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SHORT COMMUNICATION

Developmental Changes of Thresholds for Cortical Epileptic Afterdischarges

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Summary

Threshold intensities for elicitation of movements and of epileptic afterdischarges by rhythmic stimulation of the sensorimotor cortex were estimated in 90 rats with implanted electrodes. Four age groups were studied – animals 12, 18, 25 and 90 days old. Both thresholds exhibited significantly higher values for adult animals in comparison with all groups of young pups. Whereas no differences were found among the rat pups in thresholds for movements accompanying stimulation, epileptic afterdischarges demonstrated a lower threshold in 18-day-old in comparison with 25-day-old animals. The development of cortical excitability is rather complicated and deserves further studies.

Key words

Epileptic afterdischarges – Cerebral cortex – Rat – Development

Epileptic afterdischarges (ADs) characterized by a spike-and-wave rhythm may be reliably elicited by low-frequency (8 Hz) electrical stimulation of the sensorimotor cortical area since the age of 15 days, exceptionally in 12-day-old locally anaesthetized and immobilized rats (Mareš *et al.* 1980). The experiments in animals with implanted electrodes gave us an opportunity to study not only EEG afterdischarges but also motor phenomena (Kubová *et al.* 1990, Mareš *et al.* 1992). A method of electrode implantation in rat pups introduced in our laboratory some years ago (Schickerová *et al.* 1984) allows us to use cortical ADs for study of electrical excitability of the sensorimotor cortex during development. Two different measures can be exploited – a) movements asociated with stimulation, i.e. direct activation of the motor system, the same measure as used by Voskuyl *et al.* (1989), and b) elicitation of epileptic ADs accompanied by motor phenomena.

Experiments were performed in 90 albino rats of the Wistar strain in four age groups – 12, 18, 25 and 90 days old. Each age group consisted of 20-26 animals. Surgical preparation was different in adult (90 days old) and young rats. Adult animals were anaesthetized with pentobarbital in a dose of 50 mg/kg i.p. Trephine openings were made by a dental drill, two stimulation silver ball electrodes were placed over the right sensorimotor area (coordinates AP -1 and +1; L 2.5 mm in relation to the bregma). Recording flat silver electrodes (squares with sides of approximately 0.5 mm) were localized over the contralateral sensorimotor area (AP 0; L 2.5 mm) and over occipital visual areas of both hemispheres (AP 6; L 4 mm). An indifferent electrode was on the nasal bone. The electrodes were fixed to the skull by means of dental acrylic. The animals were allowed to recover for at least one week and only then the experiments started.

Young rats were surgically prepared under ether anaesthesia. The skull was cut by a razor blade and flat silver epidural electrodes were used for stimulation as well as for recording. The coordinates were recalculated according to the actual bregma-lambda distance (the value for adult rats taken as reference was 8 mm), only the AP coordinates of

stimulation electrodes (−1 and +1 mm) were the same in all age groups. Duration of anaesthesia was 10 min on the average. After the fixation of electrodes by means of dental acrylic, the rat pups were allowed to recover for at least one hour, then if their reflexes (righting, placing and suckling) were normal, they were fed and the stimulation started. Rat pups were used only once, after the experiment they were killed by an overdose of ether anaesthesia.

Stimulation was performed by means of a constant current stimulator of our own construction. Stimulation series lasted 15 seconds and consisted of biphasic rectangular pulses of 1 ms duration and 8-Hz frequency. The intensity of stimulation was changed in the following steps: 0.8, 1, 1.2, 1.5, 1.8, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12 and 15 mA. An interval between two stimulation series was at least 10 min. In rat pups the intensities used never exceeded 6 mA because of the low thresholds.

The following phenomena were recorded: clonic movements accompanying stimulation; electroencephalographic ADs, their pattern and duration; clonic seizures accompanying ADs; all other behavioural phenomena appearing during stimulation, ADs and 1 min after the end of ADs. The threshold intensities necessary for evoking stimulation-bound movements and for elicitation of ADs were used for statistical evaluation with ANOVA. Comparison of pairs of age groups was performed with Tukey studentized range method (Miller 1981). The level of statistical significance was set at 5 %.

Rhythmic stimulation of the sensorimotor cortex elicited fine movements of the vibrissae, then clonic movements of head and forelimbs. The contralateral forelimb was activated first, the homolateral one started to jerk somewhat later. The movements were synchronous with the stimuli. If stimulation intensity increased further, both forelimbs started to jerk simultaneously; in some cases rearing was observed. Exceptionally the rats fell but they righted themselves immediately. The clonus of forelimbs was taken as an endpoint for threshold evaluation. The average threshold intensity did not differ significantly among the three groups of youngs whereas the value for adult animals was significantly higher (Fig. 1).

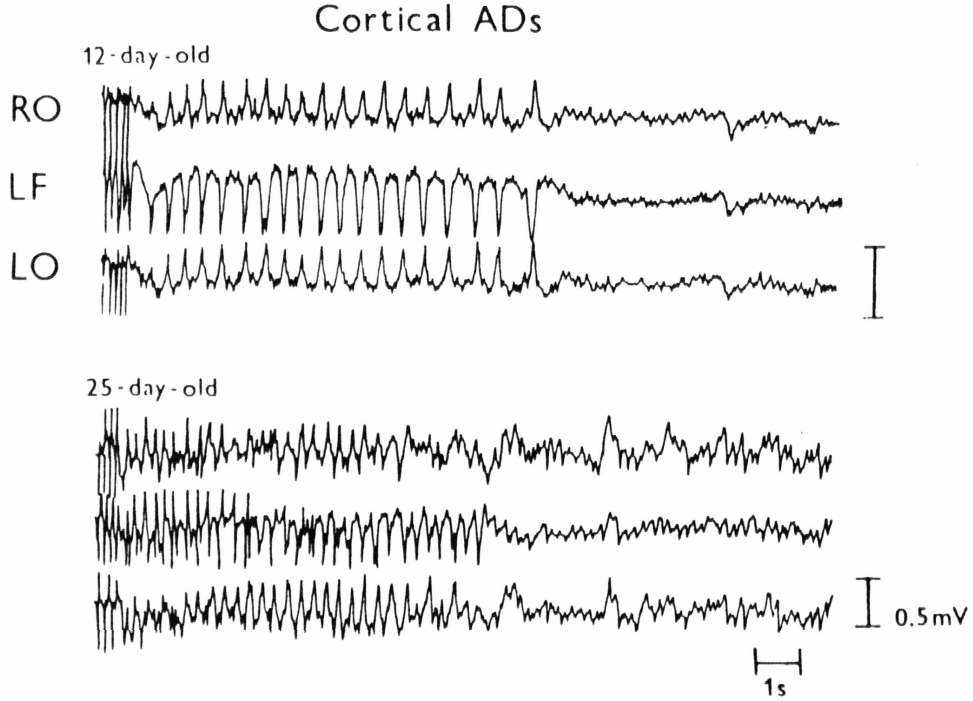

Fig. 1
Electrocorticographic recordings of epileptic afterdischarges in 12-day-old (upper part) and 25-day-old rat (lower part). The three traces in both parts from top to bottom: RO – right occipital, visual cortical area; LF – left frontal, sensorimotor, LO – left occiptal, visual area. Stimulation electrodes were placed on right sensorimotor cortex. Time mark 1 s, amplitude calibration 0.5 mV.

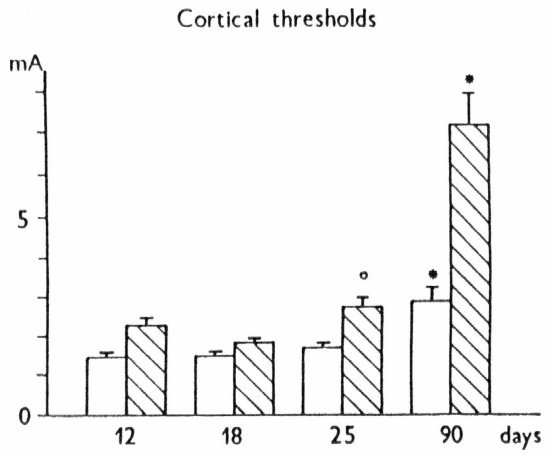

Fig. 2
Average thresholds (mean + S.E.M.) for eliciting movements accompanying stimulation of sensorimotor cortex (white columns) and of epileptic afterdischarges (hatched columns). Abscissa – age in days; ordinate – current intensity in milliampers. An asterisk denotes a significant difference in comparison with all younger groups, a triangle a significant difference in comparison with the 18-day-old group.

An epileptic afterdischarge was elicited in all rats studied (Fig. 2). The currents necessary for ADs elicitation were higher or, less frequently, the same as threshold currents for evoking stimulation-bound movements. ADs were formed by a spike-and-wave rhythm with a 4-5 Hz frequency in rats aged 18 and more days. Twelve-day-old rat pups exhibited rhythmic sharp delta waves with a frequency of about 2 Hz. Motor correlates of ADs consisted of clonic jerks of head and forelimbs identical with those accompanying stimulation, only their frequency was lower – the jerks were synchronous with sharp elements in the EEG. The intensity of clonic seizures was lower or the same as that of movements directly elicited by stimulation. The mean threshold intensity was lowest in 18-day-old rats, the difference against 25-day-old and adult animals being significant. Again the values for all three groups of young rats were significantly lower than that for adult rats. A specific phenomenon was observed in adult animals in which high intensities of stimulation had to be used. One half of animals, i.e. 13 rats, exhibited a transition of spike-and-wave ADs into another type characterized by fast spikes of slow waves with superimposed fast activity ("serrated waves") accompanied by behavioral arrest followed by automatisms – mostly wet dog shakes or orienting reaction.

Epileptic ADs were elicited in all rats studied including the 12-day-old ones. This is a substantial difference in comparison with earlier acute experiments, where this age group exhibited ADs only exceptionally (Mareš *et al.* 1980). It will be necessary to test even younger rats in future experiments.

The EEG pattern of ADs was formed by a spike-and-wave rhythm since the age of 18 days. Younger rats were unable to generate a cortical spike-and-wave rhythm (Mareš *et al.* 1982, Schickerová *et al.* 1984, Snead 1992) due to the immaturity of the cerebral cortex or reciprocal thalamo-cortical connections (Scheibel *et al.* 1976). The finding that clonic jerks are synchronous with spikes in 18-day-old and older rats and with sharp delta waves in 12-day-old pups suggests that these sharp waves have the same significance as spikes. The 12-day-old rats did not exhibit any correlate of the slow component of the spike-and-wave rhythm similarly as in other thalamocortical phenomena at this developmental stage (Mareš *et al.* 1982).

Clonic movements accompanying both stimulation and ADs represent the same motor pattern. It is identical with that described by Racine (1972) in cortical kindling and therefore his scale may be used for quantification in pharmacological experiments (Kubová *et al.* 1990, Mareš *et al.* 1992). The same motor pattern can be elicited by many other epileptogenic agents - convulsant drugs (for review see Mareš 1991), corneal electroshock (Browning and Nelson 1985), rhythmic thalamic stimulation (Chocholová and Kolínová 1975), ictal activity originating from neocortical foci (Soukupová *et al.*, unpublished data). It thus represents a common seizure pattern, generator of which is probably localized in the basal forebrain (Browning and Nelson 1986), probably in "area tempestas" (Gale 1990).

The aim of our study was to compare the cortical excitability at different stages of maturation. The results with direct activation of the motor cortex and with the ADs are not fully identical. The lowest sensitivity of adult rats is common for both measures and it is comparable with data of Voskuyl *et al.* (1992) in spite of substantial differences in the methods of stimulation and the cortical area studied. The absence of differences among the three groups of young animals using direct activation of the motor system is in contrast to the developmental changes observed with ADs thresholds. In any case there is no smooth decrease of cortical excitability from the youngest to the adult rats. The highest sensitivity of 18-day-old rats in ADs testing has no correlate in ontogenetic literature. Michelson and Lothman (1991, 1992) found the highest threshold for elicitation of hippocampal afterdischarges in 7-day-old rat pups (either freely moving with implanted electrodes or under urethane anaesthesia). Similar data were published by Moshé *et al.* (1981) for amygdala – AD thresholds were highest in 15-day-old rat pups, i.e. the youngest animals studied. The discrepance with our data might be due to the different structures stimulated. Nevertheless, the

development of cortical excitability should be addressed by other methods in the near future.

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