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## EDITORIAL

# Epileptic Phenomena in the Immature Brain

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## 1. Introduction

I have started my studies of epileptogenesis in the immature brain more than 20 years ago. During this time I and my collaborators studied models of all basic types of epileptic seizures – focal (simple, complex and secondarily generalized) as well as generalized (both grand mal and absence type). It is possible from results of these studies to outline some common features of the epileptic phenomena in developing animals which may reflect general rules of maturation of the nervous system. Developmental neurophysiology is studied in different animal species; the most frequently used are rats, rabbits and cats. In spite of the fact that all three

species belong to those born very immature, the level of maturation at birth and the rate of postnatal development is different (for review see Himwich 1970). Our systematic studies on epileptogenesis in the immature brain were performed in rats; they reach the level of brain maturation comparable with full-term human newborn approximately at the age of 10 days (Mareš *et al.* 1979), sexual maturation starts at the age of 35 days. That means that the developmental rate in this species is extraordinarily high. Since the very beginning of our studies standard age groups were used: 7, 12, 18, 25 days old and adult animals (always signed as 90 days old). If necessary, additional age groups were studied: 3, 5, 9, 15, 21, 30 and 45 days old. Using the basic groups the main stages of development are covered so that analogies to the development of other species including man could be made.

## 2. Long duration of the epileptic phenomena in the immature brain

### *2.1. Discharges of epileptic foci*

Bishop (1950) studied as the first the maturation of neocortical epileptogenic foci. Discharges of strychnine foci induced in rabbits lasted substantially longer in immature than in adult animals. All papers describing the development of epileptic foci in experimental animals came to the same conclusion (Volanschi 1960, Goldensohn *et al.* 1963, Wright and Bradley 1968, Caveness 1969, Mareš 1973a,b, Staudacherová *et al.* 1978, Mareš *et al.* 1987). The focal discharges in the rat sensorimotor cortex could be classified as spikes starting from the age of 12 days (Mareš 1973a). Long duration of discharges in the immature cortex is due to a low level of synchronization of individual neurons in an epileptic aggregate based on poor interconnections among these cells (Prince and Gutnick 1972).

### *2.2. Electroencephalographic recordings of seizures*

Electroencephalographic seizures in mature animals are formed predominantly by spikes, although spike-and-wave and polyspike-and-wave complexes are present too. On the contrary, slow waves in the delta range represent the main graphoelement of seizures in the immature cortex. This was demonstrated in both acute experiments in immobilized animals (Cadilhac *et al.* 1960, Mareš *et al.* 1980, Zouhar *et al.* 1980) and semichronic experiments in animals with implanted electrodes (Schickerová *et al.* 1984, Mareš *et al.* 1987, Zouhar *et al.* 1989). Our data cited above demonstrated also that EEG recordings of seizures were practically the same when various convulsant drugs were used. EEG spikes could be described for the first time in 12-day-old rats in this situation as well. The physiological background is not known, the poor synchronization of activity of individual neurons described in immature epileptic foci is highly probable, but the longer duration of postsynaptic potentials cannot be excluded.

### *2.3. Motor seizures*

Generalized tonic-clonic motor seizures (major seizures, model of human grand mal) induced by various convulsant drugs or by electroshock are formed by three phases: wild running, tonic phase and clonic phase (Swinyard 1973). The first

and the third phase are formed by rapid movements of all limbs, the only difference is the absence of righting reflexes during the clonic phase. The rat pups exhibit very slow movements resembling swimming during the first week of life, with increasing age the movements become faster (for review see Vernadakis and Woodbury 1969, Mareš and Schickerová 1980, Mareš and Zouhar 1988). The immaturity of the whole motor system must be taken into account, the exact determination of the responsible structure or at least level of the motor system is not yet possible.

## 3. Imperfect synchronization of the epileptic activity in the immature brain

### *3.1. Projection of focal discharges*

Cortical epileptic focus elicited in adult animals exhibits a regular projection to all structures which are neuronally connected to the site of the primary focus (for review see Prince 1978). Projected discharges are absent in 3- and 5-day-old rat pups, where the epileptic activity remains restricted to the site of application of penicillin (Mareš 1973a). Projection to the opposite hemisphere transmitted through callosal fibers appears towards the end of the first postnatal week but it becomes regular only in 12-day-old rats in spite of the fact, that interhemispheric (transcallosal) evoked responses could be reliably elicited since the age of 5 days (Mareš *et al.* 1975) and the callosal fibers could be demonstrated since the age of 4 days (Seggie and Berry 1972). An immature epileptic aggregate is probably incapable to activate sufficiently its own efferent pathways due to the time dispersal of activation of neurons in the primary focus. Propagation of focal discharges through associational pathways to other areas of the "primary" hemisphere was recorded in 7-day-old animals too, but there are no data on morphological development of these connections at disposal.

### *3.2. Electroencephalographic recordings of seizures*

There are two different phenomena: synchronization, i.e. the time coincidence of individual epileptic graphoelements (spikes, sharp waves) recorded, and generalization, i.e. the simultaneous incidence of epileptic activity in the whole brain. The synchronization of individual graphoelements matures again towards the end of the second postnatal week, it is practically perfect at the age of 12 days (Zouhar *et al.* 1980). It is due to intracortical as well as interhemispheric (callosal) connections and its development is in full agreement with the development of projected focal discharges. Non-generalized epileptic activity might be recorded at such developmental stages where the perfect synchronization is present, even in 18-day-old rats (Schickerová *et al.* 1984, Mareš and Velíšek 1986). Ictal activity which is always generalized in adult rats under control conditions may start as localized under the influence of clonazepam (Velíšek and Mareš 1987). Generalization of EEG seizure activity may be due to a mechanism different from that responsible for synchronization, probably a subcortical one. The inability of clonazepam to disturb synchronization of discharges of two symmetrical epileptic foci (Mareš *et al.* 1987) speaks in favor of such different mechanisms.

### *3.3. Electroclinical correlations*

Correlations between EEG and motor phenomena depend mostly on the type of epileptic seizures in patients as well as in experimental animals. Generalized tonic-clonic seizures exhibit relatively high but not absolute correlation in adult animals, but it diminishes with decreasing age. Two types of discordance may be seen: constant EEG activity with changing motor pattern of seizures and different EEG activities during the stable motor pattern – mostly during the clonic phase (Schickerová *et al.* 1984, Mareš *et al.* 1987, Zouhar *et al.* 1989). The dissociation between EEG and motor epileptic phenomena is especially marked in N-methyl-D-aspartate-induced seizures (Mareš and Velíšek – submitted for publication).

### *3.4. Motor seizures*

Running as well as clonic phase of major seizures is formed by well coordinated movements of all limbs in adult animals. In youngs the slow swimming movements are rather erroneous and their locomotor effects are nearly nil (Mareš and Schickerová 1980, Zouhar *et al.* 1989).

## 4. Age-specific epileptic phenomena

### *4.1. Phenomena present since a certain stage of maturation*

#### *4.1.1. Epileptic foci induced by acetylcholine*

Local application of acetylcholine to cerebral cortex elicits an epileptogenic focus in adult animals (Purpura *et al.* 1972). There is a lack of this effect in rat sensorimotor cortex up to 12 days of age and in visual cortex up to the 15th postnatal day (Staudacherová *et al.* 1978, Marešová *et al.* 1981). Both these cortical areas could be excited by penicillin as well as bicuculline to form an epileptogenic focus much earlier (Mareš 1973a, Mareš *et al.* 1987, Velíšková *et al.* – submitted for publication), i.e. the cerebral cortex is able to generate an epileptic focus, but the specific mechanism, by which it has to be set in action by acetylcholine, matures later. Therefore we examined the sensitivity of neurons of the sensorimotor cortex to microiontophoretically applied acetylcholine and we found that between the 9th and 12th postnatal day there is a steep increase in a percentage of cortical neurons responding excitatorily to acetylcholine (Strejčková *et al.* 1987). Thus the development of cholinoceptivity of nerve cells forms a background of this age-specific phenomenon.

#### *4.1.2. Spike-and-wave rhythm*

Spike-and-wave rhythm represents a model of human absences (Purpura *et al.* 1972) – an age-specific type of epileptic seizures. It might be induced by low doses of metrazol in adult rats and it posses the characteristics of absence seizures (Mareš and Velíšek 1986, Schickerová *et al.* 1989). Spike-and-wave rhythm is also characteristic for experimental epileptic seizures resembling human myoclonic seizures – minimal clonic seizures (Schickerová *et al.* 1984, Zouhar *et al.* 1989) and cortical afterdischarges (Kubová *et al.* 1990). This rhythm may be induced in an

imperfect form since the age of 12 days without any relation to the seizure type modelled, in a complete form since the third postnatal week (Zouhar *et al.* 1980, Schickerová *et al.* 1984). Similar activity could be induced by other convulsant drugs (e.g. bicuculline – Zouhar *et al.* 1989, convulsant benzodiazepine RO 5-3663 – Mareš *et al.* 1987a). There is a time coincidence with the development of rhythmic thalamocortical phenomena (sleep spindles – Jouvet-Mounier 1968, rhythmic afterdischarges, incremental responses – Mareš *et al.* 1982) but the primary role of cortical mechanisms in generation of the spike-and-wave rhythm is highly probable. Thalamus might participate in triggering the spike-and-wave rhythm as a response of an abnormally excited cortex (Gloor 1984).

*4.1.3. Motor phenomena*

The tonic phase of major seizures is composed from tonic flexion and extension of the forelimbs and hindlimbs. The tonic extension of the hindlimbs is taken as the most severe phenomenon (Swinyard 1973), but it cannot be observed before the third week of postnatal life in rats (for review Mareš and Schickerová 1980). Vernadakis (1962) demonstrated that tonic extension of the hindlimbs could be induced by electrical stimulation of the spinal cord even in very postnatal rats. Spinal and peripheral mechanisms are thus able to generate tonic extension of hindlimbs, but the central mechanisms (descending part of the reticular formation of the brainstem ?) are incapable to trigger them. Minimal metrazol seizures are of predominantly clonic nature involving muscles of the head and forelimbs. They could not be induced under control conditions up to the age of 18 days (Mareš and Schickerová 1980). On the contrary the same pattern of motor seizures might be induced by other convulsant drugs starting from the first postnatal week (Mareš 1988). The same explanation as above is suggested: the generator of minimal seizures hypothetically localized in the basal forebrain (Browning and Nelson 1985) is mature in the first week, but metrazol cannot trigger it till the third postnatal week. Wet dog shakes represent a phenomenon similar to human automatisms accompanying complex partial (temporal) seizures. It can be elicited by drugs (e.g. kainic acid) or by electrical stimulation of limbic structures (hippocampus, amygdala, entorhinal cortex) in adult animals (for review Mareš and Zouhar 1988). During the ontogenetic development it could not be induced by hippocampal stimulation before the postnatal day 7 (Marešová and Mareš 1988) and by kainic acid before the end of the second postnatal week (Velíšková *et al.* 1988). There is a combination of maturation of the generator towards the end of the first week and of the triggering mechanism of kainic acid (5 days later).

*4.2. Phenomena appearing only at a certain stage of development*
*4.2.1. Lack of postictal depression*

Epileptic seizures are stopped by an active mechanism (Jasper *et al.* 1969). The activity of this mechanism probably overlasts for some time and therefore an epileptic seizure is followed by a period of full refractoriness. Using hippocampal afterdischarges (AD) as a model we demonstrated that an interval of at least 3 min has to be left in adult animals before the second stimulation is able to induce an AD which is substantially shorter than the first one (Velíšek and Mareš 1991). The

12-day-old animals exhibit the second AD even in the case when only a 30-s interval after the end of the first AD is left. The youngest age group studied (7-day-old rats) exhibits a refractoriness after the seizures probably as a sign of high fatigability of the central nervous system. Similar lack of postictal depression in 12- and 15-day-old rat pups was demonstrated in cortical afterdischarges (Makal *et al.* unpublished data) demonstrating thus a period of increased excitability as a common feature of the immature brain. An active mechanism which blocks the possibility to induce a new seizure in the mature brain is absent at the end of the second postnatal week and matures only later. The nature of this mechanism is still unknown, it might be connected with the mechanism allowing amygdala kindling with short interstimulation on intervals in 15-day-old rats (Moshe and Albala 1983).

*4.2.2. Motor phenomena – emprosthotonic seizures*

Emprosthotonic seizures as the first stage of generalized seizures induced by systemic administration of the N-methyl-D-aspartate in rat pups could be observed in 7, 12 and 18 days old rats but not later (Mareš and Velíšek 1990). A regression of a mechanism present at early stages of development (decreasing activation of flexors) or an overweighting of an older mechanism by a later developing one (increasing activation and/or strength of extensors) might explain this situation.

## 5. Conclusions

The actual level of maturation is decisive for the expression of epileptic phenomena in developing animals. Development of many phenomena may be explained on the basis of morphological studies. It is clear especially in the case of cortical epileptogenesis – development of synapses and whole cortical circuitry was repeatedly described (e.g. Aghajanian and Bloom 1964, Caley and Maxwell 1968, Scheibel and Scheibel 1969, Seggie and Berry 1972, Wise *et al.* 1979). Many times is such explanation impossible because of lack of knowledge where certain epileptic phenomena are generated. This is the case of all motor phenomena described – they have to have a generator which matures on its own and ways how this generator might be set into action. Wet dog shakes (WDS) represent a motor automatism which is generated somewhere in the motor system. This generator matures towards the end of the first postnatal week when hippocampal afterdischarges are able to activate it. Systemic administration of kainic acid with a target structure again in the hippocampus (Ben-Ari 1985) as well as systemic administration of sodium valproate with a presumed serotonergic mechanism of elicitation of WDS (Mareš *et al.* 1981) cannot induce wet dog shakes before the postnatal day 12. This example as well as minimal clonic seizures elicited by different convulsant drugs (for review see Mareš and Zouhar 1988) demonstrate the possibility of activation of generators of epileptic activities by different mechanisms which must not mature at the same time. The most interesting data represent the development of age-specific epileptic phenomena. In addition to a simple and logic development of acetylcholine-induced cortical foci and EEG spike-and-wave rhythm which appear since certain level of maturation is reached there are other phenomena which appear and with further development again disappear. Emprosthotonic seizures elicited by NMDA and a lack of postictal depression as examples of these phenomena represent the most

important phenomena to be analyzed. In addition they remain us that maturation of the nervous system is not a smooth progression from a simple system to a highly complicated one but that there are some detours instead of a straight way and that even regression forms a part of normal development.

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