# EPSP-Spike coupling due to endogenous neuraminidase inhibition

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**Abstract:** Endogenous neuraminidase (NEU) is an essential factor that regulates the abundance of negatively charged sialic acid residues on the outer cell membrane. Earlier research on the effects of NEU blockage on hippocampal plasticity demonstrated a significant decrease in long-term potentiation (LTP) in CA3-to-CA1 network. Furthermore, deficiency of NEU activity facilitated short-term depression at Schaffer collateral inputs to CA1 layer. However, presynaptic release probability, evaluated through paired-pulse ratios, was not affected. Although there is clear evidence that LTP can be altered by blocking NEU, it is still unclear whether this modification is solely due to changes in synaptic efficacy or if intrinsic excitability, such as the EPSP-spike (E/S) coupling, also contributes to the effect. We have addressed this question by examining the changes in E/S curves from rat hippocampal CA1 region due to NEU inhibition before and after LTP induction. We show that downregulation of NEU activity leads to the E/S depression, indicating a decreased ability of EPSPs to elicit action potentials as a result of changes in activity-dependent hippocampal plasticity.

**Keywords:** neuraminidase blocker, synaptic plasticity, EPSP-spike coupling, hippocampus.

## 1. Introduction

Polysialic acid (PSA) is a linear homopolymer of sialic acid residues on the cell membrane, that modify the alpha subunit of sodium channels and the neural cell adhesion molecule (NCAM) (Zuber et al., 1992). By controlling the negatively charged PSA population, neuraminidase (NEU) is involved in numerous physiological functions including activity-dependent synaptogenesis, cell differentiation, neurogenesis, migration, axonal sprouting, plasticity and neuronal excitability (Bonfanti and Theodosis, 2009; Rougon, 1993; Isaeva et al., 2011; Schauer, 2004). Enzymatic removal of PSA from sodium channels causes large depolarizing shift of voltage-gated sodium channel activation/inactivation and revealed strong reduction of neuronal excitability (Isaev et al., 2007). Several studies demonstrate that PSA cleavage from NCAM inhibited long-term potentiation (LTP) and long-term depression (LTD) in the hippocampal network (Becker et al., 1996; Muller et al., 1996). The NEU blocker N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (NADNA) has been shown to promote PSA accumulation in neuronal tissue (Isaeva et al., 2010). over sialylation by NADNA has the opposite effect on sodium channels, causing a powerful facilitation of neuronal excitability. An increase in neuronal synchronization, amplitude, and frequency of spontaneous recurrent oscillations in the CA3-CA1 synapses of the rat hippocampus was previously shown (Isaeva et al., 2010; Usami et al., 2008). However, downregulation of NEU suppresses induction and magnitude of LTP (Savotchenko et al., 2015; Minami et al., 2018). We hypothesize, that effect of NEU on synaptic plasticity could be explained by analyzing EPSP-spike (E/S) coupling, which reflects the relationship between synaptic and cellular events.

## 2. Materials and methods

All experimental procedures were performed in accordance with the requirements of the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes and approved by the Committee on Biomedical Ethics of the Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine.

### 2.1. Slice preparation and Electrophysiology

Hippocampal slices (400 µm) were obtained from postnatal day 19-21 rats according to the technique described previously (Kopanitsa et al., 2006). Slices were cut in an ice-cold oxygenated (95% O2-5% CO2) solution containing (in mM): NaCl 119, KCl 2.5, CaCl2 2.0, MgSO4 1.3, NaHCO3 26, NaH2PO4 1.2, and glucose 11 (pH 7.35). Slices recovered (2 hr) in fresh bubbled artificial cerebrospinal fluid (ACSF) at a room temperature prior to the beginning of the experiments.

Individual slices were transferred to a temperature-controlled recording chamber (22-24° C) and kept submerged under the continuous flow (2 ml/min) of oxygenated ACSF. Field potentials were elicited simultaneously in stratum radiatum and stratum pyramidale of CA1 hippocampus to measure field excitatory postsynaptic potentials (fEPSP) and population spikes (PS) respectively by stimulation of Schaffer collaterals using a concentric bipolar stimulating electrode (FHC Inc., Bowdoin, ME) with a flexible stimulus isolator (ISO-Flex, A.M.P. Instruments, Jerusalem, Israel). Recordings were performed using glass microelectrodes (3-4 MΩ) filled with ACSF and patch-clamp amplifier (PC501A, Warner Instruments Corp., Hamden, CT). Stimulating electrode were placed approximately 400 µm apart from each recording electrode on the slice surface.

E-S curves were obtained by the series of ascending-descending stimulations ranging from 100 to 500 µA, covering the extracellular potentials from subthreshold to supramaximal. Input/output (I/O) curves were generated before and 30 min after high-frequency tetanic stimulation (HFS) consisted of 100 pulses at frequency of 100 Hz. Recordings were digitized at 10 kHz and filtered at 3 kHz using an analogue-to-digital converter (National Instruments, Austin, TX) and stored on a computer using the WinWCP program (Strathclyde Electrophysiology Software, University of Strathclyde, Glasgow, UK).

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### 2.2. NADNA-pretreatment

Hippocampal slices were treated with NADNA during 2 hr at room temperature and then rinsed with ACSF before conducting recordings. According to our previous study (Isaev et al., 2007; Isaeva et al., 2010) we used NADNA in concentration of 500 µM purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.3. Statistical Analysis

Data were analyzed using Clamp fit (Axon Instruments, USA), Origin 8.5 (Origin Lab, Northampton, MA, USA) and GraphPad Prism 5 (GraphPad, LA Jolla, CA, USA) software. The Shapiro-Wilk test was used to examine the normality of the distribution. Data were compared using two-way repeated measures ANOVA and unpaired two-tailed Student`s t-test (with Welch`s correction). A P value lower than 0.05 was considered significant. Data were presented as Mean ± SEM.

## 3. Results

We first examined the alteration of synaptic efficacy in the CA1 stratum radiatum after neuraminidase application by measuring fEPSPs before and after HFS. Gradual increase in stimulation intensity from 100 to 500 µA resulted in an enhancement of fEPSP slope in the NADNA-pretreated group of slices (n = 10) 30 min after HFS compared to those before HFS without changes of control fEPSPs (n = 10) in both pre- and post-HFS conditions. Two-way repeated measures ANOVA revealed a significant influence of bath-application with NADNA on I/O curves of fEPSP slope before HFS ($p = 0.0037$), as well as after tetanic stimulation of the perforant pathway ($p = 0.0003$). Interaction between neuraminidase inhibition and stimulation intensity was also affected before ($p < 0.0001$; $F_{8, 131} = 7.77$) and after ($p < 0.0001$; $F_{8, 109} = 20.31$) tetanic stimulation. Bonferroni's multiple comparisons post-hoc test showed that the fEPSP slopes in NADNA-pretreated slices were significantly increased under stimulation intensity of 400 µA before the delivery of HFS ($p < 0.05$) and at stimulation intensities ranging from 250 to 500 µA ($p < 0.05$) 30 min after the delivery of HFS (Fig.1A).

Next, we investigated how increased synaptic efficacy affected pyramidal cell excitability, measured by pop-spike amplitude, which reflects pyramidal cell population firing activity. I/O curves of pop-spike amplitudes were measured simultaneously with fEPSP slopes via the same range of stimulation intensities. Analyzing data obtained by two-way repeated measures ANOVA with Bonferroni post-hoc test showed no effect of NEU blockade on pop-spike amplitude either before the delivery of HFS ($p = 0.2$) or 30 min after ($p = 0.9$). However, the effect of interaction between NADNA-treatment and stimulation intensity was significant both before HFS ($p < 0.0001$; $F\ 8, 101 = 5.39$) and 30 min after HFS ($p < 0.0001$; $F\ 8, 101 = 4.95$), which could represent a variable effect of NEU inhibition at different stimulation intensities (Fig.1B).

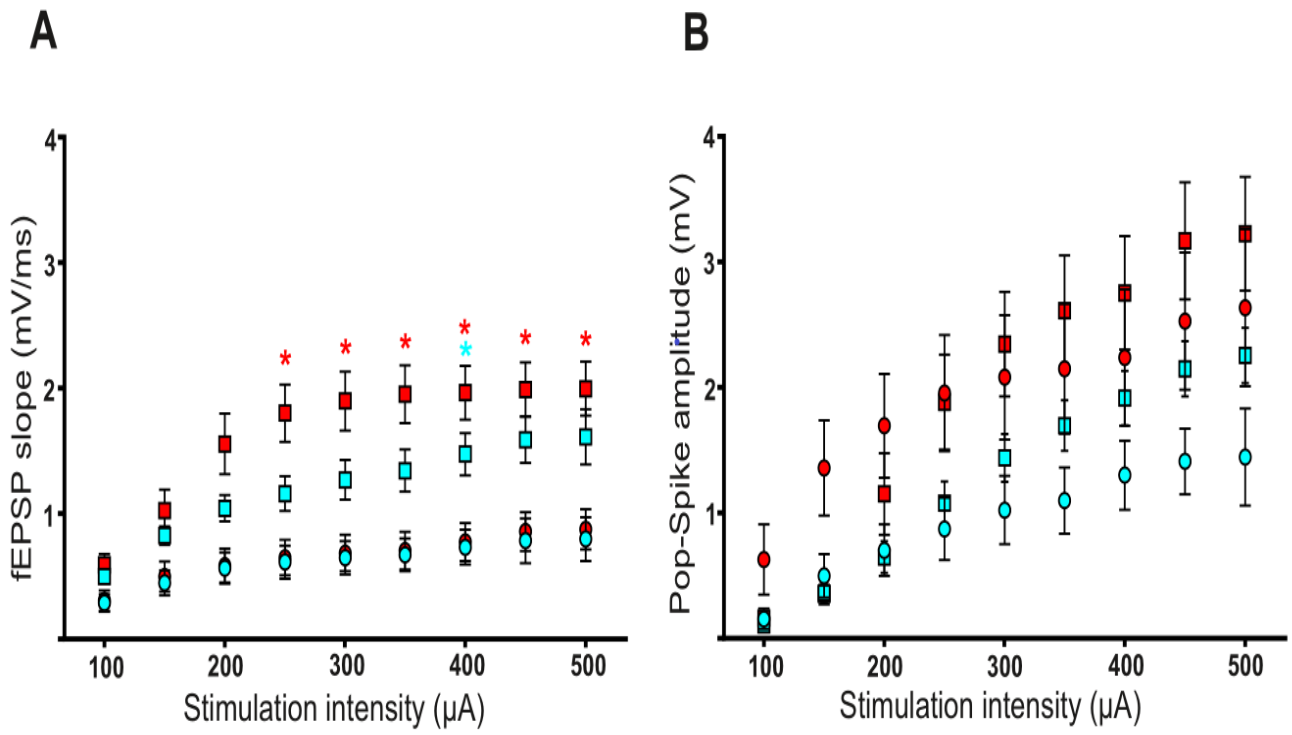


**Figure 1. Suppression of NEU activity altered excitatory synaptic transmission in the CA3-to-CA1 hippocampal synapses. I/O curves of (A) fEPSP and (B) population spike evoked by ascending stimulation intensities from 100 to 500 µA show an increase in the strength of CA3-to-CA1 synapses before (blue) and after HFS (red) in NADNA-pretreated slices (square) compared to controls (circle) without changes in pyramidal cell excitability. *P < 0.05. Data are presented as mean ± SEM.**

For subsequent investigation of interplay between synaptic efficacy and excitability, we study E/S coupling by plotting I/O curves, constructed before and 30 min after HFS. The fEPSP slopes and pop-spike amplitudes were plotted for each stimulation intensity. E/S curves of control slices ($n = 10$) were shifted left, indicating E/S potentiation. The analysis of E/S coupling after NEU blockage ($n = 10$) shows a rightward shift in the E/S curves, reflecting E/S depression. E/S curves for control and NADNA-treated slices deviated significantly along the x-axis, which we measured by examining the v50 values of the Boltzmann-fitted E/S plots for each slice before (unpaired Welch`s t-test, $p = 0.011$) and 30 min after ($p = 0.004$) HFS. On the assumption of enhancement in fEPSP slopes due to deficiency of NEU, we observed higher v50 values before (control: $0.54 \pm 0.1$ vs NADNA-treated slices: $1.13 \pm 0.2$ ($n = 10$)) and after (control: $0.62 \pm 0.1$; NADNA: $1.39 \pm 0.2$, Fig. 2B, inset) HFS.

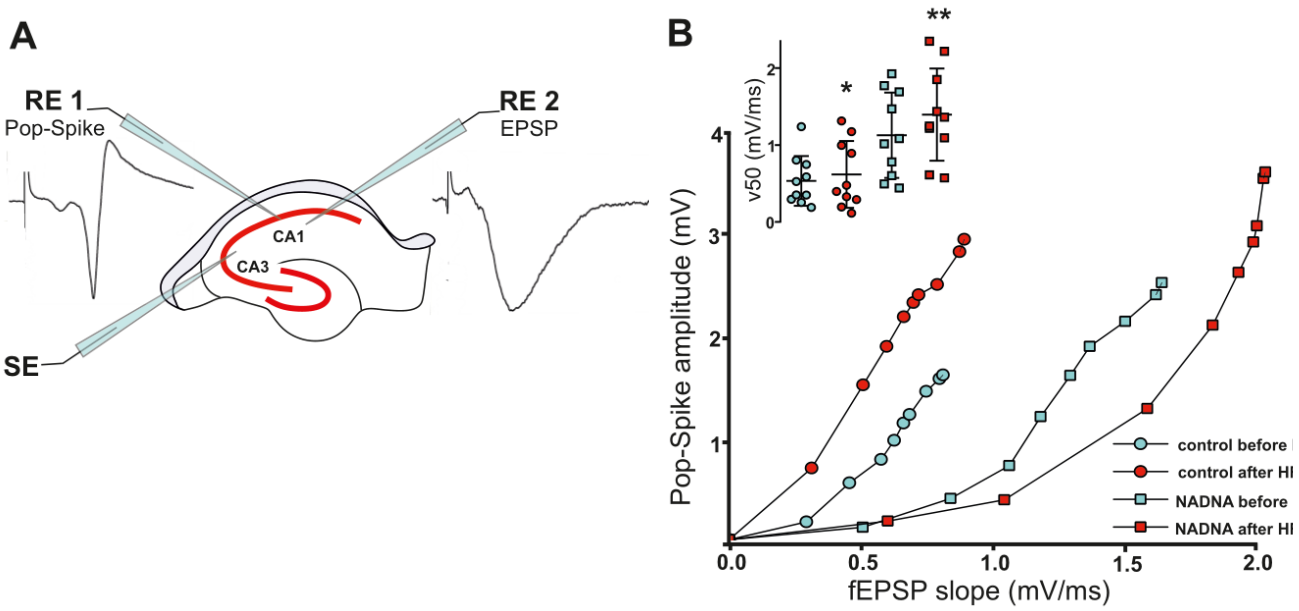


**Figure 2. The lack of NEU activity results in EPSP-Spike depression in CA3-to-CA1 hippocampal network. (A) The positioning of electrodes for recording. (B) Plotting the pop-spike amplitude as a function of fEPSP revealed a rightward shift in the E/S curves after HFS in NADNA-pretreated slices. Inset: mean values of fEPSP slope at the 0.5-spike probability point (v50 values of the Boltzman-fitted E/S curves). *P < 0.05, ** P < 0.01. Data are presented as mean ± SEM.**

## 4. Discussion

In the present study we investigated the E/S coupling following suppression of NEU activity. Using in vitro field potential recordings, we show that NEU deficiency leads to an increase in excitatory synaptic transmission at CA3-to-CA1 synapses, supporting the role of PSA in the formation and function of these synapses. These data are in agreement with previous study where the lack of NEU activity resulted in synaptogenesis and crucial accumulation of PSA in the extracellular space (Isaeva et al., 2010). On the other hand, the coupling of EPSP to the population spike is depressed due to NEU inhibition, reflecting a decreased capability of EPSPs to induce action potentials. Indeed, we have observed no changes in the firing activity of pyramidal cell population of NADNA-pretreated slices in response to fEPSP enhancement. E/S coupling may be altered through the modulation of spike threshold, which is mostly determined by voltage-gated ion channels (Xu et al., 2002), by changing the resting membrane potential (Mellor et al., 2003) or by local potentiation of synaptic transmission (Wang et al., 2003; Campanac and Debanne, 2008). Previously was shown that sialylation of the membrane surface affects gating properties of sodium channels and action potential threshold despite the absence of an effect on the resting membrane potential (Isaev et al., 2007). Such an impact of NEU inhibition reflects in enhancement of firing frequency and amplitude of spontaneous synchronous discharges (Isaeva et al., 2010), amplification of neuronal synchronization, inducement of population burst events in hippocampal network (Usami et al., 2008).

E/S depression might increase the filtering capability of pyramidal cells, so they unable to pass enlarged synaptic input and to serve a homeostatic function in the CA3-to-CA1 network. These results are in consistent with our previous study, where we show the reduction in LTP magnitude following blockage of NEU activity (Savotchenko et al., 2015), supporting an idea, that NADNA through modification of the neuronal activity produces LTP-like changes in the CA1 region of the hippocampus. It is well recognized, that LTP increases the possibility of cell firing generation in response to stimulation not just by enhancement of fEPSP strength, but although by enhancing the effectiveness of pyramidal neurons I/O function (Andersen et al., 1980). Our results demonstrate NADNA-induced enhancement in neuronal activity, which could lead to subsequent E/S depression due to saturation of synaptic responses. Previously was shown, that LTP is strongly influenced by the prior synaptic activity and could be inhibited in the synaptic pathways with the recent history of activation (Huang et al., 1992). In the experiments with synaptic depotentiation

of NADNA-pretreated slices was shown the restoration of LTP to the control values (Savotchenko et al., 2015), thus endorsing this hypothesis.

Since NEU inhibition affected both E/S coupling and LTP, this indicates the importance of strict balance for this enzyme.

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