Phasic nicotinic potentiation of frog retinotectal transmission facilitates activation of NMDA receptors of tectum column

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Introduction. We have recently demonstrated [1] that the nicotinic phasic potentiation of the retinotectal transmission enhances intrinsic activity of the tectum column (a lower activity level) reflected in the recordings of the tectal activity by a slow negative wave (sNW) and by recurrent excitatory synaptic potentials superimposed on that wave. We have also shown in our earlier study [2] that this intrinsic activity leads to the activation of the NMDA receptors and transition of the tectum column to the higher level of activity reflected in the recordings by a slow negative potential (sNP) and monophasic spikes superimposed on that potential. Firing of an efferent neuron of the tectum column occurs at that level and can lead to avoidance from a danger reaction. In the present study, we present evidences that an increase of the intrinsic excitatory activity of frog tectum column due to the nicotinic phasic potentiation of the retinotectal transmission facilitates activation of the NMDA receptors that causes the transition of the tectumn column to the higher level of activity.

Method. Experiments were performed *in vivo* on adult frogs, *Rana temporaria*. All experiments in this study were carried out in accordance with the "Principles of laboratory animal care" (NIH publication No. 86 - 23 revised in 1985) as well as with the European Communities Council Directive of 24 November 1986 (86/609/EEC), and were approved by the Animal Care and Use Committee of the State Food and Veterinary Service of Lithuania (No 0167).

Special 8-channel stimulating electrode was placed on the nasoventral quadrant of the naked retina. Single current pulses of magnitude of 10.0 - 33.4 μA and duration of 50 μs, or a train of 2 - 8 of such pulses were applied to the retina through a pair of stimulating electrode channels using World Precision Instruments' isolator. The excitation of a single ganglion cell or its axon was achieved primarily by decreasing the amplitude of the stimulating current pulse.

Responses from the F layer of the tectum were recorded using a carbon-fibre microelectrode. The responses consisted of individual retinotectal action potentials, APs, followed by individual fast synaptic potentials, fSPs, generated

by a set of synapses made up by the retinotectal fiber with the postsynaptic tectum neurons. The APs and fSPs were followed by a slow negative wave, sNW, or by a slow negative potential, sNP, depending on the intensity of discharge of an individual retinotectal axon. Amplitudes of the AP and fSP (A_{AP}, A_{fSP}), amplitude (A_{sNP}) and duration (T_{sNP}) of the sNP, and magnitude (A_{sNW}) of the sNW have been measured. The amplitude of the sNP was measured as it is shown in Fig.1 c, e. The duration of the sNP was measured as a time interval from the peak of the last fSP in the burst to the end of the sNP (see Fig.1 c, e). The magnitude of the sNW was evaluated as an average voltage of the response in the time interval from 60 to 120 ms (see Fig. 1 b). A phasic potentiation of the retinotectal transmission was calculated as a ratio of the amplitudes of potentiated and control fSPs, $A_{fSP,pot}/A_{fSP}$.

Averaged values are given as mean ±SE (standard error of the mean). Paired t-test with confidence level of 0.95 was performed for estimation of the statistical significance of the results.

Results. We have not used agonists or antagonists of the acetylcholine and NMDA receptors in our experiments, and show the relation between nicotinic phasic potentiation of the retinotectal transmission and activation of the NMDA receptors indirectly based on our earlier studies [2, 3]. Ten experiments with 7 frogs were done as follows. Responses to stimuli of moderate strength (2 – 4 action potentials with interpulse interval of 10 ms) that evoked the slow negative wave (sNW) but did not evoke the slow negative potential (sNP) were recorded (Fig. 1 b). Then, the response to a weakest stimulus (4 – 6 action potentials with interpulse interval of 10 ms) that evoked the sNP was recorded (Fig. 1 c). Next, a strong conditioning stimulus (Fig. 1 d) has been delivered to induce a substantial (2.6 ± 0.2) phasic potentiation of the retinotectal transmission that lasted more than half a minute [3]. The stimulus of moderate strength was applied again ten seconds after the delivery of the conditioning stimulus in order to test if the phasic nicotinic potentiation of the retinotectal transmission could facilitate the generation of the sNP (Fig. 1 e). The facilitation of generation of the sNP was evaluated as a difference between the strength of the weakest stimulus that had evoked the sNP in the unpotentiated (control) case and the strength of the one in the potentiated case, i. e. as difference, ΔN, between the numbers of current pulses in the corresponding stimuli (or between the numbers of action potentials in the corresponding bursts). The facilitation was observed in all of 10 experiments. In five of them $\Delta N = 2$, in the rest five $\Delta N = 1$. On average, the strength of stimulus (number of pulses, N) required to evoke the sNP has decreased from 5 ± 0.2 to 3.5 ± 0.2, $P < 8 \cdot 10^{-6}$, $n = 10$ due to the nicotinic phasic potentiation. The amplitude of the sNP was slightly smaller in the potentiated than unpotentiated (control) case in most of the experiments. In a few of them it was in opposite. However, average amplitudes and durations of the sNP in the unpotentiated and potentiated cases have not differed significantly (307 ± 16 μV and 311 ± 21 μV, $P < 0.77$, $n = 10$; 179.1 ± 13.4 ms and 182.1 ±

12.6 ms, $P < 0.7$, $n = 10$) indicating that the same event has been evoked in both of the cases.

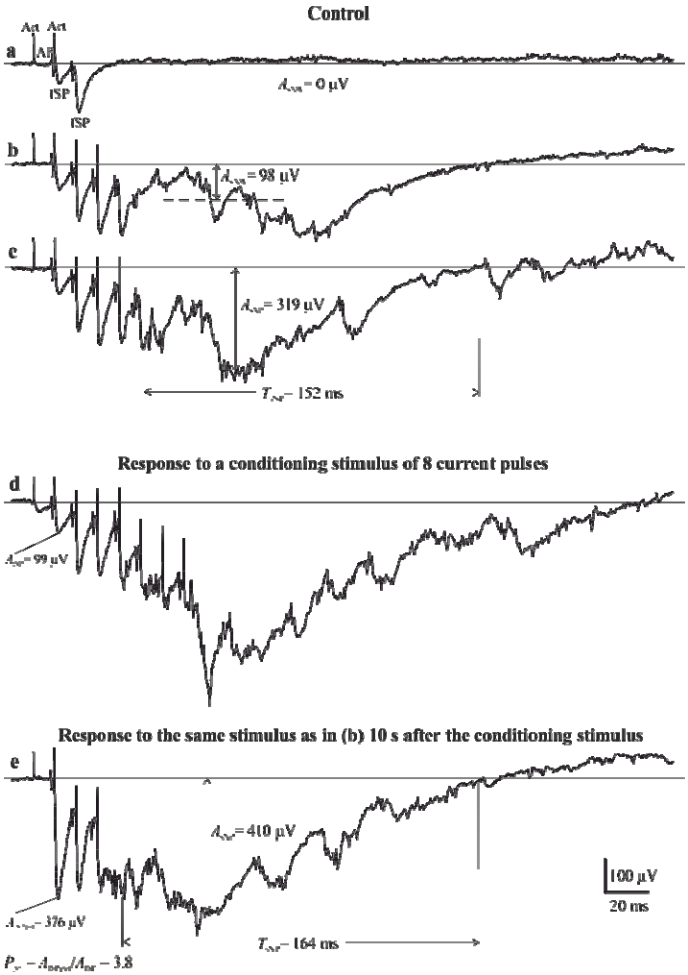

Fig.1. Phasic nicotinic potentiation of the retinotectal transmission facilitates generation of the slow negative potential (sNP) in the tectal responses. **(a)**, **(b)**, **(c)** Responses to stimuli of 2, 4, 5 current pulses with interpulse interval of 10 ms, respectively. Art - stimulus artifact, AP - retinotectal individual action potential, fSP - retinotectal individual fast synaptic potential, A_{sNW} - amplitude of the slow negative wave (sNW), A_{sNP} - amplitude of the slow negative potential (sNP), T_{sNP} - duration of the slow negative potential (sNP). **(d)** Response to conditioning stimuli of 8 current pulses with interpulse interval of 10 ms. A_{fSP} - amplitude of the fast synaptic potential. **(e)** Response to the same stimulus as in **(b)** (4 pulses with interpulse interval of 10 ms) 10 s after the conditioning stimulus. $A_{fSP,pot}$ - amplitude of the potentiated fast synaptic potential. P_{ph} - phasic (after-burst) potentiation of the retinotectal transmission.

Discussion. Experimental data presented above show that the nicotinic phasic potentiation of a frog retinotectal transmission facilitates generation of a slow negative potential (sNP) in the tectal responses by decreasing stimulus strength (number of action potentials in the burst) required to evoke the sNP from 5 ± 0.2 to 3.5 ± 0.2.

It was shown in our previous studies that the burst of moderate intensity excites the recurrent excitatory neurons of the tectum column [4] by activating dendritic L-type calcium current [5] seen in the extracellular recordings as a slow negative wave (sNW). This intrinsic recurrent excitation of the tectum column comprises a lower level of activity of the tectum column and is manifested in the recordings by the sNW and recurrent synaptic potentials superimposed on that wave (see Fig.1b). The more intense burst of spikes leads to the activation of the NMDA receptors seen in the recordings as a slow negative potential (sNP) (see Fig. 1c, d, e), and to firing of an efferent pyramidal neuron of the tectum column seen in the recordings as monophasic spikes superimposed on that potential [6, 7]. These comprise a higher activity level of the tectum column. Thus, the facilitation of generation of the sNP means the facilitation of activation of the NMDA receptors that, in turn, leads to the higher activity level of the tectum column. As the output signals from the tectum column are generated at this higher level of activity, the phasic nicotinic potentiation of the retinotectal transmission can have direct functional (behavioral) consequences. The retina ganglion cell (moving edge or darkness detector) that was stimulated during the experiments is related to a frog escape (defensive) reactions [8]. An axon of an efferent pyramidal neuron of the tectum column extends along the tectobulbospinal tract to motor nucleus, and its firing may lead to the escape from a danger reaction. Therefore, the nicotinic facilitation of activation of the NMDA receptors that elicits higher activity level of the tectum column can be considered as a mechanism of vigilance and cue detection at the level of small neuronal network. For example, a dangerous object, as it passes by, elicits strong excitation of the ganglion cell (darkness or moving edge detector) leading to the phasic nicotinic potentiation of the retinotectal transmission that lasts more than 30 seconds [3]. Within this period of time the corresponding tectum column stays in the state of enhanced responsiveness. Being in that vigilant state, the tectum column can be excited to the higher level of activity even by stimuli of moderate strength (for example by the dangerous object coming back). The output signals from the tectum column are generated at that level of activity and can lead to the motor (danger avoidance) reaction.

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References

1. Baginskas A., Kuraite V., Kuras A. Phasic nicotinic potentiation of frog retinotectal transmission enhances intrinsic activity of tectum column // Neurosci. Res. 74, 2012. –P. 42–47.

2. Baginskas A., Kuras A. L-Type Ca2+ current in frog tectal recurrent neurons determines the NMDA receptor activation on efferent neuron // Exp. Brain Res. 193, 2009. –P. 509–517.

3. Baginskas A., Kuraite V., Kuras A. Presynaptic nicotinic potentiation of a frog retinotectal transmission evoked by discharge of a single retina ganglion cell // Neurosci. Res. 70, 2011. –P. 391–400.

4. Kuras A., Baginskas A., Batuleviciene V. Suprathreshold excitation of frog tectal neurons by short spike trains of single retinal ganglion cell // Exp. Brain Res. 159, 2004. –P. 509–518.

5. Baginskas A., Kuras A. Single retina ganglion cell evokes the activation of L-type Ca^{2+}-mediated slow inward current in frog tectal pear-shaped neurons // Neurosci. Res. 60, 2008. –P. 412–421.

6. Kuras A., Baginskas A., Batuleviciene V. Non-NMDA and NMDA receptors are involved in suprathreshold excitation of network of frog tectal neurons by a single retinal ganglion cell // Neurosci. Res. 54, 2006. –P. 328–337.

7. Kuras A., Baginskas A., Batuleviciene V., Lamanauskas N. // Single retinal changing contrast (3rd) detector elicits NMDA receptor response and higher activity level of frog tectum neuron network // Exp. Brain Res. 179, 2007. –P. 209–217.

8. Ingle D. Behavioral correlates of central visual function in anurans // in: R. Llinas, W. Precht (Eds.), Frog neurobiology, Springer, Berlin Heidelberg, New York, 1976. –P. 434–451.

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We have recently demonstrated that the intrinsic recurrent excitatory activity of the tectum column increases due to the phasic (after-burst) nicotinic potentiation of a frog single axon retinotectal transmission to the tectum layer F. In the present study we show that the phasic nicotinic potentiation facilitates activation of the NMDA receptors of the tectum column. With this, a functional significance of nicotinic modulation of the activity of the tectum column has been demonstrated, since the activation of NMDA receptors leads to the higher activity level of the tectumn column at which output signals from the tectum column are generated. The nicotinic facilitation of transition of the tectum column to the higher level of activity can be considered as a mechanism of vigilance and cue detection at the level of a small neural network.