Delayed Suppression of Recurrent Excitation in the Frog Tectum Column by Endogenous Acetylcholine

A. Baginskas[1*], A. Kuras[2], A. Grigaliūnas[1]

[1]*Department of Physics, Lithuanian University of Health Sciences, Lithuania*
[2]*Lab of Neurophysiology, Lithuanian University of Health Sciences, Lithuania*
[*]*Email: armuntas.baginskas@lsmuni.lt*

Introduction. We have shown in our previous study [1] that an inhibition of the recurrent excitation in the frog tectum column is mediated by the activation of muscarinic ACh receptors, situated, presumably, in the recurrent connections of the tectum column. Results of other our study [2] have demonstrated that during firing of a single retina ganglion cell (darkness or moving edge detector) the acetylcholine (ACh) releases into the frog tectum as a co-mediator from the glutamatergic retinotectal fiber terminals. It was shown in the study [3] that this endogenous acetylcholine activates presynaptic nicotinic ACh receptors located on the retinotectal fiber terminals, causing the phasic potentiation of the retinotectal transmission. Based on these results, we have proposed that co-mediator acetylcholine should also activate muscarinic ACh receptors, presumably, located in the excitatory recurrent connections of the tectum column, leading to an inhibition of the recurrent excitation. The present study presents evidence in support of this proposition.

Method. Experiments were performed *in vivo* on adult frogs, *Rana temporaria*. All experiments in this study were carried out in accordance with the European Communities Council Directive 86/609/EEC, and were approved by the Animal Care and Use Committee of the State Food and Veterinary Service of Lithuania (No 0167**)**.

The multichannel stimulating electrode was placed on nasoventral quadrant of the naked retina. Single current pulses of magnitude of 17–43 μA and duration of 50 μs, or trains of 2–8 of such pulses were applied to the retina through a pair of the channels of the stimulating electrode. The excitation of a single ganglion cell or its axon was achieved, primarily, by decreasing an amplitude of stimulating current pulse.

Responses from the F layer of the frog tectum were recorded using carbon-fiber microelectrode. The responses consisted of individual retinotectal action potentials (APs), fast synaptic potentials (fSPs), and slow negative wave (sNW) or slow negative potential (sNP) that followed burst of the fSPs. The amplitude of the sNP (A_{sNP}), delay of the minimum (maximal absolute value) of the sNP ($t_{min,sNP}$), and amplitudes of the recurrent synaptic potentials (rSPs) superimposed on the sNW have been measured. The $t_{min,sNP}$ was considered to be equal to the delay of inhibition of the recurrent synaptic potentials.

Averaged values are given as mean ± SE. Paired t-test with confidence level of 0.95 was performed to estimate statistical significance of the results.

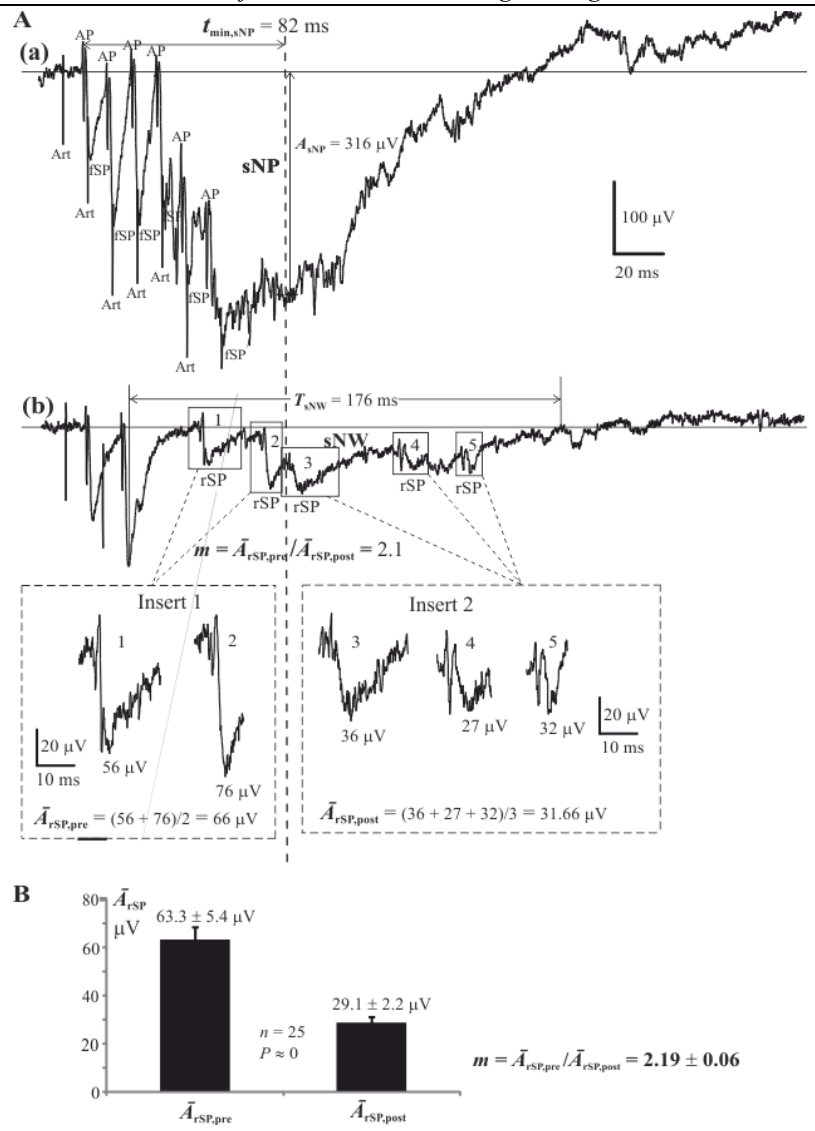

Fig.1. Delayed suppression of the recurrent excitation of the frog tectum column. **(A(a))** Response elicited in the frog tectum by the burst of 6 retinotectal action potentials in an individual retinotectal fiber. Art – stimulus artefact. AP – retinotectal action potential. fSP – retinotectal fast synaptic potential. sNP – slow negative potential. A_{sNP} – amplitude of the sNP. $t_{min,sNP}$ – delay of the sNP.
(A(b) Response elicited in the tectum by the burst of 2 retinotectal action potentials. sNW – slow negative wave. T_{sNW} – duration of the sNW. rSP – recurrent synaptic potential. $\bar{A}_{rSP,pre}$ – average amplitude of the rSPs occurring before the time moment

$t_{min,sNP}$. $\bar{A}_{rSP,post}$ – average amplitude of the rSPs accurring after the time moment $t_{min,sNP}$. Ratio of these averaged amplitudes, $m = \bar{A}_{rSP,pre}/\bar{A}_{rSP,post}$, characterizes magnitude of the delayed suppression of the rSPs. Inserts show the scaled-up rSPs associated with early and late parts of the sNW.
(B) Mean of 13 experiments.

Results. The responses elicited by strong and weak bursts of action potentials of a single retina ganglion cell (darkness or moving edge detector) were recorded from the F layer of the frog tectum. Stimulation of a single retina ganglion cell was considered reliable if an increase of the amplitude of the stimulation current pulse by up to 6 μA has not affected the response, and a decrease by less than 0.8 μA eliminated it. The F layer location of the responses was confirmed by the latency of the response being in the range of 6–10 ms, and the position of the tip of the recording microelectrode being at the depth of 240–280 μm from the tectum surface.

The strong excitation by a train of 5–8 current pulses has elicited in the tectum slow negative potential (sNP) (see Fig. 1 A(a)). The weak excitation by a train of 2–4 current pulses has elicited slow negative wave (sNW) with the recurrent excitatory synaptic potentials (rSPs) superimposed on that wave (see Fig. 1 A(b)).

Responses from 13 individual retina ganglion cells (12 frogs) have been recorded and analyzed. The amplitude (A_{sNP}) and delay ($t_{min,sNP}$) of the sNP have been measured. The delay of the sNP, $t_{min,sNP}$, was defined as the time interval from the beginning of the burst till the time moment when the sNP reaches its minimum (maximal absolute value) (Fig. 1A(a)). The mean of 13 experiments has been estimated as follows: $A_{sNP} = 370 \pm 23$ μV ($n = 13$, range of values 265–525 μV), $t_{min,sNP} = 84 \pm 1.9$ ms ($n = 13$, range 75–95 ms).

Amplitudes of the rSPs riding on the sNW (Fig. 1A(b)) have been measured, too. The average amplitude of the rSPs occurring before the time moment $t_{min,sNP}$, $\bar{A}_{rSP,pre}$, and the average amplitude of the rSPs occurring after the time moment $t_{min,sNP}$, $\bar{A}_{rSP,post}$, have been calculated. Ratio of the averaged amplitudes, $m = \bar{A}_{rSP,pre}/\bar{A}_{rSP,post}$, characterizes magnitude of the delayed inhibition of the recurrent excitation (Fig. 1A(b)). The mean of 13 experiments has been evaluated as follows: $\bar{A}_{rSP,pre} = 61.8 \pm 3.8$ μV, $\bar{A}_{rSP,post} = 28.7 \pm 1.9$ μV, $m = 2.18 \pm 0.10$ (Fig. 1B).

Discussion. Results of the present study have demonstrated the delayed inhibition of the recurrent excitation of the tectum column that has followed the activation of the tectum column by a burst of retinotectal action potentials. The delay of this inhibitory effect was considered to be equal to the delay of the minimum (maximal absolute value) of the SNP, 84 ± 1.9 ms, that, on the other hand, corresponds to the time interval needed for the transition of the tectum column to the higher activity level (the higher activity level is manifested in the recordings by a presence of the sNP [4]). After the higher activity level has been established and output from the tectum column was generated, the tectum column must be reset back to its resting state in order to respond to next

incoming stimuli. To do this, the recurrent excitation should be suppressed. This suggests a functional significance for the delayed inhibition: to establish necessary conditions for returning of the tectum column back to its resting state. This may also be true for other neuronal networks, not only for the frog tectum column.

The delayed inhibition of the recurrent excitation of the tectum column, most likely, is carried out through the activation of presynaptic muscarinic ACh receptors, located on the terminals of axons of recurrent pear-shaped neurons. The latter suggestion is encouraged by the results of other researchers (see, for example, [5]), demonstrating that activation of presynaptic muscarinic receptors generally leads to an inhibition of excitatory synaptic transmission.

Results of biochemical experiments [6] have shown that action of the acetylcholine through muscarinic ACh receptors develops with a delay of tens to hundred milliseconds, which corresponds to the value of the delay estimated in the present study.

Conclusions. Inhibition of the recurrent excitation of the tectum column occurs with a delay of ~80 ms following the activation of the tectum column by a burst of action potentials of a single retina ganglion cell. The delayed inhibition of the recurrent excitation sets necessary conditions for returning of the tectum column back to its lower activity level.

References

1. Baginskas A., Kuras A. Muscarinic inhibition of recurrent glutamatergic excitation in frog tectum column prevents NMDA receptor activation on efferent neuron // Exp. Brain Res. 208, 2011. –P. 323–334.

2. Baginskas A., Kuraite V., Kuras A. Frog retinal ganglion cells projecting to the tectum layer F release acetylcholine as co-mediator // Neurosci. Lett. 522, 2012. –P. 145–150.

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. 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.

5. Brown D. A. Muscarinic acetylcholine receptors (mAChRs) in the nervous system: some functions and mechanisms // J. Mol. Neurosci. 41(3), 2010. –P. 340–346.

6. Maier-Peuschel M., Frölich N., Dees C, Hommers L. G., Hoffmann C., Nikolaev V. O., Lohse M. J. A fluorescence resonance energy transfer-based M2 muscarinic receptor sensor reveals rapid kinetics of allosteric modulation // J. Biol. Chem. 285(12), 2010. –P. 8793–8800.

Delayed Suppression of Recurrent Excitation in the Frog Tectum Column by Endogenous Acetylcholine

A. Baginskas[1], A. Kuras[2], A. Grigaliūnas[1]
[1]*Department of Physics, Lithuanian University of Health Sciences, Lithuania*
[2]*Lab of Neurophysiology, Lithuanian University of Health Sciences, Lithuania*

Results of the present study have demonstrated the delayed inhibition of the recurrent excitation of the frog tectum column that has followed the activation of the tectum column by burst of action potentials of a single retina ganglion cell. The delay of the inhibition was estimated to be of ~80 ms, and the magnitude – more than 2 times. The inhibition of the recurrent excitation permits transition of the tectum column back to its lower activity level, suggesting a functional significance for the delayed inhibition of the activity of neuronal networks: to establish the necessary conditions for transition of a neuronal network back from the high activity state to the resting state.