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REVIEW

Models of Epileptic Seizures in Immature Rats

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Summary

Models of basic types of epileptic seizures are elaborated not only in adult but also in immature rodents. It is important because at least half of human epilepsies starts during infancy and childhood. This paper presents a review of chemically and electrically induced models of generalized convulsive and nonconvulsive (absence) seizures as well as models of partial simple (neocortical) and complex (limbic) seizures in immature rats. These models can also serve as a tool for study the development of central nervous system and motor abilities because the level of maturation is reflected in seizure semiology. Age-dependent models of epileptic seizures (absences and flexion seizures) are discussed. Models of seizures in immature animals should be used for testing of potential antiepileptic drugs.

Key words

Rat • Ontogeny • Epileptic seizures • Convulsant drugs • Electrical stimulation

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Experimental epileptology works mostly with the models of epileptic seizures induced by pharmacological or electrical epileptogenic agents. Both types of induction have some advantages and disadvantages: targets of pharmacological tools are defined in terms of transmitter or ion channel systems but their action is diffuse in the whole central nervous system. In addition, the specificity of action is always relative and depends on the dose. Electrical stimulation is usually focused on a single brain structure but all cellular elements in this structure are stimulated. These models are frequently used for testing of potential antiepileptic drugs.

Models of seizures should be adequate to human epileptic seizures. The basic classification of epileptic seizures differentiates between seizures with partial, focal origin and generalized seizures where the exact site of origin is impossible (or at least difficult) to find. There are reviews on models of epileptic seizures in adult experimental animals which are mostly focused on use of these models for testing of possible antiepileptic drugs (e.g. Loscher and Schmidt 1988, Engel 1992, White 1997, Kupferberg 2001, Sarkisian 2001, Rogawski 2006). There are two widely used models – maximal electroshock seizures adequate to human generalized convulsive (tonic-clonic) seizures and minimal metrazol seizures modeling myoclonic seizures (Loscher and Schmidt 1988). To have a model of complex partial seizures amygdala kindling is frequently used. Another model of this type of seizures can be elicited by 6 Hz electroshock (Barton *et al.* 2001).

Reviews of models of seizures in immature animals are scarce (Mareš *et al.* 1983, Mareš and Zouhar 1988, Mareš 1991) but there are some papers focused more on the pathophysiological mechanisms (Mareš *et al.* 1991, Moshé *et al.* 1991, Holmes and Ben-Ari 2001, Sanchez and Jensen 2001). Analogous to clinical epileptology some seizures are present at all stages of brain maturation like generalized tonic-clonic seizures elicited either electrically or chemically. Similarly, epileptic automatisms modeling complex partial seizures are present throughout the development. Like in pediatric patients, there are age-bound types of seizures. Some

experimental seizures can be elicited only if a certain level of maturation is reached (e.g. model of absence seizures present in rats three and more weeks old – Mareš *et al.* 1982, Schickerová *et al.* 1984, Mareš 1998). Models of childhood epileptic encephalopathies represent another developmental profile. Flexion seizures as the characteristic type of seizures in these early life epileptic syndromes can be induced by N-methyl-D-aspartate and agonists of its receptor up to the third week of postnatal life in rats but not later (Mareš and Velíšek 1992, Mareš *et al.* 1997).

The two obligatory models for a preclinical testing of perspective drugs were also studied in developing rodents.

Generator of generalized tonic-clonic seizures is localized in the brainstem (Browning and Nelson 1985) but the spinal cord isolated from the brain is also able to generate this type of seizures (Mareš 2006). They could be induced by maximal electroshock since the first postnatal day in rats (Vernadakis 1962). Not only transcutaneous or corneal stimulation is efficient, the same seizure pattern could be elicited by stimulation of the spinal cord isolated from brain by a complete transsection. The same pattern of generalized tonic-clonic seizures can be induced by high doses of many convulsant drugs – pentylenetetrazol (Mareš and Schickerová 1980, Pineau *et al.* 1999), bicuculline (Zouhar *et al.* 1989), Ro 5-3663 (Mareš *et al.* 1987), benzodiazepine receptor inverse agonist Ro 19-4603 (Kubová and Mareš 1994), strychnine (Kubová and Mareš 1995), aminophylline (Mareš *et al.* 1994), isonicotinehydrazide (Mareš and Trojan 1991). Exact pattern of generalized tonic-clonic seizures reflects the level of maturation of the motor system: clonic phase of these seizures is poorly coordinated in 7-day-old rats, movements resemble erroneous swimming movements. Coordination matures during the second and the third postnatal weeks so that P18 rats exhibit well-coordinated "running" movements. Generalized convulsant seizures elicited by N-methyl-D-aspartate and homocysteic acid exhibit a reversal of the two phases, clonic seizures precede the tonic phase.

Minimal metrazol seizures, i.e. nongeneralized clonic seizures, can be elicited since the third postnatal week in rats (Mareš and Schickerová 1980, Velíšek *et al.* 1992). Again, the same seizure pattern was elicited by other convulsant drugs – bicuculline (Zouhar *et al.* 1989), Ro 5-3663 (Mareš *et al.* 1987), isonicotinehydrazide (Mareš and Trojan 1991). Corneal electroshock with lower current intensities than those necessary for eliciting of generalized tonic-clonic seizures (Browning and Nelson 1985) and rhythmic cortical stimulation inducing cortical epileptic afterdischarges (Mareš *et al.* 2002) are also able to evoke this seizure pattern. Results with convulsant drugs demonstrated that the age when these seizures could be elicited is different for different drugs: pentylenetetrazol induced these seizures since postnatal day 18 (P18), bicuculline since P12 and isonicotinhydrazide even in 7-day-old rats. Electrical stimulation of sensorimotor cortex induced these seizures reliably since P12. These data speak in favor of different development of the generator which is mature very early and the access of triggering factors maturing differently according to the mechanism of the action of convulsant drugs. There is also a specificity in pharmacological mechanisms inducing these seizures: they could be evoked by drugs interfering with $GABA_A$ (pentylenetetrazol, bicuculline, Ro 5-3663, picrotoxin, benzodiazepine receptor inverse agonist Ro 19-4603) and adenosine (aminophylline) receptors but not by glycine receptor antagonist strychnine (Kubová and Mareš 1985) and agonists of N-methyl-D-aspartate receptors (NMDA – Mareš and Velíšek 1992, homocysteic acid – Mareš *et al.* 1997). In addition to qualitative differences in the action of individual convulsant drugs there are quantitative differences. Drugs blocking GABAergic inhibition exhibit the most potent convulsant action in the third postnatal week (e.g. Velíšek *et al.* 1992). In contrast, N-methyl-D-aspartate and its agonists are extremely potent at very early postnatal age and their efficacy decreases with maturation of the brain (Mareš and Velíšek 1992, Mareš *et al.* 1997).

Generalized nonconvulsive seizures (models of absences – Snead 1992) cannot be reliably diagnosed without EEG recording. The episodes of spike-and-wave rhythm are accompanied only by minute motor signs like rhythmic jerks of vibrissae or ears (Schickerová *et al.* 1984). Loss of consciousness, which is characteristic for human absence seizures, can be modeled by a failure of motivated behavior like drinking of thirsty rats (Schickerová *et al.* 1989). EEG and motor features of these seizures cannot be found before the third postnatal week – faint signs of these activities were observed at P15, fully developed spike-and-wave rhythm was recorded in P18 rats (Schickerová *et al.* 1984). Another possible but not generally accepted model of absence seizures – rhythmic EEG activity induced by systemic administration of gamma-hydroxybutyrate – exhibits

similar developmental profile: this activity appears in the third postnatal week (Rokyta *et al.* 1981).

Both principal types of partial seizures were also studied in immature animals. Neocortical foci were elicited by local application of penicillin (Mareš 1973) or bicuculline methiodide (Velíšková *et al.* 1991, Soukupová *et al.* 1993). They can be induced since very early stages of brain development – penicillin neocortical foci since P3 (Mareš 1973a,b), bicuculline foci at least since P5 (Velíšková *et al.* 1991). In contrast to the two above mentioned convulsants locally applied acetylcholine was found to elicit epileptogenic foci since P9 (Staudacherová *et al.* 1978), i.e. at the developmental stage when excitatory action of acetylcholine on cortical neurons develops (Strejčková *et al.* 1987). Atropine acting nonspecifically on neuronal membrane induced epileptogenic foci earlier than acetylcholine (Staudacherová *et al.* 1978). Maturation of cortical epileptogenic foci is reflected in a shift from sharp wave to spike as the EEG correlate of interictal focal discharges (it appears around P12) and especially in development of projection of focal discharges which depends on the maturation of cortical pathways (corpus callosum – Jacobson 1963). Maturation of callosal connection is clearly seen not only in development of cortical interhemispheric responses (Seggie and Berry 1972, Mareš *et al.* 1975) but also in experiments with induction of two symmetrical epileptic foci and synchronization of their activity (Mareš 1973c). Transition from interictal to ictal phases was studied with bicuculline methiodide-induced foci and was found even at P7 (Soukupová *et al.* 1993). Prince and Gutnick (1972) in their study in kittens demonstrated the basic feature of epileptogenic foci in immature cerebral cortex – low level of synchronization of firing of individual neurons in the focus. Hablitz and Heinemann (1987) described another important difference between immature and mature cortex – much higher activity of extracellular K^+ characterizing epileptic activity in developing cortex.

Hippocampal afterdischarges elicited by electrical stimulation can be used to model complex partial seizures characterized by epileptic automatisms. These afterdischarges can be evoked reliably at P7 (Velíšek and Mareš 1981, Lee *et al.* 1989). Another model is elicited by systemic administration of kainic acid. In dependence on the dose used it induces complex partial seizures without or with secondary generalization (Cherubini *et al.* 1983, Albala *et al.* 1984, Ben-Ari *et al.* 1984, Velíšková *et al.* 1988). Starting at P7 rats exhibit these seizures (Velíšková *et al.* 1988), other groups studied older animals – usually at the end of the second postnatal week. The most conspicuous automatism in both models is represented by wet dog shakes; this has a specific developmental profile. Wet dog shakes can be observed in rats since the second postnatal week, younger rats exhibit scratching as the main automatism (Ricci *et al.* 1983, Marešová and Mareš 1987). Orienting reaction (or at least its elements) in the well-known cage is also a common automatism. Among the elements sniffing is the simplest one and can be observed even in 7-day-old rats. Development of the motor system forms a background for relatively late appearance of a typical part of orienting reaction-rearing. Rat pups up to the third postnatal week are not able to rear without support (Blanck *et al.* 1967).

Amygdala is also a source of focal epileptic activity with clinical counterpart corresponding to complex partial seizures. The most important finding in amygdala-induced afterdischarges in kindling experiments is represented by a failure of postictal refractoriness (Moshé and Albala 1983). Local application of penicillin into amygdala of developing rats demonstrated P3-P4 as the youngest age when an epileptogenic focus could be induced (Kudo and Yamauchi 1988).

Conclusions and perspectives

It is possible to conclude that there are many models of epileptic seizures in immature rats. The data confirm the easy generation of epileptic seizures (ictogenesis) in the immature brain but it always depends on the epileptogenic agens used to elicit seizures. Motor as well as EEG patterns of seizures reflect the level of maturation of the central nervous system. Development of motor coordination may be studied in generalized convulsive seizures, maturation of synchronization of bioelectrical activity in interictal discharges of cortical epileptogenic foci as well as in EEG pattern of generalized seizures (Zouhar *et al.* 1980). In the last point it is necessary to differentiate between synchronization realized by intracortical connections and generalization with necessary participation of subcortical structures (thalamus as the most important structure).

As usually the data reviewed lead to many questions. Among the most important ones is the problem of age-bound type of seizures. Emprosthotonic (flexion) seizures can be observed only up to a certain level of maturation. Do they then disappear or are they masked by

other types of seizures? On the other hand, spike-and-wave rhythm characteristic for absence seizures and their models can be elicited only when some level of maturation is reached. What are the neurotransmitter mechanisms responsible for a sudden appearance of practically mature pattern? More questions should be answered including the relationship between ictogenesis and epileptogenesis – a progressive process resulting in spontaneous appearance of epileptic seizures.

Conflict of Interest

There is no conflict of interest.

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References

ALBALA BJ, MOSHÉ SL, OKADA R: Kainic acid-induced seizures: a developmental study. *Dev Brain Res* **13:** 139-148, 1984.

BARTON ME, KLEIN BD, WOLF HH, WHITE HS: Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy. *Epilepsy Res* **47:** 217-227, 2001.

BEN-ARI Y, TREMBLAY E, BERGER M, NITECKA L: Kainic acid seizure syndrome and binding sites in developing rats. *Dev Brain Res* **14:** 284-288, 1984.

BLANCK A, HÅRD E, LARSSON K: Ontogenetic development of orienting behavior in the rat. *J Comp Physiol Psychol* **63:** 327-328, 1967.

BROWNING RA, NELSON BK: Variation in threshold and pattern of electroshock-induced seizures in rats depending on site of stimulation. *Life Sci* **37:** 2205-2211, 1985.

CHERUBINI E, DE FEO MR, MECARELLI O, RICCI GF: Behavioral and electrographic patterns induced by systemic administration of kainic acid in developing rats. *Dev Brain Res* **9:** 69-77, 1983.

ENGEL J JR: Experimental animal models of epilepsy: classification and relevance to human epileptic phenomena. *Epilepsy Res* **8** (Suppl): 9-20, 1992.

HABLITZ JJ, HEINEMANN U: Extracellular K^+ and Ca^{2+} changes during epileptiform discharges in the immature rat neocortex. *Dev Brain Res* **36:** 299-303, 1987.

HOLMES GL, BEN-ARI Y: The neurobiology and consequences of epilepsy in the developing brain. *Pediat Res* **49:** 320-325, 2001.

JACOBSON S: Sequence of myelinization in the brain of the albino rat. A. Cerebral cortex, thalamus and related structures. *J Comp Neurol* **121:** 5-29, 1963.

KUBOVÁ H, MAREŠ P: Convulsant action of benzodiazepine receptor agonist/inverse agonist Ro 19-4603 in developing rats. *Naunyn-Schmiedebergs Arch Pharmacol* **350:** 393-397, 1994.

KUBOVÁ H, MAREŠ P: Different postnatal development of convulsions and lethality induced by strychnine in rats. *Pharmacol Toxicol* **77:** 219-224, 1995.

KUDO T, YAMAUCHI T: An ontogenic study of amygdala seizures induced by penicillin in rats. *Exp Neurol* **99:** 531-543, 1988.

KUPFERBERG H: Animal models used in the screening of antiepileptic drugs. *Epilepsia* **42** (Suppl 4): 7-12, 2001.

LEE S-S, MURATA R, MATSUURA H: Developmental study of hippocampal kindling. *Epilepsia* **30:** 266-270, 1989.

LOSCHER W, SCHMIDT D: Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res* **2:** 145-181, 1988.

MAREŠ P: Ontogenetic development of bioelectrical activity of the epileptogenic focus in rat neocortex. *Neuropädiatrie* **4:** 434-445, 1973a.

MAREŠ P: Ontogenesis of the cortical epileptic focus in rats. *Activ Nerv Super* **15:** 193-198, 1973b.

MAREŠ P: Symmetrical epileptogenic foci in immature rat cerebral cortex. *Epilepsia* **14:** 427-435, 1973c.

MAREŠ P: Epileptic phenomena in the immature brain. *Physiol Res* **40:** 577-584, 1991.

MAREŠ P: Ontogeny of ethosuximide action against two seizure models in rats is different. *Life Sci* **63:** PL 51-57, 1998.

MAREŠ P: Generalized seizures in rodents – only two patterns? In: *Generalized Seizures: From Clinical Phenomenology to Underlying Systems and Networks*. E HIRSCH, F ANDERMANN, P CHAUVEL, J ENGEL, F LOPES DA SILVA, H LUDERS (eds), John Libbey Eurotext, Montrouge 2006, pp 70-72.

MAREŠ P, SCHICKEROVÁ R: Seizures elicited by subcutaneous injection of metrazol during ontogenesis in rats. *Activ Nerv Super* **22:** 264-268, 1980.

MAREŠ P, TROJAN S: Ontogenetic development of isonicotine-hydrazide – induced seizures in rats. *Brain Dev* **13:** 121-125, 1991.

MAREŠ P, VELÍŠEK L: N-Methyl-D-aspartate (NMDA)-induced seizures in developing rats. *Dev Brain Res* **65:** 185-189, 1992.

MAREŠ P, ZOUHAR A: Do we possess adequate models of childhood epilepsies? *Physiol Bohemoslov* **37:** 1-9, 1988.

MAREŠ P, MAREŠ J, KOZÁKOVÁ-MÁTLOVÁ E: Development of interhemispheric response in rats. *T-I-T J Life Sci* **5:** 5-10, 1975.

MAREŠ P, MAREŠOVÁ D, TROJAN S, FISCHER J: Ontogenetic development of rhythmic thalamo-cortical phenomena in the rat. *Brain Res Bull* **8:** 765-769, 1982.

MAREŠ P, ROKYTA R, TROJAN S: Epileptic seizures during ontogenesis in the rat. *J Physiol (Paris)* **78:** 863-864, 1983.

MAREŠ P, LIŠKOVÁ-BERNÁŠKOVÁ K, MUDROCHOVÁ M: Ontogenetic development of convulsant action of Ro 5-3663 in the rat. *Life Sci* **40:** 1161-1170, 1987.

MAREŠ P, MOSHÉ SL, HOLMES GL: Epileptogenesis and the immature brain. I. Cortical mechanisms. *Exp Brain Res* **20** (Suppl): 147-149, 1991.

MAREŠ P, KUBOVÁ H, CZUCZWAR SJ: Aminophylline exhibits convulsant action in rats during ontogenesis. *Brain Dev* **16:** 296-300, 1994.

MAREŠ P, FOLBERGROVÁ J, LANGMEIER M, HAUGVICOVÁ R, KUBOVÁ H: Convulsant action of D,L-homocysteic acid and its stereoisomers in immature rats. *Epilepsia* **38:** 767-776, 1997.

MAREŠ P, HAUGVICOVÁ R, KUBOVÁ H: Unequal development of thresholds for various phenomena induced by cortical stimulation in rats. *Epilepsy Res* **49:** 35-43, 2002.

MAREŠOVÁ D, MAREŠ P: Hippocampo-cortical after-discharges during ontogenesis in rats. In: *Ontogenesis of the Brain* (4). S TROJAN, F ŠŤASTNÝ (eds), Univ. Carolina Press, Prague 1987, pp 279-283.

MOSHÉ SL, ALBALA BJ: Maturational changes in postictal refractoriness and seizure susceptibility in developing rats. *Ann Neurol* **13:** 552-557, 1983.

MOSHÉ SL, HOLMES GL, MAREŠ P: Epileptogenesis and the immature brain. II. Subcortical mechanisms. *Exp Brain Res* **20** (Suppl): 151-153, 1991.

PINEAU N, CHARRIAUT-MARLANGUE C, MOTTE J, NEHLIG A: Pentylenetetrazol seizures induce cell suffering but not death in the immature rat brain. *Dev Brain Res* **112:** 139-144, 1999.

PRINCE DA, GUTNICK MJ: Neuronal activities in epileptogenic foci of immature cortex. *Brain Res* **45:** 455-468, 1972.

RICCI GF, CHERUBINI E, DE FEO MR, MECARELLI O: Development of epileptic activity and its correlation with GABAergic and glutamatergic systems during brain maturation in the rat. In: *Epilepsy. An Update on Research and Therapy*. R NISTICO, G DI PERRI, H MEINARDI (eds), Alan R. Liss, New York 1983, pp 77-86.

ROGAWSKI MA: Molecular targets versus models for new antiepileptic drug discovery. *Epilepsy Res* **68:** 22-28, 2006.

ROKYTA R, MAREŠ P, SOBOTKA P: Electrocorticographic activity induced by gamma-hydroxybutyrate in the rat during ontogenesis. *Experientia* **37:** 750-752, 1981.

SANCHEZ RM, JENSEN FE: Maturational aspects of epilepsy mechanisms and consequences for the immature brain. *Epilepsia* **42:** 577-585, 2001.

SARKISIAN MR: Overview of the current animal models for human seizure and epileptic disorders. *Epilepsy Behav* **2:** 201-216, 2001.

SCHICKEROVÁ R, MAREŠ P, TROJAN S: Correlation between electrocorticographic and motor phenomena induced by metrazol during ontogenesis in rats. *Exp Neurol* **84:** 153-164, 1984.

SCHICKEROVÁ R, MAREŠ P, TROJAN S: Rhythmic metrazol activity in rats as a model of human absences. *Activ Nerv Super* **31:** 16-20, 1989.

SEGGIE J, BERRY M: Ontogeny of interhemispheric evoked potentials in the rat: Significance of myelination of the corpus callosum. *Exp Neurol* **35:** 215-232, 1972.

SNEAD OC 3RD: Pharmacological models of generalized absence seizures in rodents. *J Neural Transm* **35** (Suppl): 7-19, 1992.

SOUKUPOVÁ S, MIKOLÁŠOVÁ R, KUBOVÁ H, MAREŠ P: New model of cortical epileptic foci in freely moving developing rats. *Epilepsy Res* **15:** 27-33, 1993.

STAUDACHEROVÁ D, MAREŠ P, KOZÁKOVÁ H, CAMUTALIOVÁ M: Ontogenetic development of acetylcholine and atropine epileptogenic cortical foci in rats. *Neuroscience* **3:** 749-753, 1978.

STREJČKOVÁ A, MAREŠ P, RAEVSKY V: Changes of activity of cortical neurons induced by acetylcholine in adult and young rats. *Exp Neurol* **98:** 555-562, 1987.

VELÍŠEK L, MAREŠ P: Increased epileptogenesis in the immature hippocampus. *Exp Brain Res* **20** (Suppl): 183-185, 1991.

VELÍŠEK L, KUBOVÁ H, POHL M, STAŇKOVÁ L, MAREŠ P, SCHICKEROVÁ R: Pentylenetetrazol-induced seizures in rats: an ontogenetic study. *Naunyn-Schmiedeberg's Arch Pharmacol* **346:** 588-591, 1992.

VELÍŠKOVÁ J, VELÍŠEK L, MAREŠ P: Epileptic phenomena produced by kainic acid in laboratory rats during ontogenesis. *Physiol Bohemoslov* **37:** 395-405, 1988.

VELÍŠKOVÁ J, VELÍŠEK L, MAREŠ P, ROKYTA R, BUDKO KP: Bicuculline-induced neocortical epileptiform foci and the effects of 6-hydroxydopamine in developing rats. *Brain Res Bull* **26:** 693-698, 1991.

VERNADAKIS A: Spinal cord convulsions in developing rats. *Science* **137:** 532, 1962.

WHITE HS: Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. *Epilepsia* **38** (Suppl 1): S9-S17, 1997.

ZOUHAR A, MAREŠ P, BROŽEK G: Electrocorticographic activity elicited by metrazol during ontogenesis in rats. *Arch Int Pharmacodyn* **248:** 280-288, 1980.

ZOUHAR A, MAREŠ P, LIŠKOVÁ-BERNÁŠKOVÁ K, MUDROCHOVÁ M: Motor and electrocorticographic epileptic activity induced by bicuculline in developing rats. *Epilepsia* **30:** 501-510, 1989.