Memory Impairment after Incomplete Pharmacological Kindling

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In rats trained to run for food reinforcement, kindling was reproduced by daily injections of subconvulsant doses of picrotoxin until the stage of recurrent clonic seizures. Subsequent behavioral test revealed no difference between kindled and control rats in retention and retrieval of memory traces. However these groups differed significantly in the dynamics and pattern of extinction: kindled animals needed much more time to stop running under conditions of non-reinforcement. A relearning test showed that kindling did not affect learning ability. Weakened inhibition after incomplete pharmacological kindling is indicative of a congnitive dysfunction characteristic of early stages of epilepsy.

Key Words: *seizures; picrotoxin; kindling; memory*

Kindling is a phenomenon of gradual enhancement of responsiveness to subconvulsant stimuli culminating in generalized seizure. This phenomenon is considered to be the most adequate model of epileptogenesis [4, 5]. Kindling induces persistent structural and functional changes in some brain regions, in particular in the hippocampus [9], which can result in learning and memory disorders. These disorders were observed in experimental studies, and despite some controversies, it may be concluded that kindling predominantly impairs retrieval of memory traces [7,10,13], leaving memory formation unaffected [8,10,11,13]. The majority of studies applied the complete kindling inducing generalized motor convulsions and usually accompanied by pronounced morphological changes. The question arises, whether a minor decrease in the convulsive threshold is sufficient for the appearance of cognitive dysfunction in animals. The description of memory impairment in the initial stages of kindling could be useful for early diagnosis of epilepsy. The main goal of this study was to determine the nature of memory impairment in incompletely kindled animals.

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MATERIALS AND METHODS

The study was carried out on male Wistar rats (n=18) weighing initially 160-170 g. The animals were maintained under standard conditions and fed special diet during the experimental period. The study included the following stages:

1. *Learning.* Rats were trained to reach a shelf with food reinforcement for less than 10 sec. The training was performed in a special chamber for 5 days (10 trials a day). The learning procedure was described in detail [6].

2. *Kindling.* After training the rats were divided into control (n=8) and experimental (n=10) groups. Experimental rats were injected daily with intraperitoneal picrotoxin (Fluka) in a subconvulsant dose of 1.5 mg/kg. The development of seizures was visually assessed in points [4]. After 21 injections the convulsive activity manifested itself in recurrent clonic seizures (3 points). Control rats received intraperitoneal saline.

3. *Retention.* Two days after the completion of kindling all animals were tested for the retention of the acquired habit. They were placed into the training chamber and the time of reaching the target shelf was measured.

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4. *Extinction.* The time and number of conditioned reactions without reinforcement were recorded for 2 days in the same training chamber.

5. *Relearning.* The final stage of the experiment was designed to assess the learning abilities of animals with lowered seizure thresholds. Both control and experimental rats were trained for 3 days to acquire a new habit, similar to the initial one, i. e., to run to another shelf in the same chamber.

The data were analyzed statistically with Student's *t*-test.

RESULTS

The development of kindling was manifested in a gradual increase in seizure activity in response to picrotoxin, clonic seizures appeared after 21 injections. During this period the animals were not tested in the training chamber.

Retention of the acquired habit was tested 25 days after training and showed good scores in both groups (Fig. 1, *b*). Only the first run took more than 10 sec. This test showed that kindling did not affect the long-term storage and retrieval of memory. The equal level of retention in the control and experimental groups allowed us to investigate the extinction of the acquired habit.

The habit disappeared after 2 days of non-reinforcement. The time of conditioned reaction increased and after several non-reinforced trials the rats did not reproduce the reaction. The control rats showed more rapid extinction (Fig. 1, *c*): the initial extinction (duration of the first run more than 150 sec) required 6.2± 0.5 trials *vs.* 13.03±1.1 trials in the experimental group ($p<0.001$). When placed in the training chamber, experimental rats exhibited high exploratory activity with a persistent orienting reaction.

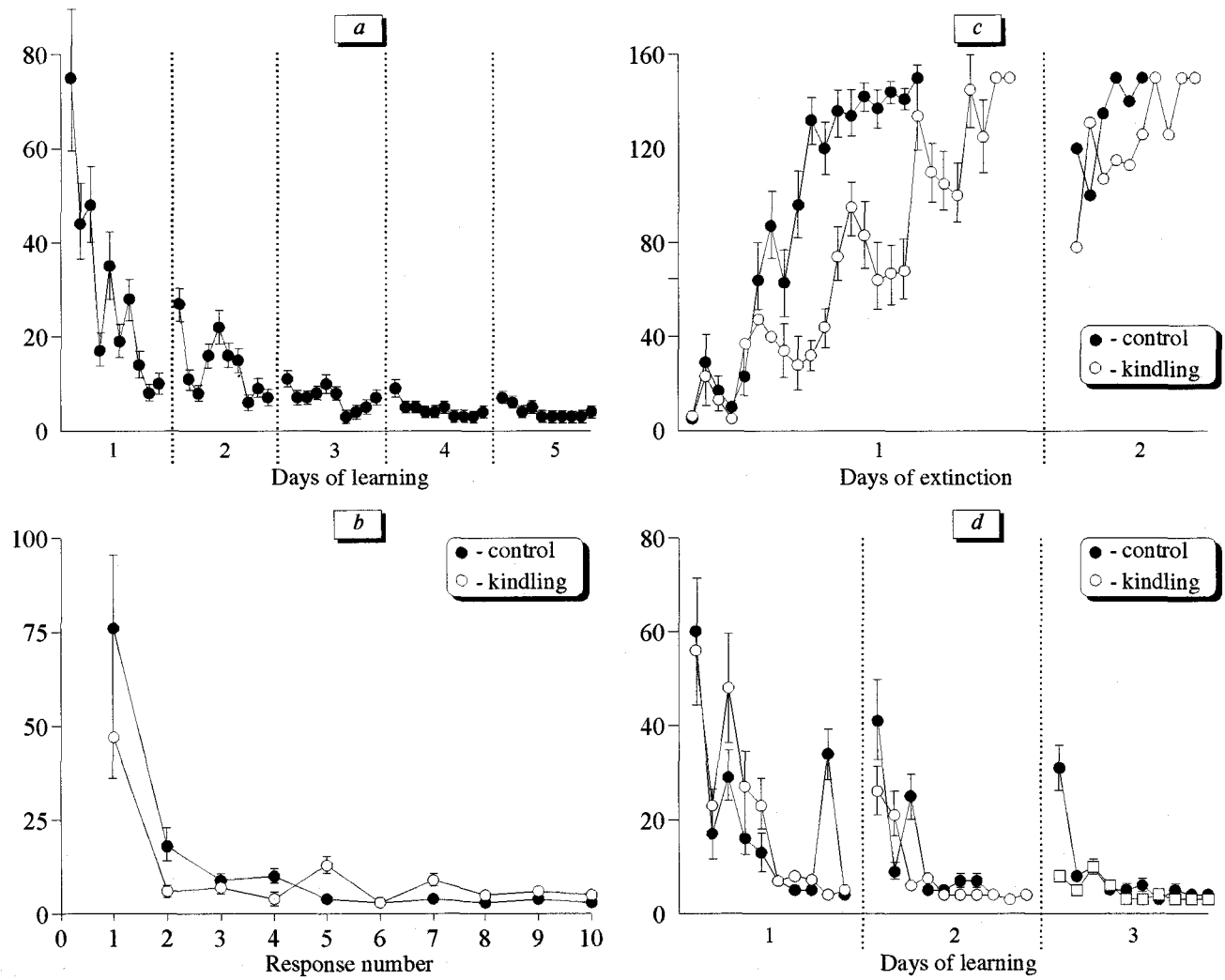

Fig. 1. Dynamics of learning (*a*), retention (*b*), and extinction of an acquired habit (c) and the dynamics of relearning (*d*) in Wistar rats. Ordinates: time of reaction, sec. Points in *a*, *c*, and *d* correspond to individual trials.

Both control and experimental rats successfully acquired a new task showing no significant difference in their scores, although experimental rats performed it even better than controls (Fig. 1, *d*). However, the number of errors (runs to previously conditioned target) significantly differed in these groups: 3.2±3.1 and 0.6±0.5 in experimental and control group, respectively ($p<0.02$), on day 1 of relearning.

Thus, daily injections of picrotoxin moderately decreased seizure threshold. This decrease caused functional disorder of a "hippocampal type", which manifested itself in weakened inhibitory reactions and intensified orienting behavior. These changes are typical of hippocampal dysfunction [3,12] and allert the formation of a kindling-induced epileptic pathological system with a determinant within lymbic structures [5,9].

Our results are in line with previous data showing minor effects of kindling on memory acquisition [8, 10,11,12]. The impairment in the retrieval of memory traces [7,10,12] can be caused by more intense kindling than in our study. The effects of kindling on cognitive functions need further investigation. Using behavioral test sensitive to hippocampal dysfunction [1,2] we showed that even moderate lowering of seizure thresholds leads to marked and specific cognitive disorders, which are primarily manifested in weakening of inhibitory processes in the brain. This finding can be used for revealing the early preconvulsive manifestations of epilepsy.

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REFERENCES

1. V. I. Arkhipov, *Zh. Vyssh. Nervn. Deyat.*, **48,** No.5, 836-845 (1998).
2. V. I. Arkhipov and A. A. Azarashvili, *Ibid.*, **35,** No. 5, 920-925 (1985).
3. O. S. Vinogradova, *Hippocampus and Memory* [in Russian], Moscow (1975).
4. G. N. Kryzhanovskii, A. A. Shandra, R. F. Makul'kin, *et al., Byull. Eksp. Biol. Med.*, **99,** No. 5, 525-532 (1985).
5. A. A. Shandra, L. S. Godlevskii, and A. M. Mazarati, *Uspekhi Fiziol. Nauk.*, **21,** No. 4, 50-67 (1990).
6. V. I. Arkhipov, *Behav. Neural. Biol.*, **57,** 244-247 (1992).
7. A. Becker and G. Grecksch, *Pharmacol. Biochem. Behav.*, **52,** 765-769 (1995).
8. S. Cammisuli, M. P. Murphy, C. J. Ikeda-Douglas, *et al., Behav. Brain Res.*, **89,** No. 1-2, 179-190 (1997).
9. J. E. Cavazos, I. Das, and T. P. Sutula, *J. Neurosci.*, **14,** No. 5, Pt. II, 3106-3121 (1994).
10. M. G. Genkova-Papazova and M. B. Lazarova-Bakarova, *Eur. Neuropsychopharmacol.*, **5,** No. 1, 53-56 (1995).
11. G. L. Holmes, A. Chronopoulos, C. E. Stafstrom, *et al., Dev. Brain Res.*, **73,** No.1, 71-77 (1993).
12. K. H. Pribram, *The Hippocampus,* Eds. R. L. Isaacson and K. H. Pribram, Vol. 4, New York (1985), pp. VII-XIII.
13. T. Sutula, S. Lauersdorf, M. Lynch, *et al., J. Neurosci.*, **15,** No. 12, 8295-8301 (1995).