Doklady Biological Sciences, Vol. 389, 2003, pp. 102–104. Translated from Doklady Akademii Nauk, Vol. 389, No. 4, 2003, pp. 555–557.

PHYSIOLOGY

Correlation between Paired Responses Confirms the Existence of a Positive Ephaptic Feedback in Central Synapses

S. V. Kulchitsky*, V. V. Maximov, P. V. Maximov**, M. S. Lemak***, and L. L. Voronin*****

Presented by Academician I.A. Shevelev December 3, 2002

Received December 4, 2002

According to the hypothesis advanced by Byzov [1, 2], excitatory chemical synapses have an electrical (ephaptic) feedback (EFB) due to the potential drop across the resistance of the synaptic cleft (*Rg*) caused by excitatory postsynaptic current (EPSC). The "supralinear" effects of postsynaptic hyperpolarization [3–6] indicate the presence of EFB in large ("perforated") synapses of the central nervous system (CNS). However, these effects could also be explained by a decrease in the potential-dependent postsynaptic release of an inhibitory retrograde messenger (the "chemical" hypothesis). A simplified ("nonquantal") model of a synapse with the number of the transmitter release sites $N = 1$ was used in the studies [3–6]. The purpose of our study was to test the hypothesis on the EFB and the alternative chemical hypothesis with the use of a more realistic model. Comparison of the predictions of this model with physiological data disproves the chemical hypothesis and provides evidence in favor of the existence of EFB, a new type of feedback, in central synapses.

Computer experiments. The model, developed in the Windows system, is available in the Internet (www.iitp.ru/projects/eq/). Paired-pulse facilitation (PPF) is included in the model as a change in the probability of transmitter release (*Pr*). In the experiments, the parameters of the model were varied so as to simulate the probable characteristics of the synapses of mossy fibers in the hippocampal CA3 region [7, 8], including *N* (10–20), *Pr* (0.04–0.1), *Rg* (up to 1.1 GΩ), the time constant (100 ms), and the PPF = EPSC2 / EPSC1, where EPSC1 and EPSC2 are the amplitudes of the first and second EPSCs, respectively, generated in the case of paired-pulse stimulation. Figure 1a shows that, on average, EPSC2 > EPSC1; however, as PPF is of presynaptic origin [7], the fluctuating amplitudes were not correlated with each other in the absence of EFB ($Rg = 0$) or when the responses did not overlap (Figs. 1a, 1b, 70 ms). In the case of response overlapping in the presence of EFB (Fig. 1b, 20 ms), the coefficient of linear correlation (r) between EPSC2 and EPSC1 was significant (Fig. 1a, 20 ms). We studied the dependence of r on the "overlapping coefficient" OC = EPSC1(m) / EPSC1($s2$), where EPSC1(m) and EPSC1($s2$) are the maximum EPSC1 and the EPSC1 measured at the moment of the application of the second stimulus pulse, respectively. We found that $r > 0.2$ if OC > 0.2 and $Rg = 0.3$–0.6 GΩ; at OC > 0.5, r was as high as 0.5–0.7.

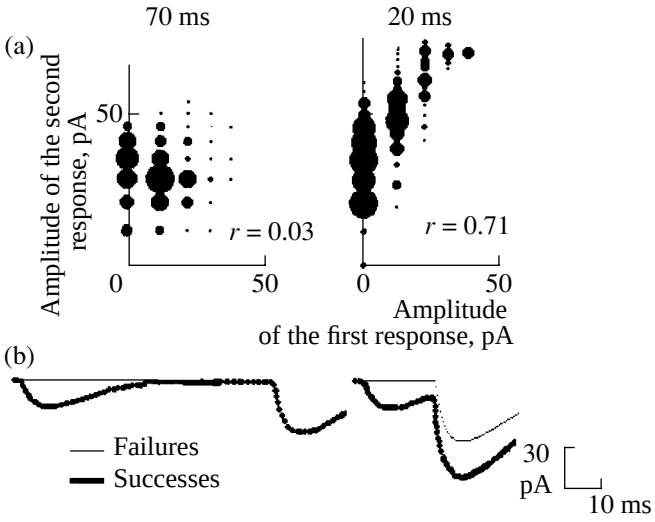

Fig. 1. Correlation between the amplitudes of the first and second EPSCs generated by the model with EFB (Rg = 0.5 GΩ) in the cases of a long (70 ms) and a short (20 ms) intervals between two presynaptic pulses (500 pairs). (a) The diameter of the symbols is proportional to the number of EPSC pulses (5–30 pairs per point); r is the linear correlation coefficient. (b) Mean EPSCs in the same model experiments separated into failures and successes in response to the first pulse in the pair.

* *Institute of Physiology, National Academy of Sciences of Belarus, ul. Akademicheskaya 28, Minsk, 220072 Belarus*

** *Institute of Information Transmission Problems, Russian Academy of Sciences, Bol'shoi Karetnyi per. 19, Moscow, 101447 Russia*

*** *Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, ul. Butlerova 5a, Moscow, 117485 Russia*

0012-4966/03/0304-0102$25.00

At low *Pr* values, both successes (EPSC1 > 0) and response failures (EPSC1 = 0, if we ignore the background noise) were observed. The comparison of mean EPSC2 values after the "failure" of the first response ($EPSC2_f$) with those after the "success" ($EPSC2_s$) provides a good illustration of the correlation between EPSC1 and EPSC2. Our simulation experiments demonstrated that, in the absence of EFB or in the case of long intervals between stimuli (Fig. 1b, 70 ms), $EPSC2_s = EPSC2_f$ ($EPSC2_s / EPSC2_f = 1$). In the presence of both EFB ($Rg > 0$) and response overlapping, $EPSC2_s/ EPSC2_f > 1$ (Fig. 1b, 20 ms). $EPSC2_s/ EPSC2_f$ was directly related to OC. For example, if $N = 15$ and $Pr = 0.047$, the correlation was close to linear with r varying between 0.99 and 0.86 at $Rg = 0.3–0.6$ GΩ.

Physiological experiments. To test the predicted dependence of EPSC2 on EPSC1, which was previously unknown, we recorded the EPSCs from pyramidal neurons of the CA3 region evoked by stimulation of mossy fibers. The experiments were performed on hippocampal slices of young (12- to 18-day-old) rats with the use of the patch-clamp recording in a whole-cell configuration (the voltage clamp mode) [3–6]. To increase the precision of the measurements, the slices were placed into a 0.05 mg/l concanavalin A solution for 15 min during EPSC overlap, which increased the EPSC duration [9]. Paired (interval, 20–70 ms) stimuli (applied every 8–16 s; 75–600 pairs per neuron) were selected so that "minimal" EPSCs with failures were evoked. The significance of differences was estimated using Student's *t*-test (at a significance level of $p < 0.05$).

Figure 2a shows EPSCs averaged over 25–70 pairs at two membrane potentials. In Fig. 2a, a pronounced PPF and a more than twofold (supralinear) increase in EPSC1 during hyperpolarization (–100 mV) expected for synapses with EFB [3–6] are seen. In Fig. 2b, the first responses are divided into those with the lowest amplitudes (failures) and the remaining (successes). When the responses did not overlap (Fig. 2b, 70 ms), $EPSC2_s$ and $EPSC2_f$ did not differ from each other; however, when the responses overlapped (Fig. 2b, 20 ms), the EPSC2 after successes ($EPSC2_s$) was considerably higher than after failures ($EPSC2_f$), reflecting the positive correlation between EPSC2 and EPSC1. The responses did not overlap in 4 out of 11 experiments (OC < 0.08), and the ratio $EPSC2_s/EPSC2_f$ was close to 1 (1.03 ± 0.02; here and below, the mean ± the error of the mean is indicated). Conversely, this ratio was greater than 1 (1.55 ± 0.06, $p < 0.01$) in seven experiments with OC = 0.2–0.8 (0.54 ± 0.05), and it was greater than 1.33 in six of them (the maximum value was 2.0), as predicted for synapses with EFB. $EPSC2_s/EPSC2_f$ was strongly correlated with OC ($r = 0.91$, $p < 0.001$, $n = 11$); this correlation was close to that obtained in the model with $Rg = 0.3–0.4$ GΩ.

Thus, our simulations demonstrated that, in the case of the overlapping of two EPSCs generated by a synapse with EFB, EPSC1 and EPSC2 were correlated; however, the correlation was absent if the EPSCs did not overlap. A positive correlation between the degrees of overlapping of two EPSCs and the ratio $EPSC2_s/EPSC2_f$ was predicted. The predictions were confirmed by the results of physiological experiments, which indicates the existence of EFB in central synapses. Within sufficiently realistic ranges of model parameters, the predictions best fitted experimental data at $Rg = 0.3–0.4$ GΩ, which is close to the *Rg* estimation in [5]. Earlier, the existence of EFB was hypothesized on the basis of the supralinear effects of postsynaptic hyperpolarization [3–6]. These effects were always observed here when EPSC2 and EPSC1 were correlated, which also confirms the EFB hypothesis. The correlation between EPSC1 and EPSC2 disproves the alternative (chemical) hypothesis, because the voltage clamp conditions in our experiments excluded potential-dependent effects. Even if the fixation was incomplete, the release of the hypothetical inhibitory messenger should have become more intense during the generation of the first EPSC, and the second EPSC should not have increased. The synapses studied are formed in the course of maturation of CA3; however, the number of perforated synapses in the neocortex increases during learning [10] and in other hippocampal regions, also during long-term potentiation, which is an experimental model of memory [2, 7]. In this case, the formation of EFB could represent one of the possible mechanisms of memory storage traces. Our data also indicate that EFB not only controls the information processing in the case of slow shifts of postsynaptic potentials [6], but also increases *Pr* in the case of high-frequency (bursting) neuronal activity, which is characteristic of the hippocampus, e.g., during the behavior related to cognitive functions [11].

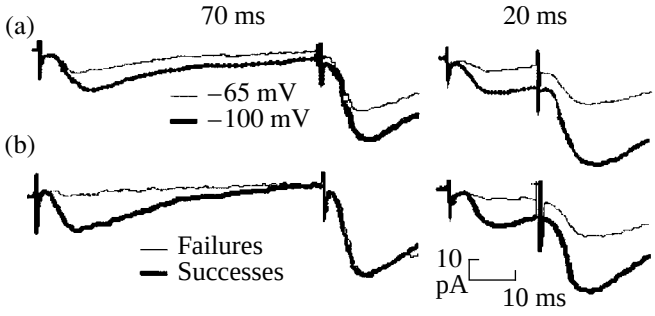

Fig. 2. Correlation between the amplitudes of the first and second EPSCs of hippocampal neurons. (a) Mean EPSCs of two neurons at different intervals between paired stimuli and the specified membrane potentials. (b) The same EPSCs at a membrane potential of –65 mV shown as in Fig. 1b.

ACKNOWLEDGMENTS

We are grateful to D.A. Sakharov and T.L. D'yakonova for valuable advice and for providing us with some of the necessary drugs.

This study was supported by INTAS, Wellcome Trust, and the Russian Foundation for Basic Research.

REFERENCES

1. Byzov, A.L. and Maximov, V.V., *Vision. Res.*, 1996, vol. 36, pp. 4077–4087.
2. Voronin, L., Byzov, A., Kleschevnikov, A., *et al.*, *Behav. Brain Res.*, 1995, vol. 66, pp. 45–52.
3. Voronin, L.L., Volgushev, M., Sokolov, M., *et al.*, *Dokl. Akad. Nauk*, 1999, vol. 369, pp. 126–129.
4. Voronin, L.L., *Ross. Fiziol. Zh.*, 1999, vol. 85, pp. 729–742.
5. Kasyanov, A.M., Maximov, V.V., Byzov, A., *et al.*, *Neuroscience*, 2000, vol. 101, pp. 323–336.
6. Berretta, N., Rossokhin, A.V., Kasyanov, A.M., *et al.*, *Neuropharmacology*, 2000, vol. 39, pp. 2288–2301.
7. Voronin, L.L., *Neuroscience*, 1993, vol. 56, pp. 275–304.
8. Henze, D.A., Urban, N.N., and Barrionuevo, G., *Neuroscience*, 2000, vol. 98, pp. 407–427.
9. Mayer, M.L. and Vyklicky, L., Jr., *Proc. Natl. Acad. Sci. USA*, 1989, vol. 86, no. 4, pp. 1411–1415.
10. Calverley, R.K.S. and Jones, D.G., *Brain Res. Rev.*, 1990, vol. 15, pp. 215–249.
11. Buzsaki, G. and Chrobak, J.J., *Curr. Opin. Neurobiol.*, 1995, vol. 5, pp. 504–510.