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FRONTAL CORTEX FUNCTIONAL ACTIVITY MODULATION IMPACT ON THE STEREOTYPIC, EMOTIONAL AND POSTURAL BEHAVIOR IN RATS DURING THE INTERICTAL PERIOD OF PILOCARPINE-INDUCED CHRONIC EPILEPTOGENESIS

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Abstract

The cholinergic mechanisms role determination in epileptogenesis attracts the attention of researchers. Pilocarpine administration in rats contributes to chronic form of epileptiform activity development characterized by the presence of a pronounced acute stage and an interictal period - free from behavioral convulsive reactions. We consider the most important feature of the pilocarpine-induced seizures interictal period might be the change of various forms of nonconvulsive behavior. Attempts to investigate the animals' behavioral reactions details during the seizure-free interictal period, as well as to determine the mechanisms of similar types of behavior formation, are interesting. The purpose of the work is to investigate

the motor, stereotypic and aggressive-defensive behavior of rats throughout the interictal period of pilocarpine-induced convulsive syndrome with a frontal cortex functional activity change. It was found that the severity of non-convulsive behavioral reactions in the interictal period during pilocarpine-induced chronic seizures is mostly determined by the frontal cortex functional state. At the same time, the frontal cortex hyperactivation is an important feature of pilocarpine-induced chronic epileptogenesis. The authors proved that when the frontal cortex is activated in rats, there is an increase in horizontal and vertical motor activity, as well as the expressiveness of emotional reactions in the "open field" test and the strengthening of the aggressive-defensive behavior. In conditions of this part of the cortex selective destruction the opposite behavioral effects are noted which confirms the important role of the frontal cortex in the interictal non-convulsive behavior formation. Observed behavioral effects during the frontal cortex functional activity modulation, according to the authors, indicate the reasonability of regulatory influences searching aiming forward this brain part to activate complex mechanisms aimed to pilocarpine-induced chronic epileptiform activity elimination.

Key words: pilocarpine; convulsive syndrome; chronic epileptogenesis; interictal period; non-convulsive forms of behavior; frontal cortex; pathogenetic mechanisms

It is important to study the role of cholinergic mechanisms in epileptogenesis, since the number of experimental models of chronic epileptic activity that would be relevant to clinical condition and allow to investigate the mechanisms of brain epileptization of the brain is critically reduced [15, 17, 18].

The most interesting results were obtained in trials with the agonist of cholinergic receptors – pilocarpine - use. Both systemic and intraventricular pilocarpine administration in large doses in rats and mice was found to induce behavioral and electrographic convulsive manifestations [8, 16, 19]. It has been proven that pilocarpine use at a dose of 280 mg/kg in rats contributes to chronic epileptiform activity formation characterized by an expressed acute stage and an interictal period - free from behavioral convulsive reactions [4, 5]. We consider the most important feature of the pilocarpine-induced seizures interictal period to be the change in nonconvulsive behavior. We identified the interictal period as the period of behavioural convulsive reactions absence. EEG registration revealed substantia nigra neurons activation in these conditions on the hippocampal electrical activity desynchronization background which we estimate as an integral indicator of the antiepileptic system activation [6].

Therefore, it is interesting to try to investigate the behavioral reactions peculiarities in animals during the seizure-free interictal period as well as to determine the mechanisms of similar types of behavior formation.

The aim of the work – is to investigate rat's stereotypic, aggressive-defensive and swimming behavior throughout the interictal period of pilocarpine-induced convulsive syndrome in conditions of frontal lobes functional activity changes.

Materials and Methods

Animals keeping, handling and manipulation was carried out in accordance with the "General Ethical Principles of Animal Experiments" adopted by the "General Ethical Principles of Animal Experiments" adopted by the Fifth National Congress on Bioethics (Kyiv, 2013) and was guided by the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1985) and guidelines of the State Pharmacological Center of the Ministry of Health of Ukraine on "Preclinical studies of drugs" (2001) as well as rules of humane treatment of experimental animals and conditions approved by the Committee on Bioethics of Odesa National Medical University.

Experimental trials were performed in conditions of chronic experiment on 55 male Wistar rats of sexually mature age, weighing from 180 to 250 g. To tame the animals and avoid their stress reaction in response to taking them with a forceps, the rats were held in their hands for 2-3 minutes before the start of the experiment within 5 days, which facilitated further experiments.

Monopolar electrodes (nichrome wire, tip diameter 0.10-0.15 mm, with varnish insulation, except for the tips), as well as cannulas were implanted into the frontal cortex of the large hemispheres to animals anesthetized with ketamine (2.0 mg/kg, ip/h) according to stereotaxic atlas coordinates (-AP=2.4; L=0.8; H=1.2) [11]. To prevent the development of infection, the animals were administered streptomycin at a dose of 30,000 IU/kg (i.v.) once a day for 5 days after surgery. The animals were taken to the experiment 7-10 days after the operation.

Frontal lobe activation was performed via its electrical stimulation (60 Hz, 0.1 ms, 400-450 μA, duration of stimulation - 1 s), and its destruction - by ibotenic acid solution ("Sigma-Aldrich", Germany; 2.0 μl) microinjection using a "Hamilton" microinjector ("SGE", Australia).

Pilocarpine was prepared in NaCl solution (p=7.4) immediately before the researches start and was administered i.p. at a dose of 280 mg/kg. Atropine (50 mg/kg) was administered i.v. 3 min before pilocarpine injection in a separate series of experiments.

Behavioural responses to pilocarpine-induced seizures were evaluated in animals throughout the interictal period. The number of stereotypic behavior elements was determined and the number of boluses was counted in the "open field" test within 2 min [1].

The aggressive-defensive behavior was assessed by the nature of the behavioural response of animals to an attempt to take in the hand and expressed in points according to the scale [12].

Swimming behavior was studied using the method proposed by Vrijmoed-de Vrles & Cools [20].

The data obtained were statistically calculated using both the parametric and nonparametric tests. The minimum statistical probability was determined at $p<0.05$.

Results

1. The investigation of stereotypic behaviour in rats with pilocarpine-induced chronic epileptiform activity throughout the interictal period

Intact rats in the "open field" performed an average of 3-5 defecations. This indicator in rats with pilocarpine convulsions in the interictal period was equal to 1.73±0.46, which was 2.8 times less than in the control ($P<0.05$; Fig. 1).

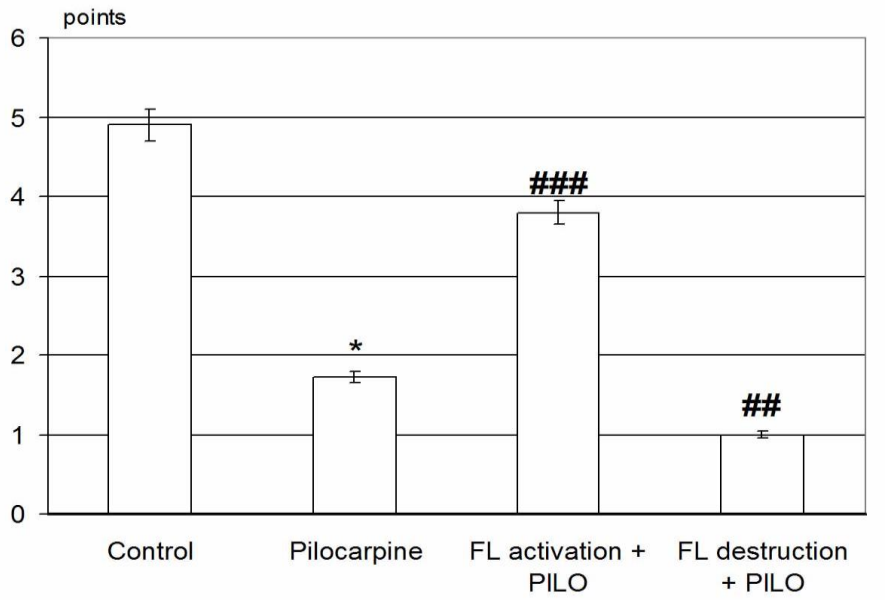

Fig. 1. Effects of frontal lobe (FL) activation and destruction on the number of defecation in the "open field" test in rats throughout the interictal period of pilocarpine (PILO)-induced chronic epileptiform activity

Notes: * - P<0.05 – statistical differences of investigated index compared with the same in control rats;

- P<0.01 and ### - P<0.001 – statistical differences of investigated index compared with the same in pilocarpine treated rats.

After the frontal cortex electrical stimulation the investigated index was equal to 3.80±0.72 which was 2.2 times higher pertaining the same in rats in the interictal period of pilocarpine-induced seizures without the frontal cortex activation (P<0.001). In rats with frontal cortex destruction the studied indicator was 42% less than that obtained in animals without the frontal lobe destruction (P<0.01; Fig. 1).

2. The investigation of aggressive-defensive behaviour in rats with pilocarpine-induced chronic epileptiform activity throughout the interictal period

The results of these trials are shown on Fig. 2.

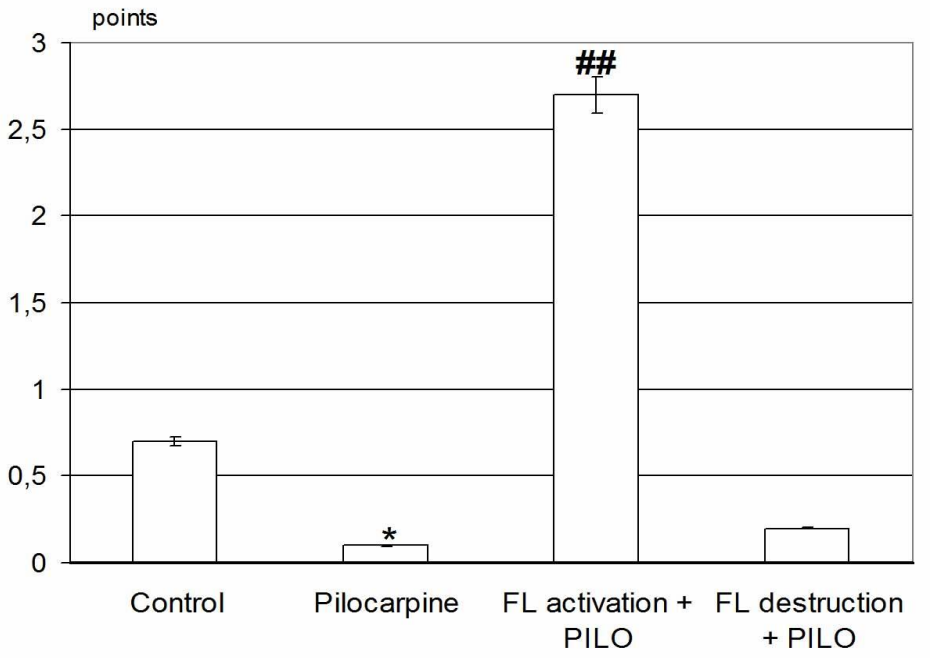

Fig. 2. Effects of frontal lobe (FL) activation and destruction on the rate of aggressive-defensive behaviour in rats throughout the interictal period of pilocarpine (PILO)-induced chronic epileptiform activity

Notes: * - P<0.05 – statistical differences of investigated index compared with the same in control rats;

- P<0.01 and ### - P<0.001 – statistical differences of investigated index compared with the same in pilocarpine treated rats.

When trying to take the rats of the experimental group in the hand in the interictal period, 12 animals did not show any resistance and continued to sit motionless in the corner of the chamber. Another 5 animals had defensive reactions in the form of withdrawal from the hand in response to an attempt to take it into the hand. The average rate of aggressive-defensive behavior in rats after the introduction of pilocarpine was 0.14±0.10 points which was 4.8 times less than the corresponding index in control rats ($P<0.05$; Fig. 2).

After the frontal cortex electrical stimulation the average rate of aggressive-defensive behavior in rats with pilocarpine was 2.70±0.24 points which was 27 times higher pertaining the same index in rats without the frontal cortex activation ($P<0.01$). When trying to grasp the hand of rats injected with pilocarpine after the frontal cortex selective destruction 9 animals did not resist at all and continued to sit motionless in the corner of the chamber. Another 5 animals had defensive reactions in the form of a deviation from the hand in response to an attempt to take it in the hand. The average rate of aggressive-defensive behavior in rats with pilocarpine was 0.20±0.10 points which did not differ from such index in rats without the frontal cortex destruction (Fig. 2).

3. The investigation of swimming behaviour in rats with pilocarpine-induced chronic epileptiform activity throughout the interictal period

7 rats out of 14 animals of the control group demonstrated 3 passive-adaptive swimming elements, the others - 2. The average number of passive-adaptive swimming forms was 2.23±0.34, the variability index was equal to 50% (Table). In rats during the interictal period of pilocarpine convulsions, the number of passive-adaptive swimming elements exceeded the same index in control observations by 2 times ($P<0.05$). At the same time, the indexes of variability and maximal variability were also higher pertaining the same indexes in control rats ($P<0.01$).

After the frontal cortex electrical stimulation 10 rats out of 12 demonstrated 6 forms of passive-adaptive swimming, the other 2 rats had 5 passive-adaptive swimming elements. At the same time, the average number of passive-adaptive swimming acts by 24% ($P<0.05$), and the index of maximal variability by 4 times ($P<0.05$) exceeded the corresponding index in rats without frontal cortex activation.

After the frontal cortex destruction 4 rats out of 13 demonstrated 2 passive-adaptive elements of swimming behavior, the other 9 rats had 1 passive-adaptive swimming element. At the same time, the average number of passive-adaptive forms of swimming was equal to 1.19±0.11 which was 3.8 times less than the similar index in rats of the control group

(P<0.05). Indexes f variability (P<0.05) and maximal variability (P<0.01) were also less pronounced compared to such data in rats without the frontal cortex destruction (Table).

Table

Effects of frontal lobe (FL) activation and destruction on swimming behaviour expression in rats throughout the interictal period of pilocarpine (PILO)-induced chronic epileptiform activity

The investigated groups	The number of passive-adaptive swimming elements, M±m	The index of variability, %	The index of maximal variability, %
1. Control (saline), n=14	2.23±0.34	50	0
2. Pilocarpine (280 mg/kg), n=14	4.48±0.35*	92**	23**
3. FL electrical stimulation + PILO, n=12	5.80±0.18#	100	91##
4. FL destruction + PILO, n=13	1.19±0.11#	0#	0##

Notes: * - P<0.05 (ANOVA + Newman-Keuls statistical test) and ** - P<0.01 (Kruscall-Wallis statistical test) – statistical differences of investigated index compared with the same in control rats;

- P<0.05 (ANOVA + Newman-Keuls statistical test) and ## - P<0.01 (Kruscall-Wallis statistical test) – statistical differences of investigated index compared with the same in pilocarpine treated rats.

4. The investigation of rat's ability to switch to active-adaptive behavior in the swimming test in conditions of pilocarpine-induced chronic epileptiform activity throughout the interictal period

The obtained data are given of the Fig. 3.

All rats in the control group escaped from the pool after visual contact with the rope. In the interictal period of pilocarpine convulsions, 13 out of 14 rats escaped from the pool after contact of the rope with the tip of the muzzle, front and hind limbs. The average index of the degree of animals contact with the rope, necessary for escape out the water, was 3 times higher than the same index in the control observations (P<0.001).

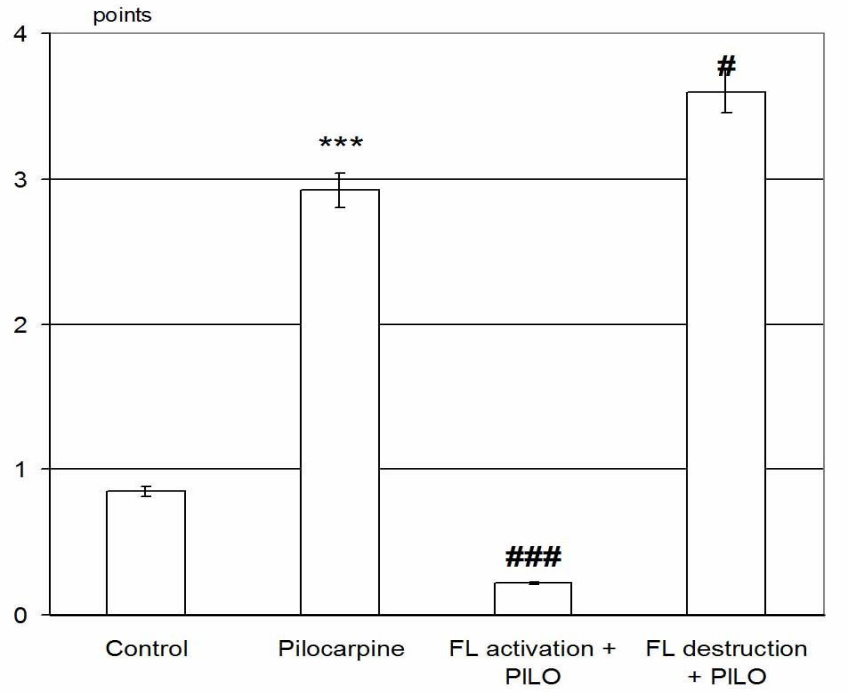

Fig. 3. Effects of frontal lobe (FL) activation and destruction on the rat's ability to switch to active-adaptive behavior in the swimming test throughout the interictal period of pilocarpine (PILO)-induced chronic epileptiform activity

Notes: *** - $P<0.001$ – statistical differences of investigated index compared with the same in control rats;

- $P<0.05$ and ### - $P<0.001$ – statistical differences of investigated index compared with the same in pilocarpine treated rats.

The average index of the animals degree of contact with the rope, necessary for escape out the water, in rats after frontal lobe activation was 13 times lower compared to same index in rats without this part of the cortex activation ($P<0.001$).

After the frontal lobe destruction, 7 rats out of 13 did not escape from the pool at all after the swimming test, another 5 rats got out of the pool on the rope after its direct contact with the face, front and hindpaws. The average index of the degree of animals contact with the rope, necessary for escaping out the water, in rats in these conditions was equal to 3.6±0.3 that 19% more compared to the same index in rats without frontal lobe destruction ($P<0.05$).

Discussion.

Thus, the conducted studies indicate that the expression of non-convulsive behavioural reactions throughout the interictal period of pilocarpine-induced chronic seizures is predominantly determined by the frontal cortex functional state.

It’s important that pilocarpine-induced chronic epileptiform activity provides an opportunity to isolate ictal convulsive moments, as well as interictal time intervals, during which it is possible to study the behavior of animals. The interictal period seems to be one of the interesting features of chronic pilocarpine-induced epileptogenesis since it is characterized by a certain decrease in the epileptogenic system activity and the antiepileptic system activation [7, 14]. At the same time, the absence of convulsive manifestations under the specified model conditions provides an opportunity to investigate the behavior of animals with a persistent form of brain epileptization, which is a unique opportunity to clarify the features, dynamics of formation, time of manifestation, dependence on convulsive episodes, etc. of non-convulsive behavior during the interictal period which can be considered as a diagnosis of approaching convulsive manifestations in clinical conditions [2].

The pilocarpine-induced model of epileptogenesis remains one of the controversial models, as there are two opposing points of view regarding the pathogenetic role of the cholinergic system in chronic seizure syndrome. The similarity of the effects observed in electrical kindling, caused by intraamygdalar carbachol microinjections, suggests the cholinergic system involvement into kindling development [21]. A decrease in cholinesterase activity in some brain structures during kindling seizures [9] was also shown, which may be a compensatory mechanisms activity that affect the level of acetylcholine synthesis. On the other hand, no changes in acetylcholinesterase activity during kindling were found [13]. It is quite likely that the cholinergic system activity specified changes are a consequence of repeated seizures and are not related to the kindling process by etiological factor.

Our data prove that pilocarpine use contributes to chronic form of epileptiform activity formation characterized by the presence of an expressed acute stage and an interictal period - free from behavioural convulsive reactions [4].

Frontal lobes hyperactivation might be an important feature of pilocarpine-induced chronic epileptogenesis. Previously, the role of the orbital cortex in seizure activity modulation was investigated, which also confirms the presence of a massive supply of descending impulses, including to the caudate nuclei. It seems that the brain cortex motor areas role in the pilocarpine-induced chronic epileptiform activity mediation should be determined more precisely. Therefore, we conducted a series of experiments devoted to the study of the frontal cortex modulation role in animal behavior during the pilocarpine-induced seizures interictal period. We chose the frontal cortex due to the presence of a large number of cholinergic receptors in this area of the neocortex, as well as taking into account its important role in the formation of descending motor commands [10].

The obtained data indicate that the expressiveness of non-convulsive behavioural reactions in the interictal period during pilocarpine-induced convulsions is predominantly determined by the frontal cortex functional state. As a result of frontal cortex activation rats showed an increase in horizontal and vertical motor activity [3], as well as emotional behaviour intensification, the strengthening of aggressive-defensive behavior and the increase of the swimming behaviour variability together with rats difficulties to switch to active-adaptive forms of swimming behaviour.

It has been proven that after frontal lobe selective destruction the opposite behavioral effects are noted, which confirms the frontal lobe important role in the formation of various forms of non-convulsive behavior during the interictal period.

Considering the sanogenic significance of non-convulsive behaviour interictal forms as well as their role in the mechanisms of epileptogenesis termination, it is possible to assume that in this aspect the frontal cortex most likely plays a "trigger" role in the development of complex mechanisms aimed to epileptiform activity suppression in conditions of pilocarpine-induced chronic seizures.

Thus, the observed behavioural effects during the frontal lobe functional activity modulation and the data we obtained testify to the feasibility of searching for regulatory influences on this brain formation in order to initiate complex mechanisms aimed to epileptiform activity suppression in conditions of pilocarpine-induced chronic seizures.

Conclusions

1. Frontal lobe hyperactivation is an important feature of pilocarpine-induced chronic epileptogenesis.

2. As a result of the frontal lobe activation one could register an emotional behaviour enhancement together with aggressive-defensive behaviour activation. There is also increase in passive-adaptive behavior of rats in the swimming test as well as the improvement in their ability to escape from the pool which indicates a better ability to switch to an active-adaptive swimming behaviour.

3. In conditions of frontal lobe selective destruction an opposite behavioral effects are noted, which confirms the frontal part of the cortex important role in various non-convulsive behavior formation throughout the pilocarpine-induced interictal period.

4. The frontal cortex most likely plays a "trigger" role in the development of complex mechanisms aimed at epileptiform activity suppression in conditions of pilocarpine-induced chronic seizures.

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Author Contributions

Conceptualization, (Kashchenko O.A., Stoyanov O.M., Zayats L.M. & Tatarko S.V.); methodology, (Kashchenko O.A., Volokhova G.O. & Pryshchepa O.O.); formal analysis, (Kashchenko O.A., Stoyanov O.M. & Berbek V.L.); data curation, (Volokhova G.O. & Voloshchuk D.A.); writing—original draft preparation, (Berbek V.L., Voloshchuk D.A. & Pryshchepa O.O.); writing—review and editing, (Zayats L.M. & Tatarko S.V.); supervision, (Kashchenko O.A., Stoyanov O.M. & Volokhova G.O.).

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Institutional Review Board Statement

The experimental studies were carried out in the conditions of a chronic experiment in accordance with international standards of humane treatment of vertebrate animals and approved by the Ethics Committee of Odesa National Medical University (N7/21, 11 October 2021)

Informed Consent Statement

The data of experimental studies are given. Written informed consent from the patients was not necessary to publish this paper.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.