central blood flow, but they also organize the rapid absorption of the liquid fraction of the chyme, thereby taking part in autoregulation of the circulating blood volume when that is deficient.

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ROLE OF LIMBIC STRUCTURES IN THE MECHANISMS OF POST-TRAUMATIC EPILEPSY

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The epileptic syndrome is a very widespread aftereffect of brain trauma (BT) [6, 7]. Meanwhile the neurophysiological mechanisms of post-traumatic epilepsy have not yet been adequately studied. It was shown previously that an epileptic syndrome is formed through the appearance of an epileptic system, whose determinant structure is located differently in focal and generalized forms of the epileptic syndrome [3, 4]. It is not yet clear whether the formation of post-traumatic epilepsy likewise is connected with activity of an epileptic system, or what brain structures can play the role of pathological determinant in this form of epilepsy. We know that hyperactive determinant structures differ from dependent components of the epileptic system and other brain formations in their high level of excitability and their ability to respond first to relatively weak provoking stimuli. For their detection, the usual method is to increase brain excitability very slightly through

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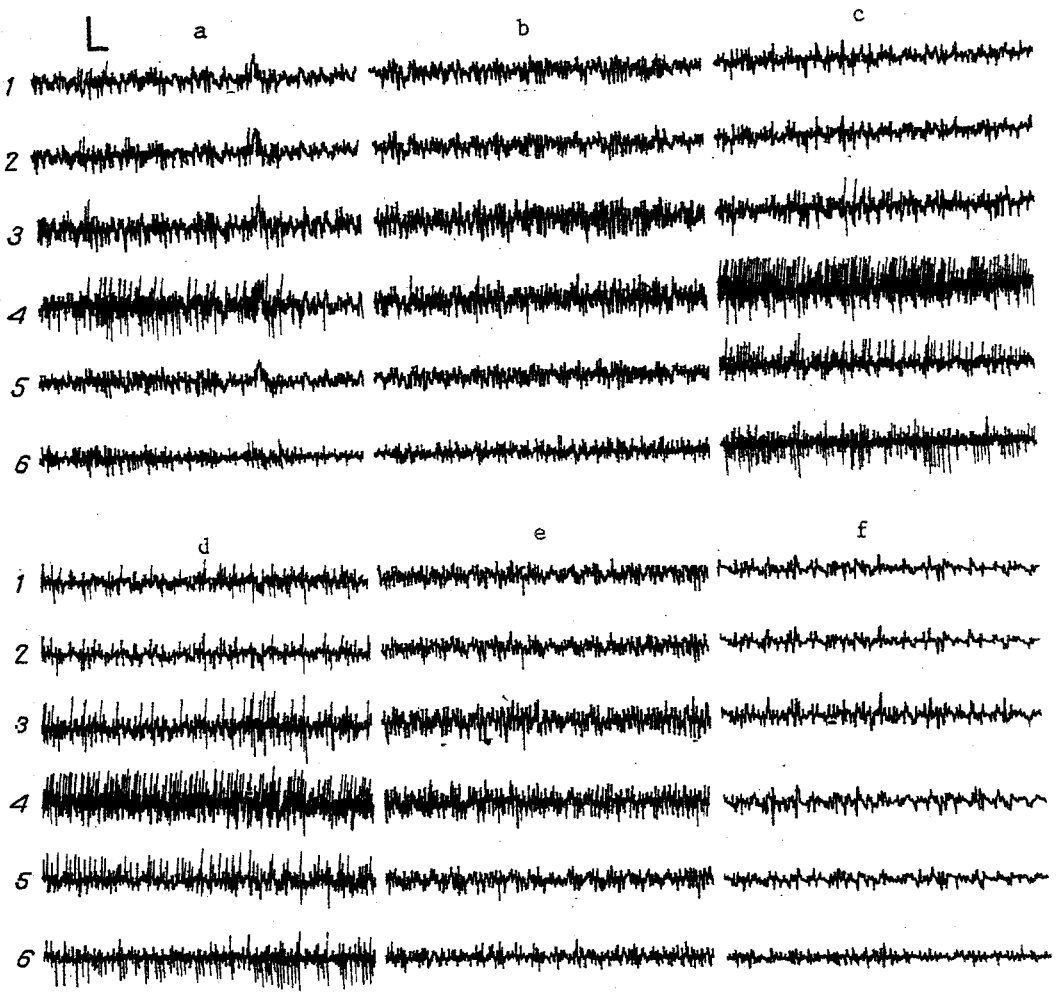

Fig. 1. EEG changes in rat No. 2 after brain trauma and intraventricular injection of kainic acid (0.01 μg): a) 4 min after BT, b-f) 3, 13, 21, 45, and 72 min respectively after injection of kainic acid. Legend: 1) left, and 2) right ventral hippocampus, 3) superior colliculus, 4) enterrhinal cortex, 5) reticular part of substantia nigra, 6) caudate nucleus. Calibration: 250 μV, time marker: 1 sec.

the use of substances with a disinhibitory or direct excitatory action [2]. Considering that the formation of generators of pathologically enhanced excitation (GPEF) can be associated both with a deficit of inhibition and with primary intensification of excitation processes [2], in the investigation described below we studied the effects of picrotoxin, which disturbs GABA-ergic inhibition, and kainic acid, an agonist of the excitatory mediator, glutamate [5], on the development of seizure activity after BT.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-210 g. Each group consisted of at least 10 animals. Graded brain trauma was inflicted by the method in [1], modified for rats. Kainic acid ("Sigma," USA, 0.01-0.05 μg in 2 μl of phosphate buffer solution (pH 7.4) was injected under open ether anesthesia into the lateral cerebral ventricle 40-60 min after BT. Picrotoxin ("Sigma," USA, 1.5-3.0 mg/kg) was injected intraperitoneally 40-60 min after BT. Animals not exposed to trauma, but receiving the above-mentioned substances in similar doses, served as the control. The intensity of the seizure responses was assessed by the scale in [4] and expressed in points. To record electrical activity of the brain structures, the animals were anesthetized with ether and, taking coordinates from a stereotaxic atlas [13], constantan electrodes 0.15 mm in diameter were implanted in the ventral hippocampus, caudate nucleus, reticular part of the substantia nigra, superior

TABLE 1. Effect of Brain Trauma on Seizure Activity Induced in Rats by Kainic Acid and Picrotoxin (M ± m; n = 10)

Experimental conditions	Intensity of seizures, points	No. of animals with generalized convulsions
Kainic acid, 0.01 μg		
Experiment	4,0±0**	12*
Control	0,9±0,2	0
0.05 μg experiment	4,0±0**	10*
Control	1,0±0,3	0
Picrotoxin 1.5 mg/kg		
Experiment	0,4±0,2	0
Control	0,3±0,2	0
3.0 mg/kg experiment	4,3±0,2	10
Control	3,9±0,4	10

Legend. Experiment) Rats with BT, control) rats without BT. *$p < 0.025$ (Fisher's exact method), **$p < 0.001$ (Student's test) denote significant differences compared with control.

colliculi, and entorrhinal and sensomotor cortex. Electrical activity was recorded by a monopolar technique (the reference electrode was fixed in the nasal bones) on an EEG-8S electroencephalograph (Hungary). The results were subjected to statistical analysis by parametric and nonparametric tests.

EXPERIMENTAL RESULTS

Immediately after infliction of BT the animals showed intensification of their locomotor activity, as shown by running about, jumping high in the air, and making uncoordinated rapid limb movements. Some rats (nine of 20 animals) exhibited brief (up to 3 min) periods of akinesia, accompanied by vestibular disturbances and by depression of the response to external (nociceptive, photic, acoustic) stimulation. In addition, five animals developed repeated clonic convulsions of the whole trunk, lasting from 1 to 3 min. An EEG study showed that four of the 10 animals after infliction of BT developed spike discharges in the entorrhinal cortex with an amplitude of 300-350 μV (Fig. 1a, zone 4).

In rats subjected to BT, exophthalmos, increased tone of the tail, and episodes of akinesia lasting 10-15 sec, interrupted by normal locomotor activity, were observed 5-7 min after intraventricular injection of kainic acid (0.01-0.05 μg). After a further 3-5 min, the rats developed paroxysmal twitches of individual muscle groups, changing into clonic convulsions of the whole trunk. All the animals developed generalized clonicotonic convulsions, with falling on their side and postictal depression, 15-20 min after injection of kainic acid. Injection of kainic acid (0.01-0.05 μg) into animals of the control group caused nonconvulsive behavioral disturbances (exophthalmos, hypertonus of the tail, episodes of akinesia), the seizure reactions were characteristically single myoclonic twitches, and their intensity was significantly less than in rats with BT (Table 1). Injection of kainic acid in a larger dose (0.1 μg) caused not only the behavioral changes described above, in rats without BT, but also the development of generalized convulsions. The average intensity of the convulsions was 4.0 points, similar in severity to seizure reactions found in rats subjected to BT, and receiving injection of kainic acid in doses of 0.01 and 0.05 μg ($p < 0.05$).

The study of EEG changes in rats subjected to BT showed that 2-3 min after injection of kainic acid (0.01 μg) seven of the 10 rats developed seizure discharges with a frequency of 5-6/sec and with maximal amplitude in the entorrhinal cortex (300 μV, Fig. 1b, zone 4). After 10-15 min the amplitude of epileptic activity (EpA) in the entorrhinal cortex increased to 450-500 μV, and it also developed in other brain structures (Fig. 1c). Fast (5-8/sec) synchronized potentials, whose amplitude was greatest in the entorrhinal cortex (500-600 μV, Fig. 1d), were recorded in all structures 15-20 min after injection of kainic acid. The duration of the periods of generalized EpA was 80-120 sec, after which seizure discharges with maximal amplitude in the entorrhinal cortex (up to 350 μV, Fig. 1e, zone 4) were recorded in the EEG for a further 30-45 min. Injection of kainic acid in a dose of 0.01 μg was accompanied in three of 10 animals by initiation of EpA in structures of the ventral hippocampus, where seizure activity with an amplitude of 250-300 μV appeared 5-7 min after in-

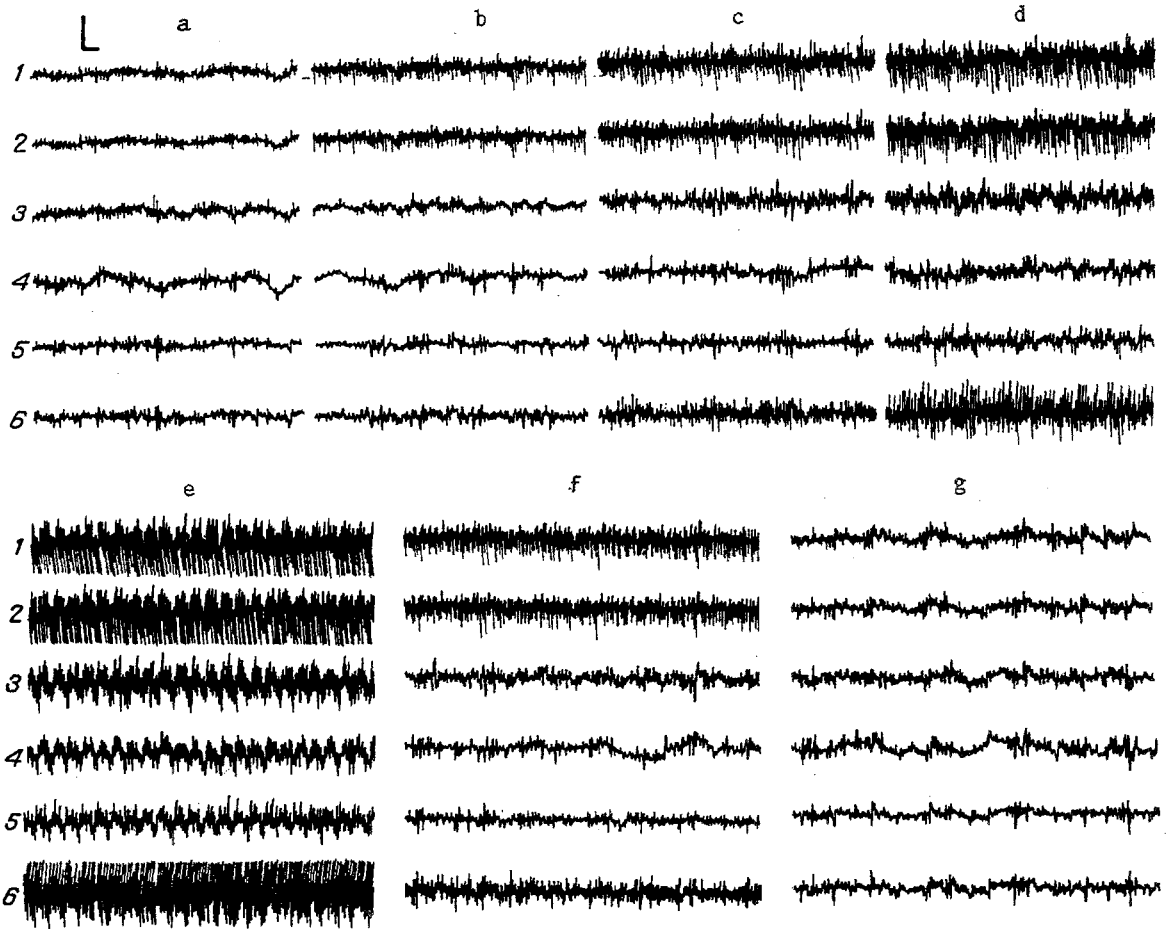

Fig. 2. EEG changes in rat No. 6 after brain trauma and intraventricular injection of kainic acid (0.01 μg): a) 3 min after BT, b-g) 4, 13, 24, 30, 45, and 75 min respectively after injection of kainic acid. Legend: 1) left and 2) right ventral hippocampus, 3) superior colliculus, 4) sensomotor cortex, 5) caudate nucleus, 6) entorrhinal cortex. Calibration: 250 μV, time marker: 1 sec.

jection of the convulsant (Fig. 2b, zones 1 and 2). The amplitude of discharges 5-7 min after injection of kainic acid increased to 400 μV in the hippocampus and in the entorrhinal cortex also (Fig. 2c). EpA was recorded 20-40 min after injection in all structures tested (Fig. 2d). Synchronized potentials were observed in all structures 35-45 min after injection of kainic acid, and in the hippocampus and entorrhinal cortex their frequency was 9-14/sec and their amplitude 400-500 μV (Fig. 2e). The duration of generalization of EpA was 2-3 min, after which spike-wave complexes were recorded for a further 25-30 min in the EEG, with maximal amplitude in the hippocampus and entorrhinal cortex (200-300 μV, Fig. 2f, zones 1, 2, and 6).

After injection of kainic acid (0.05 μg) into animals of the control group, seizure discharges with an amplitude of 200-250 μV were observed on the BEG in all structures (Fig. 3I, b), and were recorded for 35-60 min after which they disappeared (Fig. 3I, c). After injection of kainic acid in a dose of 0.1 μg, epileptic discharges with a frequency of 3-5/sec appeared in the animals of the control group; their amplitude was maximal in the hippocampus and entorrhinal cortex (400-450 μV, Fig. 3II, b).

In a separate series of experiments the effect of picrotoxin on EpA was studied in traumatized rats. The use of picrotoxin in a dose of 1.5 mg/kg led to the appearance of myoclonic spasms in animals of the experimental and control groups, whereas in a dose of 3 mg/kg it caused generalized clonicotonic fits with the animals falling on their side. The mean intensity of the seizure reactions in the animals of the two groups did not differ significantly (Table 1).

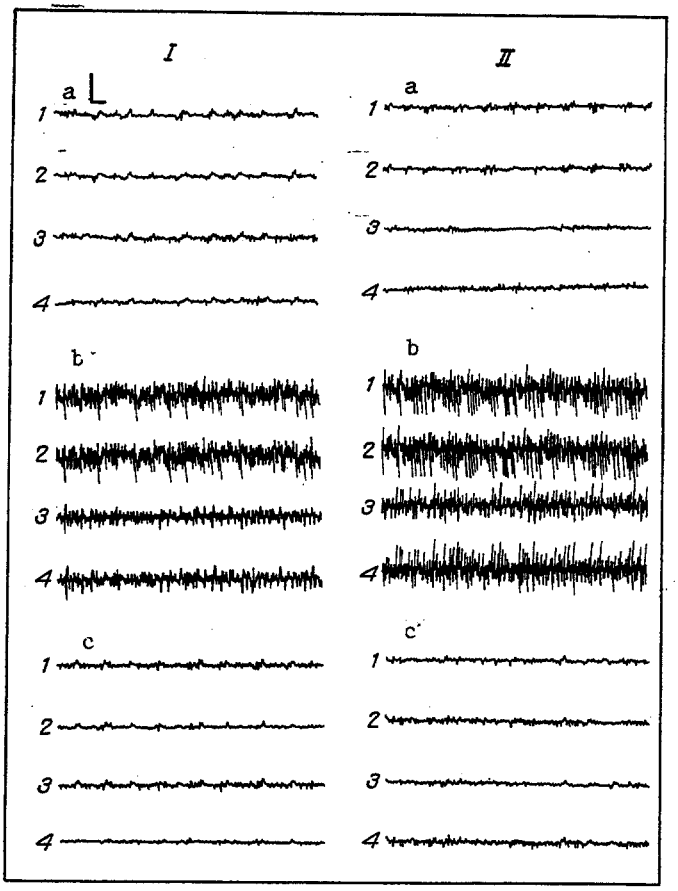

Fig. 3. BEG changes in intact rats after intraventricular injection of different doses of kainic acid I: a) Spontaneous activity, b, c) 30 and 60 min respectively after injection of kainic acid in a dose of 0.05 μg. Legend: 1) right and 2) left ventral hippocampus, 3) caudate nucleus, 4) sensomotor cortex. II: a) Spontaneous activity, b, c) 25 and 100 min respectively after injection of kainic acid in dose of 0.1 μg. Legend: 1) right and 2) left ventral hippocampus, 3) caudate nucleus, 4) entorrhinal cortex. Calibration: 250 μV, time marker: 1 sec.

The investigation thus showed that injection of relatively low doses of kainic acid into rats caused the development of marked behavioral and epileptiform EEG changes. The increase in sensitivity to the epileptogenic action of kainic acid is evidence of the involvement of excitatory neurotransmitter systems of the brain in the mechanism of post-traumatic epilepsy. This hypothesis is in agreement with our ideas on the important role of excitatory amino acids in the mechanisms of the epileptic syndrome [5, 10, 12] and of their involvement in the development of posttraumatic neuropathological syndromes [15]. EEG data revealing maximal intensity of EpA in the entorrhinal cortex or in structures of the hippocampus are evidence of the involvement of limbic structures in the formation of post-traumatic epilepsy. We know that the limbic structures play a key role in the mechanisms of kainate-induced epilepsy [9]. It has also been shown that the development of EpA during kindling is the most adequate model of epileptogenesis, due to hyperactivation of the amygdala, hippocampus, and entorrhinal cortex [4, 8, 11]. After brain trauma, the limbic structures are evidently the first to respond to BT, as formations distinguished by exceptionally high excitability [14]. The additional increase of brain excitability caused by kainic acid facilitates maximal manifestation of activity in the zone of the GPEE thus formed, and this reflects the important property of hyperactive determinant structures, which is to be the first to respond to specific pathogenic influences [2].

On the other hand the investigations showed that BT did not affect the sensitivity of the animals to the convulsant action of GABA. These findings are evidence that disturbance of GABA-ergic inhibition was not involved in the genesis of posttraumatic epilepsy, and also of the specific character of the post-traumatic strengthening of the predisposition to seizures.

Thus, after brain trauma and injection of kainic acid, a GPEE is formed in structures of the limbic system as a result of the intensification of glutamatergic neurotransmission, and it plays the role of hyperactive determinant structure in activity of the epileptic system [2, 4].

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