

Effect of Antibodies against Serotonin, Bovine Serum Albumin, and Complete Freund's Adjuvant on Mice Subjected to Acute Immobilization Stress

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Nonspecific immunomodulators (complete Freund's adjuvant and bovine serum albumin) produced an antistress effect on mice during immobilization stress. Immunization with a serotonin-protein conjugate produced no antistress effect.

Key Words: *immobilization stress; immunization; serotonin*

Various neurotransmitters, including serotonin (5-hydroxytryptamine, 5-HT), are involved in the development of psychoemotional stress-syndromes. Alcohol- or morphine-withdrawal syndrome serves as a psychophysiological correlate of stress syndromes [2]. Our previous studies showed that immunization with a conjugate of 5-HT and bovine serum albumin (BSA) alleviates symptoms of withdrawal in experimental animals [3,4]. Production of antibodies (AB) against 5-HT and BSA produces opposite effect on alcohol withdrawal. AB against serotonin inhibited, while AB against BSA stimulated alcohol consumption by alcohol-positive C57Bl/6 mice. Our experiments demonstrated increased production of AB against 5-HT in animals subjected to immobilization stress [1].

It remains unclear whether polyclonal stimulator of the immune system complete Freund's adjuvant (CFA) produces a psychoimmunomodulatory effect. However, published data suggest that CFA directly modulates higher nervous activity (*e.g.*, slow wave sleep and paradoxical sleep in rats) [8].

MATERIALS AND METHODS

Experiments were performed on 174 C57Bl/6 mice. Psychoemotional stress was produced by 14-h immobilization in tight cylinders. We performed 3 series. The animals were divided into 2 groups. Behavioral reactions were studied in group 1 mice. Immunoreactivity was estimated in group 2 mice.

In series I the effect of immunization with 5-HT-BSA conjugate on the stress response was evaluated. The effect of immunization with BSA was estimated in series II. The influence of CFA on signs of stress syndrome was determined in series III.

The animals were immunized routinely. 5-HT-BSA conjugate and BSA (2 mg/kg) were injected in combination with CFA (0.1 ml antigen solution and 0.1 ml CFA, subcutaneously in the back). Repeated intraperitoneal injections were made after 2 and 3 weeks (5 and 10 mg/kg, respectively). In series III the mice were injected with CFA (0.1 ml CFA and 0.1 ml physiological saline, subcutaneously in the back). Two intraperitoneal injections of physiological saline (0.2 ml) were made after 2 and 3 weeks. Control animals received subcutaneous and intraperitoneal injections of 0.2 ml physiological saline. 5-HT-BSA conjugate was synthesized as described elsewhere [7]. The mice were stressed 1 week after the last immunization.

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Production of AB against 5-HT and BSA was estimated by solid-phase enzyme immunoassay on a Dynateck mini-reader. A conjugate of 5-HT and BSA were used as the test antigens. This conjugate was synthesized by the same method using a heterologous protein carrier (equine γ -globulin).

Behavioral activity of mice was studied in a dark/light shuttle-box [6]. The latency of the first entry into the light compartment, time spent in the light compartment, frequency of entries into the light compartment, and number of crossed squares and vertical rearing postures in the light compartment were evaluated. The retention of conditioned passive avoidance response acquired 6 h before immobilization was tested in 42 mice on day 1 after learning (criterion of retention 300 sec) [5].

Immunoreactivity was determined by the weight of stress-marker organs (thymus and spleen) and functional state of lymphocytes (blast transformation with mitogens) and peritoneal macrophages (phagocytic activity). Splenocytes were cultured in RPMI-1640 medium containing 10% fetal bovine serum (Serva) and antibiotics for 3 days. Concanavalin A (Con A) and pokeweed mitogen were added in the last stage of culturing. ^3H -Thymidine (1 μCi) was added to samples 6 h before the end of culturing. Radioactivity was measured on an Intertechnique counter. Functional activity of peritoneal macrophages was estimated by the phagocytic number and phagocytic index after 45-min incubation with the culture of *Staphylococcus aureus* strain Zhaev.

The results were analyzed by Student's test (SWP-4 and PRIMER softwares).

RESULTS

Immunization of mice with antigens 5-HT-BSA and BSA induced production of AB against 5-HT (titer 1:32-1:64) and BSA (titer 1:64).

Behavioral activity of stressed mice far surpassed that of unstressed animals. They exhibited a greater number of entries into the light compartment (by 1.5 times, $p<0.01$), crossed squares (by 1.6 times, $p<0.05$), and rearings (by 1.8 times, $p<0.05$). The latency of the first entry into the light compartment tended to decrease in stressed mice. Moreover, the total time spent in the light compartment also increased in these animals (Fig. 1). When studying the effect of immobilization stress on memory we found that conditioned passive avoidance response retention time significantly decreased in stressed mice (Fig. 1).

Behavioral tests were performed to study the effect of immunization on stress-induced changes in higher nervous activity.

Series I showed that immunization with serotonin conjugate has no effect on stress-induced changes in behavioral activity (Fig. 1). Immunization with 5-HT-BSA did not prevent stress-induced changes in retention of the conditioned passive avoidance response (Fig. 2).

Series II showed that the total time spent by BSA-immunized mice in the light compartment decreased compared to that for stressed animals of other groups (Fig. 1). Immunization with BSA prevented stress-induced changes in the retention of conditioned response (Fig. 2). These data show that AB against BSA counteracted the effect of stress on behavioral activity of animals.

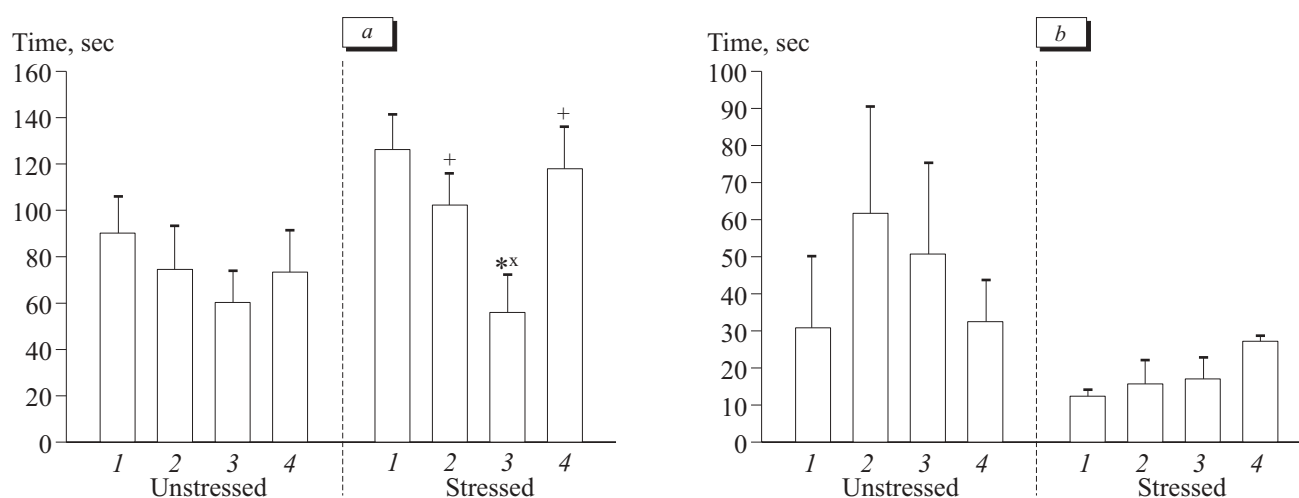


Fig. 1. Effect of stress and immunization on mouse behavior in a dark-light shuttle-box. Total time spent in the light compartment (a); latency of the first entry into the light compartment (b). Here and in Fig. 2: control mice (1); immunization with 5-HT-BSA (2) or BSA (3); treatment with complete Freund's adjuvant (4). * $p<0.01$ compared to the control; * $p<0.05$ compared to immunization with BSA; * $p<0.05$ compared to CFA.

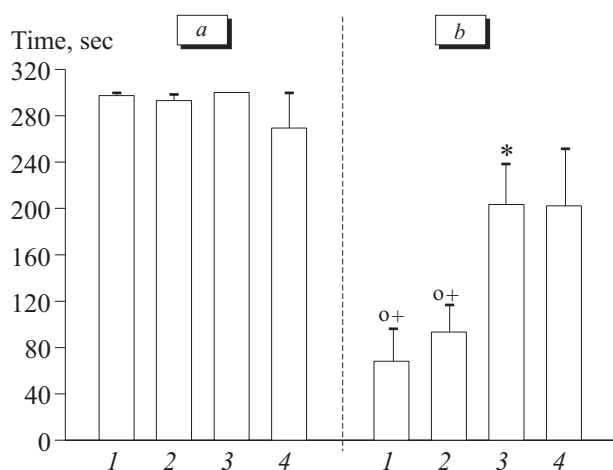


Fig. 2. Effect of stress and immunization on retention of conditioned passive avoidance response in unstressed (a) and stressed animals (b). $^{\circ}p < 0.001$ compared to unstressed mice.

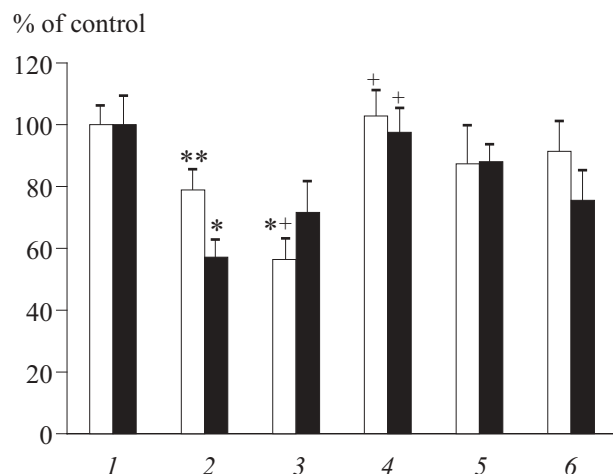


Fig. 3. Effect of stress and immunization with 5-HT-BSA and CFA on blast transformation of lymphocytes stimulated with Con A (light bars) and pokeweed mitogen (dark bars). Control (1); stress (2); 5-HT-BSA immunization and stress (3); CFA and stress (4); 5-HT-BSA immunization (5); CFA (6). $^{\circ}p < 0.001$ and $^{**}p < 0.05$ compared to the control; $^{+}p < 0.05$ compared to stress.

Series III showed that single treatment with CFA produces an antistress effect and contributes to retention of a conditioned passive avoidance response. The test parameters in animals of this group did not differ from those in unstressed mice (Fig. 2).

Immobilization stress significantly decreased functional activity of T lymphocytes estimated by the reaction of blast transformation to pokeweed mitogen and Con A (Fig. 3). Preimmunization of animals with 5-HT-BSA increased the stress reaction of splenocytes to Con A, but slightly improved the response to pokeweed mitogen. Stress exposure had a negative impact on phagocytic activity of peritoneal macrophages. The phagocytic index and phagocytic number in stressed mice were lower compared to the control (Table 1). Immunization

with serotonin conjugate decreased the severity of stress-induced changes. The stress reaction in animals exhibiting production of AB against 5-HT was accompanied by a relative decrease in the weights of the thymus and spleen (Table 2).

Pretreatment with CFA also prevented the adverse effect of immobilization stress on the proliferative response of lymphocytes to Con A (helpers) and pokeweed mitogen (suppressors) and phagocytic activity of peritoneal macrophages (Table 1).

Our results suggest that immunization with serotonin conjugate produced an unexpected effect on mice subjected to immobilization stress. The effect of immunization under these conditions was opposite to that observed in morphine- or alcohol-withdrawal syndrome. Probably, neurotransmitters play different roles under various stress conditions. This is true for the neuroimmunomodulatory effect of AB against neurotransmitters. Our results are consistent with published data that active immunization of animals with serotonin conjugate increases the severity of neuropathic pain syndrome (auto-

TABLE 1. Effect of Stress and Immunization with 5-HT-BSA Conjugate on Phagocytic Activity of Peritoneal Macrophages in C57Bl/6 Mice ($M \pm m$)

Group	Phagocytic number, %	Phagocytic index
Control ($n=7$)	51.6 ± 1.7	3.6 ± 0.1
Stress ($n=7$)	$35.3 \pm 1.7^*$	$3.2 \pm 0.1^{**}$
5-HT-BSA immunization ($n=7$)	$68.5 \pm 3.5^*$	$4.5 \pm 0.2^*$
5-HT-BSA immunization and stress ($n=7$)	$44.3 \pm 1.7^{**}$	$3.6 \pm 0.1^{++}$
CFA ($n=6$)	$59.1 \pm 2.6^{**}$	4.1 ± 0.3
CFA and stress ($n=6$)	$51.6 \pm 1.7^{++}$	$4.0 \pm 0.1^{***}$

Note. Here and in Table 2: n , number of animals. $^{\circ}p < 0.001$ and $^{**}p < 0.05$ compared to the control; $^{+}p < 0.01$ and $^{++}p < 0.05$ compared to stressed animals.

TABLE 2. Effect of Stress and Immunization with 5-HT-BSA Conjugate on the Weight of Lymphoid Organs in C57Bl/6 Mice ($M \pm m$, % of control)

Group	Thymus	Spleen
Control ($n=7$)	0.26 ± 0.02	0.38 ± 0.02
Stress ($n=7$)	$0.19 \pm 0.01^{**}$	$0.30 \pm 0.02^{**}$
5-HT-BSA immunization ($n=7$)	0.22 ± 0.02	0.39 ± 0.04
5-HT-BSA immunization and stress ($n=7$)	$0.14 \pm 0.02^*$	$0.31 \pm 0.01^*$
CFA ($n=6$)	0.20 ± 0.01	0.34 ± 0.02
CFA and stress ($n=6$)	$0.19 \pm 0.01^{**}$	$0.29 \pm 0.02^{**}$

tomy) [6]. The psychoprotective and immunoprotective effect of AB against BSA and CFA is of particular interest. This phenomenon should be analyzed in details to evaluate the role of various immune factors, including antibodies (immune complexes) and cytokines.

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