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Delayed audiogenic seizure development in a genetic rat model is associated with overactivation of ERK1/2 and disturbances in glutamatergic signaling

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ABSTRACT

Krushinsky-Molodkina (KM) rats genetically prone to audiogenic seizure are characterized by age-dependent expression of audiogenic seizures (AGS). It is known that the critical period of enhanced seizure susceptibility in rodents occurs at 2nd–3rd weeks of postnatal development. However, KM rats do not express AGS at this time-point, but start to demonstrate a stable AGS only after the age of 3 months. We hypothesized that this delay in AGS susceptibility in KM rats is genetically determined and may depend on some alterations in the development of the hippocampal glutamatergic system during the early postnatal period. We analyzed the expression and activity of seizure-related proteins, such as vesicular glutamate transporter 2 (VGLUT2), extracellular signal-regulated kinases 1 and 2 (ERK1/2), synapsin I, and NR2B subunit of the *N*-methyl-D-aspartate (NMDA) receptor (NR2B) in the hippocampus of KM rats during postnatal development. A significantly higher activity of ERK1/2 in KM rats was observed at 14th, 30th, and 60th days of postnatal development (P14, P30, P60) in comparison with control Wistar rats of the corresponding ages, while in adult (P120) KM rats it was at the same level with Wistar rats. Despite the increased activity of ERK1/2 at P14 and P30, the phosphorylation of synapsin I at Ser62/67 was significantly lower in the hippocampus of KM rats than in Wistar rats of the same ages; however, at P60 and P120, the phosphorylation of synapsin I was enhanced. Our data also revealed the increase of VGLUT2 and NR2B expression at P14, which dramatically decreased at the later stages. Our data indicate that a genetically determined increase in ERK1/2 kinase activity during postnatal ontogenesis in KM rats may be associated with the disturbances in synthesis and activity of the proteins, which are responsible for glutamatergic transmission in the KM rat hippocampus during the seizure susceptibility development.

1. Introduction

Epilepsy is common worldwide, affecting approximately 1% of the population. The hippocampus is often involved in epileptogenesis [1–3]. Reflex epilepsy is a particular epilepsy syndrome in which seizures are triggered by identifiable factors such as flashing light. In order to study reflex epilepsy, several audiogenic rat strains were created to exhibit seizure activity in response to intense acoustic stimulation [4]. Hyperexcitation of the hippocampal formation was demonstrated for genetically epilepsy-prone rats (GEPR) and Wistar audiogenic rats (WAR) even without audiogenic seizure (AGS) experience [5–7], but less is known about the involvement of the Krushinsky-Molodkina (KM) rat hippocampal formation in the expression of AGS.

Audiogenic seizure susceptibility in KM rats, which were selected from Wistar rats, is genetically determined, but KM rats are not able to exhibit stable seizure activity until they reach the age of 3 months; however, the robust sound sensitivity can be observed thereafter [8]. The critical period of enhanced seizure susceptibility in rodents occurs at weeks 2–3 of postnatal development as previously demonstrated in the experiments with proconvulsant drug administration and electrical stimulation of the amygdala or hippocampus [9]. However, KM rats do not express AGS at these time-points. This suggests that there exists in

Abbreviations: AGS, audiogenic seizure; KM, Krushinsky-Molodkina; NR2B, NR2B subunit of the *N*-methyl-D-aspartate (NMDA) receptor; ERK1/2, extracellular signal-regulated kinases 1 and 2; VGLUT2, vesicular glutamate transporter 2; DG, dentate gyrus.

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KM rats a genetically determined switch during their postnatal development, which results in expression of AGS in adulthood. It is known that, in the case of AGS, initially, epileptiform activity localizes in the brainstem structure, such as the inferior colliculus (IC) [10]. Previously, we demonstrated a significant delay in the development of the IC in KM rats and the increase of extracellular signal-regulated kinases 1 and 2 (ERK1/2) activity and glutamatergic neurotransmission in the IC during early postnatal development [11,12]. On the other hand, the hippocampus of audiogenic rats was also suggested to play a role of a threshold for propagation of seizure activity [6,7]. Verma-Ahuja and coauthors demonstrated increased excitability of the hippocampus at early postnatal ontogenesis of GEPRs [6]. In the hippocampus of WARs, increased susceptibility to seizure expression was associated with a decrease of GABAergic inhibition [7]. According to this idea, alterations in the hippocampus during KM's ontogenesis can also be a reason for seizure susceptibility along with IC. Such alterations may cause a misbalance in excitatory and inhibitory control of neuronal activity, which is supposed as general reason for seizures [13,14]. Glutamatergic cells present the major cell type in the hippocampus, and aberrant glutamatergic neurotransmission may modify the neuronal circuit and contribute to epileptogenesis [15]. The balance may also be disturbed by defective intracellular regulation. It was demonstrated that ERK1/2 cascade is involved in processes associated with seizure activity [16–18]. Previously, we demonstrated that ERK1/2 inhibition is able to prevent AGS expression in KM rats [19]. In line with these findings, we assumed that abnormalities in ERK1/2 activity may underlie high seizure susceptibility in KM rats.

Epileptogenic dysfunctions in genetic models of epilepsy can also occur at the level of neurotransmitter exocytosis. Mutations in gene of synapsin I, which is regulated by phosphorylation, are shown to be associated with epilepsy [20,21]. Synapsin I has several specific sites for various kinases, including ERK1/2 [22]. This suggests that impaired ERK1/2 regulation can affect the release of the neurotransmitters, resulting to excitation–inhibition shifts in the neuronal activity.

In order to investigate the age-dependent development of seizure susceptibility in KM rats, we analyzed the glutamatergic systems, as well as ERK1/2 activity and expression of exocytosis proteins in different areas of the hippocampus of naïve KM rats on different postnatal days, P14, P30, P60, and 4-month-old rats, in comparison to Wistar rats of the corresponding age. We found age-dependent differential changes in the expression and distribution of ERK1/2, vesicular glutamate transporter 2 (VGLUT2), NR2B subunit of the *N*-methyl-D-aspartate (NMDA) receptor (NR2B), and synapsin I in the hippocampus, especially at the 14th and 30th postnatal days. Our data proposed that these aberrations at the early stages of postnatal development can be a background for epilepsy in adulthood.

2. Materials and methods

2.1. Animals

Male and female KM rats of different ages (Moscow State University, Russia) genetically prone to AGS were used in the experiments. The KM rats were selected from Wistar rats in Moscow State University about sixty years ago. These rats are characterized by an age-dependent development of audiogenic epilepsy when the stable tonic–clonic seizures to sound stimulation are completely formed at the age of 3–3.5 months [8]. Wistar rats were recruited as controls. The rats were housed in individual cages under natural light–dark cycle with free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee at the Sechenov Institute of Evolutionary Physiology and Biochemistry. To study the biochemical changes in the hippocampus during postnatal development of KM rats, we compared KM rats with Wistar rats. The hippocampus of KM and Wistar rats both male and female were analyzed at the 14th (P14), 30th (P30), 60th (P60), and 120th (P120) days of postnatal development ($n = 10$ for each group of Wistar rats and each group of KM rats).

2.2. Sample preparation

One-half of the animals ($n = 5$) from each group were deeply anesthetized (chloral hydrate, 400 mg/kg, approved by Institutional Animal Care and Use Committee only for nonrecovery anesthesia). Animals were perfused transcardially with phosphate buffered saline (PBS) followed by 4% buffered formalin and then decapitated. The brains from all animals were removed, postfixed in 4% buffered formalin at +4 °C for 5 days, and subsequently immersed in 15% sucrose for cryoprotection. The other 5 animals from each group were decapitated; the hippocampi were dissected and homogenized for further biochemical analysis.

2.3. Immunohistochemistry

Immunohistochemical analysis was carried out according to standard biotin-streptavidin protocol. Cut cryosections (10 μm) containing the hippocampus were incubated with primary antibodies overnight at room temperature. Used antibody against: p-ERK1/2 (Thr202/Tyr204; 1:250; Cell Signaling Technology,#4376); ERK1/2 (1:250; Cell Signaling Technology, #9102); VGLUT2 (1:200; Abcam plc, #MAB5504); NR2B (1:500; Abcam, ab65875); p-synapsin I (Ser62/67; 1:500; Millipore, #AB9848); synapsin I (1:500; Millipore, #AB1543P). After incubating with primary antibodies the sections were washed in PBS and then incubated for 1 h with biotinylated secondary antibodies (1:300, Vector Laboratories Inc.), and then with peroxidase-streptavidin complex (1:500, Vector) for 1 h. The peroxidase reaction was revealed in the buffer containing 3,3′ diaminobenzidine (DAB; 0.05%) and hydrogen peroxide (0.01%). Additionally, reactions lacking primary antibodies were done to ensure the specificity of the observed staining. Finally, the sections were dehydrated and coverslipped.

2.4. Western blot analysis

The hippocampi were homogenized in lysis buffer containing protease inhibitors and phosphatase inhibitor cocktail (both from Sigma-Aldrich, St. Louis, MO, USA) using tissue grinder at 4 °C. Insoluble materials were removed by centrifugation. Total protein concentrations were determined by Bio-Rad protein assay (Bio-Rad Laboratories Inc., Hercules, CA, USA). Equal amounts of samples (10-μg protein per line) were loaded for electrophoresis, and proteins were separated on 10% polyacrylamide gel and then transferred to a nitrocellulose membrane (Santa Cruz). The membranes were incubated in 5% nonfat milk or 3% Bovine Serum Albumin (BSA) in Tris Buffered Saline with Tween (TBST) buffer (0.1% Tween 20, 20-mM Tris, 137-mM NaCl; pH 7.4) for 40 min and then incubated overnight with primary antibodies. Used antibodies: p-ERK1/2 (Thr202/Tyr204, 1:250, Cell Signaling Technology, # 4376); ERK1/2 (1:250; Cell Signaling Technology, #9102); VGLUT2 (1:200; Abcam plc, #MAB5504); NR2B (1:500 Abcam, #ab65875); p-synapsin I (Ser62/67, 1:500; Millipore, #AB9848); synapsin I (1:500; Millipore, #AB1543P); β-tubulin (1:1000; Cell Signaling Technology, #2148). Then the membranes were washed in TBST buffer and incubated with secondary anti-rabbit (1:10000, Sigma-Aldrich, #A5420) or anti-mouse (1:50000, Sigma-Aldrich, #A 9044) antibodies for 1 h at room temperature. SuperSignal@West Dura Extended Duration Substrate (#34075, Thermo Scientific) was used to produce chemiluminescent reaction.

2.5. Evaluation of sections and statistical analysis

A semiquantitative analysis of protein amount in the hippocampus was done by measurement of optical density of immunoreactive substance. Five to ten sections at the same level of the hippocampus were

analyzed from each KM and Wistar rats. Activity of ERK1/2 was estimated by immunohistochemical and Western blot detection of phosphorylated form of ERK1/2. Expression of pERK1/2 was calculated as optical density of immunopositive substance in the each selected zones of the hippocampus minus background. Optical density of the background was estimated at the same slice in nonimmunoreactive brain tissue field. For immunohistochemistry, values are expressed as the mean percentage difference ± SD. Optical density of each immunoreactive substance were estimated for each selected zone of the hippocampus of Wistar rats and taken as 100%.

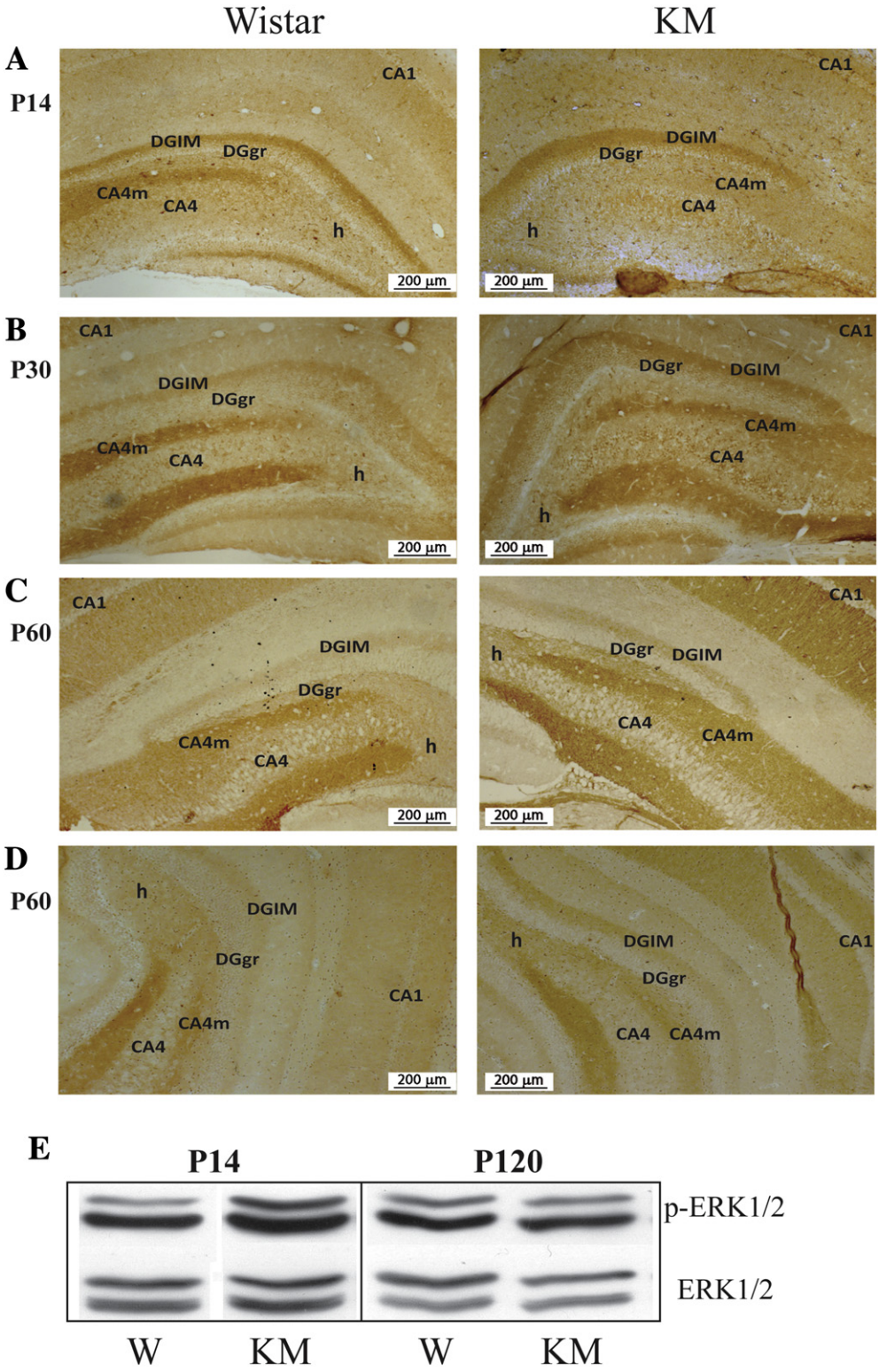

Fig. 1. pERK1/2 detection by immunohistochemistry and Western blot in the hippocampus of KM and Wistar rats at different stages of postnatal development. **A–D** - Representative images of pERK1/2 immunostating in the hippocampus of KM and Wistar rats at P14, P30, P60, and P120. **A** – P14, 14th postnatal day; **B** – P30, 30th postnatal day; **C** – P60, 60th postnatal day; **D** – P120, 4- month-old rats; DG IM – inner zone of the dentate gyrus molecular layer; DGgr – granular layer of the dentate gyrus; CA4m – molecular layer of CA4; CA4 – pyramidal layer of CA4; h – the hilus, CA1 – CA1 layer **E** – Representative image of pERK1/2 immunoblot of the hippocampus of Wistar (W) and KM rats at P14 and P120 demonstrated strong upregulation of pERK1/2 expression in two-week-old KM rats (P14).

For Western blot, expression of VGLUT2 or NR2 was estimated by of ratio between each studied protein and β-tubulin. Activity of ERK1/2 or synapsin I was estimated as ration pERK1/2/ERK1/2 or p-synapsin I/ synapsin I. Values are expressed as mean ± SD.

Statistical analysis for immunohistochemistry and Western blot data was carried out by the Mann–Whitney *U* test.

3. Results

3.1. Increased ERK1/2 activity in the hippocampus of KM rats

Firstly, we analyzed activity and distribution of ERK1/2 in the hippocampus of Wistar and KM rats during postnatal development. A pronounced p-ERK1/2 immunostaining was observed in the hilus, dentate gyrus (DG) granule cell layer, and in pyramidal cell layers of CA1 and CA4 of the two-week-old KM rats (Figs. 1A, 2A). Obtained data demonstrated a significant increase of ERK1/2 phosphorylation in two-week-old KM pups (Figs. 1A; 2A), as well as in 1- and 2-month-old KM rats (Fig.1B, C). In 1- and 2-month-old KM rats a significant increase of ERK1/2 activity was detected in the hilus, DG inner molecular layer, and in the molecular layers of the CA4 and CA1 (Fig. 1B, C; Fig. 2B, C). In 4-month-old KM rats, pERK1/2 immunoreactivity was not changed in comparison with Wistar rats of the same ages in all hippocampal layers analyzed, except the DG inner molecular layer, where p-ERK1/2 expression was increased (Fig. 1D; Fig. 2D).

3.2. Altered VGLUT2 expression in the hippocampus of KM rats

To study the alterations in the glutamatergic signaling in the hippocampus during AGS susceptibility development, we analyzed VGLUT2 expression. In P14 KM rats, the expression of VGLUT2 was significantly increased in the hilus, DG granular cell layer, and in the pyramidal layer of CA1, but decreased in the molecular layer of CA4 (Fig. 3A; Fig. 4A). This variable expression of VGLUT2 had similar trend as the changes of ERK1/2 activity in the studied zones (Fig.2A). Beginning from P30, the expression of VGLUT2 in KM rats was lower than in Wistar (Fig. 3B–D; Fig.4B-D).

3.3. Evaluation of NR2B subunit of NMDA receptor

Glutamate receptors regulate glutamate-mediated excitation and consequently may play a role in excitotoxicity, which contributes to neurodegeneration during epileptogenesis [23]. Here, we analyzed expression of NR2B subunit of NMDA receptor. Obtained results demonstrated that NR2B expression was noticeably higher in the hilus, DG, and CA4 of P14 KM rats in comparison to P14 Wistar rats (Fig. 5A, C). At P30 KM rats, NR2B expression was elevated only in the hilus and DG molecular layer (Fig. 5B, D). However, expression of NR2B in the hippocampus of four-month-old KM rats was significantly decreased (Fig. 5 E, F) that associated with a decrease of VGLUT2 expression (Fig. 3D, Fig. 4D).

3.4. Analysis of synapsin I phosphorylation

Finally, we studied the expression of phosphorylated form of synapsin I at Ser 62/67. Analysis of KM rats at P14 and P30 demonstrated a significant decrease of synapsin I phosphorylation in the hippocampus (Fig. 6A, B, C), despite the demonstrated upregulation of ERK1/2 activity (Fig.1A, B; Fig. 2A, B). On the other hand, at P60 and in four-month-old KM rats, the obtained results showed significant upregulation of synapsin I phosphorylation in comparison to control Wistar rats of the corresponding ages (Fig. 6A, D, E).

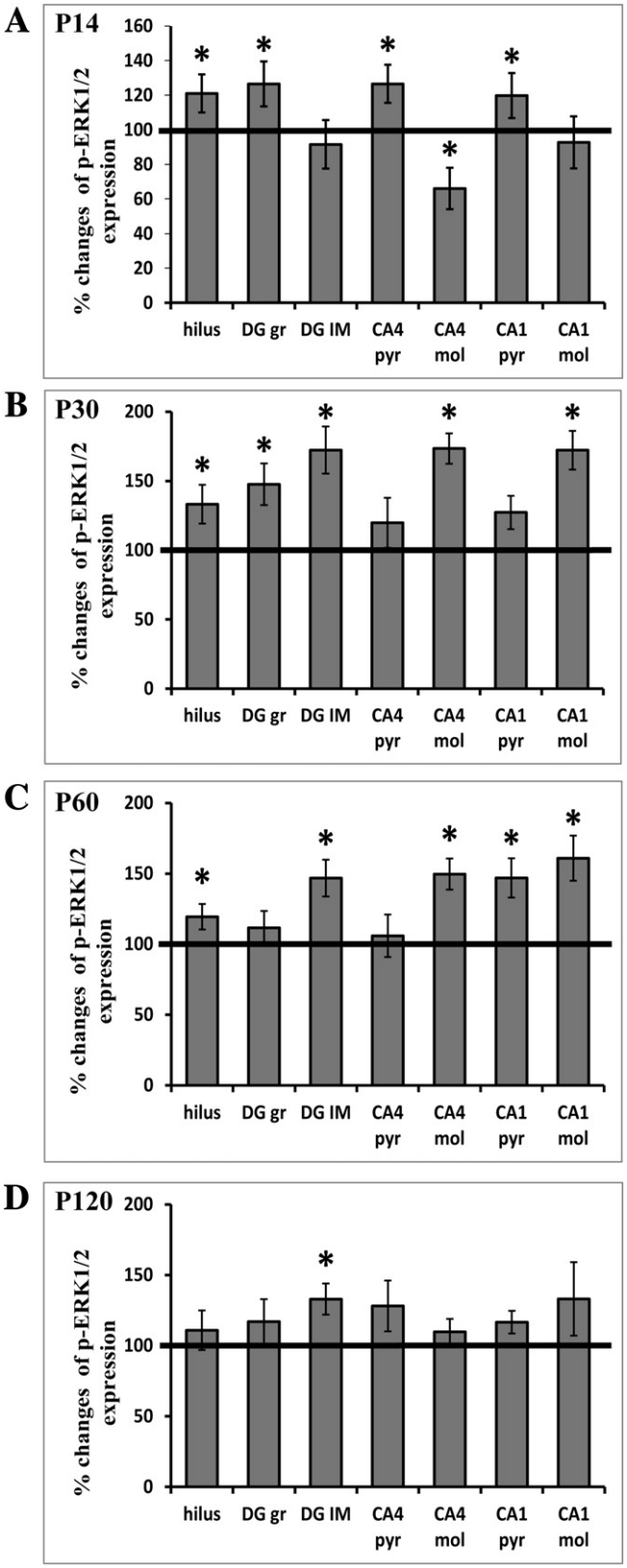

Fig. 2. Increased ERK1/2 activity in the hippocampus of KM rats. **A–D** - The optical density of immunopositive pERK1/2 was evaluated and obtained data demonstrated significant increase of pERK1/2 in the dentate gyrus and the hippocampal formation of KM rats at P14 (**A**), P30 (**B**), P60 (**C**), and P120 (**D**) in comparison with control Wistar rats. Optical density of pERK1/2 staining was estimated for each selected zone of the hippocampus of Wistar rats of corresponding ages and taken as 100%. Data are shown as % ± SE (in %). * $p < 0.05$ KM vs Wistar for each studied zone.

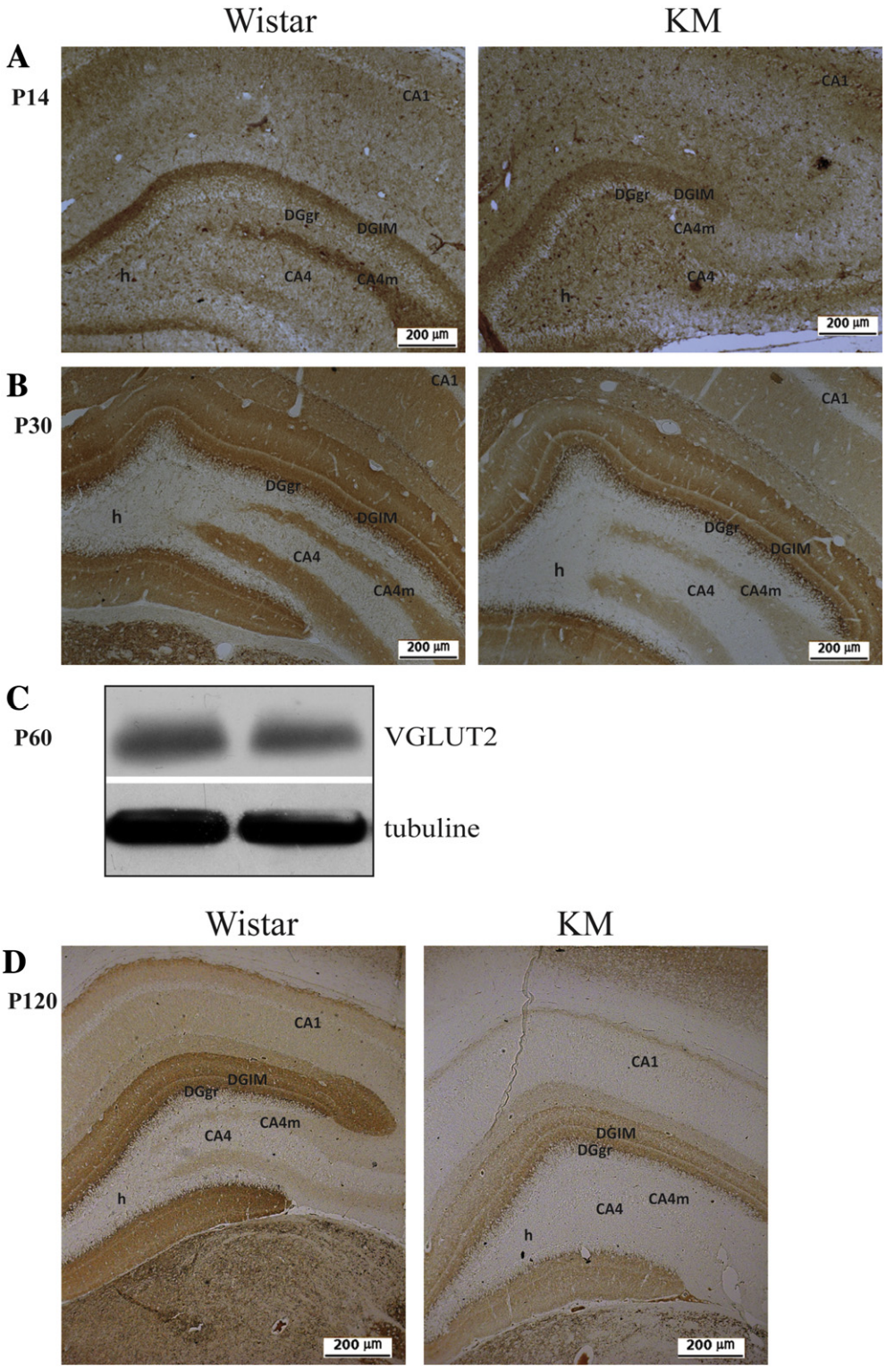

Fig. 3. Detection of VGLUT2 by immunohistochemistry and Western blot in the hippocampus of KM and Wistar rats. **A, B, D** - VGLUT2 immunostaining showed attenuation of VGLUT2 immunoreactivity in the hippocampus of KM rats at P14 (**A**), P30 (**B**), and P120 (**D**) in comparison with control Wistar rats of corresponding ages. DG IM – inner zone of the dentate gyrus molecular layer; DGgr – granular layer of the dentate gyrus; CA4m – molecular layer of CA4; CA4 – pyramidal layer of CA4; h – the hilus, CA1 – CA1 layer. **C** – At P60 VGLUT2 immunoblot demonstrated a decreasing of VGLUT2 expression in the hippocampus of KM rats.

4. Discussion

In this study, we analyzed the expression of the key proteins ERK1/2, VGLUT2, NR2B, and synapsin I involved in the glutamatergic neurotransmission in the hippocampus to check if there are any alterations in the expression and/or activity of these proteins during postnatal development in KM rats. The expression of AGS in KM rats is age-dependent, and the AGS susceptibility is known to be completely formed only after P90. In spite of the data that increased ERK1/2 activity being associated with seizure expression [16–19], we found that the

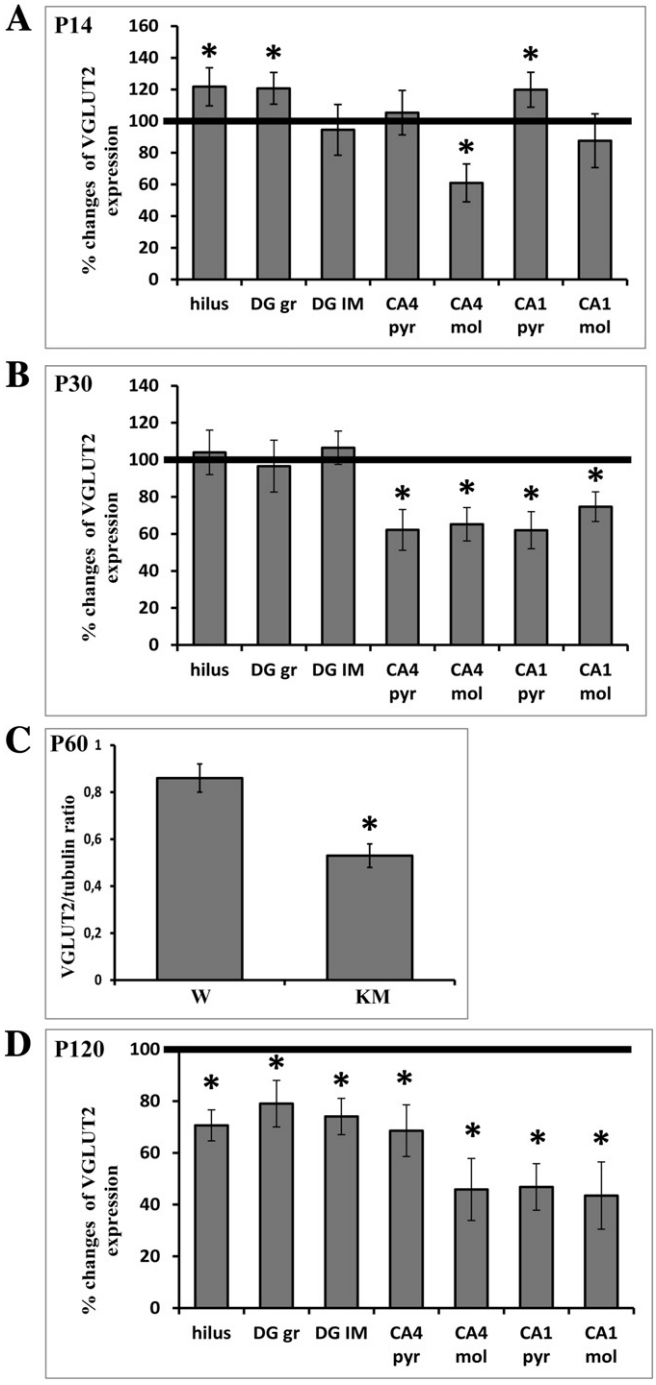

Fig. 4. KM rats are characterized by decreased expression of VGLUT2 in the hippocampus. The optical density of VGLUT2 staining in the hippocampal formation was evaluated, and the data demonstrated significant increasing of VGLUT2 in the hilus, dentate gyrus granular layer, and in pyramidal layer of CA1 in KM rats at P14 in comparison with control Wistar rats (**A**), while at P30 (**B**) and P120 (**D**) expression of VGLUT2 was decreased. At P60, (**C**) Western blot analysis also revealed significant decreasing of VGLUT2 expression in the hippocampus of KM rats. Optical density for immunohistochemistry is presented in percentages. Optical density of VGLUT2 staining was estimated for each selected zone of the hippocampus of Wistar rats of corresponding ages and taken as 100%. Data are shown as % ± SE (in %). Western blot data are presented in arbitrary units and expressed as mean ± SE. * $p < 0.05$ KM vs matched Wistar control.

AGS-free P14, P30, and P60 KM rats demonstrated a significantly upregulated ERK1/2 activity in the hippocampus. Extracellular signal-regulated kinases 1 and 2 are known to be involved in regulation of proliferation, differentiation of neurons and glia, and are also responsible for the growth of dendrites and axons and synaptogenesis [24]. Obviously, the increased activity of ERK1/2 kinases in the early stages of postnatal life (P14, P30, P60) can be associated with their participation in the regulation of the hippocampal development in KM rats. Our unpublished morphological data showed that the hippocampus of KM rats develops later then in Wistar rats. Moreover, we also demonstrated a delayed development of the IC of KM rats [11]. These data suppose the significant alterations and the delay in the postnatal development of the whole brain in these rats. At the late stages of postnatal life, an increased activity of ERK1/2 was observed only in the molecular layers of the hippocampus that can be connected with active synaptogenesis or with the growth of the fibers.

The activity of the glutamatergic system is often estimated by the expression of vesicular glutamate transporters VGLUT1/2, which mediate glutamatergic neurotransmission [25]. It was demonstrated that VGLUT2 heterozygous knockout mice are more sensitive to a proconvulsant agent pentylenetetrazole, and demonstrate reduced amplitude of the postsynaptic response [26]. We also showed a significant reduction of VGLUT2 in the hippocampus of adult KM rats in comparison with Wistar rats as well as at P60. Opposite to adults, at early stages of postnatal life of KM rats, we revealed an enhanced VGLUT2 expression in the hilus, DG, and CA1. The increase in VGLUT2 was identified in the same hippocampal regions where we detected enhanced ERK1/2 activity. Earlier, we identified active ERK1/2 kinases in the glutamatergic neurons of the hippocampus, where VGLUT2 was considered as a potential target for these kinases [19]. Altogether, these data propose that ERK1/2-dependent pathological abnormality in maturation of glutamatergic neurons leads to the development of seizure susceptibility.

Activation of ERK signaling leads to increased expression of NR2B [16]. It was proposed that NR2B-containing NMDA receptors play a crucial role in epileptic disorders [16,23,27–29]. The NMDA receptor blockers showed anticonvulsant activity in the pentylenetetrazole induced seizure model in KM rats [30]. Mutations in the *GRIN2A* gene, encoding an NMDA receptor, were detected in patients with epilepsy-aphasia disorders, which start during childhood [31]. Epileptiform discharges were also displayed in young *Grin2a* knockout mice that reproduce similar anomalies found in patients with epilepsy-aphasia disorders [32]. In this way, it can be suggested that anomalies in the maturation of the neocortical and thalamocortical systems appear at the early stage of postnatal development. It was demonstrated that in adult WAG/Rij rats with inherited absence epilepsy, the expression of NR2B in CA1 of the hippocampus was significantly lower in comparison with Wistar rats of the same ages [33]. We showed that at P14 in KM rats, the expression of NR2B in the hilus, DG, and hippocampal CA4 layer was significantly higher than in Wistar rats. The elevated expression of NR2B can induce excitotoxicity that followed by neurodegeneration, and as a result, may modify the neuronal circuit [23]. In opposite, in adult KM rats, we observed significant reduction of NR2B expression in the hippocampus. It should be noted that age-dependent changes of NR2B were associated with changes of VGLUT2 and pERK1/2, when the expression of all these proteins was upregulated in KM pups and decreased in adulthood. These data proposed a significant activation of the hippocampal glutamatergic system at the early stages of postnatal development, which may contribute to the rearrangement of neuronal connections in the hippocampus, as well as between hippocampus and other parts of the brain, leading to epileptogenesis.

How ERK1/2 kinases can be involved in the regulation of glutamatergic neurotransmission? An imbalance of inhibitory and excitatory neurotransmission arises from various factors, and some of them are defects in exocytosis process. The ERK1/2 cascade is known to be involved in the control of functional activity of neurons by acting at both presynaptic and postsynaptic sites, phosphorylating different proteins, including exocytosis proteins [34,35]. Yamagata with coauthors showed that electroconvulsive seizure is accompanied by the phosphorylation of synapsin I at Ser 62/67, which are ERK1/2 specific sites [22]. It is

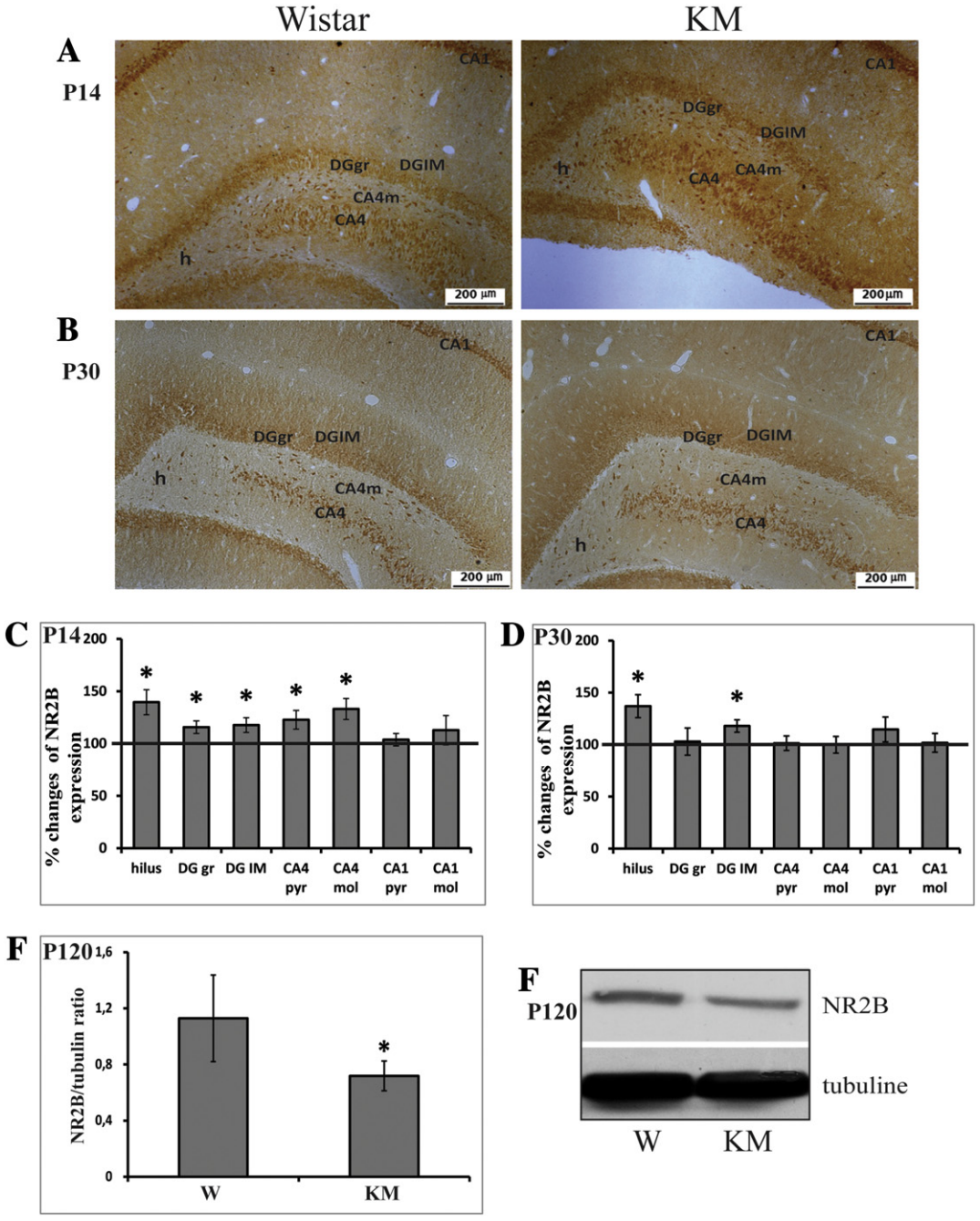

Fig. 5. Analysis of NR2B expression. **A, B** – Representative images of NR2B staining of the hippocampus of Wistar and KM rats at P14 (**A**) and P30 (**B**). DG IM – inner zone of the dentate gyrus molecular layer; DGgr – granular layer of the dentate gyrus; CA4m – molecular layer of CA4; CA4 – pyramidal layer of CA4; h – the hilus, CA1 – CA1 layer **C, D** – analysis of NR2B showed increasing of immunostaining in the hilus, dentate gyrus, and CA4 layer of KM rats at P14 (**C**), and in the hilus and inner zone of the dentate gyrus molecular layer at P30 (**D**) in comparison with control Wistar rats. Optical density of NR2B staining was estimated for each selected zone of the hippocampus of Wistar rats of corresponding ages and taken as 100%. Data are shown as % ± SE (in %). * $p < 0.05$ KM vs Wistar for each studied zone. **E, F** – Western blot analysis demonstrated significant decreasing of NR2B expression in the hippocampus of KM rats at P120. Data are expressed as mean ± SE. * $p < 0.05$.

known that synapsins play significant role in the development of epilepsy. In human, mutation of synapsin I is associated with family epilepsy [20]. Synapsin I, II, and III triple knockout mice are characterized spontaneous seizures that start to express only in adult animals [20, 21]. Synapsin triple knockout initiates "secondary adaptations in synaptic properties" during postnatal development that results in rearrangement of excitatory and inhibitory neurotransmission and onset of epilepsy in adulthood [21].

Based on these data, we hypothesized that in young KM rats an increase of ERK1/2 activity should be accompanied by an increase of synapsin I phosphorylation at Ser62/67. However, the phosphorylation of synapsin I in the hippocampus at P14 and P30 in KM rats was significantly lower than in Wistar rats of the same ages. In opposite, at the later stages of postnatal development, at P60 and P120, synapsin I phosphorylation was significantly increased. It was demonstrated that kainite-induced seizures led to activation of ERK1/2 in the hippocampus, but at the same time phosphorylation of synapsin I at ERK1/2-dependent sites Ser62/67 was dramatically decreased [36]. These results indicate that phosphorylation of synapsin I is dynamically regulated by the balance between kinase and phosphatase activities, and may indicate irreversible pathological changes during epilepsy development [36].

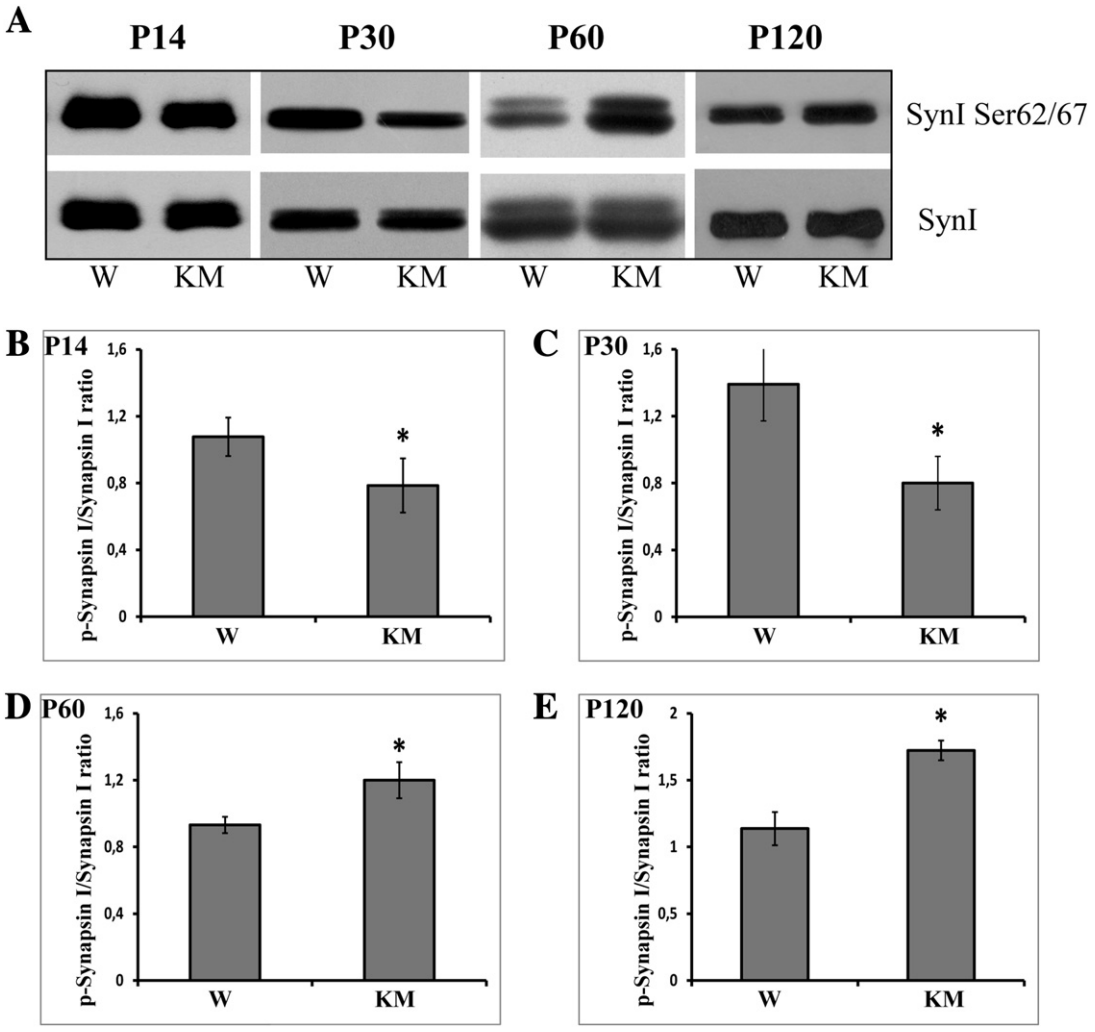

Fig. 6. Analysis of synapsin I phosphorylation at Ser62/67. A – immunoblot representative images of synapsin I of the hippocampus of Wistar (W) and KM rats at P14, P30 > P60, and P120. B–E – Western blot analysis demonstrated decreasing of phosphorylation of synapsin I in the hippocampus of KM rats at P14 and P30. At P60 and P120 phosphorylation of synapsin I at Ser62/67 was significantly increased. Data are expressed as mean ± SE. * $p < 0.05$.

5. Conclusion

Our data demonstrate a correlation between the development of seizure susceptibility in KM rats with age-dependent alterations in activity and expression of the proteins, ERK1/2, VGLUT, NR2B, and synapsin I, which tightly participate in the regulation of glutamatergic neurotransmission in the hippocampus. The most dramatic changes in the hippocampus of KM rats were observed during the first month of postnatal development. We supposed that overactivation of ERK1/2 at this period causes an increase of glutamatergic neurotransmission, which in turn may modify the development of the neuronal circuit as epileptic. Previously, we also demonstrated the same pathological aberrations at early postnatal development of the IC of KM rats [11,12]. Taken together, these data propose the first month of postnatal development in KM rats as a critical period for the formation of the brain epileptic network.

Acknowledgments

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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