Time course of repetitive heterosynaptic facilitation in *Aplysia californica*

The conditioning effect of a strong priming afferent stimulation of one nerve on the excitatory postsynaptic potential (EPSP) which follows a weaker test stimulus of another nerve was demonstrated in single nerve cells of the abdominal ganglion of *Aplysia* by Kandel and Tauc[2–4]. This was termed heterosynaptic facilitation (HSF). The present paper focuses on the exhaustion–recovery cycle of HSF and presents support for the view that some of the test neurons can be exhausted by stimulation of the priming nerve alone.

The experiments were conducted in over 50 specimens of *Aplysia californica*. Intracellular recordings were made from the giant cell of the right upper quadrant of the visceral ganglion in the usual way with micropipettes. For the test and priming stimulation, a combination of two of the five nerves or connectives of the ganglion were used and the stimulus polarity reversed after each stimulus.

If the preparation was allowed to rest for 15 min between the periods of stimulation and if the single priming periods did not exceed 1 min, HSF could be repeated over 25 times without gross decay. After 5–10 min, continuous paired stimulation without intervals of rest led to a reversible exhaustion, during which the priming effect on the test response decreased to almost zero. Exhaustion could also be obtained by continuous priming alone, *i.e.* without stimulating the test nerve. A comparatively strong and frequent priming stimulus continuously applied for 15 min was able to prevent subsequent HSF completely (Fig. 1). A continuous priming stimulation of submaximal strength, producing excitation of a smaller number of afferent and internuncial fibres, had a different effect. The amplitude of the HSF in this case was initially even larger than the effect of pairing in the fresh preparation or the first test response after a long period of rest, but the duration during which the peak amplitude was maintained was significantly shortened (Fig. 2). The test response increased again as soon as the test stimulation or the priming stimulation or both were temporarily stopped.

Exhaustion of the priming unit as an explanation for the exhaustion of the HSF seems to be unlikely for several reasons: (1) Switching the priming stimulation to another 'fresh' nerve did not restore an already exhausted HSF. (2) The priming shock still produced spikes at the recorded neuron. (3) After prolonged priming, the test response finally failed or fell beneath control levels. It appears more probable that test units were fired by collaterals from the priming side and an exhaustive amount of transmitter was consumed. Under the circumstances of long periods of submaximal priming, some of the test terminals apparently escaped being fired and exhausted, but were potentiated instead perhaps under the influence of presynaptic graded events. Since the amplitude of the test response is an indicator of transmitter release[1], it could be assumed that within the terminals of these test units transmitter was mobilized from a prestage into a more active form during the prolonged priming period. This accumulated transmitter would then be fully activated and liberated at once during subsequent paired stimulation, causing a large but short-lived increment of the test response.

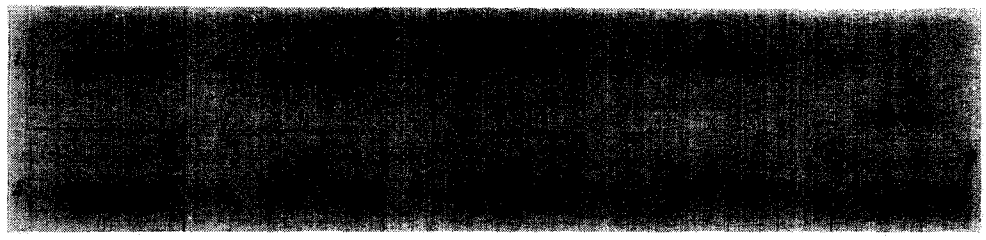

Fig. 1. Heterosynaptic facilitation and its exhaustion by priming alone. Right upper quadrant giant cell. Test stimulus: branchial nerve; priming stimulus: siphon nerve. 1, Control test response. 2, Augmented test response 10 sec after third pairing. Note priming response at end of trace. 3, 1 min after pairing, priming stimulus switched off, test response remains large. 4, 3.5 min after pairing, test response reaches control level. 5, Priming alone. 6, Unspecific facilitation. First test response immediately after 10 min of priming alone (4 shocks every 5 sec). 7, Test response immediately after 15 min priming alone, augmented test response starts to decay. 8, Test response after 20 min of priming alone. The exhausted test units no longer show facilitation. 9, Even paired stimulation (10 sec after third pairing) does not produce HSF.

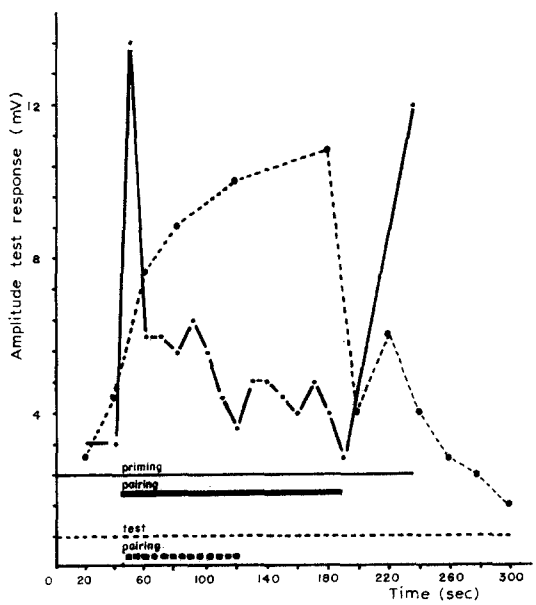

Fig. 2. Comparison between HSF in a fresh preparation and after 15 min of priming alone. Dotted line: Amplitude of test response during and after pairing trials in a fresh preparation. Note prolonged facilitation. Solid line: Amplitude of test response during and after pairing trials, following an exhaustive period of 10 min of submaximal priming alone. As soon as paired shocks are applied, a strong facilitation appears but only for a short period. After stop of the test stimulation for 25 sec —but with continued priming—recovery of some test units appears and a subsequent test stimulation indicates facilitation again.

In Fig. 3 the HSF obtained by pairing a test stimulus with a priming shock of submaximal strength is compared with the HSF after priming alone. The pairing caused noticeably better HSF than priming alone, a result which was reproducible in several preparations when the strengths of the priming stimulus and the test

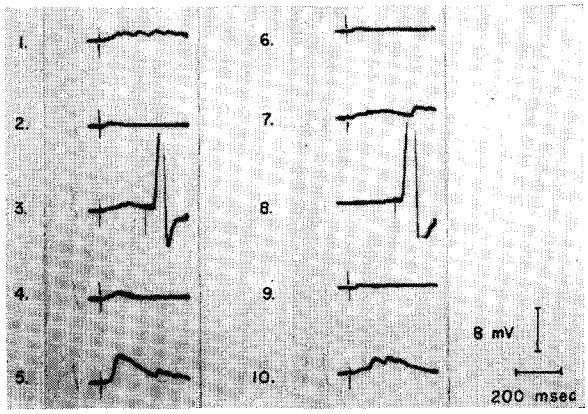

Fig. 3. Comparison between the size of HSF after paired and unpaired stimulation. 1, Test response control (after reaching steady state). 2, Test response control with reversed polarity causes smaller EPSP. 3, Paired stimulation. Only one single priming shock which triggers a spike is applied. 4, 5 sec after pairing. Reversed test stimulus. The small EPSP has grown compared with 2. 5, Response of test stimulus with original polarity: large HSF. 6, Control of the test response after 10 min. 7, Control with reversed polarity. 8, Single priming shock alone. 9, Test response 5 sec later, no effect. 10, Test response 10 sec later. The HSF is smaller than after pairing in 5.

stimulus were carefully adjusted before and then kept constant during the experiment.

Our findings support the hypothesis that the degree of specificity or unspecificity of HSF may partially depend, respectively, upon the lack or upon the presence of excitatory synaptic connections between the priming nerve and the test units. HSF would then require in both cases the discharging of the priming and the test unit in close temporal relationship. In the specific cases of 'true conditioning' the interconnecting fibres would be absent, but the test unit could be fired by paired stimulation in close temporal relation to the priming shock. In the unspecific cases of 'pseudo-conditioning', stimulation of the test nerve is not necessary because anatomical collaterals of the priming units would always fire the test units at the right time. In a third group of cells, fewer interconnections allow only a quantitative difference between sensitization and conditioning (Fig. 3). Most of the cells with HSF we encountered in the abdominal ganglion have been of this transitional type.

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1 ECCLES, J. C., *The Physiology of Synapses*, Springer, Berlin, 1964, pp. 82–100.
2 KANDEL, E. R., AND TAUC, L., Mechanism of prolonged heterosynaptic facilitation, *Nature (Lond.)*, 202 (1964) 145–147.
3 KANDEL, E. R., AND TAUC, L., Heterosynaptic facilitation in neurones of the abdominal ganglion of *Aplysia depilans*, *J. Physiol. (Lond.)*, 181 (1965) 1–27.
4 KANDEL, E. R., AND TAUC, L., Mechanisms of heterosynaptic facilitation in the giant cell of the abdominal ganglion of *Aplysia depilans*, *J. Physiol. (Lond.)*, 181 (1965) 28–47.

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