改善记忆的方法：使用超低剂量的S-100B抗原抗体

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根据众多研究的资料，S-100B抗原是神经系统中的一种抗原，影响着神经系统可塑性和记忆。超低剂量的S-100B抗原（6C稀释度，根据家庭药物典籍）被用于研究三种学习行为模型，这些模型以Wistar大鼠为对象，分别是抑制性回避、选择装有糖溶液的碗和抑制性行为。在所有这些任务中，参数在口服给药后立即得到改善。因此，支持S-100B抗体对记忆形成的影响。讨论了可能的机制。

关键词：S-100B抗原–抗体–超低剂量，记忆

引言

目前，神经药理学和现代药物学对低和超低剂量药物的研究一直保持着浓厚的兴趣[1–6]。这些药物的主要优势在于它们不会直接引起毒性效果，这使得它们在广泛使用方面具有明显的优势。针对脑等神经特定抗原制备的抗体具有很高的研究价值，因为这些抗原对神经系统的基本功能起着调节作用。S-100B抗原是神经系统中的一种抗原，它在调节神经胶质关系方面起着重要作用，并且影响着神经系统可塑性（7–15）。

在分子生物学和生物物理研究所，S-100B抗原抗体对学习-记忆机制的影响进行了多年的研究[16]。一个新阶段开始研究低浓度的S-100B抗体，它们往往具有与高浓度抗体相反的效果[3,4]。本研究是对这些研究的继续。

目的

研究超低剂量的S-100B抗体（‘Proprote-100’）对大鼠记忆形成的影响。三种不同类型的任务被用作学习模型：(i) 抑制动物从安全平台下降到电网，(ii) 选择装有糖溶液的碗，(iii) 在声音信号后抑制进食行为。

方法

抗体

为了制备活性化抗体，使用鼠单特异性多价血清对S-100B抗原制备的抗体。抗体通过CNBr-sepharose柱分离的S-100B蛋白制备。免疫球蛋白溶液用0.15 M NaCl进行透析并浓缩。抗体未对肝脏、肺、肾等器官产生影响。超低浓度的抗体通过常规的制药方法在‘Materia Medica Holding’公司（研究和生产公司，莫斯科）制备。将12 mg ml⁻¹的抗体溶液与乳糖以1:100的比例混合（0.05 ml溶液，5 g乳糖）制成1:100的稀释度C1。稀释度C2通过将9.9 g乳糖加入0.1 g C1制备。加入等体积的水，不少于10次搅拌，制成1:99的稀释度C3。稀释度C4通过将9.9 g乳糖加入0.1 g C1，加入等体积的水，不少于10次搅拌来制备。
Effects of Anti-S-100 on learning

542

water instead of antibody solution. 6C dilution (ratio 1:1012) and mechanical stirring. Potentiated water was prepared using and then C6 were prepared using addition of distilled water obtained by adding 50% ethanol solution (0.25 g/24.75 g). C5 and then C6 were prepared using addition of distilled water and mechanical stirring. Potentiated water was prepared using water instead of antibody solution. 6C dilution (ratio 1:1012) was used in the experiments.

Rats

Experiments were performed on adult male Wistar rats weighing 200–300 g from the animal house of the Novosibirsk National Academy of Medical Sciences. Rats were kept in pairs with a free access to water and food, under a 12 h illumination per day. Solution of potentiated antibodies to S-100B was administered to rats per os (0.5 ml) immediately after three tasks learning sessions described above. During learning to respond to auditory stimulus, solution was additionally administered 24 and 1 h before training. Control animals were given potentiated water (0.5 ml). During learning to choose a bowl with sucrose, dexametasone was administered per os to an additional comparison group in a dose of 0.3 mg kg⁻¹, which influence memory formation (19,20) due to affecting amygdale, which is one of the key structures of brain functioning (21,22).

Development and Testing of Inhibitory Avoidance

Training of inhibitory avoidance was carried out in a veneer box of 50 × 25 × 25 cm. The floor in the testing chamber consisted of parallel bronze bars with a diameter of 3 mm, the distance between bars being 1 cm. On the left a 7 cm wide and 2.5 cm high wooden platform was located on which an animal was delicately placed (23). During learning avoidance reaction, the animal was descended from the platform, and when all the four paws were on the grid, electric current was automatically switched on (0.5 mA, 50 Hz, 2 s). The animals were tested in 24 h time, with the electric current switched off. Learning session was repeated in 4 weeks, conservation of the difference between experimental groups of control group (by 44%). The next testing in 7 days showed a significant increase of time of staying on the safe platform after cessation of sound.

Learning to Choose Sucrose Bowls

Experiments on choosing bowls with sucrose solution were carried out in the testing chamber made of organic glass (40 × 20 × 20 cm), and a floor made of metal plates. At each side of the chamber 4 cm above the floor there were two bowls, each 3 cm in diameter, the distance between the bowls was 5 cm. The bowls contained 20% sucrose solution. The animals were previously kept in the chamber for 10 min for four consecutive days. Those with low consumption of sucrose (<5% of exposition time) were excluded from the experiment. During training session, the bowls on the left were put under electric current of 0.15 mA, 50 Hz with a latent period of 0.1 s. Learning sessions lasted 15 min and were repeated in 2 days. In experiments numbers of animals’ contacts with the bowls separated by no less than 3 s intervals were estimated.

Avoidance Reaction by Auditory Signal

Experiments were carried out in a chamber similar to those described above, with bowls containing sucrose, and an auditory signal. Animals were previously kept in the chamber for 10 min for four consecutive days. Those with low consumption of sucrose (<5% of exposition time) were excluded from the experiment. During learning sessions, with the beginning of animal’s consumption of sucrose solution the following method was used: in 7 s time a sound of 800 Hz, 20 dB was switched on. Three seconds later all the bowls were under electric current of 0.15 mA, 60 Hz. Combined action of the stimuli lasted for 5 s. The session lasted 20 min and included 10 auditory signals that animal could obtain. Testing was made under switched-off current in 24 h and in 7 days. Testing lasted 20 min. Estimated were latent periods of cessation of drinking behavior after its start, completion of drinking with sound signal and resuming of drinking behavior after cessation of sound.

Stimulation and Registration of Reactions of Rats Were Carried Out Automatically Using a Computer

Statistics

Results were expressed as the mean ± SEM. Comparison of data among three groups was performed using the one-way analysis of variance (ANOVA) with Bonferroni’s post-test. Comparison of data between two groups was performed by Student’s test based on the variance of data examined by F-test. When the P-value was <0.05, the difference was considered to be significant.

Results

Ultralow Doses of Antibodies to S-100B and Inhibitory Avoidance

In the experiment on development of inhibitory avoidance, the latent period of descent from the platform in control and experimental groups was about 4 s (see Table 1). When tested in 24 h, the differences between the groups were not significant. After 4 weeks the animals were trained repeatedly. Testing of the reaction reproduction in 24 h indicated significant increase of time of staying on the safe platform (P < 0.05) of animals of the experimental group versus the control group (by 44%). The next testing in 7 days showed a conservation of the difference between experimental groups of animals. The rats that were administered with antibodies stayed on the platform 254 s on average, and those that were given water stayed 144 s (P < 0.01).
Ultralow Doses of Antibodies to S-100B and Reaction of Choosing Bowls with Sucrose Solution

Analysis of dynamics of learning to choose ‘safe’ bowl with sucrose solution demonstrated that there were no considerable differences in the number of contacts with bowls on the right side (no punishment) between control and experimental, experimental and dexamethasone groups, although in the former two groups there was a strong tendency to increase the number of such contacts from 10–11 to 19–20 as the training went on, whereas in animals administered with dexamethasone there were no changes (see Table 2).

Different dynamics were registered for bowls on the left side with ‘punishment’ (see Table 3). As early as 3rd–4th session (the data of each two consecutive trainings were pooled), the rat group that were given dexamethasone showed a significant decrease of numbers of contacts as compared with the control (11.6 and 4.2), whereas in the group that was given potentiated antibodies there was only a tendency to 6.1. By the 5th–6th training sessions, the decrease in number of contacts with bowls on the left side as compared to the control was significant ($P < 0.05$) in the two experimental groups (control, 9.7; antibodies, 4.7; dexamethasone, 4.0 contacts).

Ultralow Doses of Anti-S-100B Antibodies and Avoidance Reaction to Auditory Signal

In the development of avoidance reaction to auditory signal, the operational conditioning reflex developed after two or three combinations, so that conditioned reactions appeared as soon as after 10 exposures to the stimulus. The animals that had been previously administered antibodies to S-100B antigen showed a higher speed of learning than the control, which was indicated as significant reduction of avoidance latency (7.42 and 4.59 s, $P < 0.05$, see Fig. 1) and decrease of time of consuming sucrose solution when exposed to auditory stimulus (2.28 and 0.78 s, $P < 0.01$, see Fig. 2).

In 24 h after learning session, the avoidance latency remained reduced in animals that had been administered with antibodies as compared to the control (7.86 and 6.17 s, $P < 0.05$, Fig. 1). Time of sucrose consumption on the background of the auditory stimulus also decreased (3.27 and 1.37 s, $P < 0.01$, see Fig. 2).

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### Table 1. Latencies of rats’ descent from the platform

| Group of animals | Control, $n = 14$ | Antibodies $6c$, $n = 14$ |
|------------------|-------------------|---------------------------|
| Learning         | $4.2 \pm 1.1$     | $4.1 \pm 1.8$             |
| Test in 24 h     | $28.7 \pm 10.4$   | $49.2 \pm 23.2$           |
| Repeated learning| $40.5 \pm 21.0$   | $64.6 \pm 29.4$           |
| Test in 24 h     | $188.2 \pm 34.2$  | $272.4 \pm 19.4^*$        |
| Test in 7 days   | $144.5 \pm 25.9$  | $254.4 \pm 10.7^{*\*}$    |

* $P < 0.05$, ** $P < 0.01$ versus control.

### Table 2. Time courses of animals’ attempts to consume sucrose from the right-hand drinking bowls

| Groups of animals | Control, $n = 8$ | Antibodies, $n = 8$ | Dexamethasone, $n = 7$ |
|-------------------|------------------|---------------------|------------------------|
| Prior to learning | $11.75 \pm 3.03$ | $10.35 \pm 2.49$    | $12.71 \pm 1.56$       |
| Sessions 1 + 2    | $14.50 \pm 3.13$ | $11.50 \pm 3.47$    | $7.85 \pm 1.94$        |
| Sessions 3 + 4    | $14.62 \pm 2.88$ | $13.00 \pm 4.45$    | $8.42 \pm 3.25$        |
| Sessions 5 + 6    | $19.62 \pm 5.45$ | $19.75 \pm 5.25$    | $12.85 \pm 3.40$       |

### Table 3. Time courses of animals’ attempts to consume sucrose from the left-hand drinking bowls

| Groups of animals | Control, $n = 8$ | Antibodies, $n = 8$ | Dexamethasone, $n = 7$ |
|-------------------|------------------|---------------------|------------------------|
| Prior to learning | $12.62 \pm 3.11$ | $10.75 \pm 2.75$    | $13.28 \pm 1.68$       |
| Sessions 1 + 2    | $12.37 \pm 2.51$ | $7.62 \pm 1.64$     | $7.14 \pm 1.03$        |
| Sessions 3 + 4    | $11.62 \pm 2.40$ | $6.12 \pm 1.35$     | $4.28 \pm 1.84^*$      |
| Sessions 5 + 6    | $9.75 \pm 1.94$  | $4.75 \pm 0.94^*$   | $4.00 \pm 0.69^*$      |

* $P < 0.05$ versus control.
Animals in the present study were exposed to three types of learning tasks. First one required inhibition of motor activity, the second required choosing one of two objects and the third involved learning a skill to stop feeding behavior by auditory signal. The tasks included processing of information obtained through various sensory channels, proprioceptive, visual and auditory. In all three cases, administration of potentiated antibodies to S-100B improved learning skills and facilitated long-term and short-term (working) memory formation in the task of avoiding of auditory signal.

When discussing possible mechanisms of effects of potentiated antibodies on memory, it is expedient to remember that the amount of S-100B protein in the brain increases during learning and the anti-S-100 antibodies in usual doses disturb the processes of memory consolidation (11,24). The influence of S-100B on memory seems to be associated with the regulation of transcription process by these proteins (12,14). Therefore, the effect of S-100B is associated with memory improvement.

The effect of antibodies to S-100B in ultralow doses may be directly opposite to that of usual doses of antibodies, therefore improving the learned skills reproduction (25). It was demonstrated in the present work as well that Anti-S-100 antibodies in usual doses changed the frequency of action potential generation in spontaneously active neurons and blocked formation of long-term potentiation (model of memory) in mossy fiber synapses, but 20 min pre-incubation of snail ganglia or hippocampal slices with ultralow dose (6C) anti-S-100 abolished the effects of the same antibodies in high concentrations (3).

The following explanations of this situation are possible. First, usual doses of antibodies decrease the functional activity of S-100B antigen molecules, inhibits the electrical activity of neurons (26) and the stimulation of adenylate cyclase (27), whereas its ultralow doses enhance the functional activity of the protein, e.g. by changing of S-100B binding with neuron and glial membranes (16). Second, low doses of antibodies via regulatory mechanisms enhance the release of S-100B molecules from glial cells, changing membrane electric potential. There are receptors of 5-HT on glial cells. Stimulation of these receptors enhanced the release of S-100B from these cells (9,28). It may be regarded as one of the mechanisms of modulation memory via serotonergic system (29). Ultralow doses of anti-S-100B antibodies probably may affect in similar way, enhancing the release of S-100B. All this ultimately can result in a more efficient influence of S-100B on the transcription of genes (12,14) involved, as it is believed, in the memory trace formation.

The effect of ultralow doses of antibodies to S-100B on the mechanisms of brain plasticity is determined presumably by their interaction to the antigen on the outer surface of neuronal membranes and glial cells, by influencing its release via glial cells or penetration into neurons. Finally, the ultralow doses of anti-S-100B may directly affect autoantibodies (3), possibly involving physical–chemical properties and structure of water solution (30). Memory disorders in psychiatric patients are...
characterized by high levels of autoantibodies to S-100 protein (31,32). Thus, yet another possibility of getting control over memory mechanisms of men and animals is shown. At present, the authors of the paper are in the process of studying the influence of ultralow doses of antibodies to S-100B in the experiments on animals with learning and memory disorders, e.g. rats genetically predisposed to catalepsy (33).

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