

Longitudinal magnetic resonance evaluation of the schizophrenia model of neonatal lesion in the ventral hippocampus

Evaluación longitudinal mediante resonancia magnética del modelo de esquizofrenia de lesión neonatal en el hipocampo ventral

Alma D. Genis-Mendoza^{1,2}, Carina Elizalde-Martínez¹, José J. Martínez-Magaña², Brenda Cabrera-Mendoza¹, Axayacatl Morales-Guadarrama³, Emilio Sacristán³, Isabel Beltrán-Villalobos⁴, Carlos A. Tovilla-Zarate^{4,5}, and Humberto Nicolini^{1*}

¹ National Institute of Genomic Medicine, Ministry of Health, Mexico City; ² Psychiatric Care Service, Children's Psychiatric Hospital Juan N. Navarro, Ministry of Health, Mexico City; ³ National Center for Medical Imaging and Instrumentation (CI3M), Metropolitan Autonomous University, Iztapalapa, Mexico City; ⁴ Autonomous University of Mexico City, Mexico City; ⁵ Comalcalco Multidisciplinary Unit, Juárez Autonomous University of Tabasco, Comalcalco, Mexico

Abstract

Objective: To evaluate the progression by means of nuclear magnetic resonance of the lesion in the schizophrenia model of lesion of the ventral hippocampal nucleus (LVNH). **Method:** Magnetic resonance imaging (MRI) were performed in male Wistar rats, from 8 days postnatal to 139 days, in animals with LVNH and without lesion (sham). The MRI were carried out on a Variant 7 T equipment. The data were analyzed with the Amira software, for a voxel-based morphometric analysis. **Results:** We observed the presence of hypersignals with a significant enhancement in the structures analyzed in the group with LVNH, and greater volume in the lateral ventricles, presenting a larger size of the lesion on day PD96 and significantly reducing on day PD139. **Conclusions:** We found a cell rearrangement during the progression of the lesion, which could be the effect of the activation of immune cells.

Keywords: Schizophrenia. Neonatal ventral hippocampal lesion. Magnetic resonance imaging.

Resumen

Objetivo: Evaluar mediante resonancia magnética (RM) la progresión de la lesión en el modelo de esquizofrenia de lesión del núcleo del hipocampo ventral (LNHV). **Método:** Se realizaron RM en ratas Wistar macho, desde los 8 días posnatales hasta los 139 días, en animales con LNHV y sin lesión (sham). Las RM se realizaron con un equipo Variant de 7 T. Los datos se analizaron con el software Amira para un análisis de morfometría basada en vóxeles. **Resultados:** Observamos hiperseñales con un realce significativo en las estructuras analizadas en el grupo con LNHV, y mayor volumen en los ventrículos laterales, presentando un mayor tamaño de la lesión el día PD96 y significativamente reducido en el día PD139. **Conclusiones:** Encontramos un reacomodo celular durante la progresión de la lesión, lo cual podría ser efecto de la activación de las células inmunitarias.

Palabras clave: Esquizofrenia. Lesión neonatal del hipocampo ventral. Resonancia magnética.

Correspondence:

*Humberto Nicolini

Periférico Sur 4809

Arenal Tepepan, Tlalpan

C.P. 14610, Mexico city, Mexico

E-mail: adgenis@inmegen.gob.mx

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Introduction

Schizophrenia is a neuropsychiatric disorder that affects more than 21 million people worldwide¹. In Mexico, 1,100,000 people suffer from schizophrenia, ranking fifth in the list of conditions with the greatest disability². Despite the relevance of this disorder, its etiology has not been elucidated to date. However, several hypotheses have been proposed, such as that of neurodevelopment, which postulates that at least a part of people with schizophrenia present structural alterations in the central nervous system produced in early stages of development³. Some structural abnormalities have been identified in *postmortem studies*, while others have been identified in living individuals diagnosed with the disorder, primarily by neuroimaging technologies, such as computed tomography and magnetic resonance imaging (MRI). A consistent finding in MRI studies in patients with schizophrenia is enlargement of the lateral ventricles³. Structural MRI studies suggest that the patients' brain abnormalities are present from the onset of the disease. However, longitudinal studies are useful for assessing progression and morphological changes, but have been limited by small sample sizes and short or infrequent follow-up intervals⁴.

However, in order to test treatments and explore neurobiological aspects that could be of interest in schizophrenia, models must be available to translate the symptoms. Among the models of schizophrenia are, among others, the neonatal ventral hippocampal lesion model (LNHV) and the Disc1 mutation model⁵. The LNVH model is widely used, as it has provided reliable reproductions of behavioral and chemical abnormalities observed in patients with schizophrenia, including positive and negative symptoms⁶. One of the relevant aspects of this model is the reproduction of anatomical brain abnormalities observed in humans⁷. Due to the above, the LNVH model offers the opportunity to assess the progression of brain morphological changes associated with schizophrenia.

The objective of this study was to evaluate the longitudinal morphological changes in the brains of male Wistar rats with lesion in the ventral hippocampus, including size and existence of cavitation, especially in the hippocampus and ventricles from day 1 after the lesion (PD) until on day PD139 from birth, through seven MRI measurements.

Method

Animal model

30 male Wistar rats were included and divided into two groups: one with LNVH and the other as control. The first group of 15 rats received an intracranial administration of 0.3 μ l of ibotenic acid at the age of 7 days (LNVH group), while the 15 rats in the control group underwent an intracranial administration of 0.3 μ l of phosphate buffered saline solution. Both procedures were performed with the procedure described by Genis-Mendoza et al.⁸. The neonatal lesion was performed in the ventral hippocampus: the 5-7 day old pups were anesthetized by hypothermia by placing them on ice for 12-15 min; subsequently, they were placed in a stereotaxic cage with a special adapter for neonatal animals. An incision was made in the skin of the skull to each calf and they were administered 0.3 μ l of ibotenic acid (dissolved in 0.1 M phosphate buffer, pH 7.4) directly into the ventral hippocampus for 2 min with a 30 Ga thick stainless steel cannula. The coordinates were: anteroposterior 2.0 mm, mediolateral $\pm$ 2.5 mm relative to the bregma, and ventrodorsal -3.3 mm from the dura mater. Subsequently, the animals were sutured and allowed to recover under the indirect heat of a 40 W lamp until they recovered their temperature. The operated animals were returned to their mothers until weaning at 21 days of age.

The care of the animals was carried out in accordance with the Official Mexican Standard NOM-062-ZOO-1999 and the International Guide for the care of animals NRC 2002. Animals were kept under the following conditions: 12/12 hour light/dark cycles, 45-60% relative humidity, 12 to 15 ventilation cycles, and a temperature of 18-26 °C.

This project was reviewed and approved by the Research Ethics Committee of the National Institute of Genetic Medicine, with registration number 120/2014/I.

In vivo lesion monitoring by magnetic resonance imaging

The MRI evaluation was performed at the following ages: PD8, PD27, PD48, PD69, PD96 PD120 and PD139. Starting from PD27, the animals were anesthetized with a mixture of 1.7 mg/kg of ketamine and 1.8 mg/kg of xylazine weight, while animals at the age of PD8 were anesthetized with ice. The rodents were placed in an immobilizing cylinder to enter the MRI equipment. The images were obtained with a Varian 7T unit at the

Table 1. Comparison of volumes (μm^3) obtained by segmentation in the seven magnetic resonance sessions

	Group	PD8	PD27	PD48	PD69	PD96	PD120	PD139
Brain	LNHV	1375.07	1325.18	1718.15	1719.01	1724.41	1736.28	1744.98
	Control	1461.86	1473.66	1626.59	1645.57	1696.35	1657.51	1688.03
Fourth ventricle	LNHV	2.19	2.53	3.56	6.79	5.8	4.81	6.06
	Control	1.45	1.48	2.64	3.56	5.87	6.06	6.92
Third ventricle	LNHV	2.41	1.54	9.43	10.02	11.67	10.81	6.92
	Control	1.91	2.14	2.31	2.83	3.16	5.67	5.73
Right hippocampus	LNHV	33.68	28.8	31.84	38.43	39.68	37.24	38.89
	Control	15.93	25.73	29.99	31.42	37.05	37.18	42.19
Left hippocampus	LNHV	29.45	26.98	32.04	39.81	39.29	36.91	40.08
	Control	17.19	25.24	25.38	30.98	37.9	40.21	43.24
Right lateral ventricle	LNHV	12.77	2.58	7.71	3.03	3.36	6.92	9.03
	Control	0.55	0.88	1.71	2.24	2.97	3.56	3.69
Left lateral ventricle	LNHV	9.6	2.63	6.86	3.49	7.58	6.79	10.42
	Control	0.66	1.04	2.5	2.86	3.16	3.23	3.76
Right lesion	LNHV	0	0.77	0.46	3.1	3.56	1.91	0.99
	Control	0	0	0	0	0	0	0
Left lesion	LNHV	0.66	1.38	0.73	3.1	4.48	1.78	0.99
	Control	0	0	0	0	0	0	0

LNHV: neonatal lesion in the ventral hippocampus.

Data from PD8 are means of only two animals that continued the study, while at PD8 time they are means of all included animals.

National Center for Research in Medical Imaging and Instrumentation of the Autonomous Metropolitan University. T2-weighted images were obtained using a FSEMS acquisition with the following parameters: echo time 65 ms, repetition time 8,200 ms, six averages per cycle, and 180° roll angle. The field of view was 22.5 × 20 mm², on a 256 × 256 matrix which resulted in a resolution of 88 × 78 μm pp. 45 contiguous cuts (400 μm thick) and the approximate exploration time was 25 minutes per animal.

Analysis of magnetic resonance imaging data

The analysis of the MR images focused in different regions of interest, including the hippocampus, ventricles, and lesion in the ventral hippocampus. Due to the T2-weighted measurements used in the present study, the lateral ventricles and injured tissue were seen as hyperintense due to cerebrospinal fluid and the presence of free water in these areas. Each hemisphere and hippocampus per animal were analyzed

by segmenting the images with the Amira 5.3.39 software, in order to obtain the measurements of the lesion (of the LNHV group and the control group), the hippocampus, the third ventricle and the lateral ventricles by using voxel-based morphometry.

Results

In order to compare the different volumes of the brain, the following measurements were obtained: fourth ventricle (4th V), third ventricle (3rd V), hippocampus (H), lateral ventricles (right and left VL) and size of the lesion promoted by ibotenic acid (Table 1). The segmented parts presented in Figure 1 are the third and fourth ventricles, the hippocampus, the lesion, and the lateral ventricles for ages PD8, PD27, PD48, and PD69, and Figure 2 presents ages PD96, PD120 and PD139.

The volume of the lateral ventricles was qualitatively greater in the animals of the LNHV group than in those of the control group at the seven different ages evaluated, in both hemispheres. With respect to the hippocampus, the volume was also qualitatively greater in

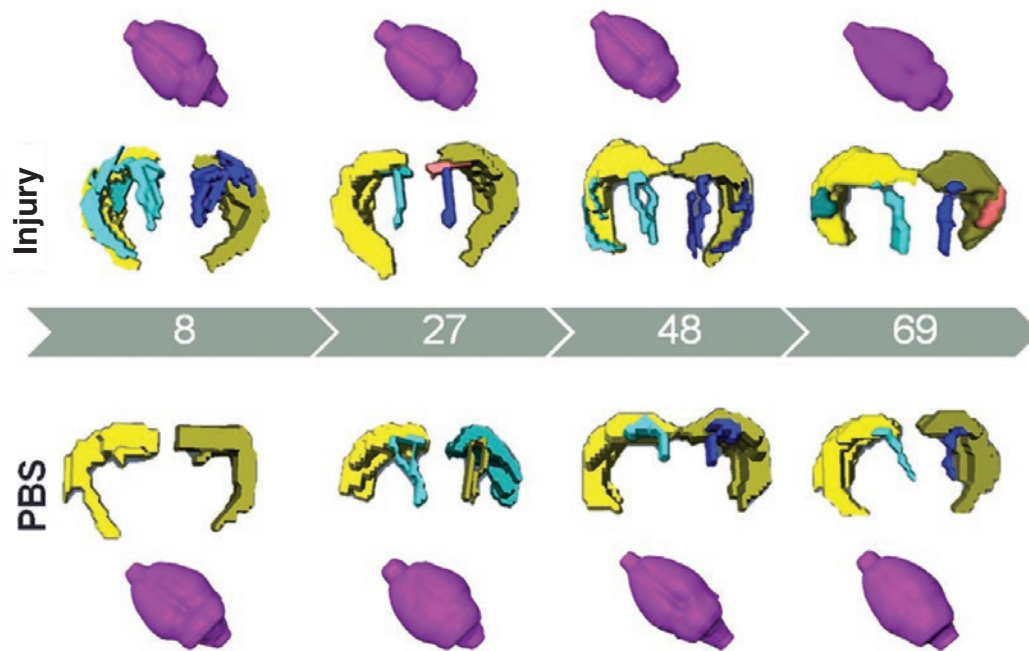


Figure 1. Segmented magnetic resonance images of the brain of the group of animals with neonatal lesion in the ventral hippocampus and in the control group (PBS) in different evaluations according to age (PD8, PD27, PD48 and PD69). The complete brain can be distinguished in purple, the right and left hippocampus in yellow, the right lesion in green and the left lesion in pink, the left ventricle in turquoise, and the right ventricle in royal blue.

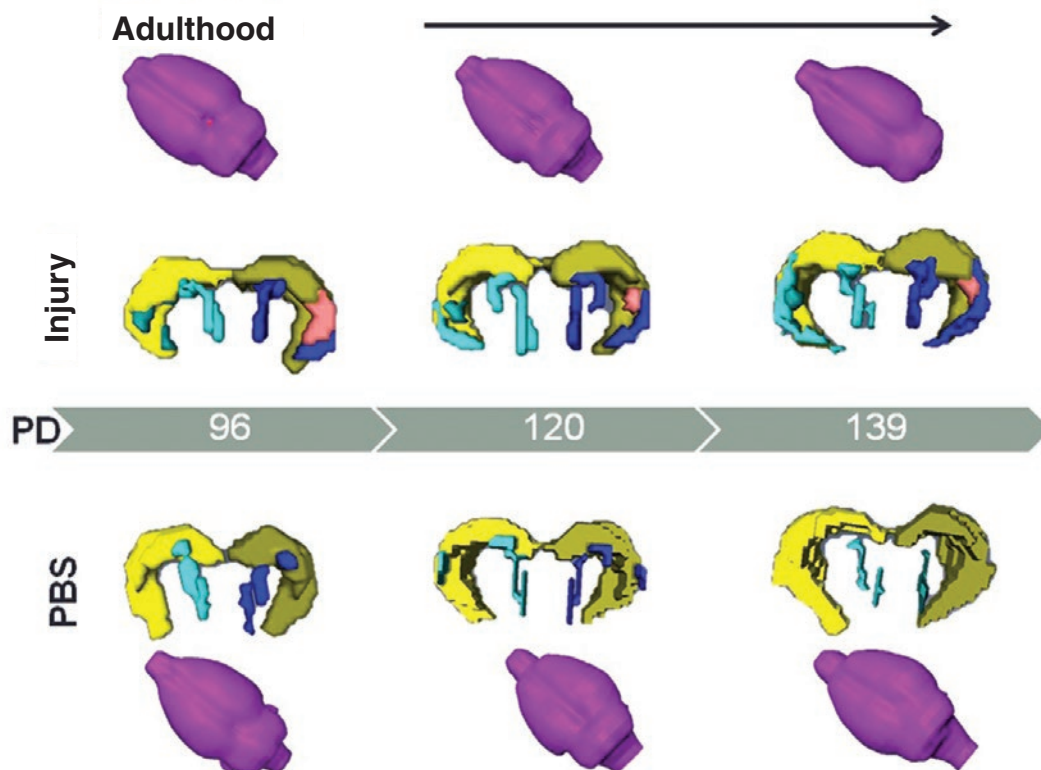


Figure 2. Segmented magnetic resonance images of the brain of the group of animals with neonatal lesion in the ventral hippocampus and the control group (PBS) in different evaluations according to age (PD96, PD120 and PD139). The complete brain can be distinguished in purple, the right and left hippocampus in yellow, the right lesion in green and the left lesion in pink, the left ventricle in turquoise and the right ventricle in royal blue.

the LNHV group than in the control group for all regions, although at first measurements there were apparently no detectable morphological changes (Table 1).

Regarding the lesion, in the first measurements it was imperceptible. However, from the second measurement in PD27, the lesion begins to be glimpsed, specifically in the left ventricle. For PD48 the lesion was more severe, with differences in size (for example, $0.73 \mu\text{m}^3$ for the left lesion and $0.46 \mu\text{m}^3$ for the right lesion) (Fig. 1), reaching its maximum size on day PD96 (with $4.48 \mu\text{m}^3$ in the left lesion and $3.56 \mu\text{m}^3$ for the right lesion), restructuring the tissue of the lesion in the two following measurements (Table 1).

Additionally, one of the most interesting findings was the presence of hypersignals with significant enhancement in the structures analyzed in the group with LNHV. The absence of such hypersignals in the control group suggests that they are unlikely to be due to accumulation of the injected fluid. The observed hypersignals could reflect gliosis and edema¹⁰, two processes that often occur simultaneously, or in a serial pattern, after neuronal damage.

Discussion

In this work, a longitudinal qualitative comparison of the lesion was made in the LNHV model of schizophrenia. Hypersignals were found in the LNHV group, but not in the control group, which could correspond to areas in which a severe glial reaction occurs, possibly due to a break in the blood-brain barrier¹⁰ (Figs. 1-2). The glial increase could also be related to the neuroinflammation process in individuals with schizophrenia^{11,12}, characterized by a complex cascade involving microglial activation by cytokine signaling¹². Increased neuroinflammation has been characterized in numerous studies of individuals with schizophrenia, and has been proposed as a factor possibly involved in the etiology of the disease^{13,14}. Another finding that could be related to a neuroinflammatory process is the greater ventricular volume in the LNHV group, compared to the control group, which could be the effect of neonatal exposure to proinflammatory cytokines. A significant positive correlation has been observed between fetal exposure to elevated levels of interleukin-8, a proinflammatory cytokine, in maternal serum during the second and third trimesters, and increased ventricular cerebrospinal fluid volume, as well as decreased left entorhinal cortical volume, characteristic of schizophrenia¹². These studies support the hypothesis that microglia are induced

by prenatal inflammation involved in the pathophysiology of schizophrenia¹⁵.

Despite the fact that the lesion in the ventral hippocampus was performed bilaterally, we observed a qualitatively larger lesion in the left hemisphere in most measurements. Possibly the change in the size of the lesion is a compensatory mechanism in the NHLV group, delaying the symmetric destruction of regions surrounding the ventral hippocampus and restoring the tissue surrounding the lesion at older ages, after the onset of schizophrenia symptoms¹⁶⁻¹⁸. In addition to the asymmetry observed in the lesion, we found that possibly the maximum peak in its size occurs on day PD96, and from there the size of the lesion reverses. This finding may suggest the presence of aberrant neurogenesis, as we already discussed in another study¹⁹, when schizophrenia-like behavior was characterized and brain slices were performed; however, to support this idea, other types of tests would have to be carried out. The mechanism of this increase in neuronal density has been hypothesized to involve the entrapment of neurons migrating from the germinal matrix to the cortex, due to lesion^{20,21}. Qualitative differences in ventricular volumes, as well as in the hippocampus, were identified from the PD69 in the LNHV group, compared to the control group. In the animal model, this day marks the end of adolescence and the beginning of adulthood, which is consistent with the onset of schizophrenia symptoms predominantly at this stage in humans⁸. In addition to the possible temporal homology of symptom onset between the model and humans, we also found qualitative differences in the hippocampus that could be involved in the development of cognitive symptoms and memory disturbances that can be observed in some patients with schizophrenia^{22,23}. In this sense, the NHLV model could be used to test new therapeutic approaches, such as deep brain stimulation, for the future treatment of patients with schizophrenia²⁴⁻²⁶.

The results highlight the importance of using the animal model as a viable and valuable approach to the study of psychiatric illness. The features of schizophrenia that have been successfully reproduced with the LNHV model provide insight into the neural mechanisms that might be involved in the symptomatology of the disease. In addition, they could be a source of future translational research and possible imaging markers of psychosis. As an example we could cite the medial temporal lobe crisis, for which many patients present manifestations of psychosis (characteristic symptoms of schizophrenia and which are emulated by the LNHV model)^{27,28}. We consider it

important to assess whether patients affected by medial temporal lobe seizures and psychosis could present cerebral alterations homologous to those of the model, in the sense of having a marker of psychosis in this particular group of patients.

Limitations and perspectives

It is important to mention that, although MRI is the best technique to structurally study the brain *in vivo*, during the performance of this study we encountered some difficulties that could be considered by other work groups when carrying out similar studies; for example, the death of some rodents during or after our experiments, probably due to their young age, or the hypothermia induced by the measuring equipment, not taken into account, until the animals were exposed during the acquisition of the images. Regarding this point, we were only able to continue monitoring two animals throughout the entire study, making it impossible to make statistical estimates, transforming our work into a qualitative and exploratory study. Another limitation, which could have a direct impact on the observed asymmetry of the lesion, could be due to a systematic error, which is why we recommend randomizing the hemispheres during surgery, in addition to being able to randomize two people who perform the surgery to minimize the possible mistake.

Conclusions

We observed a possible homology in the qualitative enlargement of the ventricles in the NHLV animal model, as previously reported in patients with schizophrenia, in addition to the presence of a possible gliosis around the lesion in the NHLV model.

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Conflicts of interest

The authors declare not to have any conflicts of interest.

Ethical disclosures

Protection of people and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and in accordance with the World Medical Association and the Declaration of Helsinki.

Data confidentiality. The authors declare that they have followed the protocols of their work center regarding the publication of patient data.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the corresponding author.

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