STRUCTURAL AND METABOLIC CHANGES IN THE BRAIN OF ANIMALS WITH DISTURBANCES OF HIGHER NERVOUS ACTIVITY FOLLOWING REPEATED ADMINISTRATION OF CHLORPROMAZINE

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Chlorpromazine is given clinically in the form of repeated courses of the drug. A factor of considerable importance to the elucidation of the mechanism of action of chlorpromazine on the central nervous system (CNS) is the study of the structural and metabolic changes in the brain tissues of animals with disturbances of higher nervous activity caused by repeated administration of this drug.

EXPERIMENTAL METHOD

Experiments were conducted on albino male rats weighing 170-250 g, in which a system of positive and inhibitory motor-food conditioned reflexes was first created by Kotlyarevskii's method. An injection of 1 ml of an aqueous solution of chlorpromazine in a dose of 5 mg/kg was given into the femoral muscles once daily for 30 days. Fifteen control rats received repeated intramuscular injections of 1 ml of distilled water. The experiments were conducted on alternate days until the 60th day after the first injection of chlorpromazine. Experiments performed on the 7th, 14th, 30th, 45th, and 60th days, i. e., at times when the dynamics of the changes in higher nervous activity was most marked, are described in this article.

For microscopic investigation, 7 experimental and 3 control animals were sacrificed by decapitation at each of the above times. Frontal sections of the brain were stained by several neurohistological techniques. In addition, histochemical reactions were carried out: for protein by Danielli's method, for protein SH-groups by Yakovlev and Nistratova's method, for RNA by Brachet's method, for acid phosphatase by Gomori's method, and for succinate dehydrogenase by Shelton and Schneider's method.

EXPERIMENTAL RESULTS

After the first days on which chlorpromazine was injected, the animals, when not inside the experimental chamber, lay motionless with their skeletal muscles relaxed. Most of the rats, if placed in an unnatural position, remained thus for a long time. The principal results of the investigation of the changes in general behavior and conditioned-reflex activity are given in the table.

On the 7th and 14th day all the experimental animals showed a sharp decrease in general motor activity, amounting in some cases to complete immobility (Fig. 1). The amplitude of the respiratory movements was greatly reduced. Inter-signal reactions were absent. All the conditioned and unconditioned reflexes were completely lost. The rats also failed to react to excessive stimuli. The control animals showed no changes in conditioned-reflex activity at this or subsequent times.

Changes in General Behavior and Conditioned Reflex Activity of Animals at Various Periods after Repeated Injection of Chlorpromazine in a Dose of 5 mg/kg

Time after injection of chlorpromazine	General motor activity	No. of inter-signal reactions	Eating time (in sec)	Deviation from mean values[1]		Unconditioned reflexes	Differentiation	Successive inhibition
				Latent period	Conditioned reflex			
Before injection	Moderate	8	33	±0.21	±2.8	Preserved	Preserved	Moderate
On 7th and 14th day	Sharply depressed	—	2	100%[3]		2	2	2
On 30th day	Depressed	5	44	+0.30	−7.6	Preserved	Preserved	Increased
On 45th day	Sharply depressed	36	27	−0.10	+20.2	Preserved	De-inhibited	Not exhibited
On 60th day	Moderate	7	32	−0.20	+2.9	Preserved	Preserved	Moderate

[1]The deviation from the mean results of 10 experiments before injection of chlorpromazine is shown.
[2]The indices were not determined because of the sharp disturbance of the animals' conditioned-reflex activity.
[3]Number of animals with complete loss of conditioned reflexes.

Microscopic investigation of the brain of the experimental rats showed the presence of hyperchromia of the of the pyramidal cells in layers II, III, and V of the cerebral cortex (Fig. 2, a) and a few shrunken neurons with cork-screw-like, tortuous processes. In the cortex of the cutaneo-motor analyzer, for each 100 unchanged pyramidal cells there were 30-35 hyperchromic, compared with 2-4 cells per 100 in the control animals. These changes were less marked in the auditory and optic cortex. Histochemical tests revealed a greatly increased concentration of proteins, SH-groups and RNA and a decreased acid phosphatase activity in the hyperchromic pyramidal cells. On the apical dendrites of the pyramidal cells of layers II, III, and V of the cortex, irregular thickenings and fusiform or spherical swellings were found by staining by Golgi's method; these dendrites were almost completely without spines. The pyramidal cells of the cortex also contained an increased number of argyrophilic granules and showed a central tinctorial acidophilia. In many of the nerve cells of the specific nuclei of the diencephalon and of the reticular nucleus of the thalamus, vacuolation of the cytoplasm was observed (Fig. 2, b).

Vacuolated nerve cells were also found in the nuclei of the hypothalamic region and in the reticular formation of the brain stem, although the modified cells in these structures were predominantly cells with moderate or severe hypochromia of the cytoplasm (Fig. 2, c). In isolated nerve cells of the reticular formation signs of cytokaryolysis were found, with the formation of shadow cells. All these changes were commonest in the small and middle-sized neurons of the reticular formation. The modified cells did not appear to be localized predominantly in any particular nucleus of the reticular formation. Histochemically, corresponding to the hypochromia in the cytoplasm of the nerve cells of the reticular formation a considerable decrease was observed in the concentration of proteins, SH-groups, and RNA, together with a marked fall in acid phosphatase activity. Signs of central tinctorial acidophilia developed in the modified neurons of the reticular formation, the number of argyrophilic granules diminished, and the succinate-dehydrogenase activity fell sharply (Fig. 2, d).

The reaction of the neuroglia was productive and degenerative in character. The number of astrocytes increased, although cells were present with shrinking of their bodies and a shortening and decrease in the number of their processes (Fig. 2, e).

Hyperplasia of the compact branched forms of oligodendroglia was observed (Fig. 2, f). The microglia showed diffuse proliferation with an increase in the number of cells to 10-15 per field of vision. Meanwhile the number of secondary ramifications on the processes of the microgliocytes diminished and some of the processes were fragmented (Fig. 2, g). In some places, both in the cortex and in the subcortical structures, irregularly collapsed and tortuous capillaries and precapillaries were found (Fig. 2, h).

On the 30th day the general motor activity, the amplitude of the respiratory movements, and the number of inter-signal reactions remained slightly below their original values. The eating time was increased. The conditioned and unconditioned reflexes were restored, and the latent periods of the conditioned reactions were increased while their amplitude was decreased. As a rule, differentiation was not de-inhibited. Successive inhibition was found in most rats in the form of a clear lengthening of the latent period of the conditioned reflex following the inhibitory stimulus.

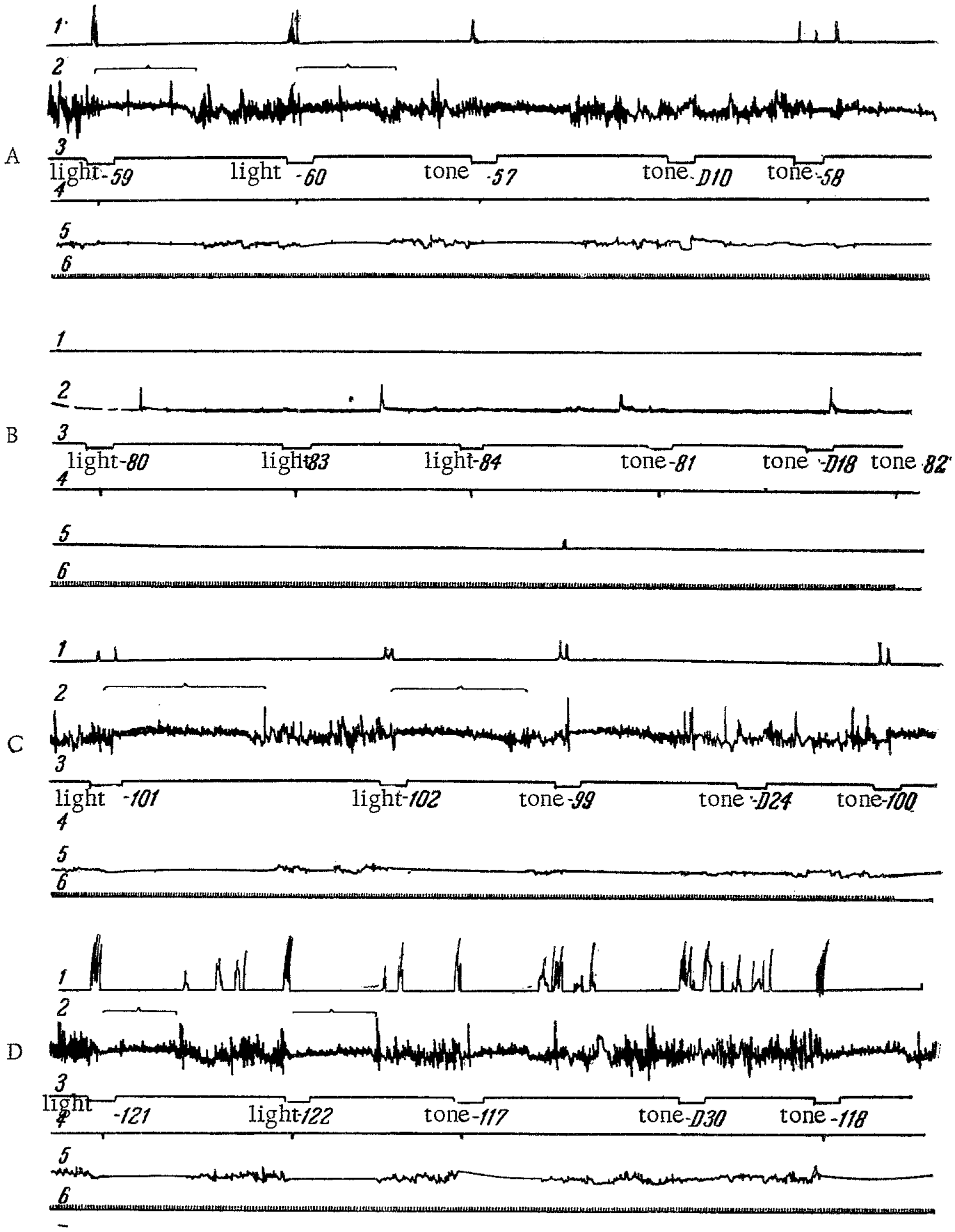

Fig. 1. Dynamics of changes in conditioned-reflex activity in No. 52 during repeated injections of chlorpromazine in a dose of 5 mg/kg. A) Before injection of chlorpromazine (experiment No. 20 on September 27, 1960); B) on 14th day after first injection of chlorpromazine (experiment No. 28 on November 1); C) on 30th day (experiment No. 34 on November 16); D) on 45th day (experiment No. 40 on December 1). 1) Conditioned motor reaction; 2) respiration; 3) time of action of conditioned stimulus; 4) marker of reinforcement; 5) actogram; 6) time marker (1 sec); the braces denote the eating times.

Microscopic investigation of the brain of the animals with the disturbances of higher nervous activity as described above revealed an increase in the number of hyperchromic cells in the cortex to 15-20 per 100 unchanged cells. Occasional shrunken cells were also found. The system of spiny projections from the dendrites was well developed in most of the cortical neurons. In some places pyramidal cells were found with irregular thickenings along the course of the apical dendrites and with a decrease in the number of spines. In some nerve cells of the specific nuclei of the diencephalon and of the reticular nucleus of the thalamus, vacuolation of the cytoplasm was observed. The nuclei of the hypothalamic region and reticular formation of the brain stem contained nerve cells with moderate hypochromia of their cytoplasm, and occasional shadow cells. Corresponding to these histological changes in the neurons, histochemical changes were found, similar to those described during preceding periods. The reaction of the neuroglia was productive and degenerative.

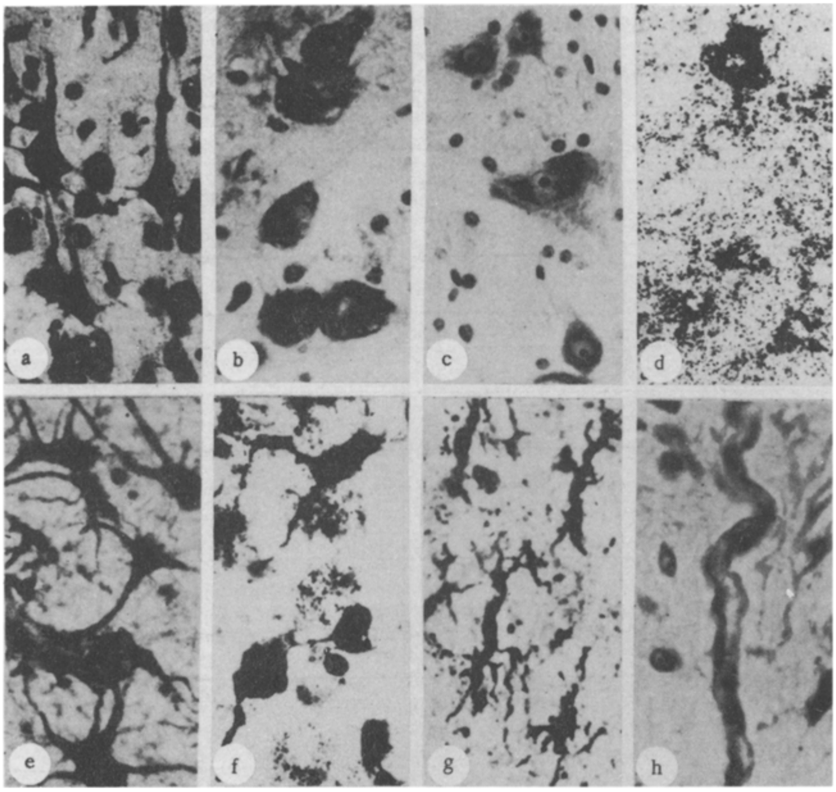

Fig. 2. Changes in structural elements of brain tissues of albino rats after repeated injection of chlorpromazine in a dose of 5 mg/kg. a) Hyperchromatosis of pyramidal cells of layer V of the cortex of the cutaneo-motor analyzer; stained by Nissl' method, objective 40, ocular 7; b) vacuolation of nerve cells of the ventral nucleus of the thalamus; stained by Nissl's method, objective 40, ocular 7; c) hypochromia of nerve cells of the caudal nucleus of the reticular formation of the pons; Nissl's stain, objective 40, ocular 7; d) decrease in succinate dehydrogenase activity in small neurons of caudal nucleus of the reticular formation of the pons; objective 40, ocular 7; e) productive-degenerative reaction of astrocytic glia: stained by Miyagawa–Aleksandrovskaya method, objective 40, ocular 10; f) hydroplasia of compact, branched forms of oligodendroglia; stained by Miyagawa –Aleksandrovskaya method, objective 40, ocular 15; g) increase in number of microglial cells with shortening and fragmentation of processes; stained by Miyagawa –Aleksandrovskaya method, objective 40, ocular 12.5; h) irregular collapse of walls and tortuosity of precapillary in cortex of cutaneo-motor analyzer; Nissl's stain, objective 40, ocular 10.

On the 45th day the general motor activity of the rats was considerably more marked than initially, and most animals actually showed motor excitation. The amplitude of the respiratory movements and the number of intersignal reactions were obviously increased, while the eating time was shortened. The latent periods were of shorter duration and the magnitude of the conditioned reflexes was appreciably greater than the initial and corresponding values in control rats. Differentiation was de-inhibited in all the rats, and no successive inhibition was found.

Microscopic investigation of the brain of the experimental animals revealed individual hyperchromic pyramidal cells and solitary shrunken neurons in the cerebral cortex. Most of the nerve cells of the cortex, especially the pyramidal cells of layers II, III, and V, stained slightly hypochromically. Corresponding to this weak hypochromia of the cortical neurons, a slight decrease was found in the concentration of cytoplasmic proteins, SH-groups, and RNA, while the acid phosphatase activity was increased. As a rule, the apical dendrites of the cortical pyramidal cells were abundantly supplied with spines. In the nuclei of the diencephalon and of the reticular formation of the brain stem a few vacuolated and hypochromic cells and occasional shadow cells were encountered. Some astrocytes were ameboid in form. The cells of the oligodendroglia and microglia were characterized by shortening and fragmentation of their processes.

On the 60th day no appreciable difference could be observed between the general behavior and the state of the conditioned-reflex activity of the experimental and control rats. Microscopic investigation revealed only isolated changed nerve cells in the cerebral cortex and subcortical structures. On the whole at this period there was no visible difference between the structural elements of the brain tissues in the experimental and control animals.

Hence, after repeated injection of chlorpromazine into albino rats in a dose of 5 mg/kg, definite disturbances of conditioned-reflex activity were revealed, together with histological changes and changes in the histochemical reactions in the cortex and the subcortical structures of the brain. During the first 3 weeks after the beginning of the injections a marked depression of the higher nervous activity of the animals was observed. This is in agreement with the findings reported by other authors [1-3, 6, 7, 9, 15, 16, 18, 21, 23], who have observed a depressant action of chlorpromazine (in dose of 1 mg/kg and above) on the conditioned-reflex activity of rats.

Microscopic investigation of the brain of the animals at these periods revealed changes in different tissue structural elements—nerve cells, neuroglia, and blood vessels. Our observations do not confirm reports [11, 13, 20] describing the absence of histological changes in the brain of animals after administration of fairly high doses (4, 15, and 300 mg/kg) of chlorpromazine. Nor can we agree with the view that administration of chlorpromazine leads to diffuse changes in the brain of animals [5, 8] or that a lesion is found predominantly in the adjacent subcortex [4], the thalamohypothalamic region [12, 19], or the brain stem [14, 17, 22]. After the repeated injection of chlorpromazine in a dose of 5 mg/kg, structural and metabolic changes were found in the cerebral cortex, the nuclei of the diencephalon, and the reticular formation of the brain stem.

On the 30th day after the first injection the disturbances of higher nervous activity and the microscopic changes in the brain of the animals were less severe, although administration of the drug continued. This indicates the development of tolerance to chlorpromazine during repeated injection of drug.

During the 3 weeks after the cessation of chlorpromazine injections a considerable elevation of the excitability of the higher divisions of the CNS of the animals were observed. Correspondingly, the changes in the structure and metabolism of the brain assumed a different character, and they were coordinated mainly with the cortical neurons. This demonstrates that the action of chlorpromazine on the higher divisions of the CNS is biphasic: a phase of depression is followed by a phase of increased excitability.

On the 60th day after the beginning of the injections the normal conditioned-reflex activity of the rats was restored, and microscopic investigation of the brain revealed no visible changes. It may be concluded from these observations that the functional, metabolic, and structural changes in the higher divisions of the CNS of animals receiving repeated injections of chlorpromazine in a dose of 5mg/kg are reversible.

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