

Temporal relations between hippocampal unit activity and phases of the theta-waves were studied in unanesthetized rabbits immobilized with tubocurarine. When spontaneous theta-rhythm potentials were recorded layer by layer from the dorsal hippocampus, a change in their polarity was observed 0.15-0.2 mm deeper than the pyramidal layer (i.e., in the radial layer). Most hippocampal neurons fired synchronously with the extracellular theta-waves. The numerous pyramidal cells, in which the spontaneous activity was inhibited during stimulation of the contralateral hippocampus and sciatic nerve and a hyperpolarization potential developed, were excited mainly during the positive phase of the theta-waves. Intracellular recording showed that their membrane potential decreased during the positive phase and increased during the negative phase. The less numerous basket cells, whose response to stimulation of the contralateral hippocampus consisted of a short high-frequency volley of spikes, and whose response to sciatic nerve stimulation was marked by a prolonged increase of frequency of the spontaneous discharges, were activated mainly during the negative phase of the theta-waves. It is concluded from these findings that inhibitory mechanisms play a role in the theta-activity of the hippocampus. It is postulated that the extracellular theta-waves are integral EPSPs of the basal dendrites and of the proximal segments of the apical dendrites, and that IPSPs of the pyramidal cell bodies play the principal role in the generation of the "intracellular theta-rhythm."

INTRODUCTION

It has often been stated [13, 15, 19, 21] that hippocampal neurons discharge in a definite phase of the theta-waves. The frequency of fluctuations of resting potential in its pyramidal cells was 3-6/sec; these waves were synchronous with waves of the extracellular theta-rhythm [5, 15]. Unit activity of this type has been called the "intracellular theta-rhythm." The results with respect to the phase in which hippocampal pyramidal cells are activated, obtained by these workers are contradictory.

There is likewise no general agreement regarding the nature of the extracellular theta-wave potentials. Green et al. [18] considered that they were an expression of "inactivation processes." Fujita and Sato [15] concluded that EPSPs and IPSPs play a part in their generation, but rejected any participation of inactivation in these processes. Some investigators [4, 8, 21, 25] consider that an important, if not the leading, role in generation of the hippocampal theta-rhythm potentials is played by recurrent inhibitory pathways and the IPSPs arising through their participation on the bodies of the pyramidal cells.

The author has studied extracellular theta-waves at different layers of the hippocampus and has correlated the activity of the pyramidal and basket cells with the phases of these waves in order to obtain additional evidence on the nature of the electrical potentials of the hippocampal theta-rhythm.

METHOD

Unanesthetized rabbits were immobilized with tubocurarine. Potentials were recorded from neurons of the dorsal hippocampus (areas CA₁ and CA₂) by means of glass microelectrodes filled with 4 M NaCl or 2 M potassium citrate and inserted through the neocortex. Global extracellular potentials were recorded

A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. Translated from *Neirofiziologiya*, Vol. 4, No. 5, pp. 531-539, September-October, 1972. Original article submitted March 29, 1972.

© 1973 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for \$15.00.

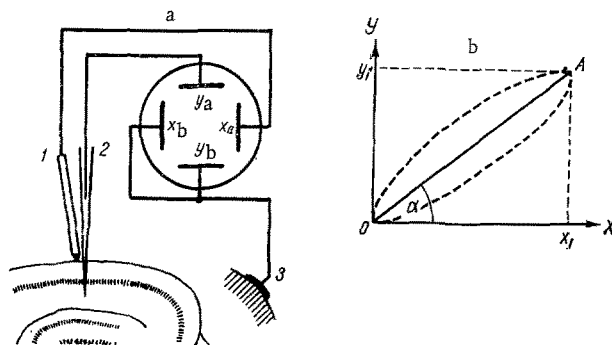


Fig. 1. Layer by layer vector analysis of hippocampal theta-waves: a) simplified scheme of recording system; b) scheme of vector analysis.

by a macroelectrode consisting of a Nichrome wire, 0.1 mm in diameter, insulated except at its tip. The macroelectrode was inserted into the layer of neocortex to a depth of 1.5-2 mm directly above the hippocampal area to be studied. The potentials recorded simultaneously by the micro- and macroelectrodes were amplified and photographed on film or recorded on paper.

Transcommissural responses were obtained by stimulating the symmetrical point of the contralateral hippocampus. Peripheral stimulation consisted of stimulation of the undivided sciatic nerve. Details of the method were described previously [1,2].

To investigate the relationship between unit activity and fluctuations in the global extracellular potential recorded at the same time, a method of graphic superposition of cuts of the records in which identical phases of the theta-waves in each record coincided was used. The complete cycle of the averaged theta-wave was broken up into time cuts of 20 msec each so that the number of spike discharges could be counted. Histograms of their frequency distributions were then plotted.

The layer by layer analysis of amplitude of the theta-waves was carried out by comparing the amplitudes of the potentials recorded by means of a microelectrode at intervals of 0.1 mm during its insertion, and by means of a macroelectrode from the surface of the hippocampus. Measurements of the amplitude of 10-15 waves were averaged and plotted on a graph against the depth of recording. However, this is a very laborious way of processing the data, and in most experiments a method of vector analysis was used. It consists essentially of the following. Potentials recorded by means of the microelectrode 2 (Fig. 1), inserted into the brain relative to the reference electrode 3, were amplified and led to the plates y_a and y_b causing vertical deviation of the beam of a cathode-ray tube; potentials recorded by the macroelectrode 1 from the hippocampal surface relative to the reference electrode 3 were led to the plates x_a and x_b producing horizontal deflection. Since the phase difference between the signals in the channels of the buried and surface electrodes in most cases was not a multiple of 2π , the beam of the cathode-ray tube described a figure which was not a straight line but resembled an elongated ellipse. The ratio between the amplitude of the theta-wave recorded by the buried electrode (y_1) and its amplitude recorded from the surface of the hippocampus (x_1) (Fig. 1b) is constant and equal to the tangent of the angle between the major axis OA of the ellipse and the abscissa OX, which coincides with the direction of movement of the beam if no signal is applied to the plates y_a and y_b (the channel of the buried electrode): $\tan \alpha_1 = y_1/x_1$. If the mean amplitude of the theta waves arising on the surface of the hippocampus (x_m) is constant in each case, the mean value of the amplitude y_m at each given depth will be proportional to $\tan \alpha_n \cdot y_{m,n}$:

$$y_{m,n} = \frac{y_{m,1} \cdot \tan \alpha_n}{\tan \alpha_1}.$$

The mean value $y_{m,1}$ of the amplitude of the theta-waves for an arbitrarily chosen depth was determined from the sum of the variants.

RESULTS

The form of the theta-waves recorded from the hippocampal surface differed from sinusoidal (Fig. 5). The positive phase of most waves was flattened or truncated by comparison with the dome-shaped or

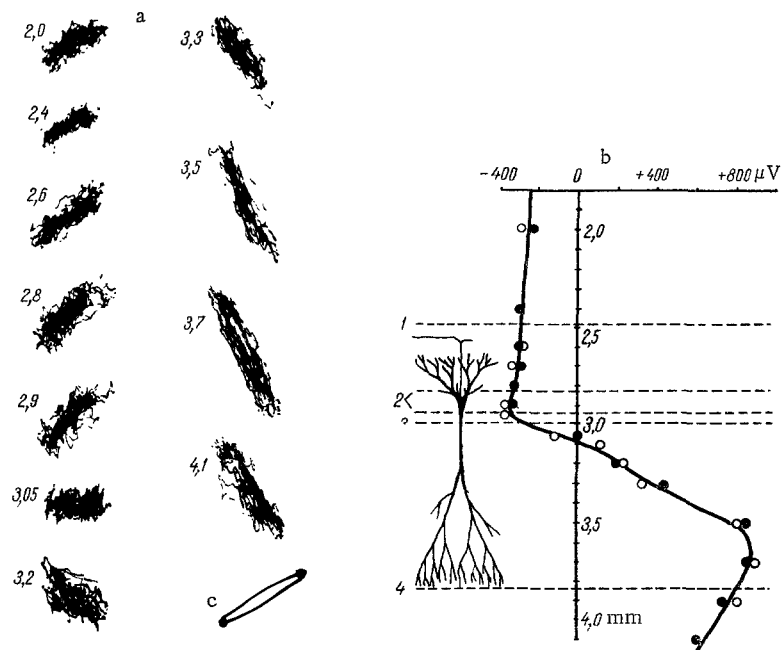


Fig. 2. Layer by layer analysis of hippocampal theta-waves: a) vector diagrams of theta-waves recorded from various depths (numbers indicate depth in mm) from surface of neocortex over hippocampus; c) calibration (sinusoid voltage of $250 \mu V$, 50 Hz applied in phase to inputs of amplifiers of both channels); b) amplitude of theta-waves as a function of depth of recording. Ordinate, depth of recording, mm; abscissa, average amplitude of potentials, μV . Graph plotted from results obtained by statistical averaging (empty circles) and vector analysis (filled circles) in same experiment; 1) dorsal surface of hippocampus; 2) boundaries of pyramidal layer; 3) level of reversal of transcommissural EP; 4) hippocampal fissure.

triangular negative phase. The nonsinusoidal shape of the theta-waves has also been described by other workers [6,20]. Faster waves (up to 15/sec) were superposed on these waves. Comparatively high-amplitude theta-waves, it will be noted, were recorded in an area of neocortex located above the hippocampus (Fig. 2a, depth 2 mm) and even in the layer of agar-agar over the surface of the neocortex (see also [19]). It is considered that theta-waves recorded in such cases may be conducted purely physically from the underlying hippocampus [19,4].

The results for the layer by layer distribution of potentials during hippocampal theta-activity obtained in four experiments by the method of vector analysis show good agreement with the results of analysis of three experiments in which the method of averaging was used (Fig. 2b).

As the recording microelectrode was inserted deeper into the brain the amplitude of the theta-waves gradually increased, to reach a maximum (300-500 μV) at approximately the lower border of the pyramidal layer. It then began to decrease rapidly, down to zero, and at the level of the radial layer (about 0.2 mm below the bodies of the pyramidal cells) the sign of the potential was reversed. The amplitude of the reversed theta-waves reached its maximum (600-1000 μV) at a depth of 0.6-0.8 mm below the pyramidal layer, in the region of the branched areas of the apical dendrites of the pyramidal cells. Deeper, their amplitude was reduced. The fastest rate of change in amplitude of the theta-waves with depth was observed in the region of the bodies of the pyramidal cells and the distal parts of their apical dendrites (Fig. 2b). Evidently the density of the extracellular currents was maximal in this region. The theta-waves presumably arise on account of longitudinal electric currents between the bodies of the pyramidal cells and their basal dendrites, on the one hand, and the most proximal segments of the apical dendrites, on the other hand; this hypothesis is in agreement with earlier conclusions [19].

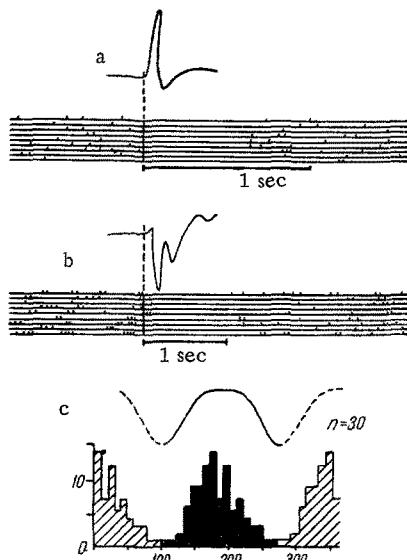


Fig. 3

Fig. 3. Analysis of spontaneous and evoked activity of a hippocampal neuron with responses of inhibitory type: a) responses to stimulation of contralateral hippocampus; b) responses to stimulation of sciatic nerve; above — averaged hippocampal EP, below — superposition of nine unit responses. Here and in Figs. 4 and 5 broken line denotes moment of stimulation; c) histogram of distribution of spontaneous unit discharges depending on phases of theta-wave. In center: complete cycle of theta-rhythm. Averaged theta-wave shown above histogram. Ordinate, number of discharges in each 20 msec; abscissa, time, msec; n) number of cycles of spontaneous activity. Here and in Figs. 4 and 5 upward deviation corresponds to positivity beneath the recording electrode.

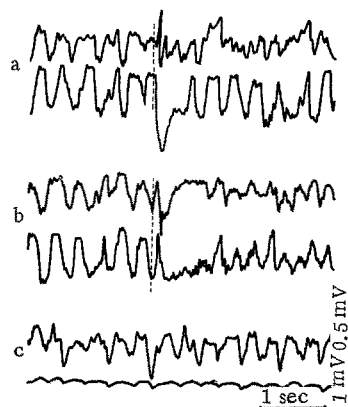


Fig. 4

Fig. 4. Fluctuations in membrane potential of pyramidal neuron during spontaneous activity and after stimulation: a) IPSP in response to stimulation of contralateral hippocampus; b) response to stimulation of sciatic nerve; c) electrical activity recorded immediately after withdrawal of microelectrode from cell. Top curve shows potentials recorded by macroelectrode from hippocampal surface, bottom curve potentials recorded by microelectrode.

The maximal amplitude of the potentials below the level of their reversal was 2-2.5 times greater than the maximal absolute amplitude above this level, possibly because of the curvature of the hippocampal cortex, on the basis of the solid angle theorem [26, 16].

In the next series of experiments the activity of various single units in the hippocampus was investigated in different phases of the theta-waves. The eight neurons tested responded neither to transcommisural nor to peripheral sciatic nerve stimulation, although their spontaneous discharges were synchronous either with the negative (five neurons) or with the positive (three neurons) phase of the extracellular theta-waves. In the character of their responses to these stimuli, the remaining 75 neurons were subdivided into two groups.

Fifty neurons were placed in the first group. In 15 of them inhibition of spike discharges (Fig. 3a, b) and an increase in the membrane potential (Fig. 4a, b) were observed (intracellular recording). Neurons which responded to stimulation of any of the direct inputs into the hippocampus by the appearance of IPSPs and by suppression of discharges were classed as pyramidal [10, 12]. The duration of the periods of inhibition in these cells was 200-600 msec during stimulation of the contralateral hippocampus, and several seconds during stimulation of the sciatic nerve (see also [1]). Inhibition evoked by peripheral stimulation in some cells was preceded by a short period of excitation, which was not observed during stimulation of the contralateral hippocampus.

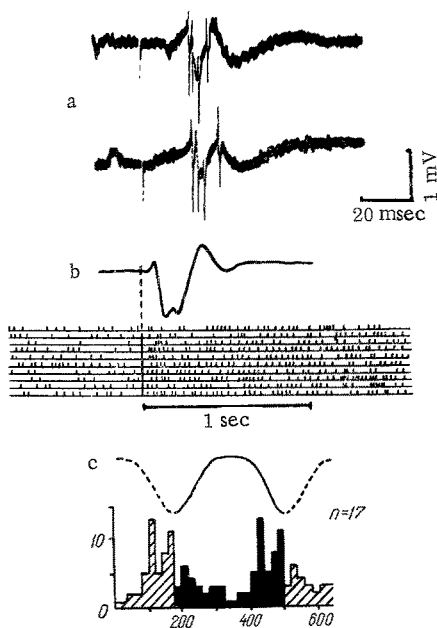


Fig. 5. Spontaneous and evoked activities of a hippocampal neuron with responses of excitatory type. Legend as in Fig. 3.

In 15 neurons of the first group, recorded intracellularly, clearly rhythmic waves of resting potential with a frequency of 3-5/sec were obtained (Fig. 4a, b); this has been called the "intracellular theta-rhythm" [15]. The amplitude of these oscillations reached several millivolts. Nearly always the wave consisted of a rounded, dome-shaped potential, in a direction increasing the membrane potential, and a flat potential of varied amplitude decreasing the membrane potentials. Sometimes the second phase was so shortened that the flat area at its apex disappeared completely. In addition, the shape of the waves was complicated by fast low-amplitude oscillations. The hyperpolarization phases of most waves of all 15 cells tested coincided with the development of the negative phase of the waves in the pyramidal layer and on the hippocampal surface. The poststimulation increase in membrane potential in these cells was accompanied by suppression of the next waves of the "intracellular theta-rhythm." After stimulation of the sciatic nerve they were restored much more slowly than after stimulation of the contralateral hippocampus.

Neurons of the second group (basket cells) discharged mainly in the negative phase of the theta-waves (Fig. 5c).

After stimulation of the sciatic nerve, during the period of increased frequency of the theta-rhythm for 2-3 sec, together with an increase in the intensity of spike activity in many neurons of this group there was a definite tendency for discharges to be generated in the earlier phases of the negative wave.

The frequency of discharges of both pyramidal and basket cells in the hippocampus is thus apparently modulated by its extracellular theta-rhythm. The activity of these various groups of cells shows mutually reciprocal relationships. When the negative phase develops on the surface of the hippocampus and in its pyramidal layer the pyramidal cells are in an inhibited state and the basket cells in an excited state. In the positive phase of the theta-waves the basket cells are relatively passive and the pyramidal cells active. These results show satisfactory agreement with those of other investigations [13, 20, 14] but they are in disagreement with the results obtained by Fujito and Sato [15]: depolarization and excitation of the pyramidal cells took place in the negative phase.

DISCUSSION

Because of their large number and the strict orientation of their axons and dendrites the pyramidal cells are the most likely sources of extracellular currents in the hippocampus. Layer by layer investigation of the hippocampal theta-waves shows that the pyramidal cells behave as electrical dipoles whose polarity varies synchronously and rhythmically. The individual currents of these dipoles undergo summation.

The spontaneous activity of this group of neurons was of very low intensity. Many neurons were initially silent, and they began to discharge as a result of injury by the microelectrode.

This group included 25 neurons whose responses to stimulation of the contralateral hippocampus consisted of a volley of high-frequency discharges (Fig. 5a), the number of which depended on the parameters of the stimulating current. During stimulation of the sciatic nerve the spontaneous firing rate of these neurons increased appreciably (Fig. 5b) for a considerable period of time (up to several seconds, see also [1]). Because of the activation character of their responses the cells of this group were classed as inhibitory interneurons [10, 12, 1]. All were recorded extracellularly at the level of the pyramidal layer or at the lower border of the layer of basal dendrites, and as a rule, their spontaneous firing rate was higher than that of the other neurons.

The spontaneous discharges of all neurons of the first group (pyramidal cells) appeared mainly in the positive phase of the theta-waves (Fig. 3c). The maximal probability of discharges, which for most pyramidal cells occurred at about the middle of the positive phase, was shifted to the right after sciatic nerve stimulation, i.e., it was delayed slightly by comparison with before stimulation.

The problem of the active phase (or phases) of the theta-waves can be solved to some extent in connection with their nonsinusoidal shape. In many records (of potentials from the hippocampal surface) the positive phase of the theta-waves was apparently not developed, by contrast with the negative phase. Other evidence in support of the view that the surface-negative phase of the theta-waves is active is the fact that during the increase in spike activity of the overwhelming majority of neurons in the pacemaker nucleus in the septum, the layer of basal dendrites of the hippocampal pyramidal cells is electrically negative [19]. The amplitude of the negative phase of the hippocampal evoked potential in response to sciatic nerve stimulation, when it develops against the background of the negative phase of the theta-wave, is reduced [7]. From this it could be concluded that the nature of the negative phases of the evoked potential and of the theta-waves is similar [6], in agreement with the hypothesis expressed above regarding the active character of the surface-negative phase of the hippocampal theta-waves. Its positive phase, on the other hand, is evidently relatively passive.

Reversal of the sign of the theta-waves at the level of the apical dendritic trunks suggests that EPSPs arising not only on basal dendrites [17], but also probably on the proximal segments of the trunks of the apical dendrites, are among the sources of genesis of the theta-waves in the hippocampus. The synaptic endings of axons arriving from the septum are known to be situated on the basal dendrites and on the proximal segments of the apical dendrites [9,23,25].

A possible explanation of the, at first sight, paradoxical mismatching between the activities of the hippocampal pyramidal neurons and the extracellular potentials of the theta-rhythm may be as follows. Hyperpolarization observed in the negative, active phase of the theta-waves is due to IPSPs, as is also shown by the distinctive shape of the fluctuations of membrane potential. Considering the strategically favorable position of the inhibitory synapses on the bodies of the pyramidal cells, they can very effectively hyperpolarize the axon hillock and suppress its excitation [11]. If the potentials are recorded from the cell body (the most likely type of recording) the microelectrode will be in the most favorable position for recording an IPSP. Perhaps the EPSPs recorded some distance away from the cell body, on the basal and apical dendrites, are weakened when conducted electrotonically into the soma. By undergoing algebraic summation with the high-amplitude IPSPs they are masked and suppressed by them.

Depolarization waves, otherwise known as "inactivation processes" [20], synchronized with the hippocampal theta-rhythm and capable of appearing without preceding cell discharges, can make a significant contribution to the rhythmic fluctuations of membrane potential recorded by means of the microelectrode. Their role in the extracellular theta-waves [14], on the other hand, cannot be regarded as proved, for as a rule they evidently arise in the positive phase.

The basket cells, which are active in the negative phase of the theta-wave, are evidently sources of impulses reaching the inhibitory synapses on the bodies of the pyramidal cells also during the development of hyperpolarization in the pyramidal cells [10]. Excitation of the basket cells in the active phase of the theta-waves can take place through recurrent collaterals of axons of the pyramidal cells [10,12,21] or by direct afferent fibers [2], which probably come from the septum. Since the spike activity of the pyramidal cells is low in any event, and it is inhibited in the negative phase of the theta-waves, activation of the inhibitory neurons through recurrent collaterals seems improbable.

Because activity of the hippocampal pyramidal cells is suppressed during the active phase of the theta-rhythm generated by the septum, it can be concluded that the pacemaker nucleus of the septum exerts its inhibitory action predominantly on impulses leaving the hippocampus.

In the experiments described above, in which potentials were recorded from successive layers, evidence of a powerful "source" of current at the level of the pyramidal cell bodies was never detected during the active phase of the theta-waves, which is identified with the extracellular manifestation of the IPSP [10,12]. On the contrary, at that moment this region was electrically negative. Activity of the inhibitory synapse thus evidently makes a negligible contribution to the generation of the extracellular potentials of the theta-rhythm.

LITERATURE CITED

1. D. P. Artemenko, "Inhibition in the hippocampal pyramidal neuron during peripheral stimulation," *Neirofiziologiya*, 1, 278 (1969).
2. D. P. Artemenko, "Layer by layer analysis of hippocampal evoked potentials to peripheral stimulation," *Neirofiziologiya*, 2, 434 (1970).

3. D. P. Artemenko, E. T. Tal'nova, and V. M. Shaban, "Layer by layer analysis of transhippocampal evoked potentials," Proceedings of a Conference of Junior Scientists of the Institute of Physiology, Academy of Sciences of the Ukrainian SSR, to commemorate the 50th Anniversary of the Foundation of the Academy of Sciences of the Ukrainian SSR [in Russian], Kiev (1969), pp. 4-5.
4. V. I. Gusel'nikov and A. Ya. Supin, Rhythmic Activity of the Brain [in Russian], Moscow University Press, Moscow (1968).
5. E. Kandel and W. Spencer, "Electrophysiological properties of neurons of the paleocortex," in: Current Problems in Electrophysiology [Russian translation], Mir, Moscow (1964), pp. 241-276.
6. P. M. Syerkov, "Electrophysiology of the hippocampus," Fiziol. Zh. (Ukr.), 12, 814 (1964).
7. F. N. Serkov and R. F. Makul'kin, "Layer by layer analysis of hippocampal evoked potentials," in: Electrophysiology of the Central Nervous System [in Russian], Metsniereba, Tbilisi (1966), pp. 274-275.
8. J. C. Eccles, The Physiology of Synapses, Berlin (1964).
9. P. Andersen, H. Bruland, and B. R. Kaada, "Activation of the field CA₁ of hippocampus by septal stimulation," Acta Physiol. Scand., 51, 29 (1961).
10. P. Andersen, J. C. Eccles, and Y. Løynning, "Recurrent inhibition in the hippocampus with identification of the inhibitory cell and its synapses," Nature, 198, 540 (1963).
11. P. Andersen, J. C. Eccles, and Y. Løynning, "Pathway of postsynaptic inhibition in the hippocampus," J. Neurophysiol., 27, 608 (1964).
12. P. Andersen, J. C. Eccles, and Y. Løynning, "Location of postsynaptic inhibitory synapses on hippocampal pyramids," J. Neurophysiol., 27, 596 (1964).
13. A. Arduini and O. Pompeiano, "Correlazioni tra onde elettriche e scariche di singole unita dell'ippocampo del coniglio cervello isolato," Boll. Soc. Ital. Biol. Sper., 30, 938 (1954).
14. C. von Euler and J. D. Green, "Excitation, inhibition and rhythmical activity in hippocampal pyramidal cells in rabbits," Acta Physiol. Scand., 48, 110 (1960).
15. Y. Fujita and T. Sato, "Intracellular records from hippocampal pyramidal cells in rabbit during theta-rhythm activity," J. Neurophysiol., 27, 1011 (1964).
16. P. Gloor, C. L. Vera, and L. Sperti, "Electrophysiological studies of hippocampal neurons. I. Configuration and laminar analysis of the resting potential gradient, of the main-transient response to perforant path, fimbrial and mossy fiber volleys and of spontaneous activity," Electroenceph. Clin. Neurophysiol., 15, 353 (1963).
17. G. Gogolak, C. Stumpf, H. Petsche, and J. Sterc, "The firing pattern of septal neurons and the form of the hippocampal theta wave," Brain Res., 7, 201 (1968).
18. J. D. Green, D. S. Maxwell, and H. Petsche, "Hippocampal electrical activity. III. Unitary events and genesis of slow waves," Electroenceph. Clin. Neurophysiol., 13, 854 (1961).
19. J. D. Green, D. S. Maxwell, W. Shindler, and C. Stumpf, "Rabbit EEG theta rhythm: its anatomical source and relation to activity in single neurons," J. Neurophysiol., 23, 403 (1960).
20. J. D. Green and H. Petsche, "Hippocampal electrical activity. II. Virtual generators," Electroenceph. Clin. Neurophysiol., 13, 847 (1961).
21. H. Noda, S. Manogar, and W. R. Adey, "Spontaneous activity of cat hippocampal neurons in sleep and wakefulness," Exptl. Neurol., 24, 217 (1969).
22. H. Petsche, G. Gogolak, and C. Stumpf, "Die Projektion der Zellen des Schrittmachers für den Thetarhythmus auf den Kaninchenhippocampus," J. Hirnforsch., 8, 129 (1966).
23. H. Petsche and C. Stumpf, "Topographic and toposcopic study of origin and spread of the reticular synchronizing arousal pattern in the rabbit," Electroenceph. Clin. Neurophysiol., 12, 589 (1960).
24. G. Raisman, W. M. Cowan, and T. P. S. Powell, "The extrinsic, afferent, commissural and association fibers of hippocampus," Brain, 88, 963 (1966).
25. W. A. Spencer and E. R. Kandel, "Hippocampal neuron responses to selective activation of recurrent collaterals of hippocampofugal axons," Exptl. Neurol., 4, 149 (1961).
26. J. W. Woodbury, "Potentials in a volume conductor," in: T. C. Ruch, H. D. Patton, J. W. Woodbury, and A. L. Towe, Neurophysiology, W. B. Saunders, Philadelphia and London (1965), pp. 73-91.