INVESTIGATION OF HETEROSYNAPTIC INTERACTIONS IN HIPPOCAMPAL AREAS CA1 AND CA3 IN VITRO

A. G. Bragin and N. A. Otmakhov

Heterosynaptic interactions between synapses located at a considerable distance from the cell body (perforant path) and lying close to the body of the neuron (synapses of Schaffer's collaterals and axons of the dentate fascia) on guinea pig hippocampal neurons were investigated in vitro. It was shown by the paired stimulus method that, using stimulation of subthreshold intensity for action potential generation, spatiotemporal summation takes place in both pairs of synaptic systems. If above-threshold stimulation was used, afferents lying close to the cell body suppressed responses evoked by stimulation of distant afferents for a longer time (up to 20 msec in area CA1 and up to 300 msec in area CA3) than during the opposite combination of stimuli (up to 3-8 msec). After tetanization of the dentate fascia depression of responses of area CA3 neurons to stimulation of the perforant path was observed for 2-30 min. In the remaining cases, no significant prolonged heterosynaptic posttetanic changes were observed. The possible mechanisms of these interactions are discussed.

INTRODUCTION

The functional role of direct and relayed influences of the cortical input (perforant path -- PP) of the hippocampus has not yet been explained (Fig. 1). Uninterrupted fibers of PP are known to form synapses on terminal apical dendrites of neurons in areas CA1 and CA3. Relayed influences are transmitted to "strategically more effective" regions -- the principal inputs of the apical dendrites (stratum lacunosum in area CA1 -- the sites of endings of Schaffer's collaterals -- SC -- and the stratum lucidum in area CA3 -- the sites of endings of giant synapses from the dentate fascia -- DF).

Several authors [9, 19, 20] consider that the relayed cortical input of the hippocampus is functionally the most important, that all information from the cortical regions runs along the trisynaptic DF--CA3--CA1 chain, and that the direct cortical input has only a weak modulating influence. More recent work has shown, however, that the direct cortical input
Fig. 1. Scheme of hippocampal slice with location of electrodes and additional incisions. $S_1, S_2, S_3$) stimulation, $R_1, R_2$ recording electrodes, $i, 2, 3$) additional incisions. PP) perforant path, SC) Schaffer's collaterals, MF) mossy fibers, DF) dentate fascia.

Fig. 2. Spatiotemporal summation between area CA3 afferents (DF and PP). Abscissa, interval between conditioning (DF) and testing (PP), stimuli (logarithmic scale), msec; ordinate, probability of generation of AP by neurons in response to testing stimulation. C) Testing stimulation of PP. Stimulation of both inputs at subthreshold level (see text).

can evoke action potential generation in hippocampal neurons [2, 3, 11]. It is accordingly interesting to study the role of interaction between this input and the functionally powerful indirect input which duplicates it.

Data on heterosynaptic interaction between these inputs would evidently help to shed light on these problems.

In the present investigation interaction between three groups of connections on single hippocampal neurons was studied (Fig. 1): different synapses from the same source (DF), making contact with area CA3 neurons at a point where the soma joins the dendrite; synapses from different sources, some of which are located near the neuron soma (synapses of DF), others on terminal dendrites at a distance of 400-500 $\mu$ from the cell body (synapses of PP fibers on area CA3 neurons); synapses from different sources, some of which are 300-400 $\mu$, others 500-600 $\mu$ away from the cell body (synapses of SC and PP on area CA1 neurons).

**EXPERIMENTAL METHOD**

Experiments were carried out on surviving slices of the guinea pig hippocampus. The animals were killed by a blow on the neck, after which the brain was removed and the hippocampus isolated. Slices 300-500 $\mu$ thick were cut manually, perpendicularly to the longitudinal axis of the hippocampus. All operations involved in preparing the slices lasted not more than 3-5 min. The slices were incubated in an experimental continuous-flow chamber in Ringer-Krebs solution saturated with carbogen (95% O$_2$ and 5% CO$_2$); the incubation temperature was $36 \pm 1^\circ$C and the pH of the solution $7.4 \pm 0.2$.

Before the experiment, to abolish indirect influences of PP on area CA3, transmitted through DF, PP running to DF was divided in the slice, and to rule out any possible antidromic influences and accompanying excitation of the commissural fibers during electrical stimulation, incisions were made between CA1 and CA3, as far as the pyramidal layer, and also between the subiculum and CA1 down to the molecular layer. Stimulating electrodes (nichrome, diameter 100 $\mu$) were located in the granular layer of DF, in the region where PP passes through the subiculum, and into str. radiatum in area CA2 (to stimulate SC).