# LONG-TERM POST-TETANIC POTENTIATION IN HIPPOCAMPAL SLICES FROM MICE WITH "KINDLING"

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The phenomenon of long-term hippocampal post-tetanic potentiation (LPP), observed both in vivo and in vitro, provides a good model with which to study the mechanisms regulating synaptic transmission in the presence of changes of its effectiveness which, it has been suggested, constitutes the physiological basis of learning [2]. Another distinctive plastic phenomenon is that of "kindling"(KP), which consists of the appearance and progression of electrographic and behavioral seizures in response to below-threshold electrical stimulation or to a subconvulsive dose of an epileptogen, not previously giving rise to these effects [3]. Goddard [6] described a similar type of formation of LPP and kindling, and he considered that a relationship of cause and effect exists between these two phenomena. The present investigation is devoted to the study of this connection.

## EXPERIMENTAL METHOD

Experiments were carried out on inbred BALB male mice weighing 23-25 g. Kindling was induced by daily (for one month) intraperitoneal injection of metrazol in a subconvulsive dose (30 mg/kg in a volume of 0.1 ml); these mice constituted the experimental group. Animals of the control group were given an intraperitoneal injection of the same volume of physiological saline.

Investigations on hippocampal slices began not earlier than one year after the last injection of metrazol, at a time when KP had developed. After decapitation of the animals the brain was exposed and hippocampal slices were cut to a thickness of 300-400 μ, and they were immediately transferred into a special chamber. A continuous flow of Yamamoto's balanced salt solution of the following composition was used (in mM): NaCl – 124, KCl – 2.5, $KH_2PO_4$ – 1.24, $MgSO_4$ – 1.3, $CaCl_2$ – 2.4, $NaHCO_3$ – 26, glucose 10; the solution was saturated beforehand with a gas mixture (95% $O_2$, 5% $CO_2$), and the temperature of the solution was kept at 25-27°C. The slice was fixed to a nylon grid by means of a bipolar glass stimulating electrode, filled with Yamamoto's solution, which was inserted into the stria radialis, through which Schaffer's collaterals run; the latter are axons of neurons in area CA3, running to neurons in area CA1. The recording glass microelecrode (resistance 1-5 MΩ) also was filled with Yamamoto's solution and inserted into the layer of pyramidal neurons of area CA1.

Recording of electrical activity began 1-1.5 h after cutting of the slices. Focal responses, evoked by double ($\Delta t$ = 20, 30, 40, 50, 80, and 100 msec) periodic (1/3 sec) stimulation with square pulses (0.1 msec), were recorded by means of an FOR-2 camera. Four or five series (until stabilization of the response) of ten stimuli (the interval between the series was 10 min) ended with tetanization (20 Hz, 5 sec). After tetanization, the testing series followed in the same order.

The measure of reactivity of the pyramidal neurons in area CA1 was the amplitude of the population spike (PS), reflecting the synchronous discharge of pyramidal neurons. The dynamics of changes in the amplitude of PS before and after tetanization was studied. In the course of one experiment hippocampal slices from experimental and control mice were studied consecutively.

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TABLE 1. Ratio $A_{tr}/A_{cr}$ before and after Tetanization in Slices from Control and Experimental Groups (M ± m)

| Delay between stimuli, msec | Before tetanization | | After tetanization | |
|---|---|---|---|---|
| | control | experiment | control | experiment |
| 20 | 2,5±0,6 | 4,4±0,7* | 2,5±0,4 | 2,2±0,3 |
| 30 | 2,7±0,6 | 3,6±1,1 | 2,3±0,5 | 2,5±0,3 |
| 40 | 2,3±0,5 | 3,1±0,4 | 2,3±0,6 | 2,7±0,4 |
| 50 | 3,0±1,1 | 3,3±0,3 | 2,8±0,8 | 2,7±0,3 |
| 80 | 3,1±1,6 | 2,6±0,3 | 2,2±0,6 | 2,2±0,2 |
| 100 | 2,8±1,6 | 2,0±0,3 | 2,0±0,6 | 2,3±0,2 |

Legend. *$p < 0.05$.

The results were subjected to statistical analysis by Student's t test and by Mann–Whitney U test.

## EXPERIMENTAL RESULTS

Experiments were carried out on hippocampal slices of 10 control mice and of 19 mice with KP. The field potential arising in area CA1 of the hippocampus in response to stimulation of Schaffer's collaterals with pairs of pulses, is an informative electrophysiological parameter of integral activity of neurons. By analysis of this parameter, processes of excitation manifested as enhancement of the field response to a testing stimulus, or processes of inhibition, manifested as a decrease of the response to the testing stimulus, can be evaluated. In the investigation we analyzed the ratio of the amplitude of the testing response ($A_{tr}$) to the amplitude of the conditioning response ($A_{cr}$) in control and experimental groups, before and after tetanization. In all the experiments facilitation was observed on presentation of paired stimuli (the ratio $A_{tr}/A_{cr}$ was greater than 1). Before tetanization the ratio $A_{tr}/A_{cr}$ (with a delay of 20 msec between stimuli) in the experimental group was 4.4 ± 0.7 and was significantly higher than the ratio $A_{tr}/A_{cr}$ in the control group (2.5 ± 0.6). If the delay between stimuli was 30, 40, and 50 msec, an increase also was observed in the ratio $A_{tr}/A_{cr}$ in the experimental group compared with the control, but the differences are not significant. After tetanization, against the background of developing LPP, differences between the experiment and control (for all delays between stimuli) likewise are not significant.

The development of LPP was judged according to the increase (in %) in $A_{tr}$ and $A_{cr}$ after tetanization. The mean amplitude before tetanization in the control and in the experimental was taken as 100%. A developed LPP was spoken of only in the case when the amplitude of the potentiated response was more than 20% greater than the control value and this change lasted 40 min. In the remaining cases it was a matter either of the absence of LPP (amplitude of the potentiated response from 100 to 120%) or of a prolonged depression (amplitude of the response after tetanization less than 100%). The results in sections of the control and experimental groups with developed LPP were analyzed (Table 2). The calculated mean amplitudes of the conditioning and testing responses after tetanization (time of analysis 40 min) were calculated. In the control group LPP was observed in 7 sections out of 10 (70%), and on the average for $A_{cr}$ it was 250.3 ± 62.8%, for $A_{tr}$ 239.2 ± 95.5%. In the experimental group LPP was observed in 13 sections out of 19 (68%), and on the average for $A_{cr}$ was equal to 488.2 ± 146.7%, and for $A_{tr}$ 243.1 ± 63.7%. The differences between the values of the amplitudes of the conditioning responses in the control and experimental groups were statistically significant ($p < 0.05$, U criterion), which indicates a facilitation of the development of LPP in sections of the hippocampus of mice with KP.

As the writers showed previously [1], a considerable lowering of the threshold of the evoked seizure discharge is observed in area CA1 in sections through the hippocampus of mice with KP in response to stimulation of Schaffer's collaterals, evidence of a lasting change in the efficacy of the synaptic pathways. Pathological excitability of neurons in hippocampal slices from rats with KP also has been observed by other workers [9, 10], who observed in particular, that GABA-ergic inhibition in area CA1 was depressed [9] 24 h after the last seizures. During a study of changes in local evoked potentials in the hippocampus (CA1) of rats during development of KP induced by electrical stimulation, progressive weakening of the depression arising in response to paired stimuli was observed [8]. The authors cited note

TABLE 2. Intensity of LPP in Slices from Control and Experimental Groups

| Control | | | Experiment | | |
|---|---|---|---|---|---|
| Exp. No. | $A_{cr}$, % | $A_{tr}$, % | Exp. No. | $A_{cr}$, % | $A_{tr}$, % |
| 1 | 352 | 164 | 1 | 147 | 121 |
| 2 | 194 | 156 | 2 | 136 | (109) |
| | | | 3 | 148 | (112) |
| | | | 4 | 1278 | 331 |
| 3 | 492 | 590 | 5 | 168 | (115) |
| | | | 6 | (100) | 122 |
| 4 | 199 | 186 | 7 | 1721 | 543 |
| 5 | 139 | 193 | 8 | 137 | (113) |
| | | | 9 | 193 | 153 |
| 6 | 137 | (99) | 10 | 228 | (106) |
| | | | 11 | 353 | 162 |
| 7 | 239 | 146 | 12 | 126 | (100) |
| | | | 13 | 849 | 347 |
| | | | 14 | 863 | 166 |

Legend. Responses in control in both groups taken as 100%. Figures in parentheses indicate that LPP is absent and the data are not included in the analysis.

that a progressive disturbance of relations between excitatory and inhibitory processes arises as a result of weakening of inhibitory control in CA1, which is accompanied by lowering of the threshold of excitation of the pyramidal neurons. As was shown in [11], evocation of LPP is facilitated in the hippocampus during blockage of inhibition by the specific GABA antagonists bicuculline and picrotoxin. Meanwhile investigations [4] have shown that the amplitude of PS and of the population excitatory postsynaptic potential is 5 and 3 times less respectively in hippocampal slices of rats subjected to electroconvulsive excitation than in slices of the control group. The authors cited suggest that the worsening of memory as a result of ECT observed under clinical conditions may be linked with inhibition of LPP in the hippocampus. A lasting learning deficit has been found in rats after a series of spontaneous epileptic fits induced by intrahippocampal injection of tetanus toxin [7]. Another group of workers has found [5] that characteristics of LPP in hippocampal slices (area CA1) of mice (genetic epileptic mutants) are the same as those of phenotypically normal mice. Our own investigation and analysis of data in the literature suggests that KP may have different mechanisms depending on how the "kindling" is induced. During the development of KP, induced by metrazol, the decisive role is evidently played by weakening of inhibitory influences. The strengthening of facilitation which we found in hippocampal slices from mice with metrazol-induced KP in response to paired stimulation and the increase in LPP may be due to weakening of inhibition in intrahippocampal connection.

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