Effect of Cycloheximide on the Initiation and Maintenance of Increased Seizure Readiness in the Rat Sensorimotor Cortex

G. N. Kryzhanovskii, V. K. Lutsenko, and N. N. Khlebnikova

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Cycloheximide does not affect the thresholds of elicitation of either direct or transcallosal responses of the sensorimotor cortex, the poststimulation elevation of brain excitability immediately after termination of the 20th series of rhythmic electrostimulations, or the diminishment of its excitability 24 h later. Cycloheximide administration does not change the latency or the duration of the first seizure discharge but does abolish the potentiation of the duration of the seizure discharges. When tested again a day later, control rats exhibit a marked decrease of the latency of the first seizure discharge and an increase of discharge amplitude as compared to the corresponding parameters in the first series of rhythmic electrostimulations.

Key Words: *increased seizure readiness; cycloheximide; sensorimotor cortex*

Repeated rhythmic electrical stimulations (RES) of the sensorimotor cortex of the rat brain induce trace seizure discharges (SD) of the peak-wave type which increase in amplitude and duration during subsequent RES. Increased seizure readiness develops after 1-2 h of RES, when all RES series without exception induce trace discharges of the same amplitude and duration [1]. The second RES session performed 24 h later attests to the preservation of the state of increased seizure readiness during this period. The elicitation of the first SD requires far fewer stimulations in the second RES, and all SD themselves induced in this case are of higher amplitude than the SD recorded during the first RES session [2].

Inhibitors of protein synthesis inhibit RES-induced long-term modifications of the neuronal excitability, attesting to the key role of protein synthesis in consolidating the heightened excitability of neurons. Thus, the inhibitor of protein synthesis cycloheximide (CH) obstructs SD potentiation in the amygdala and markedly delays the development of kindling [6,8]. Anisomycin-induced blockade of protein synthesis keeps the hippocampus from developing the late phase of long-term potentiation [5]. The present study was undertaken to explore the possible role of protein synthesis in eliciting and maintaining the state of increased seizure readiness in the sensorimotor cortex. For this purpose the effect of CH on SD parameters was studied in two identical RES sessions conducted 24 h apart.

MATERIALS AND METHODS

Experiments were carried out on 50 outbred male rats weighing 280-350 g. Control animals (38 rats) were injected i.p. with 0.5 ml physiological saline, while the 12 experimental rats received i.p. 0.5 ml CH (Serva, 2 mg/kg) 1.5-2 h prior to the operation. Electrodes placed on the undamaged dura mater were used to stimulate a region of the sensorimotor cortex of the right hemisphere and to record the electrocorticogram from this region as well as from the symmetrical one in the contralateral hemisphere [1]. The experiment was begun by determining the thresholds of the direct and transcallosal responses to a single electrical pulse. Then 20 series of RES were performed with a

Research Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow

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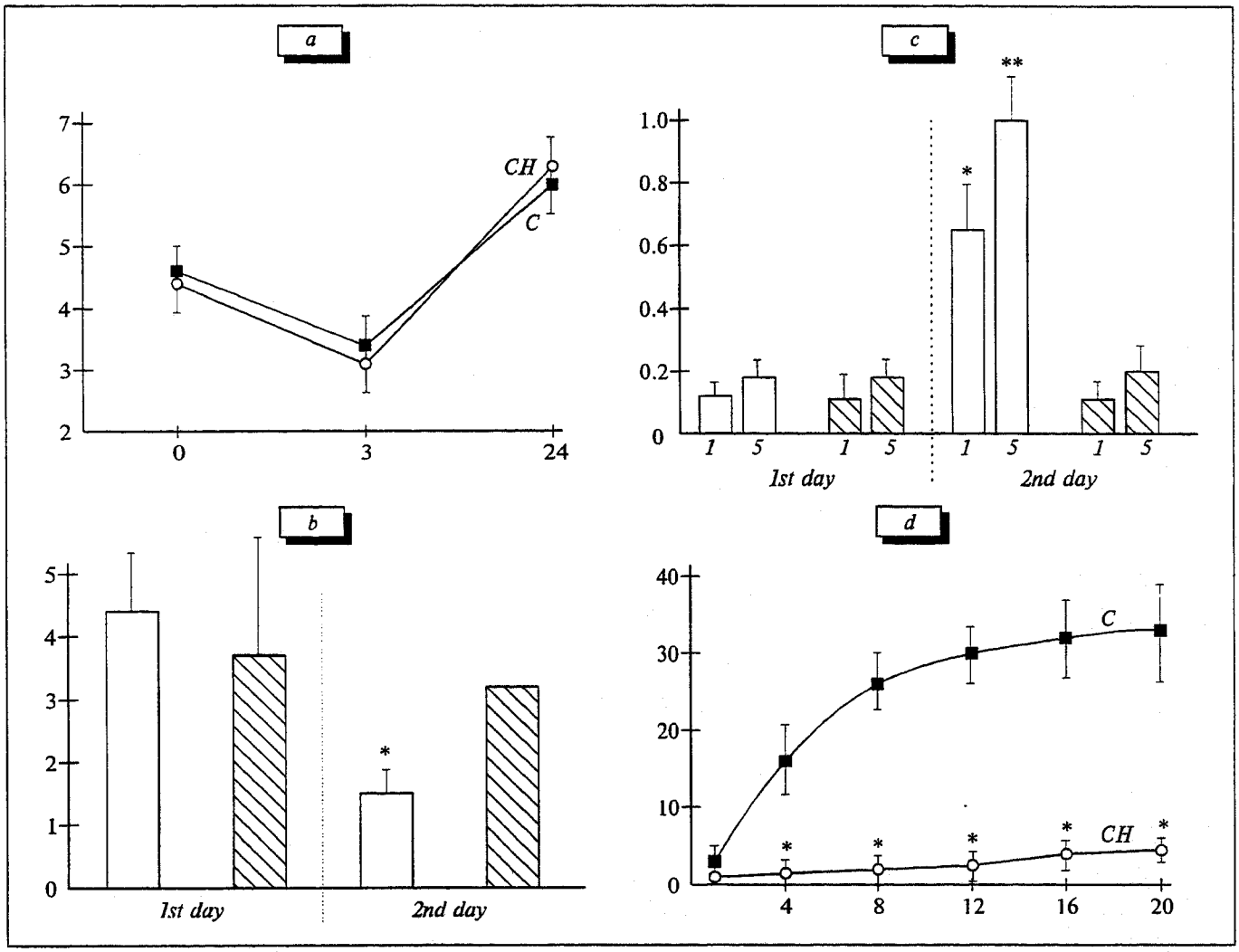

Fig. 1. Effect of CH (2 mg/kg) on thresholds of transcallosal responses (*a*), latency of the first SD (*b*), and amplitude (*c*), and duration (*d*) of SD in the rat sensorimotor cortex. *a*) ordinate: value of threshold for eliciting transcallosal response (V); abscissa: time points of threshold measurement (h): *0*, before RE; *3*, immediately after the end of 20 series of RES; *24*, 24 h after the end of the first RES session. Here and on *d*) *C*: control, *CH*: rats injected with CH. *b*) ordinate: number of RES series necessary to induce first SD (SD latency) on the 1st and 2nd day of stimulation. Here and on *c*: light bars refer to the control, hatched bars to the experiment, $^{*}p<0.05$. *c*) ordinate: amplitude of SD (mV) induced after 1st and 5th RES (indicated below bars) on the 1st and 2nd day of stimulation. $^{*}p<0.05$, $^{**}p<0.01$. *d*) ordinate: SD duration (sec); abscissa: number of RES. *indicates that the difference between experiment and control is reliable for all SD, beginning from the second ($p<0.01$).

pulse duration of 0.2 msec, frequency 10 cps, duration of RES series 10 sec, interval between series 10 min, and an intensity of stimuli of twice the threshold of the transcallosal response. The development of increased seizure readiness was indicated by the appearance of prolonged SD with stable parameters [1,2]. The thresholds of the direct and transcallosal responses were measured again after 20 series of RES to assess the changes in excitability of the sensorimotor cortex induced by the long-term stimulation.

A shortened RES session (5 series) was performed on the next day with the same stimulation parameters as the day before. The parameters compared were the latency of the first SD and the dynamics of SD duration and amplitude in repeated RES in the experiment and control. The data were processed using Statgraf software.

RESULTS

The intensity of the threshold stimuli measured before the first RES session was found to be the same in the control and in the test group both for the direct and for the transcallosal response. A decrease of the threshold stimulus was noted after 20 RES, whereas 24 h later it was increased significantly in both test and control as compared to the prestimulation level for the transcallosal response ($p<0.01$) and for the direct one ($p<0.05$, Fig. 1, *a*).

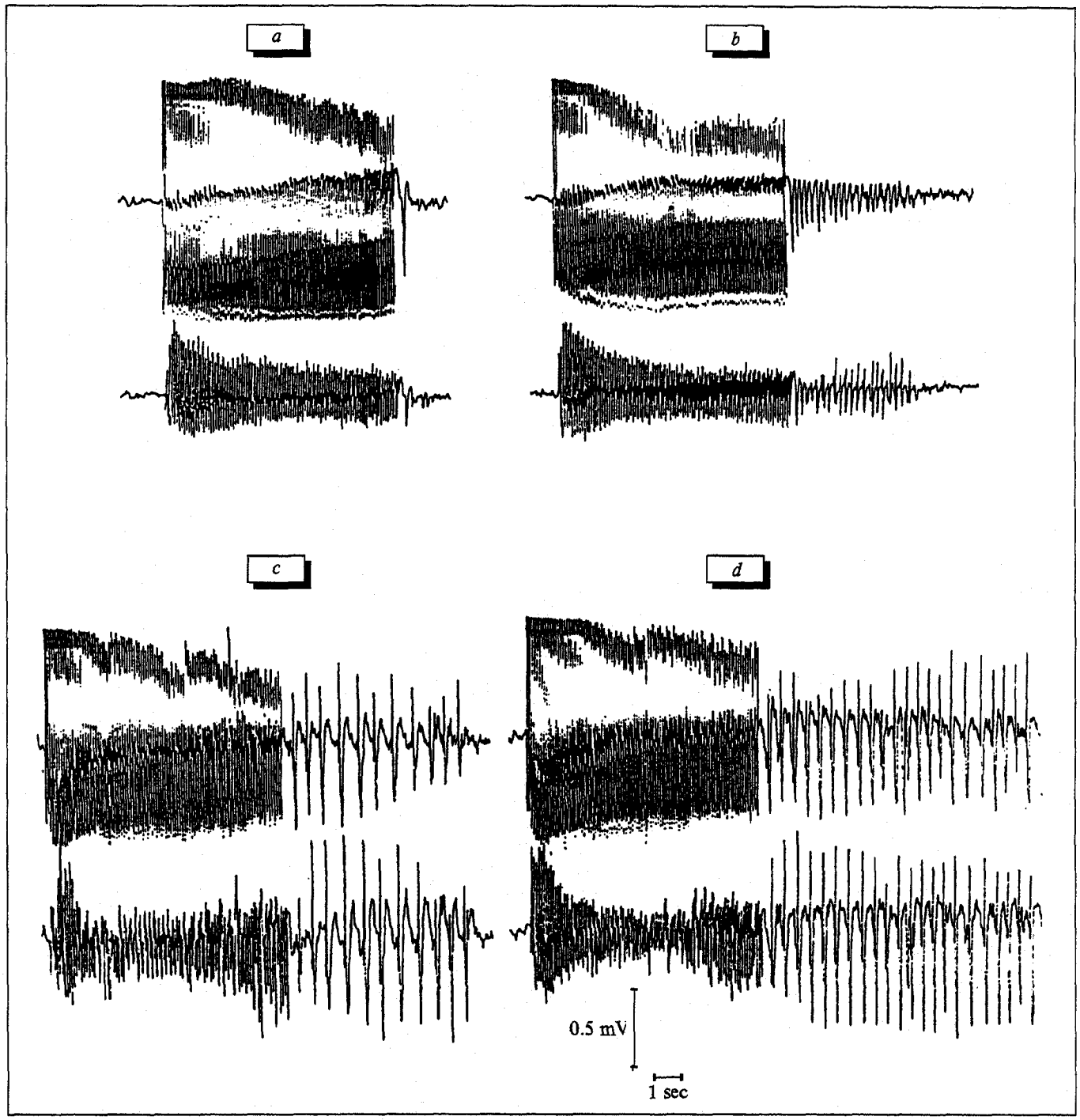

Fig 2. Electrocorticograms of the sensorimotor cortex of a rat not treated with CH during and after a single RES series (first: *a* and *c*; fifth: *b* and *d*). Here and on Fig. 3: upper tracings indicate the direct response and SD in the region of electrical stimulation, lower tracings indicate the transcallosal response and SD in the synaptically activated region of the contralateral hemisphere. *a* and *b*) the first day; *c* and *d*) the second day of brain stimulation.

In the first RES session CH did not affect the latency or duration (1-2 sec usually) of the first SD (Fig. 1, *b*). Nevertheless, the potentiation of SD amplitude and duration was subsequently suppressed by CH. An increase of the SD amplitude and duration occurred in the control during repeated RES (Figs. 1, *d*; 2). The stable SD in the control lasted an average of 25-30 sec and their maximal duration attained 80-150 sec. The duration of SD in rats pretreated with CH practically did not increase with repeated RES (Fig. 1, *d*, bottom curve; Fig. 3). SD duration in test animals was markedly lesser ($p<0.01$) beginning with the second SD (Fig. 1, *d*). In this case stable SD

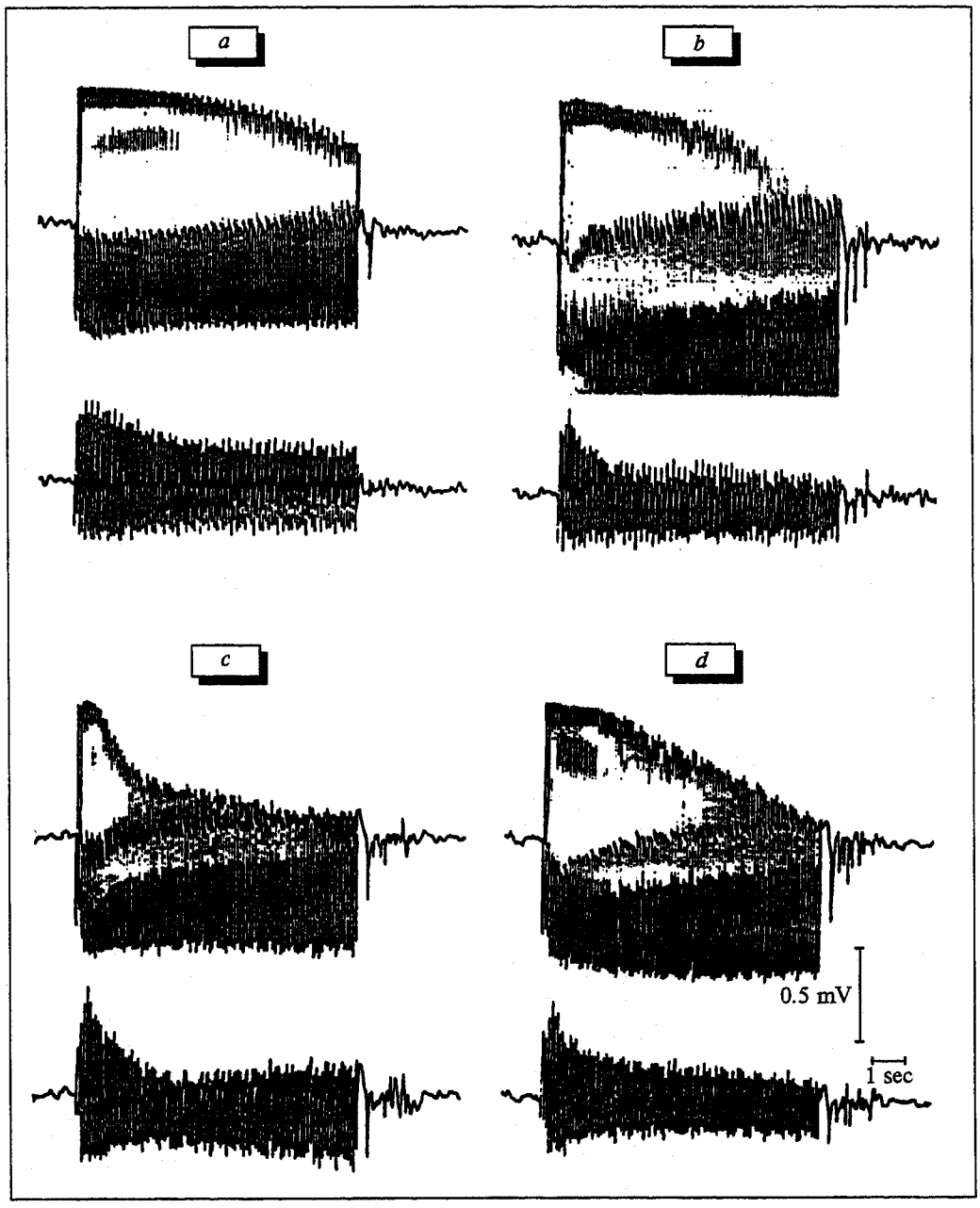

Fig. 3. Electrocorticograms from the sensorimotor cortex of a rat pretreated with CH at 2 mg/kg 2 h prior to the first RES series.

lasted 3-5 sec only and maximal SD duration was not over 10-12 sec (Figs. 1, *d*; 3). SD amplitudes progressively increased during repeated RES similarly in the control and in the test (Fig. 1, *c*).

Seizure readiness was assessed one day after the end of the first RES session using an analogous shortened RES session (Figs. 1, *b*, *c*; 2, 3). Animals not administered CH exhibited a marked decrease of the number of RES necessary to elicit the first SD (4.5±0.9 on the 1st day and 1.6±0.2 on the 2nd day, $p<0.05$, Fig. 1, *b*). In addition, the duration and, in particular, the amplitude of SD appearing in the control rats significantly exceeded the corresponding parameters of SD found in the same animal during the first stimulation session the day before (Figs. 1, *c*; 2). The latency of the first SD as well as the SD parameters practically did not differ on the 1st and 2nd days of the experiment and the potentiation of the amplitude and duration of SD during test RES which was characteristic for control animals (Figs. 1, *c*; 2) did not take place in CH-treated rats (Figs. 1, *c*; 3).

The data above attest to the unequal sensitivity of the functional processes in the sensorimotor cortex to a protein synthesis inhibitor. The thresholds of the direct and transcallosal responses, the dynamics of their parameters during an RES series, as well as the poststimulation stepped-up brain excitability after 20 RES are virtually the same in control and test rats. These findings and

evidence of the resistance of baseline brain rhythms to CH action [4] indicate that the processes maintaining ongoing brain activity are resistant to the inhibition of protein synthesis.

The latency and duration of the first SD are not sensitive to CH, whereas the cumulative trace processes governing the progressive prolongation of SD in repeated RES were abolished after CH administration (Figs. 1, *d*; 3). These data are reminiscent of the same effect of protein synthesis inhibitors on posttetanic potentiation in the hippocampus, the early stage of which is resistant to the action of CH and anisomycin, whereas the long-term stage is abolished by them [7,11]. Abolished potentiation of SD duration as well as delayed development of the generalized seizure syndrome have been described previously in studies of classical amygdalar kindling [8,10].

Experiments on the frog hippocampus have shown that CH does not affect SD that have already developed, not being an anticonvulsant in the ordinary sense [9]. It is assumed that the development of seizure readiness in kindling is due to the synthesis of new proteins [8,10]. Data on the effect of protein synthesis inhibitors on long-term potentiation of the exciting postsynaptic potential in the dentate gyrus attest that the synthesis of proteins required for the maintenance of heightened neuronal excitability terminates 15 min after the end of tetanization. Since the RES series in our experiments are separated by a comparable interval (10 min) and the prolongation of SD after each RES series is canceled by CH, the new protein synthesis may well be involved in the development of seizure readiness. This assumption is in agreement with direct evidence of the synthesis of new polypeptides after the completion of RES [14] as well as with the detection of new proteins in the extracellular space after the establishment of long-term potentiation in the hippocampus [4,5]. The functional importance of poststimulation chemical modifications in the brain is confirmed by our data on the proconvulsive action of mixed peptides extracted from the primary and secondary foci of SD generation at the stage of increased seizure readiness.

A state of seizure readiness is preserved in the sensorimotor cortex of CH-free rats for at least 24 h (the period of observation). This is indicated by the smaller number of the RES required for the initiation of the first SD and the increased amplitude of SD induced during the second RES session performed 24 h after the end of the first session (Figs. 1, *b*, *c*; 2). Administration of CH prevents the increased seizure readiness trace from developing, namely, the latency and amplitude of SD induced during the second RES session does not differ from those in the first (Figs. 1, *b*, *c*; 3). The differences in SD parameters recorded in the two RES sessions cannot be explained just by the preservation ("memory") of the high level of seizure readiness induced by the first RES session for 24 h and CH action cannot be considered as absolute proof of the role of protein synthesis in the fixation of this state. The SD amplitude in the second session is several times greater than that in the first session in the same rat free of CH. Therefore, not only is seizure readiness preserved, but some kind of process develops during the 24 h that favors the increase of neuron mass generating SD (Figs. 1, *c*; 2) [2].

The most likely cause of the proliferation of neurons involved in SD is RES-induced abrogation of neuron inhibition. As has been shown in acute experiments on the hippocampus, rhythmic stimulation of the afferent tract markedly weakens GABA-ergic inhibition [12,13]. The decreased inhibition of neurons observed in kindling is probably due to the selective death of interneurons [10].

The potentiation of SD duration is inhibited in CH-treated rats not only in the first but also in the second RES session (Fig. 3). The progressive action of CH on the mechanism of SD potentiation may underlie the observed similarity of its effects. It is worth noting that a lowered convulsive response to a sound stimulus is still retained in rats with audiogenic seizures 24 h after CH administration (at 2 mg/kg) [3].

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