Physiol. Res. 46: 397–402, 1997

SHORT COMMUNICATION

Two Models of Epileptic Spike-and-Wave Rhythm in Rats Are Differently Influenced by Ethosuximide

P. MAREŠ, H. KUBOVÁ, M. MOCKOVÁ

Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Received April 29, 1997
Accepted August 13, 1997

Summary

Epileptic afterdischarges induced by electrical stimulation of the sensorimotor cortex as well as minimal metrazol seizures are characterized by EEG spike-and-wave rhythm and nearly the same motor pattern of clonic seizures. The action of ethosuximide on these two models was tested in adult rats with implanted electrodes. Cortical afterdischarges remained practically uninfluenced by ethosuximide (62.5 or 125 mg/kg i.p.) whereas minimal metrazol seizures were suppressed in a dose-dependent manner (doses of 31.25, 62.5 and 125 mg/kg i.p. were used). Present results in connection with recent data on the abolition of spike-and-wave rhythm elicited by low systemic doses of pentylenetetrazol suggest that spike-and-wave rhythm does not represent a single entity.

Key words
EEG - Spike-and-wave - Seizures - Ethosuximide - Rat

Rhythmic electrical stimulation of the sensorimotor cortex elicited epileptic afterdischarges (ADs) characterized by electroencephalographic spike-and-wave rhythm and clonic seizures of facial and forelimb muscles (Kubová *et al.* 1990). Motor pattern of these seizures was nearly identical with that of minimal metrazol seizures, i.e. predominantly clonic seizures of head and forelimb muscles with preserved righting reflexes induced by systemic pentylenetetrazol administration. Tonic component, if present, was formed by rearing and torsion of the trunk (Swinyard 1973, Velíšek *et al.* 1992). Another similarity was found in EEG pattern of both animal models (Fig. 1) – minimal metrazol seizures are also accompanied by spike-and-wave activity. Minimal metrazol seizures could be taken as a model of human myoclonic seizures (Löscher and Schmidt 1988), the clinical counterpart of cortical afterdischarges might be the same (Kubová *et al.* 1996). Spike-and-wave rhythm characteristic for these two models is generated by thalamocortical system (Steriade and Deschenes 1984, Snead 1988, Marescaux *et al.* 1992). An important role in the generation of spike-and-wave rhythm is played by low-threshold calcium current which is markedly expressed in thalamic neurons and which is a target of ethosuximide (Coulter *et al.* 1989). Because of the efficacy of ethosuximide against models of absence seizures characterized also by spike-and-wave rhythm (Guberman *et al.* 1975, Sasa *et al.* 1988, Snead 1988, Marescaux *et al.* 1992) we decided to study the action of this antiepileptic drug against two models mentioned above. Efficacy of ethosuximide against motor minimal metrazol seizures is known (Swinyard 1973) but the data on its action on an EEG correlate of these seizures are missing. Suppression of motor seizures elicited by pentylenetetrazol was also confirmed in our strain of rats (Mareš and Velíšek 1983). In addition, we have for comparison our own data on the marked action of ethosuximide against a model of absence seizures – spike-and-wave episodes induced by low doses of pentylenetetrazol (Brabcová *et al.* 1993). In spite of the similarity of the EEG pattern of these models their pharmacological sensitivity might be different as indicated by results with ketamine (Velíšek *et al.* 1989, 1993, Mareš *et al.* 1992).

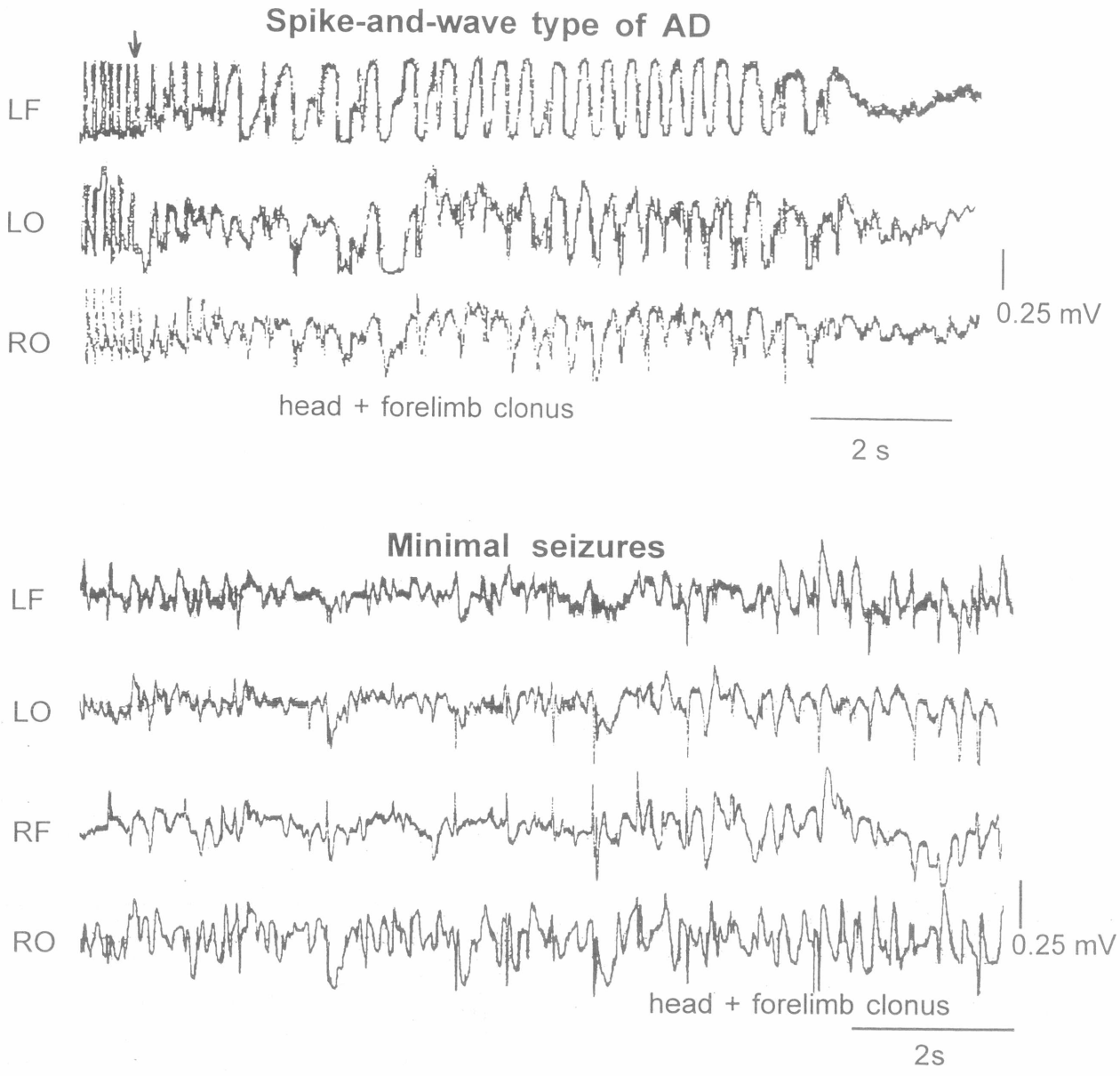

Fig. 1. *EEG recording of cortical epileptic afterdischarge (upper part) and minimal, clonic seizures (lower part) in adult rats. Individual leads: LF – left frontal, LO – left occipital, RF – right frontal, RO – right occipital area in reference connection. Time marks 2 s, amplitude calibrations 0.25 mV. An arrow in the upper graph marks the end of stimulation. Behavior of animals is described under EEG recordings.*

Cortical afterdischarges

Adult rats of the Wistar strain (Velaz, Prague) were surgically prepared under pentobarbital anaesthesia (Nembutal^{R} Abbott, 50 mg/kg i.p.). All electrodes were implanted epidurally: two silver ball electrodes for stimulation over the right sensorimotor area at coordinates AP = −1 and +1 and L = 2.5 mm in relation to bregma. Flat silver electrodes (1x1 mm, L-shaped) served for recording; one of them was placed over left sensorimotor area (AP = 0, L = 2.5 mm) and two over occipital, visual areas of both hemispheres (AP = 6, L = 4 mm). An indifferent electrode (a stainless screw) was put into the nasal bone and served for all EEG leads and also for anchoring the whole assembly (including a six-pin connector) fixed to the skull by means of a dental acrylic. The rats were then allowed to recover for at least one week.

Biphasic pulses of 1-ms duration (0.5 ms pulse of one polarity and 0.5 ms pulse of opposite polarity) and 8-Hz frequency were generated by a constant current stimulator. These pulses were applied in series

lasting 15 s. During the experimental session the rats were connected to the input of an EEG apparatus and placed in isolation in a plexiglass box. The first stimulation session was always a control one. After establishing the threshold intensity necessary for elicitation of epileptic afterdischarges, the stimulation was repeated four times with 10-min intervals between the end of AD and the beginning of the next stimulation with an injection of physiological saline (1 ml/kg i.p.) between the first and second stimulation. During the experimental session ethosuximide (ESI, a generous gift of Gerot Pharmazeutika, Wien) was injected in a dose of 62.5 or 125 mg/kg i.p. (five min after the end of the first AD), so that the first AD was always a control one. Water solution of ESI was always freshly prepared in such concentration that the volume injected corresponded to 1 ml/kg. Nine out of 12 animals underwent all three session, the remaining three lost their electrodes after the first ethosuximide session. The movements accompanying stimulation as well as incidence, duration, EEG pattern and motor correlates of ADs were recorded. Severity of motor phenomena was scored according to a five-point scale (Kubová *et al.* 1996). The incidence of ADs was evaluated statistically using Fisher's exact test, the duration of ADs and severity of motor correlates of both stimulation and ADs by means of ANOVA with dose of ESI and ordinal number of AD as factors. Subsequent comparison of individual values was performed by means of Holm sequential analysis (Holm 1979). The level of statistical significance was set at 5 %.

The incidence of afterdischarges (ADs) was not changed by either dose of ethosuximide. Lower dose of ESI significantly shortened the second and third ADs, whereas the 125 mg/kg dose did not exhibit any effect (Fig. 2). Motor correlates of stimulation remained unchanged by ESI; clonic seizures accompanying ADs tended to be less intense after ESI administration, the level of statistical significance was reached only exceptionally – in the third AD after the 62.5 mg/kg dose and in the fourth AD after the 125 mg/kg dose (Fig. 2).

Minimal metrazol seizures

Surgical preparation of animals was the same as in the first experimental series with the only exception that one recording electrode was implanted over the right sensorimotor area instead of two stimulation electrodes. A group of 20 rats was used in this study.

The first session was always a control one, when only pentylenetetrazol (PTZ, Sigma, freshly dissolved 10 % water solution) was injected in a dose of 60 mg/kg i.p. If minimal seizures were not elicited, the control session was repeated three days later with higher doses (65 or 70 mg/kg, respectively). The effective dose was then used in all subsequent sessions. Ethosuximide (31.25, 62.5 or 125 mg/kg i.p. in a random order) was injected 15 min before PTZ administration in each session. EEG was recorded for two min before and 30 min after PTZ injection. Behavior of rats was registered directly into EEG recording.

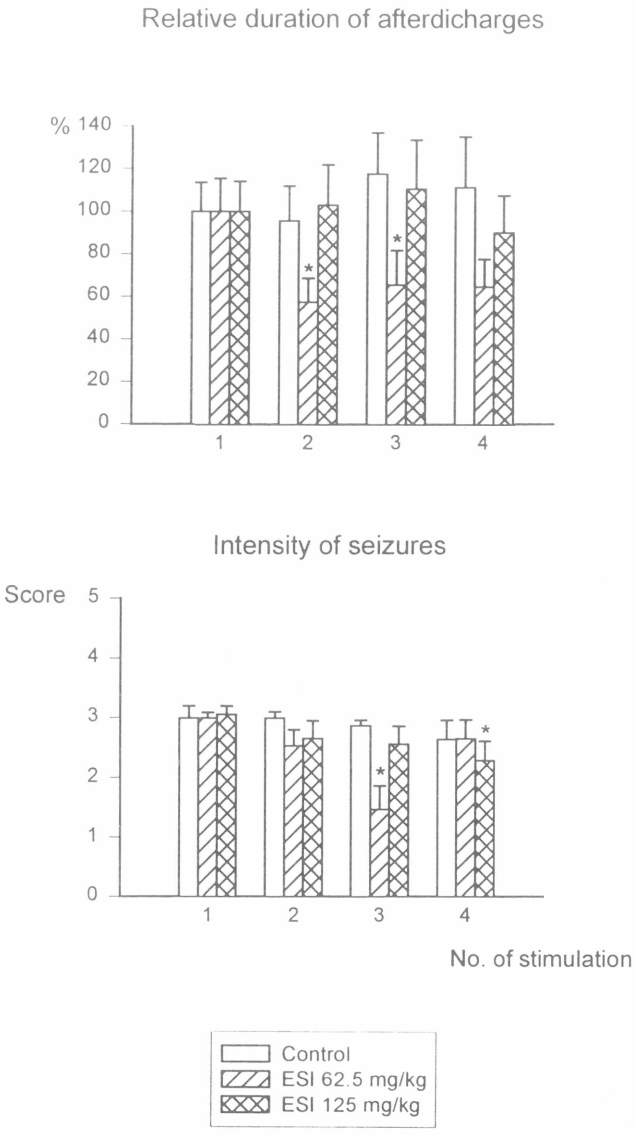

Fig. 2. *Influence of ethosuximide on relative duration (mean ± S.E.M.) of cortical afterdischarges (upper part) and on severity of clonic seizures accompanying ADs (lower part). Abscissa – first to fourth afterdischarge; upper ordinate – relative duration of ADs (the duration of the first one is always taken as 100 %), lower ordinate – five-point scale quantifying motor phenomena (Kubová et al. 1996). Individual columns – see inset. Asterisks denote statistically significant difference in comparison with the first, control AD.*

An interval between two sessions was at least three days. Only nine animals received all three doses of ESI, majority of the rats underwent two ethosuximide sessions because of a loss of electrodes during seizures. Incidence, latency and duration of minimal seizures was counted, their EEG and motor patterns were evaluated. The results were analyzed statistically - incidence by Fisher's exact test, latency and duration by ANOVA with subsequent pairwise comparison (SigmaStat Jandel). The level of statistical significance was again put on 5 %.

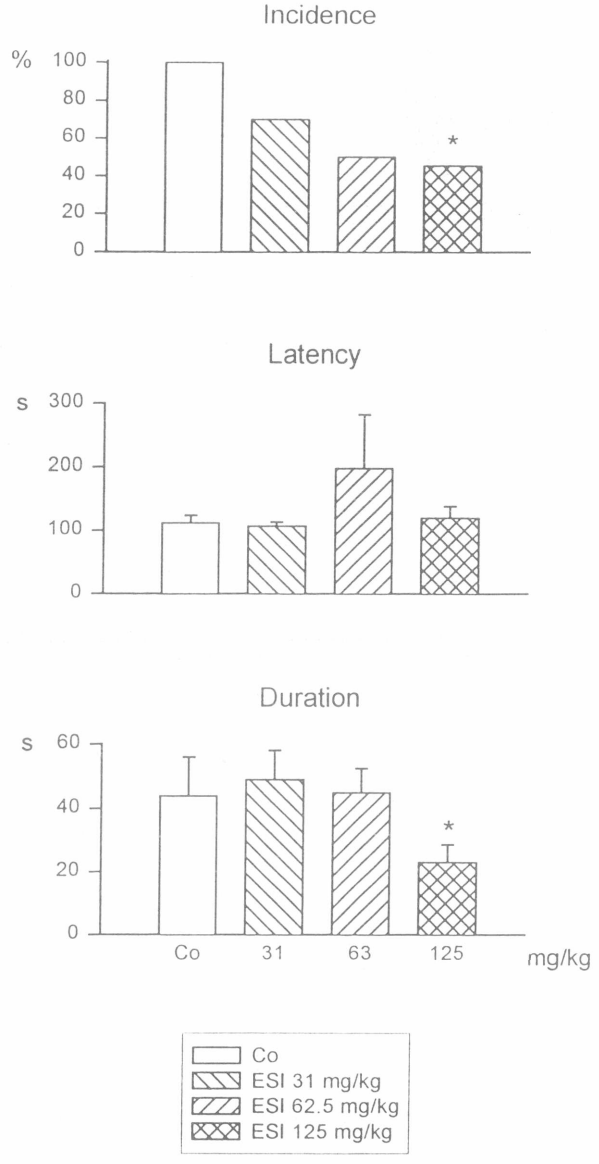

Fig. 3. *Influence of ethosuximide on incidence (upper graph), latency (middle graph) and duration (lower graph) of minimal metrazol seizures. Abscissae – doses of ethosuximide, C – control sessions; ordinates from top to bottom – percentage of animals exhibiting minimal seizures, latency in seconds, duration in seconds. The columns represent mean ± S.E.M. in the two lower graphs.*

Motor (predominantly clonic seizures of facial and forelimb muscles) and EEG pattern (spike-and-wave rhythm or rhythmic spikes) correlated perfectly. They were not changed by any dose of ESI. Neither the incidence nor the time characteristics (latency and duration) of minimal seizures were changed by the 31.25 mg/kg dose of ESI. The 62.5 mg/kg dose decreased the incidence to 53 % leaving untouched pattern, latency and duration of seizures. The highest dose used (125 mg/kg) decreased significantly the incidence to 46 %, tended to prolong the latency and significantly shortened the duration of seizures from 44±12 s in control sessions to 24±5 s after this dose of ESI (Fig. 3).

Ethosuximide is a drug of choice against human absence seizures characterized by an EEG spike-and-wave rhythm; it did not exhibit anticonvulsant action against any other type of human epileptic seizures (Rogawski and Porter 1990, Sherwin 1995). ESI dose-dependently suppressed spike-and-wave episodes elicited by a low dose of PTZ (Brabcová *et al.* 1993), decreased the incidence of minimal metrazol seizures again in a dose dependent manner; in addition, the highest dose used (125 mg/kg) shortened duration of these seizures. On the other hand, it only inconsistently changed cortical epileptic afterdischarges, the 125 mg/kg dose was without effect. The transient effect of the lower dose of ESI against afterdischarges cannot be explained at present; all other published data on ESI (including our own results) invariably demonstrated dose-dependent effects of this antiepileptic drug. Action of ESI in the three types of seizures is opposite to that of ketamine, which suppressed cortical afterdischarges and did not change the other two models (Velíšek *et al.* 1989, 1993, Mareš *et al.* 1992).

Pharmacological data for both ESI and ketamine suggest that spike-and-wave EEG rhythm does not always represent the same model. There is no doubt that this activity is in all cases generated by a thalamocortical oscillator (Steriade and Deschenes 1984). Alternation of excitatory and inhibitory postsynaptic potentials was recorded in cortical neurons; wave component of the spike-and-wave rhythm represents an inhibitory period (Pollen and Sie 1964, Quesney and Gloor 1978). The only difference in the spike-and-wave rhythm among the three models mentioned is in the frequency which is higher in the absence model than in the two remaining models (Kubová *et al.* – unpublished data). Shorter waves in the absence model may be due to shorter IPSPs and thus ethosuximide might exert stronger action in this model than in the two others. This conclusion is in agreement with Fromm's data that ethosuximide influences mainly inhibitory mechanisms (Fromm 1992). Which factors are decisive for the rate of thalamocortical oscillations and thus for generation of slow or faster spike-and-wave rhythm as well as for

different influencing of spike-and-wave rhythm in the three models used in our laboratory remains to be addressed in future research.

As far as the mechanism of action of ethosuximide is concerned, our present results are in agreement with the data on thalamocortical action of this antiepileptic drug (Englander *et al.* 1977, Nowack *et al.* 1979, Pellegrini *et al.* 1989). A possible brainstem component of ethosuximide action (Kästner *et al.* 1968, Mareš *et al.* 1994) could not be demonstrated in present experiments. Our data cannot add anything to the described mechanism of action of ethosuximide on subcellular level – reduction of low-threshold calcium currents in thalamic neurons (Coulter *et al.* 1989), but they may be used as a background for testing the function of this type of Ca^{2+} channels in different models of seizures.

Acknowledgement
This study was supported by grants No. 2210-3 and 4036-1 of the Grant Agency of the Czech Ministry of Health.

References

BRABCOVÁ R., KUBOVÁ H., VELÍŠEK L., MAREŠ P.: Effects of a benzodiazepine, bretazenil (Ro 16-6028), on rhythmic metrazol EEG activity: comparison with standard anticonvulsants. *Epilepsia* **34:** 1135–1140, 1993.

COULTER D.A., HUGUENARD J.R., PRINCE D.A.: Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann. Neurol.* **25:** 582–593, 1989.

ENGLANDER R.N., JOHNSON R.N., BRICKLEY J.J., HANNA G.R.: Effects of antiepileptic drugs on thalamocortical excitability. *Neurology* **27:** 1134–1139, 1977.

FROMM G.H.: Antiepileptic actions of ethosuximide. In: *Drugs for Control of Epilepsy,* C.L. FAINGOLD, G.H. FROMM (eds), CRC Press, Boca Raton, 1992, pp. 477–484.

GUBERMAN A., GLOOR P., SHERWIN A.L.: Response of generalized penicillin epilepsy in the cat to ethosuximide and diphenylhydantoin. *Neurology* **25:** 758–764, 1975.

HOLM S.: A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6:** 56–70, 1979.

KÄSTNER I., KLINGBERG F., MÜLLER M.: Untersuchungen zum zentralnervösen Wirkung des Ethosuximids. *Arch. Psychiat. Nervenkr.* **211:** 365–376, 1968.

KUBOVÁ H., MAKAL V., MIŇOVÁ M., VAŇKOVÁ S., MAREŠ P.: Influence of clonazepam on cortical epileptic afterdischarges in rats. *Arch. Int. Pharmacodyn. Ther.* **307:** 49–59, 1990.

KUBOVÁ H., LANŠTIAKOVÁ M., MOCKOVÁ M., MAREŠ P., VORLÍČEK J.: Pharmacology of cortical epileptic afterdischarges in rats. *Epilepsia* **37:** 336–341, 1996.

LÖSCHER 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., VELÍŠEK L.: Influence of ethosuximide on metrazol-induced seizures during ontogenesis in rats. *Activ. Nerv. Super.* **25:** 295–298, 1983.

MAREŠ P., LANŠTIAKOVÁ M., VAŇKOVÁ S., KUBOVÁ, H., VELÍŠEK, L.: Ketamine blocks cortical epileptic afterdischarges but not paired-pulse and frequency potentiation. *Neuroscience* **50:** 339–344, 1992.

MAREŠ P., POHL M., KUBOVÁ H., ŽELÍZKO M.: Is the site of action of ethosuximide in the hindbrain? *Physiol. Res.* **43:** 51–56, 1994.

MARESCAUX C., VERGNES M., DEPAULIS A.: Genetic absence epilepsy in rats from Strasbourg – a review. *J. Neural Transm.* Suppl. **35:** 37–69, 1992.

NOWACK W.J., JOHNSON R.N., ENGLANDER R.N., HANNA G.R.: Effects of valproic acid and ethosuximide on thalamocortical excitability. *Neurology* **29:** 96–99, 1979.

PELLEGRINI A., DOSSI R.C., DALPOS F., ERMANI M., ZANOTTO L., TESTA G.: Ethosuximide alters intrathalamic and thalamocortical synchronizing mechanisms: a possible explanation of its antiabsence effect. *Brain Res.* **497:** 344–360, 1989.

POLLEN D.A., SIE P.G. Analysis of thalamic induced wave and spike by modifications in cortical excitability. *Electroenceph. Clin. Neurophysiol.* **17:** 154–163, 1964.

QUESNEY L.F., GLOOR P.: Generalized penicillin epilepsy in the cat: correlation between electrophysiological data and distribution of ^{14}C-penicillin in the brain. *Epilepsia* **19:** 35–45, 1978.

ROGAWSKI M.A., PORTER R.J.: Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. *Pharmacol. Rev.* **42:** 223–286, 1990.

SASA M., OHNO Y., UJIHARA H., FUJITA Y., YOSHIMURA M., TAKAORI S., SERIKAWA T., YAMADA J.: Effects of antiepileptic drugs on absence-like and tonic seizures in the spontaneously epileptic rat, a double mutant rat. *Epilepsia* **29:** 505–513, 1988.

SHERWIN A.L.: Ethosuximide: clinical use. In: *Antiepileptic Drugs.* Fourth Edition. R.H. LEVY, R.H. MATTSON, B.S. MELDRUM (eds), Raven Press, New York 1995, pp. 667–673.

SNEAD O.C.: Gamma-hydroxybutyrate model of generalized absence seizures: further characterization and comparison with other absence models. *Epilepsia* **29:** 361–368, 1988.

STERIADE M., DESCHENES M.: The thalamus as a neuronal oscillator. *Brain Res. Rev.* **8:** 1–62, 1984.

SWINYARD E.A.: Assay of antiepileptic drug activity in experimental animals: standard tests. In: *Anticonvulsant Drugs,* Vol. 1. J. MERCIER (ed.), Pergamon Press, Oxford 1973, pp. 47–65.

VELÍŠEK L., MIKOLÁŠOVÁ R., BLANKOVÁ-VAŇKOVÁ S., MAREŠ P.: Effects of ketamine on metrazol-induced seizures during ontogenesis in rats. *Pharmacol. Biochem. Behav.* **32:** 405–410, 1989.

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.

VELÍŠEK L., VONDŘIČKOVÁ R., MAREŠ P.: Models of simple partial and absence seizures in freely moving rats: action of ketamine. *Pharmacol. Biochem. Behav.* **45:** 889–896, 1993.

Reprint requests

P. Mareš, M.D., D.Sc., Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic.