Charles University in Prague
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Consequences of status epilepticus elicited in immature rats

Ph.D. Thesis

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GENERAL INTRODUCTION

I. Epilepsy

Epilepsy is a chronic neurological condition characterized by recurrent unprovoked epileptic seizures and is associated with a variety of medical conditions and neurological diseases. Epileptic seizures are paroxysmal clinical events; they occur as a consequence of sudden imbalances between the excitatory and inhibitory inputs to a network of neurons, such that there is overall excitability.

Epileptic seizures are classified into two main categories – partial (focal) and generalized. Either category contains different types of seizures according to localization (partial seizures with simple and/or complex symptomatology) or mechanisms (generalized convulsive seizures or absences).

Epilepsy has no geographical, racial or social boundaries. It occurs in men and women (at the same rate generally but with differences in individual epileptic syndromes) and can begin at any age, but is most frequently diagnosed in infancy, childhood, adolescence and old age (See figures 1). In fact, up to 5% of the world's population may have a single seizure at some time in their lives, but a diagnosis of epilepsy is reserved for those who have recurring seizures, at least two unprovoked ones.

The prevalence of a disorder is the proportion of a population with that disorder at a given point in time. From many studies around the world, it has been estimated that the mean prevalence of active epilepsy (i.e. continuing seizures or the need for treatment) is approximately 8.2 per 1,000 of the general population. However, this may be an underestimate as some studies in developing countries (such as Colombia, Ecuador, India, Liberia, Nigeria, Panama, United Republic of Tanzania and Venezuela) suggest a prevalence of more than 10 per 1,000.

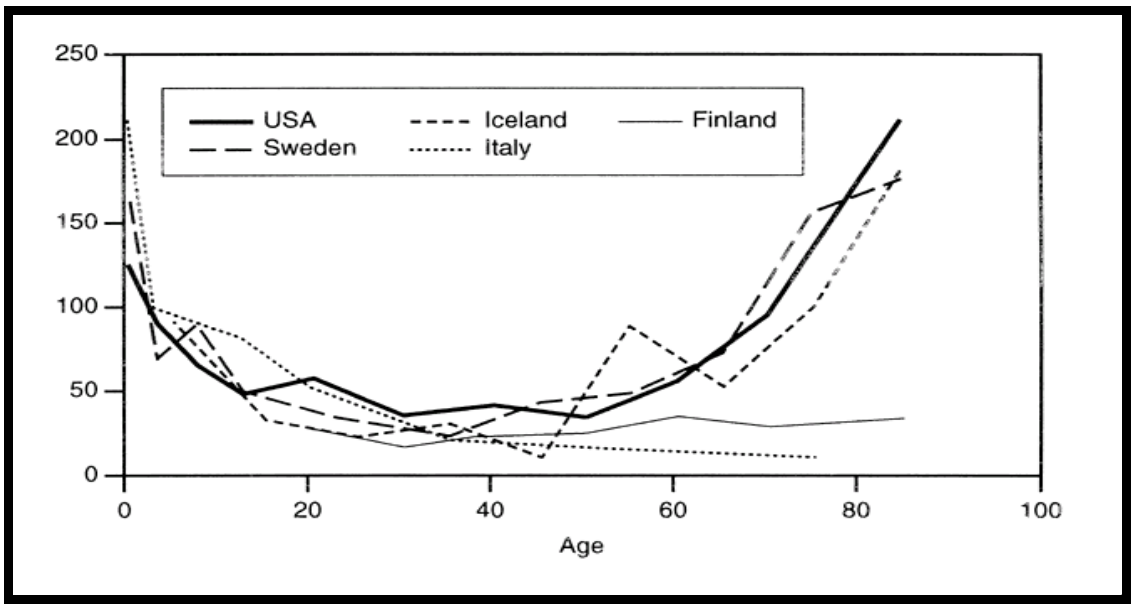

Fig.1. Incidence (per 100.000) of first epileptic seizure in addition to age (*Hauser W.A. and Hessdorfer D.C. 1990*).

The incidence of a disorder is the number of new cases at a given time. Studies in developed countries suggest an annual incidence of epilepsy of approximately 50 per 100,000 of the general population. However, studies in developing countries suggest that this figure is nearly double that at 100 per 100,000.

One of the main reasons for the higher incidence of epilepsy in developing countries is the higher risk of experiencing a condition that can lead to permanent brain damage. These conditions include neurocysticercosis, meningitis, malaria, pre and perinatal complications and malnutrition.

The above-mentioned data from WHO (World Health Organization) and from studies in the USA and European counties showed that epilepsy is frequently starting in infancy. The response of the developing brain to epilepsy is age-specific. Studies from *Hauser & Hessdorfer* reported that the first epileptic seizures mostly present during the first year of life. This fact was also confirmed in animal models. Childhood epilepsy is a problematic issue not only in developing countries but also in developed

countries. Studies reported that children with epilepsy have a significant risk for problems with attention, learning and memory.

Epileptic seizures are arrested by activation of intrinsic inhibitory systems. There is no general agreement which systems participate in this arrest; experimental data support opioid peptides *(Hammers et al 2007; Rocha et al 1991*) as well as adenosinergic system (*Dragunow 1986*), but a role of other transmitter systems is possible. Role of other factors cannot be excluded. If these mechanisms fail epileptic seizures may last tens of minutes, and they transgress into *status epilepticus* (*SE*).

Status epilepticus

The term *status* was used "whenever a seizure persists for sufficient length of time (subsequently defined as at least 30 to 60 minutes) or is repeated frequently enough to produce a fixed or enduring epileptic condition." This definition is enshrined within the World Health Organization dictionary of epilepsy (*Gastaut 1973*), as well as the Handbook of clinical neurology (*Roger et al 1974*) and Handbook of electroencephalography and clinical neurophysiology (*Gastaut et al 1975*). Today, a widely accepted operational definition of *SE* is that of a "condition in which epileptic activity persists for 30 minutes or more, causing a wide spectrum of clinical symptoms, and with a highly variable pathophysiological, anatomical and an etiological basis." It is important to note that this definition implies that status is not simply a rapid repetition of seizures (in fact the word "seizure" is no longer retained), and as such an iterative version of ordinary epilepsy, but is a condition (or group of conditions) in its own right with distinctive pathophysiological features.

The justification for this duration (at least 30 minutes) is that this is the time at which *SE* may become self-sustaining, pharmacoresistance may have developed, and seizure-induced neuronal injury may take place

(*Chen and Wasterlain 2006*). On its last proposal of terminology, the International League Against Epilepsy (ILAE) defined *SE* as "a seizure that shows no clinical signs of arresting after a duration encompassing the great majority of seizures of that type in most patients or recurrent seizures without interictal resumption of baseline central nervous system function," but did not set a temporal criterion as had previously been the case (*Blume et al 2001; Commission on Epidemiology and Prognosis 1993; Engel, Jr. 2001*). This definition, which may be judged as somewhat ambiguous, is more in accordance with recently proposed "operational" definitions that aim to define the time when patients should be treated as if they were in established *SE*; this includes seizure durations as low as 5 min (*Lowenstein et al 1999*) (Fig. 2).

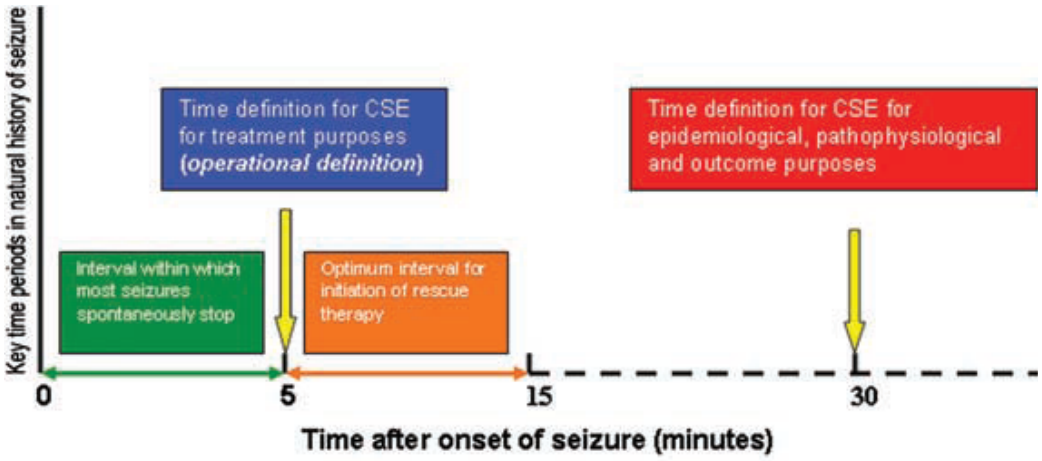

Fig.2. Duration of seizure activity against key time periods in the natural history of a prolonged seizure (from (*Raspall-Chaure et al 2007*)).

There is probably a need for different definitions, having different specifications for duration of seizures or recovery of consciousness, which are tailored to a particular research question. Operational definitions serve as much better guides for treatment and should be those used in clinical trials. In contrast, there are no clear reasons to modify the "traditional" 30-min-duration definition to evaluate the incidence and outcome of

convulsive *SE* until a better understanding of the pathophysiological and prognostic determinants of convulsive *SE* has been reached.

Today it is estimated that there are between 65,000 and 150,000 cases of *SE* in the United States each year (*Treiman 1996*), and that approximately 25% are nonconvulsive (*Cascino 1993; Jagoda 1994; Kline et al 1998*). At least 10% of epileptic patients suffer a *SE* during the course of their disease, and 50% of *SE* appears in patients with no known history of epilepsy (*Salas-Puig et al 1996*). *SE* is more frequent in symptomatic epilepsies, particularly those arising from trauma, tumor, or infection involving the frontal lobe. Both acute and remote cerebral insults can cause *SE*, as can severe systemic disease that causes *SE* secondary to a toxic-metabolic encephalopathy. *Status epilepticus* is present in nearly all epileptic syndromes, even idiopathic ones, although it is more frequent in cryptogenic and symptomatic forms. Whereas tonic-clonic *SE* is the best-known type and its diagnosis is simple, partial *SE*, more than psychomotor *SE*, presents a diagnostic challenge. Particularly difficult is the differential diagnosis of psychomotor (complex partial) *SE* and absence *SE*, above all the form termed "late-onset *de novo* absence *SE*," which presents as confusional syndrome in the elderly (*Salas-Puig et al 1996*).

Nonconvulsive confusional status epilepticus

This is classically separated into two forms on the basis of (1) ictal EEG (i.e., absence status) and (2) psychomotor (complex partial) *SE*. The diagnosis is difficult on the basis of clinical semiology alone. Absence status (or 'petit mal' status) can complicate many epileptic syndromes and is the most frequently encountered form of nonconvulsive *SE*. It is characterized by confusion of varying intensity and associated in 50% of cases with bilateral myoclonia (*Thomas 2000*). The EEG shows ictal generalized paroxysmal activity; normalization is obtained after

benzodiazepine injection. In absence status, there is nosographic heterogeneity. Four groups can be distinguished (a) 'typical' absence status in patients with generalized idiopathic epilepsies; (b) 'atypical' in patients with symptomatic or cryptogenic generalized epilepsies; (c) 'de novo' absence status of late onset characterized by toxic or metabolic precipitating factors in middle-aged subjects with no previous history of epilepsy; and (d) absence status with focal characteristics in subjects with a pre-existing or newly diagnosed partial epilepsy, mostly of extratemporal origin. The majority of cases are in fact 'transitional' forms between these four groups.

Psychomotor (complex partial) *SE* is characterized by continuous or rapidly recurring psychomotor (complex partial) seizures that may involve temporal or extratemporal regions. Cyclic disturbance of consciousness is characteristic of psychomotor *SE* of temporal lobe origin. The diagnosis of complex partial *SE* of frontal lobe origin remains a challenge *(Licht and Fujikawa 2002*). In one third of cases, a frontal lesion is revealed (*Thomas 2000*).

Temporal lobe or psychomotor or complex partial or limbic status epilepticus

The former conventional classification of *SE* was designed to parallel the seizure type classification scheme (*Commission on Classification and Terminology of the International League Against Epilepsy 1981; Commission on Classification and Terminology of the International League Against Epilepsy 1989*). It has been questioned with regard to its appropriateness to adequately describe the plurality of the clinical forms of status. The first International Classification of Seizure Type (*Gastaut 1969 ;Gastaut 1970*) and its revision (*Commission on Classification and Terminology of the International League Against Epilepsy 1981*) divided partial seizures, and consequently, partial *SE* into "simple" and "complex"

according to whether or not consciousness is retained or lost. Therefore, the older term psychomotor status or temporal lobe status was replaced by complex partial *SE* and simple partial *SE*. The following classification of epilepsies and epileptic syndromes (*Commission on Classification and Terminology of the International League Against Epilepsy 1989*) included a few syndromes that might conform to the widened definition of status, e.g., *epilepsia partialis continua*, electrical *SE* during slow-wave sleep (*Patry et al 1971*), now called continuous spike-wave discharges during sleep (*Morikawa et al 1989*), or the Landau-Kleffner syndrome; otherwise, it is lacking a synoptic view. In 1994, *Shorvon* proposed a new scheme in his monograph, grouped by age, and tried to encompass the various nonconvulsive and myoclonic forms that fit uneasily into the "seizure type approach" (*Shorvon 1994*).

Other peculiar conditions are electroencephalographic *SE* with subtle clinical signs (*Wieser et al 1985*) and "epileptic" behavioral disturbances up to "epileptic" psychosis. In such cases, the EEG recorded in the epileptogenic zone might show 'high-frequency tonic spike discharges,' 'regular clonic' or 'clonic-tonic' discharge patterns. In the vicinity of such an epileptic discharging focus, the EEG might exhibit signs of attenuation ('critical aplattisement') and the surface EEG a regional or even generalized attenuation with disappearance of interictal spikes described as 'forced normalization'. It is obvious that such stages or conditions bear similarities to spike suppression prior to seizures (*Wieser 1989*), with dimensional loss in nonlinear correlation dimension analysis applied to predict seizures (*Le Van et al 1998; Le Van et al 1999; Le Van et al 2001; Weber et al 1998*) and associated with changes in neurotransmitters and neuromodulators (*Wieser 1999*).

It is difficult to deny the intriguing possibility that some abnormal mental states (in epilepsy) are due to prolonged seizure activity. Although there is

undisputed evidence that prolonged epileptiform EEG discharges (characteristic of status) in hippocampal and amygdaloid regions can be associated with behavioral abnormalities and can occur with or without clear-cut scalp EEG changes, it is quite unknown to what extent the generality of "interictal behavioral peculiarities" might be associated with such "subclinical EEG status activity" in deep structures.

Since limbic *SE* implies seizure discharges in the limbic system, it is often not detectable without intracranial recording from the core structures of the limbic system, such as hippocampal formation and amygdala. This might be one reason that limbic *SE* is rarely reported in literature in comparison to generalized convulsive *SE* and absence *SE*.

Moreover, psychomotor status often evolves from or alternates with aura continua, what was called simple partial *SE,* (*Wieser 1980; Wieser 1997*), so that many overlaps between aura continua and psychomotor status exist in literature and cases are finally categorized according to their full blown semiology (i.e., as psychomotor status, although for a certain period of time they would fulfill the criteria of simple partial *SE*).

Consequences of *SE* are best documented with convulsive *SE*, but might also be associated with certain types of nonconvulsive *SE*, particularly psychomotor (complex partial) *SE* (*Krumholz 1999*).

There is increasing experimental as well as clinical evidence that generalized convulsive *SE* produces lasting neuropathological damage in the hippocampus, neocortex, and cerebellum due to associated metabolic failure. Cerebellar (Purkinje and basket cell) damage was related particularly to hyperpyrexia and hypotension, and was prevented by control of the systemic metabolic derangements, i.e. hyperpyrexia, hypotension, hypoxia, acidosis, and hypoglycemia (*DeGiorgio et al 1992; Meldrum et al 1973*). Convulsive *SE* is the most common form of *SE*, but

its relative frequency is difficult to document because the various types of status (*Raspall-Chaure et al 2007*).

Temporal Lobe Epilepsy

Genetic factors in the causation of epilepsy have been recognized since the time of Hippocrates. However, until the second half of the 20th century, generalized epilepsies were thought to be genetic in origin, whereas focal or partial epilepsies were largely attributed to environmental factors, such as birth injuries, infections, postnatal head trauma, and brain lesions such as tumors and vascular insults. In a series of publications (*Andermann 1991; Andermann and Andermann 1992; Andermann and Metrakos 1969; Andermann et al 1980*) based on patients operated for partial epilepsy at the Montreal Neurological Hospital, Eva Andermann was able to demonstrate that genetic factors were important in patients with partial epilepsy, particularly temporal lobe epilepsy, and that both generalized and partial epilepsies fit a model of multifactorial inheritance (now termed complex inheritance), with interaction of one or more genes and environmental factors.

Temporal lobe epilepsy (TLE) is the main cause of refractory epilepsies and is associated to atrophy and sclerosis of the hippocampus. The physiopathology of this disease is unknown but early brain injury, with ensuing neuronal death and loss, triggered by mechanisms of excitotoxicity has been put forward as an explanation. TLE presents a clinical picture, which is heterogeneous in childhood and homogeneous in adulthood that is characterized by the presence of simple partial seizures and complex partial seizures. These seizures can become generalized.

Diagnosis is based on the results from the electroencephalogram and from the cranial magnetic resonance, which is currently considered the standard

diagnostic method. The pharmacological treatment of TLE only achieves complete control over the seizures in less than 20% of patients. Surgical methods, such as anterior temporal lobectomy and amygdalo-hippocampectomy, reach control rates in 67-85% of patients, with low morbidity and mortality rates. Although prognosis depends on a number of factors, surgical treatment improves the quality of life of these patients (*Volcy 2004*).

TLE is probably more difficult to recognize in children than in adults. In fact, ictal symptoms in children are less stereotyped and less obvious, and the neuropathological substrate is more heterogeneous than in adults (*Fontana et al 2006*).

II. Epileptogenesis

Different factors e.g. massive electric stimulations, chemical drugs, changes of ions balance and many others can provoke epileptic seizures. All epileptic seizures arise from neuronal hyperexcitability and hypersynchrony that occurs when a balance between the excitatory and inhibitory inputs to a network of neurons had been changed. Three main parts can be differentiated in epileptic activities:

Ictogenesis - process of generation, spread and generalization of epileptic activity (seizure) though if seizure is spontaneous or reactive;

Epileptogenesis – phase of initiation of epilepsy as a chronic disorder that is characterized by recurrent (spontaneous) unprovoked seizures;

Progressive epileptogenesis – a condition when an aggravation of epilepsy and impairment of brain function (especially memory) appears with repeated seizures.

Epileptogenesis included various changes at systemic, cellular, molecular and genomic level that play role in becoming epilepsy. Many studies

described these changes in human patients and animal models of epilepsy. During TLE, these pathological changes appear in structures of temporal lobe in humans or limbic structures in rats (most commonly used species in animal models of epilepsy). Epileptogenesis is not only a time-dependent process but also it has the age-specific character (*Vining 1990; Wasterlain et al 2002; White 2002*).

Lithium-Pilocarpine status epilepticus as model of Temporal Lobe Epilepsy

There are innumerable animal models of epilepsy or epileptic seizures (*Loscher 1997*), but only few models of epilepsy are currently used for pharmacological studies of epilepsy or epileptic seizures (Table 1). Models of epilepsy can be divided into models of acquired (symptomatic) epilepsy and models of genetic (idiopathic) epilepsy. The first category includes models, in which epilepsy or epilepsy-like conditions are induced by electrical or chemical methods in previously healthy (non-epileptic) animals, mostly rats. Models with electrical induction of epilepsy or epilepsy-like conditions include the kindling model, which can progress up to the appearance of spontaneous seizures, and models in which recurrent spontaneous seizures develop after a self-sustained *status epilepticus* (*SSSE*), which is elicited by sustained electrical stimulation of the hippocampus (via stimulation of the perforant path, the angular bundle, or the CA3 of the ventral hippocampus), the lateral or basolateral nucleus of the amygdala, or other limbic brain regions (*Goodman 1998*).
Using experimental models, in which *SE* is induced in healthy immature rat brain, is a direct approach to address the question of whether *SE* without underlying pathology (e.g., genetically programmed malformation) can lead to a reorganization of neuronal circuits, epileptogenesis, and cognitive decline.

Table 1. Overview of chronic models of epilepsy (*Loscher 2002*).

Models of acquired (symptomatic) epilepsy	
With electrical induction of epilepsy	Kindling
With chemically induction of epilepsy	Post-status models in which epilepsy develops after a chemically induced status epilepticus (e.g. pilocarpine and kainate models of epilepsy)
Models of genetic (idiopathic) epilepsy	
Spontaneous mutations in diverse animal species	Mutant animals with reflex epilepsy Mutant animals with spontaneous recurrent seizures
Induces mutations in mice	Transgenic or knock-out mice

Only the currently most popular models in epilepsy research are shown.

Data available from such studies are controversial. There are several reports, however, of neuronal damage, mossy fiber sprouting, cognitive impairment, and development of epilepsy in rats exposed to *SE* at around P20, which corresponds to early childhood in humans (*Cha et al 2002; Cilio et al 2003; Dube et al 2000; Fernandes et al 1999; Kostakos et al 1993; Liu et al 1994; Raol et al 2003; Rutten et al 2002; Sankar et al 1998; Sankar et al 2000; Stafstrom et al 1992; Stafstrom et al 1993*). Marked acute morphological damage was demonstrated in thalamus (*Druga et al 2005*) and in basal ganglia (*Druga et al 2007*) of rat pups with *SE* induced at P12. Only recently, Wu and collaborators (*Wu et al 2001*) found spatial memory deficits in rats surviving lithium-pilocarpine induced *SE* at 14th postnatal day. Importantly, several elegant studies

demonstrated hyperexcitability, even without spontaneous seizures, after exposure of immature brain, even before P12, to clinically relevant epileptogenic conditions, including hyperthermic seizures (*Chen et al 1999; Dube et al 2000*), perinatal hypoxia (*Jensen et al 1992; Jensen et al 1998*), or recurrent brief seizures (*Holmes et al 1999; Huang et al 1999*). These data suggest that immature brain is also susceptible to activity - dependent plasticity leading to the molecular and network reorganization underlying hyperexcitability.

To receive relevant information about effects of long-term epileptic activity on developing brain, it is important correctly choose not only an adequate age group of animals, but also the experimental procedure of elicitation of epileptic activity. Pharmacological as well as electrical ways can be used in adult animals; however, for developmental studies the possibilities are rather restricted. Long-term stimulation in young animals is unusual, mainly because of technical reasons, but repetitive long-term hippocampal stimulation in 15-16-day-old rats (*Thompson et al 1988*) and perforant path stimulation in 21-day-old and older animals (*Sankar et al 2000*) were used.

The chemical way is more usual for eliciting of *SE*. Two models of *SE* were described in detail in immature rats: systemic administration of kainic acid (*Ben-Ari et al 1984*) and pilocarpine (*Priel et al 1996*) or combination lithium-pilocarpine (*Hirsch et al 1992*). Sensitivity of different age groups to elicitation of convulsive *SE* and its EEG correlates was described in these studies; however experimental animals were monitored only a few hours and detailed comparison of individual age-groups in those models is missing.

The response of the developing brain to *SE* is highly age-specific. Previously, published data demonstrated that *SE* can cause acute neurodegeneration followed by morphological changes already in 12th

postnatal day (*Druga et al 2005; Nairismagi et al 2006*) or 14th postnatal day (Sankar et al 1998). In the same age groups, *SE* leads to permanent functional impairment as cognitive deficits or development of epilepsy (*Kubova et al 2004; Sankar et al 2000; Wu et al 2001*). Extension of *SE*-induced neuronal damage increases with age. In animals younger than 2 weeks, neuronal loss is relatively mild and unable to explain completely functional sequelae seen in these age groups. This suggests that factors other than neuronal cell death might also have a significant role in the development of functional impairment or epilepsy later in the life.

Today the pilocarpine or lithium-pilocarpine models represent the modulation of pharmacoresistant *SE* and there is no drug that can completely block pathological activity and lead to fast recovery to normal EEG and behavioral manifestations if status persists for more than one hour.

III. Why to analyze neocortex in Temporal Lobe Epilepsy

Immature brain

Brain electrical activity recorded as electroencephalogram (EEG) matures during ontogeny and this is reflected in expression of epileptic seizures. Published data demonstrated that noncontinuous EEG could be registered in immature rats from 5th-6th postnatal day. It is formed by slow waves with low amplitude interrupted by sections of isoelectric line. *Ellingson* and Rose (*Ellingson and Rose 1970*) showed that periods of isoelectric line could be recorded up to 10th postnatal day; it corresponds to "trace alternant" described in premature infant but never found in full-term newborns (*Dreyfus-Brisac 1966*).

In spite of the difficulties with comparison of development of different species, this finding demonstrates that the newborn rat is less mature than

newborn human baby, but postnatal maturation of rats is much faster than that of human babies. Based on speed of brain maturation the full-term newborn child could be compared to a rat of 8-10 days old (*Dobbing and Sands 1979*), nevertheless rats EEGs still have premature profile (*Mares et al 1979; Tuge et al 1960*).

Excitability of the immature brain

In the last three decades clinical and experimental data proved that immature brain easily produce epileptic activity and the spectrum of epileptic seizures is more extensive than in mature brain including specific age-dependent forms of epileptic seizures and epilepsy (*Aicardi et al 1997*).

The results of experimental studies clearly confirmed that the specific "*ontogenetic window of higher sensitivity to produce epileptic seizures*" is persisting during infancy and that there are multiple reasons of this higher sensitivity. One of the possible factors can be an imbalance between inhibition and excitation, with predominance of the latter. This prevalence of excitation was found in nearly all types of seizures (*Schwartzkroin 1993*) except the absences (*Gloor et al 1990*). In rats, the sensitivity of central nervous system to excitatory aminoacids is highest at the end of first postnatal week and can be demonstrated by systemic administration of different agonists of excitatory aminoacids. Doses necessary to induce seizures are very low at early developmental stages and increase with maturation (*Albala et al 1984; Mares and Velisek 1992; Schoepp et al 1990*). A role of inhibitory systems can be tested in a similar way. Administration of antagonists of inhibitory receptors (e.g. of GABA-A receptors) induces seizures most easily in the third postnatal week in rats (*Mares et al 1982*).

The existence of "*ontogeny window of higher sensitivity to produce epileptic seizures*" was shown not only for chemical models but also for electrical stimulation of different brain structures. Kindling induced by electrical stimulation of amygdala can be elicited in 15-day-old rats with higher stimulation intensities and with high number of stimulations than in adult rats (*Moshe et al 1981*). However threshold for 21-39 day-old (prepubertal and pubertal rats) was lower than in adult and young animals (*Gilbert and Cain 1981; Moshe et al 1981*). In contrast, a study from our laboratory (*Mares et al 2002*) demonstrate that the threshold for elicitation of cortical epileptic afterdischarges in sensorimotor cortex is lowest around the 18th postnatal day.

Results from ontogenetic studies thus demonstrated that age-dependent changes of sensitivity to epileptogenic agents could exhibit different developmental profiles.

Ictogenesis – generation of epileptic seizures – includes not only the initiation of epileptic activity, its spread and generalization, but also the termination of epileptic activity. It is clear that easy initiation of epileptic seizures in developing brain is due to many factors. However, it is highly improbable that epileptic activity spread and generalized easily during infancy because the connections between individual brain structures are immature. *Moshe et al*. (*1981*) showed that behavioral manifestation of single afterdischarges during kindling was the same in prepubertal and pubertal rats (25-35 days old) and adult animals, whereas they found marked differences in progress of kindling phases in immature animals. The youngest rats exhibit poor transition of activity from limbic into motor structures and EEG-motor correlation is very poor.

Arrest of epileptic activity represents an important phenomenon. Generally, seizures in the immature brain are longer than in adult brain (e.g. *Mares et al. 2002*). Development of postictal depression in

connection with level of brain maturation was for the first time discussed by *Moshe and Albala* (*Moshe and Albala 1983*). They described that 16-day-old rats were unable to stop afterdischarges elicited by short time repetitive electrical stimulation of amygdala. Similar results were received in our laboratory for a model of repetitive hippocampal stimulation in 12-day-old rats, whereas 25-day-old animals had this mechanism fully developed (*Velisek and Mares 1991*); the same situation was observed during stimulation of sensorimotor cortex (*Mares et al 1992; Mares et al 2002*).

Ontogenetic changes in brain excitability

There is an imbalance between excitation and inhibition in the brain at early developmental stages. It is characterized by the relatively slow maturation of inhibitory neurotransmitter systems on the one hand and the rapid, exuberant maturation of excitatory systems on the other. Gammaaminobutyric acid (GABA) is the main inhibitory neurotransmitter in the adult brain. Potentiation of GABA receptor activity represents the main mechanism of action of commonly prescribed antiepileptic drugs (AEDs), such as phenobarbital, the benzodiazepines, and to a lesser extent valproate, topiramate, and levitiracetam. GABAergic system consists of three main receptor subtypes: $GABA_A$, $GABA_B$, and $GABA_C$. Ionotropic $GABA_A$ receptors are primarily located postsynaptically and mediate most of the fast synaptic inhibition in the brain due to its permeability for chloride anions; under certain circumstances, they may also gate bicarbonate.

$GABA_B$ receptors are G-protein-linked metabotropic receptors located both presynaptically and postsynaptically. Their activation results in slower and longer (hundreds of milliseconds) inhibitory currents.

Ionotropic $GABA_C$ receptors are localized mainly in retina.

The function of the GABAergic system differs markedly in the mature and immature brain. Whereas $GABA_A$ receptor activation results in neuronal hyperpolarization and an inhibition of cell firing in the mature brain, receptor activation results in membrane depolarization and excitation at very early development (*Ben-Ari et al 1990; Cherubini et al 1990; Mohler 2007*). The switch from GABA-mediated excitation to inhibition can be related to changes in the chloride gradient that form a part of normal development (*Clayton et al 1998; Ganguly et al 2001; Lu et al 1999; Payne et al 2003; Plotkin et al 1997; Rivera et al 1999*) (see Fig. 3).

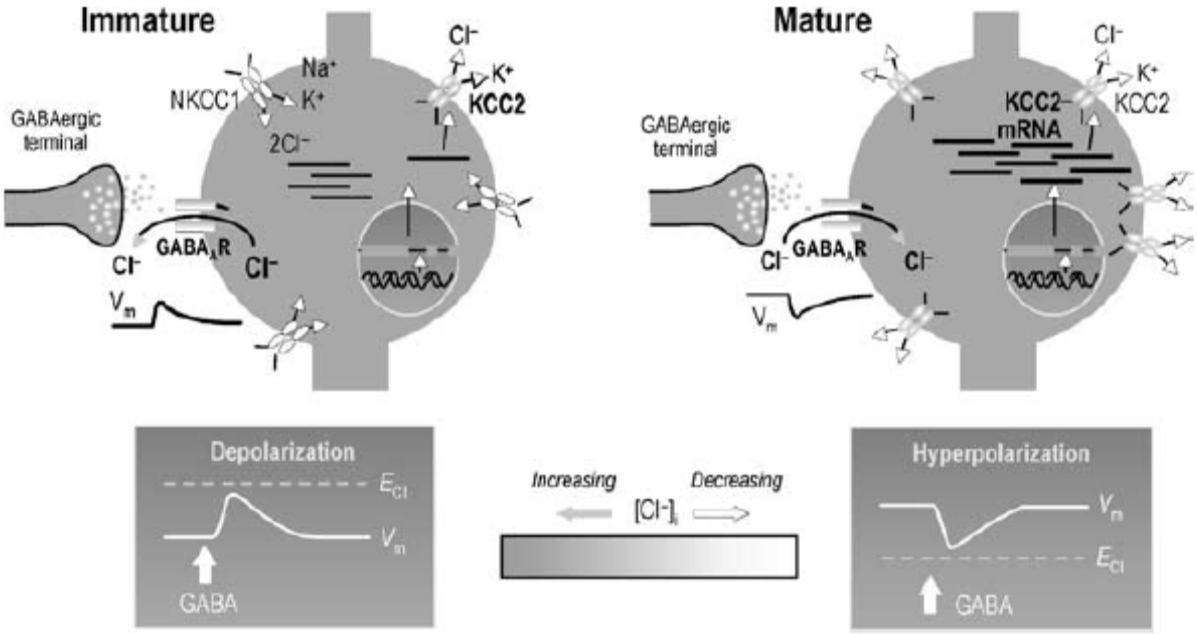

Fig.3. This schematic illustrates how the developmental shift in the chloride gradient (Cl^-) affects GABAergic transmission in the immature and mature brain. $GABA_AR$ - $GABA_A$ receptor; KCC2 - transports Cl^- out of the cell; NKCC1 - transports Cl^- into the cell; mRNA - messenger RNA; V_m - membrane voltage; E_{Cl} - chloride equilibrium potential [from *Rivera et al 1999; Plotkin et al 1997; Clayton et al 1998; Lu et al 1999; Ganguly et al 2000*].

Depolarizing excitatory GABA currents are critical for the development of various calcium-dependent processes, e.g. neuronal proliferation, migration, targeting, and synaptogenesis (*Barbin et al 1993; Ben-Ari et al*

1997; Leinekugel et al 1999; LoTurco et al 1995; Owens et al 1996). In addition, GABA currents were suggested to play a critical role in the generation of ictal activity in the developing brain. Synchronous neuronal activity in the hippocampus can be driven by $GABA_A$ receptor activation and inhibited by $GABA_A$ receptor blockade (*Ben-Ari et al 1989*). More recent evidence, however, suggests that GABA-mediated excitation may drive ictal activity in the developing hippocampus as well (*Dzhala and Staley 2003; Khalilov et al 2003*). *Dzhala and Staley* (*2003*) found that the ictal-like epileptiform activity induced by high extracellular potassium levels in cultured hippocampal slices from juvenile rats was exacerbated by the $GABA_A$ receptor agonist, muscimol, and inhibited by the antagonist, bicuculline. This is the opposite of what is typically observed in the hippocampus of adult rats.

Neurologists are concerned that a secondary focus may develop after repetitive seizures. *Khalilov* and collaborators demonstrated formation of a mirror focus in immature rat hippocampi and identified GABA-mediated excitation as one of the mechanisms underlying its induction (*Khalilov et al 2003*).

A growing body of evidence indicates that early-life seizures can alter the functions of both inhibitory and excitatory neurotransmitter systems and intrinsic neuronal properties, what possibly contributes to cognitive deficits (*Mohler 2007*) or development of epilepsy (*Marsh et al 2006*). Alteration of the major inhibitory system, the GABAergic system, can affect both learning abilities and brain excitability. In lithium-pilocarpine-induced model of *SE*, changes in $GABA_A$ receptor expression were observed in both adult and immature (10-day-old) rats. Importantly, the pattern of $GABA_A$ changes was highly related to the age at *SE*. In the dentate gyrus of the hippocampus, rats that experienced *SE* in adulthood have reduced level of α1 subunit expression whereas selective increase in

the α1 subunit occurred in 10-day-old rats both immediately and later after seizures (*Brooks-Kayal et al 1998; Raol et al 2006; Zhang et al 2004*). The increase of the α1 subunit was accompanied by enhanced type 1-benzodiazepine (BDZ) augmentation in hippocampus when the animals reach adulthood (*Brooks-Kayal et al 1998*). Similar age-dependent changes of BDZ receptor binding was also found in rats exposed to kainate-induced *SE* in 15- and 35-day-old rats (*Rocha et al 2000*).

Anatomical evidence indicates that GABAergic and opioidergic systems are closely linked and that the expression of the μ-opioid receptors by GABAergic neurons is common in several brain areas of rat (*Kalyuzhny et al 2000; Kalyuzhny and Wessendorf 1998*). Close relationship between these two systems was confirmed by electrophysiological studies (*Duggan and North 1983*). These data suggest that in a large proportion of cases, the effects of μ-opioid receptors are mediated by GABAergic neurons.
Opioid receptors were found to be functional at early stages of ontogeny, and to modulate specific developmental functions. Systemic administration of opioids alters the seizure threshold of convulsants such as the volatile agent flurothyl (*Cowan et al 1979*), and under certain conditions opioids themselves are epileptogenic (*Snead, III 1986*). Electroencephalographic and behavioral manifestations of convulsive activity occur with low doses of opioids and are inhibited by opioid antagonists, implying a receptor-mediated mechanism. Membrane binding data indicate that the affinities of *μ*-, *κ*- and *δ*-sites for radiolabeled drugs are similar in neonatal and adult rats. *μ*- and *κ*-receptors are present in significant densities during early neonatal periods (*μ*-receptors are present at levels at least as high as those found in the adult), while *δ*-receptors appear much later (*Milligan et al 1987*). Autoradiographic data indicate that *μ*- and *κ*-receptors appear very early in the development of several brain regions, including the neostriatum, olfactory tubercle and rostral midbrain, and later in other regions such as the thalamus and

hypothalamus. Whereas the densities of κ-binding sites remain relatively constant throughout development, there is a transient appearance and/or redistribution of μ-receptors in several brain areas. *Delta*-receptors are present in low densities in the basal forebrain at birth. The level of δ-receptor binding increases markedly during the third postnatal week in all brain areas examined (*Kornblum et al 1987*).

Previous studies demonstrate that *SE* induces changes in expression of μ-opioid receptors. Similarly to GABA receptors also changes of μ-opioid receptor system were related to the age on *SE* onset (*Perez-Cruz and Rocha 2002*).

At present, *SE*-induced changes in GABAergic and opioid receptor binding were never studied in the same animals. Also, there are no detailed information concerning changes in specific brain areas of the immature rat brain at short and long intervals after severe epileptic seizures or *SE*. Lithium-pilocarpine induced *SE* causes changes of both BDZ- and μ- receptor binding in selected brain structures (amygdala complex, hippocampus, basal ganglia and thalamic nuclei) of immature as well as adult rats (*Rocha et al 2007*). The patterns of changes were less expressed in immature rats and were highly related to the age at *SE*, to the time after *SE* and to the brain structure.

In addition to excitatory action of GABA, glutamate as the primary excitatory neurotransmitter in the brain plays a role. It acts on two major receptor subtypes: ionotropic receptors that are ligandgated, cation-selective channels (of NMDA, AMPA, and kainate types) and metabotropic receptors that are linked to G-proteins (*Johnston 1996; Raol et al 2001*).

Work from a number of laboratories on the ontogeny of receptor development in rat brain from birth to adulthood (*Insel et al 1990; Miller and Ferrendelli 1990; Sims and Robinson 1999; Swann et al 1992*)

showed that rapid growth of both NMDA and AMPA receptor function during the first two postnatal weeks, and peak functional levels, overshoot those seen in adulthood by approximately 50%. This sets the stage for the functional predominance of excitation in the neonatal brain. In addition, there are qualitative changes (subunit composition of individual receptor) that further enhance excitatory neurotransmission (*Johnston, 1996; Raol, Lynch et al 2001*).

Experimental data received from numerous laboratories describe the main changes of the glutamatergic system during early development:

• Expression of the most important glutamate transporter, GLT1, is very low; the postsynaptic response is potentiated due to longer presence of glutamate in the synaptic cleft (*Sims and Robinson 1999*).

• NMDA receptors have different subunit compositions than in the adult rodents and therefore different functional properties. They are more permeable to calcium and magnesium blockade is less efficient. NMDA receptors also depolarize more easily and the resultant excitatory postsynaptic currents last longer (*Flint et al 1997; Hestrin 1992*).

• More AMPA receptors are permeable to calcium due to a low expression of the GluR2 subunit. AMPA receptors also desensitize more slowly and stay open longer (*Pickard et al 2000*).

• Metabotropic receptors have increased turnover of inositol triphosphate (IP3P), which enhances their signaling (*Catania et al 1994*).

Taken together, these results support the hypothesis that the increased seizure susceptibility of the immature brain results, at least in part, from the slow maturation of GABAergic inhibition relative to glutamatergic excitation.

Cumulative evidence suggests that the developmental imbalance between excitation and inhibition may contribute to the increased susceptibility of

the immature brain to seizures. Similarly, early-life seizures can disrupt normal activity-dependent patterns of receptor development in both excitatory and inhibitory neurotransmitter systems in the brain. Although some of these changes may be protective, others may enhance limbic excitability and increase the probability that epilepsy will develop at a later time.

IV. Methods used to study excitability and consequences after SE

There are many different methods allowing researchers to study the consequences of experimentally induced *SE*, and the choice is determined by the model or aims to study specific structures or phenomena. Our laboratory is focused on development of cerebral cortex, therefore we studied possible changes of cortical excitability after *SE* elicited in early ontogeny. Four electrophysiological methods were used for evaluation of these changes:

Interhemispheric (transcallosal) evoked potentials are mediated by corpus callosum and – as late components are concerned - by subcortical structures. The development of cortical interhemispheric response was repeatedly described (*Hatotani and Timiras 1967; Mares et al 1975; Seggie and Berry 1972*). The first two components of these potentials (first positive and first negative wave) represent true transcallosal potentials, i.e. monosynaptic responses to an influx of action potentials from the opposite hemisphere (*Grafstain 1959*).

- The next two methods allow the study of dynamics of cortical responses:

Paired-pulse paradigm elicits the simplest potentiation phenomenon. Paired-pulse potentiation (and/or depression) of cortical interhemispheric responses develops during the third postnatal week in rats (*Mares et al 1993*) therefore possible developmental delay could also be recognized.

Low-frequency stimulation results in another simple potentiation phenomenon. Frequency potentiation develops a little later than paired-pulse potentiation (*Mares et al 1993*) and thus represents a phenomenon with different mechanism of generation.

Cortical epileptic afterdischarges elicited by low-frequency rhythmic electrical stimulation of the sensorimotor area of cerebral cortex represent model epileptic seizures of cortical origin. This model allows the determination of five different phenomena:

- 1. Movements elicited by individual stimuli, i.e. by direct activation of motor cortex.
- 2. Spike-and-wave afterdischarges, generated by thalamocortical system (*Avanzini et al 1992*).
- 3. Clonic movements accompanying spike-and-wave afterdischarges, i.e. spread of epileptic activity into the generator of this pattern of convulsions localized in the basal forebrain (*Browning and Nelson 1986*).
- 4. Transition into another type of afterdischarges identical with those elicited by stimulation of limbic structures (hippocampus - *Dyer et al 1979*), i.e. spread of epileptic activity to the limbic system.
- 5. Recurrent afterdischarge occurred in result of epileptic activity circulation in the limbic system and spreading this activity into other brain region across neuronal pathways.

AIMS OF WORK

The questions to be answered were if status epilepticus elicit changes in cortical excitability after *SE* elicited in immature rats. What is the role of developmental stage when status is elicited and what is the time course of possible consequences? The changes could be expected because of an important role of neocortex in early childhood epilepsies (*Dulac 1994*). To study these problems four different electrophysiological methods were used at different intervals after SE:

1. Transcallosal, i.e. simple monosynaptic, responses of sensorimotor cortex were evoked by single pulses and input-output curve (intensity of stimulus vs. amplitude of responses) was constructed to have basic information about excitability of sensorimotor area.

2. Paired-pulse stimulation paradigm was applied to know potentiation and/or depression of the second response. Aftereffects of the first response will inform about the state of excitatory transmission.

3. Frequency potentiation and/or depression was used to study dynamics of cortical activity in a more complicated situation.

4. Epileptic afterdischarges induced by stimulation of cortical sensorimotor area were studied to have a direct measure of generation of epileptic seizures, their spread and duration. May the changes of this model epileptic seizure form a background for possible increased seizure susceptibility?

GENERAL DISCUSSION

Summarizing our data, we have to discriminate early and late consequences of *SE*.

Early period after SE (up to 6 days)

Degenerating neurons with characteristic of interneurons were demonstrated in both SE12 and SE25 rats. FluoroJade B-positive neurons were not numerous in SE12 rats, whereas substantial numbers of positive neurons were found in SE25 animals.

Evoked potentials exhibited at first transient changes tended towards lower amplitudes of single responses and a potentiation of responses to the 5-stimuli train three days after *SE* in the SE12 group, and an increased amplitude of individual waves as a consequences of frequency stimulation in SE25 rats. Paired-pulse potentiation of interhemispheric responses was abolished specifically at intervals from 100 to 160 ms, but not at shorter and longer intervals. This effect was permanent in the P12 group, but only transient (three days after *SE*) in SE25 animals.

Cortical epileptic afterdischarges exhibited abolition of the second (limbic) type of afterdischarges and recurrent afterdischarges in both age groups. Duration of ADs was shortened 3 days after *SE* in P12 rats at 3 and 6 days after *SE* in P25 group. P12 group then exhibited a transient increase in duration of ADs 6 days after *SE*.

Long-term effects (13 and 26 days after SE)

Single responses exhibited opposite effects in the two age groups: 26 days after *SE* younger group exhibited lower amplitudes than control rats whereas SE25 animals generated responses with higher amplitude than controls, their input/output curve was steeper. Paired-pulse potentiation failed to appear at interstimulation intervals from 100 to 160 ms in SE12 group only. This age group also exhibited a tendency to lower responses to frequency stimulation whereas SE25 rats generated higher responses than controls 13 days after *SE*.

None of the parameters of epileptic afterdischarges was changed in either age group.

Results of individual parts of the study are discussed in individual papers; here I will discuss the relations between individual results and the principal question about increased excitability of cerebral cortex as a consequence of *SE* elicited in developing rats. Methodological considerations have to be mentioned at first.

Methodological considerations

1. Choice of age groups: The two ages at which *SE* is elicited were selected to represent important developmental stages – level of brain (or at least cortical) maturation in 12-day-old rats corresponds to very early postnatal development of human brain, 25-day-old ones to schoolchildren. *Status epilepticus* has different effects on immature brains at these two stages: there is a general agreement in the literature that *SE* elicited after postnatal day 21 is deleterious to the brain. On the contrary, data on rats with *SE* in the first two postnatal weeks are contradictory. Some papers conclude that there are no marked consequences of *SE* (*Sperber 1996; Sperber et al 1999*), others including results from our laboratory demonstrate serious effects of *SE* even at this age (*Babb et al 1996; Druga et al 2005; Kubova et al 2001; Kubova et al 2004; Sankar et al 1998*). These effects are not only age but also model specific (*Sankar et al 2000*). To avoid differences due to different timing in individual studies our ontogenetic experiments always analyze several intervals after *SE*. Because of substantial changes among the groups used in behavioral and morphological experiments (usually one day, one week and one month - (*Druga et al 2005; Kubova et al 2000; Kubova et al 2001; Kubova et al 2004*) present study was extended to more intervals (3, 6, 9, 13 and 26 days).

2. Evoked potentials stimulation protocols were arranged according to the age of animals. Longer interval between single as well as paired pulses had to be used in youngest rats because of higher fatigability of responses (*Myslivecek 1970*); to avoid effects of rhythmic repetition stimuli were always irregularly generated with intervals of 10±2 s in 15-day-old rats and of 5±2 s in older animals. Intervals between series in frequency stimulation were also longer in the youngest group studied. Double threshold intensity used in paired-pulse and low frequency stimulations was chosen as equivalent of biological entity of stimulation.

3. The 8 Hz frequency stimulation was used for elicitation of cortical epileptic afterdischarges. Kindling studies use high frequencies for elicitation of afterdischarges (*Sato et al 1990*) but previous study from our laboratory (*Mares et al 2002*) demonstrated a marked difference between 50- and 8-Hz stimulation frequencies. High frequency did not allow the study of motor phenomena related to individual stimuli and sharp EEG elements and more easily elicited afterdischarges of the limbic type. Due to our interest in cortical changes the 8-Hz frequency was selected in addition it was close to stimulation parameters used in the first description of ontogeny of cortical afterdischarges (*Mares et al 1980*). The duration of stimulus trains was chosen to have similar number of stimuli (120 in the 8 Hz stimulation) to literary data because there is an inverse relation between the number of stimuli and the threshold intensity. This relation was demonstrated up to the 'ceiling' number which is approximately 100 pulses (*Lothman and Williamson 1992*).

Summary

Status epilepticus represents a severe insult for the mature as well as the developing brain; consequences of this insult are usually studied in limbic structures. We started to study possible consequences of lithium-pilocarpine *SE* elicited in two age groups of immature rats in the cerebral cortex. The reason for this study was the clinical experience that in childhood epilepsies the cerebral cortex plays an important role.

Status epilepticus was induced in rats 12 and/or 25 days old. Three, 6, 9, 13 and 26 days after status electrophysiological testing was performed. Interhemispheric (transcallosal) evoked responses were elicited by single stimuli and input/output (intensity of stimulus/amplitude of responses) curve were constructed, paired-pulse and frequency potentiation of these responses was studied; cortically induced epileptic afterdischarges were used to test seizure susceptibility of the cerebral cortex.

Changes at early intervals after *SE* can be interpreted mostly as a sign of decreased cortical excitability; this might be due to the poor state of animals after such a severe insult as *SE* lasting for hours. Paired-pulse potentiation was replaced at nearly all intervals after *SE* by depression; frequency potentiation was diminished in comparison with controls. Thresholds for elicitation of cortical epileptic afterdischarges, characterized by spike-and-wave rhythm in the EEG (and accompanied by clonic seizures of head and forelimb muscles), remained uninfluenced by *SE*. Transition into the other type of epileptic afterdischarges, spread of epileptic activity into limbic structures, was markedly delayed in both age groups. It is probably due to damage of the thalamic mediodorsal nucleus that represents the preferred relay for spread of epileptic activity from thalamocortical system into limbic structures. Changes at late intervals (13 and 26 days) are different: SE12 animals exhibited lower amplitude of single pulse responses whereas the in SE25 animals the input/output curve

was steeper than in controls. It was the only finding at late intervals after *SE*, which can be interpreted as a sign of increased excitability.

Special attention has to be focused on a finding of suppressed paired-pulse potentiation specific for interpulse intervals from 100 to 160 ms at all stages after *SE* in the SE12 group. This finding indicates a permanent change whose functional significance is not known and has to be analyzed in future.

We can conclude that there are no clear signs of neocortical hyperexcitability, in contrast to this phenomenon in limbic structures, after different types of seizure activity in immature rats as demonstrated in the literature. The neocortex is affected by severe seizure activity in a different way than hippocampal formation. A substantial difference between old and new cortical structures is not surprising because differences among various parts of hippocampal formation were demonstrated in the literature.

Our study clearly demonstrated that different intervals after *SE* must be studied because the consequences exhibit dynamic changes even qualitatively different. Furthermore, with data for different intervals after *SE* is it possible to discriminate between transient and permanent changes.

In addition to studies in adult animals, ontogenetic studies have to differentiate between developmental delay and qualitatively altered development. Majority of our findings at short intervals after *SE* can be interpreted as developmental delay; the best example is delayed appearance of transition of epileptic afterdischarges into the limbic system.

As far as the mechanisms of described changes are concerned, we can hypothesize that interplay of glutamatergic and GABAergic system in the developing brain responsible for studied phenomena is specifically altered at different intervals after *SE*. Further studies that are necessary represent future programs of work.

REFERENCE LIST

1. Aicardi,J., Dulac,O., Blume,TW., Dreifuss,FE., Wolf,P., Dam,M., Moshé,SL., Engel,J.Jr., Pedley,T. & Eds. (1997) Epilepsic syndromes. In: Epilepsy: A Comprehensive Textbook. Eds. Engel, J. Jr., Pedley, TA, Lippincott-Raven (Philadelphia), pp. 2243-2514. pp. 2243-2514.

2. Albala,B.J., Moshe,S.L. & Okada,R. (1984) Kainic-acid-induced seizures: a developmental study. *Brain Res.*, **315**, 139-148.

3. Andermann,E. (1991) Genetic studies of epilepsy in Montreal. *Epilepsy Res.Suppl*, **4**, 129-137.

4. Andermann,E. & Andermann,F. (1992) The children of mothers with epilepsy: recent trends in research, management and prospect. *Brain Dev.*, **14**, 423.

5. Andermann,E. & Metrakos,J.D. (1969) EEG studies of relatives of probands with focal epilepsy who have been treated surgically. *Epilepsia*, **10**, 415-420.

6. Andermann,F., Keene,D.L., Andermann,E. & Quesney,L.F. (1980) Startle disease or hyperekplexia: further delineation of the syndrome. *Brain*, **103**, 985-997.

7. Avanzini,G., deCurtis M., Marescaux,C., Panzica,F., Spreafico,R. & Vergnes,M. (1992) Role of the thalamic reticular nucleus in the generation of rhythmic thalamo-cortical activities subserving spike and waves. *J.Neural Transm.Suppl*, **35**, 85-95.

8. Babb,T.L., Mathern,G.W., Leite,J.P., Pretorius,J.K., Yeoman,K.M. & Kuhlman,P.A. (1996) Glutamate AMPA receptors in the fascia dentata of human and kainate rat hippocampal epilepsy. *Epilepsy Res.*, **26**, 193-205.

9. Barbin,G., Pollard,H., Gaiarsa,J.L. & Ben-Ari,Y. (1993) Involvement of GABAA receptors in the outgrowth of cultured hippocampal neurons. *Neurosci.Lett.*, **152**, 150-154.

10. Ben-Ari,Y., Cherubini,E., Corradetti,R. & Gaiarsa,J.L. (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J.Physiol*, **416**, 303-325.

11. Ben-Ari,Y., Khazipov,R., Leinekugel,X., Caillard,O. & Gaiarsa,J.L. (1997) GABAA, NMDA and AMPA receptors: a developmentally regulated 'menage a trois'. *Trends Neurosci.*, **20**, 523-529.

12. Ben-Ari,Y., Rovira,C., Gaiarsa,J.L., Corradetti,R., Robain,O. & Cherubini,E. (1990) GABAergic mechanisms in the CA3 hippocampal region during early postnatal life. *Prog.Brain Res.*, **83**, 313-321.

13. Ben-Ari,Y., Tremblay,E., Berger,M. & Nitecka,L. (1984) Kainic acid seizure syndrome and binding sites in developing rats. *Brain Res.*, **316**, 284-288.

14. Blume,W.T., Holloway,G.M. & Wiebe,S. (2001) Temporal epileptogenesis: localizing value of scalp and subdural interictal and ictal EEG data. *Epilepsia*, **42**, 508-514.

15. Brooks-Kayal,A.R., Shumate,M.D., Jin,H., Rikhter,T.Y. & Coulter,D.A. (1998) Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat.Med.*, **4**, 1166-1172.

16. Browning,R.A. & Nelson,D.K. (1986) Modification of electroshock and pentylenetetrazol seizure patterns in rats after precollicular transections. *Exp.Neurol.*, **93**, 546-556.

17. Cascino,G.D. (1993) Nonconvulsive status epilepticus in adults and children. *Epilepsia*, **34 Suppl 1**, S21-S28.

18. Catania,M.V., De,S.H., Penney,J.B. & Young,A.B. (1994) Metabotropic glutamate receptor heterogeneity in rat brain. *Mol.Pharmacol.*, **45**, 626-636.

19. Cha,B.H., Silveira,D.C., Liu,X., Hu,Y. & Holmes,G.L. (2002) Effect of topiramate following recurrent and prolonged seizures during early development. *Epilepsy Res.*, **51**, 217-232.

20. Chen,J.W. & Wasterlain,C.G. (2006) Status epilepticus: pathophysiology and management in adults. *Lancet Neurol.*, **5**, 246-256.

21. Chen,K., Baram,T.Z. & Soltesz,I. (1999) Febrile seizures in the developing brain result in persistent modification of neuronal excitability in limbic circuits. *Nat.Med.*, **5**, 888-894.

22. Cherubini,E., Rovira,C., Gaiarsa,J.L., Corradetti,R. & Ben,A.Y. (1990) GABA mediated excitation in immature rat CA3 hippocampal neurons. *Int.J.Dev.Neurosci.*, **8**, 481-490.

23. Cilio,M.R., Sogawa,Y., Cha,B.H., Liu,X., Huang,L.T. & Holmes,G.L. (2003) Long-term effects of status epilepticus in the immature brain are specific for age and model. *Epilepsia*, **44**, 518-528.

24. Clayton,G.H., Owens,G.C., Wolff,J.S. & Smith,R.L. (1998) Ontogeny of cation-Cl- cotransporter expression in rat neocortex. *Brain Res.Dev.Brain Res.*, **109**, 281-292.

25. Commission on Classification and Terminology of the International League Against Epilepsy (1981) Proposal for revised clinical and electroencephalographic classification of epileptic seizures. *Epilepsia*, **22**, 489-501.

26. Commission on Classification and Terminology of the International League Against Epilepsy (1989) Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia*, **30**, 389-399.

27. Commission on Epidemiology and Prognosis (1993) Guidelines for epidemiologic studies on epilepsy. , International League Against Epilepsy. *Epilepsia*, **34**, 592-598.

28. Cowan,A., Geller,E.B. & Adler,M.W. (1979) Classification of opioids on the basis of change in seizure threshold in rats. *Science*, **206**, 465-467.

29. DeGiorgio,C.M., Tomiyasu,U., Gott,P.S. & Treiman,D.M. (1992) Hippocampal pyramidal cell loss in human status epilepticus. *Epilepsia*, **33**, 23-27.

30. Dobbing,J. & Sands,J. (1979) Comparative aspects of the brain growth spurt. *Early Hum.Dev.*, **3**, 79-83.

31. Dragunow,M. (1986) Endogenous anticonvulsant substances. *Neurosci.Biobehav.Rev.*, **10**, 229-244.

32. Dreyfus-Brisac,C. (1966) The bioelectrical development of the central nervous system during early life. In: Human Development. Ed. Flakner, F., Saunders (Philadelphia). pp. 286-305.

33. Druga,R., Mares,P. & Kubova,H. (2007) Neuronal degeneration induced by status epilepticus in the nucleus accumbens of immature rats. In: Abstracts from the 2007 Annual Meeting of the American Epilepsy Society. *Epilepsia*, **48**, 257-258.

34. Druga,R., Mares,P., Otahal,J. & Kubova,H. (2005) Degenerative neuronal changes in the rat thalamus induced by status epilepticus at different developmental stages. *Epilepsy Res.*, **63**, 43-65.

35. Dube,C., Chen,K., Eghbal-Ahmadi,M., Brunson,K., Soltesz,I. & Baram,T.Z. (2000) Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. *Ann.Neurol.*, **47**, 336-344.

36. Duggan,A.W. & North,R.A. (1983) Electrophysiology of opioids. *Pharmacol.Rev.*, **35**, 219-281.

37. Dulac,O. (1994) Epilepsy in children. *Curr.Opin.Neurol.*, **7**, 102-106.

38. Dyer,R.S., Swartzwelder,H.S., Eccles,C.U. & Annau,Z. (1979) Hippocampal afterdischarges and their post-ictal sequelae in rats: a potential tool for assessment of CNS neurotoxicity. *Neurobehav.Toxicol.*, **1**, 5-19.

39. Dzhala,V.I. & Staley,K.J. (2003) Excitatory actions of endogenously released GABA contribute to initiation of ictal epileptiform activity in the developing hippocampus. *J.Neurosci.*, **23**, 1840-1846.

40. Ellingson,R.J. & Rose,G.H. (1970) Ontogenesis of the electrocorticogram. In: Developmental Neurobiology. Ed. Himwich, W.A., Charles C Thomas (Springfield). pp. 441-474.

41. Engel,J., Jr. (2001) Mesial temporal lobe epilepsy: what have we learned? *Neuroscientist.*, **7**, 340-352.

42. Fernandes,M.J., Dube,C., Boyet,S., Marescaux,C. & Nehlig,A. (1999) Correlation between hypermetabolism and neuronal damage during status epilepticus induced by lithium and pilocarpine in immature and adult rats. *J.Cereb.Blood Flow Metab*, **19**, 195-209.

43. Flint,A.C., Maisch,U.S., Weishaupt,J.H., Kriegstein,A.R. & Monyer,H. (1997) NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. *J.Neurosci.*, **17**, 2469-2476.

44. Fontana,E., Negrini,F., Francione,S., Mai,R., Osanni,E., Menna,E., Offredi,F., Darra,F. & Bernardina,B.D. (2006) Temporal lobe epilepsy in children: electroclinical study of 77 cases. *Epilepsia*, **47 Suppl 5**, 26-30.

45. Ganguly,K., Schinder,A.F., Wong,S.T. & Poo,M. (2001) GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell*, **105**, 521-532.

46. Gastaut,H. (1969) Classification of the epilepsies. Proposal for an international classification. *Epilepsia*, **10**, Suppl-21.

47. Gastaut,H. (1970) Clinical and electroencephalographical classification of epileptic seizures. *Epilepsia*, **11**, 102-113.

48. Gastaut,H. (1973) *Dictiony of epilepsy. Geneva: World Health Organization.*

49. Gastaut,H., Tassinari,CA. & eds (1975) *Handbook of Electroencephalography and Clinical Neurophysiology* Elsevier - Netherlands, Amsterdam - 1975 - (104 pages).

50. Gilbert,M.E. & Cain,D.P. (1981) A developmental study of kindling in the rat. *Brain Res.*, **254**, 321-328.

51. Gloor,P., Avoli,M. & Kostopulos,G. (1990) Thalamocortical relationships in generalized epilpesy with bilaterally synchronous spike-and-wave discharge. In: Generalized Epilepsy. Eds. Avoli, M., Gloor, P., Kostopulos, G., Naquet, R., Birkhauser (Boston), 190-212. pp. 190-212.

52. Goodman,J.H. (1998) Experimental models of status epilepticus.In: Peterson, S.L., Albertson, T.E. (Eds.). *Neuropharmacology Methods in Epilepsy Research.*, 95-125.

53. Grafstain,B. (1959) Organization of callosal connections in suprasylvian gyrus of cat. *J.Neurophysiol.*, **22**, 504.

54. Hammers,A., Asselin,M.C., Hinz,R., Kitchen,I., Brooks,D.J., Duncan,J.S. & Koepp,M.J. (2007) Upregulation of opioid receptor binding following spontaneous epileptic seizures. *Brain*, **130**, 1009-1016.

55. Hatotani,N. & Timiras,P.S. (1967) Influence of theroid function on the postnatal development of the transcallosal response in the rat. *Neuroendocrinology*, **2**, 147.

56. Hauser W.A. & Hessdorfer D.C. (1990) Epilepsy: Frequency, Causes and Consequences. *Landover, Maryland: Epilepsy Foundation of America.*.

57. Hestrin,S. (1992) Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. *Nature*, **357**, 686-689.

58. Hirsch,E., Baram,T.Z. & Snead,O.C., III (1992) Ontogenic study of lithium-pilocarpine-induced status epilepticus in rats. *Brain Res.*, **583**, 120-126.

59. Holmes,G.L., Sarkisian,M., Ben-Ari,Y. & Chevassus-Au-Louis,N. (1999) Mossy fiber sprouting after recurrent seizures during early development in rats. *J.Comp Neurol.*, **404**, 537-553.

60. Huang,L., Cilio,M.R., Silveira,D.C., McCabe,B.K., Sogawa,Y., Stafstrom,C.E. & Holmes,G.L. (1999) Long-term effects of neonatal seizures: a behavioral, electrophysiological, and histological study. *Brain Res.Dev.Brain Res.*, **118**, 99-107.

61. Insel,T.R., Miller,L.P. & Gelhard,R.E. (1990) The ontogeny of excitatory amino acid receptors in rat forebrain--I. N-methyl-D-aspartate and quisqualate receptors. *Neuroscience*, **35**, 31-43.

62. Jagoda,A. (1994) Nonconvulsive seizures. *Emerg.Med.Clin.North Am.*, **12**, 963-971.

63. Jensen,F.E., Holmes,G.L., Lombroso,C.T., Blume,H.K. & Firkusny,I.R. (1992) Age-dependent changes in long-term seizure susceptibility and behavior after hypoxia in rats. *Epilepsia*, **33**, 971-980.

64. Jensen,F.E., Wang,C., Stafstrom,C.E., Liu,Z., Geary,C. & Stevens,M.C. (1998) Acute and chronic increases in excitability in rat hippocampal slices after perinatal hypoxia In vivo. *J.Neurophysiol.*, **79**, 73-81.

65. Johnston,M.V. (1996) Developmental aspects of epileptogenesis. *Epilepsia*, **37 Suppl 1**, S2-S9.

66. Kalyuzhny,A.E., Dooyema,J. & Wessendorf,M.W. (2000) Opioid- and GABA(A)-receptors are co-expressed by neurons in rat brain. *Neuroreport*, **11**, 2625-2628.

67. Kalyuzhny,A.E. & Wessendorf,M.W. (1998) Relationship of mu- and delta-opioid receptors to GABAergic neurons in the central nervous system, including antinociceptive brainstem circuits. *J.Comp Neurol.*, **392**, 528-547.

68. Khalilov,I., Holmes,G.L. & Ben-Ari,Y. (2003) In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. *Nat.Neurosci.*, **6**, 1079-1085.

69. Kline,C.A., Esekogwu,V.I., Henderson,S.O. & Newton,K.I. (1998) Non-convulsive status epilepticus in a patient with hypocalcemia. *J.Emerg.Med.*, **16**, 715-718.

70. Kornblum,H.I., Hurlbut,D.E. & Leslie,F.M. (1987) Postnatal development of multiple opioid receptors in rat brain. *Brain Res.*, **465**, 21-41.

71. Kostakos,M., Persinger,M.A. & Peredery,O. (1993) Deficits in working but not reference memory in adult rats in which limbic seizures had been induced before weaning: implications for early brain injuries. *Neurosci.Lett.*, **158**, 209-212.

72. Krumholz,A. (1999) Epidemiology and evidence for morbidity of nonconvulsive status epilepticus. *J.Clin.Neurophysiol.*, **16**, 314-322.

73. Kubova,H., Druga,R., Lukasiuk,K., Suchomelova,L., Haugvicova,R., Jirmanova,I. & Pitkanen,A. (2001) Status epilepticus causes necrotic damage in the mediodorsal nucleus of the thalamus in immature rats. *J.Neurosci.*, **21**, 3593-3599.

74. Kubova,H., Haugvicova,R., Suchomelova,L. & Mares,P. (2000) Does status epilepticus influence the motor development of immature rats? *Epilepsia*, **41 Suppl 6**, S64-S69.

75. Kubova,H., Mares,P., Suchomelova,L., Brozek,G., Druga,R. & Pitkanen,A. (2004) Status epilepticus in immature rats leads to behavioural and cognitive impairment and epileptogenesis. *Eur.J.Neurosci.*, **19**, 3255-3265.

76. Le Van,Q.M., Adam,C., Baulac,M., Martinerie,J. & Varela,F.J. (1998) Nonlinear interdependencies of EEG signals in human intracranially recorded temporal lobe seizures. *Brain Res.*, **792**, 24-40.

77. Le Van,Q.M., Martinerie,J., Baulac,M. & Varela,F. (1999) Anticipating epileptic seizures in real time by a non-linear analysis of similarity between EEG recordings. *Neuroreport*, **10**, 2149-2155.

78. Le Van,Q.M., Martinerie,J., Navarro,V., Boon,P., D'Have,M., Adam,C., Renault,B., Varela,F. & Baulac,M. (2001) Anticipation of epileptic seizures from standard EEG recordings. *Lancet*, **357**, 183-188.

79. Leinekugel,X., Khalilov,I., McLean,H., Caillard,O., Gaiarsa,J.L., Ben-Ari,Y. & Khazipov,R. (1999) GABA is the principal fast-acting excitatory transmitter in the neonatal brain. *Adv.Neurol.*, **79**, 189-201.

80. Licht,E.A. & Fujikawa,D.G. (2002) Nonconvulsive status epilepticus with frontal features: quantitating severity of subclinical epileptiform discharges provides a marker for treatment efficacy, recurrence and outcome. *Epilepsy Res.*, **51**, 13-21.

81. Liu,Z., Gatt,A., Werner,S.J., Mikati,M.A. & Holmes,G.L. (1994) Long-term behavioral deficits following pilocarpine seizures in immature rats. *Epilepsy Res.*, **19**, 191-204.

82. Loscher,W. (1997) Animal models of intractable epilepsy. *Prog.Neurobiol.*, **53**, 239-258.

83. Loscher,W. (2002) Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Res.*, **50**, 105-123.

84. Lothman,E.W. & Williamson,J.M. (1992) Influence of electrical stimulus parameters on afterdischarge thresholds in the rat hippocampus. *Epilepsy Res.*, **13**, 205-213.

85. LoTurco,J.J., Owens,D.F., Heath,M.J., Davis,M.B. & Kriegstein,A.R. (1995) GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron*, **15**, 1287-1298.

86. Lowenstein,D.H., Bleck,T. & Macdonald,R.L. (1999) It's time to revise the definition of status epilepticus. *Epilepsia*, **40**, 120-122.

87. Lu,J., Karadsheh,M. & Delpire,E. (1999) Developmental regulation of the neuronal-specific isoform of K-Cl cotransporter KCC2 in postnatal rat brains. *J.Neurobiol.*, **39**, 558-568.

88. Mares,J., Mares,P. & Trojan,S. (1980) The ontogenesis of cortical self-sustained after-discharges in rats. *Epilepsia*, **21**, 111-121.

89. Mares,P., Haugvicova,R. & Kubova,H. (2002) Unequal development of thresholds for various phenomena induced by cortical stimulation in rats. *Epilepsy Res.*, **49**, 35-43.

90. Mares,P., Makal,V. & Velisek,L. (1992) Increased epileptogenesis in the immature brain. *Epilepsy Res.Suppl*, **9**, 127-129.

91. Mares,P., Mares,J. & Kozakova-Matlova,E. (1975) Development of the interhemispheric response in rats. *TIT.J.Life Sci.*, **5**, 5-10.

92. Mares,P., Rokyta,R. & Trojan,S. (1982) Epileptic seizures during ontogenesis in the rat. *J Physiol (Paris)*, **78**, 863-864.

93. Mares,P., Seidl,J. & Pohl,M. (1993) Paired-pulse and frequency potentiation of cortical responses in developing rats. *Brain Res.Bull.*, **32**, 107-111.

94. Mares,P. & Velisek,L. (1992) N-methyl-D-aspartate (NMDA)-induced seizures in developing rats. *Brain Res.Dev.Brain Res.*, **65**, 185-189.

95. Mares,P., Zouhar,A. & Brozek,G. (1979) Ontogenetic development of electrocorticogram in the rat. *Act.Nerv.Super.(Praha)*, **21**, 218-225.

96. Marsh,E.D., Brooks-Kayal,A.R. & Porter,B.E. (2006) Seizures and antiepileptic drugs: does exposure alter normal brain development? *Epilepsia*, **47**, 1999-2010.

97. Meldrum,B.S., Vigouroux,R.A. & Brierley,J.B. (1973) Systemic factors and epileptic brain damage. Prolonged seizures in paralyzed, artificially ventilated baboons. *Arch.Neurol.*, **29**, 82-87.

98. Miller,J.W. & Ferrendelli,J.A. (1990) Characterization of GABAergic seizure regulation in the midline thalamus. *Neuropharmacology*, **29**, 649-655.

99. Milligan,G., Streaty,R.A., Gierschik,P., Spiegel,A.M. & Klee,W.A. (1987) Development of opiate receptors and GTP-binding regulatory proteins in neonatal rat brain. *J.Biol.Chem.*, **262**, 8626-8630.

100. Mohler,H. (2007) Molecular regulation of cognitive functions and developmental plasticity: impact of GABAA receptors. *J.Neurochem.*, **102**, 1-12.

101. Morikawa, T., Seino, M., Watanabe, Y., and Yagi, K. (1989) Clinical relevance of continuous spike-waves during slow wavs sleep. In Eds.: Manelis, J., Bental, E., Loeber, JN, and Dreifuss, FE. pp. 359-363.

102. Moshe,S.L. & Albala,B.J. (1983) Maturational changes in postictal refractoriness and seizure susceptibility in developing rats. *Ann.Neurol.*, **13**, 552-557.

103. Moshe,S.L., Sharpless,N.S. & Kaplan,J. (1981) Kindling in developing rats: variability of afterdischarge thresholds with age. *Brain Res.*, **211**, 190-195.

104. Myslivecek,J. (1970) [Relations between visual and auditory responses at the cortical level of rats]. *J.Physiol (Paris)*, **62 Suppl 1**, 198-199.

105. Nairismagi,J., Pitkanen,A., Kettunen,M.I., Kauppinen,R.A. & Kubova,H. (2006) Status epilepticus in 12-day-old rats leads to temporal lobe neurodegeneration and volume reduction: a histologic and MRI study. *Epilepsia*, **47**, 479-488.

106. Owens,D.F., Boyce,L.H., Davis,M.B. & Kriegstein,A.R. (1996) Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. *J.Neurosci.*, **16**, 6414-6423.

107. Patry,G., Lyagoubi,S. & Tassinari,C.A. (1971) Subclinical "electrical status epilepticus" induced by sleep in children. A clinical and electroencephalographic study of six cases. *Arch.Neurol.*, **24**, 242-252.

108. Payne,J.A., Rivera,C., Voipio,J. & Kaila,K. (2003) Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci.*, **26**, 199-206.

109. Perez-Cruz,C. & Rocha,L. (2002) Kainic acid modifies mu-receptor binding in young, adult, and elderly rat brain. *Cell Mol.Neurobiol.*, **22**, 741-753.

110. Pickard,L., Noel,J., Henley,J.M., Collingridge,G.L. & Molnar,E. (2000) Developmental changes in synaptic AMPA and NMDA receptor distribution and AMPA receptor subunit composition in living hippocampal neurons. *J.Neurosci.*, **20**, 7922-7931.

111. Plotkin,M.D., Snyder,E.Y., Hebert,S.C. & Delpire,E. (1997) Expression of the Na-K-2Cl cotransporter is developmentally regulated in postnatal rat brains: a possible mechanism underlying GABA's excitatory role in immature brain. *J.Neurobiol.*, **33**, 781-795.

112. Priel,M.R., dos Santos,N.F. & Cavalheiro,E.A. (1996) Developmental aspects of the pilocarpine model of epilepsy. *Epilepsy Res.*, **26**, 115-121.

113. Raol,Y.H., Lynch,D.R. & Brooks-Kayal,A.R. (2001) Role of excitatory amino acids in developmental epilepsies. *Ment.Retard.Dev.Disabil.Res.Rev.*, **7**, 254-260.

114. Raol,Y.H., Zhang,G., Lund,I.V., Porter,B.E., Maronski,M.A. & Brooks-Kayal,A.R. (2006) Increased GABA(A)-receptor alpha1-subunit expression in hippocampal dentate gyrus after early-life status epilepticus. *Epilepsia*, **47**, 1665-1673.

115. Raol,Y.S., Budreck,E.C. & Brooks-Kayal,A.R. (2003) Epilepsy after early-life seizures can be independent of hippocampal injury. *Ann.Neurol.*, **53**, 503-511.

116. Raspall-Chaure,M., Chin,R.F., Neville,B.G., Bedford,H. & Scott,R.C. (2007) The epidemiology of convulsive status epilepticus in children: a critical review. *Epilepsia*, **48**, 1652-1663.

117. Rivera,C., Voipio,J., Payne,J.A., Ruusuvuori,E., Lahtinen,H., Lamsa,K., Pirvola,U., Saarma,M. & Kaila,K. (1999) The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*, **397**, 251-255.

118. Rocha,L., Engel,J., Jr. & Ackermann,R.F. (1991) Effects of chronic naloxone pretreatment on amygdaloid kindling in rats. *Epilepsy Res.*, **10**, 103-110.

119. Rocha,L., Gonzalez-Trujano,M.E., Jimenez,G., Gaona,A. & Ondarza,R. (2000) Characterization of benzodiazepine receptor binding in immature rat brain after kainic acid administration. *Epilepsia*, **41 Suppl 6**, S44-S47.

120. Rocha,L., Suchomelova,L., Mares,P. & Kubova,H. (2007) Effects of LiCl/pilocarpine-induced status epilepticus on rat brain mu and benzodiazepine receptor binding: regional and ontogenetic studies. *Brain Res.*, **1181**, 104-117.

121. Roger,J., Lob,H. & Tassinari,CA. (1974) Status epilepticus. In: Magnus, O., Lorentz de Haas, AM., editors. The epilepsies. Handbook of clinical neurology. Vol. 15 Amsterdam: North Holland Publishing Company.

122. Rutten,A., Van,A.M., Silveira,D.C., Cha,B.H., Liu,X., Hu,Y.N., Cilio,M.R. & Holmes,G.L. (2002) Memory impairment following status epilepticus in immature rats: time-course and environmental effects. *Eur.J.Neurosci.*, **16**, 501-513.

123. Salas-Puig,J., Suarez-Moro,R. & Mateos,V. (1996) [Status epilepticus]. *Neurologia*, **11 Suppl 4**, 108-121.

124. Sankar,R., Shin,D., Mazarati,A.M., Liu,H., Katsumori,H., Lezama,R. & Wasterlain,C.G. (2000) Epileptogenesis after status epilepticus reflects age- and model-dependent plasticity. *Ann.Neurol.*, **48**, 580-589.

125. Sankar,R., Shin,D.H., Liu,H., Mazarati,A., Pereira,d., V & Wasterlain,C.G. (1998) Patterns of status epilepticus-induced neuronal injury during development and long-term consequences. *J.Neurosci.*, **18**, 8382-8393.

126. Sato,M., Racine,R.J. & McIntyre,D.C. (1990) Kindling: basic mechanisms and clinical validity. *Electroencephalogr.Clin.Neurophysiol.*, **76**, 459-472.

127. Schoepp,D.D., Gamble,A.Y., Salhoff,C.R., Johnson,B.G. & Ornstein,P.L. (1990) Excitatory amino acid-induced convulsions in neonatal rats mediated by distinct receptor subtypes. *Eur.J.Pharmacol.*, **182**, 421-427.

128. Schwartzkroin,PA. (1993) Basic mechanisms of epileptogenesis. In: The Treatment of Epilepsy: Principles and Practice. Ed: Wallie, E. Lee & Febiger (Philadelphia), 83-98. pp. 83-98.

129. Seggie,J. & Berry,M. (1972) Ontogeny of interhemispheric evoked potentials in the rat: significance of myelination of the corpus callosum. *Exp.Neurol.*, **35**, 215-232.

130. Shorvon,S. (1994) Status epilepticus. Cambridge: University Press, pp. 382.

131. Sims,K.D. & Robinson,M.B. (1999) Expression patterns and regulation of glutamate transporters in the developing and adult nervous system. *Crit Rev.Neurobiol.*, **13**, 169-197.

132. Snead,O.C., III (1986) Opiate-induced seizures: a study of mu and delta specific mechanisms. *Exp.Neurol.*, **93**, 348-358.

133. Sperber,E.F. (1996) The relationship between seizures and damage in the maturing brain. *Epilepsy Res.Suppl*, **12**, 365-376.

134. Sperber,E.F., Veliskova,J., Germano,I.M., Friedman,L.K. & Moshe,S.L. (1999) Age-dependent vulnerability to seizures. *Adv.Neurol.*, **79**, 161-169.

135. Stafstrom,C.E., Chronopoulos,A., Thurber,S., Thompson,J.L. & Holmes,G.L. (1993) Age-dependent cognitive and behavioral deficits after kainic acid seizures. *Epilepsia*, **34**, 420-432.

136. Stafstrom,C.E., Thompson,J.L. & Holmes,G.L. (1992) Kainic acid seizures in the developing brain: status epilepticus and spontaneous recurrent seizures. *Brain Res.Dev.Brain Res.*, **65**, 227-236.

137. Swann,J.W., Smith,K.L., Gomez,C.M. & Brady,R.J. (1992) The ontogeny of hippocampal local circuits and focal epileptogenesis. *Epilepsy Res.Suppl*, **9**, 115-125.

138. Thomas,P. (2000) [Status epilepticus with confusional symptomatology]. *Neurophysiol.Clin.*, **30**, 147-154.

139. Thompson,J.L., Bryan,M., Bates,T. & Holmes,G.L. (1988) Failure of kindling to alter susceptibility to kainic acid. *Brain Res.*, **466**, 149-151.

140. Treiman,D.M. (1996) Status epilepticus. *Baillieres Clin.Neurol.*, **5**, 821-839.

141. Tuge,Z., Kanayma,Y. & Chang,H.Y. (1960) Comparative studies on the developmant of EEG. *Jap J Physiol*, **10**, 211-220.

142. Velisek,L. & Mares,P. (1991) The action of clonazepam against seizures induced by N-methyl-D-aspartate in rats during ontogenesis. *Homeost.Health Dis.*, **33**, 176.

143. Vining,E.P. (1990) Chaos, balance, and development: thoughts on selected childhood epilepsy syndromes. *Epilepsia*, **31 Suppl 3**, S30-S36.

144. Volcy,G.M. (2004) [Mesial temporal lobe epilepsy: its physiopathology, clinical characteristics, treatment and prognosis]. *Rev.Neurol.*, **38**, 663-667.

145. Wasterlain,C.G., Niquet,J., Thompson,K.W., Baldwin,R., Liu,H., Sankar,R., Mazarati,A.M., Naylor,D., Katsumori,H., Suchomelova,L. & Shirasaka,Y. (2002) Seizure-induced neuronal death in the immature brain. *Prog.Brain Res.*, **135**, 335-353.

146. Weber,B., Lehnertz,K., Elger,C.E. & Wieser,H.G. (1998) Neuronal complexity loss in interictal EEG recorded with foramen ovale electrodes predicts side of primary epileptogenic area in temporal lobe epilepsy: a replication study. *Epilepsia*, **39**, 922-927.

147. White,H.S. (2002) Animal models of epileptogenesis. *Neurology*, **59**, S7-S14.

148. Wieser,H.G. (1980) Temporal lobe or psychomotor status epilepticus. A case report. *Electroencephalogr.Clin.Neurophysiol.*, **48**, 558-572.

149. Wieser,H.G. (1989) Pre-ictal findings. *Epilepsia*, **30**, 664.

150. Wieser,H.G. (1997) *Simple partial status epilepticus. In: Engel J Jr, Pedley TA, editors. Epilepsy: a comprehensive textbook.* Philadelphia: Lippincott-Raven Publishers.

151. Wieser,H.G. (1999) EEG, the language of the brain, and its neurochemical souffleuse. *Schweiz.Arch.Neurol.Psych.*, **150**, 62-71.

152. Wieser,H.G., Hailemariam,S., Regard,M. & Landis,T. (1985) Unilateral limbic epileptic status activity: stereo EEG, behavioral, and cognitive data. *Epilepsia*, **26**, 19-29.

153. Wu,C.L., Huang,L.T., Liou,C.W., Wang,T.J., Tung,Y.R., Hsu,H.Y. & Lai,M.C. (2001) Lithium-pilocarpine-induced status epilepticus in immature rats result in long-term deficits in spatial learning and hippocampal cell loss. *Neurosci.Lett.*, **312**, 113-117.

154. Zhang,G., Raol,Y.H., Hsu,F.C., Coulter,D.A. & Brooks-Kayal,A.R. (2004) Effects of status epilepticus on hippocampal GABAA receptors are age-dependent. *Neuroscience*, **125**, 299-303.