

A Decrease of Reelin Expression in Neonatal Ventral Hippocampal Lesion Model of Schizophrenia

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Abstract

Schizophrenia affects 1% of population. Neonatal ventral hippocampus lesion (NVHL) model of schizophrenia designed in 1993 by Lipska is a widely studied developmental animal model of schizophrenia. NVHL rats mimic many of the symptoms of schizophrenia in detail. We studied this model in molecular level and reelin expression in it. Reelin is an extracellular matrix glycoprotein that regulates some processes in CNS development and reduces significantly in schizophrenia. For this study, animals (male pups) take into 3 groups: control, sham and experiment. The lesion made by injection of 0.3 μ l. Isotonic Neurotoxin with stereotaxic surgery in age 7 day and body weight 11-15 gr. Social behavioral and stereotypic movement assessed in age 56 day then reelin expression in frontal cortex was evaluated by western blotting. Behavioral analysis and histological studies demonstrated the schizophrenia model. Western blotting of reelin protein in frontal cortex and hippocampus showed a decrease of reelin (P value: 0.012) in experimental group as compared to control and sham group. So, in the NVHL as a common and more similar model of schizophrenia reelin expression significantly decreases in frontal cortex and hippocampus that means this model in molecular pathways is similar to the disease.

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Introduction

Schizophrenia characterized by a breakdown of thought processes and deficit of typical emotional responses [1]. Common symptoms include auditory hallucinations and paranoid delusions or thinking. It is accompanied by significant social or occupational dysfunction. Schizophrenia affects around 0.3–0.7% of people or 24 million people worldwide as of 2011 [2]. It occurs 1.4 times more frequently in males than females and resulted in 20,000 deaths in 2010. The causes of schizophrenia are not known but a combination of genetic and environmental factors play a role in the development of schizophrenia. In the pathophysiology of schizophrenia both anatomic and neurotransmitter system abnormalities have been implicated [3].

Neuroimaging studies in patients with schizophrenia show abnormalities such as enlargement of the ventricles, decreased brain volume in temporal areas, and changes in the hippocampus [4]. Abnormalities of the dopaminergic system are thought to exist in schizophrenia. It has been suggested that schizophrenia should be thought of as a collection of neurodevelopmental disorders [5]. Reelin is a large secreted extracellular matrix glycoprotein that regulates processes of neuronal migration and positioning in developing brain by controlling cell–cell interactions. Besides this important role in early development, reelin continues to work in the adult brain. It modulates synaptic plasticity by enhancing the induction and maintenance of long-term potentiation [6]. It also stimulates dendrite and dendritic spine, development and



regulates the continuing migration of neuroblasts generated in adult neurogenesis sites like subventricular and subgranular zones. Reelin is found not only in the brain, but also in the spinal cord, blood, and other body organs and tissues [7]. Reelin has been suggested to be implicated in pathogenesis of several brain diseases. The expression of the protein has been found to be significantly lower in schizophrenia and psychotic bipolar disorder, but the cause of this observation remains uncertain as studies show that psychotropic medication itself affects reelin expression [8].

Several models have been developed for schizophrenia. These models take in four basic categories: pharmacological models, developmental models, lesion models, and genetic models. The neurodevelopmental and neurodegenerative aspects of schizophrenia have led to the use of lesion models in studies.

Neonatal lesion of the ventral part of the hippocampus in rats (NVHL rats) is a widely studied developmental animal model of schizophrenia designed in 1993 by Lipska [9]. NVHL rats mimic many of the symptoms of schizophrenia in detail [10]. The behavioral deficits caused by NVHL are seen after puberty and include aggression and social interaction abnormalities [11]. Because of importance of this model we studied reelin expression in it and our hypothesis is that reelin decreases after lesion in hippocampus of animals and plays important role in NVHL modeling. In addition it reveals other aspects of pathophysiology occurred in schizophrenia.

Materials and Methods

All of the surgical procedures were approved by Iran University Animal care committee. (Guide for the care and use of laboratory animals, national research council, 1996)

Animals

Pregnant rats were obtained at the last days of pregnancy and individually housed in controlled environment (humidity and temperature) on a 12/12 hours light/dark cycle with free access to standard food and water. Following delivery of the pups, the rats were left undisturbed for seven days. On the PD = 7, body weight 11-15 gr, the male pups randomized to 3 groups: sham, control and test, 8 pups in each group. All efforts made to minimize animal suffering and to reduce the number of animals.

Surgery

Pups anesthetized by hypothermia were placed on wet ice for 10-15 min. Anesthesia was verified by non-responsiveness to tactile stimulation. An incision was made in midline longitudinally on skull and 0.3 µl of biogenic acid or an equal volume sterile physiological serum injected via a 30 gauge track needle. The rate of injection was 0.15 µl/min. Surgery had been done by a Stoeber Stereotaxic apparatus modified by a platform through the coordinates: Anteroposterior = -3.0 Lateral – medial = +3.5. Dorsoventral = 5.0 mm from the skull, relative to bregma. The needle left in the place for 2-4 minutes after injection to prevent backflow. Finally the skin sutured [12]. The animals placed under a warming

lamp and returned to their mothers after normal activity and consciousness.

Stereotypic movement test and social behavioral test

At the PD = 56-58, the animals were undergone 90 minutes Stereotypic movement test then was observed for social behavioral test 7 days [13].

Histology

NVHL model was controlled by Nissle staining. Perfused brain of 4 animals of each group was removed from the skull and post fixed by immersion in the same fixative for at least two days before histological processing. Parietal cortex was dehydrated and embedded in paraffin. A series of 10 µm thick was cut and stained with crystal violet [14].

Tissue harvesting and Western blotting

At the PD = 56-58 after injection of over doses of ketamin and xilosin , brain of the 4 animals of each group sampled and frontal lobe was removed to lysis buffer on ice (Ripa buffer: Sigma R0278, Cocktail Ant protease: Sigma P8340). Tissue samples homogenized in 40°C then the samples were placed on ice 45 minutes and centrifuged for 20 minutes at 1200 Rpm in 4°C. Supernatants were collected and assayed for total protein (Radford method). After extraction the total protein was mixed with denaturing loading buffer (Basic Tris PH: 6.8 1/25 ml, glycerol 1ml, SDS 0.2 gr, Mercaptoethanol 0.1 ml). The mixture was placed on G10 SDS-poly acryl amid gel and run at 75 v 15 min followed by 60 min at 150 V at room temperature. The gel was transferred to nitrocellulose membrane at 150 mA for 1.5 h in 4°C [14]. The membrane was blocked 1 h at room temperature with 3% nonfat dry milk in TBS and then was reacted with mouse antibody of reelin (G10 abcam) diluted 1/1000 for 2 h at room temperature. The immunoreactive band was detected by using a peroxidase conjugated goat anti mouse IgG diluted 1: 5000 for 1 h followed by the application of the ECL plus chemiluminescence western blotting kit (Amersham , ECI).

Results

Stereotypic movement test and social behavioral test at the PD = 56-58 confirmed schizophrenia in the animals. The Nissle staining showed a dilatation in ventricles and glycolysis in hippocampus of NVHL model indicating that lesion led to schizophrenia (Fig. 1, 2 & 3).

Analysis of reelin protein expression in frontal and hippocampus with western blotting shows a significantly decrease of reelin in frontal and hippocampus of test animals in contrast with control and sham groups. Figure 4 shows the SDS-poly acryl amid gel after running that is quantified in Figure 5.

Discussion

Developing reliable, predictive animal models for complex psychiatric disorders, such as schizophrenia, is important to increase understandings of the neurobiological basis of the disorder and development of new drugs with improved therapeutic efficacy. Increasing information on the neurochemical and structural CNS changes accompanying each model will also help assess to treatments that prevent

the development of schizophrenia rather than treating the symptoms [15].



Figure 1. A section of normal hippocampus (Nissle staining).

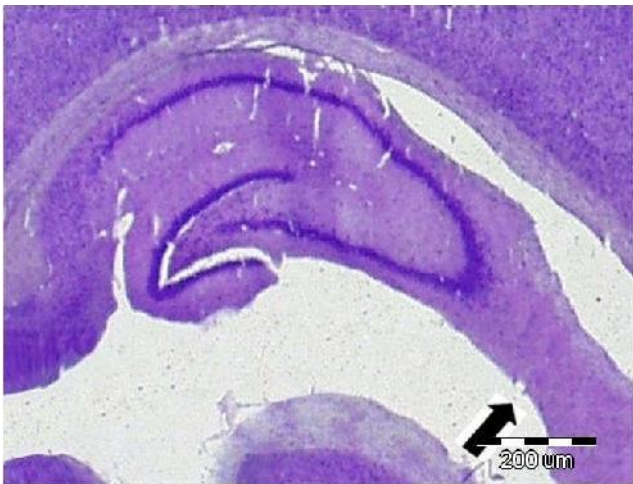


Figure 2. A section of hippocampus of NVHL model show dilatation in ventricle (Nissle staining).

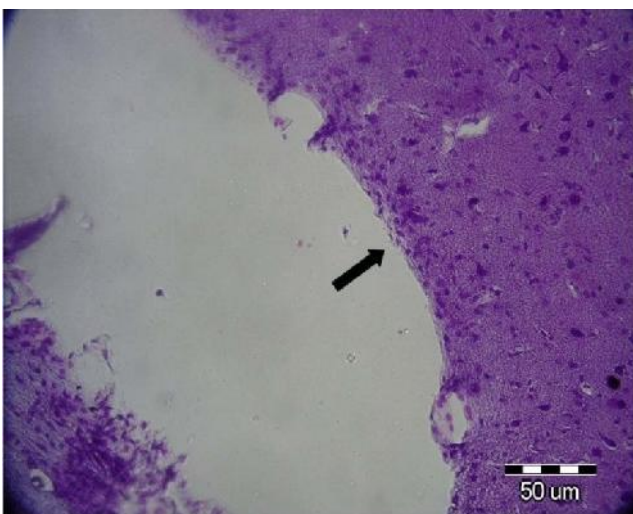


Figure 3. Glycosis in hippocampus of NVHL model (Nissle staining)

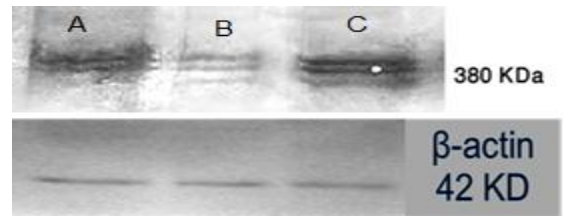


Figure 4. SDS-poly acryl amid gel and reelin expression in frontal region at sham (A), test (B) and control (C) groups.

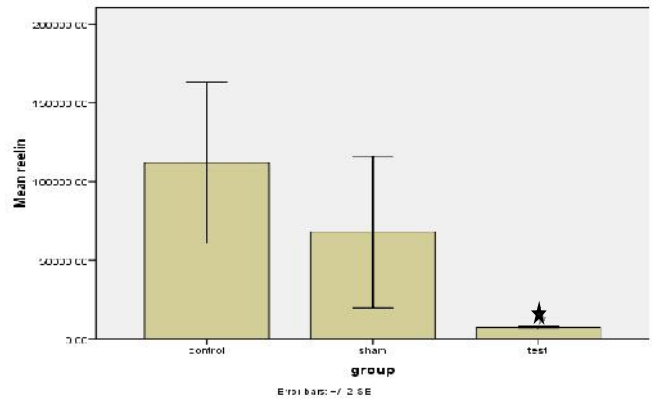


Figure 5. Pixel density quantification in reelin western blotting study showed a significant decrease in reelin protein expression in frontal and hippocampus in test group compared to control and sham groups (P value: 0.012).

The most thoroughly characterized heuristic neuro developmental animal model of schizophrenia available today is the neonatal ventral hippocampal lesion (NVHL) model. This model was inspired by an attempt to capture prominent aspects of schizophrenia unaddressed by pharmacological models. Emerging brain imaging evidence pointed to a lateral ventricular enlargement and hippocampal changes in schizophrenia patients. In this study histological assay with Nissle staining showed that dilatation in ventricles and glycosis in hippocampus have been occurred in schizophrenia.

In the NVHL model there are a number of behavioral, molecular and physiological changes reminiscent of a variety of aspects of schizophrenia that emerge at a particular time in development, around young adulthood/adolescence that we observed by stereotypic movement test and social behavioral test at the PD = 56-58 and confirmed schizophrenia in the animals. Kuei et al indicate that in NVHL rats, not only are multiple clinical features of the syndrome manifest, but the development and function of multiple brain systems are affected, including the frontal and medial temporal lobes, the ventral striatum, and the mesocorticolimbic dopamine system so we focused on frontal lobe and hippocampal regions. Genetic background, environmental factors and

pharmacological interventions can all modulate the behavioral and neurobiological aspects of the NVHL syndrome and demonstrating the utility of this model for exploring the interaction of diverse causative factors in disease pathogenesis. The findings on the NVHL model highlight its advantages and limitations, and how it may offer clues about the extent to different aspects of schizophrenia represent inter-related pathophysiological mechanisms [15]. This assay tried to increase those findings and reveal other genetic aspects of this model. Reelin is one of important gene that its manipulation can make a new model of schizophrenia. Knockout mice in which the reelin gene is disrupted are called reeler mice. In homozygous reeler mice, extreme changes in gait and other behavioral anomalies are seen; these changes go beyond those that are associated with schizophrenia [10]. Our findings show a decrease of reelin expression in the NVHL model after puberty that means this model have other unrevealed aspects and more similarity to the disease. In other words, lesions in NVHL decreases reelin expression and via this may led to schizophrenia symptoms.

Conclusion

In the NVHL as a common and more similar model of schizophrenia reelin expression significantly decreases in frontal cortex and hippocampus that means this model in molecular pathways is similar to the disease.

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