Incorporation of [³H]Thymidine into Brain DNA after Cerebellar Damage

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Extract

Changes of incorporation of [³H]thymidine into brain DNA were studied in C57BL/6J mice after perinatal neocerebellar lesion. The destruction of part of the left neocerebellar cortex caused temporary increase of the specific radioactivity of DNA extracted from neocerebellum (15.22 ± 0.57 cpm/mg DNA vs. 4.83 ± 0.40 cpm/mg DNA in controls), from left hemisphere (9.86 ± 0.45 cpm/mg DNA in operated vs. 4.22 ± 0.40 cpm/mg DNA in controls), and from right hemisphere (11.75 ± 0.52 cpm/mg DNA in operated vs. 4.78 ± 0.39 cpm/mg DNA in controls). The labeled DNA was localized both in glia and in neurons in different brain areas. In animals operated upon in adult age, no changes in labeling of brain DNA were observed.

Speculation

It is possible that the described changes of the metabolism of the brain DNA are necessary and precede the "spontaneous nervous reorganization" necessary for the restitution of functions of the damaged brain.

The mechanism of functional recovery of the damaged brain are only partially known. Full anatomical restitution of the lost parts of the brain is not possible. It is probable that the remaining undamaged parts of the central nervous system take over the function of the destroyed areas. This plastic change is probably accompanied by functional, structural, and biochemical restructuring of the remaining parts. Previously, we have described a few of these changes which take place in the brain after perinatal surgical damage. We found an increase in the brain temperature, blood flow and vascularity, oxygen consumption, and activity of several enzymes after destruction of different cortical and subcortical areas performed in the early perinatal period. These metabolic changes were detected even 1 year after the lesion and appeared at the time of almost complete functional recovery (19-21). The increase of tissue metabolism was found not only in the immediate vicinity of the lesion, but also throughout the whole brain.

These experiments indicated that there is a whole brain reaction to even minimal damage of brain tissue and that these changes are probably permanent and probably associated with the process of recovery.

Pursuing this idea further, we attempted to find other, possibly more important components of this massive brain reaction. Thus, in this paper we studied the changes of DNA synthesis in different parts of the brain after focal lesion of the neocerebellum. DNA synthesis indicates whether there are new cells produced in the damaged brain, and where they are localized. The migration of new cells may indicate which areas of the brain are structurally modified after the lesion.

MATERIAL AND METHODS

ANIMALS AND SURGICAL PROCEDURE

Mice of the C57BL/6J strain whose parents were purchased from Jackson's Laboratories (32) were used in this study. The

number of newborn mice in each litter was adjusted to six, and at the age of 24-39 hr, the left cerebellar cortex was penetrated with a sharp needle introduced through the occipital plane to a depth of about 1-1.5 mm. The damage to the neocerebellar cortex caused an asymmetry of the position of hind limbs. The asymmetry of the position of the hind limbs was clearly apparent about 20 sec after the lesion and indicated the success of the operation. Three animals in each litter served as controls.

In another series of control experiments, cerebellar lesions were performed in adult C57BL/6J mice, both males and females. These mice were narcotized with ether, the skin of the head cut in the midline, and a small hole drilled into the occipital plane. The left cerebellar cortex was lesioned electrolytically with a current 1.5 ma for 3 sec. Asymmetry of the position of the hind limbs was tested immediately after recovery from anesthesia.

TESTING OF REFLEXES

Asymmetry of the position of the limbs was tested weekly up to the day of the killing of the animals. We did not test the behavior of the growing animals more than once a week, because we did not want to handle the pups too often.

The reflexes described by Fox (8) were tested in each litter twice during the first 16 days of life. The following reflexes were evaluated: righting, crossed extensor, forelimb placing, hindlimb placing, acceleration righting, postural flexion, postural extension, forelimb grasp reflex, hindlimb grasp reflex, head turning response, pivoting, straight line walking, rooting reflex, positive supportive reaction, vibrissa placing, negative geotropism, cliff drop aversion, visual placing reflex, and sucking reflex for description of the reflexes, see Fox (8). The animals were also weighed weekly.

BIOCHEMICAL PROCEDURES

Groups of 10 operated and 10 control young mice were killed by decapitation 48 hr after birth, and 1, 2, 3, 4, 8, and 16 weeks after birth. The head was frozen immediately in liquid nitrogen and kept at -60°. The adult male and female mice were killed 24 hr, 48 hr, and 1, 2, 4, and 8 weeks after the lesion. Twenty-four hours before killing, the mice were injected intraperitoneally with 2 μ Ci [³H]thymidine/g of body weight (Amersham-Searle, specific activity 23 Ci/mmol).

The brains were divided into three parts: cerebellum with part of the brain stem up to corpora quadrigemina, left hemisphere, and right hemisphere. Each of these parts was extracted separately.

The brain DNA was extracted by the method of Zamenhof *et al.* (31). Each brain was homogenized with 3 ml cold 6% trichloroacetic acid (TCA); the homogenate was centrifuged at $18,000 \times g$ in a Sorvall RC-2 refrigerated centrifuge for 40 min. The radioactivity in the TCA supernatant was measured in a Beckman liquid scintillation counter. The remaining pellet was suspended in 1 M perchloric acid and was digested in a 70° water bath for 15 min. The suspension was centrifuged again and the supernatant was taken off. The extraction was repeated once more. Part of the extract was taken for diphenylamine determination of DNA concentration, another part for the measuring of radioactivity.

The results of the DNA determination by Zamenhof's method gave us a general picture of the DNA metabolism after brain damage. The differences which were found were verified once more by lithium chloride extraction of DNA. For this purpose, the animals with damaged cerebellum were injected with 2 μ Ci/g body weight of 3 H-labeled thymidine 1 hr after the lesion. The mice were killed 24 hr after the injection of the label, and 10 newborn brains or 4 adult brains were pooled and DNA extracted. Extracted DNA was purified by Pronase and ribonuclease and extracted again from the solution by the lithium chloride method. Finally, DNA was purified on a methylated albumin-kieselguhr (MAK) column. Amount of the remaining DNA was measured by diphenylamine method and the radioactivity detected in the Beckman liquid scintillation counter. The test of significance used was Student's *t*-test.

AUTORADIOGRAPHY AND MORPHOLOGICAL CONTROL

The mice used in this part of our work were injected by 2 μ Ci 3 H-labeled thymidine/g body weight exactly 24 hr after the perinatal neocerebellar lesion. Groups of mice were killed 24 hr later, or at the age of 7, 14, 21, or 42 days. There were four control and four operated mice in each of these groups. The mice operated on in adult age were killed at the same intervals.

The brains were fixed in Carnoy fluid for 3 hr, embedded in paraffin, and cut into 7 μ m thick sections. After deparaffination the sections were covered with autoradiographic emulsion NTB-2 (Kodak) and exposed for 30 days. The developed sections were stained with Giemsa stain.

RESULTS

BODY WEIGHT AND DEVELOPMENT OF REFLEXES

The changes of the body weight during postnatal development of the operated and control group did not differ substantially. There was also no delay in the appearance and disappearance of all tested reflexes during the postnatal development. The asymmetry of the position of the hind limbs was present in all animals operated after

the birth, disappeared in 2 animals out of 75 (2.7%) within 1 week, in 3 out of 52 in 3 weeks (5.8%), in 8.3% at the age of 6 weeks, in 26.3% at the age of 8 weeks, and in 38.4% at the age of 16 weeks. Although we did not measure the extent of the asymmetry, it was obvious that, in the remaining animals, the asymmetry was not well expressed.

In the adult mice, the asymmetry disappeared in 2 out of 30 mice (6.7%) within 8 weeks after the lesion.

CHANGES OF AMOUNT AND SPECIFIC ACTIVITY OF DNA

The results summarized in Tables 1–3 show that the lesion did not cause any delay in the development of the brain weight, total amount of DNA both in cerebellum and in hemispheres, or amount of DNA per mg of tissue. Whereas the brain weight as well as total amount of DNA increased during the postnatal ontogeny, the amount of DNA per unit weight decreased considerably in telencephalon and did not change much in the cerebellum. This reflects the unequal development of different parts of the brain and relative decrease of the number of the nerve cells per unit volume during the ontogeny.

In spite of the absence of the quantitative changes in the brain development, there was an increased incorporation of the radioactive thymidine into brain DNA after the cerebellar lesion (Table 4). The differences were not only statistically significant in the second day of postnatal life, but also present 1 week later. From 2 weeks of life to the adult age, there were no differences in the incorporation of [3 H]thymidine into brain DNA. The differences are apparent not only in the extracts prepared by the method of Zamenhof *et al.* (1972), but also in DNA extracted with the lithium chloride method and purified on the MAK column (Table 5).

There is some difference in the amount of TCA-soluble radioactivity measured 24 hr after the lesion, but not later. This shows that there may be some difference in the passage of the radioactive thymidine into the damaged brain and/or some difference in the overall metabolism of [3 H]thymidine (Table 6).

All of the changes were detected not only at the site of the lesion, in the cerebellum, but also in both cerebral hemispheres. This

Table 1. *Changes of weights of some parts of mouse brains after neocerebellar damage*¹

Age	Cerebellum		Left hemisphere		Right hemisphere	
	Operated	Control	Operated	Control	Operated	Control
48 hr	23.39 $\pm$ 3.57	32.36 $\pm$ 4.81	41.89 $\pm$ 2.93	40.36 $\pm$ 1.74	32.22 $\pm$ 3.01	35.00 $\pm$ 3.52
1 week	46.50 $\pm$ 4.23	35.68 $\pm$ 4.16	85.93 $\pm$ 5.32	65.87 $\pm$ 8.18	74.79 $\pm$ 6.69	56.06 $\pm$ 5.79
2 weeks	75.73 $\pm$ 5.08	58.50 $\pm$ 4.83	128.82 $\pm$ 4.64	125.61 $\pm$ 5.14	129.45 $\pm$ 5.95	146.33 $\pm$ 6.18
3 weeks	74.23 $\pm$ 5.65	76.93 $\pm$ 3.23	132.33 $\pm$ 3.17	142.93 $\pm$ 6.05	128.27 $\pm$ 6.10	139.32 $\pm$ 5.81
4 weeks	79.85 $\pm$ 3.14	73.71 $\pm$ 5.62	144.30 $\pm$ 8.38	153.57 $\pm$ 4.84	143.00 $\pm$ 7.88	137.43 $\pm$ 7.44
8 weeks	78.60 $\pm$ 8.07	83.42 $\pm$ 6.14	149.90 $\pm$ 2.82	161.50 $\pm$ 5.36	125.40 $\pm$ 9.13	143.50 $\pm$ 9.01
16 weeks	96.65 $\pm$ 4.12	102.38 $\pm$ 6.77	159.05 $\pm$ 4.27	147.56 $\pm$ 10.10	151.20 $\pm$ 3.55	149.50 $\pm$ 4.18

¹ Values in milligrams $\pm$ SE.

Table 2. *Development of total amount of DNA in cerebellum and both hemispheres after neocerebellar damage*¹

Age	Cerebellum		Left hemisphere		Right hemisphere	
	Operated	Control	Operated	Control	Operated	Control
48 hr	92.07 $\pm$ 13.75	115.57 $\pm$ 14.29	145.24 $\pm$ 21.51	152.06 $\pm$ 13.42	129.97 $\pm$ 26.78	142.27 $\pm$ 16.85
1 week	209.15 $\pm$ 44.43	181.03 $\pm$ 31.66	189.67 $\pm$ 22.52	183.30 $\pm$ 25.53	154.13 $\pm$ 33.22	152.21 $\pm$ 18.69
2 weeks	492.23 $\pm$ 33.34	404.07 $\pm$ 23.01	262.00 $\pm$ 17.55	249.03 $\pm$ 18.03	274.00 $\pm$ 13.93	282.18 $\pm$ 16.02
3 weeks	425.10 $\pm$ 34.65	418.28 $\pm$ 16.37	258.20 $\pm$ 16.89	254.60 $\pm$ 15.86	245.91 $\pm$ 17.50	262.71 $\pm$ 14.36
4 weeks	508.35 $\pm$ 22.91	484.67 $\pm$ 32.90	345.93 $\pm$ 49.38	277.37 $\pm$ 32.33	277.28 $\pm$ 21.00	272.50 $\pm$ 26.96
8 weeks	468.55 $\pm$ 25.35	543.16 $\pm$ 49.38	268.63 $\pm$ 12.80	321.73 $\pm$ 24.61	214.18 $\pm$ 21.22	280.25 $\pm$ 40.10
16 weeks	496.49 $\pm$ 37.60	570.40 $\pm$ 23.54	284.93 $\pm$ 9.66	274.35 $\pm$ 20.46	257.42 $\pm$ 17.40	288.50 $\pm$ 22.25

¹ Amounts in micrograms $\pm$ SE.

Table 3. *Relative amount of DNA in cerebellum and both hemispheres after perinatal damage to neocerebellum*¹

Age	Cerebellum		Left hemisphere		Right hemisphere	
	Operated	Control	Operated	Control	Operated	Control
48 hr	5.00 ± 0.63	4.80 ± 0.67	3.58 ± 0.49	4.39 ± 1.06	4.18 ± 0.81	5.30 ± 1.51
1 week	4.33 ± 0.69	5.50 ± 0.87	2.24 ± 0.30	2.84 ± 0.34	2.05 ± 0.29	2.70 ± 0.34
2 weeks	6.66 ± 0.46	7.12 ± 0.44	2.03 ± 0.11	1.97 ± 0.10	2.10 ± 0.12	1.92 ± 0.10
3 weeks	5.91 ± 0.48	5.77 ± 0.31	1.96 ± 0.13	1.73 ± 0.08	1.93 ± 0.13	1.85 ± 0.06
4 weeks	6.41 ± 0.29	6.79 ± 0.65	1.94 ± 0.24	1.95 ± 0.11	2.00 ± 0.19	1.96 ± 0.11
8 weeks	6.11 ± 0.37	6.55 ± 0.43	1.79 ± 0.08	2.00 ± 0.15	1.70 ± 0.09	1.92 ± 0.45
16 weeks	5.21 ± 0.38	5.69 ± 0.28	1.78 ± 0.09	1.75 ± 0.08	1.73 ± 0.10	1.93 ± 0.15

¹ Values in micrograms per mg wet weight ± SE.Table 4. *Specific radioactivity of DNA extracted from cerebellum and brain hemispheres after perinatal neocerebellar damage*¹

Age	Cerebellum		Left hemisphere		Right hemisphere	
	Operated	Control	Operated	Control	Operated	Control
48 hr	15.22 ± 0.57	4.83 ± 0.40	9.86 ± 0.45	4.22 ± 0.40	11.75 ± 0.52	4.78 ± 0.39
1 week	18.84 ± 0.64	16.24 ± 0.16	9.25 ± 0.78	6.27 ± 1.01	11.83 ± 1.01	5.30 ± 0.36
2 weeks	6.41 ± 0.51	5.90 ± 0.24	9.47 ± 0.71	9.26 ± 1.33	9.07 ± 0.78	9.31 ± 0.24
3 weeks	1.23 ± 0.20	0.78 ± 0.10	4.03 ± 0.41	4.39 ± 0.26	4.81 ± 0.38	4.40 ± 0.25
4 weeks	0.97 ± 0.09	0.86 ± 0.20	4.69 ± 0.48	4.29 ± 1.07	4.96 ± 0.44	3.76 ± 0.50
8 weeks	0.85 ± 0.06	0.83 ± 0.08	4.77 ± 0.43	4.48 ± 0.66	4.78 ± 0.17	4.10 ± 0.53
16 weeks	1.02 ± 0.09	0.82 ± 0.12	4.48 ± 0.36	4.93 ± 0.39	4.58 ± 0.51	4.85 ± 0.33

¹ Values in counts per min per mg DNA ± SE.Table 5. *Specific radioactivity in brain DNA extracted by lithium chloride method*

	Operated		Controls	
	No. of observations	DNA, cpm/mg	No. of observations	DNA, cpm/mg
Newborn	7 × 10 brains	13.34 ± 0.56	8 × 10 brains	6.33 ± 0.42
Adult females	4 × 4 brains	2.88 ± 0.60	4 × 4 brains	2.53 ± 0.79
Adult males	4 × 4 brains	2.38 ± 0.43	6 × 4 brains	2.01 ± 0.38

shows that the reaction of the central nervous system to the damage probably spreads into several areas of the central nervous system.

The cerebellar lesion performed in adult mice did not cause any increased incorporation of the radioactive thymidine into brain DNA. Two independent groups of adult mice, males and females, were tested without detecting any significant difference. There were, of course, no differences in the brain weights, total amounts of DNA, and amount of DNA per mg brain weight.

In adult males, specific radioactivity in cerebellum after the lesion was 1.22 ± 0.18 cpm/ μ g DNA and in controls 1.23 ± 0.10 . There was no further difference up to 8 weeks after the lesion. In both hemispheres, the specific radioactivity of DNA from operated animals did not differ from that of the controls (4.86 ± 0.27 and 4.52 ± 0.30 in the left hemispheres, 4.69 ± 0.18 and 4.32 ± 0.60 in the right hemispheres of mice killed 24 hr after the lesion). There were no differences recorded up to 8 weeks after the lesion.

In females, the values are very similar and there were no statistically significant differences between groups. In the cerebellum, there were 1.36 ± 0.19 cpm/ μ g DNA in operated and 1.23 ± 0.20 in controls. In the left hemisphere, the specific radioactivity was 4.54 ± 0.40 cpm/ μ g DNA (operated) and 5.03 ± 0.75 in controls. In the right hemisphere, the values are 4.85 ± 0.35 in

operated and 4.50 ± 0.60 in controls, all values detected 24 hr after the lesion. Up to 8 weeks after the lesion, these values did not change substantially.

The only differences were found in the level of TCA-soluble radioactivity. The TCA-soluble radioactivity after the cerebellar lesion in the adult mice was increased for at least 48 hr after the operation (Tables 7 and 8). This probably reflects the changes of the blood-brain barrier. However, increased TCA-soluble radioactivity was not accompanied by increased labeling of DNA.

AUTORADIOGRAPHY AND MORPHOLOGY OF BRAINS

Morphological control of the lesions in both adult and young animals showed that the molecular layer, the Purkinje cell layer, and the granular layer of the cerebellar cortex were damaged in the area of lobulus ansiform and lobulus paramedianus (27). Only about one-fourth of the left lobulus ansiformis and paramedianus were destroyed.

In control animals examined 24 hr after the injection of labeled thymidine, most radioactivity was found in the germinative areas around lateral ventricles, but some labeled cells were already scattered in different other areas of the brain. In the cerebellum, most labeled cells were in the external granular layer, and some in the stratum granulosum and in the stratum moleculare. Labeled cells were also scattered all over the medulla, pons, and midbrain (mostly reticular formation, but also colliculi anterior and posterior). Few labeled cells were found in different nuclei of the thalamus. In the germinative zone around the lateral ventricle, numerous cells were labeled. Many cells were also labeled in different layers of the hippocampus and fascia dentata. The cortex, septum, and caudate nucleus contained few labeled cells. Many cells were labeled in the tractus olfactorius, nucleus olfactorius, and in entorhinal cortex.

The distribution of cells in the brains of operated animals did not differ substantially from the control brains. Already at this early stage of development, the number of labeled cells in different cortical areas (parietal, occipital, temporal) seemed to be higher in operated than in control animals. Precise evaluation of the numbers and localization of labeled cells in different brain areas will be published separately.

Table 6. *Trichloroacetic acid-soluble radioactivity extracted from cerebellum and brain hemispheres after perinatal cerebellar damage*¹

Age	Cerebellum		Left hemisphere		Right hemisphere	
	Operated	Control	Operated	Control	Operated	Control
48 hr	207.54 ± 14.07	157.51 ± 11.76	227.40 ± 21.77	150.35 ± 11.00	195.37 ± 16.61	116.99 ± 20.45
1 week	246.97 ± 14.59	284.86 ± 59.30	251.80 ± 15.40	256.13 ± 51.18	248.44 ± 14.90	271.44 ± 51.84
2 weeks	410.45 ± 50.30	351.74 ± 30.40	400.79 ± 35.66	447.32 ± 34.95	467.41 ± 26.89	441.36 ± 35.05
3 weeks	538.55 ± 44.01	483.62 ± 27.14	521.26 ± 44.82	467.82 ± 30.29	498.85 ± 30.12	469.76 ± 23.18
4 weeks	480.86 ± 60.65	393.16 ± 80.40	433.35 ± 52.74	320.25 ± 67.06	492.84 ± 70.41	470.10 ± 42.62
8 weeks	457.39 ± 49.38	401.28 ± 38.00	377.70 ± 65.98	409.60 ± 36.79	466.10 ± 60.86	410.00 ± 40.99
16 weeks	439.80 ± 37.43	476.84 ± 51.52	444.06 ± 37.79	553.73 ± 95.74	439.32 ± 35.53	468.46 ± 48.56

¹ Values are in counts per min per mg tissue ± SE.Table 7. *Trichloroacetic acid-soluble radioactivity extracted from cerebellum and brain hemispheres after neocerebellar damage in adult males*¹

Time after lesion	Cerebellum		Left hemisphere		Right hemisphere	
	Operated	Control	Operated	Control	Operated	Control
24 hr	712.32 ± 48.60	481.13 ± 32.11	716.74 ± 41.46	469.61 ± 30.94	729.52 ± 49.06	462.30 ± 32.24
48 hr	635.46 ± 55.29	456.19 ± 29.63	643.02 ± 53.39	458.15 ± 33.78	620.68 ± 54.13	455.40 ± 27.60
1 week	473.77 ± 38.86	444.21 ± 28.26	478.92 ± 38.77	489.21 ± 39.44	439.54 ± 31.47	448.23 ± 30.40
2 weeks	491.72 ± 15.84	454.51 ± 30.04	469.33 ± 16.10	470.66 ± 28.93	461.73 ± 13.50	468.97 ± 30.02
4 weeks	495.00 ± 24.98	466.14 ± 20.16	488.89 ± 36.28	492.25 ± 27.58	495.91 ± 34.59	459.12 ± 22.59
8 weeks	509.56 ± 75.93	450.09 ± 52.78	514.66 ± 75.61	496.96 ± 55.66	516.06 ± 73.43	481.91 ± 49.44

¹ Values are in counts per min per mg wet weight ± SE.Table 8. *Trichloroacetic acid-soluble radioactivity extracted from cerebellum and brain hemispheres after neocerebellar damage in adult females*¹

Adult females						
Time after lesion	Cerebellum		Left hemisphere		Right hemisphere	
	Operated	Controls	Operated	Controls	Operated	Controls
24 hr	705.50 ± 63.69	422.95 ± 38.78	777.52 ± 80.28	441.23 ± 38.72	702.04 ± 47.76	429.03 ± 43.18
48 hr	602.38 ± 33.54	467.47 ± 49.07	686.07 ± 44.29	557.31 ± 25.64	686.61 ± 38.34	551.82 ± 18.61
1 week	488.28 ± 41.01	442.38 ± 19.37	426.12 ± 50.32	455.44 ± 29.74	417.26 ± 36.59	467.84 ± 29.70
2 weeks	415.02 ± 13.72	480.33 ± 21.07	428.00 ± 11.62	463.10 ± 22.54	401.76 ± 11.00	453.38 ± 26.66
4 weeks	395.89 ± 31.81	435.11 ± 19.80	408.86 ± 35.19	442.02 ± 18.63	410.98 ± 34.32	436.32 ± 17.97
8 weeks	421.82 ± 68.30	440.1 ± 19.90	439.94 ± 70.55	421.90 ± 42.30	447.40 ± 70.07	429.20 ± 41.10

¹ Values are in counts per min per mg wet weight ± SE.

In very young animals, it often was difficult to distinguish whether the labeled cells were neurons or glia. The cells were usually small, with very distinct labeling covering the whole nucleus.

In further developmental periods we did not notice substantial differences in the localization of the labeled cells. The external granular layer in the cerebellum of both groups of mice disappeared between the 14th and 21st day of life. The subependymal germinative layer around the lateral ventricles was present in all observed brains.

In mice 2 and 3 weeks old we were able to recognize many labeled cells as neurons. In mice 42 days old, most labeled cells were neurons.

In the animals operated on as adults, few labeled cells were found in the brain, cortex, basal ganglia, thalamus, and mesencephalon. Some of these were neurons.

DISCUSSION

The damaged central nervous system can recover considerably its function in spite of the almost complete absence of the

regeneration of differentiated neurons and their parts, axons, dendrites, etc. Although there is extensive literature concerning the theories of the functional recovery (*cf.* References 3 and 23) little is known about the cellular mechanisms underlying this process.

The morphological and biochemical substrate of the functional recovery has usually been attributed to vaguely defined "plasticity," "spontaneous functional reorganization," etc. In mammals, the recovery is considerably better in younger animals.

At present, there are two main possibilities considered explaining the spontaneous nervous reorganization: (1) sprouting of collaterals from the remaining axons, and (2) increase of excitability of the partially denervated areas. However, the collateral sprouting in the fibers of the remaining pathway may actually prevent the recovery of function (18). Thus, although the sprouting of axons was demonstrated in several areas of the brain (1, 2, 7, 11, 12, 16, 17, 22, 24, 28, 31), its real functional significance is uncertain.

Another possibility, an increase of the sensitivity of the partially denervated areas of the brain, has already been emphasized by Stavratsky (29). The partial isolation of the cerebral cortex or hemitransection of the spinal cord has been widely studied, mostly

in connection with a possible explanation of the activity of epileptogenic focuses in the central nervous system (5, 26). In the partially isolated parts of the brain there are several permanent changes of the neuronal unit activity, sensitivity to the electrical stimulation, enzymatic activity, etc. (4-6, 9, 10, 13-15, 25, 30).

Although one or another mechanism of the functional recovery of the damaged brain is theoretically acceptable, there is no convincing evidence anywhere demonstrating the real importance of these processes for recovery.

We suppose that a fruitful approach to the understanding of recovery may be the study of the cellular control mechanisms directing the "spontaneous nervous reorganization." Permanent metabolic changes in the damaged brain which we found earlier (19-21) are probably preceded by the changes of the metabolism of the nucleic acids, first probably DNA (cell division, activation of different parts of the genome), then RNA and proteosynthesis. This was the reason why we were interested in the changes of DNA.

The described temporary increase of DNA synthesis after the perinatal brain lesion is not just a simple local glial reaction. Many labeled cells are neurons, and they remain in the tissue permanently. The number of labeled glia cells with age decreases, both absolutely and relatively to the number of neurons. The increased DNA synthesis also does not indicate a regeneration process in a usual sense of word. The labeled cells are not localized around the lesion, but migrate and find their final localization in many different areas of the brain.

Thus, it is possible that the described changes of DNA, particularly those associated with neurogenesis, are necessary for the "spontaneous nervous reorganization" after brain damage. Changes in the number and spatial organization of the cells may precede the changes of synaptic connections and sensitivity of the tissue to different neurotransmitters as well as the changes of tissue respiration which we detected earlier. The final result then may be the compensation of the lost functions of the damaged parts of the brain.

We found full recovery in only some of our animals. This may mean that sometimes these biochemical processes are not sufficient for a full recovery.

SUMMARY

The changes of the amount of DNA and incorporation of the ³H-labeled thymidine into brain DNA were studied after unilateral lesion of the neocerebellar cortex. In newborn mice the lesion was followed by increased labeling of the DNA extracted both from cerebellum and of the cerebral hemispheres. In adult animals, TCA-soluble radioactivity after the lesion increased, but there were no changes of the incorporation of the radioactive thymidine into DNA. Immediately after the lesion, the label is localized in different types of cells throughout the brain. Later, 6 weeks after the lesion, the label is found mostly in neurons.

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