PROLONGATION OF SLEEP AS THE RESULT OF THE CREATION OF A GENERATOR OF PATHOLOGICALLY ENHANCED EXCITATION IN THE ORBITAL CORTEX

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In acute experiments on cats injection of tetanus toxin, which disturbs various types of inhibition, into the orbital cortex caused the formation of a local generator of pathologically enhanced excitation in that region. In chronic experiments on cats with such a generator in the orbital cortex pathological changes of sleep appeared: a decrease in the duration of wakefulness and the development of a prolonged sleep state, while the normal ratio between slow-wave and paradoxical stages in the sleep continuum was preserved. The results confirm the view that the orbitofrontal cortex participates in the induction of sleep and they develop the general concept of the role of determinant structures in the activity of the nervous system and the theory of generator mechanisms of neuropathological syndromes characterized by hyperactivity of its systems.

KEY WORDS: determinant; orbital cortex; sleep–waking cycle; tetanus toxin, generator of pathologically enhanced excitation.

When a functional structure determining the behavior of a physiological system and so playing the role of determinant dispatch station (DDS) or determinant [3], becomes hyperactive and acts as a generator of pathologically enhanced excitation (GPEE) [9, 10], it makes the physiological system hyperactive also and endows it with a pathological character [3, 4]. The activity of such systems is manifested as the corresponding neuropathological syndromes [4-7]. These observations form the basis of the present investigations whose object was to study pathological changes in sleep associated with the appearance of a hyperactive determinant structure, namely an excitation generator in the somnogenic system. One of the components of that system is the orbitofrontal cortex, with whose activity the induction of sleep is associated [14, 17]. It might be supposed that the creation of a GPEE in the orbitofrontal cortex and its consequent conversion into a hyperactive determinant structure may lead to changes in the sleep–waking continuum.

EXPERIMENTAL METHOD

Chronic and acute experiments were carried out on 25 cats. Operations were performed under open ether anesthesia. Access to the frontal pole of the hemisphere was obtained through a burr hole in the frontal sinus. For manipulations on the orbital gyrus under visual control the frontal pole of the hemisphere was displaced superiorly and posteriorly by means of a spatula. The GPEE was produced by means of tetanus toxin (TT) which disturbs various types of inhibition [2, 8, 13, 15, 16] and makes it possible to use both acute and chronic experiments and to maintain prolonged observations on animals under conditions of free behavior [5-7]. TT was injected into the orbital cortex by means of a microinjector at 3 and 4 points to a depth of 500-1000 μ, in a total dose of between 60 and 100 MLD for mice (dried toxin, diluted in a 30% solution of glycerol in physiological saline, was used). The volume of fluid at each injection was $2 \cdot 10^{-4}$-$1 \cdot 10^{-3}$ ml. Animals receiving an injection of a similar solution of TT but inactivated by antitetanus serum served as the control.

Potentials were recorded by implanted silver electrodes from the frontal and occipital cortex of both hemispheres by a monopolar technique (the reference electrode was fixed in the nasal bones) and from the antigravity muscles of the neck by a bipolar technique.

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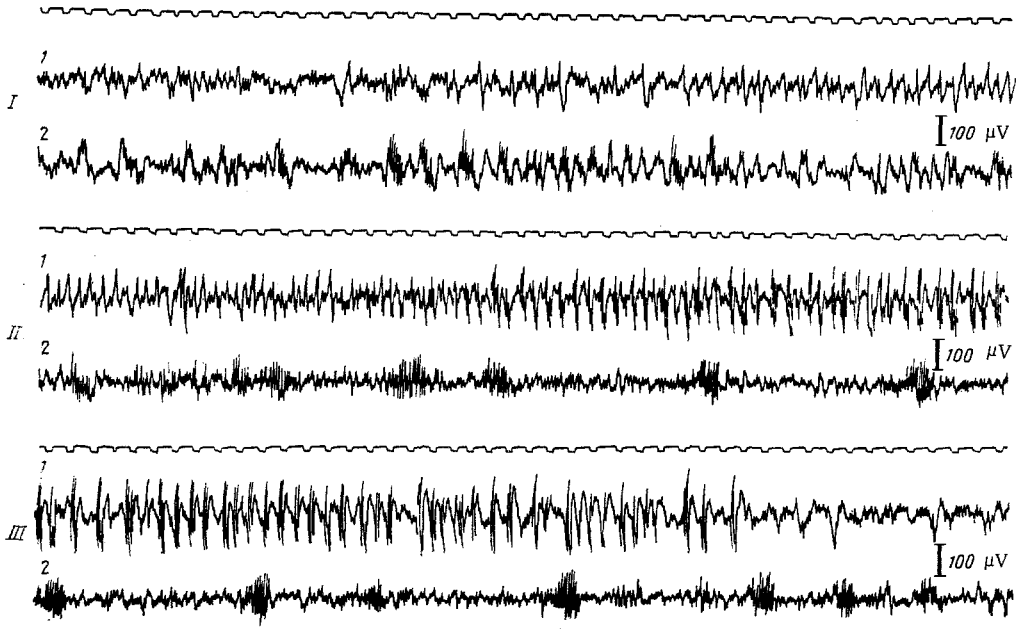

Fig. 1. Electrical activity in orbital cortex (at site of injection of TT) and visual cortex 22 h after injection. I, II, and III) Successive fragments of record of potentials in orbital (1) and ipsilateral visual cortex (2). Calibration: time 1 sec, signal amplitude 100 μV.

Observations on the animals' behavior began immediately after their recovery from the anesthetic and the EEG was recorded periodically (every 3 h).

From 22 to 24 h after injection of TT, when persistent signs of prolonged sleep appeared, continuous recording of the potentials began and extended over periods of 8 h (period 1) and 4 h (period 2), for 12 h altogether, with an interval of 10 h. On the following days until sleep returned to normal, observations were concentrated on the animals' behavior. On the 7th-9th day potentials were recorded continuously for 12 h. At the end of the experiments (after 10-12 days) the animals were killed to determine the precise site of the injection. Observations on the behavior of the control animals and the recording of their biopotentials were carried out at the same times and under similar conditions.

In acute and semichronic experiments the potentials were recorded from the site of injection of TT. In the acute experiments activity was recorded before and every hour after the injection of TT (30-100 MLD at 3 or 4 points) into the orbital cortex for 24-26 h. Throughout this period the animals were kept in a frame. In the semichronic experiments the operation was performed in the same way as the chronic experiments, but the animals were fixed to the bench 24 h after the injection and the orbital gyrus exposed. Potentials were recorded from the orbital cortex by means of bipolar electrodes.

For the statistical analysis of the data the Wilcoxon – Mann – Whitney criterion was calculated [1].

EXPERIMENTAL RESULTS

Persistent behavioral signs of deep sleep appeared 18-22 h after the injection of TT into the orbital cortex. The cats could be awakened only by strong stimuli and only for a short time (1-5 min). To satisfy their physiological needs (eating, defecation, etc.) they awoke for a little longer (up to 10 min). The cats spent most of the day asleep.

The behavioral features of sleep, awakening, and wakefulness correlated with the electrographic indices.

The behavioral and electroencephalographic pictures of prolonged sleep continued for 3-4 days, after which the normal sleep – waking cycle was restored; periods of wakefulness increased in duration and the animals responded more actively to external stimuli. Three of the five cats developed convulsions of clonic type, which stopped after a few days. These convulsions appeared in the late stages (on the 4th-9th days) in the period of normalization of sleep.

Analysis of the duration of the phases of the sleep–waking cycle based on the results of continuous 12-hourly recording of the potentials showed that the duration of wakefulness in the experimental animals was significantly ($U = 0$, $P < 0.001$) reduced compared with the control (1-1.5 h and 5.5-8 h respectively). The duration of the sleep state was increased significantly ($U = 0$, $P < 0.001$) from 5-6.5 h in the control animals to 10.5-11 h in the experimental, mainly on account of lengthening of slow-wave sleep (SS). In the experimental cats the duration of SS was 3-6 h longer than in the control. The duration of paradoxical sleep (PS) also was increased (by 20-90 min compared with the control), so that the relative proportions of these phases in the total duration of sleep remained almost the same as in the control animals (in the experimental group PS accounted for 7.3-15.8% of the duration of sleep, compared with 7.6-16.4% in the control; $P > 0.05$, differences not significant).

At the site of injection of TT in the orbital cortex, activity characterized by the appearance of irregular spike potentials with an amplitude of 100-200 μV (Fig. 1, curves I) against the background of the α and β rhythms was recorded after 16-18 h. Epileptiform activity, characterized initially by an increase in the frequency and amplitude of the spike potentials and the appearance of pointed slow waves, appeared periodically in the orbital cortex. At the height of this activity potentials of the spike-wave type were observed (Fig. 1, curves II). The paroxysmal potentials could disappear either gradually (they became less frequent, their amplitude decreased, and their previous rhythm was restored) or suddenly (Fig. 1, curves III). This type of activity at this stage of the process, which corresponded in time with the appearance of a persistent sleep state in the cat during the chronic experiments, was local and was recorded only in the region of injection of TT into the orbital gyrus. In other parts of the cortex studied (occipital and frontal lobes of both hemispheres), bursts of spindles and slow θ and Δ waves were recorded parallel with the epileptiform activity in the orbital cortex (Fig. 1, curves I-III).

Under these experimental conditions the total duration of sleep in cats with a GPEE in the orbital cortex was significantly increased compared with the control; in absolute values this increase took place chiefly on account of lengthening of SS. However, as already stated, since the total duration of PS also increased, the structure of the sleep continuum was undisturbed and the ratio of SS to PS remained the same as in the control animals. These results agree basically with the results of clinical observations [10] showing that in patients with epileptic foci in the prefrontal regions of the brain the total duration of sleep is increased, and the duration is attributable to an increase in the periods of SS. The dynamics of the EEG changes with deepening of SS and the transition from SS to PS and vice versa in the experimental animals was similar to that in intact cats and corresponded to the description of this process by other workers [11, 18]. No new components were observed in the sleep–waking cycle and no stages were omitted.

The sleep of the experimental animals was thus typical, with all the characteristic electrographic and behavioral elements. Its pathological feature was a considerable increase in its duration. This description shows that by the time of appearance of a stable sleep state in the animal, the GPEE had formed in the orbital cortex, and the appearance of slow waves and bursts of spindles was connected with the functional discharge from the hyperactive determinant, which the orbital cortex had now become.

Besides its purely neurological significance, the syndrome discussed above provides a promising model for the study of the mechanisms of sleep.

The results obtained with this new model develop the general concept of the role of determinant structures in CNS activity and the theory of generator mechanisms of neuropathological syndromes characterized by hyperactivity of brain systems [3, 4].

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EXPERIMENTAL INVESTIGATION OF THE IMMUNOTHERAPY OF BURNS

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The effect of convalescent burn serum on the toxic properties, level of activity of proteolytic enzymes, and morphological changes after burns was studied in experiments on rats. After burns the serum and organ extracts were found to acquire toxic properties, proteolytic enzyme activity was increased, and marked morphological changes developed. Injection of convalescent burn serum promoted detoxication, reduced the proteolytic enzyme activity distinctly, and reduced the severity of the morphological changes. Serum of healthy animals gave a much smaller therapeutic effect.
KEY WORDS: burns; convalescent burn serum; immunotherapy.

The acute period of burns is characterized by toxic manifestations, disturbances of the function of various organs, and predominance of catabolic processes [1, 3, 7, 8].

Because of the combination of disturbances associated with burns, a method of treatment must be used which can influence the pathogenetic basis of the functional changes.

The pathogenesis and methods of treatment of burns have now been studied for many years. The theory of the noninfectious immunology of burns has been formulated and a method of immunotherapy, consisting of treatment of burned patients with the serum of persons recovering from burns, has been developed. Although the method of immunotherapy has been widely acclaimed [2, 4-10], the study of the mechanism of action of convalescent serum still continues. The investigation of whether the processes of increased catabolism can be influenced by immunotherapy is of great interest, for increased proteolysis may be one of the sources of the toxic effects.

The object of the present investigation was to study the effect of convalescent burn serum on the toxic properties, the level of proteolytic enzyme activity, and the morphological changes in the organs of rats after burns.

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