

Experimental Study of the Mechanism and Indices of Harmful Effects of Certain Chemical Substances on the Central Nervous System

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The task of the second stage of Soviet-American cooperation on the problem of environmental health science was to explain the question of the comparative sensitivity of methods used in both countries, as well as the indices of harmful effects for the same toxic substance (carbon disulfide), with the purpose of determining the most informative methods of assessing the influence of atmospheric pollutants on organisms.

The application of neurophysiological research methods (recording total electrical activity of the cortex and cortical structures of the brain, studying amplitude-time characteristics of averaged evoked potentials of the optical cortex, investigating sensory and convulsive thresholds) has made it possible to explain the neurophysiological basis of the effect of carbon disulfide on the central nervous system—the perturbation of cortical inhibition processes and the increase of excitation in amygdalate structures, both of which play an important role in the fixation process of temporary connection.

The compilation of data from neurophysiological and neurochemical investigations show that neurophysiological changes are associated primarily with a decrease in enzymic breakdown of free neuraminic acid.

The study of the average evoked potentials in humans during exposure to carbon disulfide concentrations of 0.09 mg/m³ revealed a tendency to decrease the short latent amplitude components and increase the long latent amplitude components of the averaged evoked potentials.

The study of operant behavior in rats revealed a characteristic change in the instrumental alimentary reactions under long-term (3 months) exposure of carbon disulfide to a concentration of 16 mg/m³.

In this manner, the following were developed in experiments with animals and research on humans: indices of the harmful effects of neurotropic toxic substances, a change in operant behavior, a decrease in the amplitude of total electrical activity, a change in time-amplitude parameters of evoked potentials, and a decrease in post-discharge convulsion thresholds in the cortical-medial nucleus of the amygdala.

Neurophysiological and neurochemical research methods have proven to be the most sensitive and informative of the methods used.

These criteria and methods are recommended for determining threshold levels of various neurotropic toxic substances which pollute the atmosphere.

The previous stage of joint Soviet-American research on the mechanism of the effects of pollutants on the CNS (central nervous system) showed that the methods used in the U.S.S.R. for functional electroencephalography and the multiple recording of biopotentials from various brain structures give specific advantages in evaluating the condition and

character of such reactions of the various functional systems of the brain. Such methods made it possible to discover the specifics of the reactions, a high selectivity of the effects of toxic substances, and to reveal abnormal forms of activity.

The next stage of Soviet-American cooperation (1975-1976) examined the question of comparative sensitivity of methods used in both countries, as well as indices of the harmful effects of the same toxic substance—carbon disulfide—as an example.

In this connection, the Soviet researchers con-

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ducted a study on the toxicology of low carbon disulfide concentrations using approved as well as completely new methods and approaches. In studying the chronic effects of carbon disulfide, the method of primary and secondary responses of evoked potentials of the visual center of the brain of experimental animals was applied, which made it possible to characterize in detail the processes of cortical inhibition (1, 2) as well as a method for studying sensory thresholds widely used in both domestic and foreign toxicological studies. At the same time, in one neurophysiological experiment, neurochemical research was also conducted, including the study of the metabolism of sial-containing glycoproteins and lysosomal enzymes in the brain tissue of the animals. According to current postulates, the expression of a series of highly specialized functions of the CNS is determined by structural integrity and permeability of neuronal membranes. This is related, to a great extent, to the condition of the volume of sial-containing glyco- and lipoproteins, which become gangliosides, cerebroside, nerve endings and other functional structures of the brain (3-5).

One of the possible metabolic mechanisms of the neurotropic effect of carbon disulfide (a lipotropic organic compound) could depend on its influence on the cell structures of the central nervous system, particularly lysosome membranes. However, the biological role of lysosomatic enzymes in the brain in the neurotropic effect of carbon disulfide still remains unclear at this time.

We were the first in environmental research to study evoked potentials from human visual cortex under macroconcentrations of carbon disulfide in a short-term experiment. This method was used because there is a basis for assuming that a direct evaluation can be made of the neurotropic effect of this substance by using evoked potential time-amplitude characteristics and since the correlation is known between the parameters of the evoked potential and the various functional states of the CNS (6-9). This is of both theoretical and practical importance in setting standards.

The comparatively widespread use of rats in evaluating the toxic effect of chemicals in the laboratories of both countries has made it advisable to test operant conditioning methods with rats. Operant conditioning was used to study operant behavioral reactions of rats on equipment supplied for our use by the American groups.

Methodology

Rabbits weighing 2.5-5.0 kg were used in the chronic experiments. Constantan electrodes 0.8 mm

in diameter were used to record the evoked potential. The active electrode was placed in the maximum activity point of the visual projection region and the passive electrode on the frontal bone of the cranium. The visual evoked potential was evoked by light stimuli from an Orion EEG photostimulator, using unilateral stimulation (1.4 joules flash energy and 1.2 μ sec duration). The distance from the head of the animal to the lamp was approximately 30 cm. The evoked potential take-off was monopolar. After amplification, the evoked potential was averaged on a Nixon-Koden ATAK-201 computer (two averages with 50 events each). The following evoked potential components were analyzed: primary response (latent periods of positive and negative phases and peak amplitudes) and the slow negative wave (latency, maximal amplitude and duration).

The sensory threshold to sound stimulation was determined according to the manifestation of an arousal reaction in occipital cortex in the form of a clearly expressed theta rhythm. A 500 cps tone was used that was produced by a 3G-10 sound generator. The tone duration was 5 sec with a steady increase in loudness from 10 to 80 db. The reaction was considered threshold, if, in 5 of the 10 sequentially presented sounds, a clearly defined theta rhythm was present.

The chronic six-week exposure to carbon disulfide (0.2 and 2.0 mg/m^3) was conducted in 200-liter exposure chambers in which the air was changed 30 times/hr. The control animals were placed in the same kind of chambers with circulating air free of dust and gaseous impurities.

The constancy of a given concentration during exposure was determined twice a week by colorimetric analysis, based on the interaction of CS_2 with diethylamine and copper acetate with the subsequent formation of copper diethyldithiocarbamate, which colors the solution yellow-brown. The sensitivity of the method is 0.5 μg . In parallel, the CS_2 concentration was followed by a gas-chromatography method, based on the chromatographic isolation of CS_2 and its qualitative determination with the aid of an electron capture detector. The sensitivity of this method is 0.2 mg/m^3 . The neurophysiological studies were conducted on 24 rabbits, 18 from the test group and 6 from the control group.

The metabolism of the sial-containing glycoproteins in various sections of the brains of the animals was studied after the termination of exposure by determining neuraminic acid aldolase, which participates in the catabolic phase of neuraminic acid metabolism. The neuraminic acid content of the blood serum of the animals was de-

terminated at various times after the start of CS₂ (2 mg/m³) exposures. Neuraminic acid concentration was determined by the thiobarbiturate method (10) and by the modification of Merkur'yeva and Bazanova (11). Enzyme activity is expressed by the decomposition percentage of free *N*-acetylneuraminic acid added as a substrate.

The activity of four types of lysosomal glycanohydrolase was studied biochemically in rabbit brain tissue: hyaluronidase and *N*-acetyl- β -D-glucosaminidase and β -galactosidase (12, 13). The biochemical studies were conducted on 32 rabbits, 11 from the control group and 21 from the test group.

In studying the evoked potential in man, the active electrode was located on the scalp, 2–3 cm above the median junction; the inactive electrode was located on the right ear lobe; the ground was located on the back part of the lower third of the right antebrium. The subject under study, with eyes closed, sat in a comfortable position on a chair in front of a cylinder through which either air or experimental gas flowed at a rate of 20 l./min. The photostimulator lamp was placed 50 cm from the subject at eye level. The ambient lighting of the chamber was 15 lux.

After a 3-min adaptation period, the subject was given a series of 100 aperiodic flashes with a mean frequency of 0.5 Hz and at an intensity of 0.27 joules. After amplification by the UBP2-03 amplifier, the evoked potential was averaged on the ATAK-201 computer, whose scan onset was synchronized with the stimulator onset. After summation, the average evoked potential was photographed.

The subject was given the following work program during the 10-min exposure to pure air in order to maintain an alert state: recognition of geometric figures and solution of arithmetic problems, whose order of presentation was controlled by the experimenter and varied from test to test. The figure recognition time, the number of solutions, and errors were registered on Orion EEG tape. At the end of the work program, averaged evoked potentials based on 100 repetitions were again completed. A 10-min exposure to gas followed (clean air in the

control tests). During the exposure to gas, the subject was presented with an analogous work program. Concentrations of CS₂ from 0.03 to 0.09 mg/m³ were studied.

The constancy of CS₂ microconcentrations in the observations on man was determined by Aptukhin's diffusion capillary cell (14), modified by Daylidovich et al. (15) with automatic heat control. The concentration was determined at the end of each experimental day by computation and control assessed by the calorimetric method (10% error). The results of the electrophysiological tests were statistically processed by using a modified *t*-criterion (16). Student's *t* method was used for processing the results of the biochemical studies.

Results

Statistical processing of data from the control group of animals for the eight-week observation period showed the limits of physiological variability of the evoked potential parameters as listed in Table 1. Average values by weeks of observation for the control are given in Table 2.

During the six-week period of continuous exposure to CS₂, the evoked potential parameters did not differ statistically from those for the control group and background values. Only during the sixth week of exposure was it possible to note a tendency towards an increase in the amplitude of the primary response and the slow negative wave (124.6 ± 2.8 and $231.5 \pm 110.5 \mu V$ as opposed to 56.2 ± 1.1 and $95.8 \pm 20.3 \mu V$ in the background, respectively).

CS₂ at a concentration of 2 mg/m³ during the fourth week of exposure produced a statistically significant reduction in the primary response amplitude and an increase in the duration of the slow negative wave at constant amplitude. By the sixth week, all parameters of the evoked potential were within background value limits except for the primary response amplitude and the duration of the half-wave of the slow negative wave, which remain statistically unchanged with respect to the background (Table 3).

At all lengths of time, this concentration of CS₂ was shown to have no statistically significant effect

Table 1. Limits of physiological variability of evoked potential parameters.

Parameter	
Latency of the positive phase of the primary response, msec	30.2 ± 0.9
Latency of the negative phase of the primary response, msec	39.0 ± 1.1
Peak amplitude of the primary response, μV	48.3 ± 5.4
Maximum amplitude of the slow negative wave, msec	134.0 ± 4.3
Duration of the half wave of the slow negative wave, msec	78.0 ± 5.1
Amplitude of slow negative wave, μV	94.0 ± 6.2
Duration of the primary response (slow negative wave complex), msec	199.0 ± 6.2

Table 2. Evoked potential parameters of the visual cortex for control group animals for an eight week period of observation (average data based on 5 rabbits each).

	Primary response			Slow negative wave			
	Latent period		Amplitude μV	Maximum amplitude time, msec	Duration of halfwave, msec	Amplitude μV	Primary response slow negative wave duration, msec
	Positive phase, msec	Negative phase, msec					
2 weeks	30.2 \pm 2.4	39.0 \pm 2.8	35.8 \pm 9.0	136.4 \pm 12.2	80.6 \pm 13.9	89.0 \pm 17.6	204.2 \pm 10.5
4 weeks	31.6 \pm 2.6	40.4 \pm 3.0	47.8 \pm 14.8	127.4 \pm 14.4	71.8 \pm 11.2	90.4 \pm 18.9	188.4 \pm 21.9
6 weeks	33.2 \pm 2.4	42.4 \pm 3.2	58.6 \pm 24.2	124.4 \pm 16.2	70.6 \pm 17.4	86.8 \pm 13.1	181.8 \pm 17.8
8 weeks	32.0 \pm 2.1	40.6 \pm 3.4	50.6 \pm 11.8	131.6 \pm 18.4	78.8 \pm 19.3	86.6 \pm 21.5	201.8 \pm 15.7

Table 3. Evoked potential parameters of the visual cortex of a 1.5-month continuous exposure of rabbits to CS₂ at a concentration of 2.0 mg/m³ (average data based on 5 animals each).

	Primary response			Slow negative wave			
	Latent period		Amplitude, μV	Maximum amplitude time, msec	Duration of halfwave, msec	Amplitude, μV	Primary response slow negative wave duration, msec
	Positive phase, msec	Negative phase, msec					
Background	24.8 \pm 2.1	36.2 \pm 2.8	95.0 \pm 23.6	110.4 \pm 14.4	58.4 \pm 13.9	89.8 \pm 27.9	189.8 \pm 16.1
2 weeks	27.2 \pm 2.1	36.0 \pm 1.9	51.2 \pm 20.4	138.4 \pm 15.0	93.0 \pm 15.0 ^a	83.0 \pm 32.2	206.4 \pm 13.5
4 weeks	27.8 \pm 1.1	36.0 \pm 1.7	41.0 \pm 5.8 ^a	129.4 \pm 6.9	82.4 \pm 5.8 ^a	65.8 \pm 20.8	221.4 \pm 8.6 ^a
6 weeks	25.6 \pm 1.7	33.8 \pm 2.8	40.0 \pm 4.3 ^a	124.4 \pm 10.7	88.0 \pm 11.8 ^a	74.0 \pm 16.1	203.0 \pm 9.6

^a $p < 0.05$.

Table 4. Sensory threshold values for sound stimulation during the course of exposure to CS₂ at a concentration of 0.2 mg/m³.

	Sensory threshold values, arbitrary units ^a
Background	24.0 \pm 0.3
Week 1	33.0 \pm 10.7
Week 2	37.0 \pm 10.7
Week 3	37.0 \pm 7.5
Week 5	26.0 \pm 10.7
Week 7	28.0 \pm 10.7

^a Average values based on five rabbits.

on the level of sensory threshold in sound stimulation (Table 4).

A study of neuraminic acid metabolism in brain tissue after six weeks of exposure to CS₂ at a concentration of 0.2 mg/m³ showed that in one third of the animals, neuraminic acid content decreased to 90 \pm 4 mg % on the average, which is 21% lower than that for the control group (122 \pm 2 mg %, $p < 0.001$). No change was observed in neuraminic acid aldolase activity in brain tissue for the majority of the animals studied (Table 5).

Significant changes in neuraminic acid metabolism were observed in the gray matter of the brain after CS₂ at a concentration of 2 mg/m³. After one week of exposure, 75% of the animals showed a 36% ($p < 0.001$) decrease in neuraminic acid level in comparison to the control group (103–137 mg %). (As the control animals did not show differences

between gray matter and white matter with respect to content of neuraminic acid and neuraminic acid aldolase, we combined values for gray and white matter for reference of experimental animals to controls). Simultaneously, the neuraminic acid aldolase activity in gray matter was reduced to 11 \pm 2% on the average, in comparison with a value for the animals of the control group of 18 \pm 2% ($p < 0.01$). As shown in Table 5, an opposing and statistically verifiable increase of 35% ($p < 0.05$) occurred in the neuraminic acid content of the olfactory bulb, while neuraminic acid aldolase activity remained unchanged. No changes in biochemical indices were observed in white matter. After 6 weeks of the experiment, neuraminic acid content in all areas of the brain did not differ from the values for the control group; nevertheless, there is no basis for assuming that neuraminic acid metabolism normalized completely, since neuraminic acid aldolase activity remained at a lower level in gray matter and, on the average, reached 10 \pm 2% in comparison to the values for the control group (18 \pm 2%, $p < 0.01$). Parallel determination of *N*-acetylneuraminic acid in brain and blood serum made it possible to establish an identical trend in its level during all periods of exposure to the maximum dose of carbon sulfide.

Neurochemical studies also made it possible to reveal the ambiguity of changes in lysosomal enzyme activity of the brain at various stages of expo-

Table 5. Content of *N*-acetylneuramine acid and activity of aldolase *N*-acetylneuraminic acid on various sections of the brain and blood serum of rabbits with inhalation exposure of carbon disulfide (2 mg/m³)^a.

	Neuraminic acid, mg%				Activity of aldolase <i>N</i> -acetylneuraminic acid, %		
	White matter	Gray matter	Olfactory bulbs	Blood serum	White matter	Gray matter	Olfactory bulbs
Control	122 ± 2 (13)	122 ± 2 (13)	97 ± 8 (5)	71 ± 2 (21)	18 ± 2 (11)	18 ± 2 (11)	17 ± 3 (4)
1 week	119 ± 5 ^b (4)	92 ± 19 ^b (4)	131 ± 6 ^b (4)	68 ± 2 ^b (4)	19 ± 4 ^b (4)	11 ± 2 ^d (4)	21 ± 1 ^b (4)
2 weeks	109 ± 6 ^b (4)	123 ± 17 ^b (4)	123 ± 6 ^b (3)	94 ± 4 ^c (7)	9 ± 2 ^d (4)	13 ± 3 ^b (4)	18 ± 4 ^b (3)
6 weeks	107 ± 6 ^b (5)	67 ± 7 ^b (5)	92 ± 2 ^b (4)	83 ± 5 ^b (5)	13 ± 2 ^b (5)	10 ± 2 ^d (5)	14 ± 2 ^b (5)

^a Values given are means ± SD; values in parentheses are number of animals (*n*).

^b *p* > 0.05.

^c *p* > 0.02.

^d *p* > 0.01.

sure to carbon sulfide at 2 mg/m³. The lysosome most sensitive to the enzyme system during the early periods of exposure (one week) turned out to be the pattern enzyme of lysosome- β -galactosidase, the activity of which was increased to twice that of the control level. These changes have a stable character and persisted during the 6 weeks carbon sulfide exposure.

At a later period (after two weeks), we observed a significant change in the activity of all the assayed enzymes; however, the direction of change differed among them. Thus, analogous to β -galactosidase, the activities of β -glucosidase and *N*-acetyl- β -D-glucosidase were correspondingly noticeably higher—on the average, 2.2–2.5 times—than the value of the control group of rabbits. In contrast, the activity of hyaluronidase in the brain tissue of test animals in these periods was sharply reduced (on the average 3.2 times, *p* < 0.001). The subsequent action of carbon sulfide (during 6 weeks) promoted the activation of hyaluronidase, whereas the activity of *N*-acetyl- β -D-glucosaminidase and β -glucosidase fluctuated within the limits of normal values.

It is necessary to note that, along with the above mentioned neurophysiological and neurochemical disturbances, we also found behavioral reactivity of test animals during the last stage of chronic exposure to CS₂ at a concentration of 2 mg/m³. The rabbits were excited and reacted sharply to the procedure of being placed in the experimental set-up.

Discussion of Results

Since the primary response of the evoked potential changes ambiguously when there is a change in the state of activation/inhibition (17), the

simultaneous analysis of primary and secondary components of the evoked potential allows us to interpret the results with great confidence. As is known, the amplitude of the primary response depends on the functional state of the brain. It decreases with an increase in tonic effects from the reticular formation of the brain stem (18) and increases when they increase or under the influence of anesthetics (19, 20). In accordance with the most common point of view, the slow negative wave of the evoked potential reflects hyperpolarization (true inhibitory postsynaptic potentials) synchronously developing in the pyramidal cells of the visual cortex (21–23), so that the amplitude of the slow negative wave and the steepness of its rise is related to the intensity of the processes of cortical inhibition. In light of this literature, the tendency we discovered for increased amplitude of the primary response and of the slow negative wave with a 6-week exposure to CS₂ at concentrations of 0.2 mg/m³ can be seen as an intensification of cortical inhibition.

Taking into account that only half of animals in the group displayed this tendency, and, also, the absence of disturbances in the behavior of the animals, one should consider a concentration of 0.2 mg/m³ as the threshold concentration for this species of animals from the hygienic point of view.

The observed lowering of the amplitude of the primary response and the increased duration of the slow negative wave (without change of the amplitude of the latter under the influence of CS₂ at the 2.0 mg/m³ concentration) suggests weakening of the processes of cortical inhibition, probably at the expense of the presynaptic discharge (24, 25). The most distinct and stable changes were found in the primary response of the evoked potential, which reflects the advent of excitation along specific

pathways (26). This leads us to assume a great sensitivity of the visual analyzer to the influence of the substance. This agrees with the literature on injuries to the visual system of man and animals under chronic inhalation of carbon disulfide. It is possible that, along with nonspecific reticular influences, disturbances in the receptor mechanisms and conducting pathways are conditioned by the observed lowering of the amplitude of the primary response under chronic exposure to carbon sulfide at a concentration of 2 mg/m³.

The most substantial metabolic disturbances with carbon disulfide at concentration of 2.0 mg/m³ were in the gray matter of the rabbit brains, and were manifested by a significant decrease in the aldolase *N*-acetylneuraminic acid for various periods of intoxication. The fact that a decrease in activity of this enzyme was observed in the cortex of test animals even six weeks after the carbon disulfide exposure indicates a change in the metabolism of sial-containing glycoproteins, which was manifested, in part, by the depression of the enzyme decay of free *N*-acetylneuraminic acid. The situation early in inhalation exposure to carbon disulfide, when these disturbances were accompanied by a statistically significant accumulation of *N*-acetylneuraminic acid in the olfactory bulbs, indicates systematic disturbance of the metabolism of this compound in various sections of the brain. Taking into account the important biological role of glycoproteins on cell surfaces and, specifically, those of neurons. The phenomenon discovered in the olfactory bulbs can be considered a manifestation of a compensatory effect by the chemical structures of this section of the brain due to the toxic influence of carbon disulfide.

The change in the activity of the enzymes of the subcellular structures of the brain at the lysosome with exposure to carbon disulfide, which we discovered, indicates that one of the biochemical mechanisms of the neurotropic effect, paralleling the disturbance of the metabolism of glycoproteins, can be the disturbance of the functional state of the cellular organelle of the central nervous system.

Comparative analysis of the electrophysiological and neurochemical data made it possible to establish the parallel of their changes. With exposure to the maximum concentration of carbon disulfide, a decrease in the activity of aldolase *N*-acetylneuraminic acid in the cortex and white matter of the brain, with a simultaneous increase in the duration of the slow negative wave was found and is seen as a weakening of inhibition processes in the visual cortex of the brain. On the other hand, the tendency towards increasing amplitude of the slow negative wave induced by exposure to the threshold

dose of carbon disulfide, which is viewed as an expression of intensified inhibition processes, was accompanied, in a number of animals, by a decrease in the level of sialic acids in brain tissue. The observed correlation of the disturbance in the enzyme-substrate system, characterized by the intensity of one reaction of the catabolism of sial-containing glycoproteins of the brain, with the nature of neurophysiological processes, apparently indicates the great importance of neurochemical mechanisms in the development of the neurotropic effect of carbon disulfide. Since the electrophysiological and neurochemical results correlated with the behavioral changes in the animals, we are inclined to consider a concentration of 2.0 mg/m³ as having a harmful effect on the given species of animals.

Research on evoked potentials in humans and the study of operant behavioral responses in animals are now being completed.

Conclusions

Chronic inhalation exposure to carbon disulfide gives rise to different changes in the process of cortical inhibition, depending on the level of concentration: 0.2 mg/m³ intensifies, while 2.0 mg/m³ weakens the cortical processes of inhibition. Correspondingly, there is a lowering of the level of sialic acids in the brain tissue with a low concentration, and an intensification of their synthesis with exposure to the high concentration of CS₂.

Neurochemical study of the activity of lysosomal enzymes and the exchange of sial-containing glycoproteins has shown that one of the metabolic mechanisms of the neurotropic effect of carbon disulfide is its influence on the state of the cellular structures (lysosome) and the metabolism of the neuraminic acid of various sections of the brain.

Comparative analysis of the neurophysiological (research on the evoked potential of the cortex) and neurochemical (study of the metabolism of *N*-acetylneuraminic acid and the activity of lysosomal enzymes in a number of brain structures) methods for determining the nature of chronic intoxication of CS₂ in low concentrations showed the sensitivity of these methods to be about equal.

Comparison of methods for studying the visual evoked potential and sensory thresholds to sound revealed a greater sensitivity of the first method in contrast to the second; this finding is probably related to the specific effect of carbon disulfide on the visual system.

Comparison of the results of research on the mechanisms of the neurotropic action of carbon disulfide with data from ozone and formaldehyde showed that neurotropic substances at low levels

have a nonspecific harmful effect on cortical processes; amplitude parameters of the primary and secondary responses of the evoked potential change in an identical way, indicating intensification of activation and weakening of inhibition. Thus, experience with research on a number of chemical substances made it possible to show a certain nonspecific indicator of a harmful neurotropic effect. It would seem that the evoked potential method, being the most simple and reproducible method, can be recommended as a comprehensive method for hygienic evaluation of the harmful effect of atmospheric pollutants on the central nervous system.

Summary

A study was conducted on the chronic effect of carbon disulfide in concentrations of 0.2 and 2.0 mg/m³ using the method of investigating the primary and secondary responses of the evoked potential of the visual cortex in brains of rabbits. At the same time, neurochemical research was conducted on the sial-containing glycoproteins and lysosomal enzymes in brain tissues of animals.

Study of the evoked potentials of the visual cortex of the human brain was done with microconcentrations of carbon disulfide (0.03 and 0.09 mg/m³) in short-term exposures.

An evaluation of the method of operant conditioning, used by American specialists on pigeons, was conducted on rats in conditions of chronic CS₂ exposure.

The inhalation exposure of CS₂ was seen to elicit different disturbances in the process of cortical inhibition depending on concentration. One of the metabolic mechanisms of the neurotropic action of CS₂ is its effect on the state of cellular structures (lysosomes) and the metabolism of neuraminic acid.

Comparative analysis of neurophysiological and neurochemical methods in determining the character of chronic intoxication by CS₂ showed the sensitivity of these methods to be about equal.

Comparison of these methods for studying the visual evoked potential and the sensory thresholds showed a greater sensitivity of the first method, which seems to be related to the specific action of CS₂ on the visual system.

The method of evoked potentials, being the most simple and reproducible, can be recommended as a comprehensive method for the hygienic evaluation of the harmful effects of atmospheric pollutants on the CNS.

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