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Physiological and Pathological Aspects of Neuroontogenesis

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In Czechoslovakia, neuroontogenetic research in physiology begins shortly after the Second World War. Together with older colleagues – probably under the influence of strong domestic traditions (Babák 1873–1926) – a group of newly qualified physicians in the Institute of Physiology of the First Faculty of Medicine of Charles University began to study problems of developmental physiology. This has ever since been highly characteristic both of this department and the Institute of Physiology of the Czechoslovak Academy of Sciences (to which some of the workers of the department went after its foundation in 1953) and also of the work of several physiologists who emigrated (especially between 1968 and 1971, when various other scientists were also obliged to leave their research). Although the developmental physiologists who left for the Academy were not explicitly concerned with neuroontogenesis, their work brought them very close to this subject in many respects, as confirmed by their monographs (Fifková and Maršala 1960, Jelínek 1961, Křeček 1962, Bureš 1962, Hahn 1966, Koldovský 1968). In 1960, in Liblice, they organized an exceptionally well-attended symposium (Adolph, Anokhin, Verley, Widdowson) on development, part of which was likewise devoted to neuroontogenesis.

At first, actual neuroontogenetic research dealt with a traditional problem of evolutionary neurophysiology, i.e. the question of the high resistance of newborn mammals to oxygen deficiency. The work was successful and so, in 1960, the results were presented in monograph form in a book edited by Mourek (Hahn *et al.* 1960). Complex functional, electrophysiological, biochemical and morphological study of the developing CNS became characteristic and was summed up in subsequent papers (Mysliveček 1963, Sedláček 1959, 1967, Jílek 1966, Mourek 1966).

The departure of Mysliveček (the founder of neuroontogenetic research in the Institute of Physiology of the First Faculty of Medicine in Prague – see Mysliveček 1991) to the Medical Faculty in Plzeň extended this research (Hasmannová-Myslivečková, Chaloupka, Rokyta, Sobotka, Šafanda, Záhlava). In 1960 the first international neuroontogenetic symposium was held in Plzeň and it is worth mentioning that, despite the restrictions on contacts with other countries, the number of visitors and papers from abroad was flattering (the Flexners, Scherrer, the Himwiches, Volokhov, Koshtoyants, Minkowski, Krebs, Tuge and Dreifuss-Brissac, etc.). These neuroontogenetic symposia were continued successfully in Prague, in 1967, 1973, 1979 and 1989 (Ontogenesis of the Brain, vol. I, II, III and IV).

Neuroontogenetic research was also pursued elsewhere – Nováková, Šterc, Lodin *et al.*, Fischer, Křivánek and Lisý *et al.* in the Institute of Physiology of the Czechoslovak Academy of Sciences, Sedláček in the Research Laboratory of Psychiatry of the First Faculty of Medicine of Charles University (Mysliveček 1991) and Pressl in the Institute for the Care of Mother and Child in Prague, etc.

Many new findings on the structural development of the brain, in particular on the early formation of neurones and glial cells, on changes in their chemical composition and on cellular and extracellular components formed in response to numerous physiological and pathological stimuli. These results were obtained in the Laboratory of Cellular Neurobiology in the Institute of Physiology of the Czechoslovak Academy of Sciences in collaboration with various universities and academic institutions at home and abroad.

In 1967, in cooperation with the Centre de Neurochimie in Strasbourg, a method for the cultivation of dissociated brain cells, which is still used all over the world, was evolved (Sensenbrenner *et al.* 1969). In Czechoslovakia, this method was modified to allow the development, metabolism and ultrastructural organization of nervous tissue cells to be studied *in vitro*, and the interrelationship between glial cells and neurones (Lodin *et al.* 1973, 1974). Fruitful collaboration was carried out with the laboratories of the late Prof. Hašek and Academician J. Šterzl, which led to new and valuable contributions concerning transplantation immune reactions of the brain and the basis of the defence mechanism of nervous tissue (Hašek *et al.* 1985, Štědrá *et al.* 1988).

Histochemical studies of the brain of young small laboratory rodents have helped to elucidate changes in the proliferation rate during morphogenesis of the cerebellum (Lodin *et al.* 1968, Mareš and Lodin 1970). The time sequence with which the division of germ cells is arrested and the formation of nerve and glial cells in the various projection centres was determined, using a visual analyser of the brain as the model (Mareš 1979). X-ray irradiation of animals in the late stages of prenatal development made it possible to induce, in these stages of cytogenesis, the formation of selective cellular lesions and to describe the subsequent regeneration reaction of the neuroepithelium. Persistent discrete neuronal lesions which might form a basis for minimal brain dysfunction in adulthood were found in the forebrain cortex (Bruckner *et al.* 1980). Acute and late changes of a similar type were found in the cerebellum of animals given the cytostatic cisdichlorodiaminoplatinum II (Mareš 1980).

In some parts of the brain of laboratory rodents, the formation of new cells begins to decline soon after birth. The source of this decline in the glial cell population was qualified in various parts of the brain by means of autoradiographic detection of DNA synthesis. A significantly lower rate of residual gliogenesis was found in the visual centres of animals kept in the dark after birth, i.e. with a low degree of sensory feedback in the relevant projection regions of the brain. A quantitative evaluation of residual proliferation of glial and endothelial cells in the brain of adolescent and adult animals showed that the majority of newly formed cells died soon after they were formed. It was suggested that disturbances of the rate of cell death in the minority pole of proliferating glial cells might represent a new pathogenetic mechanism for the development of dysplastic states of the brain (for a review see Mareš 1986).

A series of new findings was likewise obtained by studying developmental changes in the chemical composition of nervous tissue (Lodin and Mareš 1968). Elucidation of the causes of the protracted controversy over the actual DNA content of the large projection neurones of the cerebellum and the forebrain and a description of the distribution and general activity of the ribosomal RNA genes in the Purkyně cell nuclei of young and adult laboratory rats are important contributions (Mareš *et al.* 1985a, 1987). Cellular and topographic differences in the rate of glycoprotein synthesis in the immature and adult mouse brain were described (Mareš and Muller 1987) and the presence of alpha-foetoprotein (and several other serum proteins) was demonstrated at sites of intense cell and tissue differentiation of the embryonic CNS of the laboratory rat and the pig (Mareš *et al.* 1985b).

The Institute of Physiology of the First Medical Faculty of Charles University traditionally remained the centre of neuroontogenetic research, however, and so it became more or less automatically the national guarantor of this research. Because of its scientific results and the attractiveness of the subject matter, it was joined in time – and quite spontaneously – by institutions working on clinical problems (epilepsy, prenatal brain damage and rehabilitation of its consequences, reflex motor activity, the balneotherapy of cerebral palsy).

Research in the Institute of Physiology was concentrated mainly in two laboratories, one under Jílek (later Trojan) and the other under Mourek. The first of these laboratories concentrated initially on the principles of the immediate response and the adaptive reaction of immature nervous tissue to changes in the internal and external environment – in particular to oxygen deficiency (Jílek *et al.* 1962, 1964, 1970, Fischer *et al.* 1962, Krulich *et al.* 1962). Later it concentrated on the study of adaptation of the developing brain to various forms of oxygen deficiency. It was demonstrated that, for the development of adaptation processes, the duration of the intervals between individual stimuli (i.e. hypoxia or anoxia) was at least as important as their total number or the total time of exposure. Neither the somatic nor the reflex development of adapted animals is impaired (only their learning capacity is lower for a short time after exposure). On further exposure to oxygen deficiency, adapted animals display greater resistance. In this, the metabolic reaction of the brain (lower energy metabolism, a greater role of anaerobic glycolysis in total energy balance and more economic metabolism) is of primary adaptive significance (Trojan 1978).

For clinical practice, it is important to note that the resistance of immature nervous tissue can be raised still further (Trojan and Trojanová 1983). Morphologically, oxygen deficiency in the postnatal period affects the development of dendrites and their spines, formation of the myelin sheaths of axons and development of the ultrastructure of synapses. The changes are partly repaired, but persist to some extent into adulthood, indicating that the integration process in the individual circuits of the brain may have been impaired (Pokorný and Trojan 1986). This was confirmed by electrophysiological findings. Changes in evoked potentials (especially interhemispheric responses) testify to some slowing down of development as a consequence of chronic hypoxia. This retardation might be an important adaptive mechanism, since it could keep the CNS in a "younger" phase of development, when the brain is significantly less sensitive to oxygen deficiency. Animals exposed to chronic hypoxia after birth are liable in adulthood to more severe and longer epileptic seizures evoked by electric stimulation of the cerebral

cortex (self-sustained after-discharges). Since other electrophysiological parameters (evoked potentials, the spontaneous EEG, focal discharges) are unchanged, this can be assumed to be a sign of minimal brain damage (Mareš *et al.* 1985c).

Since an objective – and mainly specific – indicator of the intensity and duration of perinatal hypoxia is lacking in clinical practice, the release of cytosol enzymes (LDH, aminotransferase, creatine kinase, neurospecific enolase) into the plasma was studied. This is a valuable indicator of tissue ischaemia, as it detects destructive changes in the cells, but for the evaluation of perinatal hypoxia its sensitivity is too small (Štípek *et al.* 1987). The study of purine and purine nucleotide catabolites, and particularly gamma-glutamyl transpeptidase, proved to be more satisfactory (Trojan and Šťastný 1988). The determination of plasma antibodies to nervous tissue offers good prospects, especially as their titre rises with an increase in the number of exposures to hypoxia (Trojan and Ježková 1979).

These results were followed by a study of the plasticity of the brain in pathological hyperfunction. A successful model of the initial phases of acute "kindling" was elaborated. Hyperexcitability begins to appear after 10 min, but its further development depends on how many seizures have occurred before. A morphological analysis showed that a seizure was followed almost at once by exhaustion of the synaptic vesicles (Langmeier *et al.* 1983). The same kindling model can also be used to study the transmission of plastic changes to further areas of the cerebral cortex (Mareš *et al.* 1981a). It likewise permits a study of the effects of various antiepileptic drugs and basic research on short-term plasticity of the CNS (Heidler and Trojan 1985). Plasticity of the CNS was found to be determined genetically. On the other hand, paradoxically the experimental use of inbred strains of rats allows the situation encountered in clinical practice in the genetically heterogeneous human population to be modelled more exactly (Kunz *et al.* 1988).

Several groups of complementary questions, in a mutual causal relationship, have been resolved in Mourek's laboratory. One of the first problems was the question of metabolic substrates for oxidative processes (oxidative phosphorylation) in the brain (or individual compartments of the brain) in the early stages of ontogenesis, with the finding of a given substrate pluralism due probably to hitherto incomplete tissue differentiation. It is interesting that the phylogenetically oldest parts of the CNS retain this substrate nonspecificity (lactate, acetoacetate, glucose, pyruvate, glutamate) (Mourek 1965, 1966, 1970). This finding was also demonstrated at the mitochondrial level.

Another group of problems concerns the stability and/or variability of the internal environment. It was found that the internal environment values in the early stages of ontogenesis were not only significantly less stable than in adult individuals, but that their oscillations determined by changes in the external environment were very often completely different. These changes are indicative of a secondary effect on enzymatic activity, or on oxidative processes in general. The effect of pH on the metabolism of immature tissue (Mourek and Trojan 1963) is regarded as a basic finding.

It was demonstrated that hypoxic stress led to a significant decrease in oxygen consumption (and in body temperature), which was greater in the presence of greater hypoxia and in younger organisms (Mourek 1959). This phenomenon was demonstrated *in vitro* as well as *in vivo*. Furthermore, after hypoxia had been discontinued, the tissue of newborn rats (as opposed to adult animals) showed

an over-shoot in oxygen consumption reminiscent of oxygen debt (i.e. of a compensatory nature). Changes in cortical impedance during hypoxia were described in newborn and young rats for the first time (Mourek 1961).

Starvation (i.e. nutritional stress) is likewise a risk factor of the perinatal period. Systematic studies have been done on the effect of this treatment on brain metabolism, nerve cell differentiation, amino acid spectra (Mourek *et al.* 1970), enzyme activities and reflex or electroencephalographic parameters (Mourek 1967), confirming the premise and hypothesis that the newborn mammal and its nervous tissue suffers from nutritional deficiency significantly more, with the possibility of long-term consequences (Mourek *et al.* 1974).

Hypoxia and malnutrition are clearly very serious perinatal hazards. This logically led to cooperation with obstetricians, chiefly as regards the question of changes in fatty acid spectra – during development in the serum and brain, in hypoxic states, or in premature births and immature foetuses, etc. (Mourek *et al.* 1987). These studies were supplemented by model studies in rats, which led, *inter alia*, to the conclusion that retarded development is manifested primarily by an increase in the proportion of short chain fatty acids and in a decrease in polyene fatty acids, chiefly of the order n-3 and with further details relating to arachidonic or eicosopentaenic acid.

The view (previously only a conjecture) was expressed – supported by numerous experiments – that the hypoxic state can lead to the deposition of hydrogen ions in the fatty acids of immature brain tissue and thereby induce their elongation. That would explain, at least in part, the high resistance of newborns to hypoxia and anoxia (Mourek 1989), due to many factors some of which were identified (e.g. lower energy requirements, tolerance to pH changes in the internal environment, to temperature changes and changes in the energy content, maybe with the possibility of utilizing fatty acids or lactate as the substrate).

Lately, attention has been paid in this laboratory to peroxidative processes (and hence to oxygen radicals) and to protective mechanisms, especially in relation to perinatal risks (chiefly as regards the CNS).

An important aspect concerns the ontogenetic research of epilepsy. This was started in the Institute of Physiology of the Czechoslovak Academy of Sciences, where Servít's team studied the development of audiogenic seizures (Bureš 1953). This field was not studied systematically, however; developmental studies were concerned chiefly with phylogenesis and data on ontogenesis in the literature were used only for comparison (Servít 1962). The ontogenetic approach in research on the pathophysiology of epilepsy was not extensively applied until P. Mareš joined Servít's laboratory in 1967. After 1968 for reasons, which had nothing to do with science, Academician Servít's original plan – parallel study of the phylogenetic and ontogenetic laws of epileptogenesis – was abandoned, but the ontogenetic problems survived. From the very outset of ontogenetic research on epilepsy in the Institute of Physiology of the Academy, the problems were resolved in close collaboration with clinical institutions of child neurology in Brno (Zouhar) and Prague (Dolanský). The first studies, dealing with the development of the epileptic focus (Mareš 1973a,b), in which the projection of focal discharges in the immature brain was described systematically for the first time, were followed by studies on the model of metrazol-induced generalized seizures (Mareš and Schickerová 1980, Zouhar *et al.* 1980). By then the circle of those interested in combining epilepsy and ontogenesis

had spread to further institutions – the Department of Pathological Physiology of the Medical Faculty of Charles University in Plzeň and the Institute of Physiology of the First Medical Faculty of Charles University in Prague, in both of which the thalamocortical mechanisms of epileptic phenomena were studied (Rokyta *et al.* 1981, Mareš *et al.* 1982). In addition, epileptic after-discharges induced by electric stimulation of the cortex were investigated (Mareš *et al.* 1980). When, after 1981, developmental epileptology became the main theme in what had been Servít's laboratory, not only was collaboration with other workplaces continued, but the range of problems was also extended. More attention was paid to the development of motor seizures (Mareš *et al.* 1983) and to the correlation between the EEG and the motor manifestations of the seizures (Schickerová *et al.* 1984, Zouhar *et al.* 1989), and the study of the effects of antiepileptics in the immature brain was expanded (Mareš *et al.* 1981b, 1989, Kubová and Mareš 1989). In studies on the development of limbic epileptic after-discharges, dissociated development of the EEG manifestations and automatisms in behaviour (Marešová and Mareš 1988) and absence of postictal depression at a given stage of maturity – or rather immaturity – of the brain (Velíšek and Mareš 1990) were described. This developmental law – incomplete maturation of the mechanisms preventing rapid repetition of a seizure – was also demonstrated in the cerebral cortex and can be regarded as a general property of the immature brain (Makal *et al.* 1990). These last experiments were carried out at the Third Medical Faculty of Charles University, where workers of the Institutes of Physiology and Pathological Physiology joined in the study of the development of epilepsy. When enumerating the problems which have been and are being studied in our institutions, we must not forget research on the role of amino acids in epileptogenesis in the immature CNS. After GABA, interest has also centred on excitatory amino acids, and their antagonists, as possible antiepileptics. The high effectiveness of the latter in the immature CNS (Velíšek *et al.* 1989, 1990) is at present marked by undesirable side-effects.

Neuroontogenetic research in Czechoslovakia has unquestionably proved its viability. Now the younger generation of neurophysiologists is beginning to be involved, so that we can put our trust in the prospects of this field of neuroscience.

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