3. V. K. Mazo, I. E. Mitin, and E. V. Petrova, Proceedings of the 14th Congress of the I. P. Pavlov All-Union Physiological Society [in Russian], Vol. 2, Leningrad (1983), p. 288.
4. I. L. Medkova, K. V. Smirnov, V. K. Mazo, and V. A. Shaternikov, Lab. Delo, No. 3, 142 (1978).
5. I. L. Medkova, K. V. Smirnov, and B. P. Surikov, Lab. Delo, No. 3, 157 (1981).
6. B. V. Pitran, V. F. Beker, I. A. Morozov, et al., Membrane Digestion and Absorption [in Russian], Riga (1986), pp. 110-112.
7. A. A. Pokrovskii, E. A. Malakhova, and L. D. Boltunova, Handbook on the Study of Nutrition and Health of the Population [in Russian], Moscow (1964), pp. 103-106.
8. A. L. Ryzhova, Lab. Delo, No. 11, 673 (1965).
9. I. L. Tarvid, R. I. Kushak, A. A. Tara, and Yu. R. Siigur, Transport and Metabolic Processes in the Animal Intestine [in Russian], Riga (1984), pp. 136-145.
10. N. M. Timofeeva, V. V. Egorova, V. A. Tsvetkova, et al., Fiziol. Zh. SSSR, No. 7, 960 (1987).
11. V. A. Tsvetkova, D. A. Sirota, B. V. Pitran, et al., Zh. Évol. Biokhim. Fiziol., **22**, No. 6, 596 (1986).
12. V. A. Shaternikov, Vopr. Med. Khim., No. 1, 103 (1966).
13. G. K. Shligin, Intestinal Enzymes under Normal and Pathological Conditions [in Russian], Leningrad (1967).
14. P. De Laey, Narung, **10**, 649 (1966).
15. W. Pule, Metabolism, 24, 93 (1975).

USING FLUORESCENT CARBOCYANINE DYES TO STUDY PATHWAY ORGANIZATION IN AUTOPSY MATERIAL OF THE HUMAN BRAIN

P. V. Belichenko

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Key Words: neuromorphology; fluorescent dyes; retrograde and anterograde axonal transport; terminal ramifications.

One of the main trends in modern neuromorphology is the study of the human brain with its highly complex structural organization. Methods of axonal transport of substances (enzymes, salts of metals, fluorochromes) in association with operations, which are widely used to study pathways of the animal brain, naturally are not fully applicable for investigations of this kind of the human brain. The few methods available to study pathways of the human brain by teasing nerve fibers [3], by retrograde fiber degeneration and anterograde chromatolysis of cells, using clinical material [2], axonal transport of horseradish peroxidase in autopsy material [6]; and axonal iontophoresis of cobalt salts in weakly fixed autopsy material [1], also have a number of important limitations. The main problems which have to be solved for work on autopsy material from the human brain when the last two methods (both giving the best results) are used to ensure adequate preservation of the tissue throughout the experiment and to choose a transported material and the appropriate mechanisms of transport of that material along axons for use with it. Much progress in the study of human brain pathways may be expected by the use of prefixed material. However, axonal transport of materials is impaired by complete fixation of brain tissue, and this problem can be overcome only by the use of weakly fixed material and using an iontophoretic current to transport the materials [1]. When axonal iontophoresis of cobalt salts is used, when studying central projections of cranial nerves, afferent fibers are revealed much more clearly than efferent cells and their processes. This is because of the decrease in concentration of cobalt ions during their passage through the axon and cell body, which have a considerable volume [1].

Laboratory of Neuronal Structure of the Brain, Brain Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. S. Andrianov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 5, pp. 500-501, May, 1990. Original article submitted September 25, 1989.

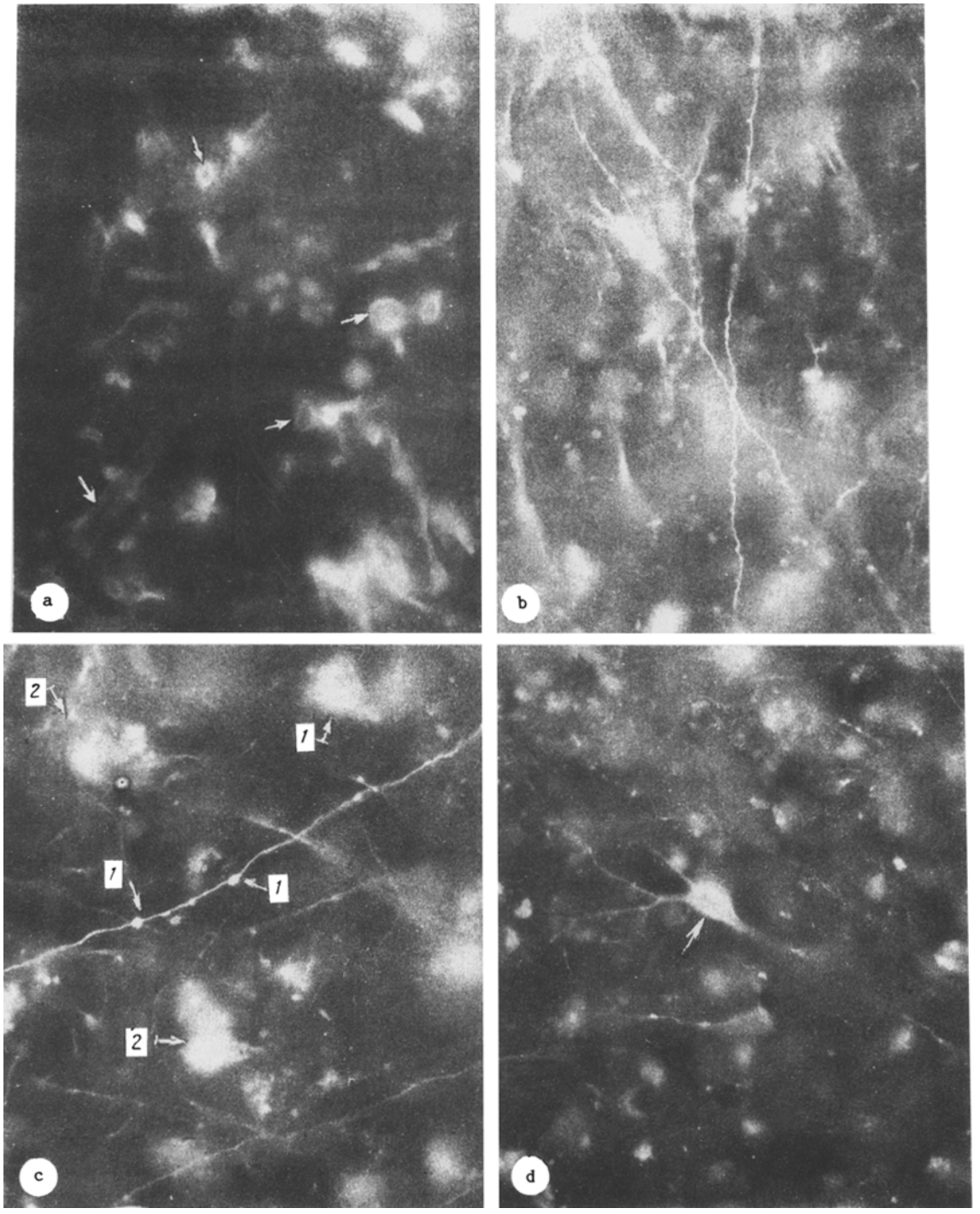

Fig. 1. Autopsy material from the human brain: a) section through white matter of human motor cortex. Arrows indicate myelin sheaths of nerve fibers. Scale 25 μ. b) Efferent sensory fibers (arrows) in layer III of human motor cortex. Scale 50 μ. c) Single thin afferent fiber in layer III of human motor cortex. Arrows (1) indicate large beads along course, and some neurons contain lipofuscin granules (2). Scale 25 μ. d) Pyramidal neuron in layer III of human motor cortex. Scale 50 μ. a-d) Stained with carbocyanine dye dil.

Our experiments showed that the use of fluorescent carbocyanide dyes as the transported materials provides much improved facilities for the demonstration of pathways in fixed autopsy material from the human brain. These substances are soluble in lipids and are used to label lipoproteins in order to study lipid mobility in plasma membranes [7]. They were first used for nerve tissue in order to study sympathetic preganglionic spinal neurons [7]. Only little experience has so far been gained of

the use of carbocyanine dyes to study morphology of the retinotectal system in chick embryos [8], retinal ganglion cells in rats [9], and the organization of the human visual cortex [4]. The mechanism of axonal transport of these substances is evidently longitudinal diffusion in the lipid bilayer of the plasma membrane, resulting in staining of the cell membrane only [5, 7]. This fact suggested to us that when carbocyanine dyes are used, the dye will not be greatly diluted as it passes from the axon into the body of the neuron, as is the case when cobalt ions are used. Another important fact is that substances of this class have a high degree of emission, which is stable in aldehyde fixatives and at room temperature.

EXPERIMENTAL METHOD

Autopsy material from the brain of a person (aged 68 years, with cirrhosis of the liver) taken 12 h after death was used. The cerebral cortex was kept in cold (4°C) 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brain was denuded of its vascular membrane and cut into small blocks (3 × 2 × 1 cm). The following fluorescent carbocyanine dyes were used as materials for transport: diI (1,1′-dioctadecyl − 3,3,3′,3′-tetramethylindocarbocyanine perchlorate) and diO (3,3′-dioctadecyloxacarbocyanine perchlorate; "Molecular Probes," OR, USA). Small crystals (100 μm) of one of the dyes were introduced into the white matter in layer I or III of the motor cortex. The brain fragment was then transferred into fresh 2% paraformaldehyde in PB and allowed to stand at room temperature and protected against light. After 2 months the brain was cut into serial vibratome sections 150 μm thick, which were mounted under a coverslip in PB or 90% glycerin solution in PB. The sections were analyzed under a "Zeiss ASM" luminescent microscope (West Germany), using appropriate filters (maximum of excitation for diI 546 nm, emission 563 nm, for diO 489 and 499 nm, respectively). Some of the sections were stained by Nissl's method to determine the boundaries of the layers.

EXPERIMENTAL RESULTS

Microscopic analysis of the histological sections showed that stained afferent fibers and neurons were visible within a radius of 2 cm from the site of introduction of the dye. In transverse section of an axon bright fluorescence of the myelin sheath could be seen (Fig. 1a), evidence of transport of the dye in the lipid layer. Afferent fibers together with collaterals, terminal ramifications, and beads along their course (Fig. 1b, c) were stained due to anterograde transport of the dye from the site of its injection. Axons with their collaterals and neurons with their dendrites were stained by retrograde transport, and intense fluorescence of the membrane of the cell body and dendrites was observed (Fig. 1d). Throughout the region of spread of the dye (radius of 2 cm) no reduction of fluorescence could be observed from the site of application of the crystal of dye to the periphery, and this is strong argument for the use of this method when studying more extensive pathways of the human brain. According to our data, the rate of transport of diI and diO was about the same, on average 1 cm per month. If there was no decrease in the rate of transport of the dye with increasing duration of the experiment (and there are as yet no weighty arguments in support of such a view), it will be possible to calculate the necessary duration of the experiment in order to study long pathways and their initial neurons. In our experiments single thin terminal fibers (Fig. 1b) stained very clearly, the beads on such fibers also had clear outlines (Fig. 1c), so that it can be reasonably confidently concluded that transsynaptic transport of dyes does not take place, at least for 2 months. However, when long brain pathways are studied, with a corresponding increase in the length of exposure of the applied dye, this problem may arise and may require further study.

The use of this staining technique provides wide opportunities for the study of the construction of the human brain. First, the use of fixed autopsy material prevents destruction of the nerve tissue and guarantees stable results; second, the visibility of the initial neurons is much improved on account of transport of the dye along the membrane of the neuron body and its dendrites only. The use of two carbocyanine dyes with different parameters of fluorescence (diI and diO) makes it possible to investigate not only single pathways, but also fibers and cells with extensive collaterals and terminating in different brain structures.

LITERATURE CITED

1. P. V. Belichenko, "Method of demonstrating structures of nerve cells in a histological preparation," Author's Certificate 1303879, Lodged June 30, 1986, No. 3824752/28-14. Byull. Izobret., No. 14 (1987).
2. L. V. Blumenau, The Human Brain [in Russian], Moscow (1925).
3. S. B. Dzugaeva, Pathways of the Human Brain during Ontogeny [in Russian], Moscow (1975).

4. A. Burkhalter and K. L. Bernardo, Proc. Nat. Acad. Sci. USA, **86**, 1071 (1989).
5. P. Godement, J. Vanselow, S. Thanos, and F. Bonhoeffer, Development, **101**, 697 (1987).
6. S. Haber, Neuroscience, Suppl. 22, 799 (1987).
7. M. G. Honis and R. L. Hume, J. Cell Biol., **103**, 171 (1986).
8. S. Thanos and F. Bonhoeffer, J. Comp. Neurol., **261**, 155 (1987).
9. M. Vidal-Sanz, M. P. Vellesas-Perez, G. M. Bray, and A. J. Asuayo, Exp. Neurol., **102**, 92 (1988).

EFFECT OF EARLY MONOCULAR DEPRIVATION BY LEUKOMA OR CATARACT ON RETINAL DEVELOPMENT

V. G. Taktarov and A. N. Bekchanov

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The cellular mechanisms of plasticity of the brain in visual deprivation have been studied in detail in structures of the visual cortex and basal ganglia [3, 6]. The receptor section (the retina) has received far less study, and information on the character of the changes taking place is extremely contradictory. Some workers [1, 5] have described marked changes of a degenerative character, whereas others deny the existence of any significant morphological disturbances in occlusive amblyopia [4]. The study of the retina in unilateral deafferentation is particularly interesting because changes at the cortical level in this case are maximal [2].

The aim of this investigation was to study the action of early unilateral deprivation on maturation of the retina. Occlusion resulting from induced leukoma or cataract was used as a completely unstudied model.

EXPERIMENTAL METHOD

Experiments were carried out on male kittens (*Felis domestica*) reared in the laboratory under ordinary conditions of lighting and with monocular deprivation at the age of 2-3 days. Cataract was induced by division of the capsule of the lens, and leukoma of the cornea was formed after a burn inflicted with a 25% aqueous solution of ammonia. The retina of the kittens was studied at the age of 1, 4, 7, 14, 28, 56, 84, and 120 days of postnatal development. Material was fixed in Carnoy's fluid and embedded 5-6 μm thick were stained with fast cresyl violet by Nissl's method in a modification. Total protein was determined by staining with Naphthol yellow S, followed by photometry on an SMP-01 scanning microscope ("Opton"). The results were analyzed by "Wang-720C" microcomputer, by the "Areascan Print" program. The area of cross section of the cell bodies was measured with the "Microvideomed-11" attachment. The intensity of protein metabolism was assessed with the aid of ^{3}H-lysine labeling. Labeled lysine ("Amersham," specific radioactivity 10 MBq/mmole), was injected into the vitreous body in vivo in a dose of 12×10^5 Bq, and enucleation was carried out 20 min later. In a series of experiments in vitro, the enucleated eye was opened and placed in incubation medium containing 3.7×10^{-5} Bq/ml of ^{3}H-lysine at 37°C for 15 min. Autoradiographs were obtained by the usual method.

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