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Kierownik: prof. dr hab. n. med. Zdzisław Kleinrok

Paweł MILART, Waldemar TURSKI,
Stanisław CZUCZWAR, Zdzisław KLEINROK

Effects of Atropine, Pilocarpine and Morphine on Footshock-induced Aggressive Behaviour in Rats after Lesion of Hippocampal Pyramidal Cells with Kainic Acid

Wpływ atropiny, pilokarpiny oraz morfiny na agresywne zachowanie się szczurów z uszkodzonymi kwasem kainowym piramidalnymi komórkami hipokampa

Влияние атропина, пилокарпина и морфина на агрессивное поведение крыс с поврежденными каиновой кислотой пирамидальными клетками гиппокампа

It is generally considered that central cholinergic pathways influence the aggressive behaviour in rats (1). Electrolytic lesions of the dorsal hippocampus attenuated the footshock-induced behavioral suppression (6, 8, 23), but medial septal lesions greatly increased aggressiveness and resulted in hyperreactivity (24). The hippocampal pyramidal cells which contribute to the feedback regulation of septal cholinergic neurons (18, 19, 30) project also to the hypothalamus (27). Septal (9) and hypothalamic (2) cholinergic mechanisms have been shown to be mainly involved in the control of aggressive behaviour. However, it has been implicated that septal-hippocampal cholinergic neurons have an inhibitory role in emotional behaviour (26). On the other hand, following kainic acid-induced lesion of the hippocampal pyramidal cells, the activity of the cholinergic spetal-hippocampal neurons was unaltered (30). In this respect the possibility arises that the septum can display its activity in the absence of hippocampal feedback control (31). Thus, the changes in the septal activity and its behavioural consequences, following acute stimulation in hippocampally lesioned animals with the use of kainic acid, appear to be an open question. Since the role of the hippocampal glutaminergic pro-

jection in the regulation and control of agressive behaviour is unclear, the present studies were designed.

MATERIALS AND METHODS

Animals. Experiments were performed on male "Wistar" rats weighing from 180 to 230 g. The animals were housed in colony cages, being maintained with a natural light-dark cycle. Chow pellets (Murigram®, Bacutil) and tap water were continuously available.

Electrical Foot Shock Aggression. "Foot-shock" aggression was evaluated by means of the procedure described by Tedeschi et al. (29), with minor modifications. Paired, male Wistar rats were placed in a glass circular compartment, 20 cm in diameter and 20 cm high. Its floor was constructed with stainless-steel rods (0.2 cm in diameter) spaced in parallel (0.5 cm apart, center to center). Electric shocks (0.9 mA; 0.1 sec) were delivered through the grid floor every 1 sec for 10 min. Upright boxing posture of both rats standing face to face, and pushed with their forepaws has been accepted as a fighting position. Aggressive behaviour has been recorded by two separate observers. Aggression intensity was assessed as a total time of fighting expressed in seconds. Moreover, the number of fighting positions, lasting 1—5, 6—10, 11—20, 21—30, 31—40, 41—50 and 51—60 sec during the whole test, has been considered. Each pair of rats was used only once. Sham-control rats were treated by means of the identical procedure and were always tested at the same time.

Kainic acid administration. Kainic acid (KA; Sigma, St. Louis, Mo., USA) was freshly dissolved in sterile buffered saline ($pH=7.35$) and administered in a dose of 0.1 μg and in a volume of 10 μl into the lateral brain ventricle of unanesthetized rats, using Hamilton microsyringe, according to Herman (10), procedure in details described by Kleinrok and Turski (11) elsewhere. Control animals received saline injections by intracerebroventricular route.

Drugs. The following drugs were used: atropine sulfate (Polfa, Warsaw, Poland), pilocarpine hydrochloride (Polfa, Warsaw, Poland) and morphine hydrochloride (Polfa, Kutno, Poland). All drugs were dissolved in sterile saline and were given intraperitoneally (IP) in a volume of 0.5 ml/100 g body weight. Atropine was given in a dose of 5.0 $mg \cdot kg^{-1}$, pilocarpine — 1.0 $mg \cdot kg^{-1}$ and morphine — 1.5 $mg \cdot kg^{-1}$. Drugs were administered 30 min before the test, after 240 h from the time of KA injection. Control rats received saline only. All doses of the drugs refer to the salt forms.

Histological examination. After completion of the behavioral experiments the lesioned animals were sacrificed by decapitation. The brains were dissected carefully and fixed in 10% neutral buffered formalin. Then the brains were embedded in paraffin and 10 micron sections were taken. The brains were cut coronally and every fifth section was collected and stained with hematoxylin and eosin.

Statistics. The data collected from the behavioural experiments were treated by means of Mann-Whitney U-test.

RESULTS

Histology

Unilateral intraventricular injection of kainic acid in a dose of 0.1 μg was found to cause neuronal degeneration of hippocampal pyramidal cells in the subfield CA 3/CA 4. Additional damage included small decrease of neuronal density in the hippocampal subfield CA_1 and the layers V and VI of the neocortex above the injection site. The histological documentation was presented elsewhere (13).

Aggressive behaviour

Effects of kainic acid lesion on electrical footshock aggression. Relatively selective lesion of the hippocampal pyramidal cells with kainic acid (0.1 μg) considerably prolonged the total fighting time and increased the number of fighting positions lasting from 51 to 60 sec. These effects have been shown from 24 h up to 480 h after kainic acid injection (Figs. 1 and 2).

Effect of atropine, pilocarpine and morphine on the aggressive behaviour in sham-rats (Figs. 3 and 4). Atropine in a dose of 5.0 $mg \cdot kg^{-1}$, administered IP 30 min before the

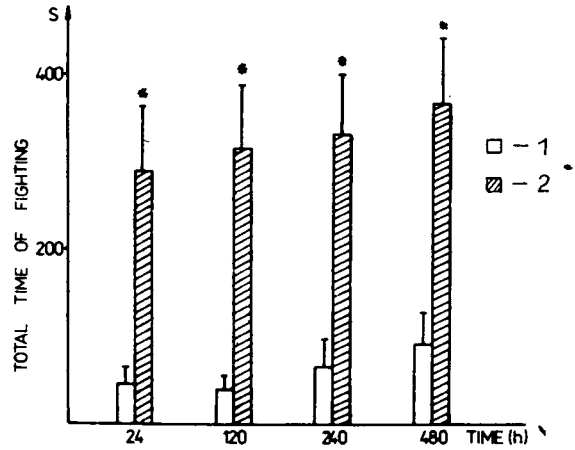

Fig. 1. Footshock-induced aggressive behaviour in the rats after lesion of the hippocampal pyramidal cells with kainic acid; kainic acid was administered 24, 120, 240 and 480 h before the test; each value: mean of the total time of aggression in 8 pairs of the animals; 1 — sham-lesioned; 2 — kainic acid 0.1 μg; values of kainic acid were compared with those found in sham-lesioned; * $P<0.01$ for the entire period of observations

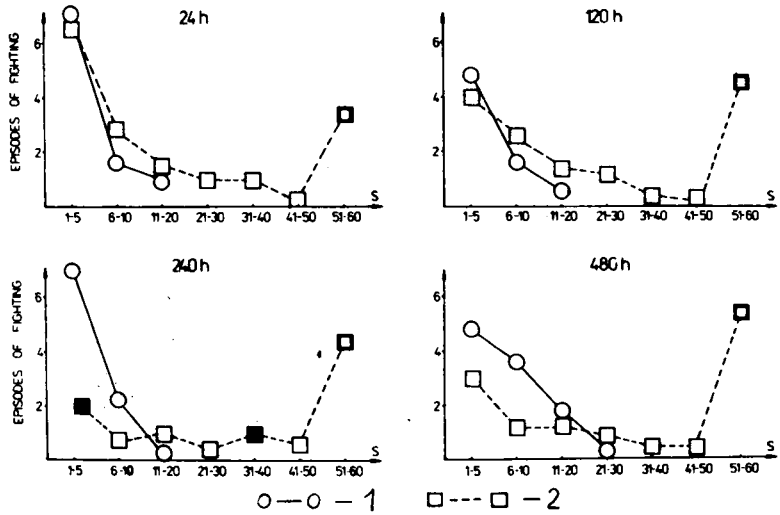

Fig. 2. Effect of the lesion of the hippocampal pyramidal cells with kainic acid on the time-schedule of the episodes of fighting; episodes lasting 1—5, 6—10, 11—20, 20—30, 31—40, 41—50 and 51—60 were scored during 10 min. test; kainic acid was administered 24, 120, 240 and 480 h before the test; each point: mean of 8 determinations; 1 — sham-lesioned, 2 — kainic acid 0.1 μg; values of kainic acid in both doses were compared with those found in sham-lesioned; $P<0.05$, filled squares; $P<0.01$, doubled squares

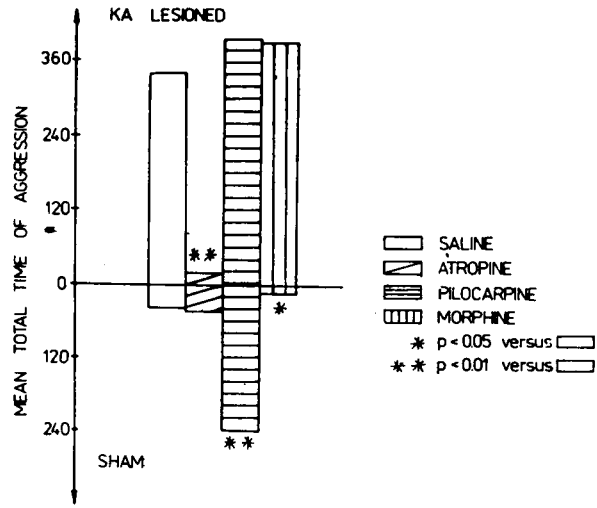

Fig. 3. Effect of atropine, pilocarpine and morphine on footshock induced aggressive behaviour in the rats after lesion of the hippocampal pyramidal cells with kainic acid; kainic acid at the dose of 0.1 μg was administered 10 days before the test; atropine (5.0 $mg \cdot kg^{-1}$), pilocarpine (1.0 $mg \cdot kg^{-1}$) and morphine (1.5 $mg \cdot kg^{-1}$) were administered 30 min before test; each value: mean of measurements in 8 pairs of the animals; values of the kainic acid-lesioned rats treated with atropine, pilocarpine or morphine were compared with those of the kainic acid-lesioned+saline; values of sham-lesioned rats treated with the above drugs were compared with those of sham-lesioned+saline; values of sham-lesioned rats treated with the above drugs were compared with those of sham-lesioned+saline; * $P<0.05$, ** $P<0.01$ (Mann-Whitney U-test)

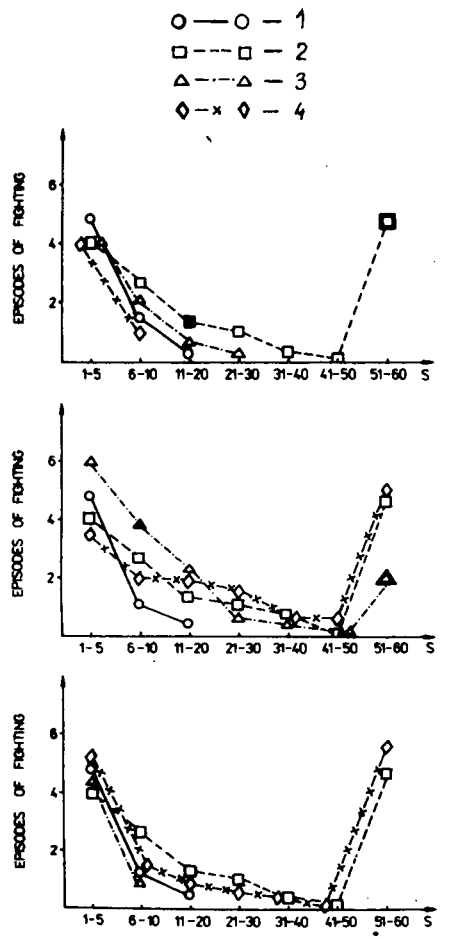

Fig. 4. Effect of the lesion of hippocampal pyramidal cells with kainic acid on the time-schedule of the episodes of fighting in the rats treated with atropine, pilocarpine and morphine; episodes lasting 1—5, 6—10, 11—20, 21—30, 31—40, 41—50 and 51—60 s were scored during 10 min test; kainic acid at the dose of fore the test; each point: mean of 8 fore the test; each pint: mean of 8 determinations. Upper panel: 1 — sham-lesioned+saline, 2 — kainic acid+saline, 3 — sham-lesioned+ +atropine 5.0 mg · kg^{-1}, 4 — kainic acid+atropine; values of sham-lesioned+atropine were compared with those of sham-lesioned+saline (filled triangles); values of kainic acid+saline were compared with those of kainic acid+atropine ($P<0.05$ filled squares, $P<0.01$ doubled squares). Middle panel: 1 — sham-lesioned+saline, 2 — kainic acid+ +saline, 3 — sham-lesioned+pilocarpine 1.0 mg · kg^{-1}, 4 — kainic acid+pilocarpine; values of sham-lesioned+pilocarpine were compared with those of sham-lesioned+ +saline ($P<0.05$ filled triangles, $P<0.01$ doubled triangles); values of kainic acid+pilocarpine ($P<0.05$ filled squares). Lower panel: 1 — sham-lesioned+saline, 2 — kainic acid+saline, 3 — sham-lesioned+morphine 1.5 mg · kg^{-1}, 4 — kainic acid+morphine; values of sham-lesioned+morphine were compared with those of sham-lesioned+saline ($P<0.05$ filled triangles); values of kainic acid+saline were compared with those of kainic+morphine ($P<0.05$ filled squares)

test did not affect the intensity of the aggressive behaviour regarding the total fighting time and the number of fighting positions in sham-operated rats. On the other hand, pilocarpine at the dose of 1.0 mg · kg^{-1} (IP, 30 min before the test) considerably increased the fighting time ($P<0.01$), and the number of fighting positions lasting 6—10 sec and 51—60 sec. Morphine, administered IP at the dose of 1.5 mg · kg^{-1} (30 min) significantly shortened the total fighting time in the sham-lesioned animals but failed to influence the number of fighting positions.

Effect of atropine, pilocarpine and morphine on the aggressive behaviour in the kainic acid-lesioned rats (Figs. 3 and 4). Atropine significantly shortened the total fighting time ($P<0.01$) and considerably decreased the number of fighting positions lasting 51—60 sec in the KA-lesioned rats. However, pilocarpine did not increase aggression scores in the KA-lesioned rats. Moreover, morphine was also ineffective regarding the inhibition of the aggressive behaviour in the KA-lesioned rats.

DISCUSSION

The most characteristic feature of the KA administration was a degeneration of the pyramidal cells within the hippocampal area. It is of particular interest that the initial symptoms of the degeneration were reported to occur as early as 3 h after injection of the neurotoxin (22). The results shown in the present experiments indicate that relatively selective lesion of the hippocampal pyramidal cells with KA markedly increases the aggressive behaviour in the rats. Specifically, the total time of fighting has been greatly prolonged in the KA-lesioned rats which additionally exhibited a significant increase in the long-lasting fighting positions. However, the apparent increase of the aggressive behaviour was attenuated by atropine which was ineffective regarding electric footshock-induced behaviour in the sham-rats. These observations may suggest that cholinergic mechanisms have been involved in the facilitation of aggression in the KA-lesioned rats.

Hippocampal pyramidal cells project to the lateral septum (5, 20) where they contribute to the feedback regulation (via GABA-ergic interneurons) of the cholinergic cells (17, 18, 19). A partial liberation of the cholinergic neurons from the feedback control, involving the intact pyramidal projection, might be responsible for the increase of the aggressive behaviour in the KA-lesioned rats. In a separate paper, we reported on a decrease in the activity of glutamic acid decarboxylase (an enzyme synthetizing GABA) and in the level of GABA in the hippocampus of the KA-lesioned rats (14). On the other hand, GABA-ergic stimulation was shown to inhibit the aggressive behaviour (16) so the GABA deficit in the KA-treated rats might be another factor potentiating the aggressive response.

Furthermore, pilocarpine was shown to enhance significantly the fighting time and the number of fighting positions in the sham-operated rats, which is in a good agreement with the results obtained by Roliński and Herbut in mice (24), while it failed to influence aggression scores in the KA-lesioned animals. One may assume, that in

the KA-treated rats the septal-hippocampal system, deprived of the feedback inhibition, is totally activated and this might be the reason for the lack of any pilocarpine effect.

In addition, morphine was ineffective regarding aggressive behaviour in the KA-lesioned rats but significantly reduced the total time of fighting in the sham-control rats. There is a concurrence of opinion that β-endorphin, among other neurotransmitters, may affect the activity of the septal cholinergic neurons (21). Moreover, it has been shown that enkephalins may inhibit intrahippocampal inhibitory interneurons (15) and increase a discharge of the hippocampal pyramidal cells (7). These findings directly demonstrated that opiate system exhibited inhibitory effects upon the septal cholinergic neurons not only at the level of the medial septum but also in the hippocampus. The attenuation of the inhibitory effects of morphine in the KA-lesioned rats fits well this hypothesis. On the other hand, it should be emphasized that the hipocampal pyramidal cells project also to the hypothalamus (28). The hypothalamus has been suggested to be of importance in the control of aggressive behaviour in rats (1). Taken together, it is not clear whether the septal-hippocampal system is fully responsible for the observed behavioural changes following KA-lesion. Moreover, recent biochemical data gave evidence for the existence of a specific cholinergic system in the dorsal hippocampus responsible for stress-induced behavioural suppression (26).

It is of particular interest that the KA-lesioned mice exhibited higher susceptibility to chemoconvulsions (3, 12), and the activity of some anticonvulsant agents was distinctly reduced in such animals (4). These results were also interpreted in terms of a disturbed feedback control of the septal cholinergic cells. In summary, it is noteworthy that the septal region has complex neuronal inputs involving different neurotransmitters which may modulate behavioural responses of different types (30). Even so, further investigations, possibly with the neurotoxins more selectively destroying neuronal cell bodies, are required to elucidate the common places and neurotransmitters involved in producing the behavioural phenomena reported in the present paper.

REFERENCES

1. Allikmets L. H.: Cholinergic Mechanisms in Aggressive Behaviour. Med. Biol. **52**, 19, 1974.
2. Bandler R. J.: Facilitation of Aggressive Behaviour in Rats by Direct Cholinergic Stimulation of the Hypothalamus. Nature (London) **224**, 1035, 1969.
3. Czuczwar S. J. et al.: Atropine Reversal of Kainic Acid-induced Decrease in the Leptazol Convulsive Threshold. J. Pharm. Pharmacol. **33**, 44, 1981.

4. Czuczwar S. J. et al.: Effects of some Antiepileptic Drugs in Pentetrazol-induced Convulsions in Mice Lesioned with Kainic Acid. Epilepsia **22**, 407, 1981.
5. De France J. F. et al.: Electrophysiological Analysis of the Hippocampal-septal Projections: II. Functional Characteristics. Exp. Brain. Res. **17**, 63, 1973.
6. Douglas R. J.: The Hippocampus and Behavior. Psychol. Bull. **67**, 416, 1967.
7. Dunwiddie T. et al.: Electrophysiological Interactions of Enkephalins with Neuronal Circuitry in the Rat Hippocampus. I. Effects on Pyramidal Cell Activity. Brain Res. **184**, 311, 1980.
8. Glickman S. E. et al.: Some Effects of Hippocampal Lesions on Behavior of Mongolian Gerbils. Physiol. Behav. **5**, 931, 1970.
9. Hernandez-Peon R. et al.: Limbic Cholinergic Pathways Involved in Sleep and Emotional Behavior. Exp. Neurol. **8**, 93, 1963.
10. Herman Z. S.: Effects of Noradrenaline on Rats Behaviour. Psychopharmacologia **16**, 369, 1980.
11. Kleinrok Z., Turski L.: Kainic Acid-induced Wet-dog Shakes in Rats: The Relation to Central Neurotransmitters. Naunyn-Schmiedeberg's Arch. Pharmacol. **314**, 37, 1980.
12. Kleinrok Z. et al.: Effect of Intracerebroventricular Injection of Kainic Acid on Electrically and Chemically Induced Convulsions in Mice. Pol. J. Pharmacol. Pharm. **33**, 265, 1980.
13. Kleinrok Z. et al.: The Locomotor and Exploratory Activities in Rats after Lession of Hippocampal Pyramidal Cells with Kainic Acid. Pol. J. Pharmacol. Pharm. **32**, 625, 1980.
14. Kleinrok Z., Turski L.: Biochemical Consequences of Kainic Acid Injection into the Lateral Brain Ventricle in Rat. Acta Biochem. Pol. **28**, 111, 1981.
15. Lee H. K. et al.: Electrophysiological Interactions of Enkephalins with Neuronal Circuitry in the Rat Hippocampus. II. Effects on Interneuron Excitability. Brain Res. **184**, 331, 1980.
16. Mandel P. et al.: Molecular Aspects of a Model of Aggressive Behavior. [In:] Interactions between Putative Neurotransmitters in the Brain. Ed. by Garattini S. Pujol J. F. and Samanin R. Raven Press, New York 1968, 285.
17. McLennan H., Miller J. J.: The Hippocampal Control of Neuronal Discharges in the Septum of the Rat. J. Physiol. (London) **237**, 607, 1974.
18. McLennan H., Miller J. J.: γ-Aminobutyric Acid and Inhibition in the Septal Nuclei of the Rat. I. Physiol. (London) **237**, 625, 1974.
19. McLennan H., Miller J. J.: Frequency-related Inhibitory Mechanisms Controlling Rhythmical Activity in the Septal Area. J. Physiol. (London) **254**, 827, 1976.
20. Meibach R. C., Siegel A.: Afferent Connections of the Hippocampal Formation in the Rat. Brain Res. **124**, 197, 1977.
21. Moroni F. et al.: Inhibition of Acetylcholine Turnover in Rat Hippocampus by Intraseptal Injections of β-endorphin and Morphine. Naunyn-Schmiedeberg's Arch. Pharmacol. **299**, 149, 1977.
22. Obeta H. L. et al.: The Effects of Small Doses of Kainic Acid on the Area Ca 3 of the Hippocampal Formation. An Electron Microscopic Study. Arch. Histol. Jap. **44**, 135, 1981.
23. Olton D. S.: Shock Motivated Avoidance and the Analysis of Behavior. Psychol. Bull. **79**, 243, 1973.
24. Roliński Z., Herbut M.: The Role of the Cholinergic System in Foot-shock-induced Mouse Aggression. Pol. J. Pharmacol. Pharm. **33**, 177, 1981.

25. Russell R. W., Macri J.: Central Cholinergic Involvement in Behavior Hyper-reactivity. Pharm. Bioch. Behav. **10**, 43, 1978.
26. Schmidt D. E. et al.: Strain Specific Alternations in Hippocampal Chol nergic Function Following Acute Footshock. Pharm. Bioch. Behav. **12**, 277, 198
27. Stark P., Henderson J. K.: Central Cholinergic Suppression of Hype reactivity and Aggresion in Septal-lesioned Rats. Neuropharmacology **11**, 83 1972.
28. Storm-Mathisen J., Opsahl M. W.: Aspartate and/or Glutamate Ma be Transmitters in Hippocampal Efferents to Septum and Hypothalamu Neurosci. Lett. **9**, 65, 1978.
29. Tedeschi R. E. et al.: Effects of Various Centrally Acting Drugs (Fighting Behavior of Mice. J. Pharmacol. Exp. Ther. **28**, 125, 1959.
30. Weiskrantz L.: Functions of the Septo-hippocampal System. Ciba Fo Symp. Elsevier Exerpta Media, Elsevier, (Holland) **58**, 1978.
31. Wood P. L. et al.: The Turnover Rate of ACh in the Hippocampus aft Lesion of Hippocampal Pyramidal Cells with Kainic Acid. Neuropharmacolo **18**, 519, 1979.
32. Yokoto T., Jujimori B.: Effects of Brain-stem Stimulation upon Hipp campal Electrical Activity, Somatomotor Reflexes and Autonomic Functior Electroenceph. Clin. Neurophysiol. **16**, 375, 1964.

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STRESZCZENIE

Wywoływane stymulacją elektryczną agresywne zachowanie się szczurów by nasilone po dokomorowym podaniu kwasu kainowego (0,1 μg na szczura), wywoł jącego uszkodzenie komórek piramidalnych hipokampa. Atropina hamowała wzro agresywności u szczurów poddanych działaniu kwasu kainowego, natomiast n wpływała na parametry agresji u zwierząt kontrolnych. Natomiast pilokarpi wzmagała agresywne zachowanie się szczurów kontrolnych, lecz nie zmieniała u zwierząt, którym uprzednio podano kwas kainowy. W przeciwieństwie do te morfina nie wpływała na agresywność zwierząt uszkodzonych, ale silnie hamowa ją u szczurów kontrolnych. Uzyskane wyniki wskazują na rolę komórek piram dalnych hipokampa w regulacji agresywnego zachowania u szczurów.

РЕЗЮМЕ

Вызванное электрической стимуляцией агрессивное поведение крыс усил вается после применения в латеральный желудочек мозга каиновой кисло (0,1 μг на крысу), вызывающей повреждение пирамидальных клеток гипп кампа. Атропин тормозит усиление агрессивности крыс подвергнутых действ каиновой кислоты, а не влияет на параметры агрессии контрольных животны Зато пилокарпин усиливает агрессивное поведение контрольных животных, а влияет на агрессивность крыс подвергнутых действию каиновой кислоты. В пр тиворечии с этим морфин не влияет на агрессивность животных подвергнут действию каиновой кислоты, но отчетливо тормозит ее у крыс контрольны На основе проведенных исследований можно сделать вывод, что пирамидал ные клетки гиппокампа участвуют в регуляции агрессивного поведения кры