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**Short communication**

## Effect of neuraminidase treatment on persistent epileptiform activity in the rat hippocampus

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**Abstract:**

Negatively charged sialic acid residues located close to pores of voltage-gated sodium channels substantially influence their gating properties. The *in vitro* low Mg<sup>2+</sup> seizure model is used to emulate difficult-to-treat status epilepticus. Using this model on cultured hippocampal slices, we examined the effectiveness of desialylation in reducing persistent seizure-like activity. We show that desialylation in cultured hippocampal slices effectively suppresses seizure-like activity induced by low Mg<sup>2+</sup>. These findings suggest that targeting negatively charged sialic acids may be an effective strategy to treat status epilepticus.

**Key words:**

neuraminidase, surface charge, seizures, hippocampus

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**Abbreviations:** ACSF – artificial cerebrospinal fluid, AP – action potential, NEU – neuraminidase, NMDA – N-methyl-D-aspartate, SLA – seizure-like activity

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### Introduction

Epilepsy is a serious neurological disorder characterized by recurrent, unprovoked seizures [8]. Almost 1% of humans suffer from epilepsy during their lifetime, most commonly children and people over the age of 65 years. While in many cases epilepsy is a mild condition with a favorable outcome, epilepsy can be also a life threatening disorder if the seizure is prolonged (status epilepticus) [15, 18, 21]. Because of

the many causes and types of epilepsy, treatment of this disorder can be quite difficult [8, 12]. Over the past several decades, over 20 new antiepileptic drugs have been approved [4, 14, 16]. Many of the currently available antiepileptic drugs have adverse side effects, such as alterations in cognition and behavior that impair the quality of patients' lives, thus limiting their use [4, 7, 10]. In addition, nearly one third of patients are refractory to antiepileptic drugs and have continued seizures despite appropriate dosing [13]. Thus, searching for novel therapeutic strategies remains a priority in drug development. One of the promising new ways to regulate abnormal neuronal excitability is to alter sodium channel activation through the modification of the negative surface charge of the cellular membrane from polysialic acid.

In a previous report, we showed that desialylation of hippocampal slices with neuraminidase (NEU), a specific enzyme that cleaves negatively charged sialic acid residues from the cellular membrane, altered the action potential threshold, delayed the onset of epileptiform activity and reduced the population spike frequency of the 10 mM [K<sup>+</sup>]<sub>o</sub>/low [Mg<sup>2+</sup>]<sub>o</sub> model of seizure in the CA3 zone of rat hippocampus [9]. Here, we examined if desialylation is effective against low Mg<sup>2+</sup>-induced epileptiform activity in cultured rat hippocampal slices. The self-sustained epileptiform activity in this *in vitro* model resembles human status epilepticus [1, 5].

## Materials and Methods

### Animals

Albino Wistar rat pups were housed with their dams under a constant 12/12 h light/dark cycle at 22–24°C in the institutional animal facility and removed from the litter no more than half an hour before sacrifice. All procedures used in this study were approved by the Animal Care Committee of Bogomoletz Institute of Physiology and conform to the Guidelines of the National Institutes of Health on the care and use of animals.

### Chemicals

All chemicals were purchased from Sigma (St. Louis, MO, USA).

### Organotypic culture

Organotypic hippocampal slice cultures were prepared from 7-day-old rats using the method of Stoppini and co-authors [22], with modifications. Rat pups were anesthetized with isoflurane and decapitated; the brains were aseptically removed and placed into ice-cold dissection medium consisting 50% Minimum Essential Medium, 10 mM Tris, 2 mM NaHCO<sub>3</sub>, 12.5 mM HEPES, 15 mM glucose, 25% Hanks' balanced salt solution (HBSS), 100 U/ml penicillin and 100 µg/ml streptomycin, pH 7.3. The hippocampi were rapidly isolated, and transversal slices 350 µm in thickness were cut from the middle part of each hippocampus using a tissue chopper (McIlwain, Surrey, UK). The slices were transferred to sterile porous

membrane inserts (Millicell, Bedford, MA, USA), which were placed in a 6-well plate containing 1 ml culture medium/well (50% of MEM, 25% horse serum (HS), 25% HBSS, 5 mM Tris, 2 mM NaHCO<sub>3</sub>, 12.5 mM HEPES, 15 mM glucose, 100 U/ml penicillin and 100 µg/ml streptomycin, pH 7.2). Four slices were placed on each insert and cultivated at 35°C at air atmosphere with 5% CO<sub>2</sub>. The culture medium was changed the day after preparation of the slices and twice a week thereafter. All *in vitro* experiments with organotypic hippocampal slice cultures were performed at 12–14 days.

### Electrophysiological recordings

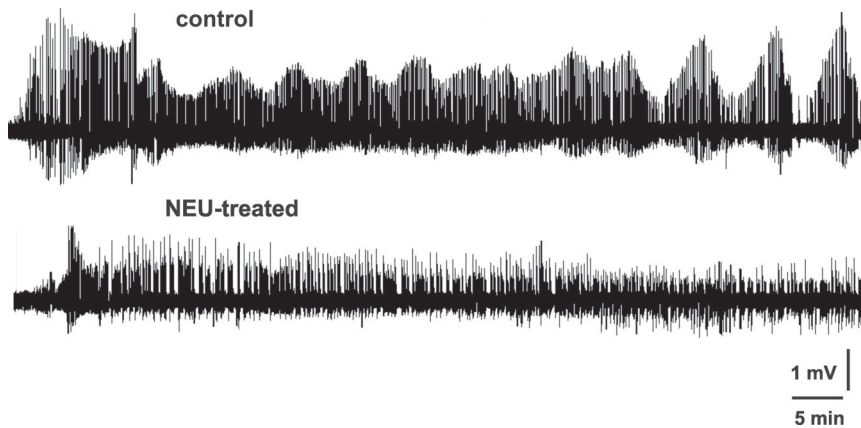
Extracellular field potential recordings were made from the pyramidal CA1 layer of hippocampus at 12–14 days *in vitro* [18]. For recordings, slices were transferred to a submersion-type thermostatic chamber (Warner Instrument Corp., Hamden, CT, USA) mounted on an Olympus BX51WI (Oberkochen, Germany) microscope. The slices were continuously superfused at a rate of 2–4 ml/min at 30–32°C with oxygenated (95% O<sub>2</sub>–5% CO<sub>2</sub>) artificial cerebrospinal fluid (ACSF) consisting of the following: 125 mM NaCl, 3.5 mM KCl, 2 mM CaCl<sub>2</sub>, 1.3 mM MgCl<sub>2</sub>, 24 mM NaHCO<sub>3</sub>, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub> and 11 mM glucose. To induce seizure-like activity (SLA) in the hippocampus, we omitted Mg<sup>2+</sup> from the extracellular solution. To increase neuronal activity in the low Mg<sup>2+</sup> model, we also reduced the amount of extracellular calcium ([Ca<sup>2+</sup>]<sub>o</sub>) in ACSF to 1 mM [3]. Extracellular recordings were made using a patch-pipette filled with ACSF solution. Pipette resistances ranged from 1–3 MΩ. Field potential recordings were made using a two-channel AC differential amplifier (A-M Systems, Carlsborg, USA) (bandpass 0.1 Hz–1 kHz; 100). Recordings were digitized at 10 kHz using an analogue-to-digital converter (NI PCI-6221, National Instruments, Austin, TX, USA) and stored on a computer using the WinWCP program (Strathclyde Electrophysiology Software, University of Strathclyde, Glasgow, UK). Off-line analysis of the recordings was performed using Mini Analysis software (version 5.5; Synaptosoft, Decatur, GA, USA) and Origin 7.0 (OriginLab, Northampton, MA, USA). SLA was defined as brief (80–200 ms), high amplitude spikes in the EEG. For each experimental group, no more than two slices per each rat were used. Statistical analysis was performed using Statistica (version 6.0, Tulsa,

OK, USA) and Origin (OriginLab, version 7.0, Northampton, MA, USA) software using the unpaired Mann-Whitney test and Kolmogorov-Smirnov test. A  $p$  value less than 0.05 was considered significant. Results were expressed as the mean  $\pm$  SEM;  $n$  is the number of recordings.

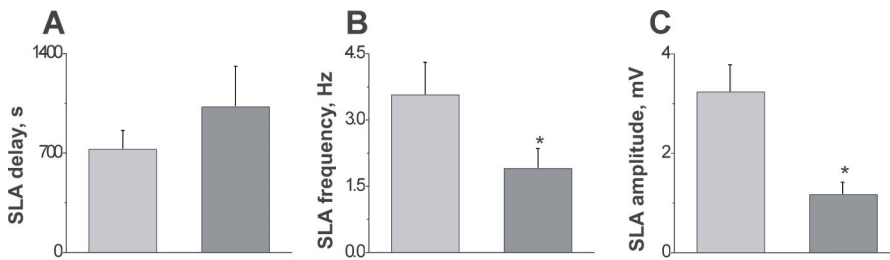
## Results

Extracellular field potential recordings were made from the pyramidal CA1 layer at 12–14 days *in vitro*. Hippocampal slices were incubated in culture me-

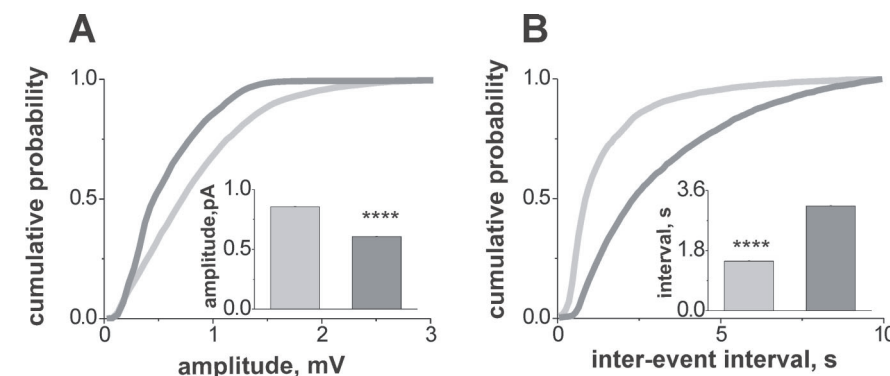
dium with and without NEU from *Vibrio cholerae* (Sigma) at a concentration of 0.08 U/ml for 20–24 h at 35°C before recordings. SLA was consistently generated in all slices omitting  $Mg^{2+}$  from the ACSF (Fig. 1). This activity persisted in both groups as long as the low  $Mg^{2+}$  ACSF was maintained. In NEU-treated slices, we observed non-significant increases in the delay of SLA initiation compared to the controls (delay: 725.5  $\pm$  126.1 s ( $n = 10$ ) for the controls and 1025  $\pm$  283.7 s ( $n = 11$ ) for the NEU-treated group,  $p > 0.05$ , Mann-Whitney test, Fig. 2A). We also found a significant decrease in the frequency of SLA, from 3.6  $\pm$  0.7 Hz ( $n = 10$ ) in the controls to 1.9  $\pm$  0.4 Hz in NEU-treated slices ( $n = 11$ ) ( $p < 0.05$ , Mann-Whitney test, Fig. 2B), and decreases in the amplitude of SLA, from 3.2



**Fig. 1.** Effect of desialylation on epileptiform activity *in vitro*. Extracellular field potentials were recorded from the CA1 pyramidal cell layer of cultured hippocampal slices from P12–14 rats. Epileptiform activity was induced by omitting  $Mg^{2+}$  from the extracellular solution. Recordings were obtained from control (upper panel) and NEU-treated (lower panel) slices



**Fig. 2.** Effects of NEU pretreatment on different characteristics of low- $Mg^{2+}$ -induced synchronous oscillations in CA1 pyramidal layer. Summary plots show the delay (A), amplitude (B), and frequency (C) of SLA in the control (light grey) and NEU-pretreated (dark grey) slice. All values are the mean  $\pm$  SEM, \*  $p < 0.05$  vs. control, Mann-Whitney U test



**Fig. 3.** Effect of NEU treatment on hippocampal field activity recorded in the CA1 pyramidal layer in a low- $Mg^{2+}$  extracellular solution. Representative cumulative probability plots and bar graphs (the mean  $\pm$  SE) show a decrease in amplitude (A) and an increase in the inter-event interval (B) of field potentials recorded in NEU-treated slices (dark grey) vs. control slices (light grey). All values are the mean  $\pm$  SEM, \*\*\*\*  $p < 0.0001$  vs. control, Kolmogorov-Smirnov test

$\pm 0.5$  mV ( $n = 10$ ) for the controls to  $1.2 \pm 0.2$  mV ( $n = 11$ ) for NEU-treated slices ( $p < 0.05$ , Mann-Whitney test, Fig. 2C). Comparative analyses of all event cumulative data revealed highly significant decreases in amplitude and inter-event intervals of the field potentials recorded during the 10–20 min application of the low-Mg<sup>2+</sup> solution in NEU-treated slices compared to controls ( $p < 0.0001$ , Kolmogorov-Smirnov test, Fig. 3).

## Discussion

The present study shows that although NEU treatment did not stop the SLA induced by lowering Mg<sup>2+</sup> levels in the extracellular solution, it considerably suppressed persistent epileptic activity in this model. The low-Mg<sup>2+</sup> model of seizures is a classical model, which is often used to test antiepileptic drugs [1]. The generation of seizures in this model depends on the facilitated activation of N-methyl-D-aspartate (NMDA) receptors. In addition, omitting positively charged Mg<sup>2+</sup> ions from the extracellular solution decreases the screening effect on the surface charge, thereby increasing its influence on voltage-sensitive channel mechanisms. It was shown that treatment with NEU did not affect NMDA receptors; therefore, we excluded the possibility that the antiepileptic action of NEU is related to its action on NMDA receptors [6]. NEU is a specific enzyme, which cleaves sialic acid moieties from the extracellular membrane [19]. Polysialic acids are large negatively charged molecules, which, along with other hydrophilic residues such as charged amino acids, lipids, and phosphates, create a surface charge on the cellular membrane. It has been shown that sodium channels, which play a vital role in cellular excitability, are heavily glycosylated with high levels of sialic acid residues on the extracellular region [17]. Three independent methods to reduce channel sialylation showed very similar depolarizing shifts in the gating of sodium channels [2, 9]. The depolarizing shift in sodium current activation implies that a stronger depolarization is needed to activate sodium channels, thereby increasing the action potential (AP) threshold. We confirmed this assumption by examining the effect of desialylation on the AP threshold in CA3 pyramidal cells [9]. The AP threshold recorded from NEU-treated cells was 4 mV lower than in control slices. In the biological systems,

such a small shift in the AP threshold by itself is sufficient to cause substantial changes in neuronal excitability [11]. We suggest that the most likely mechanism responsible for the anti-seizure effect of NEU treatment in the low-magnesium model of seizures is secondary to an effect on gating of sodium channels due to the influence of desialylation on the electric field in the region of the channel voltage sensor.

In conclusion, in the present study, we show that decreasing the number of negatively charged sialic acid moieties on the extracellular membrane suppresses low-magnesium-induced seizures in the model of seizures that is resistant to conventional anticonvulsant drugs. Although the method that we used to manipulate the amount of extracellular negatively charged compounds has limitations, because desialylation can affect various cellular functions besides channel activity, modulating surface charges opens new avenues for the development of novel anticonvulsant compounds.

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