

# Effect of altered extracellular magnesium concentration on the neuronal activity in different hippocampal regions of immature rats

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*Using acute hippocampal slice preparation dissected from immature rats, we investigated the effect of lowering  $Mg^{2+}$  concentration in the extracellular solution on the probability to evoke epileptiform activity in different regions of hippocampus. Electrophysiological recordings revealed different patterns of such activity in hippocampal CA1 region. In agreement with studies on the adult rat hippocampus probability to induce epileptiform activity by means of lowering  $Mg^{2+}$  concentration was increased in the ventral part of hippocampus (VH) compared to dorsal part (DH). We also found a significant increase in probability to induce ictal-like activity (ILE) in both hippocampal regions compared to results obtained in adult rats with a higher rate of ILE incidence in VH (90 %) compared to DH (50 %). Our experiments with cuts of hippocampal fiber pathways showed that in VH, ILE originated in CA1 region and propagated to CA3 region and dentate gyrus, while interictal-like activity originated in CA3 region. Possible mechanisms of the region specificity of the development of epileptiform activity in the immature hippocampus discussed. Key words: epileptiform activity; dorsal and ventral hippocampus; immature rat.*

## INTRODUCTION

Temporal lobe epilepsy (TLE) is the most common type of partial epilepsy [1]. TLE is associated with a high mortality rate and various psychiatric and behavioral comorbidities [2]. The histopathological studies suggest that different temporal lobe regions, including the hippocampus, are frequently damaged in human drug-refractory TLE [3]. It was shown that the surgical removal of hippocampus results in a decrease or complete cessation of seizures [3]. These data reveal a crucial role of this structure in pathophysiology of TLE. So understanding the mechanisms of generation and propagation of seizure activity in the hippocampus is of key importance in the antiepileptic drug discovery.

Although in the majority of epilepsy research studies the hippocampus is regarded as a unitary structure, there are a lot of evidences suggesting that separate parts of hippocampus

have substantial difference in molecular, cellular and histological organization and, as a result, play distinctive functional roles [4]. Regional differences in susceptibility to seizure generation as well as sensitivity to seizure-induced damage was also reported when studied in the adult animals [5–7]. It was shown that ventral hippocampus (VH) is more vulnerable to the abnormal synchronization and dorsal part of hippocampus (DH) is more resistant to seizure-induced injury and neuronal death than VH [5,6,8,9]. The difference in seizure susceptibility of DH and VH could be at least partly explained by the difference in the receptor expression in different parts of hippocampus [8,10].

It is well established that the immature brain is more susceptible to seizures compared to the adult brain. Among the factors, which may contribute to the increased vulnerability of immature brain to seizure generation, are developmental changes in the expression and

molecular composition of glutamate receptors and maturation of functional properties of GABA<sub>A</sub> receptors. We hypothesized that neuronal network excitability in VH and DH in the immature brain may differ from that one reported in the mature brain. To test this hypothesis in the present study we examined the regional differences in susceptibility to seizure generation between the VH and DH of immature rats. Using an acute hippocampal slice preparation dissected from rats at postnatal ages 12-14, we investigated the effect of lowering Mg<sup>2+</sup> concentrations in the extracellular solution on the probability to evoke seizure-like activity, its delay time and patterns of discharges in different parts of hippocampus.

## METHODS

All experimental procedures were performed in accordance with the guidelines set by the National Institute of Health for the humane treatment of laboratory animals and approved by the Animal Care Committees of Bogomoletz Institute of Physiology. Wistar rats (postnatal day 12-14) were deeply anesthetized using sevoflurane, decapitated, and both hippocampi were dissected out from the brain immersed in cold (2-4°C) carboxygenated (95% O<sub>2</sub> and 5% CO<sub>2</sub>) artificial cerebrospinal fluid (ACSF) of the following composition (mmol/l): NaCl 125, KCl 3.5, CaCl<sub>2</sub> 2.0, MgCl<sub>2</sub> 1.3, NaHCO<sub>3</sub> 24, NaH<sub>2</sub>PO<sub>4</sub> 1.25 and glucose 11 (pH 7.35). Hippocampal slices (400 μm) were prepared using a vibroslicer and then held submerged in oxygenated ACSF at room temperature (22-24°C) for at least 1.5 hours before the transfer to the recording chamber.

For experiments the 2<sup>d</sup> to 4<sup>th</sup> slices from dorsal and ventral ends of hippocampus (DH and VH) were used. Slices were placed in the recording chamber and perfused with oxygenated ACSF (2 ml/min, 30°C). All experiments were started 10-15 min after transferring slices into recording chamber to allow equilibration of the temperature inside the slice. Extracellular

field potential recordings were obtained from pyramidal CA1, CA3 regions and dentate gyrus (DG) using glass microelectrodes filled with ACSF (1-2 MΩ). The signals were filtered between 0.1 Hz and 1 kHz using differential amplifier (A-M Systems 1700, Sequim, WA, USA). Epileptiform activity (EA) was induced by application of modified ACSF of the following composition (mmol/l): NaCl 125, KCl 3.5, CaCl<sub>2</sub> 2.0, NaHCO<sub>3</sub> 24, NaH<sub>2</sub>PO<sub>4</sub> 1.25 and glucose 11 (pH 7.35) (Mg<sup>2+</sup>-free solution). We did not observed EA in any slices before the application of pro-epileptic solution.

Data were analyzed using Origin 7.5 (Microcal Software, Northampton, MA, USA) software. The proportion of slices with and without EA was compared using the Fisher's exact test. The difference between means was compared using Student's t-test.

## RESULTS AND DISCUSSION

Perfusion of hippocampal slices with Mg<sup>2+</sup>-free solution evoke EA. Field potential recordings from hippocampal CA1 layer were used to compare EA from DH and VH slices. Two patterns of EA induced by Mg<sup>2+</sup>-free solution were observed in both DH and VH slices: ictal-like events (ILE) and interictal-like epileptiform discharges (IED) (Fig. 1A, B). IED had spontaneous nature with changing frequency throughout the application of Mg<sup>2+</sup>-free solution (data not shown). ILE were characterized by the similar frequency and time between bursts in precise slice (data not shown). Extracellular recordings showed that 65 % of VH (n = 20) slices generated EA under Mg<sup>2+</sup>-free conditions, compared to 28.6 % of DH (n = 21) slices (P = 0.05, Fig. 1C). Data obtained for the VH slices showed a higher rate of ILE incidence (90 %) compared to 50 % in DH slices while no regional difference was observed in occurrence of IED (60 % in VH slices vs 66.7 % in DH slices, Fig. 1D).

The onset of EA in VH (10 ± 4 min) was faster than in DH (23 ± 10 min) (P < 0.01).

Restoring normal ACSF after one hour of exposure of slices to  $Mg^{2+}$ -free solution resulted in the complete suppression of EA in all DH and VH slices. Ictal activity had a varying frequency between slices from 4 to 10 Hz and ictal bursts occurred every 2-10 min with no difference between recordings from DH and VH slices. IED occurred with a low frequency from 0.01 to 0.09 Hz no difference were observed in both hippocampal regions.

Considering a higher probability of VH to generate ILE, we performed a series of fiber pathways cuts in VH to understand better an origin of ictal-like activity in CA1 region of hippocampus (Fig. 2). ILE was evoked by application of low  $Mg^{2+}$  solution in the intact VH slice and recording was made simultaneously

from CA1, CA3 and DG regions (Fig. 2A). After first cut through Shaffer collaterals original ILE disappeared in all regions and new patterns of activity developed: ILE in CA1 and IED in CA3 regions (Fig. 2B). DG expressed both patterns of activity simultaneously with CA1 and CA3. Isolated CA1 continued to generate the same pattern of ILE (Fig. 2C). Frequency of ILE in CA1 region changed slightly from 5 to 4 Hz after the first cut while average time between bursts (6 min) remained unchanged. Cut through mossy fibers revealed that separated DG did not generate EA in a response to the application of low  $Mg^{2+}$  solution (Fig. 2D). These data indicate that in VH, ILE originates in CA1 region and propagates to CA3 and DG, where it seems to have a suppressive action on IED. IED origina-

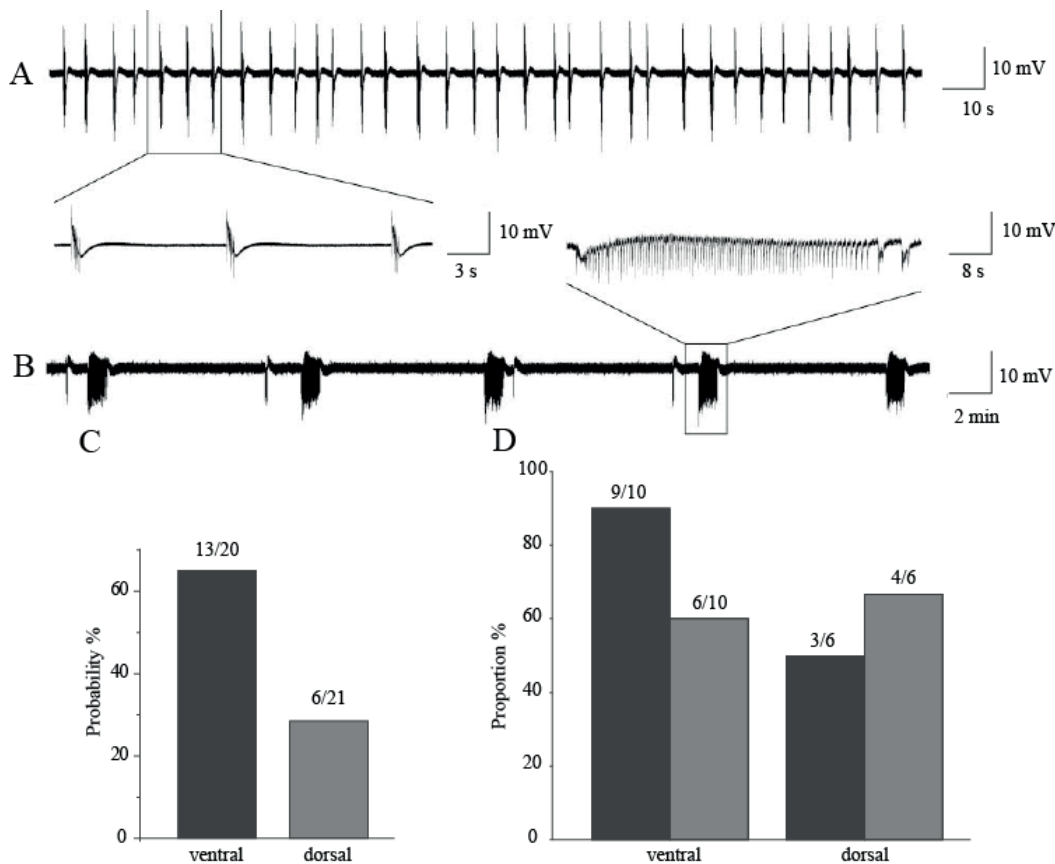


Fig. 1. Epileptiform activity (EA) induced by  $Mg^{2+}$ -free solution in VH and DH slices. Example of evoked interictal epileptiform discharges (IED) in VH slice (A) and ictal-like events (ILE) generated in DH slice (B) with parts of traces on an expanded time scale. C. Probability of EA induction in VH (1) and DH (2). D. Proportion of ILE (black) and IED (grey) evoked in VH and DH. Number of slices used for experiments are indicated above the each column

tes in CA3 region and invades to DG.

Although regional specificity of hippocampal formation to generate EA has been studied extensively, surprisingly little is known about seizure susceptibility of different regions of hippocampus in the immature rats. Our data

show that the VH is more vulnerable to seizure generation compared to its dorsal counterpart in immature animals. These data are in agreement with previous studies conducted on the adult animals [7]. In our study the propensity to generate ILE in both hippocampal regions of

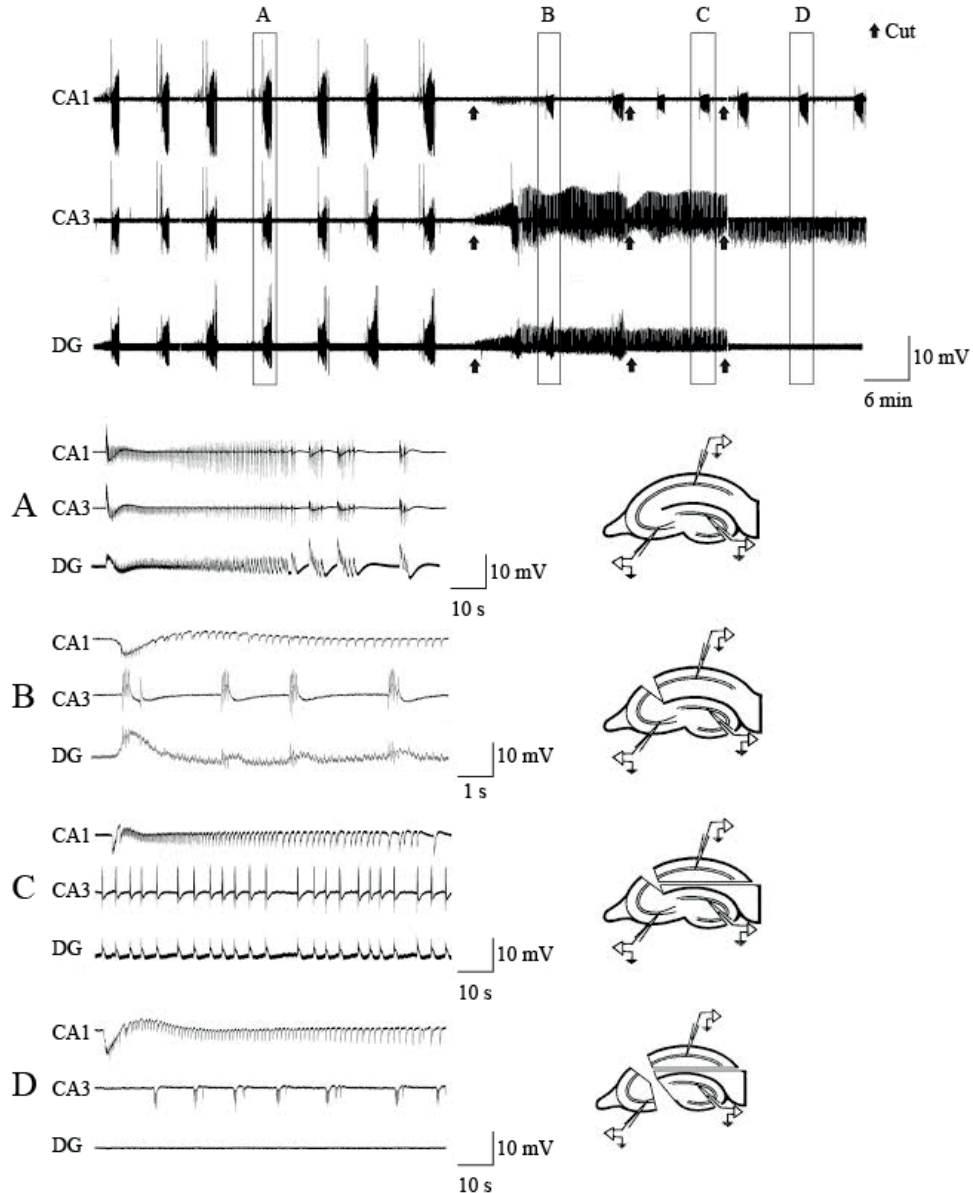


Fig. 2. Effects of fiber pathways cuts in VH on the propagation of ILE. The top panel shows simultaneous extracellular recordings from hippocampal CA1, CA3 and DG regions with arrows specifying of cut locations. Representative recording of ILE induced by  $Mg^{2+}$ -free solution in the intact hippocampal slice (A) and in the slice with a cut through the Schaffer collaterals (B). Note that in the latter case ILE occurred in CA1 while EA of CA3 was changed to IED. At the same time, DG expressed both activities together. C. When CA1 area had been severed from the rest of the slice, IED remained coupled with CA3 region and DG. D. The separated DG area did not generate any EA

immature animals was higher compared to one previously obtained for adult rats [7]. Also VH demonstrate a higher rate of ILE incidence compared to DH. To induce EA in our study we used a low  $Mg^{2+}$  solution. Lowering  $Mg^{2+}$  in an extracellular solution has been shown to create a hyperexcitable environment conducive for seizure generation by decreasing membrane surface charge screening, lowering  $Mg^{2+}$ -sensitive  $Ca^{2+}$  channel block, and decreasing the  $Mg^{2+}$  blockade of N-methyl-D-aspartate (NMDA) receptors [11–13]. Although, lower levels of mRNA and protein expression for NR2A, NR2B subunits was reported in CA1 region of VH, compare to CA1 region of DH [10], it was suggested that in the low  $Mg^{2+}$  model of ictogenesis the contribution of NMDA receptors was greater in VH than in DH in adults rats [5]. This discrepancy could be at least partly explained by the difference in the ratio of NMDA receptor subunits NR2A and NR2B in VH and DH [10], with NR2A subunit prevailing in the DH. The decreased ratio of NR2A/NR2B in VH compared to DH implies the prolongation of the decay time of the NMDAR-mediated synaptic current and a weaker sensitivity of the NMDA receptor channel to  $Mg^{2+}$  [14]. It is known that in the immature brain, a ratio of NR2A/NR2B in hippocampal neurons is increased compared to one in adult rats [14]. We suggest that developmental changes in the NMDA receptors subunit composition can at least partly explain the increased probability to ILE induction in the immature hippocampus. Increased expression of AMPA receptor and underdeveloped inhibitory synaptic system in the immature brain could be among other contributing factors [15].

Previous studies indicate that the origin of seizure-like activity onset may depend on the postnatal age [16]. Our experiments reveal that both CA1 and CA3 regions in VH are able to produce the EA in response to the  $Mg^{2+}$ -free solution. In the intact slice ILE occurs simultaneously in both CA1 and CA3 regions. However, our experiments with cuts of fiber pathways show that, when separated, CA1

and CA3 regions express different patterns of activity. ILE originates in CA1 region and propagates to CA3 and DG, while IED originates in CA3 region and invade to DG. Using  $Mg^{2+}$ -free model of seizure-like activity in 22-32 days old rats Lewis et al. [17] have obtained very similar results. They showed that low  $Mg^{2+}$ -induced interictal-like activity originates in an area CA2-3 and propagates to area CA1, and CA1 did not generate the interictal-like activity when isolated from CA2-3, but produced ILE more readily than CA2-3 [17]. A cellular mechanism of development of ictal-like activity in CA1 region is not completely understood. It has been recently reported that different forms of  $Mg^{2+}$ -free induced EA in CA1 region of hippocampus have a different sensitivity to NMDA receptor antagonists [18]. ILE can be blocked by application of D-APV. In contrast, NMDA antagonist does not affect interictal activity (late recurrent interictal discharges) [18]. Also it has been reported that there is a greater expression of NR2B in the CA1 region compare to the CA3 or DG [20]. As NR2B subunit has a greater affinity for glutamate, also it is more selective to  $Ca^{2+}$  entry, and has slower desensitization than NR2A subunit [20], we hypothesize that the increased expression of this subunit of NMDA receptors in CA1 neurons, at least partly, may contribute to the development of ILE in this region of hippocampus.

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#### **ВПЛИВ ЗМІН ПОЗАКЛІТИННОЇ КОНЦЕНТРАЦІЇ МАГНІЮ НА НЕЙРОННУ АКТИВНІСТЬ У РІЗНИХ ДІЛЯНКАХ ГІПОКАМПА МОЛОДИХ ЩУРІВ**

Досліджували вплив зниження концентрації  $Mg^{2+}$  у позаклітинному розчині на ймовірність ініціації епілептиформної активності в різних зонах гіпокампа щура. Електрофізіологічні дослідження показують різний характер епілептиформних явищ в зоні CA1 гіпокампа. Відповідно до експериментальних результатів, отриманих на моделі дорослих щурів, у дослідженнях на молодих щурах вірогідність виникнення епілептиформної активності в вентральній частині гіпокампа була вище, ніж у дорсальній. Також було показано істотне підвищення



ймовірності ініціації іктальноподібної активності в обох зонах гіпокампа щодо значень у дорослих щурів. При цьому вірогідність виникнення епілептиформної активності у вентральних зрізах була вище порівняно з дорсальними зрізами на 40 %. Наші експерименти з розрізанням провідних шляхів показують, що іктальноподібна активність у вентральних зрізах виникає в зоні CA1 і поширюється в зону CA3 і зубчасту звивину, водночас інтеріктальна активність бере початок в зоні CA3. Механізми генерації епілептиформної активності в різних зонах гіпокампа молодих щурів потребують подальших досліджень.

Ключові слова: епілептиформна активність; дорсальна і вентральна ділянки гіпокампа; молоді щури.

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### **ВЛИЯНИЕ ИЗМЕНЕНИЙ ВНЕКЛЕТОЧНОЙ КОНЦЕНТРАЦИИ МАГНИЯ НА НЕЙРОННУЮ АКТИВНОСТЬ В РАЗЛИЧНЫХ УЧАСТКАХ ГИППОКАМПА МОЛОДЫХ КРЫС**

Исследовали влияние снижения концентрации  $Mg^{2+}$  во внеклеточной растворе на вероятность инициации эпилептоподобной активности в различных зонах гиппокампа крысы. Электрофизиологические исследования показывают различный характер эпилептоформных явлений в зоне CA1 гиппокампа. Согласно экспериментальным результатам, полученным на модели взрослых крыс, вероятность возникновения эпилептоподобной активности в вентральной части гиппокампа выше, чем в дорсальной. Также было показано существенное повышение вероятности возникновения иктальноподобной активности в обеих зонах гиппокампа по сравнению с значениями, полученными на взрослых крысах. При этом вероятности инициации эпилептоподобной активности в вентральных срезах была выше относительно дорсальных срезов на 40%. Наши эксперименты с перерезанием проводящих путей показывают, что иктальноподобная активность в вентральных срезах возникает в CA1 зоне и распространяется в зону CA3 и зубчатую извилину, в то время как интеріктальная активність берег начало в зоне CA3. Механізми виникнення епілептиформної активності в різних зонах гіпокампа молодих щурів потребують подальших досліджень.

Ключевые слова: эпилептиформная активність; дорсальные и вентральные отделы гиппокампа; молодые крысы.

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