COMBINED EFFECT OF RETICULAR STIMULATION AND PHYSOSTIGMINE ON HIPPOCAMPAL THETA ACTIVITY

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Abstract. The effect of electrical stimulation of the mesencephalic reticular formation and of physostigmine on hippocampal theta activity was combined in rats with implanted electrodes. The effect was not additive. The threshold for evoking motor activity by means of reticular stimulation decreased after physostigmine administration.

The hippocampal EEG theta activity — TA (5) — is functionally related in a complex way to reticular activation during orienting reaction, motivational process and paradoxical sleep (4, 9, 10, 13, 14, 15, 22). TA can be induced experimentally both by arousing stimuli and direct electrical stimulation of the reticular formation — RF (5, 17, 24). Clear-cut TA may also be evoked (without the usual behavioral correlates of activation) by some drugs, physostigmine (Ph) being the most typical one (see 3, 20).

The aim of the present experiments is to analyse whether the neuronal network activated by direct electrical stimulation of RF is identical with that influenced by the cholinergic drug Ph. We attempted to combine both factors inducing TA in the hippocampus. The influence

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1 A preliminary report was read at the 1981 meeting of the Czechoslovak Society for the Study of Higher Nervous Activity (6).
of Ph upon the occurrence, duration and frequency of TA evolved by increasing stimulation voltage has been investigated.

As Ph could change the readiness of the animals to react to RF stimulation with movements, which might in turn evoke TA (11, 23), the influence of Ph upon the threshold of motor reaction has been also established.

Experiments have been performed on unanesthetized freely moving rats with stereotaxically (2) implanted chronic bipolar electrodes (tip distance about 1 mm) in the dorsal hippocampus (coordinates A 3.5, L 3.5, V 4 for recording TA) and in the mesencephalic reticular formation coordinates A-P 7, L 1.5, V 7.5 for stimulation by means of trains of 2 ms rectangular, electrical pulses 200/s). Eight second stimulation and nonstimulation periods alternated 10 times regularly at each stimulating voltage. This regime of stimulation has been found adequate in preceding experiments (7). The stimulating voltage was increased in 0.5 or 0.25 V steps from 0.5 V to about 2 V (till the threshold for evoking motor activity has been reached). The intervals between the series of stimulation with different voltage were about 1 min. The EEG activity from the dorsal hippocampus has been recorded on paper in native and band-path filtered form (Fig. 1). The incidence of TA (the

Fig. 1. Hippocampal activity (3-12 Hz band-path filtered). C, control (without any influence), St, during stimulation of RF (full line under recording), Ph, 10 min after administration of physostigmine (0.5 mg/kg); Ph + St, combined influence of St and Ph.
proportion of stimulation period covered by TA) and its frequency were measured and evaluated statistically (1 way ANOVA). The same measurements were taken before and 10 and 60 min after Ph (0.5 mg/kg i.p.) administration. In control experiments either saline was administered instead of Ph, or Ph was not accompanied with electrical stimulation.

It was found in 9 out of 12 rats that the threshold for evoking motor activity (usually escape reaction) by means of electrical stimulation of the reticular formation decreased significantly \((P < 0.05)\) 10 min after Ph administration (by 0.46 V on the average).

TA has been analysed in detail in 3 rats. Only experiments without any sign of movements were evaluated.

The incidence of TA increased (as it has been shown before, see (1)) after Ph. The gradual increase of TA incidence, typical for ascending stimulation voltages (24), was apparent only at values evoking, in control experiments, a higher TA incidence than that corresponding to Ph administration. Average maximal values of TA incidence were practically equal for electrical stimulation and electrical stimulation combined with Ph. Electrical stimulation never decreased the incidence of TA induced by Ph (Fig. 2).

The average frequency of TA induced by Ph was, irrespectively of its high incidence, significantly lower \((P < 0.05)\) than that after electrical stimulation or the one appearing spontaneously. TA frequency could be increased above the average Ph level only when higher voltage

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**Fig. 2.** Average incidence of theta activity (see text) before (left, A), 10 min (middle, B) and 60 min (right, C) after physostigmine administration. Full line represents combined physostigmine and reticular stimulation, dashed line physostigmine only, dot-and-dash line reticular stimulation. The number below the groups represents stimulation voltages.
(2 V) stimuli were used. It remained, however, somewhat (although unsignificantly) lower ($P < 0.1$) in this case in comparison with the control experiments, when no Ph was given (Fig. 3).

It follows from the results that although in the above experimental conditions both factors: RF stimulation and Ph, act upon the system responsible for hippocampal TA generation, their effect is not additive. It may be assumed that different subsystems might be responsible for, TA generation. The results concerning TA frequency suggest that the subsystem activated by Ph might even, to a certain degree, inhibit that driven by reticular stimulation-activation (probably through occlusion in the RF neuronal network).

It has been found in previous experiments performed on curarized unanesthetized rats (18, 19), that somewhat higher doses of Ph (1 mg/kg i.p.) increase the voltage of RF stimulation necessary for evoking TA of a certain frequency or for maintaining a stable preselected frequency of TA by means of a feedback set-up in which RF stimulation voltage increases when hippocampal TA frequency decreases and vice versa (16, 21). Our present findings confirm these results and the results concerned with a dissociation of hippocampal TA and behavior (14). TA induced by higher doses of Ph is difficult to influence by RF or external sti-

![Fig. 3. Average frequencies of hippocampal theta activity (see Fig. 2). Changes of the unit activity in visual and motor cortex during the behavioral act of taking of food after the “occluders” are put on. The cases when the activations persisted, but their merkedness changed are not included in the Table](image-url)
ulation and is hardly comparable to any physiological state.

We have found in other experiments that massive activation by phystostigmine of cholinergic synaptic transmission in the RF causes a certain desorganization of the neuronal network, which is manifested by changes in the communication between nerve cells by means of nerve impulse trains (8). However, it has been also shown in the above experiments (18, 19) that subthreshold doses of Ph (0.1 mg/kg i.p.) might facilitate the effect of RF stimulation. Similar findings have been reported from another laboratory (12). The above results obtained in curarized rats have to be repeated in freely moving unanesthetized animals, as in the former case the component of reticulo-hippocampal system, related in normal conditions to movements, could play a role at higher stimulation voltages and higher TA frequencies that those possible in freely moving animals without escape reaction (23).

The positive influence of Ph on the descendent reticular system was not the subject of study, and the range of stimulation voltages was selected for the reason of being below the threshold of influencing the motor system. The decrease of the threshold for evoking movement by RF stimulation after Ph administration points, however, to the possibility that this drug has a descendent activating effect.


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