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Post-synaptic *N*-methyl-D-aspartate signalling in hippocampal neurons of rat: spillover increases the impact of each spike in a short burst discharge[☆]

Sergei E. Grebenyuk, Natasha A. Lozovaya, Timur S. Tsintsadze, Oleg A. Krishtal*

Bogomoletz Institute of Physiology, 4, Bogomoletz St., 01024 Kiev, Ukraine

Abstract

High-frequency burst discharges in hippocampus typically consist of less than ten spikes fired at frequencies too high to be followed by a post-synaptic neuron. How significant are these numbers for synaptic signalling? We have measured the *N*-methyl-D-aspartate (NMDA) component of the excitatory post-synaptic current (EPSC_{NMDA}) in hippocampal CA1 neurons of rat after burst discharge of variable duration. The synaptic facilitation is accompanied by a slow-down of the EPSC_{NMDA} which develops on a spike-to-spike basis. Consequently the charge transferred by the after-burst EPSC_{NMDA} is increased with each spike. The phenomenon is most probably due to the spillover-mediated recruitment of extrasynaptic NMDA receptors. In terms of post-synaptic signalling it dramatically increases the impact of each spike in a short burst discharge.

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Natural spiking patterns in the brain are composed of relatively short periods of high-frequency activity. From hippocampus through cortex, high-frequency burst discharges are believed to be associated with information processing and memory consolidation. Specifically in hippocampus, they have two to nine action potentials fired at frequencies up to 200 Hz [7,16]. How significant is every spike within this small number? This question seems to be especially important since at high frequencies of firing the post-synaptic neuron cannot follow pre-synaptic burst discharge with an equivalent number of spikes. To address

this question, we analyzed *a*-amino-3-hydroxy-5 methylisoxazole-4-propionic acid (AMPA) and an *N*-methyl-D-aspartate (NMDA) component of the excitatory post-synaptic currents (EPSCs) in CA1 pyramidal neurons elicited by short trains of high-frequency stimulation of Schaffer collaterals.

This study was carried out on 21-day-old Wistar rats (WAG/GSto, Moscow, Russia). After rapid decapitation, rat brains were immediately transferred to a Petri dish with a chilled (4 °C) solution of the following composition: 120 mM NaCl, 5 mM KCl, 26 mM NaHCO₃, 2 mM MgCl₂ and 20 mM glucose. The solution was constantly bubbled with a 95% O₂/5% CO₂ gas mixture to maintain pH 7.4. During the pre-incubation and experiments, the slices (300–400 μm thick) were kept fully submerged in the extracellular solution: 135 mM NaCl, 5 mM KCl, 26 mM NaHCO₃, 1.5 mM CaCl₂, 1.5 mM MgCl₂, 20 mM glucose (pH 7.4, bubbled with 95% O₂/5% CO₂) at 30–31 °C. Picrotoxin (25–50 μM) was added to the extracellular solution during the experiments to suppress the inhibitory activity of interneurons.

Standard whole-cell patch clamp techniques were used to record EPSCs from CA1 pyramidal neurons in situ in response to stimulation of the Schaffer collateral/commissural pathway. To prevent the spread of electrical activity from area CA3, mini-slices were prepared by making a cut orthogonal to the *stratum pyramidale* and extending to the

[☆] **Laudation in honour of Professor Zimmermann** Great thanks to Professor Zimmermann from the senior author, Oleg A. Krishtal: I cannot find a better word than 'visionary' to describe the Editorial activities of Professor Zimmermann. At least on two occasions his openness to non-paradigmatic findings was crucial to the first publication on the 'proton receptor and the ATP receptor in sensory neurons' O.A. Krishtal, V.I. Pidoplichko, A "receptor" for protons in small neurons of trigeminal ganglia: possible role in nociception. *Neurosci. Lett.*, 24 (1981) 243–246 O.A. Krishtal, S.M. Marchenko, V.I. Pidoplichko, Receptor for ATP in the membrane of mammalian sensory neurones. *Neurosci. Lett.*, 35 (1983) 41–45. Both papers dealt with unusual findings and this was a penetrating intuition of the Editor to say 'Yes'. Subsequent history demonstrated that he was right: the first paper resulted in the cloning of ASICs and in establishing their role as nociceptors, while the second one contributed to the role of the P2X receptor family in sensory function..

* Corresponding author. Tel.: +38-044-2532466; fax: +38-044-2562590.

E-mail address: krishtal@serv.biph.kiev.ua (O.A. Krishtal).

mossy fibres layer. The intracellular solution for patch pipettes contained 100 mM CsF, 40 mM NaH_2PO_4 , 10 mM HEPES-CsOH, and 10 mM Tris-Cl (pH 7.2). *N*-(2,6-Dimethyl-phenylcarbamoylmethyl)-triethylammonium bromide (QX-314) (2–3 mM) was routinely added to the intracellular solution to block voltage-gated sodium conductance. Patch pipettes were pulled from soft borosilicate glass on a two-stage horizontal puller. When fire-polished and filled with the intracellular solution, they had a resistance of 2–3 M Ω . To visualize cell bodies of CA1 pyramidal neurons, the *stratum oriens* and *alveus* were removed by a saline jet from a micropipette. Currents were digitally sampled at 400 μs intervals by a 12-digit ADC board, filtered at 3 kHz, and stored on a hard disk for further analysis. Access resistance was monitored throughout the experiments and ranged typically from 6 to 9 M Ω . When the access resistance changed by more than 25% during the experiment, the data were discarded. To stimulate a Schaffer collateral/commissural pathway, a bipolar Ni/Cr electrode was positioned on the surface of the slice. Current pulses (10–100 μA) of 0.1–1 ms duration were delivered through the isolated stimulator HG 203 (Hi-Med, London, UK) at 0.066–0.2 Hz.

Sodium bicarbonate and CsF were obtained from Merck (Darmstadt, Germany); 4-AP, lidocaine and picrotoxin were purchased from RBI (Natick, MA); 6-nitro-7-sulphamoylbenzo[*f*]quinoxalin-2,3-dione (NBQX) was obtained from Tocris Cookson (Bristol, UK). All other chemicals were from Sigma (St. Louis, MO).

When measured after a short train of stimuli delivered at high frequency, kinetics of the AMPA component of EPSC are the same as after a single stimulus, while the NMDA-receptor mediated component (EPSC_{NMDA}^{train}) decay undergoes a dramatic slow-down (Fig. 1A). The changes in the EPSC_{NMDA}^{train} kinetics were quantified by normalizing the charge transfer with the peak amplitude of the EPSC_{NMDA} (measured as the mean over a 10 ms window around the peak). A larger charge transfer corresponds to slower decay kinetics and vice versa. The normalized charge transfer of the EPSC_{NMDA}^{train} was $355 \pm 32\%$ of the same parameter for the EPSC_{NMDA}^{single} induced by a single stimulus (EPSC_{NMDA}^{single}) ($P < 0.01$, $n = 10$).

The after-train slow-down of EPSC_{NMDA} cannot be attributed to inadequate voltage clamp. A biophysical model of CA1 pyramidal cells [5,13] was adapted to our experimental conditions in order to estimate the extent to which the quality of the voltage clamp affects the EPSC decay. The density of synapses in the model of CA1 neuron was varied and the EPSC_{NMDA} amplitude, as well as the changes in EPSC_{NMDA} kinetics, were analyzed. Fig. 1D demonstrates the theoretical relationship between the amplitude and kinetics of EPSC_{NMDA}. The experimentally obtained changes in EPSC_{NMDA} amplitude (~ 1.5 -fold increase) can be associated with $\sim 5\%$ changes in the calculated charge transfer vs. 355% changes in the experimentally detected charge transfer. Fig. 1C demon-

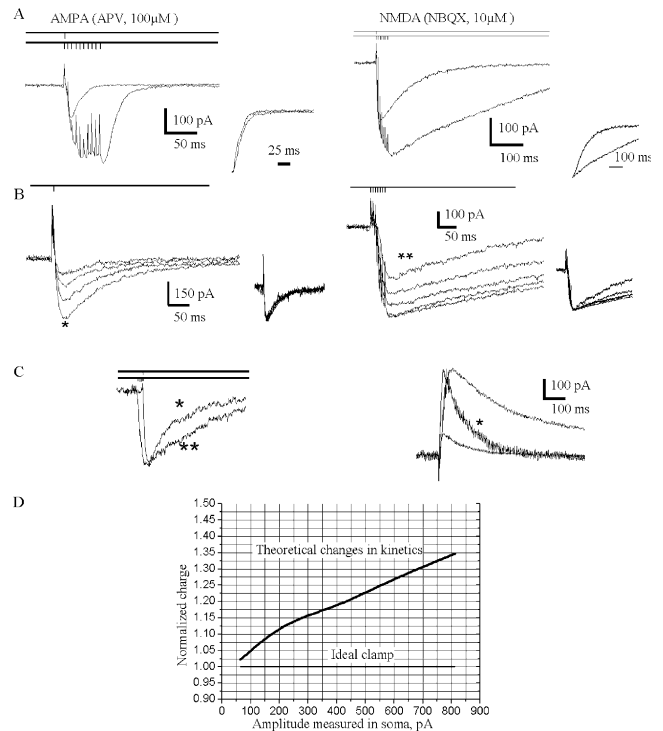


Fig. 1. Facilitation of EPSC by short trains of stimulation is accompanied by a selective slow-down of the NMDA component. (A) (Left) Pharmacologically isolated EPSC_{AMPA} evoked by a single stimulus applied to Schaffer collaterals is superimposed with the EPSC_{AMPA} evoked by a ten pulses long (200 Hz) train ($n = 7$). Here and below: stimulation protocols are schematically represented over the traces; the insets demonstrate normalized time courses of the currents. (Right) The same for EPSC_{NMDA} ($n = 30$). (B) The kinetics of EPSC_{NMDA} evoked by a single stimulus does not depend on the strength of a single stimulus (left), but becomes stimulus-dependent after seven pulses at 200 Hz (right) ($n = 10$). The stimulus intensities varied by a factor of six. (C) (Left) The slowdown of EPSC_{NMDA} is not determined solely by the current amplitudes: the traces from (B) elicited by the largest single stimulus (*) and by the smallest stimulus in the train (**) have equal amplitudes (superimposed, not normalized). The kinetics of the post-train EPSC_{NMDA} is evidently slower. (Right) Slowdown of EPSC_{NMDA} decay under high-frequency stimulation is clearly seen at positive voltage +20 mV ($n = 4$), when the voltage-dependent conductance is inactivated and voltage-dependent Mg^{2+} block of NMDA channels is removed. The small current elicited by a single pulse is normalized (*) to the peak value of current elicited by seven pulses at 200 Hz. (D) Theoretical changes in the EPSC_{NMDA} kinetics due to the change in the current amplitude. The plot shows the dependence of charge transferred by EPSC_{NMDA} on the EPSC_{NMDA} amplitude measured in the soma (modelling according to Refs. [5,13]).

strates the responses of the same amplitude obtained after a single stimulus and a burst stimulation. Their kinetics are obviously different. Therefore, at least qualitatively, we can rely on the experimental data: it is not the increase in the amplitude of the current that solely determines the slow-down of EPSC_{NMDA}, though, for a given number of stimuli, the larger the stimulus applied, the greater this effect becomes (Fig. 1B).

The behaviour of EPSC components after the train (Fig. 1A) remarkably resembles the findings previously made in conditions of enhanced transmitter release: the AMPA

component has unaltered kinetics, while the NMDA component slows down and acquires stimulus-dependent kinetics [10]. This phenomenology is consistent with the spillover of Glu from the synaptic cleft resulting in a cross-talk between neighbouring synapses [1,2,6,15] and/or activation of extrasynaptic NMDA receptors [1,10]. If this picture is correct, the enhanced release of Glu should have the following consequences: (i) the contribution of extrasynaptic receptors should increase; (ii) these receptors should ‘see’ considerably lower concentrations of Glu as compared to the case of the receptors in the post-synaptic density.

The efficacy of low-affinity competitive NMDA receptor antagonist D-amino adipate (D-AA) is known to depend on the synaptic concentration of glutamate [3]. It has been used to distinguish receptors activated within active synapses from those activated by spillover [6]. Fig. 2 demonstrates the experiments in which the prolonged after-train EPSC_{NMDA} was elicited in the presence of D-AA. The blocker leaves practically unaltered the kinetics of EPSC_{NMDA} in the control, but markedly speeds up the decay of EPSC_{NMDA} induced by the train. Taken as a measure of the change in the kinetics, normalized charge transfer produced by the EPSC_{NMDA}^{train} in the presence of the D-AA was $65 \pm 2\%$ of control, while the corresponding value for the EPSC_{NMDA}^{single} was $88 \pm 8\%$ ($P < 0.005$, $n = 4$). Preferential inhibition of a slower component of the EPSC_{NMDA} suggests that the NMDA receptors responsible for this component are activated by lower [Glu] than those contributing to the peak of the current.

We have found an indication of a dramatic increase in the transmitter concentration acting at the peak of after-train EPSC_{NMDA}. Inhibition of current by $50 \mu\text{M}$ D-AA in the control ($29.7 \pm 8.7\%$) is much decreased ($80 \pm 18\%$, $n = 4$) at the peak of EPSC_{NMDA} recorded after a train of seven stimuli (Fig. 2). The 1.25-fold decrease in EPSC_{NMDA} amplitude observed in the D-AA experiments should result in a 4% decrease in the theoretical normalized charge transfer, whereas the actual changes comprised 23%. The most straightforward explanation of this phenomenon is the induction of multivesicular transmitter release in the course of high-frequency firing [4,14,17]. Such a possibility can serve as a functional ‘justification’ for the lack of synaptic

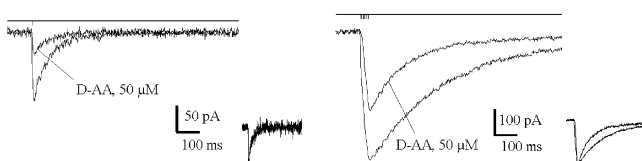


Fig. 2. D-AA accelerates after-burst EPSC_{NMDA}. Block of EPSC_{NMDA} by D-AA induced by a single pulse (left) and by seven pulses at 200 Hz (right). Holding voltage -45 mV . The insets demonstrate that D-AA does not alter the kinetics of the current elicited by a single pulse, but speeds up the after-train EPSC_{NMDA} kinetics.

NMDA receptor saturation by single quanta [11,12] and provide a basis for synaptic integration.

Thus, the synaptic facilitation by a short train of stimulation imitating the natural pattern of burst discharge leads to the increased impact of spillover-activated NMDA receptors. For a given number of stimuli in the train, the higher the frequency used (Fig. 3A) and the larger the stimulus applied (Fig. 1B), the greater is this effect.

Fig. 3B demonstrates the spike-to-spike changes in the EPSC_{NMDA}. The kinetics becomes notably altered starting already from the second stimulus. The charge transferred by EPSC_{NMDA} allows the estimation of the dependence of Ca^{2+} entering the cell on the number of spikes in the pre-synaptic burst discharge (Fig. 3C). When compared with a hypothetical dependence for the case of kinetics which would remain unaltered by the burst discharge (only the peak value of EPSC_{NMDA}^{train} is facilitated), it is dramatically steeper. Therefore, the information on the pre-synaptic activity (the number of spikes and of activated synapses) is

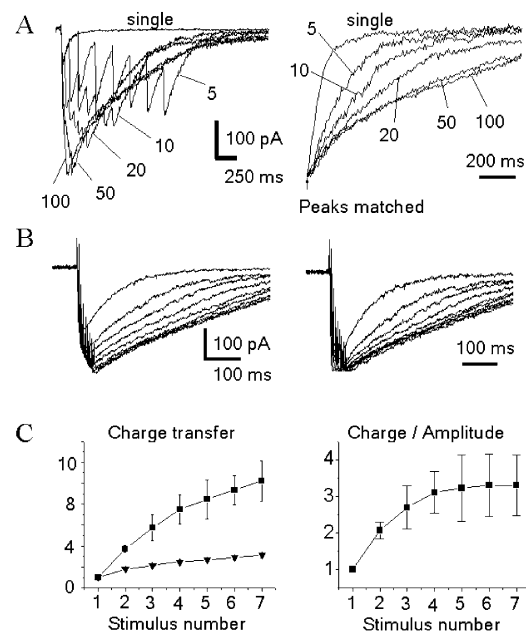


Fig. 3. The after-train EPSC_{NMDA} depends on the frequency of stimulation and on the number of spikes in a train. (A) The slow-down of EPSC_{NMDA} decay depends on the frequency of stimulation within the train. The responses to seven stimuli at 5, 10, 20, 50 and 100 Hz are superimposed on the left graph. Their normalized decays are on the right graph. (B) The slow-down of EPSC_{NMDA} on a spike-to-spike basis. (Left) Family of EPSC_{NMDA} evoked by the progressively increased number of pulses (one to nine) in the train (200 Hz). Holding voltage -45 mV . (Right) Corresponding traces are normalized and superimposed. (C) (Left) The charge transferred by after-burst EPSC_{NMDA} depends on the number of spikes (one to seven) in the train. The data obtained for five neurons stimulated at 200 Hz (squares) are compared with the values obtained for the hypothetical case of unaltered kinetics (only amplitude facilitation is accounted for; triangles). (Right) The charge transferred by EPSC_{NMDA}, normalized to the peak amplitude. The dependence saturates after the 5th to 6th stimulus. In the case of unaltered kinetics this dependence would become just a unity.

reflected in the changes in the EPSC_{NMDA} kinetics. Fig. 3C (right) demonstrates that the gradual change in the EPSC_{NMDA} kinetics is saturated by the 5th to 6th stimulus.

The experiments with Ca²⁺ imaging reveal that NMDA receptors play a leading role in creating the post-synaptic Ca²⁺ signal [8] and in controlling plasticity [9]. Spillover seems to play an important role in shaping this signal by enhancing its dependence on the number of spikes in the high-frequency burst discharge.

Acknowledgements

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