SPATIAL DISTRIBUTION AND CHARACTER OF ACTIVITY OF EXCITED NEURONS AFTER PENICILLIN INJECTION INTO THE NUCLEUS RETICULARIS GIGANTOCELLULARIS

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The formation of generators of pathologically enhanced excitation (GPEE) lies at the basis of the development of typical pathological processes in the nervous system, it has a universal mechanism, and is expressed as hyperactivation of the corresponding systems [1, 3-6, 8, 9]. The study of the conditions and mechanisms of formation and activity of the GPEE is thus a very urgent problem from both theoretical and practical points of view.

The object of this investigation was to study the spatial distribution and character of activity of excited neurons in the zone of GPEE formation in the nucleus reticularis gigantocellularis (NRG) after injection of penicillin, used to create a GPEE in different parts of the CNS.

EXPERIMENTAL METHOD

Experiments were carried out on 48 anesthetized (chloralhydrate, 40 mg/kg, intraperitoneally) male Wistar rats weighing 250-300 g. The animals were kept under animal house conditions on a standard diet with food and water ad lib., and with alternations of 12 h daylight and 12 h darkness. An area was chosen in NRG (Fig. 1a), into the central part of which 1 μl of a 10% solution of the sodium salt of benzylpenicillin (200 U), made up in physiologicalsaline, was injected (at the rate of 1 μl/min) by means of an oil microinjector through a glass cannula (diameter of tip 50 μ). Twitching of the forelimb, neck, and head muscles of the animal began to appear 5-10 min after the penicillin microinjection. The action of penicillin, in accordance with data in the literature [17], continued for 1.5-2.5 h and was sufficient to allow probing of the test area of NRG. By means of a stereotaxic apparatus, nine insertions of the electrode (I-IX) were made in this area in accordance with the following coordinates: A = 1.1-1.8; L = 0.5-1.2; H = 1.5-2.7 (counting from the obex). Unit activity was recorded extracellularly by means of glass microelectrodes (diameter of tip 3-5 μ, resistance 5-10 MΩ) by the standard method. The signal recorded was led through a cathode follower to a VC-9 oscilloscope (Nihon Kohden, Japan). Unit activity was analyzed on an interspike interval counter, and the momentary average frequency and tachogram and histogram of interspike intervals were recorded on a graph plotter. When the number of active neurons was counted each track was divided by depth at four counting points. The spatial distribution of active neurons was analyzed in six vertical planes A, B, C, D, E, and F, including tracks I-III, IV-VI, VII-IX, I-VI-VII, II-V-VIII, III-IV-IX, respectively. and also in four horizontal planes, G, H, I, and K, where K and G correspond to the upper and lower surfaces of a conventional volume (the probed region). Unit activity was recorded under normal conditions (992 neurons) and after injection of penicillin (908 neurons). After the experiment the position of the electrode was verified relative to the obex, and the region of injection was labeled by injection of 1 μl of India ink. The animal's brain was removed and fixed in 10% formalin solution, and sections were cut (5-10 μ) and stained with hematoxylin or with cresyl violet by Nissl's method.

EXPERIMENTAL RESULTS

The morphological control confirmed that the electrode tracks corresponded to the calculated data and that 1 μl of penicillin was injected accurately into the central part of

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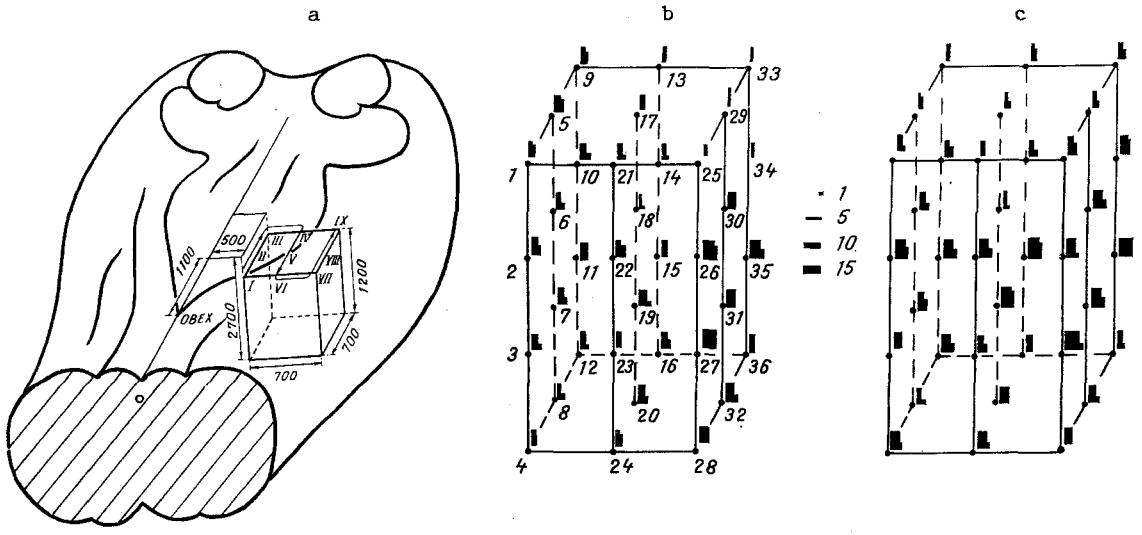

Fig. 1. Scheme showing location and size (in μ) of test region of NRG in rat medulla (a) and spatial distribution of number of active neurons in test region before (b) and after (c) penicillin injection. I-IX) Successive insertions of microelectrode during probing (tracks); 1-36) order of probing of NRG points by microelectrode. Area shaded is proportional to number of active neurons recorded at the given point.

NRG, so that it was possible to determine the volume of the structure subjected to the action of the convulsant, and it did not exceed 500 × 500 × 900 μ (Fig. 2b). The spatial distribution of excited neurons in this region under normal conditions is characterized by unevenness of arrangement of active cells. The range of their scatter at 36 points was quite wide and varied at one point from 3 to 20 (Fig. 1b). The largest number of active neurons was found on planes E and D, with a tendency toward displacement in the caudomedial direction. An increase in the number of excited neurons also was observed in the vertical direction in the central part of the probed volume compared with that in the dorsal and ventral regions.

After injection of penicillin an increase was observed in the number of active neurons on average by 21% (Fig. 1c). This increase was observed at a distance of 350-400 μ from the site of injection and was greatest in the caudal plane, and also into track IV of the rostral plane. The number of active neurons at individual points of the test volume varied from 6 to 25 (Fig. 1c). The largest number of active neurons after injection of penicillin was found in the caudal and lateral planes. Consequently, the formation of hyperactivity leads to a spatial redistribution of the active neurons of NRG and to displacement of most of them from the caudomedial into the caudolateral region.

Among neurons recorded before and after penicillin injection there were cells with different types of spike discharge: regular, irregular, periodic, and bursting. The average discharge frequency of NRG neurons was not significantly altered after penicillin injection (1.34-51.27 spikes/sec normally and 1.73-65.84 spikes/sec after injection). According to the histograms of interspike intervals, three main types of activity observed both normally and with the onset of hyperactivity were distinguished: a unimodal distribution of interspike intervals (Fig. 3a); a bi- or multimodal distribution (Fig. 3b), and an exponential distribution (Fig. 3c). After penicillin injection the percentage of neurons with exponential type of activity increased from 7 to 13%, the percentage with multimodal activity decreased from 30 to 20%, and the percentage with the unimodal type of activity remained substantially unchanged (70%).

After injection of penicillin there was thus a marked increase in the number of active neurons, spatial redistribution of the active neurons, and a change in the relative proportions of neurons with different types of activity. Let us examine the possible causes of the changes discovered. The increase in the number of active neurons in the test region arising on account of excitation of hitherto inactive cells is evidence of the establishment of a new, or manifestation of a latent, pre-existing connection between NRG neurons or groups of them, characterized by a high degree of integration of the cells [2, 18]. An increase in the number of active points (by 45%) was observed previously [4] during the creation of

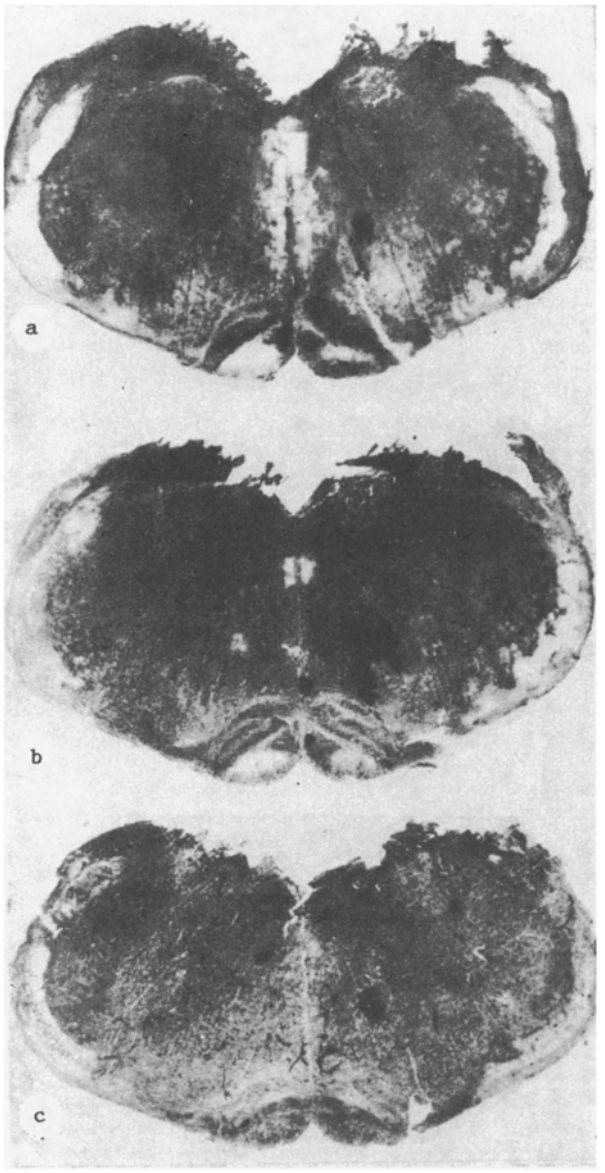

Fig. 2. Frontal sections through rat medulla after injection of 1 μl of a solution of India ink into NRG. a) Rostral part of test region of NRG, b) central part, c) caudal part. Distance between sections a-b and b-c 200 μ (light microscope, 10 ×).

a GPEE in NRG by injection of tetanus toxin. The manifestation of activity of hitherto unexcited neurons and also the increase in discharge frequency of excited neurons are undoubtedly connected with weakening of inhibitory processes [13, 15, 16]. The weaker nature of this effect in the present case (21%) may perhaps be explained by differences in the degree of action of penicillin and tetanus toxin as agents inducing GPEE formation. Penicillin and tetanus toxin not only weaken inhibitory processes, but also give rise to direct depolarizing effects [7, 10]. Incidentally, the process of formation of hyperactivity under the influence of tetanus toxin differs from that when penicillin is used by the gradual nature of the increase as the toxin spreads, and by the stability and more prolonged character of its action. Changes in the number of active neurons in the present experiments were due to the action of penicillin, for in response to injection of physiological saline into NRG no such changes were observed, and in addition, substantial tissue destruction likewise was not found.

The spatial redistribution of activity of NRG neurons after injection of penicillin is connected with a change in interneuronal synaptic interaction. Studies of the action of convulsants within the limits of neuron nets and also with respect to the spread of hyperactivity to neighboring regions [12] have been published, but the spatial distribution of active neurons in GPEE has virtually not been investigated. Activation of a certain volume of a neuron population is essential for the development of seizure activity; the seizure threshold of a structure, moreover, is determined to a large extent by the character of interaction between its individual elements [14, 19]. There is information that a crucial stage in the

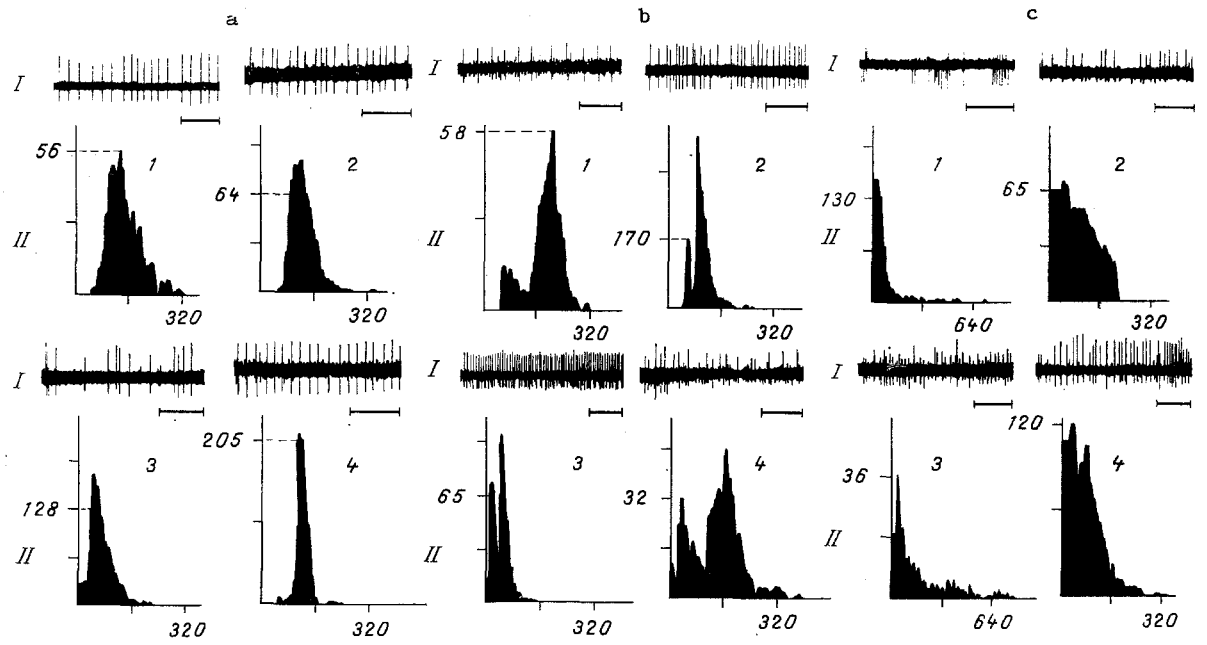

Fig. 3. Spike discharges of NRG neurons (I) with unimodal (a), bi- or multimodal (b), and exponential (c) types of distribution of interspike intervals (II). 1-4) Activity of different neurons; 1, 2) before penicillin injection, 3, 4) after injection. Abscissa) duration of interval (in msec); ordinate) number of intervals. Time marker 0.5 sec.

development of epileptiform activity in the rat cerebral cortex during electrical stimulation is "regrouping and synchronization of activity of single neurons" [11].

Distinct spatial redistribution of the volume structure of excited neurons during the creation of a GPEE took place in the present experiments through reorganization of the geometry of interneuronal synaptic interactions. Characteristically, under these circumstances, the number of excitatory connections increases mainly between neurons of type III, evidence in support of the greater proneness of neurons of this population to hyperactivation.

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