Session B. Influence of Microwave Radiation on the Nervous System and Behavior

Chairman: *A. V. Roščin*
Vice-Chairman: *W. A. G. Voss*
Rapporteurs: *W. R. Adey and E. A. Lobanova*

THE USE OF CONDITIONED REFLEXES TO STUDY MICROWAVE EFFECTS ON THE CENTRAL NERVOUS SYSTEM

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The harmful influence of radiowaves on the human organism, and particularly on the central nervous system (CNS), as established in the course of clinical investigations, required detailed studies under experimental conditions. Two closely related directions of experimental work were needed: a) studies aimed at defining the maximum permissible level of irradiation, and b) studies focussed on elucidation of the mechanism of action of radiowaves.

The aim of the experiments influenced the choice of method. It was deemed necessary that it should characterize the integrative activities of the CNS, possess so-called "hygienic" significance and be informative enough with regard to at least certain aspects of pathogenesis. Such a method is the use of conditioned reflexes analysis.

In this paper an attempt is made to answer the following questions:

— how do the functions of higher compartments of the CNS change following a single exposure to microwaves?

— what are the dynamics of these changes in the course of chronic irradiation?

— what are the possible mechanisms of the observed disturbances?

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Before going into a presentation of our own and others authors' data, it should be noted that the most thoroughly studied are the biologic effects of microwaves, particularly in the centimeter range. By this we mean data not only on the action on the CNS, but also on effects on endocrine, circulatory, and other systems.

The functions of higher compartments of the CNS as affected by this range of wavelengths were investigated using various methods. The levels of irradiation applied by different authors varied over a broad range, but we will limit ourselves to presentation of the data obtained at intensities not higher than 10 mW/cm².

In hygienic investigations of this kind the method of motor-food conditioned reflexes has been used extensively. Its modification is applied successfully for producing conditioned reflexes in dogs, rabbits, rats and mice.

The preparation of the experimental animals is a very long process and consists of elicitation and strengthening of a system of positive and negative conditioned reflexes, performing a number of functional tests indispensible for defining individual peculiarities of higher nervous activities of the animals, and finally in setting up the background conditions preceding irradiation.

For characterizing the conditioned reflex activities of the animals we made use of generally employed indicators: duration of the latent period and of motor reactions in positive conditioned reflexes directed at auditory and visual analysers; the value of these parameters before and following application of the differentiating stimulus; the state of differential inhibition. The cases in which positive conditioned reflexes and natural reflexes to the sight of food had been lacking were recorded and the behavior of the animals and of functional tests were taken into account.

To facilitate analysis of the experimental material we computed these indicators and their significance for each animal and for the entire group following each series of 10 experiments during the irradiation period; this corresponded to twenty exposures to microwave radiation.

The first series of experiments was performed on rabbits irradiated during 4 months with centimeter microwaves at an intensity of 10 mW/cm² for 60 min daily. Following irradiation the conditioned reflexes were investigated for two months.

The conditioned motor-food reflex in rabbits was produced according to the method of O. V. Malinovsky (12); upon exposure to a sound or light stimulus the animal pulled a ring attached to a feeding trough filled with food following a conditioning signal lasting 5 s.

The action of microwaves during the first month of irradiation led to an inhibition of conditioned reflexes, as apparent from a prolongation of the latent period of the reflexes to positive signals, and, in a number of cases, even the lack of conditioned reflexes (Fig. 1). More frequently these changes were found in the first half of the stereotype, preceding application of the differentiating signal. The active inhibition, as judged by the number of disturbed differentials, was somewhat intensified.

During the second and third months of irradiation the shifts in neurodynamics were preserved. In the course of the fourth month, all indicators of the conditioned reflex activity — both fine and coarse — underwent marked changes. The conditioned reactions to positive signals appeared only after a long latent period. The ratio of the latent periods of the conditioned reflexes, after and before differentiation, increased markedly. The strength of positive conditioned reflexes following differentiation decreased, and that of the differentiation increased appreciably.

Following cessation of irradiation, normalization of the conditioned reflex activity took place only after 2 months. Hence, the inhibiting action of microwaves upon the functions of higher compartments of the CNS of rabbits under the specified conditions

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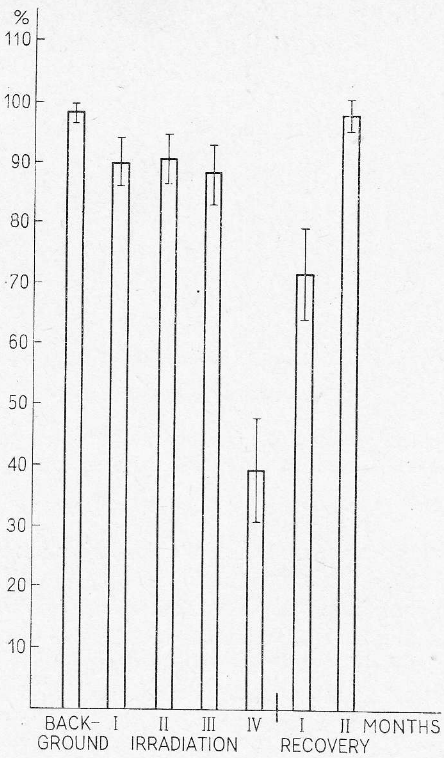

Fig. 1. Changes in strength of positive reflexes in rabbits (in percentages) and the confidence limits at 98% probability.

was apparent during the first month of irradiation and reached a maximum after 4 months.

In the second series of experiments the conditioned motor-food reflexes were established in outbred, non-stock rats by the method of L. I. Kotljarevskij (5): the conditioned animal concentrated on a feeding trough and by pushing a valve obtained its food following an appropriate stimulus of 5 s duration. Irradiation parameters for rats were the same as for rabbits.

The behavior of irradiated rats in early experiments clearly, differed from that of controls. The experimental animals were less mobile and lethargic, continuously washing and licking themselves. The performance of conditioned reactions to positive signals was impeded; frequently the animals did not react to them nor, in a number of cases, even to the sight of food. Upon application of the differentiating signal the performance of conditioned reactions. was improved in some experiments while in others the differentiating signal elicited an opposite effect. Finally, on occasions the animals rested immobile in the chamber, did not react to positive conditioning signals or to the sight of food and, following differentiation, fell asleep.

The described changes in the early stages of irradiation were reflected in quantitative changes in the indicators investigated and consisted of a significant increase in latent periods, frequent lack of conditioned reflexes to positive conditioning stimuli and the sight of food, the presence of phase phenomena, some deepening of differential inhibition and a resultant pronounced inhibition. It should be noted that in the course of the

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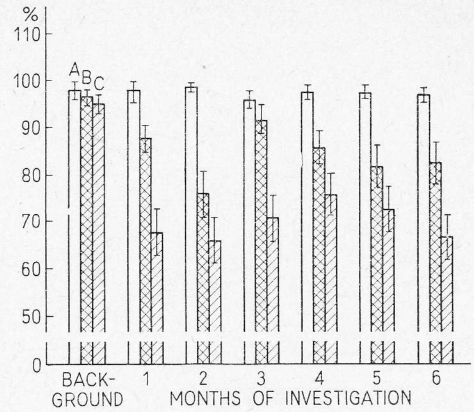

Fig. 2. Changes in strength of positive conditioned reflexes (in percentages) in control (A) and irradiated rats (inbred, B and outbred, C), and the confidence limits at 98% probability.

first month of irradiation, as compared with the following months, the changes in conditioned reflex activity were at their maximum (Fig. 2).

Upon prolongation of irradiation up to 6 months, the inhibiting influence of microwaves on the functions of higher compartments of the CNS did not change significantly except in the fourth month when they were somewhat less pronounced.

The third series of experiments was performed on K-M strain rats which, as is well known, are characterized by a higher level of excitability of the CNS and a motor reaction to strong sound stimuli; the reaction may end in convulsions.

Changes in conditioned reflexes in these rats also appeared in the course of the first month of irradiation and their character was analogous to that of outbred animals. However, the changes observed during the entire irradiation period were significantly less pronounced in outbred rats (Fig. 2).

Functional tests performed after six months of irradiation and taking the form of a strong sound stimulus and a strong inhibition of positive conditioned reflexes did not reveal significant differences in comparison with control and among the experimental groups.

It might be concluded from the data presented above that the K-M rats, that is to say, the animals with a more excitable CNS, are more resistant to the action of microwaves.

However, another trial in which the animals were starved for 24 h, revealed some differences between these two groups of animals. In outbred rats the increased excitability of the CNS due to starvation led to slight facilitation of the conditioned reflex activity, although this was not significant, while in inbred animals deterioration of the activity followed. It was less expressed in the first test after starvation, but progressed with further challenges in a way very characteristic of neurotic conditions.

In the fourth series of experiments on chronic irradiation of K-M strain rats, their reaction to an intense sound stimulus (100 dB, 90 s) was investigated systematically (once a week). During the first month of irradiation no significant changes in parameters of the motor reaction were revealed.

During the second month of irradiation, however, two out of ten rats stopped reacting to the stimulus, and in the remaining animals the period of latency of the reaction

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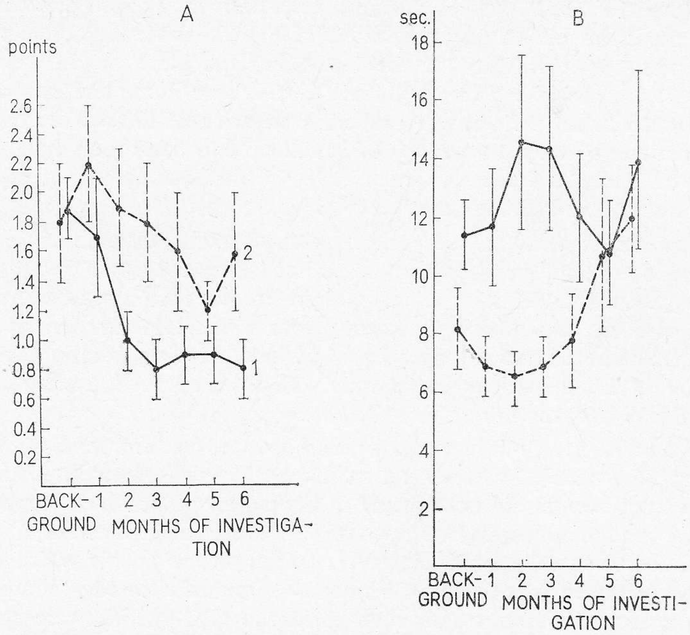

Fig. 3. Changes in the intensity of the motor reaction (A) and the latent period of the reaction in seconds (B) in irradiated (1) and control (2) K-M strain rats. Mean values and the confidence limits of the parameters at 98% probability are given.

increased significantly and the intensity of reaction, as assessed with the five-point scale of L. V. Krušinskij (8), decreased. This situation persisted during 4 months of irradiation. After 5 and 6 months, the differences between irradiated and control animals were insignificant (Fig. 3).

It follows that after microwave irradiation the excitability of the CNS, and particulary of brain formations responsible for carrying the motor reactions to sound, were lowered. However, the genetically inherited aptitude for reacting with motor excitation to an intense sound stimulus changed later and was less dependent on the influence of microwaves than the conditioned reflexes acquired during irradiation.

In the fifth series of experiments rats were irradiated with pulsed 10 cm waves of 10 and 1 mV/cm² intensity, the time of action being the same. Without dwelling upon details of the results obtained we will only mention that their characteristics at 10 mW/cm² were essentially the same as those described above and that the changes in conditioned reflex activity after 4 months of irradiation were less pronounced at 1 mW/cm². Application of a pharmacologic pretreatment (subcortical introduction of caffein at doses of 10, 50 and 100 mg/kg) substantially facilitated the appearance of conditioned reactions in rats irradiated with 1 mW/cm²; in rats irradiated with 10 mW/cm² the high dose of caffein (100 mg/kg) elicited an opposite effect.

Conversion of the positive and negative meanings of the conditioning signals was performed by irradiated rats in the same manner as by control ones.

In the sixth series of experiments, rats were irradiated with continuous 10 centimeter waves of 10 and 1 mW/cm² intensity and conditioned reflexes were investigated during irradiation. Prior to irradiation the animals were distributed into three groups according to their reaction to a strong sound stimulus, with parallel controls in each group.

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Irradiation lasted for 15, 30 and 60 min at a time and was applied at weekly intervals. Duration of the latent period and magnitude of the positive conditioned reflexes were investigated along with stability of the latter and the state of differentiation. The means of these indices and their confidence limits were computed, and the variance distribution of the latent periods was studied. Evaluation of differences in the distribution of a parameter between the groups of irradiated and control animals, and between those irradiated under different conditions, was carried out by means of a non-parametric criterion of A. N. Kolmogorov and N. V. Smirnov (16).

It has been found that, in comparison with controls, differences in distribution of latent periods of conditioned reflexes could not be considered as due to mere chance but are significant for 1 mW/cm², 15 and 60 min irradiations (λ = 1.82 and 2.37, resp.). In the first case an obvious displacement towards short latent periods took place; in the latter, in the direction of longer latent periods. Also significant was a difference in distribution of latent periods in animals with lowered food excitability irradiated with 1 and 10 mW/cm² for 15 min (λ = 1.37).

It follows that 10 mW/cm², 15 min irradiation does not influence significantly the level of excitability of the CNS, while 1 mW/cm², 15 min somewhat increases it.

With few exceptions, we did not succeed in finding any differences in reactions of rats to microwave irradiation dependent upon the level of food motivation and the character of reaction to sound stimuli. However, studies on the way in which the establishment of an orientation reaction to an incidental stimulus affected a conditioned reflex response made it possible to reveal changes in the CNS which were masked under ordinary experimental conditions, and the expression of which depended on the level of food motivation. In all three experimental variants (Tab. 1) the differences were significant in comparison with controls and also among the groups, and they clearly demonstrated a major bio-effect of microwaves at 10 mW/cm² intensity.

It follows that even a single short exposure to microwaves may result in CNS changes which could be disclosed by means of functional tests. The experiments on rabbits

Table 1

Influence of an orientation reaction on a conditioned reflex response (assessment of differences in distribution of latent periods of the response by means of the λ test)

Irradiation parameters	Level of food excitability		Control		Low level of food excitability			
					10 mW/cm², 15 min		10 mW/cm², 15 min	
			Background	Test	Background	Test	Background	Test
10 mW/cm², 15 min	high	background test	*0.29*	2.18	*0.45*	3.21		
10 mW/cm², 15 min	low	background test	*0.75*	2.82			*0.3*	2.13
1 mW/cm², 15 min	low	background test	*0.77*	1.49				

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and rats presented above show the degree to which results of such acute experiments can be extended to conditions of chronic irradiation at 10 mW/cm² intensity. The effects of exposure at 1 mW/cm² are demonstrated in the following series of experiments, in which animals were irradiated at that intensity for 60 min daily for long periods of time.

During first exposures, behavioral changes were seen: the rats washed themselves continuously, and towards the end of the experiment they frequently fell asleep; sometimes they did not react to the sight of food, conditioned reflex responses were delayed and the differential inhibition was deepened. In the majority of rats this type of behavior continued for 18 treatments, and in some of them even longer. After 18 irradiations, as seen in the graph, the picture changed and the differences between irradiated and control

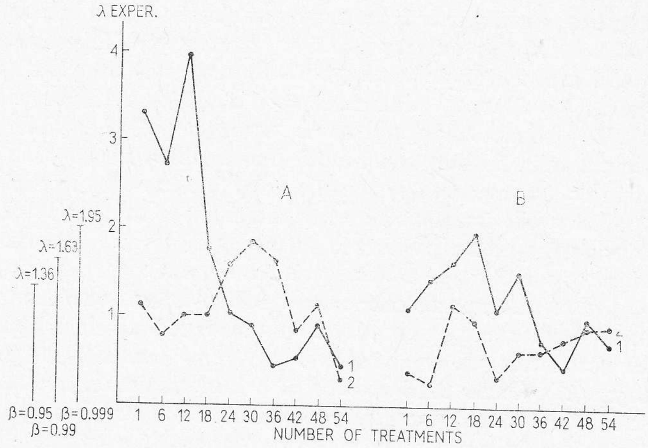

Fig. 4. Evaluation of differences in distribution of latent periods of positive conditioned reflexes (A) and time of differentiation (B) in irradiated (1) and control (2) rats as compared with the background and judged by the λ test.

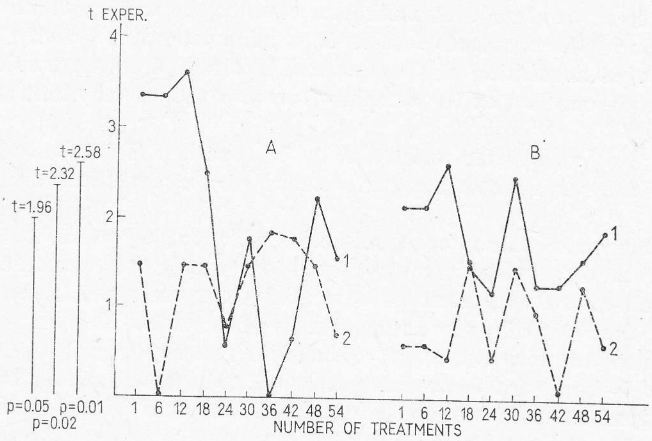

Fig. 5. Evaluation of differences in strength of a positive conditioned reflex (A) and differentiation (B) in irradiated (1) and control (2) rats as compared with the background by means of the Student-Fisher t test.

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rats became insignificant (Figs. 4 and 5). Only the employment of the functional test in the form of an incidental stimulus after 48 treatments disclosed hidden disturbances of the CNS in the irradiated animals, similar to those caused by a single irradiation. The λ value of 1.37 was found upon assessment of differences in the distributions of latent periods of conditioned reflexes in irradiated versus control rats.

To sum up the data quoted above, the method of conditioned reflexes is sensitive enough; it allows demonstration of functional changes in the higher compartments of the CNS and their various patterns, which depend upon microwave intensity, duration of irradiation, its character (single, chronic), species of experimental animals, and the initial state of the CNS.

The data presented deal with conditioned motor-food reflexes. Other authors have studied salivary-nutritive, vegetative and defensive conditioned reflexes in dogs, rabbits, rats and mice exposed to centimeter wavelengths of closely similar characteristics — 10.5 and 1 mW/cm^2. Conditioned salivary-nutritive reflexes were investigated after single or repeated 2 h microwave irradiation of 5—10 mW/cm^2 intensity (17). Reactions to single irradiation were largely dependent upon the type of higher nervous activity of these animals. In animals belonging to the "strong type" (less labile) a stimulating effect was observed, while in the "weak-type" (more labile) inactivation ensued.

Analogous changes accompanied systematic daily irradiations. In "strong type" animals the conditioned reflex secretion of saliva increased in the course of the first 4—5 sessions, and henceforth gradually returned to the initial level. In "weak type" animals oscillating changes of conditioned reflexes were observed, with alternating periods of lowered conditioned reflexes, phase phenomena and so-called normalization periods.

Investigations of cardiac and respiratory conditioned reflexes in rabbits after 30 min irradiation with centimeter wavelengths at 5 mW/cm^2 intensity did not reveal any regulatory changes (23). Under the influence of a few irradiation treatments, a number of animals disclosed a lowered expression of cardiac and respiratory conditioned reflexes lasting for a few days and followed by oscillating changes in the reflexes.

A single exposure of rats to microwaves of the same characteristics was not reflected in changes in cardiac conditioned reflexes, and only a systematic irradiation of 4—5 weeks' duration led to a weakened conditioned reflex regulation of heart action (24).

In rats irradiated *in utero* with centimeter wavelengths at 10 mW/cm^2 intensity for 20 min daily during 20 consecutive days, the conditioned reflex activity was characterized by a delayed acquisition and adaptation, and also a lowered ability of preservation of a conditioned reflex for escaping from a T-shaped labyrinth with an electrified floor (15).

Investigations of conditioned reflex activity of mice in a T-shaped labyrinth showed that after 10 daily treatments the performance of the conditioned escape reflex is worsened (6).

Therefore, the investigations on conditioned reflex activity of animals with various levels of organization of their nervous system (dogs, rabbits, rats and mice), as obtained by means of different methods, showed essentially that the waves of centimeter length suppressed the functions of higher compartments of the CNS.

The conclusion that microwaves act upon the nervous system of the litter irradiated while *in utero* is important from the theoretical, and particularly practical, points of view. The supposition that changes in the functional state of the CNS of irradiated pregnant animals are the cause of disturbed functions of higher compartments of the CNS in the postnatal ontogenesis of rats seems most probable. These data indicate the need for great caution in assuming that irreversible bioeffects of microwave irradiation occur only at thermogenic levels (15).

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It is obvious that the recorded changes in conditioned reflex activity of the animals are not specific for microwaves. Similar disturbances may appear as a result of the action of other exogenous (physical, chemical) and endogenous factors. However, it is necessary to consider the possibility that severe changes may occur in the function of higher compartments of the CNS, even though these may be reversible under the specified experimental conditions.

Academician P.K. Anohin showed that for true understanding of the conditioned reflex, which is a type of activity that characterizes the entire brain, an experimental analysis of all compartments of the CNS is necessary (1). This great goal of neurophysiology is being achieved by pupils and successors of I.P. Pavlov. With the help of new methodological approaches, the role of various deep brain structures in blocking conditioned reflexes and in effecting conditioned reflex activity is being elucidated.

The complexity and difficulty of interpreting changes in conditioned reflex activity of microwave-irradiated animals with respect to the possible mechanism of action on the CNS are obvious. Although the CNS attracts the close attention of, and is thoroughly investigated by, specialists of various sorts — physiologists, electrophysiologists, biochemists, morphologists — this problem is far from being solved.

Studies of electrical activity of the brain cortex of animals after single or repeated irradiations with pulsed or continuous centimeter waves show an inactivating effect of microwaves, as expressed by the occurrence of non-paroxysmal- and paroxysmal-type changes (3, 4, 20, 22). Moreover, reactions to microwaves involve various other structures of the brain: specific and nonspecific thalamus, hypothalamus, hippocampus, amygdalae, septum and reticular formation of the midbrain (11, 21). However, the most pronounced changes concern the hypothalamus and the sensorimotor cortex.

The syndrome of generalized inactivation is the most characteristic (2). The accompanying slow, high amplitude activity as registered in the cortex may be combined with convulsive discharges in the dorsal hippocampus. Recording of a large number of outbursts of spindle-shaped oscillations in the sensorimotor cortex of microwave-irradiated animals points to the possibility that, in addition to the mid-thalamus, the nucleus caudatus is involved (13, 25, 26).

The stimulus conduction along the specific afferent pathways (rabbits, 3—4 mW/cm^2), as judged by the character of primary responses, is even facilitated. Preservation of secondary responses to light impulses makes it possible to suppose that conduction along complex polysynaptic pathways passing through nonspecific medial thalamic nuclei is also facilitated (7).

Microwave irradiation (10—0.5 mW/cm^2) leads to a lowered activity of cholinesterase in the cortex, subcortical structures, cerebellum, stem and medulla oblongata of rabbits, and is sometimes accompanied by an accumulation of acetylcholine (14, 18).

Morphological investigations of the brain cortex of irradiated rats disclose reversible lesions of cortical neurons, synapses and neuroglia (9, 10, 19). By comparison with other investigated parts of the brain, the most labile cellular structures are those of the hypothalamus. Morphological examination of neurons of the supracortical and paraventricular nuclei and investigation of their neurosecretion reveal a phasic character of the lesions following chronic microwave irradiation.

From what has been said above we suppose that functional disturbances of higher compartments of the CNS accompanying microwave irradiation stem from changed cortical-subcortical relations due to a lowered tonus of the brain cortex. The latter is probably the cause of weakened corticofugal influences, as a result of which the tonus of structures responsible for the inhibiting and regulatory functions of the brain increases.

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PHARMACOLOGIC ANALYSIS OF MICROWAVE EFFECTS ON THE CENTRAL NERVOUS SYSTEM IN EXPERIMENTAL ANIMALS

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Earlier studies on the effects of microwave irradiation on animals carried out at the Military Institute of Aviation Medicine revealed that microwaves affect the activity of the central nervous system (2, 3, 4, 7, 8, 9).

The aim of this paper is to present an attempt to analyse the effects of chronic microwave exposure of experimental animals on the activity of different structures of the central nervous system.

Microwave exposure was carried out at doses below thermal levels. During these studies drugs stimulating or inhibiting the function of the central nervous system at different levels were used.

METHODS

Male, one-year old rabbits weighing about 3.0 kg were used. The animals were kept under constant environmental conditions and were all subjected previously to chronic irradiation with pulse-modulated 10 cm microwaves at a power density of 7 mW/cm^2. The total period of irradiation was approximately 200 hours (3 hours daily exposure).

Investigations were performed on two main experimental groups: The first group of 45 animals was subdivided into 3 subgroups (A, B and C), 15 rabbits each.

Animals from the subgroup A were given intravenous injections of chlorpromazine (Fenactil), 4 mg/kg of the body weight.

Subgroup B received intravenously a 1% solution of pentetrazole (Cardiazolum), 3 mg/kg of the body weight.

Animals from the subgroup C received intravenously an aqueous solution of phenobarbitone (Luminal), 40 mg/kg of the body weight. Unirradiated animals, receiving an identical treatment were used as controls. Subsequent experimental procedure was identical in both control and irradiated animals.

After the period of irradiation, cortical screw electrodes were implanted into the skull of all animals. Electrodes were placed symmetrically in the regions of the motor, sensory and visual cortex. Electroencephalographic recordings were made after the irradiation cycle was terminated as well as after administration of drugs.

The second experimental group consisted of 20 animals. In these animals the action of chronic irradiation on the most typical components of evoked potentials in the visual cortex was investigated. The experimental procedure was essentially the same as in the former group.

In this group 5 unirradiated rabbits served as the controls. In these animals superficial electrodes were implanted into the symmetrical points of the visual cortex, which permitted the recording of evoked visual potentials from the symmetrical points of both cerebral hemispheres.

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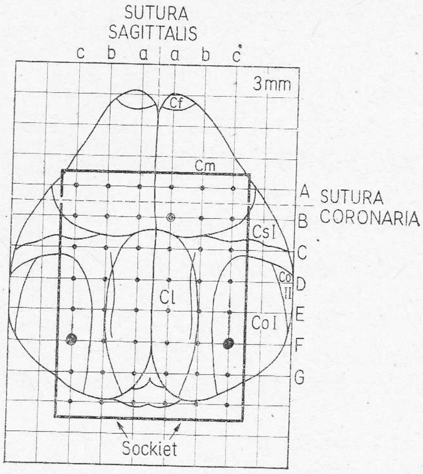

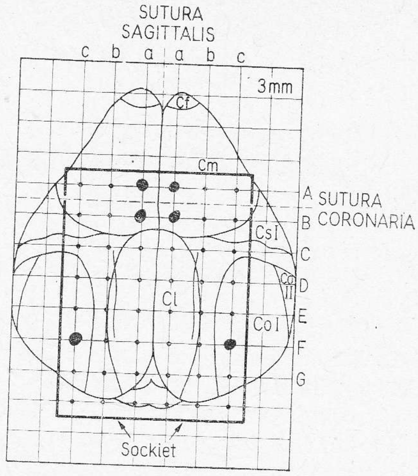

Fig. 1. The points of implantation of surface cortical electrodes.

Fig. 2. Diagram of implantation of surface cortical electrodes used during registration of evoked visual cortical potentials.

Visual potentials were evoked by the retinal stimulation with a flash of light of 700 lux intensity and 1 c/s frequency emitted by a stroboscope (Rydan). In all experiments the conditions of stimulation were kept constant.

During the experiments an "Anops" computer connected with the EEG apparatus (Elema Schönander) was employed. The analyzed period was 66 ms and comprised 128 repetitions.

RESULTS

Analysis of the electroencephalographic records shows that the effects of chronic irradiation may be detected in all of the recorded parameters. Figure 3 shows an EEG record obtained in a control animal in relaxed, wakeful stage as well as in a rabbit following chronic exposure to microwaves. In the latter case the high degree of desynchronization of the EEG pattern in all of the recorded leads was observed.

Administration of chlorpromazine (4 mg/kg i.v.) produced EEG synchronization in both irradiated and control animals.

Intravenous administration of pentetrazole (3 mg/kg) produced a long-lasting afterdischarge of rapid onset in irradiated animals, whereas in control rabbits only a series of subthreshold spindles consisting of 3—4 c/s spikes were observed.

Marked differences between the action of phenobarbitone on irradiated and control animals were noted. In rabbits exposed to chronic microwave irradiation a desynchronization of the EEG pattern was still marked after drug administration.

In a further series of experiments the effects of chronic exposure to microwaves on the main components of visual potentials were determined. Figures 7a and 7b show visual potentials in control rabbits and an animal previously exposed to microwaves. As may be seen, all the main components were markedly changed after irradiation as compared with the control response.

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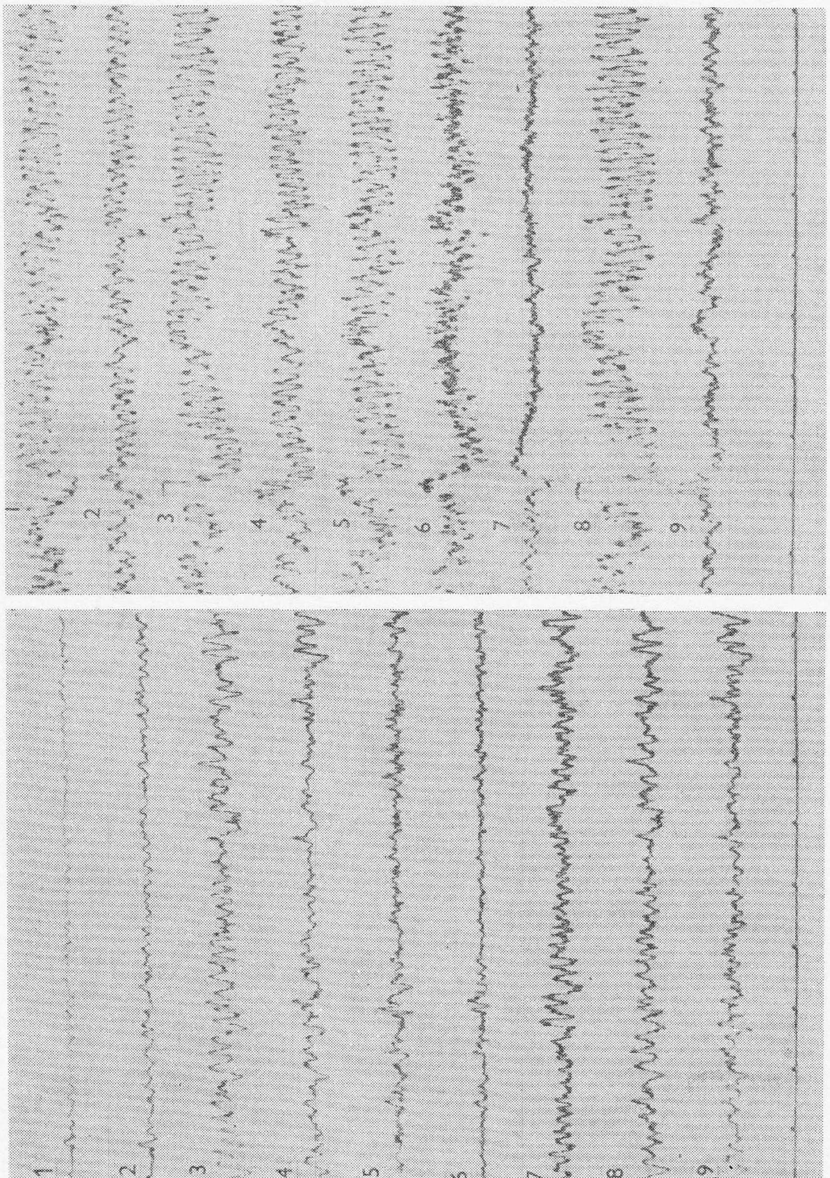

Fig. 3. Left panel: electrocorticogram in control rabbit. Right panel: electrocorticogram in animal previously irradiated. Note a desynchronization with relatively high voltage amplitude in EEG pattern. Leads: *1* — left sensorimotor, *2* — right sensorimotor, *3* — left sensorioptic, *4* — right sensorioptic, *5* — left motor-right motor, *6* — left sensory-right sensory, *7* — left optic-right optic, *8* — left motor-optic, *9* — right motor-optic.

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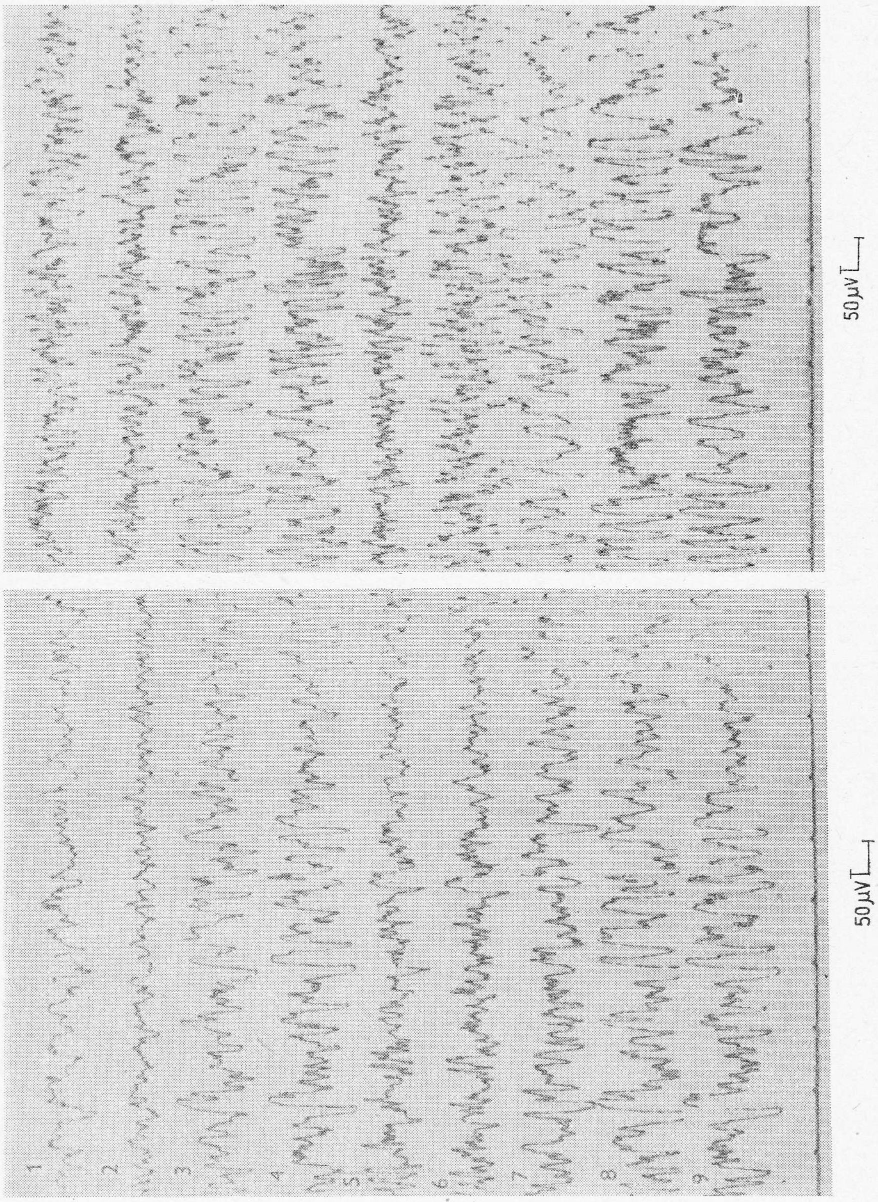

Fig. 4. On the left: effect of chlorpromazine (4 mg/kg i.v.) on the EEG pattern in control rabbit. On the right: as on the left but in animal previously irradiated. Leads: as in Fig. 3.

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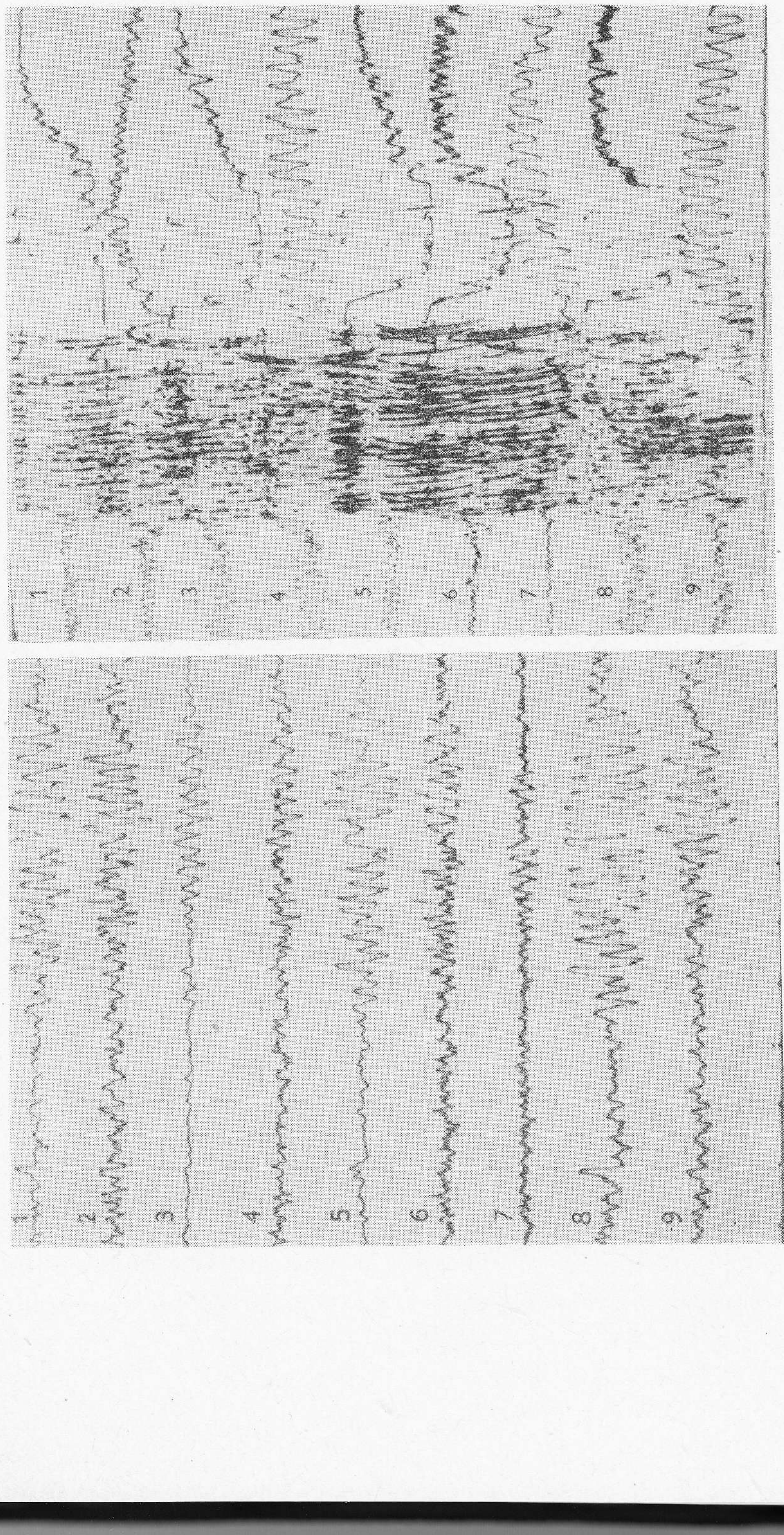

Fig. 5. Panel on the left: action of pentetrazole (3 mg/kg i.v.) on the EEG pattern in control rabbit. Panel on the right: action of pentetrazole on the EEG pattern in rabbit previously exposed to microwaves. Note the series of high-voltage spikes in all of the leads. Leads: as in Fig. 3.

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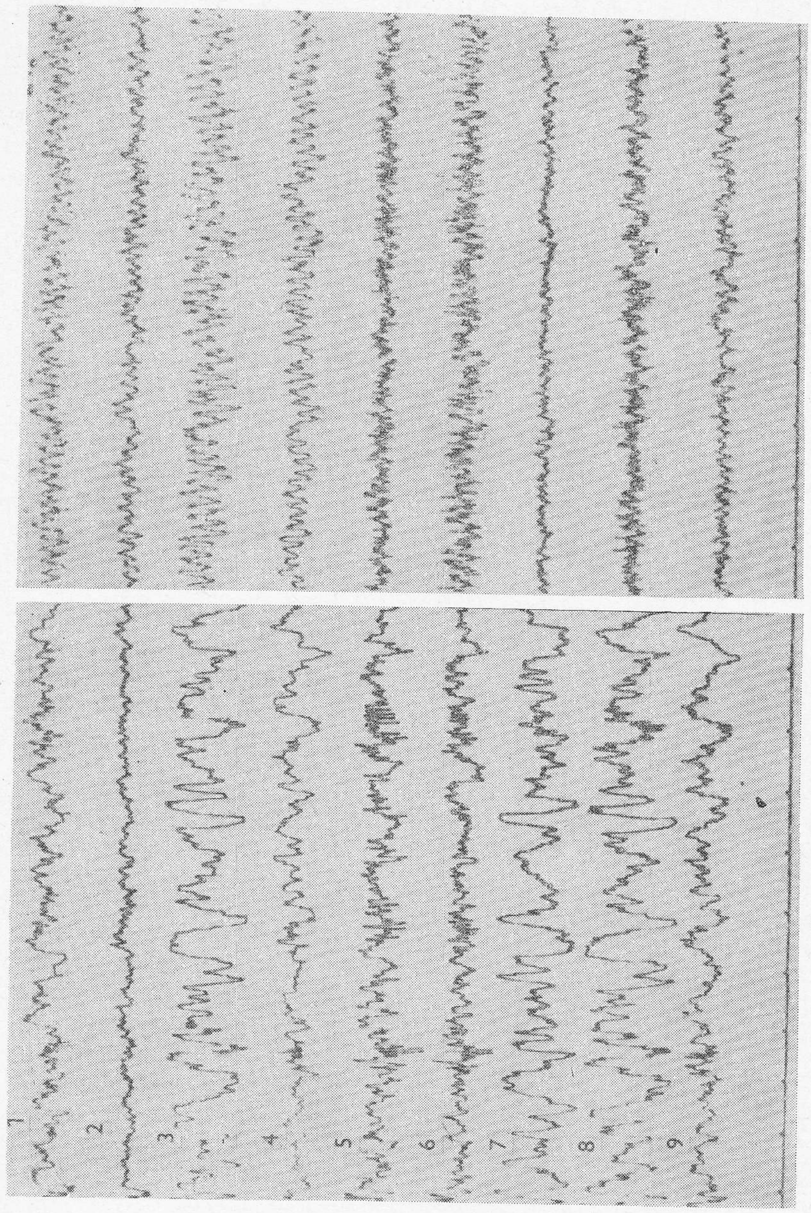

Fig. 6. On the left: effect of phenobarbitone (40 mg/kg i.v.) on the EEG pattern in control animal. On the right: same as on the left but in rabbit previously irradiated with microwaves. Note alternately occurring waves and spikes. Leads: as in Fig. 3.

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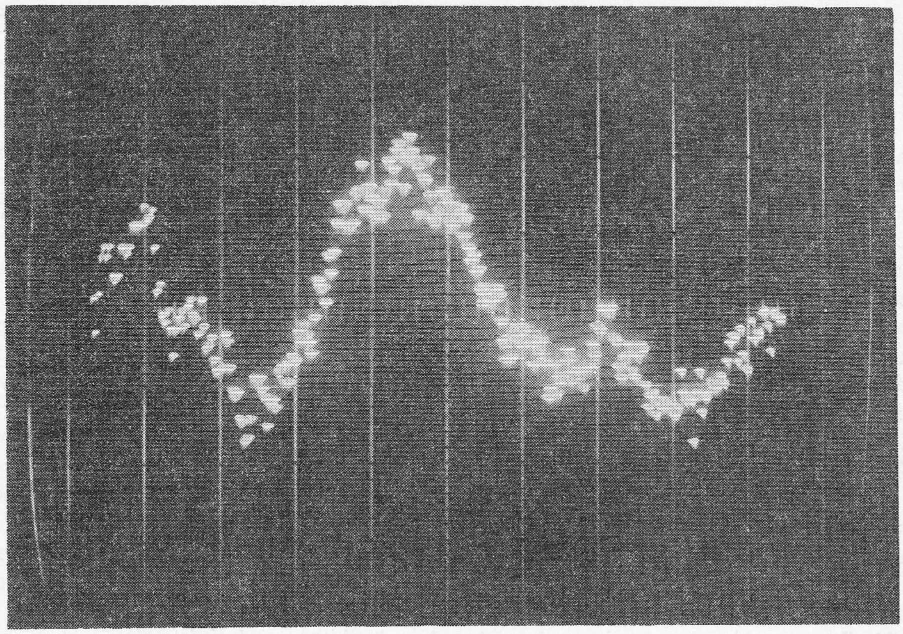

Fig. 7a. Evoked visual cortical potential in a normal rabbit.

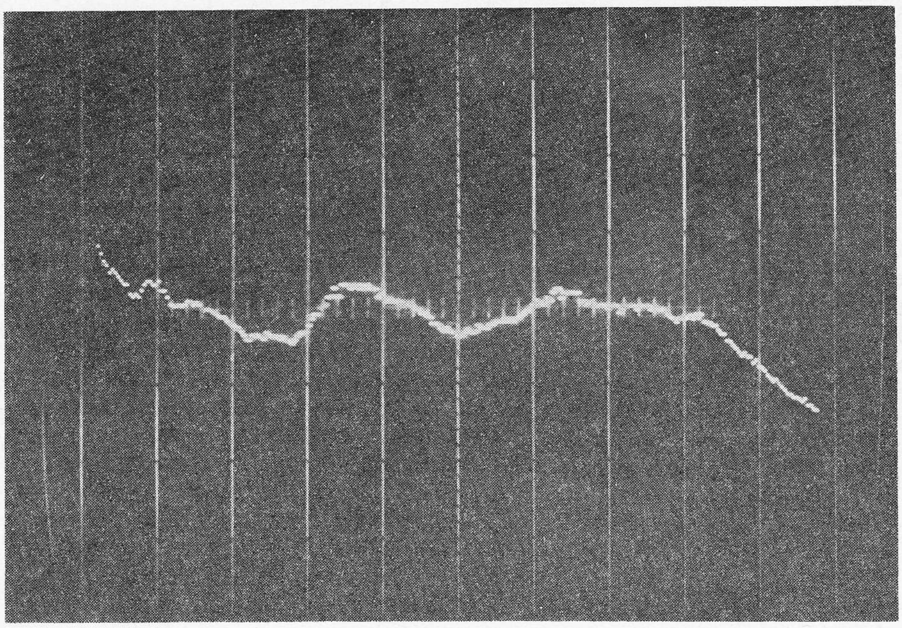

Fig. 7b. Same as in 7a but in an irradiated animal. Note the changes in all the components of the evoked visual potential.

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DISCUSSION

The above-described experiments demonstrate that chronic exposure to microwaves at subthermal doses markedly changes the EEG pattern in experimental animals.

Desynchronization of the EEG pattern observed in irradiated animals is most probably due to the activation of the ascending part of the mesencephalic reticular formation (Fig. 3). Administration of chlorpromazine induced rapid synchronization of a previously desynchronized EEG pattern, which may suggest an antagonism between the action of microwaves and chlorpromazine on the structures of the central nervous system (Fig. 4). It is commonly known that chlorpromazine acts directly on the midbrain reticular formation (14, 15) and decreases its spontaneous activity (12). Essential for the central action of chlorpromazine is also the effect of this drug on the activation produced by cortical stimulation (13) as well as the influence exerted on the synaptic connections between the collaterals of the ascending lemniscal system and unspecific reticular formation (5). It may be suggested therefore that the antagonism between the effects of chlorpromazine and microwaves depends first of all on the opposite action of these factors on the activating part of the reticular formation and cerebral cortex.

In order to estimate the sensitivity of the cortical structures and reticular formation we have used a 1% solution of pentetrazole, a drug which is known to exert a facilitating action mainly on the thalamic part of the reticular formation and motor cortex (10, 11, 16, 17). The experiments described have shown that irradiated animals react more strongly than control rabbits (Fig. 5).

Similar experiments performed with phenobarbitone administration resulted in a slight facilitating action of this drug on EEG desynchronization in irradiated animals (Fig. 6).

Marked differences in the evoked visual cortical potentials were observed in irradiated rabbits as compared with control animals (Fig. 7a and 7b). As is commonly known (1) the individual components of the evoked visual potential possess different origins and their shape is determined by the temporal correlation of two afferent activating waves determining the occurrence of positive and negative phases. It may be accepted that all changes in evoked potential parameters are due — at least partially — to the functional state of afferent systems transmitting the specific and unspecific impulses.

It is of interest to note an important role of synaptic connections within the separate cortical fields, especially both facilitating and inhibitory postsynaptic influences.

Analysis of the shape of the visual cortical potential in rabbits exposed to chronic microwave irradiation demonstrated that more pronounced changes are observed in the electronegative component. This component expresses the impulses transmitted by the unspecific afferent system. The above-mentioned data confirm our previous hypothesis that the nonthermic influence of microwaves depends on the activating effect on the ascending part of the reticular formation.

The data reported in this paper are of a preliminary character only and do not make it possible to determine precisely which level of separate afferent systems is responsibile for the microwave-induced disturbances in bioelectric activity resulting in the morphological changes of the evoked visual cortical potential.

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A QUANTITATIVE ELECTROENCEPHALOGRAPHIC STUDY OF THE ACUTE EFFECTS OF X-BAND MICROWAVES IN RABBITS

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Although many studies involving the effects of microwaves on brain function in animals have been carried out, there is still no clear agreement either on the nature of the changes produced or on the power density and wavelength at which these changes occur. This may be explained by the fact that the scientists involved in such studies have used different wave bands, different power densities, and further, that their consideration of the effects has tended to be qualitative rather than quantitative.

Our studies were performed on adult male New Zealand rabbits and involved chiefly amplitude measurements of the cortical brain waves. It is well established that one can detect and characterize objectively, through such measurements, states of sedation, arousal, stimulation and hyperstimulation, since they correspond to progressive changes in the EEG from low-frequency, high-amplitude waves to high-frequency, low-amplitude waves. Behavioral observations are also involved but become secondary rather than primary for the characterization of the functional states of the brain.

The main complicating feature of these studies is that, under everyday conditions, the EEG patterns of rabbits are quite variable. The animals oscillate between sedation and arousal unpredictably. It is therefore difficult, when a change from one state to another state occurs in animals exposed to microwaves, to be confident that the shift would not have taken place spontaneously in the absence of treatment. One way to deal with this problem is to induce a sustained, stable baseline state. Since, as will be seen, exposure to continuous microwaves in the X-band (9.3 GHz) was found to produce under our particular experimental conditions stimulant effects, we have used a simple method of pretreatment with a low dose of a barbiturate to induce sustained sedation, hence a stable base state which permits more reliable detection of stimulant effects. We found in previous studies that with the low dose of the sedative used, such a state is not different from spontaneous sedation as regards liability to environmental as well as drug-induced stimulatory effects.

In order to reduce the presence of metals in the pathway of the microwaves, we used as cortical electrodes extremely thin (10/1000 inch.) Formvar-coated stainless steel wires, anchored within the cranial bone by means of nylon screws and methacrylate cement. Three such electrodes were implanted, above the right and left somatosensory cortices and within the nasal bone for the common reference electrode. The leads were soldered to a small plug cemented to the top of the skull. Following recovery from surgery, the animals were carefuly trained to sit quietly (although unrestrained) in a custom-built anechoic chamber, 60 cm wide × 60 cm high × 90 cm length, and to accept intravenous injections administered by remote control via a catheter inserted in one of the marginal ear veins before each experimental session. The chamber was provided for forced ventilation and had openings for the passage of the coaxial cable connected to the horn antenna, EEG lead wires, temperature probe, DC light, and the objective of a TV camera, used to monitor the behavior of the animals on an adjacent receiver. The head

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of an animal was approximately 45 cm from the horn antenna. The microwaves were generated with a Hewlett-Packard signal generator and amplified by an Alfred power amplifier.

The routine of the experimental sessions was as follows: After being introduced in the chamber and having EEG leads attached and the venous catheter inserted, the animals were left undisturbed for 5—10 min. EEG recordings were then started, first for a 10 min "baseline" period and next for a 30—60 min period following administration of Na-pentobarbital (4 mg/kg in a 0.1 ml/kg volume, intravenously).

A total of 90 experiments were run on 13 animals. Forty-five percent of the experimental sessions were run without administration of microwaves during the post-pentobarbital period. In 55% of the experiments, the microwaves were turned on 5 min after injection of the barbiturate and kept on for 5 min. Thus, for inter-session comparisons, most animals served as their own controls. The changes produced by microwaves were ascertained from the comparison of the electrical activity with and without microwave treatment, plus behavioral observations.

The power densities used ranged from 0.7 to 2.8 mW/cm². The accuracy of the dosage was verified before and after each experiment with a Narda Electromagnetic Wave Monitor. The brain electrical activity was recorded directly on paper, and, at

Table 1

Levels of mean integrated amplitudes and coefficient of variation in rabbits during different behavioral states
(Intervals of 20 s; mean values from 12 experiments on 6 animals. N = 360)

Behavioral state	Mean EEG amplitudes	Coefficient of variation (S.D./mean× 100).
AROUSAL	35.0	23.1
Pentobarbital-induced sedation	67.8	26.2
Stimulation	27.5	18.6
Hyperstimulation	18.0	8.0

the same time, processed through solid-state electronic integrators. These devices measure continuously on-line the amplitudes of unfiltered brain waves, and express numerically their values over time for pre-set, fixed intervals. As would be expected from the description of the EEG patterns, during sedation the integrators display much higher values than during arousal and, especially, hyper-stimulation. In this latter state, not only are the main amplitudes at a low level but also the variances of the distributions of amplitudes are small. Table 1 lists integration values for means and coefficients of variation of the amplitudes of brain waves during selected periods corresponding to the typical behavioral states previously mentioned.

The first finding was that no detectable changes in EEG patterns, their integrated counterparts or in behavior occurred during the 5 min period of exposure to microwaves. In only 2 experiments (2%) was there a possible indication of an "on-

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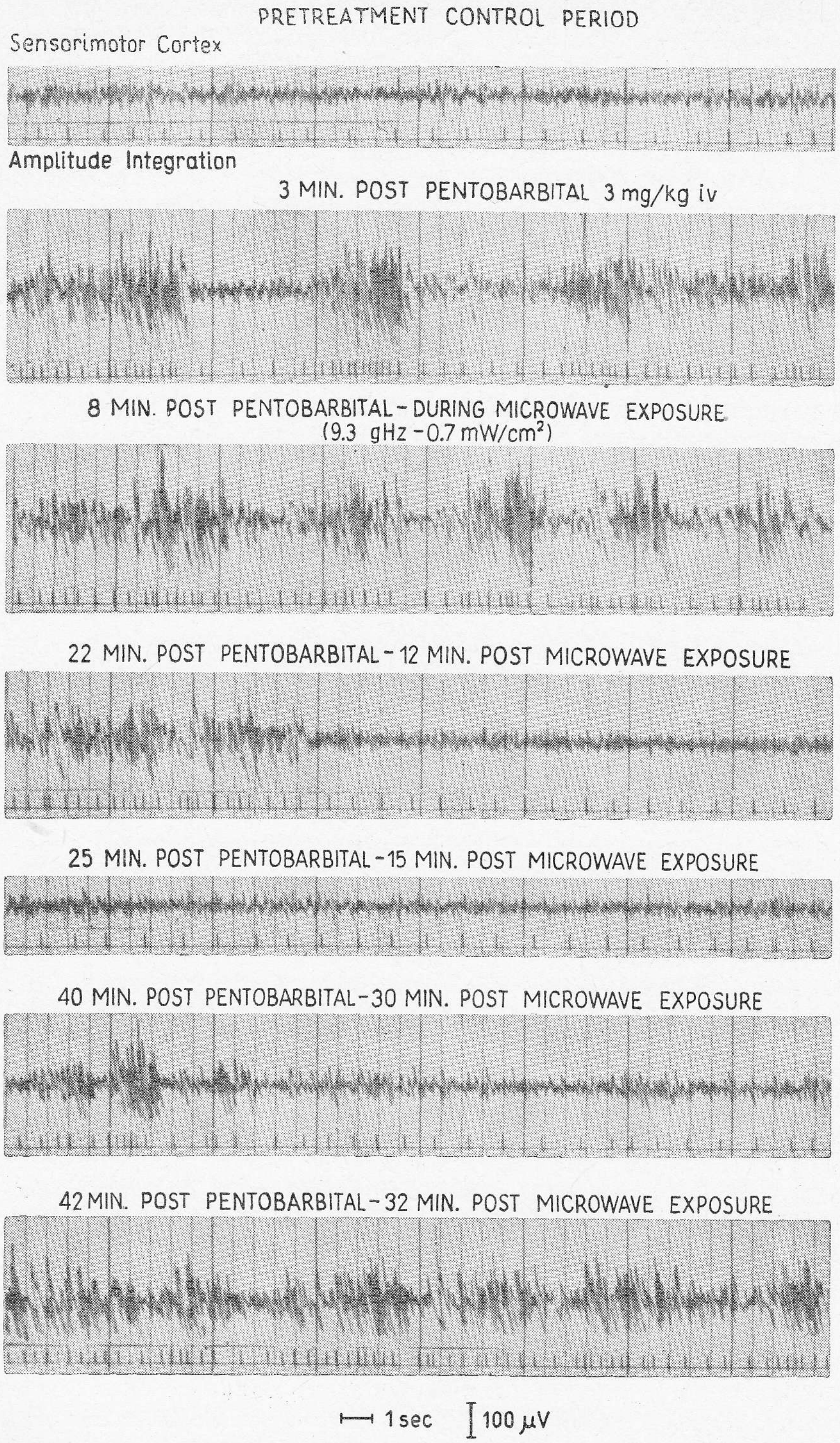

Fig. 1. Direct EEG patterns and their integrated measurements in a rabbit at various stages of a microwave experiment.

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effect" and an "off-effect", manifested by a brief period of sudden arousal when the microwaves were turned on and off. In all the other experiments, the state of sedation was not changed during the period of microwave administration.

The second finding was that following a latent period of 3 to 12 min there occurred a sudden arousal which lasted on the average 3 min. This was followed by a return to sedation. Again, 3 to 5 min later, a second arousal occurred, 2 to 10 min in duration. Such an alternation of periods of sedation and arousal occurred up to 4 times in certain experiments. In a number of cases, when arousal was present, it was sustained for as long as the animals were in the chamber.

Typical examples of the EEG patterns during the two phases of sedation and arousal can be seen in Figure 1. There were minimal changes during the period of microwave exposure and up to 12 min following exposure. However, at that time, there occurred a sudden arousal which lasted more than 10 min. This was followed by a return of the sedation-type activity, again followed by arousal lasting 12 min. Finally, during the last 5 min of the post-exposure period, sedation was quite prominent.

Under the direct tracings, one can see the integration pulses, the number of which is directly proportional to the cumulated amplitudes. When the successive numbers of integration pulses for fixed intervals are plotted versus time, one obtains chronograms of the electrical activity. For this particular experiment, the chronogram can be seen in

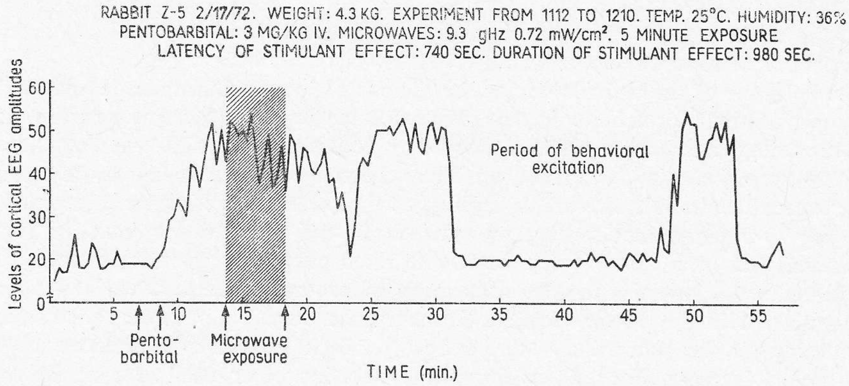

Fig. 2. Time-course changes in EEG amplitudes before, during and following microwave exposure.

Figure 2. The alternation of sedation and arousal is quite clear. It can also be seen that the variations in electrical activity during the 16 min of arousal were less pronounced than the ones present during the pretreatment control period. There was a brief arousal 6 min after microwave irradiation, but this lasted only 1 min. As a matter of fact, arousals do occur in control experiments without microwave exposure, but they are most often of short duration, at most 1 min. The power density in this particular experiment was 0.72 mW/cm^2.

In Figure 3 one can see the chronograms for another experiment with a power density of 2 mW/cm^2. The chronology of the changes is quite similar. One finds a decrease in the overall amplitudes and a 6-fold decrease in the standard deviation of the distribution of amplitudes during the period of intense behavioral excitation. The difference between the mean amplitudes during the control period and excitation period is statistically highly significant ($t = 3.1$; p smaller than 0.01). We found very similar decreases of mean amplitudes and variability in rabbits treated with hallucinogenic drugs. How-

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