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Two Types of Epileptiform Activity Induced in Rats by Repeated Injections of Subconvulsive Picrotoxin Doses

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Seizure activity in rats was induced by picrotoxin i.p. injected every 30 min for 4 h in subconvulsive doses (0.9 mg/kg in the first and 0.7 mg/kg in subsequent injections). Such picrotoxin-evoked epileptiform activity (EA) could be divided into two types, with the development of exclusively peak-wave (P-W) complexes (68.4%) and with generation of regular cortical peaks and only separate short P-W discharges (31.6%). In animals with EA of the first type, we observed the gradual development of clonic seizures of increasing intensities. In some rats of this group after the final (ninth) injection of picrotoxin, dramatic clonico-tonic seizures were observed. In animals with EA of the second type, the duration of P-W discharges after final injections of picrotoxin was shorter than in rats without generation of cortical peaks; these discharges were accompanied by clonic seizures of smaller intensity.

Keywords: epileptiform activity, picrotoxin, seizures, peak-wave EEG discharges, regular cortical peak discharges.

INTRODUCTION

In studies of the mechanisms of development of epilepsy, most researchers focus on the appearance and spreading of excessive excitation in cerebral neuronal systems and also on the search and elucidation of the role of compensatory mechanisms activated in the course of epileptization [1-3]. At present, one of the urgent fields of experimental epileptology is the development of novel models allowing experimenters to monitor changes in the course of rapidly increasing synchronization and generation of epileptiform EEG activity (EA). For the first time, it was demonstrated that rapid, or accelerated, kindling could be reproduced in studies of the effects of electrical stimulation of the hippocampus (electrostimulation kindling) where the dependence of such effects on the mode and parameters of such stimulation was examined [4]. As is known, classic kindling can be formed not only when electrical stimulation of the limbic and neocortical cerebral structures was used but also with the help of some pharmacological influences. Taking into account the phenomena of cross-sensitivity and synergism manifested upon the action of kindling stimuli of a different nature, some researchers believed that the pathogenetic mechanisms of formation of EA in different kindling models are, to a considerable extent, common [5]. Recently, a model of induction of acute convulsions using repeated injections of corazole in subconvulsive doses within only a 70-min-long interval has been proposed [6]. Under conditions of such experiments, a specific state, the so-called generalized nonconvulsive *status epilepticus*, was formed. Reproduction of neuronal hyperactivity of such a type in the experiment makes it possible to study regularities of the formation, development, and reorganization of epileptogenic and antiepileptogenic systems manifested during rather short time intervals.

Our study was aimed at elucidating the peculiarities of accelerated formation of progressive EA in rats subjected to repeated systemic injections of picrotoxin in subconvulsive doses during several hours.

METHODS

Experiments were carried out under conditions of chronic experiments on 19 male mongrel albino rats weighing 180-250 g. Preliminary surgery was performed under combined anesthesia (thiopental sodium, 70 mg/kg + calypsol, 7 mg/kg, i.p.). After trepanation of the skull, monopolar nichrome electrodes with varnish insulation (tip diameter

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0.10-0.15 mm) were stereotaxically implanted into the frontal cortical zone, ventral hippocampus, and mediodorsal thalamus according to the following coordinates: frontal cortex, AP = 2.4, L = 0.8, and H = 1.2; hippocampus, AP = –4.8, L = 5.0, and H = 7.0; and thalamus, AP = –2.8, L = 0.5, and H = 5.5 [7]. Electrodes were fixed to the skull using dental plastic (Protacryl). Recording of electrical activity, injections of the convulsant, and examination of behavioral reactions were performed not earlier than 7 days after preliminary operations. Recording of the brain electrical activity was performed using a technique allowing us to obtain such records under conditions close to free behavior of the rats, during 30 min prior to injections of the convulsant and 5-6 h after such injections. A differential amplifier, DL304 (NeiroBioLab, Russia), coupled with an ADC (L-154, L-KARD, Russsia) were used. Recording and analysis of EEG activity were performed using a multichannel oscillograph supplied with the corresponding software, PowerGraph (Russia).

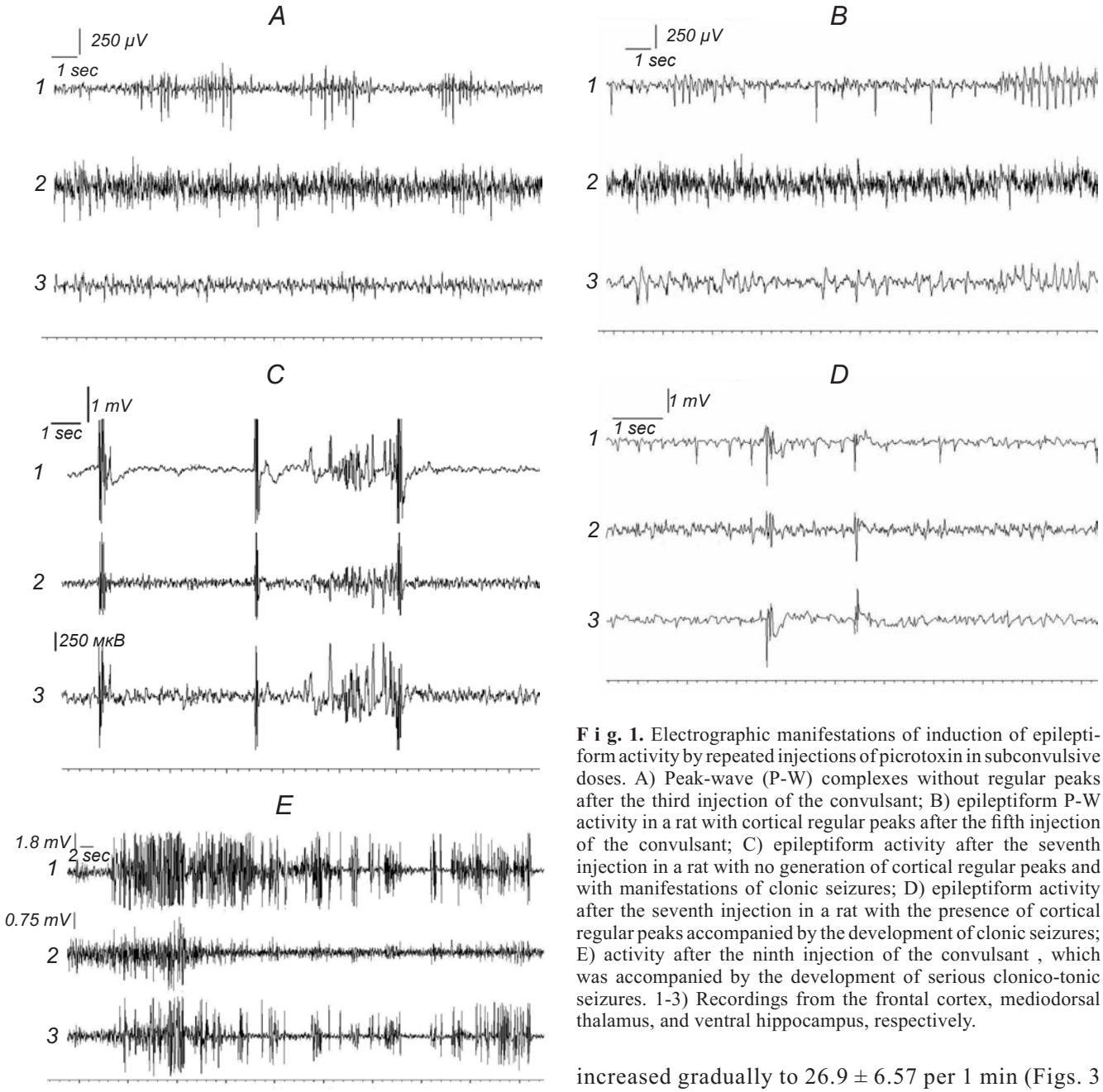
Epileptiform activity was induced by repetitive injections of picrotoxin (Sigma, USA). The convulsive effect of this agent is related to the disturbance of GABA-ergic inhibition. Picrotoxin was i.p. introduced every 30 min in doses of 0.9 mg/kg in the first and 0.7 mg/kg in the next injections. The total dose of the convulsant injected into a rat was about 6.5 mg/kg.

We analyzed the frequency-amplitude characteristics of (i) peak-wave (P-W) EEG complexes, (ii) separate peak EEG potentials generated with no accompanying behavioral convulsive manifestations, and (iii) generalized potentials recorded in the course of development of clonic and clonico-tonic seizures. The frequencies of generation of epileptiform potentials were measured within 3-min-long intervals, and their mean values were calculated. In addition, we studied the dynamics of generation of generalized P-W potentials related to the development of convulsive manifestations. We measured the total duration of 3-min-long EEG segments, within which convulsive potentials were generated, and estimated the mean value of the duration of epileptiform EEG potentials within 1-min-long intervals. High-amplitude (> 300-400 μV) rapid oscillations lasting about 50 msec were considered the EEG peaks. If these discharges were repeated with intervals from 3 to 20 sec, such EA observed after injection of picrotoxin was considered an activity with regular generation of peaks potentials. Concurrently, we monitored behavioral changes and estimated the intensity of convulsive manifestations, which were visually estimated using a 5-point scale. The absence of convulsive manifestations corresponded to 0 point; appearance of myofascial jerks, 1 point; clonic contractions of the head and body muscles, 2 points; clonic contractions of the forelimb muscles and rearings ("kangaroo poses"), 3 points; generalized clonico-tonic convulsions with falling of the animal on body side, 4 points, and repeated generalized clonico-tonic convulsions, 5 points [8].

Numerical data were processed statistically with calculation of the mean value, standard deviation, and confidence interval; the level of significance of intergroup differences was estimated using ANOVA. Such differences were considered statistically significant at $P < 0.05$.

RESULTS

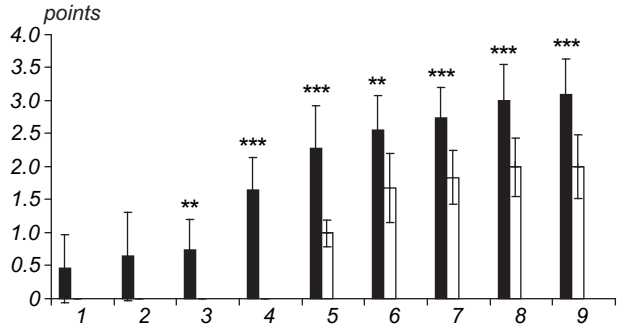
To form EA, the rats under study were injected every 30 min with picrotoxin in doses that initially were not sufficient to initiate convulsive behavioral manifestations in most animals. Three to six minutes after the first injection of the convulsant, we observed in four rats (21%) a short (about 1 min long) total suppression of motor activity, "freezing in place." Repeated injection of picrotoxin caused an increase in the duration of such time intervals to 5-7 min and gradual development of clonic seizures. The pattern of behavioral manifestations correlated with the type of EEG activity, which was recorded within this time interval. In our experiments, the development of EA was associated with the formation of two types of cortical activity. The first type of EEG phenomena was typical of 13 of 19 (68.4%) rats. In such animals after one to three injections of picrotoxin, a considerable number of P-W complexes were generated in the neocortex (Fig. 1A). The amplitude of these discharges did not exceed 400 μV, and their frequency was 6-8 sec^{-1}. After injection of the fourth dose of picrotoxin, all animals whose EA was characterized by the presence of only P-W discharges demonstrated clonic contractions of muscles of the muzzle, head, and forebody. The mean intensity of such convulsions was 1.67 ± 0.5 points (Fig. 2). Behavioral changes were recorded against the background of a gradual increase in the frequency and duration of synchronized P-W discharges (Figs. 3 and 4). After the sixth to



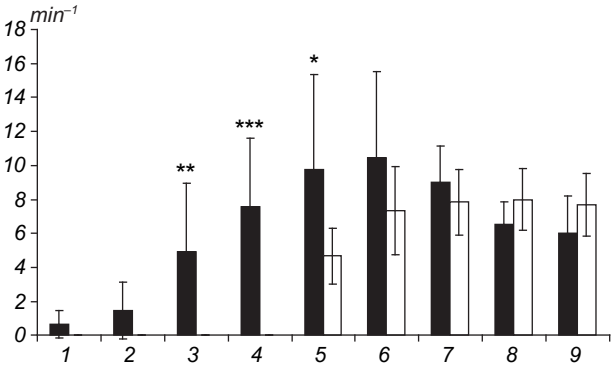
F i g. 1. Electrographic manifestations of induction of epileptiform activity by repeated injections of picrotoxin in subconvulsive doses. A) Peak-wave (P-W) complexes without regular peaks after the third injection of the convulsant; B) epileptiform P-W activity in a rat with cortical regular peaks after the fifth injection of the convulsant; C) epileptiform activity after the seventh injection in a rat with no generation of cortical regular peaks and with manifestations of clonic seizures; D) epileptiform activity after the seventh injection in a rat with the presence of cortical regular peaks accompanied by the development of clonic seizures; E) activity after the ninth injection of the convulsant , which was accompanied by the development of serious clonico-tonic seizures. 1-3) Recordings from the frontal cortex, mediodorsal thalamus, and ventral hippocampus, respectively.

eighth injections of the convulsant, the intensity of convulsions in these rats increased (maximally to 3 points; Fig. 2). At the same time, generalized rapid and slow waves, which formed a rhythmic pattern of P-W discharges alternated with periods of low-amplitude oscillations, were observed in EEG activity of the cortex, thalamus, and hippocampus (Fig. 1C). A maximum rise in the frequency of P-W complexes was observed after the sixth injection of the convulsant, when the above index reached $10.45 \pm 5.05\ min^{-1}$. Over the entire period of formation of such movements, the duration of convulsions increased gradually to 26.9 ± 6.57 per 1 min (Figs. 3 and 4). After the final, ninth, injection of picrotoxin, the development of generalized clonico-tonic seizures was obvious in 2 of 13 animals with generation of P-W activity. Such a significant increase in the intensity of seizures was accompanied by a rise in both frequency and duration of high-amplitude P-W discharges (in the cortex, 3.51 ± 0.46 mV on average, in the thalamus, 1.42 ± 0.41 mV, and in the hippocampus, 2.23 ± 0.51 mV), which lasted up to 40-50 sec (Fig. 1E).

Another specific, type of EEG activity was observed in the rest of the rats (six animals, 31.6%, Fig. 1B). After the first to third injections

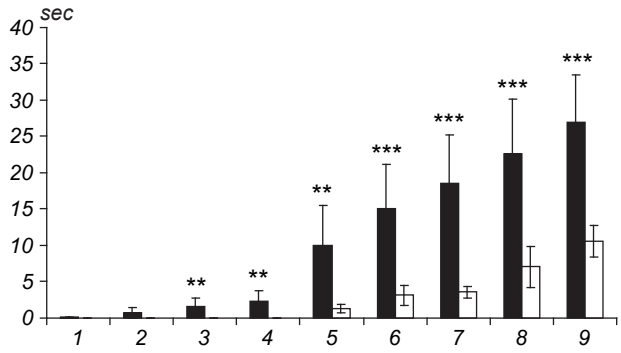


F i g. 2. Intensity of behavioral seizures in rats under conditions of accelerated induction of epileptiform activity using repeated injections of picrotoxin (1-9) in subconvulsive doses. Filled and open columns show the mean intensities of seizures, points, in animals with epileptiform EEG activity of the first and second types, respectively; the values of standard deviations are also shown. Two and three asterisks indicate cases of significant intergroup differences with $P < 0.01$ and $P < 0.001$, respectively.



F i g. 3. Frequency of generation of peak-wave discharges, min^{-1}, under conditions of accelerated induction of epileptiform seizure activity. One asterisk indicates an intergroup difference with $P < 0.05$. Other designations are the same as in Fig. 1.

of picrotoxin into such rats, a regular peak EEG activity in the cortex was formed. Examination of the processes of development of these epileptiform potentials showed that such activity, within the initial period of formation of EA, occurred in different structures in an asynchronous manner. Only in one case, there were analogous potentials in the cortex and hippocampus generated synchronously. Generation of cortical EEG peaks gradually acquired a regular pattern; such potentials were observed with 3- to 8-sec-long intervals. The mean frequency of such potentials was 14.8 ± 3.27 min^{-1}, and the mean amplitude was 468.5 ± 43.2 μV. Within the initial stages of formation, activity of this type was not accompanied by the development of convulsive behavioral manifestations. In these rats within further formation of EA, we recorded, in addition to regular cortical peak potentials, generations of synchronous P-W discharges. After the fifth injection of the convulsant into rats and



F i g. 4. Duration of peak-wave discharges, sec, within 1-min-long intervals under conditions of accelerated induction of epileptiform seizure activity. Designations are the same as in Figs. 2 and 3.

against the background of generation of the first P-W discharges and decrease in the frequency of cortical peaks to 9.6 ± 1.67 min^{-1}, we observed periods of immobilization of the animals and occurrence of myofascial jerks (Fig. 2). In animals with EA of the second type, P-W complexes were shorter than those in rats in the absence of generation of cortical peaks; these discharges were accompanied by clonic seizures whose intensity did not exceed 2 points (Figs. 1D, 2, and 4).

DISCUSSION

According to the results of our studies, repeated injections of picrotoxin in subconvulsive doses lead, within an interval of several hours, to induction of EA. The latter can, according to some peculiarities, be divided into two groups, (i) activity with the development of exclusively P-W complexes and (ii) regular cortical peak activity with generation of only sporadic short P-W discharges.

In animals of the first group, the formation of EA was accompanied by gradual development of clonic seizures of different intensities. In two rats of this group (10.5%), the final (ninth) injection of picrotoxin was accompanied by serious clonico-tonic seizures. The increasing intensity of seizures upon the action of repeated injections of picrotoxin is similar to that in the case of rapid kindling induced by electrical stimulation of structures of the limbic system [4].

In the second animal group, the development of regular peak EEG activity was accompanied by the development of only moderate behavioral seizures (mean intensity, 2 points on average). The mechanisms of generation and pathogenetic

importance of peak EA remain poorly studied [9-12]. Over a few decades, epileptologists believed that increases in the frequency and amplitude of peak discharges depend on the intensification of pro-epileptic influences on brain neuronal structures, and that this is related to activation of anticonvulsive mechanisms [10, 12]. Taking into account such data, clinicians prescribed anti-epileptic remedies and adjusted their doses in order to not only prevent the development of long-lasting ictal EEG discharges, but also to supress completely generation of interictal peak discharges.

The hypothesis that the development of interictal peak discharges represents most likely a manifestation of activation of anticonvulsive mechanisms, but not of proconvulsive ones, was, for the first time, proposed in the course of a study of changes in the amplitude and number of EEG peaks and also of thresholds for initiation of convulsive attacks during electrostimulation kindling [11]. In our study, we observed generation of regular peak activity in the cortex in aproximately one-third (31.6%) of the animals; such situation can be assumed to be a manifestation of activation of anticonvulsive mechanisms. The fact that convulsions formed in the case of just such peak cortical activity were perhaps less intense than the corresponding phenomena in animals with generation of exclusively P-W complexes confirms this hypothesis.

Recently, we also showed that, under conditions of the formation of "classic" pharmacological kindling, electrographic and behavioral manifestations of convulsive reactions can be clearly classified into two types [13]. In the inbred WAG/Rij rat strain with genetically determined susceptibility to convulsive reactions of the absence type, electrographic manifestations with different intensities of paroxysmal P-W complexes were once again divided into two types [14].

If we generalize the above-mentioned data, we should state that the formation of EA of two types in the experimental model with induction of such activity by repeated injections of picrotoxin in subconvulsive doses during several hours allows us to propose one more model of epileptogenesis; this can be useful in the studies of mechanisms of this phenomenon.

The study was carried out in accordance with the Ukrainian and international standards for use of animals in experiments (Council of Europe Convention, 1986), the Law of Ukraine "On Protection of Animals from Inhumane Treatment" (from 21.02.06, No. 3447-IV), as well as with regulations of the Ethics Committees of the Odessa National Medical University (protocol No. 28 D from 09.11.12).

The authors of this study, O. V. Denisenko and O. A. Shandra, confirm that, in the course of performance of the experiments, they have no conflict of interest pertinent to commercial or financial relations and relations with organizations or persons somehow or other related to the study, as well as to research group interaction.

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