
BIOPHYSICS AND BIOCHEMISTRY

Effect of Antibodies against S-100B Antigen in Ultralow Doses on Sucrose Consumption during Learning

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We studied the effect of potentiated antibodies against S-100B antigen on 20% sucrose consumption by Wistar rats under conditions of free-choice drinking from the bowls with sucrose and water during presentation of an acoustic pre-nociceptive or neutral signal. Peroral administration of antibodies after training sessions increased the number and duration of contacts with sucrose solution.

Key Words: *ultralow doses; antibodies against S-100 antigen; learning; memory*

Previous studies showed that antibodies against S-100B antigen in ultralow doses (Proproten-100) modulate the dynamics and effectiveness of learning and accelerate memory formation in rats [4,5]. This preparation also produces antidepressant and, probably, anxiolytic effects [1-3]. These specific features should affect the mechanisms of learning by reducing the influence of aversive stimuli and increasing the stimulatory effect of reinforcing signals.

Here we studied the effect of antibodies against S-100B antigen on learning of rats under conditions of free choice between sucrose and water during the presentation of alternating acoustic signals for nociceptive electrostimulation and discriminative stimuli.

MATERIALS AND METHODS

Experiments were performed on adult male Wistar rats weighing 200-300 g and obtained from the nursery of the Novosibirsk State Medical Academy. The animals were housed in cages (2 rats per cage)

under 12-h illumination regimen and had free access to water and food. The rats were trained in a plexi-glass chamber (the floor was made of metal plates). Two drinking bowls with 20% sucrose solution were located near each lateral wall of the chamber [5].

Before training, the animals were placed into the chamber for 10 min daily for 4 days. Rats with low level of sucrose consumption (<5% exposure time) were excluded from further experiments. In the first 3 sessions, two yellow bowls on the left side were replaced with black bowls with water. Two yellow bowls on the right side contained sucrose solution. Further sessions included the presentation of acoustic signals and electrostimulation reinforcement. The stimulation and recording of the response were performed using a computer. Animal's contact with any bowl (beginning of sucrose consumption) was accompanied by the start of a computer subprogram, which recorded the cessation of this contact (drinking) over 5 sec. During each third and fourth contacts (*i.e.* No. 3, 4, 6, 8, *etc.*), an acoustic stimulus (800 Hz, 20 dB) was presented. After 2 sec, electric current (not more than 0.15 mA, 50 Hz) was applied to all bowls. Combined action of the stimuli lasted for 3 sec.

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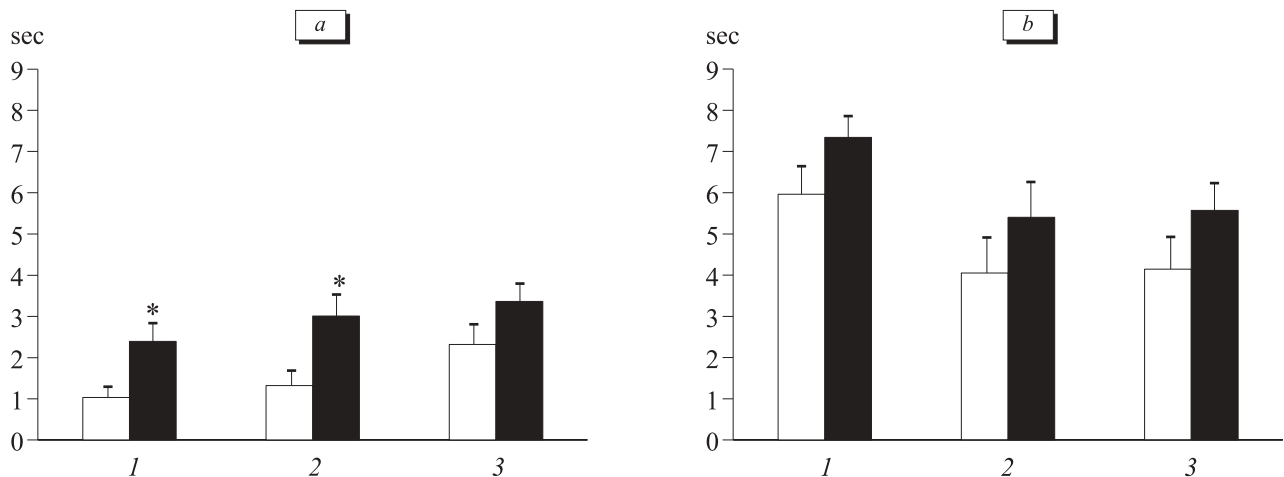


Fig. 1. Latency of response to a conditioned stimulus (a) and discriminative stimulus (b). Frequency of the conditioned stimulus: 800 Hz. Frequency of the discriminative stimulus: 0 (1), 200 (2), and 400 Hz (3). Light bars, control; dark bars, potentiated antibodies. * $p < 0.05$ compared to the control.

This stimulation regimen was repeated in 3 sessions. In the next 2 sessions, an additional acoustic signal (200 Hz, 20 dB, 5 sec) was presented during animal contact with bowls not accompanied by presentation of the conditioned stimulus (*i.e.* No. 1, 2, 5, 7, *etc.*); no electrostimulation was delivered under these conditions. In the next 2 sessions, the frequency of discriminative stimuli was increased to 400 Hz. The 800-Hz signal was always accompanied by electric stimulation.

Potentiated antibodies were obtained by repeated dilution and shaking of affinity-purified antibodies against S-100B antigen. They serve as the major constituent of the neurotropic drug Propoten-100 (according to the rules of homeopathic pharmacopoeia, Materia Medica Holding). The solution of antibodies (0.5 ml) was given to rats immediately after each training session. Control animals received potentiated water (0.5 ml).

During statistical treatment of data we evaluated the number of animal's contacts with bowls and time of contacts with sucrose solution. Experimental data obtained in the last 2 sessions under various experimental conditions were summarized.

The results were analyzed by Student's *t* test for independent samples and paired variables.

RESULTS

Experiments with free choice of bowls with water and sucrose solutions showed that rats preferred sucrose solution, which manifested in increased number ($p < 0.001$) and total time ($p < 0.001$) of contacts with sucrose bowls (Table 1). No differences were found between control rats and animals receiving antibodies.

Experiments with acoustic signals warning on electric shock upon approach to the sucrose bowls revealed differences in the behavior of control and treated rats. The rats receiving antibodies exhibited a greater number of contacts with bowls compared to animals receiving water under conditions of presentation of the conditioned signal (800 Hz) and additional discriminative stimulus (400 Hz, Table 2). The total time of contacts with bowls in rats receiving antibodies increased in the presence and absence of the conditioned stimulus (or under the influence of a 400-Hz discriminative stimulus. The

TABLE 1. Behavioral Parameters in Free-Choice Experiments ($M \pm m$)

Parameter	Group	
	control	experiment
Number of contacts with sucrose	17.7±2.4	19.6±2.8
Number of contacts with water	12.1±1.5	11.7±2.6
Time of contacts with sucrose, sec	137.2±23.7	139.3±30.9
Time of contacts with water, sec	11.7±3.5	15.0±4.4

Note. Here and in Table 2: number of contacts with bowls with 5-sec intervals is shown. Each group comprised 9 rats.

TABLE 2. Number of Approaches and Time of Contact with Bowls in Experiments with Acoustic Stimulation

Experimental conditions, groups		Number of approaches	Time of contact against the background of conditioned stimulus, sec	Time of contact against the background of discriminative stimulus, sec
One stimulus 800 Hz	control	21.7±4.8	6.37±2.32	37.04±8.31
	experiment	46.6±7.1**	34.83±8.21**	93.89±17.16**
Two stimuli 800 and 200 Hz	control	21.1±6.7	10.92±6.05	29.03±11.94
	experiment	40.7±8.0	35.40±9.74*	65.48±15.60
Two stimuli 800 and 400 Hz	control	15.5±3.8	9.32±3.68	18.98±7.70
	experiment	37.5±6.7**	33.34±7.12**	54.24±11.49*

Note. * $p<0.05$, ** $p<0.01$ compared to the control.

latency of the response to the conditioned stimulus in antibody-treated rats was much longer compared to controls in experiments with presentation of one (800 Hz) or two stimuli (800 and 200 Hz, Fig. 1).

The effect of antibodies against S-100B on behavioral characteristics was observed in experiments when feeding behavior was suppressed by electrostimulation, but not in the absence of electrostimulation (choice of bowls with water and sucrose solution). Acoustic and nociceptive stimulation during sucrose consumption is an additional stress decreasing sucrose consumption (the time of contacts with bowls decreased from 140 to 80-53 sec, $p<0.05$).

Previous experiments demonstrated the effectiveness of antibodies against S-100 B protein in rats with depression and anxiety. Administration of these antibodies decreased the time of immobility in the tail suspension test and increased the number of entrances and time spent in open arms of the elevated plus-maze [2]. Antidepressant properties of antibodies against S-100B were also described in other reports [1,3]. Moderate stress induces a depression-like state in animals accompanied by a decrease in sucrose consumption [6,8]. These changes are abolished by antidepressants [7].

We showed that potentiated antibodies against S-100B increase the number and time of contacts with sucrose bowls during stress caused by acoustic and nociceptive stimulation. Hence, this preparation possesses antidepressant activity. The simultaneous increase in the latency of the response

to conditioned stimulus suggests that antibodies against S-100B produce an anxiolytic effect.

Acoustic stimulation was delivered under alternating conditions of sucrose reinforcement: unpunished (safe) regimen and regimen associated with the risk of electrostimulation during the presentation of the conditioned stimulus. Our data suggest that administration of antibodies against S-100B immediately after training sessions improves memorization of unpunished contacts with sucrose (compared to aversive events). Different memory for favorable and adverse consequences of behavioral reactions probably serves as a mechanism for the development of depressions and effect of antidepressants.

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