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Early Disembryogenesis as an Etiological Explanation of Developmental Dyslexia

Diana Ignatova, Victoria Radeva

Department of Special Education, St. Kliment Ohridsky University of Sofia

It is well known that β-ionizing radiation as a physical factor and Nerve-Growth Factor (NGF) as an embryoactive substance influence the early neuroembryogenesis. The degree of the influence depends on the size and the duration of the treatment as well as on the distance source (regarding β-ionizing radiation). NGF takes part in the natural neuroembryogenesis. It is possible under certain circumstances NGF to be excreted larger than usually which can have an adverse effect in the developmental course.

Our hypothesis is that in children with developmental dyslexia discrete subcellular changes are apparent which have been emerged during early neuroembryogenesis.

Key words: early disembryogenesis, Nerve Growth Factor (NGF), ionizing radiation, developmental dyslexia, cognitive deficits.

Introduction

During the last three decades the question of the biological basis of minimal neuronal disabilities (which in most cases are associated with specific learning difficulties known as developmental dyslexia) has been much treated. Because of the presence of a great number of various biologically embryo-active factors the reasons for the changes that have occurred cannot always be explained. Besides, it is difficult to define the period during which these factors have exerted influence over the passing of neuroembryogenesis. That's why qualitative morphological researches are of great importance since they contribute to clarifying of the biological basis of the disabilities which can be manifested as functional long after the prenatal development of the nervous system has finished. The present article supports the concept for multi-causality of the human behaviour. On the basis of this we try to formulate integrative explanatory hypothesis as a peculiar bridge between neuromorphology and behaviour studies. That's how the idea of the three-dimensional model of human behaviour was born as an interaction between genetic data, biological factors and the social environment of the individual. We allow the possibility to make conclusions about man based on the research of animal experimental model. Our idea of the phylogenesis of the nervous system makes it plausible. Studying the influence of some biologically active factors upon the developing nervous system during early embryogenesis ultrastuctural changes have been observed which are

assessed as a prerequisite for the formation of discrete changes in the morphogenesis of the nervous system.

An embryo-active substance – Nerve Growth Factor (NGF) and a physical factor – β-ionizing radiation were chosen for the experiments.

NGF takes part in the natural neuroembryogenesis. It is possible under certain circumstances NGF to be excreted larger than usually which can have an adverse effect in the developmental course as causing discrete ultrastructural changes in the cells of the future nervous system.

On the other hand, it was assumed that increasing of the radioactive background influences the process of neurulation and sometimes it influences the gametogenesis as well. In this way the ultrastructural changes can be genetically transmitted to the future embryo after treatment with β-ionizing radiation.

Material and Methods

1. Embryos of *Triturus cristatus* obtained after artificial fertilization by HERTWIG'S method from animals provided from their natural habitats were used in the investigation. The embryos were bred in currently refreshed HOLTFRETER'S solution at a temperature of 19.2 °C. Control embryos were separated. Entire embryos of stages 16, 17 and 18 (according to the classification of Pollister and Moore, 1937) were incubated for 1 hour and for 24 hours in NGF solution (obtained by Cohen's method [2]) at a concentration of 32 μg/ml. The state of the treated and control embryos was observed under stereomicroscope after the incubation and photos were taken.

2. Embryos of *Triturus cristatus* with a preserved gelatin capsule from the period of fertilization to the stage of larva (10 days) were incubated in tap water around a fixed "hot" particle at a distance of 0.5 cm, 2.0 cm and 4.0 cm.

After mechanical decapsulation control and treated embryos were fixed. Fixation (1, 2) was carried out (according to a modification of V. Radeva [6]) in a mixture of equal parts of 10 % neutral formol in HOLTFRETER'S solution and 2.5 % glutaraldehyde in phosphate buffer at pH = 7.2 – 7.3 for 2 – 4 hours at 4°C. The neural plate, groove and tube were mechanically isolated. This was followed by postfixation with 2 % OsO_4 for 1 hour at 4°C embedding in Durcupan, cutting on an ultramicrotome "Reichert" and contrasting with uranyl acetate. Electron microscope "Hitachi" 11-A was used for the observation.

Results

Fig. 1 was used as a comparative base in order to evaluate the changes in neuroepithelial cells in experimental conditions.

Treatment with an embryo-active substance – Nerve Growth Factor (NGF)

The action of NGF causes changes during the first hour. The bodies of the embryos become longer and the sculpture of the dorsal part becomes clearly distinguished. These changes are still more marked after 24 hours. Mechanical stimulation of the embryos at the end of the 17th and the beginning of the 18th stage, treated for 24 hours with NGF provokes C-shaped unilateral contractions of the myotomes which appear consecutively on either side. It was not possible for us to provoke similar contractions in the control embryos during the respective stages.

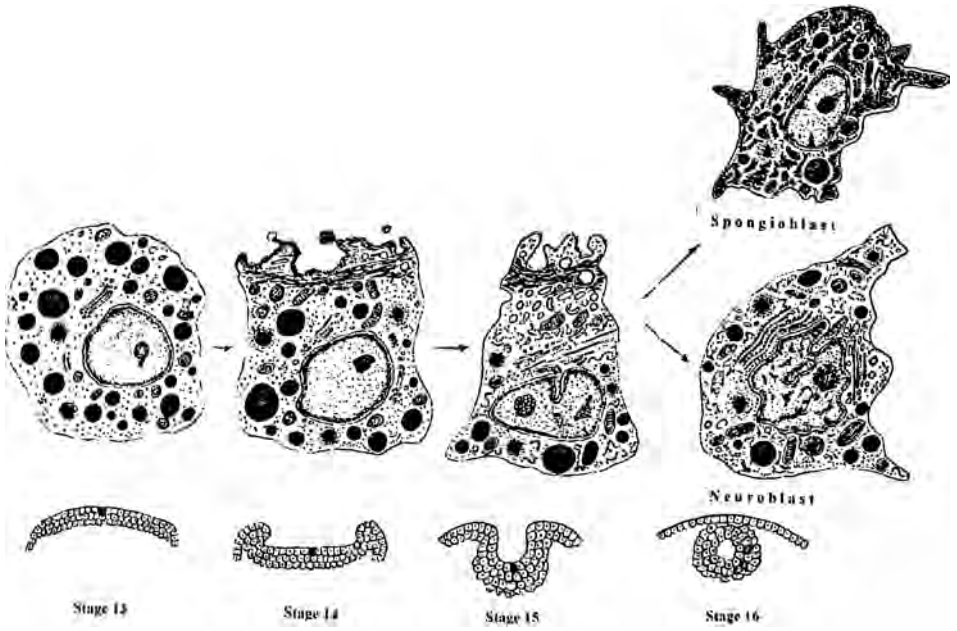


Fig. 1. Ultrastructural changes in neuroepithelial cells during normal neurulation

A clear hyperplasia of the neuroepithelial cells from the neural groove and tube has been established after 24-hour incubation in NGF. In comparison with the controls (at a histological level) clear differences are observed. A hyperplasia of the neural tube was not observed after incubation in NGF in case that the tube is already fully formed. Symptoms of accelerated neurulation respective to the macro-microscopic changes were established under electron microscope. However, an ultrastructural specificity of the changes that characterize the influence of the NGF was not apparent. The inter-phase nuclei showed structural changes characteristic of a definite stage specificity. At the initial stages of neurulation and in the first hour of incubation in NGF a marginal condensation of heterochromatin was observed. Besides, during the stages after the formation of the neural tube – in the central zone of the nucleus the invaginations of the nuclear membrane were rare for stage 14 and stage 15 and become typical for stages 16 and 17 (see Fig. 2). The granular (rough) endoplasmic reticulum (Gr.r.) has unusual ring-shaped profile which is not visible in the controls. The changes affect all cell organels but they are most evident in the nucleus and in the granular reticulum as well as in the increased assimilation of yolk material (Y). Unlike the controls (in which at the beginning stages of neurulation mainly compact nucleoli were found) after treatment with NGF at the beginning of stage 14 and the next stages of neurulation, the characteristic reticular nucleoli for the active cells were visible. Besides, they were either increased in number or hypertrophied.

The nuclei of spongioblasts also demonstrated symptoms of activation which were manifested through the numerous and deep invaginations of the nuclear membrane, increasing of the nuclear pores, peripheral position of the nucleoli.

After treatment with NGF (from the stage of the neural groove – stage 15 until and after closure of the neural tube) the number of perichromatine-like and perichromatine

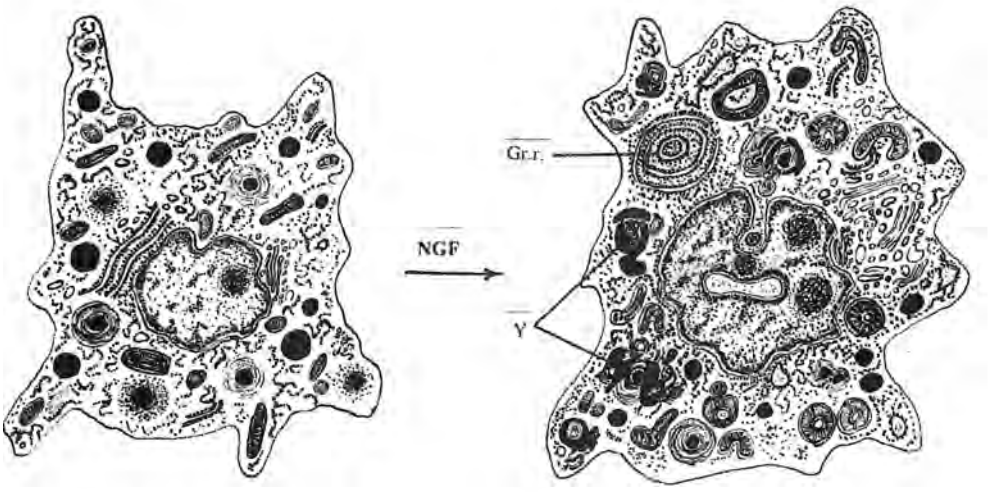


Fig. 2. Ultrastructural changes of neuroblast after treatment with NGF

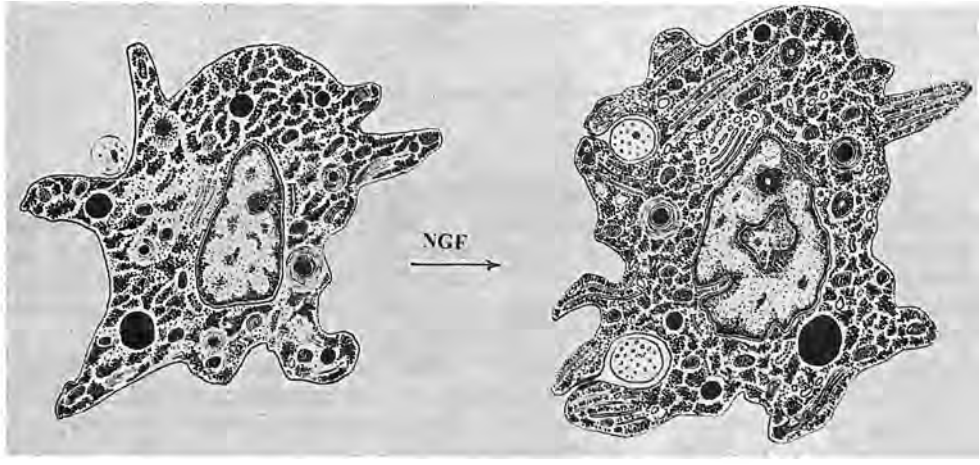


Fig. 3. Ultrastructural changes of spongioblast after treatment with NGF

granules were obviously increased and in stage 18 giant perichromatin granules were observed.

One of the common ultrastructural changes was increasing of the Golgi zone (embryonic in type) which was in immediate neighbourhood with the numerous microtubules (that was not characteristic for the controls). Highly typical was the polymorphism of the mitochondria where ring-shaped profiles predominated as well as mitohondria with longitudinal crists of peculiar outlines.

Another important effect of the treatment with NGF was the unusual production of microphilaments and microtubules. This was clearly observed after stage 15. The maximum of the effect was visible in stage 18. Under the influence of NGF an increased assimilation of yolk material was seen as well as an increased aptitude of the cells to make contacts. Besides, neuroblasts form synapsis as early as the beginning of stage 18 which was considerably earlier than the controls [11].

Treatment with a physical factor – β-ionizing radiation

The effect of β-ionizing radiation on neuroepithelial cells was studied. The source distance was 4.0 cm. Parallel with hyperplasia of Golgi complex (with elongated cisterns), two big vacuoles (see Fig. 4)(arrows) full of dense network-like substance were observed. The changes shown are discrete. The remaining cell organels were not changed in comparison with the controls.

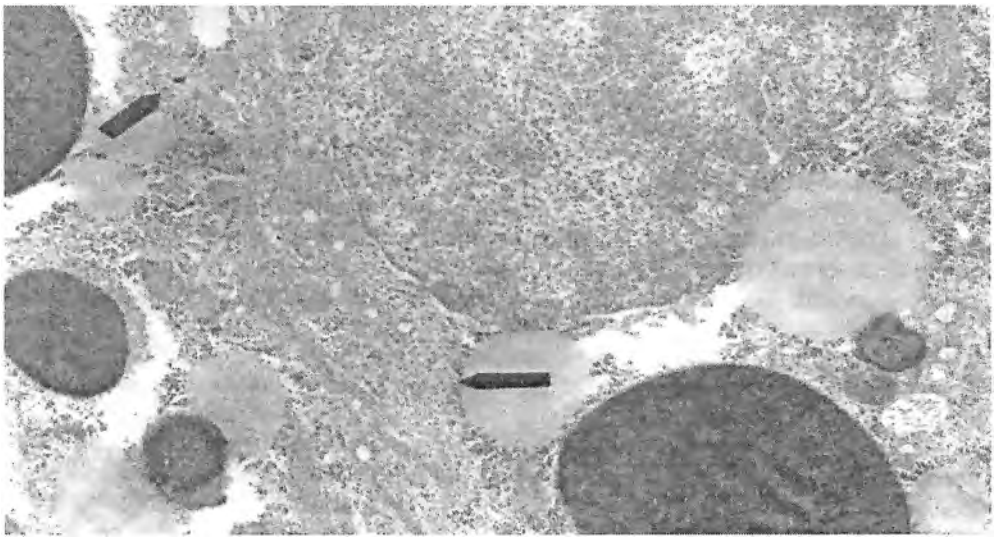


Fig. 4. Influence of β-ionizing radiation on a neuroepithelial cell (a part of cell) (× 6100)

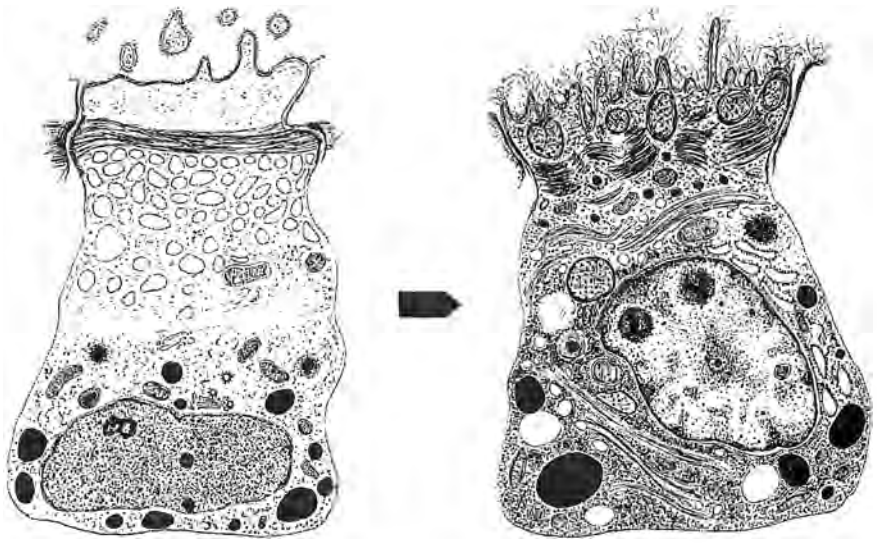


Fig. 5. Ultrastructural changes of neuroepithelial cells after treatment with β-ionizing radiation; source distance – 4.0 cm; duration of the radiation – 72 hours

The distance from the source is of great importance for the effect of β-ionizing radiation. In experimental conditions the distance of 0.5 – 2.0 cm for a short time of 72-120 hours causes death of the cells and of the embryo. However, a source distance of 4.0 cm caused the discrete ultrastructural changes mentioned above which showed peculiar stimulation of the process of neurulation. The stimulation was manifested through morphological symptoms respective to the accelerated invagination, intensive processes of environmental exchange (apical endosytozis) and changes in heterogeneous nuclear RNA processing (see Fig. 5). The ultrastructural changes that have been described resemble a tumour growth.

Discussion

It was known that during pregnancy the rate of NGF in blood increases. This fact explains some discrete subcellular changes which have taken place in the still unshaped nervous system of the embryo. These changes can be manifested long after the birth.

It should be noticed that during embryogenesis because of many reasons (for example treatment with embryo-active substances and also mechanical factors) an apoptosis can be observed. Taken alone it can influence the vital functions of neighbour tissues and organs.

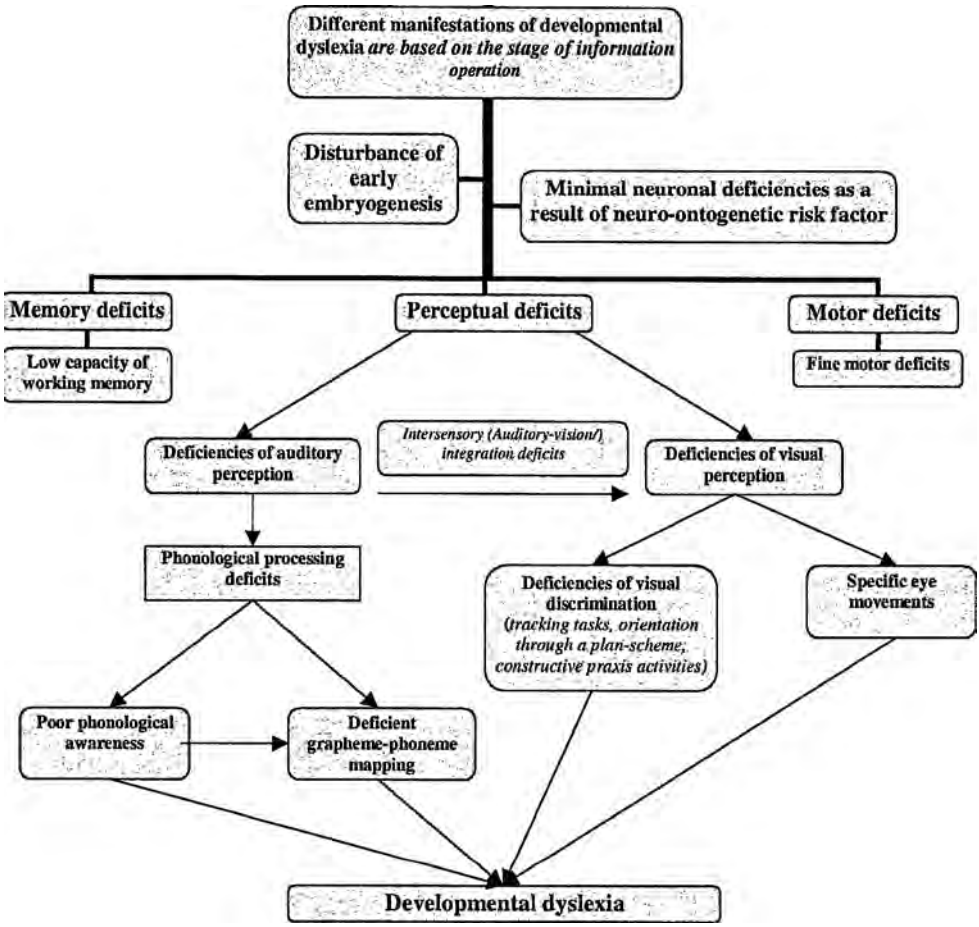
Duration of the radiation as well as distance from the source of ionizing radiation is very important – if the distance of the treatment is longer but its duration shorter – changes are very discrete.

Because of the extremely high rate of cell-division after closure of the neural tube, i.e. after 23rd–25th day from the conception, the ultrastructural changes (which are a result of the β-ionizing radiation effect) multiply in a considerable degree. After this period a cell generates 250 000 new cells per minute in which minimal intra-cell changes can be multiplied [1]. Since the ultrastructural changes mentioned multiply in the process of cell differentiation it may lead to minimal brain deficits. Later, this will reflect upon the development of learning abilities and will be manifested as so called developmental dyslexia.

Developmental dyslexia is a disorder that is defined as a difficulty in language processing. There is a wide consensus that it is a neurological disorder with a genetic origin [3, 4, 5]. On a world scale it has a prevalence estimated between 5% and 17% [8]. The difficulties are primarily at the level of phonological processing of speech sounds which is the ability to recognize and manipulate the sound structure of words (rhyming, syllable counting and spelling words) [9]. Abstraction of rules in the spelling patterns, the use of syntax and hierarchical order to extract meaning are high-level perceptual and cognitive abilities. The existing cognitive deficits in children with developmental dyslexia evolved from congenital disfunction of some areas in the brain cortex. The abnormal functioning may result in various combinations of deficits in perception, inter-sensory integration, language, working memory or control of attention, which will interfere with the reading process (see Tabl.1). A child with developmental dyslexia may exhibit any or all of these cognitive deficits. Their optional character makes sense in the light of genetic studies showing that the language/phonological deficit is highly heritable and is in a close connection with some cytoarchitectonic abnormalities of the brain [3, 4].

The results of the present study suggest ideas about assumed relation between early disembryogenesis and developmental dyslexia. An attempt to make analysis of some possible causes of dyslexia was carried out. Certainly it is not possible to speak about developmental dyslexia as a disorder with one unique cause. On the contrary, it can

Table 1. Cognitive deficits in developmental dyslexia



rather be considered as a general condition that has many possible causes. However, it seems that some dyslexic children may have deficits in the neural mechanisms responsible for cognitive processing and reading problems that emerge after treatment with some embryo-active substances and physical factors. Regarding this matter their causal role in the etiology of developmental dyslexia is strong indeed.

References

1. Angeletti, P. U., R. Levi-Montalcini, F. Caramia. Ultrastructural changes in sympathetic neurons of new-born and adult mice treated with NGF. – J. Ultrastr. Res., **36**, 1971, 24-36.
2. Cohen, S. Purification of a nerve-growth promoting protein from the mouse salivary gland and its neurocytotoxic antiserum. – Proc. Natl. Acad. Sci. U.S., **71**, 1960, 302-311.
3. Davis, C.J., J. Gayan, V. S. Knopik, S.D. Smith, L.R. Cardon, B.F. Pennington, R.K. Olson, J.C. DeFries. Etiology of reading difficulties and rapid naming: the Colorado Twin Study of Reading Disability – Behav. Genet., **31**, 2001, 625-635.
4. Galaburda, A.M. Cytoarchitectonic abnormalities in development dyslexia: a case study. – Annals of Neurology, **6**, 1979, 94-100.

5. Geschwind, N. Biological Associations of left handedness. – Annals of Dyslexia, **33**, 1983, 29-39.
6. Radeva, V., J. Taxi. Influence du facteur de croissance nerveuse (NGF) sur la genese de synapses dans le tube neural d'embryon de triton. – Arch. Anat. Micr. Morph. Exp., **64**, 1975, 135-148.
7. Radeva, V., I. Valkov, G. Roussev. On the appearance of asteroid perichromatin granules under the influence of certain biologically active substances during the neurulation of newts. – Biol. Structure and Morphogenesis., **2**, 1989, 55-59.
8. Shaywitz, S. E. Dyslexia. – New Engl. J. Med., **38**, 1998, 307-312.
9. Wagner, R. K., J. K. Torgenson, C. A. Rashotte. Longitudinal studies of phonological processing and reading. – J. Learn. Disabil., **27**, 1994, 276-186.
10. Антонов, А., И. Белоконски, Ц. Бончев, В. Босевски и сътр. Върху проблема „горещи частици" след аварията в чернобилската АЕЦ. – В: Научни доклади и съобщения. Част I. С., Селскостопанска академия, 1987, 119–132.
11. Радева, В.Р. Морфология на неврулацията. С., Университетско изд. „Св. Кл. Охридски", 1997, 96–115.