature not typical in schizophrenia and, therefore, the animals with NVHL were not expected to display behavioral dispair or depressive-type behavior in the analysis of the test[36].

Defensive burying model

For this test, an acrylic piece of equipment (34 x 16 x 24 cm) containing a 7-cm long electrode at one side of the box that emerged 2 cm above a fine sawdust bed was used. Each time the animal touched the electrode, it could receive a 0.3 mA electric shock. Since the source of the shock was an adverse stimulus, instinctively, the animal tried to hide or bury the stimulus. The session was video recorded for 10 min in order to later record the measurements and burying cumulative time as indicators of anxiety[37]. In schizophrenia, anxiety does not occur typically and, therefore, it is likely not to be found in the NVHL model.

Nociception test

Each animal was introduced in a glass cylinder (20 cm diameter by 25 cm height) and placed at the center of a metal plate at 53 + 0.5 °C. Within the first few seconds, the animal must show the behavior of licking its paws as a concrete response to the thermal stimulus. Latency marks the onset of this response and it has been established that the test must conclude before 30 s to prevent tissue damage[38]. Patients with schizophrenia are known for displaying decreased pain sensitivity[39]; hence, latencies are expected to be higher in animals with NVHL than in controls.

Results

To assess the age-dependent behavioral parameters (rotarod, open field, object recognition, SI, pre-pulse inhibition, forced swimming, defensive burying and nociception) in both groups of sham and naïve lesioned rats, the statistical pack SPSS (version 15) and the Sigma Plot program (version 10.0) were used.

The behavioral results were analyzed with the analysis of variance (ANOVA) two-way test with *post hoc* planned contrasts and Turkey's correction, in order to find out if the differences were driven by the effect of interaction between age (45 or 90 days) and the type of behavior (Table 1).

The motor coordination test showed that the majority of animals did not display locomotion problems; only 2% of the animals had balance problems and were discarded from the protocol (data not shown).

In the open field test, locomotor activity in the group of adult lesioned rats revealed an increase in locomotion compared with their control group and, in the juvenile rats group, no statistically significant differences were observed (Fig. 1).

With regard to the memory assessment, in the group of adult lesioned rats, a significant reduction in the unfamiliar object exploration was observed, which translates into a memory deficit, compared with the control groups (Fig. 2). No differences were observed in the group of juvenile rats; the unfamiliar object exploration accumulated time was no different for the three experimental groups.

In the SI test, the lesioned adult rats group was found to show a highly marked decrease in SI compared with their controls, unlike the group of juvenile rats, where

Table 1. Behavioral tests statistical results*

Test	naïve vs. sham p	sham vs. lesioned p	naïve vs. sham p	sham vs lesioned p	Juvenile lesioned vs. adult lesioned p
	Juvenile		Adults		
Open field	1.0	1.0	1.0	0.001	0.001
Object recognition	1.0	1.0	1.0	0.023	0.021
Social interaction	1.0	1.0	1.0	0.051	0.005
Pre-pulse inhibition	1.0	1.0	1.0	0.048	0.038
Forced swimming	1.0	1.0	1.0	1.0	0.556
Defensive burying	1.0	1.0	1.0	1.0	1.02
Hot plate	1.0	1.0	1.0	1.0	1.05

Behavioral tests statistical analysis. Results of the statistical analysis with the ANOVA test with planned contrasts by age and type of behavior are shown.
*p < 0.005 naïve vs. sham; sham vs. lesioned, and juvenile lesioned vs. adult lesioned (n = 8 per group).

no statistically significant differences were observed in social contact accumulated time with regard to the control groups (Fig. 3).

Regarding the pre-pulse inhibition, the adult lesioned animals group was observed to show an increase in the percentage of pre-pulse inhibition, compared with the falsely lesioned animals and their controls (Fig. 4). In the group of juvenile animals, there were no changes in the response to sounds (pre-pulse or pulse).

With regard to the depression-like behavior assessment using the forced swimming model, no statistically significant differences were found in the immobility accumulated time, compared with the sham and naïve lesioned groups in juvenile and adult animals (Table 2). Additionally, the anxiety assessment by means of the burying model did not show depresssion-like behavioral changes. The burying accumulated time for the sham and naïve lesioned groups was compared in juvenile and adult animals, with no significant differences observed for either two ages (Table 2).

Finally, nociception was assessed using the hot plate test. In this test, no statistical differences were detected between the juvenile group and the adult animals group, compared with their respective controls (falsely

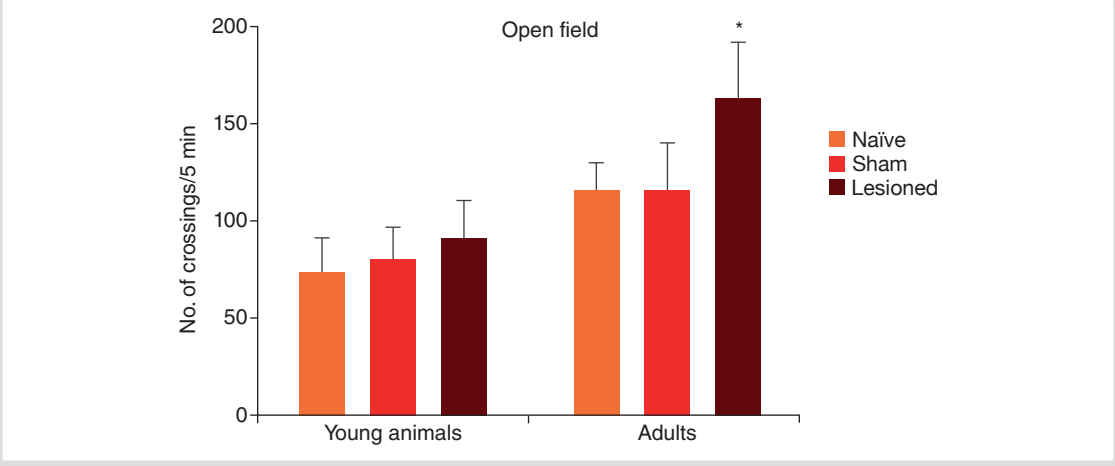

Figure 1. Open field test. Average number of crossings is shown for each 5 min in the groups of 45-day juvenile and 90-day adult rats. *p < 0.05 versus same-age control group. t-test* < 0.005 vs. control group (n = 8 per group).

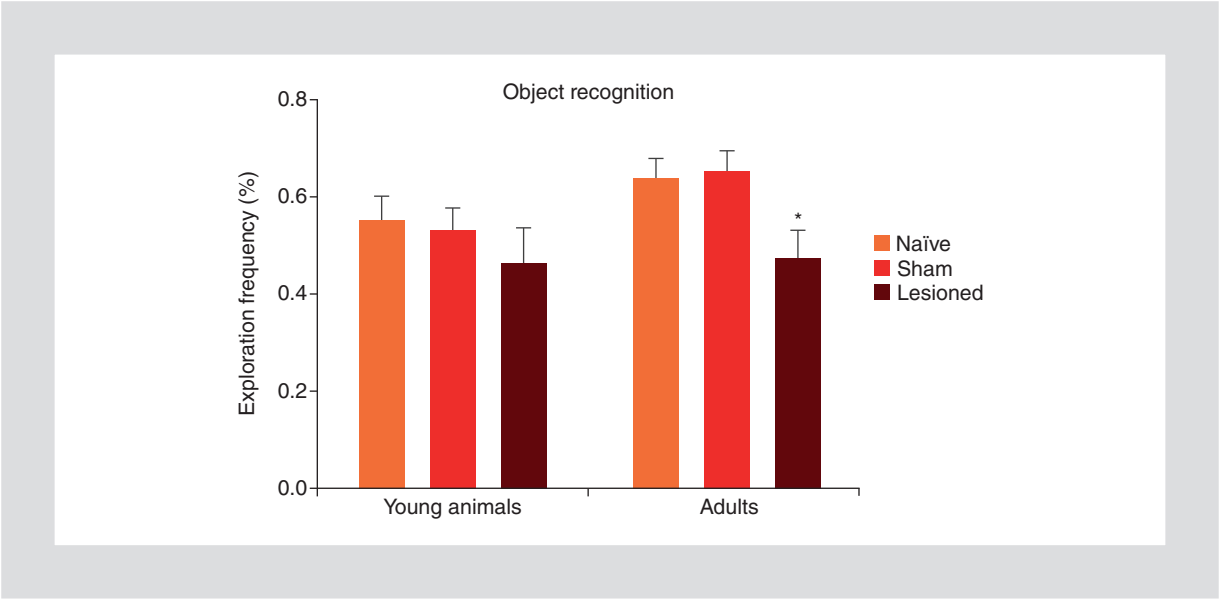

Figure 2. Effect of NVHL on the frequency of exploration in the groups of 45-day juvenile and 90-day adult rats. *p < 0.05 vs. same-age control group. t-test* < 0.005 vs. control group (n = 8 per group).

lesioned animals group and group without manipulation) (Table 3).

Additionally, a representative photomicrograph of the hippocampus from 45 day-old lesioned versus sham juvenile rats and of the hippocampus from 90 day-old lesioned and sham adult rats is shown; the ibotenic acid-induced lesion is also shown (Fig. 5).

Discussion

The purpose of this work was to analyze the age-dependent behavioral differences associated with schizophrenia in the model of NVHL. The behaviors assesd in the NVHL model demonstrate a behavioral specificity associated with schizophrenia, since in the forced

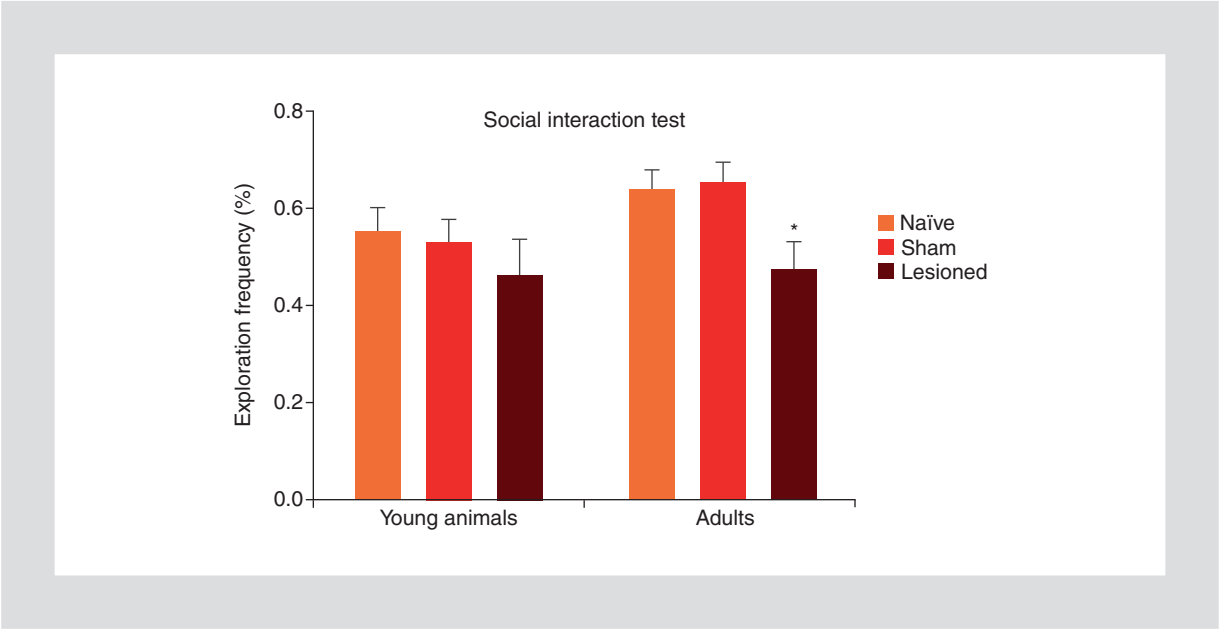

Figure 3. Effect of NVHL on social contact time in the groups of 45-day juvenile and 90-day adult rats. *p < 0.05 vs. same-age control group. t-test* < 0.005 vs. control group (n = 8 per group).

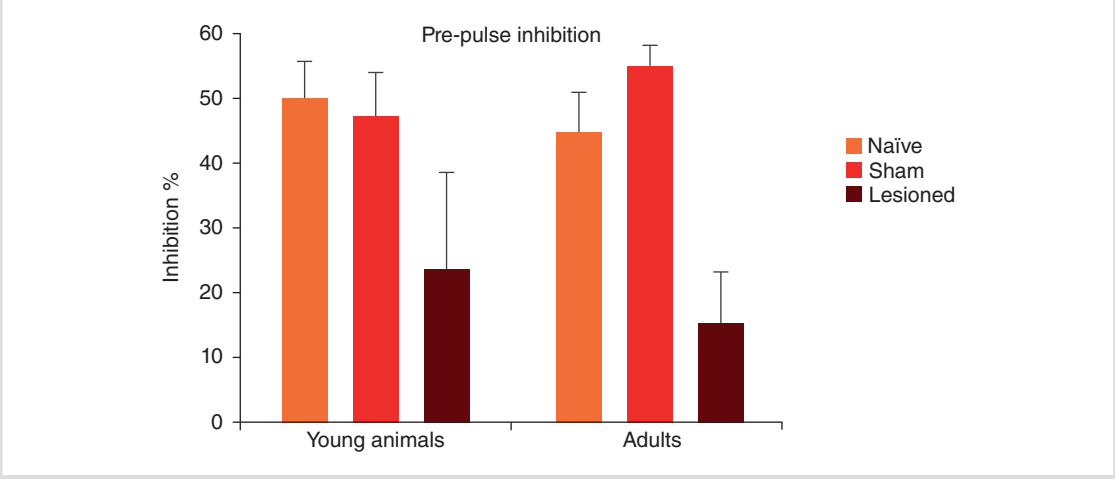

Figure 4. Effect of NVHL on the pre-pulse inhibition test in the groups of 45-day juvenile and 90-day adult rats. *p < 0.05 vs. same-age control group. t-test < 0.005 vs. control group (n = 8 per group).

Table 2. Defensive burying and forced swimming behaviors in the groups of juvenile and adult animals lesioned at the ventral hippocampus

	Juvenile	Adults
Defensive burying (s)		
Naïve	62.00 ± 29.27	99.00 ± 96.63
Sham	104.0 ± 83.93	125.75 ± 79.35
Lesioned	96.50 ± 69.70	119.45 ± 90.08
Forced swimming (s)		
Naïve	64.41 ± 41.29	54.83 ± 22.11
Sham	75.00 ± 34.46	64.13 ± 30.27
Lesioned	72.36 ± 45.04	48.41 ± 33.53

Data are expressed as average values ± S.E.M.

Table 3. Nociception in the groups of juvenile and adult rats lesioned at the ventral hippocampus on post-natal day 2 (PE 7), compared with their controls

Hot plate test	Juvenile	Adults
Climbing		
Naïve	22.00 ± 0.447	4.400 ± 1.140
Sham	2.200 ± 0.632	4.111 ± 1.364
Lesioned	2.545 ± 0.820	4.400 ± 1.265
Paw licking		
Naïve	6.600 ± 4.278	8.400 ± 1.140
Sham	6.900 ± 3.178	8.125 ± 1.553
Lesioned	5.364 ± 1.120	10.444 ± 2.404
Escape		
Naïve	8.00 ± 2.00	8.400 ± 1.140
Sham	8.30 ± 2.83	8.125 ± 1.55
Lesioned	6.81 ± 1.40	10.444 ± 2.4

Data are expressed as average values ± S.E.M.

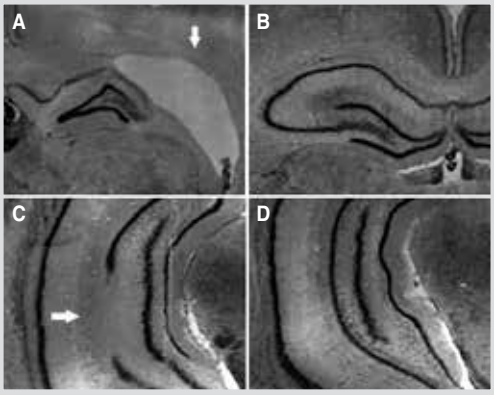

Figure 5. NVHL verification in juvenile and adult rats. In the representative lesions, located in the groups of sham and lesioned rats, histological sections are shown from juvenile (***A*** and ***B***) and adult rats (***C*** and ***D***). Photograph A shows a coronal section of the juvenile rat lesioned brain, whereas B illustrates that of a falsely lesioned animal. Photograph C shows a brain section of a lesioned adult animal and D, that of a falsely lesioned adult animal. Arrows indicate the lesion in both panels.

swimming and defensive burying tests no depression and anxiety characteristics were observed; all measurements were compared with the group of falsely lesioned animals and the group without manipulation (sham) in two stages: juvenile (45 days) and adult (90 days) (Tables 2 and 3).

In the adult stage, schizophrenia-related behaviors, such as hyperlocomotion, memory deficit, loss of SI and pre-pulse inhibition were observed; the results showed that behaviors associated with schizophrenia appeared only in the group of adult lesioned animals.

In the juvenile stage, the lesioned rats showed very similar behaviors to those displayed by the non-manipulated (naïve) and the falsely lesioned (sham) rats.

The motor coordination test was important to observe the motor skills of the animals; in this case, we used it to determine if the neonatal operation did not interfere with the motor skills and locomotion of the animal to be assessed, since if it showed abnormalities, the animal was discarded from the study[23]. The rotarod test showed that the lesion produced at the neonatal ventral hippocampus did not affect motor coordination, since the animals responded adequately to the test without falling.

Hyperlocomotion is considered a behavior associated with positive symptoms of schizophrenia (schizophrenia-like indicator)[40], one of the features that has been more profusely evaluated in schizophrenia animal models, either pharmacologically or gene silencing or gene mutation-induced[30,34].

The open field test enabled for hyperlocomotion to be observed in the lesioned animals group, whereas the group of lesioned juvenile animals only showed trends towards an increase (Fig. 1). Locomotor activity is the parameter that has been most assessed in the NVHL model. Previous works report hyperlocomotion in adult animals[41-43], but few works have studied hyperlocomotion in young animals. In our case, we observed that juvenile rats displayed trends towards increasing locomotion, but these were not significant. Noteworthy, Lipska et al. (2000) and Al-Amin et al. did also compare animals with these ages and found that both groups showed hyperlocomotion, but in these works, hyperlocomotion in young animals was lower than that observed in adult animals. Therefore, hyperlocomotion is likely to be present in juvenile animals but, depending on the form of recording, statistically significant differences may be found or not[19,45].

With regard to SI, one of schizophrenia's most representative symptoms is the deterioration of sociability or SI disability. Interestingly, reduction of this behavior has also been observed in pharmacological models of schizophrenia. For example, in a model where phenylcyclidine was chronically administered, a significant SI decrease was observed in adult animals treated with this drug[46,47]. In the NVHL model, a decrease in SI has been reported in adult lesioned rats[19], but few works have analyzed if there are age-dependant differences. In the present work, we found that subjects in the group of adult animals with NVHL lose interest for interaction with the other rodent. The statistical analysis shows a significant SI reduction in the group of lesioned adult rats. However, in young animals aged 45 days, similar SI levels to those diaplayed by control animals (sham or naïve) of the same age are observed (Fig. 3).

With regard to object recognition, cognitive deterioration, reflected as a loss of working memory, is a representative feature of schizophrenia[48]. In the model of NVHL, deterioration does occur, possibly associated with damage in the prefrontal cortex resulting from the ventral hippocampus lesion at neonatal age[47]. In this test, the group of lesioned adult animals showed memory loss; exploration time of the unfamiliar object was very similar or lower than exploration time for the familiar object (Fig. 4). Loss of memory only occurred in the group of lesioned adult animals; the group of juvenile animals showed a trend towards memory deficit, but

the data was not statistically significant. Other authors have measured memory by means of the radial arms maze or the T-maze; with these tests, the animal is likely to be exposed to more stress than with the object recognition test. Additionally, these tests measure long-term memory and, in our case, with the object recognition test, we only measured short-time working memory.

As to the pre-pulse inhibition test, schizophrenia is associated with information-processing and attention abnormalities; the pre-pulse inhibition test assesses the individual's responsiveness to an auditory stimulus, as well as its processing[19]. In our results, we observed that inhibition of the sensorial pre-pulse only occurred in the lesioned adult rats group: the group of lesioned juvenile animals did not show statistically significant differences with regard to their controls (Fig. 4). It has been established that patients with schizophrenia display a reduction of sensorial pre-pulse, probably resulting from trouble filtering sounds or from the way they translate or filter them[48]. This test has been assessed in previous works in animal models for schizophrenia, as an important proof of the disease[33,35], and in most animals, a pre-pulse inhibition deficit has been found.

The nociception test measures pain, although schizophrenia patients are known to have a reduced pain sensitivity[49]. In the NVHL, no statistically significant differences were found in comparison with controls and thus, possibly the lesion did not affect the animal's nociception. These results are similar to those previously reported by Al-Amin et al. (in spite of having found a reduction in latencies, their data were not entirely conclusive), as well as to those reported in patients with schizophrenia[50].

The anxiety and depression models were applied in the NVHL model to assess the predisposition to acquire these behaviors after the NVHL. The tests were conducted in the group of lesioned juvenile and adult animals. The results of the tests revealed that no differences were found between the lesioned and the falsely lesioned groups. Depression and anxiety are known to be features not inherent to the disease, since if these occur in patients, this usually happens in an atypical fashion and inconsistently[51]; therefore, our interest was focused on observing the lesioned animals' response to anxiety and depression paradigms. In 2003, Wood et al. reported higher anxiety in lesioned animals, as determined using the elevated maze, the same as Sams-Dodd et al. (1997) did; however, anxiety had not been studied before with the defensive burying model[52,53]. The defensive burying model is based on the instinctive feature of burying adverse objects, considering that the more the burying, the more the anxiety; evaluated animals with NVHL did not respond to this paradigm. Similarly, in 1993, Lipska et al. reported that the NVHL did not show depression characteristics with the forced swimming model in lesioned animals aged 35, 56 and 70 days[54], similar results to those obtained in this work.

The behavioral results for the animals with NVHL indicated that behavioral changes similar to schizophrenia (schizophrenia-like) were only observed in the adult stage. Possibly, the behaviors were only observed at the adult age due to the neuronal pruning phenomenon; in rats, pruning is known to occur between days 44 and 46; therefore, at 45 days, the effects of this pruning are not yet observed; however, at 90 days, the schizophrenia-like behaviors (hyperlocomotion, memory deterioration, SI reduction, pre-pulse inhibition), behaviors resembling those occurring in patients with schizophrenia, are clearly observed[55]. In this sense, in patients with schizophrenia, most of the schizophrenia symptoms have been shown to be observed more clearly after the neuronal pruning process, coinciding just with the moment this process has taken place, and this is the reason why, in adolescents, schizophrenia-related symptoms are difficult to be clearly observed, and, therefore, diagnosis of the disease is made at late stages[56]. It is important not losing sight of this phenomenon in the schizophrenia animal model; if the behaviors appeared only at adult ages, the pruning phenomenon could be possibly ocurring in the NVHL model as well. Feinberg et al. consider that schizophrenia is generated by a pruning alteration during adolescence and Steven proposes aberrant reinnervation and synaptic connection after a previous injury to be possible. In the same sense, some authors argue that, in the model of NVHL, some behaviors are better observed at adult ages[57-59].

The histological sections of the two groups of assayed animals (lesioned and sham) were compared with each other; these were exposed to the same conditions and behavioral tests. In the brains of the group of lesioned rats, a loss of cells occurred due to atrophy, probably resulting from the damage caused by the neonatal lesion produced by ibotenic acid and in response to possible regeneration, compared with the sections of the sham rats group. However, the lesion observed in histological sections of the juvenile rats group was greater. Cavitations or cell-deprived regions were observed, compared with the sham rat's groups

sections. Probably, these cavitations were observed to be bigger because the time elapsed since the lesion was shorter, and their brain had less time to regenerate other cells and, therefore, the lesion was observed to be bigger in the juvenile rats group compared with the damage observed in the adult rats group. Further experimentation is required to be available in the future (Fig. 5).

Conclusions

The use of animal models for schizophrenia is very recent and we are still far from the ideal animal model to be available. However, currently available animal models allow for some symptoms and characteristics of the disease to be modeled, a condition that will allow for the etiology and molecular, genomic and proteomic elements that might be altered in patients to be studied, thus making it possible for new candidate genes for this disease to be found, leading us to the search for new molecular diagnostic techniques and, eventually, to new therapies or to improve those already existing.

The lesion performed at the ventral hippocampus in neonates impacts not only on the brain of the lesioned rats, but also on their neurodevelopment, since schizophrenia-like behaviors do consolidate only in the group of lesioned adult rats.

The NVHL is likely to remain clinically silent until the end of adolescence, since clinical symptoms of schizophrenia in patients are observed in late adolescence or in the young adult.

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