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RELATIVE CONTRIBUTION OF SYNAPTIC AND NON-SYNAPTIC INFLUENCES TO RESPONSE DECREMENTS IN A POST-SYNAPTIC NEURONE

By CATHY LAMAR STEPHENS

Department of Physiology, Center for the Health Sciences, University of California, Los Angeles, California 90024

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INTRODUCTION

Habituation phenomena and decrements of neural responses in isolated nervous structures have been related to depression of synaptic potentials (Bruner & Kehoe, 1970; Kandel *et al.* 1970; Horn & Wright, 1970; Zucker, 1972). However, previous research (Stephens, 1973) has shown that repeated intracellular stimulation of neurones in the isolated abdominal ganglion of *Aplysia californica* produces response decrements common to trans-synaptic models of habituation (Bruner & Tauc, 1966*a*, *b*; Buchwald, Halas, & Schramm, 1965; Kupfermann, Castellucci, Pinsker & Kandel, 1970; Thompson & Spencer, 1966). The demonstration of a decremental process resulting from intracellular stimulation and independent of synaptic modification indicates that there may be two factors contributing to trans-synaptic decrement of firing probability. Both synaptic depression and a process originating in the non-synaptic membrane of the post-synaptic neurone could cause a decrease in firing of the post-synaptic cell. The research reported here examines some aspects of the relative influence of these two factors.

METHODS

The experiments were performed on the right dorsal giant cell (Frazier *et al.* 1967) in the abdominal ganglion of *Aplysia californica*. The experimental arrangement for intra-cellular recording and stimulation was the same as that used in a previous study (Stephens, 1973). Synaptic input to the giant neurone was produced by stimulating the left connective placed across a bipolar electrode positioned just above the bathing medium. A constant inter-stimulus interval was used ranging in different experiments from 2 to 10 sec. All experiments were carried out at 21–23 °C.

RESULTS

Decrement of firing probability

As with direct intracellular stimulation (Stephens, 1973), synaptic stimulation of the dorsal giant neurone produced a progressive decrement in the probability of eliciting an action potential. As indicated by Fig. 1, during the first block of ten trials every

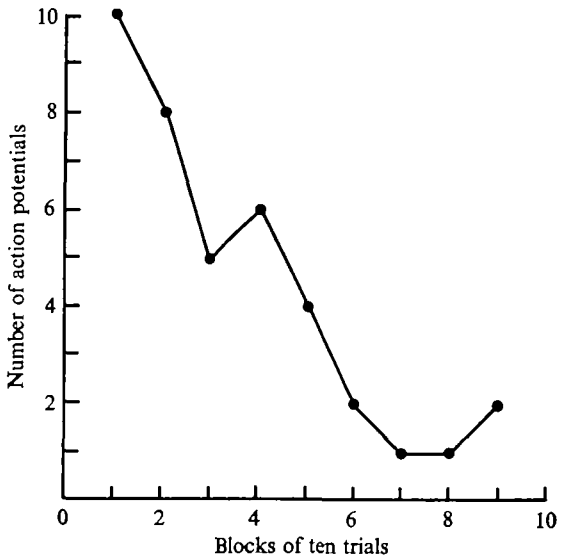

Fig. 1. Decrements in action potential production following repeated trans-synaptic stimulation. Stimulation of the left connective produced either one action potential or no action potential in the dorsal giant neurone. The trials are grouped in blocks of ten. The first ten stimulations induced action-potential discharge everytime; stimulations 81–90 (9th block) produced two action potentials.

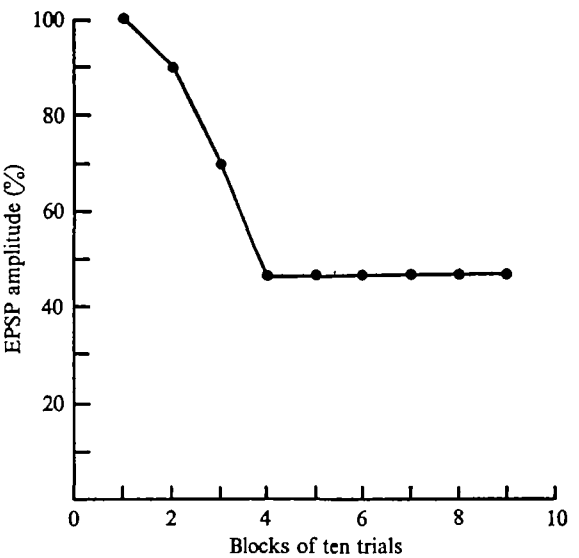

Fig. 2. Decrease of EPSP amplitude following repeated trans-synaptic stimulation in a neurone hyperpolarized to block action potentials. Each point is the average EPSP amplitude for a block of ten trials, normalized to the average amplitude of the first block.

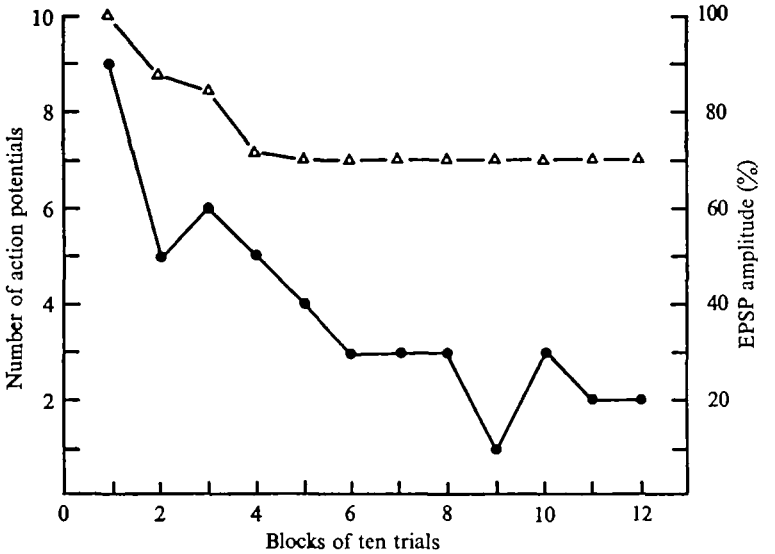

Fig. 3. Decrements of action potential and of EPSP seen in the same neurone. The EPSP amplitude was measured when an action potential was not initiated. The top graph indicates the average amplitude of the EPSPs which did not produce spikes in each block of trials; the lower graph is the number of action potentials in corresponding blocks of trials.

stimulus produced an action potential, while by the seventh block of ten trials no action potentials were elicited. Recovery took place after 10–20 min; at least 30 min elapsed between successive tests.

Decrement of excitatory post-synaptic potential (EPSP) amplitude (in a hyperpolarized cell)

The decrease in spike production following trans-synaptic stimulation has typically been attributed to depression of the EPSP (Bruner & Kehoe, 1970; Horn & Wright, 1970; Kandel *et al.* 1970). If the post-synaptic neurone is hyperpolarized to prevent action potentials and is stimulated at the same rate and intensity as in the previous experiment, then the amplitude of the EPSP can be measured (which is not possible when a impulse arises from the EPSP). Fig. 2 shows that the amplitude of the EPSP (in a different neurone from that of Fig. 1) does progressively decrease during repeated stimulation. With an inter-stimulus interval of 3 sec the EPSP amplitude reached a constant low value after four blocks of ten stimuli. Thus, the time course of decrement of the EPSP does not seem to follow that of the decrease in firing probability seen in the cell of Fig. 1.

Comparison of firing probability and EPSP decrements

In the above experiments it was possible that the difference in time-courses of decrement of firing probability and of EPSP amplitude could have resulted from a difference between the two cells. One can examine both effects in the same experiment by measuring the magnitude of the EPSPs that fail to elicit action potentials during trans-synaptic stimulation. Fig. 3 shows a typical result of measures of both the EPSPs and firing probability in the post-synaptic neurone during repeated trans-

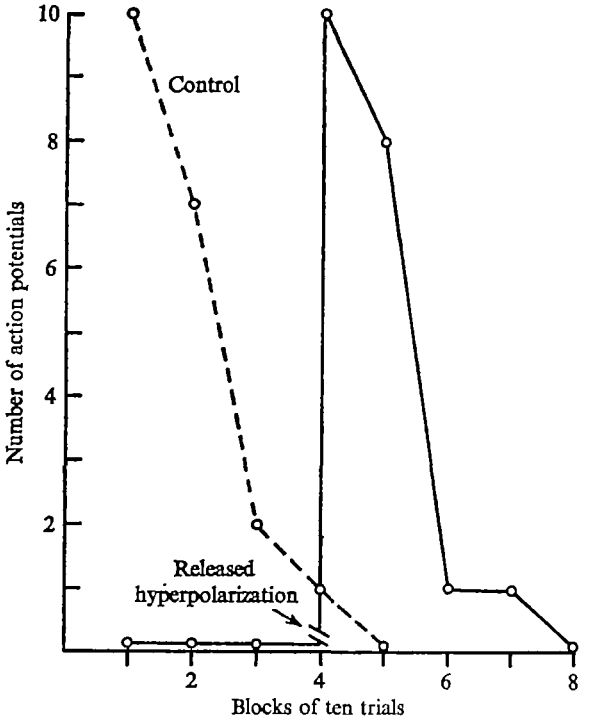

Fig. 4. Effects of trans-synaptic stimulation during intracellular hyperpolarization (and suppression of spikes) upon subsequent trans-synaptic action-potential discharge in the non-hyperpolarized cell. The inter-stimulus interval was 2 sec.

synaptic stimulation. The EPSP amplitude reached a constant low value while the firing probability continued to decrease in subsequent blocks of trials.

Decrement of firing probability following pre-decrement of EPSPs

It is possible to separate the occurrence of EPSPs from that of action potentials in a cell by stimulating trans-synaptically for several blocks of trials while the cell is hyperpolarized sufficiently to block action potential production. The resultant EPSPs will show decrements during repeated stimulation just as if there were no hyperpolarization. If the decrease of the EPSP amplitude is the major mechanism responsible for the decrement in the probability of action-potential discharge upon repeated trans-synaptic stimulation, then a repeated trans-synaptic stimulus which had previously produced, for example, a 90% decrease in number of spikes (when the post-synaptic neurone was not hyperpolarized) would be expected to produce approximately the same level (90%) of decrement in action-potential firing probability following release of the hyperpolarization. On the other hand, if the decrease of EPSP amplitude did not influence the production of spikes, then repeated trans-synaptic stimulation during the suppression of action potentials under the conditions described above should have little effect on excitability of the post-synaptic neurone (as measured by the probability of action potential discharge) or on subsequent decrements in excitability. Intermediate decrements after removing the hyperpolarization would indicate the effect of both processes.

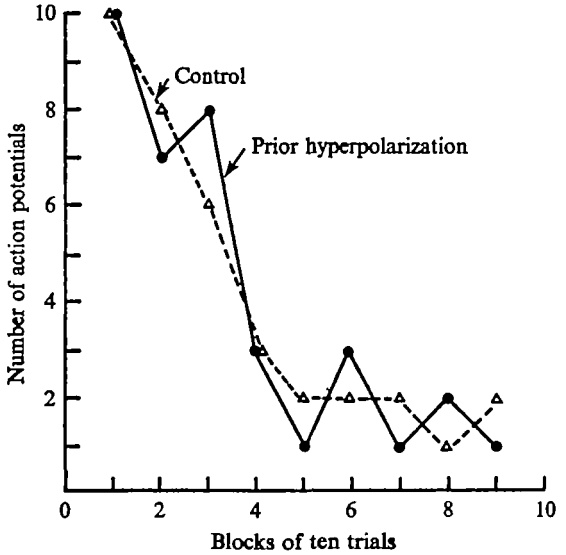

Fig. 5. Response decrements with and without prior hyperpolarization. The giant cell was hyperpolarized for 1 min before trans-synaptic stimulation. Prior hyperpolarization had no effect on action-potential probabilities induced by subsequent stimulation.

Experiments of this type were conducted by repeatedly stimulating the left connective while the right dorsal giant neurone was hyperpolarized at a level sufficient to prevent spike discharge. During the period of hyperpolarization the EPSP markedly decreased. The hyperpolarization was subsequently released while trans-synaptic stimulation continued. Fig. 4 demonstrates that the initial response level and subsequent decrease in the probability of action potential discharge during trans-synaptic stimulation were unaffected by decrement of the EPSPs. This suggests that, in this situation, EPSP decrements were not responsible for the normal decrease in action-potential production following repeated trans-synaptic stimulation. In another situation in which the decreased EPSP amplitude was just at or below threshold the decrease in EPSP amplitude would be expected to have a predominant influence.

Since hyperpolarization (Hodgkin & Huxley, 1952) has the possible effect of increasing the subsequent excitability of neurones, the effect of hyperpolarization *per se* was studied in other experiments. Prior to trans-synaptic stimulation hyperpolarization was applied intracellularly for the same period of time as that used in the above experiments. The hyperpolarization applied before the beginning of trans-synaptic stimulation had no effect on the subsequent course of the response decrements (Fig. 5).

DISCUSSION

The similar time-course of decrement of EPSPs and of firing probability with repeated trans-synaptic stimulation in the *Aplysia* giant neurone make it appear that synaptic depression may be the cause of decrement of the firing probability. However, the results of the present experiments indicate that such a mechanism cannot account fully for the decrement of firing rate; and further, that a normal time-course of decrement of firing is observed even after the EPSP has been maximally reduced.

These and previous results (Stephens, 1973) point towards the post-synaptic membrane as the site of such 'plastic' changes. Constancy of membrane conductance has been invoked by some authors (Castellucci *et al.* 1970) as an argument against post-synaptic changes. However, such tests are subject to experimental errors of current and voltage measurements which may be sufficiently large to conceal small changes in conductance. Additionally, the post-synaptic firing threshold may be increased by repeated stimulation, and only small changes in membrane potential or threshold may be needed to block production of action potentials.

Since decrements of firing probability have been shown to occur with repeated intracellular depolarization of the post-synaptic cell (Stephens, 1973), it is possible that the decrement in firing seen with trans-synaptic stimulation may be mediated by depolarization resulting from action potentials produced. The mechanism of such a reduction of post-synaptic excitability may involve Na-inactivation or K-activation, since both processes have been shown to result from depolarization (Hodgkin & Huxley, 1952; Geduldig & Gruener, 1970). However, further studies will be needed to establish the exact mechanisms responsible for the changes observed in the post-synaptic cell.

In general, the findings reported here emphasize the need to take into account the possibility of alterations in individual neurones independent of synaptic effects in developing models of habituation-like phenomena.

SUMMARY

1. Repeated trans-synaptic stimulation of the giant neurone in the abdominal ganglion from *Aplysia californica* produced decrements of nervous activity.

2. Although the EPSP amplitude decreased during the stimulation, the firing probability of the post-synaptic neurone continued to decrease after the amplitude had reached a constant low value.

3. When the cell was hyperpolarized and stimulated until the EPSP had decreased to a constant low value, the firing probability immediately after removal of the hyperpolarization was the same as with no previous stimulation.

4. These results and previous work suggest that a process occurring in the post-synaptic neurone, independent of synaptic modification, may contribute significantly to habituation-like phenomena.

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