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The effect of *Psilocybe cubensis* extract on hippocampal neurons in vitro

Действие экстракта гриба P.cubensis, *содержащего псилоцибин (ПСБ) и псилоцин, на импульсную активность нейронов пирамидного слоя зоны CA1 гиппокампа было изучено на переживающих срезах мозга крысы. У 38 (76%) из 50 исследованных нейронов наблюдалось угнетение импульсной активности, у 2 (4%) клеток - возбуждение, 10 (20%) нейронов не реагировали. Аппликация экстракта вызывала короткие групповые импульсные разряды у 12 (24%) нейронов. Все нейроны, тормозившиеся при действии ПСБ-содержащего экстракта, также тормозились серотонином (5-НТ). Длительность тормозной реакции на экстракт обычно не превышала 4 - 5 мин, а на серотонин достигала 10-43 мин при 3-минутной аппликации. Часть нейронов тормозилась при аппликации серотонина и не реагировала на экстракт. Тормозные реакции, возникавшие при действии экстракта, блокировались ритансерином у половины тестированных единиц и были обусловлены активацией 5-HT_2-серотониновых рецепторов. Экстракт угнетал возбудительные импульсные реакции, вызванные аппликацией L-глутамата. Таким образом, ПСБ-содержащий экстракт, в большинстве случаев угнетал импульсную активность нейронов пирамидного слоя зоны CA1 гиппокампа и подавлял глутаматную передачу.*

INTRODUCTION

Study of physiological action on CNS of strong indolic hallucinogens (IG) - psilocybin (PSB) and psilocine (PS) - is a vital problem due to increasingly drug abuse [8, 14, 18]. PSB as a mixed agonist of 5-HT_{2A} and 5-HT_{1A} serotonin receptors acts on CNS in a similar way as lysergic acid diethylamide (LSD) causing psychomimetic symptoms [8, 9, 18, 24]. Similar effect has also its metabolite - PS. Structures of different CNS levels: raphe nuclei (RN) and thalamic nuclei, amygdala, basal ganglia, hippocampus and neocortex are the targets for IG action [4, 5, 24]. PS reveals the strongest inhibitory action on serotonergic neurons (5-HT neurons) of RN causing a decrease in their spike activity [5, 16, 23]. Hippocampus and neocortex are the other structures where an especially high PSB concentration was observed during its systemic administration [4]. It is established that the use of PSB-containing mushrooms of *Psilocybe* genus leads to a multifocal cerebral demyelinization and dystrophic changes of neurocytes just in the hippocampus [2, 21]. Thus, the IG effect is determined mainly by functional state of the hippocampus which receives direct projections from RN [15, 16, 17]. At the same time, direct PSB and PS effects on serotonin and glutamate inputs of hippocampal neurons have been studied insufficiently.

For this reason the study of the action of PSB-containing *P.cubensis* extract on serotonin inputs of neurons and glutamate transmission in CA1 pyramidal layer of the hippocampus was the tadk of the present study.

METHODS

Wistar rats weighing 150 g were anaesthetized by ether and then decapitated. The brain was

quickly removed, and a block containing the hippocampus was excised. The tissue was immersed in an ice-cold Ringer solution. Using a vibratome, 400 mm thick coronal slices were prepared and put in an ice-cold (0-3 ^{O}C) artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl – 124,0; KCl – 3,0; $MgSO_4$ – 2,0; KH_2PO_4 – 1,2; $CaCl_2$ – 2,0; $NaHCO_3$ – 20,0; glucose – 25,0; continuously saturated with a 95% O_2 and 5% CO_2 gas mixture to maintain pH 7.4. After 4 to 6 h pre-incubation in ACSF at 30°C slices were transferred into a flow chamber. Background spike activity of single neurons in hippocampal *stratum pyramidale* (CA1 area) was recorded for 3-4 min while rinsing the brain slices in the ACSF. After stoppage ACSF rinsing, *P.cubensis* extract was applied for 2-4 min. After extract application the slice was washed out again by ACSF for 10-30 min (sometimes up to 1 hour). The flow rate in the chamber was 2 ml/min, and solution temperature was kept at 30°C. Spike activity was recorded extracellularly by glass microelectrodes filled with 3M NaCl and having tip resistance of 5-12 M. The recorded action potentials were converted to standard pulses by an amplitude discriminator. The obtained pulse flow was analyzed by a special software, which allowed statistical processing with their representation as average frequency spike activity histograms. When recording spike activity from one neuron, the extract was applied from one to seven times. Spike activity of single neurons was recorded for 1-2 hours. Neuronal responses were considered significant if the difference between the levels of spike activity before and after application of test solution was no less than 2 (+2 S.D.). Data were expressed as means +S.E.M.

Preparations used

To determine the types of neuronal receptors that were activated during extract application, the following antagonists of synaptic transmission, were used: ritanserin (100 mM) an antagonist of 5-HT_2 and 5-HT_{1C} serotonin receptors which was first diluted in DMSO ("Sigma", USA) and then in ACSF, bicuculline methiodide (10 M, Sigma) an antagonist of $GABA_A$ receptors. Serotonin (5-HT creatinine sulfate, 100 M) and L-glutamic acid (100 M, "Sigma", USA) were used also. The substances used in experiments were diluted in ACSF.

Preparation of mushroom extracts

Extracts were prepared from *Psilocybe cubensis (Earle) Singer* mushroom. To obtain extracts, dried and pulverized mushroom fruit bodies were covered with ethanol (96%) at a ratio of 1:10. The obtained suspension was kept for 10 days at 4°C. This method allowed us to extract no less than 22% of PSB containing in mushrooms [1]. The use of pure ethanol prevented the extraction of enzymes which dephosphorylate PSB into unstable PS [1, 12]. Immediately before the experiment ethanol extract was evaporated, and distilled water was added to obtain initial volume. Thus, the aqueous extract was obtained whose concentration was taken as 100%. Dilutions of required concentrations (1,2,4,6,8,10,16%) were prepared by adding ACSF to the aqueous extract. Ethanol content in the extract did not exceed 0.01% and did not cause any changes in the neuron spike activity. Taking into account that the PSB content reaches 0.6 % and PS - 0.15 % per dry weight of mushroom, the PSB content in the 10 % extract should make no less than 65 nM and that of PS - 31 nM [3, 11].

RESULTS

Application of *P.cubensis* extract caused an inhibition of spike activity in 38 (76 %) out of 50 investigated neurons, excitation - in 2 (4 %) cells, and 10 (20 %) neurons did not respond. The short burst firing during extract application was observed in 12 (24 %) neurons (fig.1). Latency of inhibitory responses was on the average 67 8 sec, duration - 273 37 sec and a maximum of the reaction - 134 18 sec. The time of extract application was 164 11 sec. The antagonist of 5-HT_2 and 5-HT_{1C} serotonin receptors, ritanserin, completely blocked inhi-

bitory responses in half of the investigated cells during *P.cubensis* extract application. Inhibitory neuronal reactions elicited upon application of 4 and 8 % extracts are shown in fig. 2 (1). Ritanserin blocked neuronal responses to the extract application which restored after washout. In other cases ritanserin suppressed these reactions partly or was ineffective.

To elucidate the peculiarities of serotonin inputs activation, the responses to *P.cubensis* extract and serotonin (5-HT) application were compared in 4 neurons. All the investigated cells reacted to serotonin by inhibition (fig.2 (2)). Inhibitory reactions to serotonin had a shorter latency (50 24 sec), a considerably large duration (1324 621 sec) and reached the maximal value much earlier (70 32 sec). Sometimes the response to serotonin lasted 43 min upon 3 min application. Some neurons were inhibited by serotonin and did not react to the extract (fig.3). However, the use of ritanserin strengthened spike activity which could even be increased with subsequent extract application. One can suggest that in this case ritanserin by blocking 5-HT_{1C} serotonin receptors, which had a tonic inhibitory effect on the background activity, caused excitation of the neuron. For elucidation of possible involvement of GABAergic neurons in the observed inhibitory responses we used an antagonist of $GABA_A$ receptors -

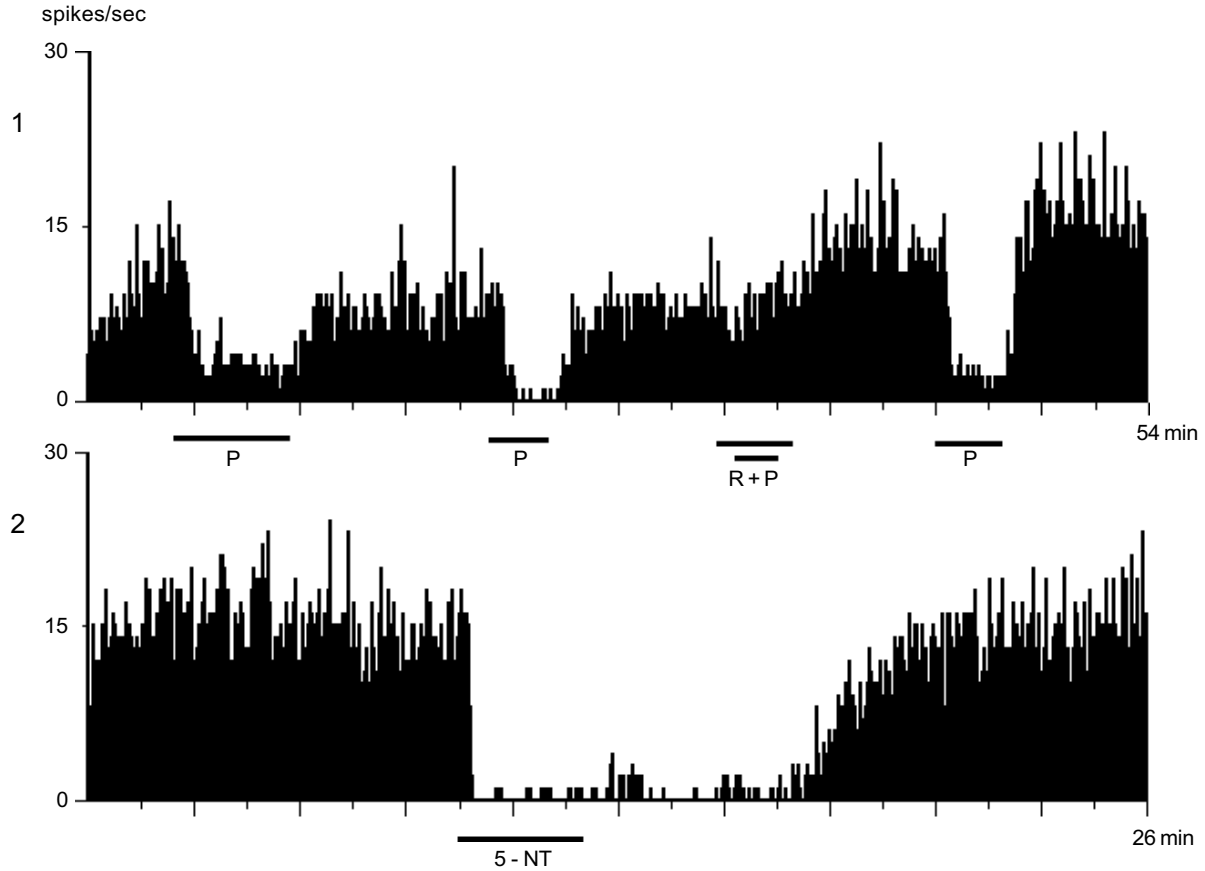

Fig. 1. Short burst firing of the neuron in CA1 pyramidal layer of hippocampus upon application of P.cubensis extract.
Histogram showing spike activity changes in mean frequency. Abscissa - time (min); ordinate - spikes frequency (per sec). Horizontal line under abscissa - interval of extract.application. P - extract from fruit bodies of P.cubensis mushroom.

bicuculline. Its application could somewhat reduce reactions to extract application, however, only ritanserin completely blocked them (fig. 4).

One of the research tasks was to study the effect of PSB-containing extract on glutamate transmission in the hippocampus. The use of the extract after L-glutamic acid application suppressed excitatory spike responses caused by its action (fig. 5 (1)). This inhibitory action of the extract remained some time after the beginning of washout. At first, the responses to L-glutamate which was applied with an interval from 3 up to 11 min after extract application did not appear at all, but then they gradually restored, reaching the initial value 34 min after. Thus, IG containing in the extract could suppress glutamate transmission for a long time.

DISCUSSION

It is known that PS as well as LSD causes a dose-dependent decrease in spike activity of 5-HT neurons of RN giving direct projections to the hippocampus [16, 23]. According to the proposed hypothesis, hallucinogens exert their psychoactive effect acting primarily on 5-HT_{1A} autoreceptors of 5-HT neurons of RN and cause inhibition of these cells [10, 16]. In addition, postsynaptic neurons (in particular, hippocampal) should show disinhibition as a result of suppression of tonic inhibitory influences from the dorsal RN.

Besides the described direct inhibitory influence of dorsal RN 5-HT neurons on hippo-

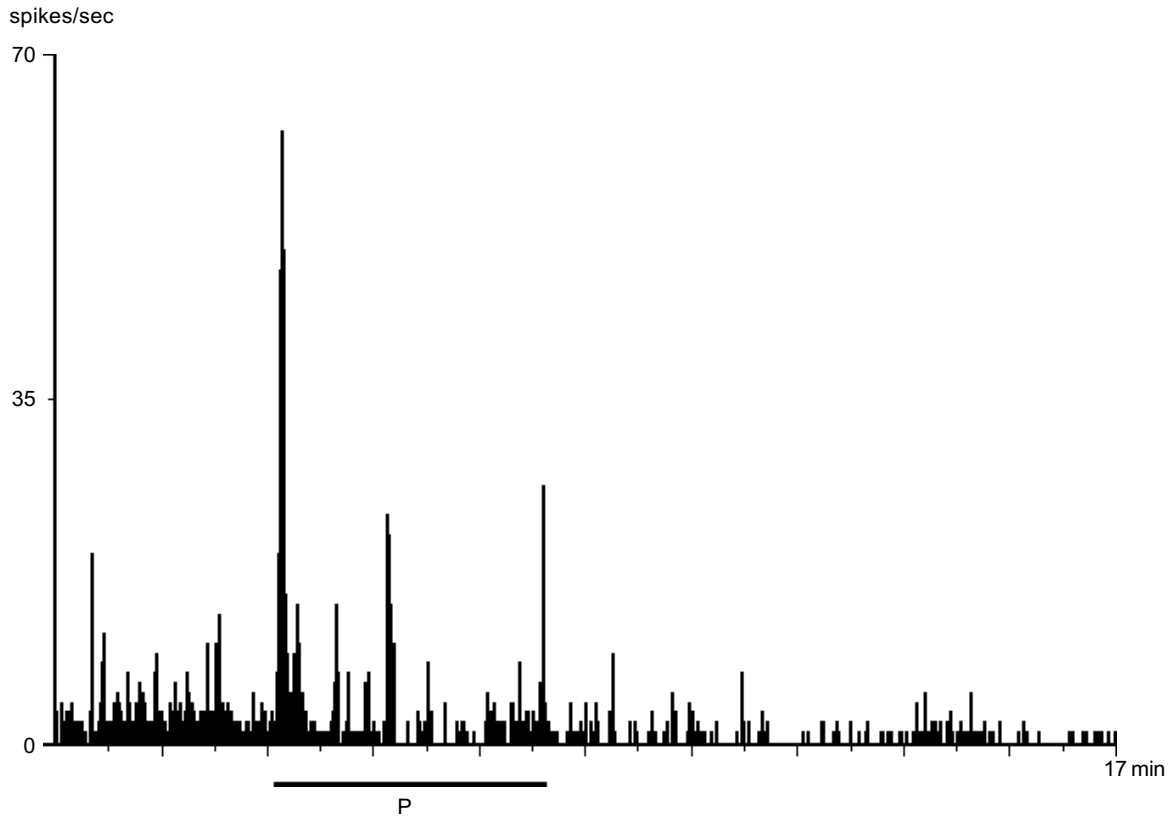

Fig. 2. Inhibitory responses of hippocampal neuron to application of P.cubensis extract and serotonin. Blocking of extract action by ritanserin.
1 - consecutive application of: P - 4 % P.cubensis extract, P - 8 % extract, R+P - extract on the background of ritanserin action, P - again 8 % extract. 2 - serotonin (5-HT) application. Other designations are the same as in Fig. 1.

campal neurons, there is the other way mediated by hippocampal interneurons. So, serotonin released from synaptic terminals of median RN neurons has a direct excitatory effect on hippocampal GABAergic interneurons located in *stratum radiatum* of area CA1 through activation of 5-HT_3 receptors [17]. These 5-HT-responsive interneurons can in turn inhibit CA1 pyramidal cells. Hence, during activation of 5-HT_{1A} autoreceptors of 5-HT neurons of median RN one should observe disinhibition of CA1 pyramidal neurons of the hippocampus (like in the first case). However, the proposed schemes consider only local effect of serotonin agonists on 5-HT_{1A} autoreceptors of RN 5-HT neurons and do not take into account direct action of these agonists on 5-HT_{1A} receptors of hippocampal cells.

If the described above effect of PSB on RN could result in disinhibition of hippocampal pyramidal neurons, direct action of PSB-containing extract from *P.cubensis* on hippocampal slices caused a decrease of spike activity in 76 % of the investigated neurons in CA1 pyramidal layer of hippocampus. This decrease can be explained by PSB action on 5-HT_{1A} receptors whose activation results in hyperpolarization and inhibition of these neurons [10, 22]. The application of ritanserin (antagonist of 5-HT_2 and 5-HT_{1C} serotonin receptors) showed that the observed inhibitory responses can appear not only due to activation of 5-$HT_{1A,}$ but by activating 5-HT_2 serotonin receptors as well.

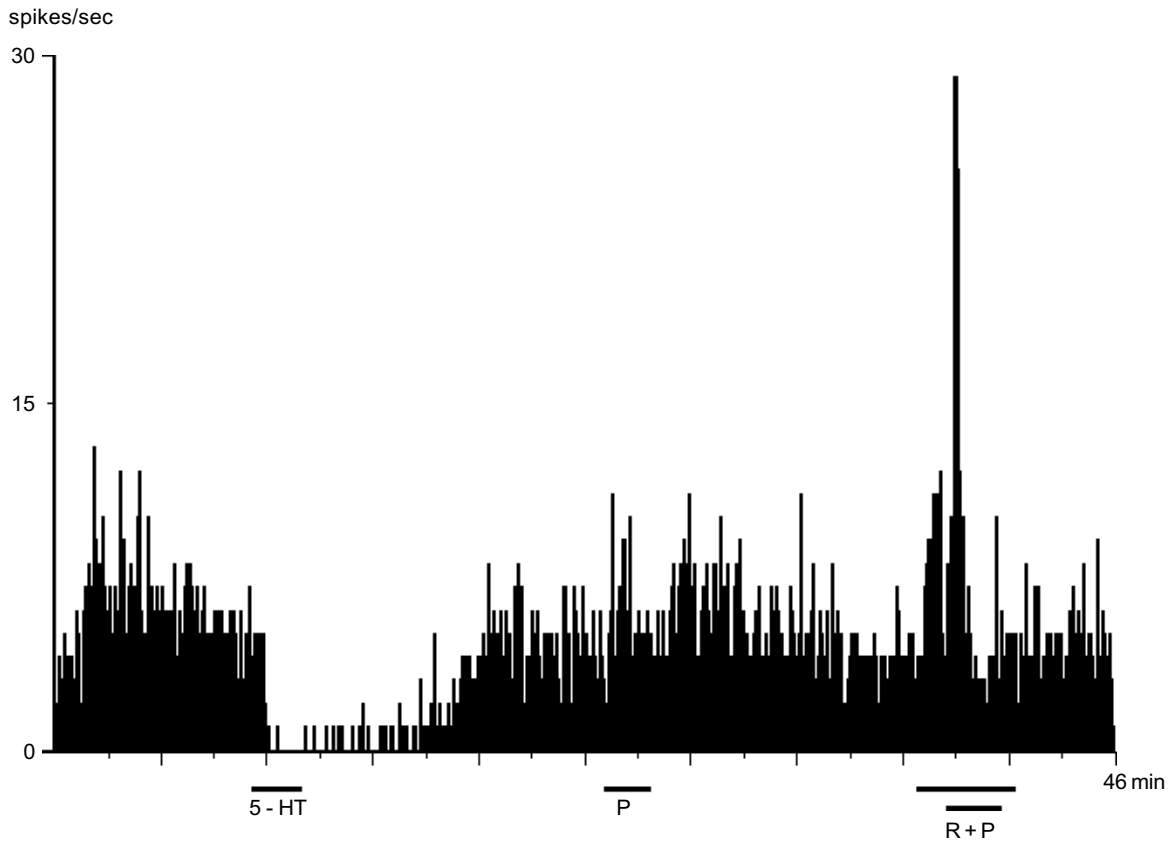

Fig. 3. Responses a hippocampal neuron which was inhibited by serotonin, did not react to P.cubensis extract application and was disinhibited by ritanserin.
Other designations are same as in Fig. 1, 2.

Experiments with bicuculline application have confirmed that inhibitory responses induced by the extract appear due to direct activation of serotonin receptors, but not as a secondary effect due to inhibition of GABAergic interneurons. There was observed much in common between the action of serotonin and PSB. However, the fact that all the tested neurons reacted to serotonin by inhibition which was much stronger and longer than that produced by application of PSB-containing extract confirms the presence of functionally different subtypes of serotonin receptors in the hippocampus [6]. It is known that serotonin application causes hyperpolarization of the soma and apical dendrites of CA1 pyramidal neurons as well as reduce of their excitability due to opening of inward rectifier K+ channels [13, 19, 20]. Apparently, suppression of spike activity of pyramidal neurons during PSB action can be accompanied by similar processes.

The analysis of bicuculline and ritanserin action also allows one to think that some pyramidal neurons are under tonic inhibitory control of GABAergic and 5-HT_{1C} serotonin inputs. The short burst firing observed in 24 % of cells in pyramidal layer with extract application could be a result of temporary disinhibition of pyramidal cells during inhibition of inhibitory interneurons.

It has also been directly confirmed that PSB containing in the *P.cubensis* extract sup-

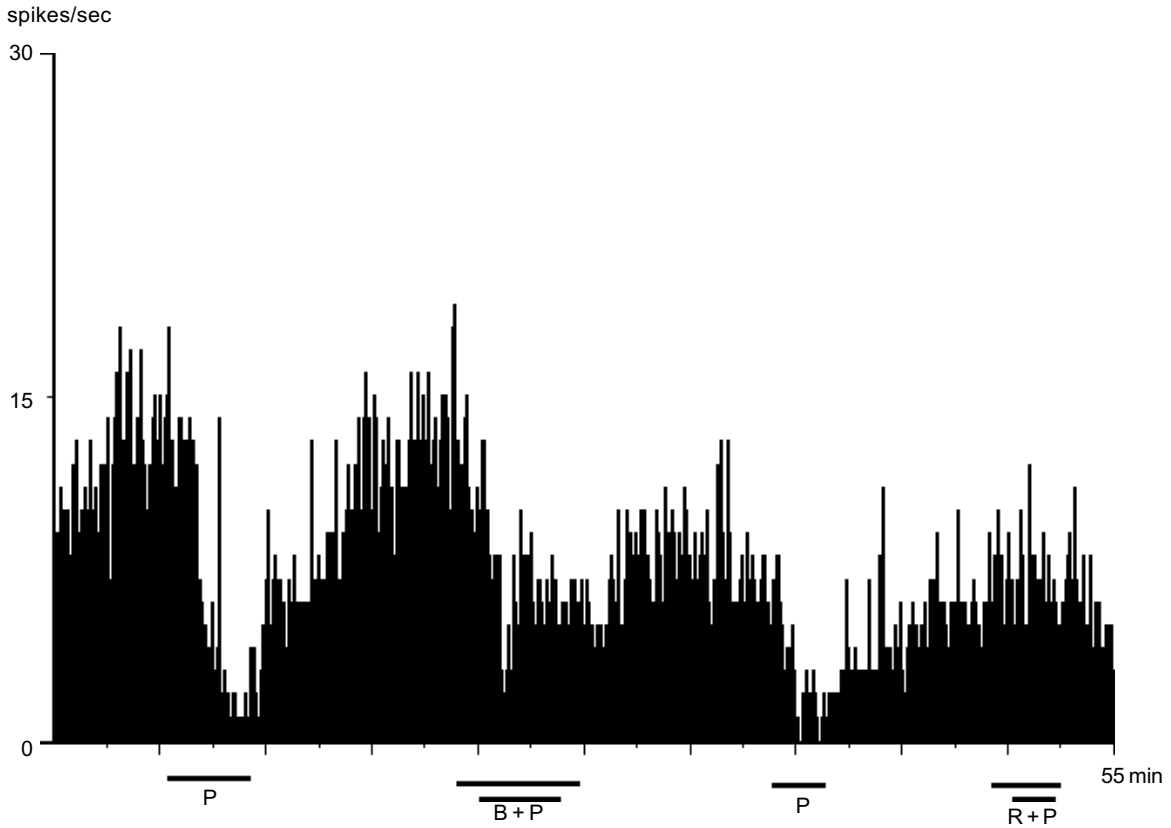

Fig. 4.Effect of bicuculline and ritanserin on inhibitory responses of hippocampal neuron induced by P.cubensis extract application..
B - bicuculline, B+P - extract application on the background of bicuculline action. Other designations are the same as in Fig. 1, 2.

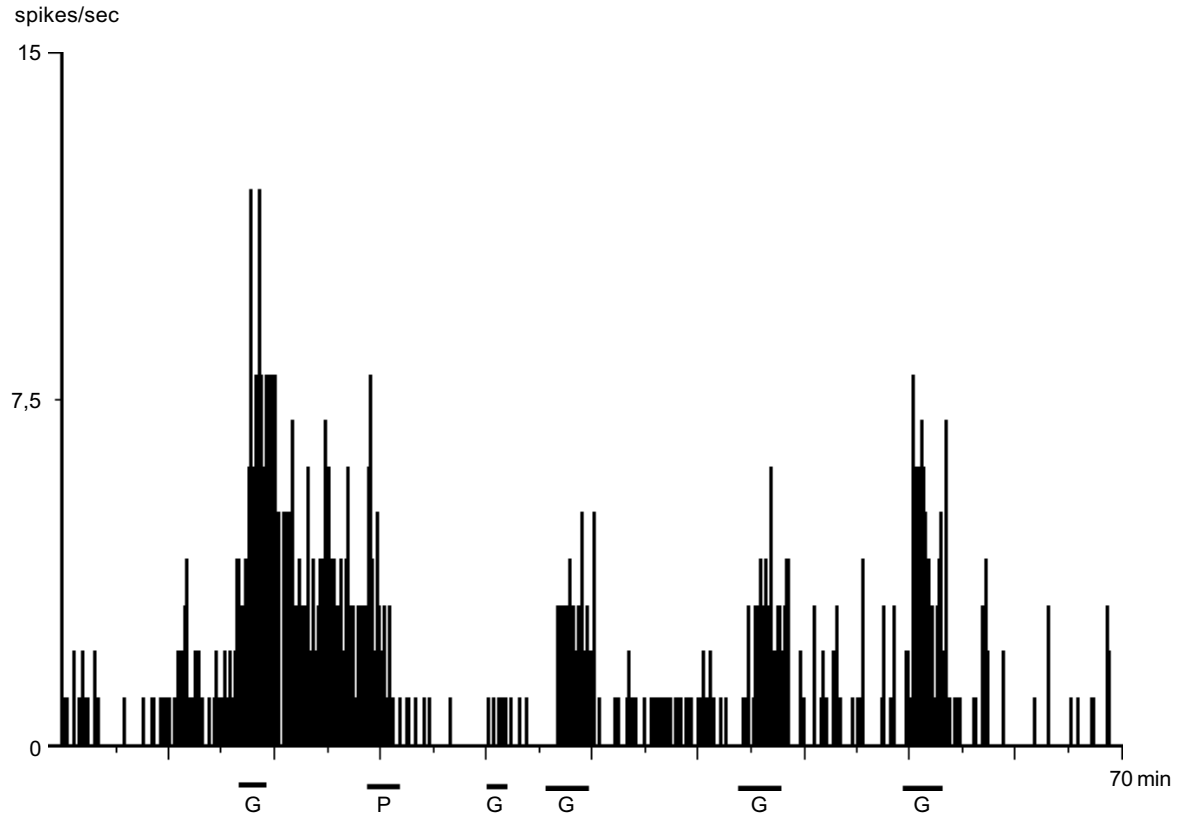

Fig. 5. Depression of excitation induced by L-glutamic acid in hippocampal neuron after P.cubensis extract application.
G - L-glutamic acid application. Other designations are the same as in Fig. 1, 2.

presses the glutamate transmission in CA1 pyramidal layer of hippocampus. This fact is well in line with the data available that 5-HT_{2A} and 5-HT_{1A} agonists of serotonin receptors (to which belongs PSB) inhibit glutamate transmission in the hippocampus and neocortex mediated by NMDA and AMPA receptors [7, 19].

Thus, it can be concluded that the systemic introduction of PSB-containing preparations may have simultaneously inhibitory and disinhibitory action on pyramidal neurons of the hippocampus the balance of which has to determine the functional state of these cells. On the one hand, this is a decrease in inhibitory influences from RN and, on the other, inhibition of hippocampal neurons due to direct action of PSB on their 5-HT_{2A} and 5-HT_{1A} serotonin receptors.

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THE EFFECT OF PSILOCYBE CUBENSIS EXTRACT EFFECT ON HIPPOCAMPAL NEURONS IN VITRO

The action of *P.cubensis* mushroom extract, containing psilocybin (PCB) and psilocin, on spike activity of hippocampal CA1 pyramidal neurons was studied in in vitro rat brain slices. In 38 (76 %) out of 50 investigated neurons spike activity was decreased, in 2 (4 %) cells it increased. There was no response 10 (20 %) neurons. Application of the extract caused short burst firing in 12 (24 %) neurons. All neurons showing inhibition during PCB-containing extract application, were also inhibited by serotonin (5-HT). Usually inhibitory reaction did not last over 4 - 5 min upon 3 min extract application and could be prolonged up to 10-43 min up on serotonin application. Part of

neurons were inhibited by serotonin and did not react to extract application. Inhibitory reactions induced by extract application were blocked by ritanserin in half of the tested units and were induced due to activation of 5-HT_2 serotonin receptors. The extract suppressed excitative spike reactions caused by application of L-glutamic acid.

It is concluded, that application of PCB-containing extract in most cases reduced spike activity in hippocampal CA1 pyramidal neurons and suppressed glutamate transmission.

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ABBREVIATION:

ACSF: artificial cerebrospinal fluid;
LSD: lysergic acid diethylamide
AMPA receptor: receptor for alfa-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
NMDA receptor: N-methyl-D-aspartic receptor
5-HT: 5-hydroxythryptamine
5-HT neurons: serotonergic neurons
5-HT_{1A}, 5-HT_{2A}, 5-HT_{1C}: the verious types of serotonin receptors
DMSO: dimethyl sulfoxide
RN: raphe nuclei (-us)
IG: indole hallucinogens
PS: psilocine
PSB: psilocybin
Key words: extract, pyramidal neurons, hippocampal slices, spike activity, rat, psilocine, psilocybin, ritanserin, L-glutamic acid, serotonin (5-HT)