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CONTRIBUTION TO THE FORMATION OF *THETA* RHYTHM IN CAT HIPPOCAMPUS *

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TRĄBKA J.: *Contribution to the Formation of Theta Rhythm in Cat Hippocampus.* Acta Physiol. Polon. 18 (6) : 857—864, 1967. — The study is contributing to the problem of *theta* rhythm formation in hippocampus (H). According to other authors it was never obtained by direct stimulation of H. The present investigation is aimed to indicate a possibility of *theta* rhythm induction using the method of direct, chemical stimulation of H. Chronic experiments were carried out on 25 cats, Carboline compound (1-methyl-6-methoxy-1,2,3,4-hydro-β-carboline) was introduced by a canula, inserted by stereotaxic method in dorsal portion of H. Chemical irritation of H was performed either by application of the crystalline compound (15—30 mcg) or by injecting the compound in solution (50—150 mcg). The results of study suggests *theta* rhythm to be an autochthonic process appearing primarily in H. The slow rhythm local formation in H under the influence of chemical irritation was stated. The gradually developing synchronization, leading to *thetha* rhythm manifestation was examined.

Among all structures of the central nervous system, the hippocampus (H) is characterized by highest abundancy of forms of EEG records. According to *Bradley* and *Nicholson* even the individual regions of H show different types of EEG activity. Among the diverse bioelectric phenomena in H the *theta* rhythm is of great interest; it was described first by *Green* and *Arduini* in their studies on arousal reaction in rabbits. It is much more difficult to induce *theta* rhythm in cats and monkeys.

In spite of many studies (*Mac Lean, Grastyan et al., Lena* and *Parmeggiani*), the question still remains to be answered of what is the importance of synchronization of bioelectric activity in *theta* rhythm for regulation processes in H and whether it is a manifestation of stimulation or inhibition.

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Theta rhythm similar in its morphology was described in the thalamus during arousal reaction of rabbit (*Petsche* and *Stumpf*) and in cat upon wake *(Aquilar et al.)* and in fast phase of sleep (*Parmeggiani* and *Zanocco*). *Rouguel* and *Benešova* also identified in some regions of cat thalamus and in the association nuclei in particular, a *theta* rhythm which slightly differed in frequency range from that found in H.

There is thus the problem whether the two *theta* rhythms coexist independently, or are correlated in spite of certain differences, or one of them is a reflection of the other. In that connection it is of interest to answer the question of where *theta* rhythm is formed and in what way it spreads. In spite of many hypotheses it remains unknown whether *theta* rhythm is formed in the thalamus and then it is secondarily reflected in H or, reversely, whether it primarily arises in H and spreads to the thalamus and other structures.

The present study is a contribution to the problem of the site and way of formation of *theta* rhythm in H. According to *Green* it was never obtained by direct stimulation of H; the present results indicate a possibility of its induction by introducing a chemical compound directly into H.

METHODS

Experiments were carried out in 25 cats of both sexes, 2500 to 3000 g of body weight. In chronic experiments, for which 18 animals were used, carboline compound (1-methyl-6-methoxy-1,2,3,4-hydro-β-carboline) was introduced by a cannula of Grossman type with double walls, which was inserted by stereotaxic method in dorsal portion of H (F = 3, L = 6, H = 7). Chemical irritation of H was performed by two methods: either by giving crystalline compound in a dose of 15—30 mcg, or by injecting through the inner tube 0·01—0·02 ml of solution containing 50—150 mcg of the compound. The inner tube was connected to a microinfusion unit or to a calibrated "Agla" syringe. Each animal was used for a series of experiments performed at irregular, on the average one week, time intervals. In all experiments, one concentration of the compound was used administered in the same fluid volume. During chemical irritation of H the bioelectric processes were observed as well as behavior of the animals. Pharmacological properties of the carboline compound, in some measure resembling those of LSD, as well as methodological details and recording instruments are described elsewhere *(Trąbka)*.

RESULTS

Indirect stimulation of a point distant but anatomically and functionally related to H induced *theta* rhythm in all segments of H. In Fig. 1 part A, C presents a typical example of *theta* activity induced by stimulation of the reticular formation of the thalamus with high frequency electric stimuli. When applied after intravenous injection of the carboline compound, stimulation with identical electric parameters was followed by a less distinct synchronization which appeared after a longer latency period (Fig. 1, B, D).

Under certain conditions a similar *theta* activity was observed after introducing carboline compound directly into H. This was observed only twice in two animals. In cat No 23 the first administration of 0·01 ml of carboline solution was without effect. After the second administration 5 minutes later in the lead from H irregular *delta* waves appeared

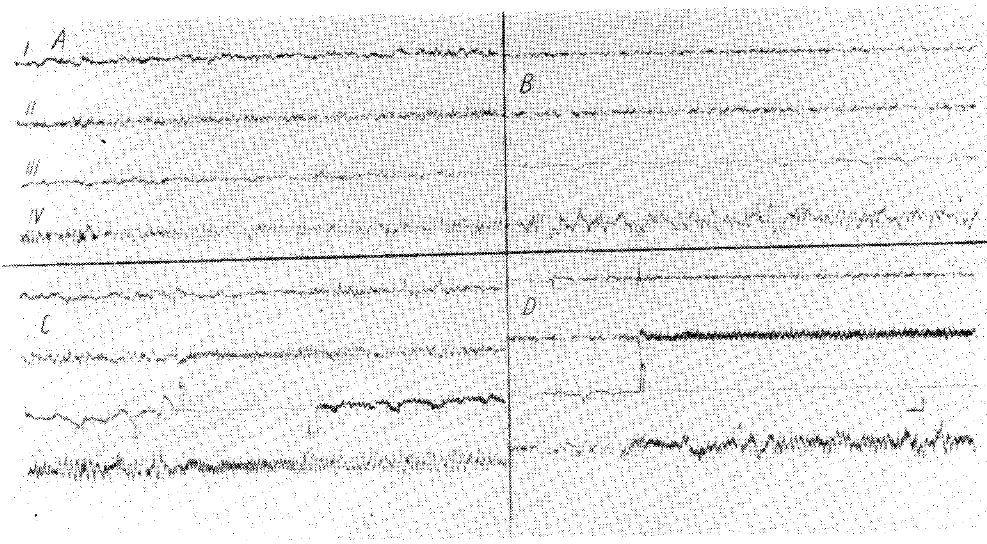

Fig. 1. Cat No. 5, immobilized with flaxedil. *A* — before, *B* — 30 min after intraperitoneal administration of 10 mg/kg of carboline. *C* — stimulation of the reticular formation of the thalamus with high frequency stimuli before, *D* — after administration of the compound. *I* — right sigmoidal and suprasylvial gyrus. *II* — left sigmoidal and suprasylvial gyrus. *III* — reticular formation of the thalamus. *IV* — left hippocampus. Long longitudinal lines — time of electric stimulation. Perpendicular section — 50 μV, horizontal section — 1 sec (this pertains also to the next figures).

(0·5/sec) superimposed on fast desynchronized waves (Fig. 2 B, C). After further 5 minutes very slow *delta* waves began to transform into irregular *delta* waves with higher frequency, accompanied by single fast and spike elements (Fig. 2 D). The third subsequent administration was followed by unilateral series of high-potential uniphasic rhythm with 2—4/sec frequency, restricted to the site of injection. Five minutes later the slow rhythmic activity showed gradual desynchronization and drop of potential. No changes were observed in the animal behavior — the cat sat motionless, with eyes open.

In the second case (cat No 13, Fig. 3) regular, long-lasting slow rhythm appeared after the first administration of 0·01 ml. During the next injections the slow rhythm disappeared, probably due to "short circuit" of the receiving electrodes by a drop of the solution. When injection was discontinued the slow rhythm reappeared in an unchanged form, being constantly restricted to the point of stimulation. Its frequency accelerated and its amplitude rose only periodically. After 13 minutes the slow acti-

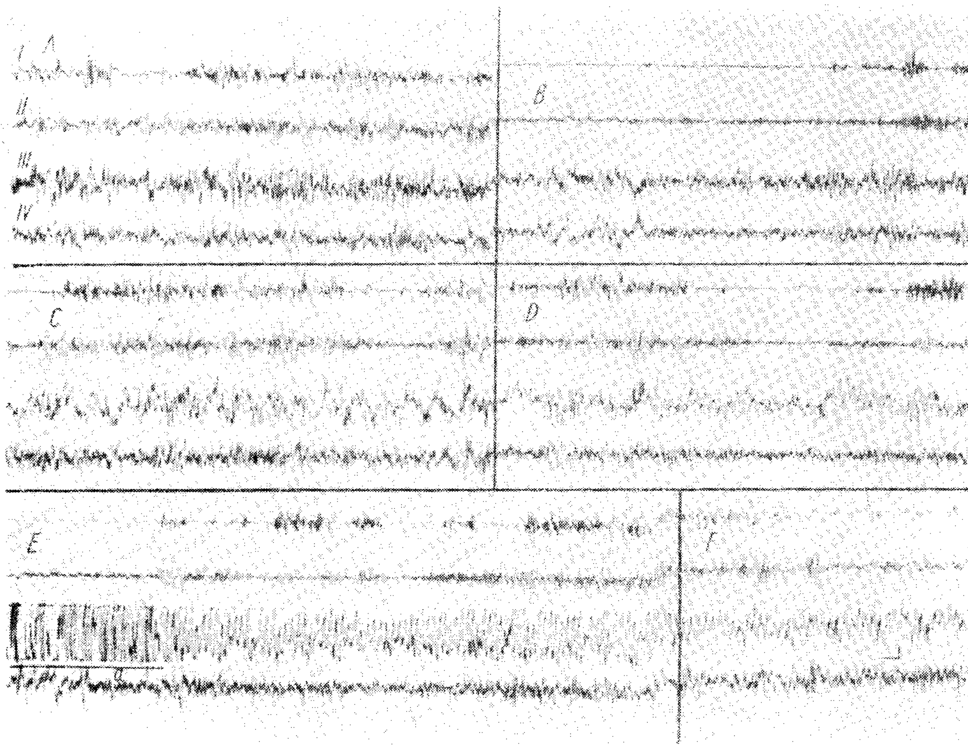

Fig. 2. Cat No. 23. *A* — initial record, *B* — record after the first administration, *CD* — after the second one (gradual synchronization), *E* — after the third one, *F* — 5 min after the third one. *I* — right sensory-association cortex. *II* — left sensory-association cortex. *III* — right hippocampus. *IV* — left hippocampus. *a* — time of infusion.

vity spontaneously disappeared. The electric activity of the cortex, which was recorded at the same time, showed the picture of unilateral (on the side of stimulation) marked flattening — desynchronization. During multiple injections the animal behaved freely-sitting position and open eyes rather indicated wake condition.

Experiments in which slow activity was recorded were grouped in medial part of series, while the first and the last experiments of series showed the reaction in the form of bursts of impulses.

Burst impulses induced by chemical stimuli differed essentially from those following electric stimulation (Fig. 4). The former were composed of spikes similar in appearance, with 30—40/sec frequency and 50 μV am-

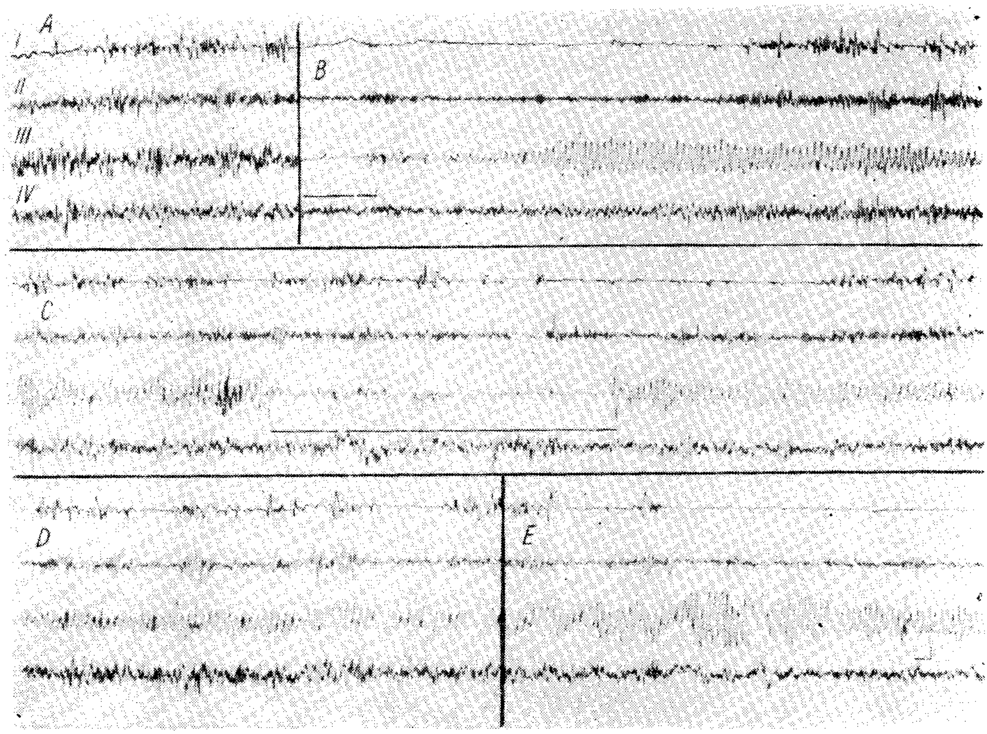

Fig. 3. Cat No. 13. Chronic experiment No. 4, chemical irritation of the right hippocampus. *A* — initial record, *B, C, D* — subsequent sections after administration of carboline, *E* — 10 min after administration of carboline. *I* — right sensory-association cortex. *II* — left sensory-association cortex. *III* — right hippocampus. *IV* — left hippocampus. Horizontal lines — time of infusion.

plitude, while the latter were more differentiated and showed a higher tendency to pass on the opposite side. High-potential spikes were grouped in series, which were separated by short isoelectric sections that prolonged as the impulses continued.

DISCUSSION

Monophasic regular slow rhythm probably arises due to synchronization of the dendritic potential variation originating from the layer of pyramidal cells — the main component of H *(Grastyan et al., Gloor)*. According to *MacLean*, rhythmic slow activity is produced in dorsal portion of H more easily than in the ventral portion neighboring the complex of amygdaloid nuclei. Slow activity with *theta* frequency was also observed in the thalamus and in other centers functionally related to H structure (*Costin et al.*). *Theta* rhythm was even supposed to be formed in brain stem centers from which it secondarily spreads to H. The present study suggests *theta* rhythm to be an autochtonic process that appears primarily in H and not a reflectory process whose origin is located in other struc-

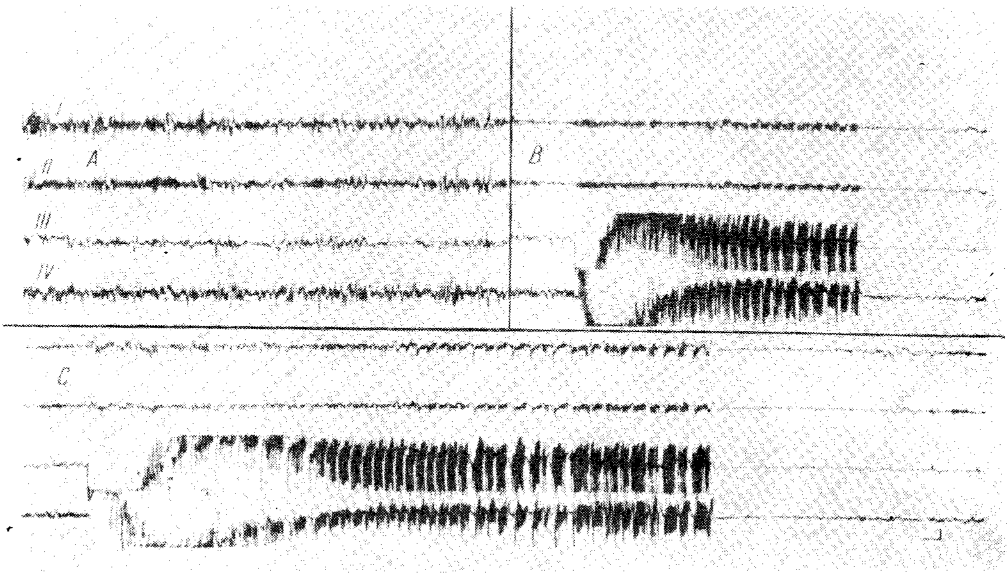

Fig. 4. Cat No. 13. Chronic experiment, electric stimulation of the right hippocampus. *A* — initial record, *B* — 5 min, *C* — 30 min after intraperitoneal carboline administration (leads as in *Fig. 6*).

tures. It cannot be excluded, however, that *theta* rhythm is generated beyond the hippocampus and that it is controlled by the septal region (*Yokota* and *Fujimori*).

It seems to be of special interest for the problem of the formation of slow rhythm in H, that gradually developing synchronization was manifested by transformation of desynchronized waves, through the phase of irregular *delta* waves with increasing frequency, into sinusoidal, homogenous slow activity.

There are various concepts as to the role of slow rhythm in H. The fact that desynchronization in the cortex and synchronization in H occur concurrently led *Green* and *Arduini* to a conclusion that slow rhythm is a particular form of activation resulting from stimulation processes. It was found in further studies that there is no close relation of cortical desynchronization and synchronization in H, that there are also other types of arousal reaction not accompanied by *theta* rhythm, and that cortical desynchronization and synchronization in H are two functional conditions differing in nature. According to *Tokizane et al.*, "the synchronous slow activity is a manifestation of H reactivity to which stimulatory character should not be ascribed".

A more resolute opinion is represented by *Grastyan et al.* who take the rhythmic slow activity — as a sign observed during development of conditioned reflexes — for a bioelectric manifestation of the inhibition. According to *Lena* and *Parmeggiani* hypnogenic activity that appears in H as the rebound effect consists in generalization of synchronization, thus it is related to gradual spreading of *theta* rhythm towards other support structures and the cortex. The role of *theta* rhythm in paradoxical phase of sleep is indicated by findings reported by *Parmeggiani* and *Zanacco.*

It is difficult to ascribe a specific physiological role to the appearance of slow activity under discussion. The animals showed no sleepiness during the experiments. Because of serial schedule of the experiments which eliminated the possibility of conditioned reflexes, it is also difficult to take the slow rhythm for a manifestation of conditioned process. The experiments rather suggest the existence of other unknown factors by which the appearance of rhythmic activity in H is induced.

Differences in bioelectric reactions, which were observed after applying carboline compound in identical concentration, seem to be due not to the properties of the compound but to different neurophysiological situations in which chemical irritation are performed.

There is a fundamental difference in the reaction of H depending on the method of irritation. The electric stimuli never induce synchronization in the slow rhythm, while rhythmic slow activity occurs under certain circumstances as a result of chemical irritation which exerts a more differentiated effect. Such a conclusion can be drawn from the present experiments as well as from data reported by *Mac Lean.*

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