GENETICS

GABA in Regulation of Communicative Activity and Sexual Motivation of Male Mice with Different Psychoemotional Status

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We studied the effects of drugs modulating GABA content in the brain on communicative activity and sexual motivation of male mice. The effects of the drug depended on animal genotype and initial psychoemotional status. Aminooxyacetic acid elevating GABA content did not modulate communicative activity of intact males, reduced it in aggressive animals, restored in anxious animals, and promoted exhaustion of sexual motivation in anxious animals. Thiosemicarbazide reducing GABA level produced an anxiogenic effect and destabilized sexual motivation in intact males.

Key Words: *anxiety; aggressiveness; sexual motivation; γ-aminobutyric acid*

Animal behavior is based on genetic peculiarities of neurochemical mechanisms of brain activity and depends on their individual experience; experimental manipulation of this experience makes it possible to form stable alternative psychoemotional statuses (highly aggressive and highly anxious) in male mice with the same genotype [5]. Acquisition of a certain psychoemotional status is paralleled by functional restructuring of the GABAergic system of the brain. More anxious mice are characterized by decreased density of central $GABA_A$-receptors [8]. In mice with low anxiety level, GABA-stimulated release of Cl^- ions in some compartments of the brain is increased in comparison with highly anxious animals [14]. In aggressive animals, activity of the GABAergic system in the brain also changes [11]. Comparisons of individuals of the same strain and of different strains revealed a positive correlation between high aggressiveness and low level and metabolism of GABA in the brain [9]. For example, C57Bl/6J mice with low GABA content characteristic of this genotype are more aggressive than DBA/2 mice with high level of this amino acid in the CNS. It is obvious that the effects of neuroactive drugs on animal behavior depend on animal genotype [12] and on their initial psychoemotional status. However, special studies in this sphere are scanty.

GABA is the major inhibitory neurotransmitter in the brain; it is involved in the regulation of behavioral reactions of animals. The majority of GABA-positive drugs reduce anxiety; many drugs reduce also manifestations of aggression [3]. Of the GABA effects on male sexual behavior, its inhibitory effect is mainly taken into consideration [7]. Diazepam treatment improved sexual behavior in anxious, but not in dominant males [10]. Increased anxiety is one of the main factors promoting the development of sexual disorders, but GABAergic mechanisms regulating these disorders are not studied. Here we

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studied changes in the level of GABA on the development of anxious, aggressive, and sexual behavioral reactions of male mice of different genotypes with different psychoemotional statuses formed within the strains.

MATERIALS AND METHODS

Experiments were carried out on adult male CBA/Lac and C57Bl/6J mice. These strains are contrast by activity of glutamate decarboxylase (GABA synthesizing enzyme) in the brain: high in CBA/Lac and low in C57Bl/6J [10]. Behavioral model of sensory contact [5] was used: the males gained daily experience of social victories (aggressors) or defeats (victims) in male-male confrontations for 20 days, which led to the formation of the corresponding psychoemotional status. Intact males kept in groups without experience of chronic social confrontations served as controls.

The communicativeness of animals was evaluated in the wall test [5]. Behavioral activity of males in the reaction to an unknown male behind the transparent wall with holes dividing the cage into parts was evaluated for 5 min.

Sexual motivation testing is used for evaluation of the intensity and stability of reaction of a male to an estrus female behind a transparent wall with holes [1]. The behavioral reaction of males was studied for 30 min by a series of 5-min sessions. The parameters were recorded using Mouse software.

Aminooxyacetic acid (AOAA; ICN; 10 and 15 mg/kg intraperitoneally), an inhibitor of the enzyme cleaving GABA increasing total GABA content in CNS starting from the specified concentrations [14] and thiosemicarbazide (TSC; ICN; 6 mg/kg intraperitoneally), an inhibitor of GABA synthesis decreasing the total content of this amino acid were used in the study. The doses were selected so as to rule out pronounced effects of the preparations on consciousness and motor activity of animals. The drugs were injected in single doses 1-1.5 h (AOAA) and 1.5 h (TSC) before testing.

The data were processed by nonparametric Mann—Whitney *U* test and Wilcoxon *T* test using Statistica software. Each group consisted of 10-12 animals

RESULTS

Intact CBA/Lac males spent more time near the wall separating them from receptive females. Victims in intermale confrontations of this strain also spent more time near the wall in this test with females, though their behavioral reactions were reduced by the absolute values in comparison with intact animals. Aggressive males had longer contacts with unknown males than control animals, the duration of contacts did not differ from the time of contact with the female (Fig. 1). The duration of behavioral reaction to an animal behind the wall is the most direct indicator of the level of motivation (social or sexual) [2,5]. Intact and submissive males were more motivated to contact with a female, while aggressive males preferred aggressive male-male contacts.

Injection of AOAA (10 mg/kg) to CBA/Lac males with different psychoemotional status did not modify the parameters of their behavioral reactions to unknown males and receptive females in com-

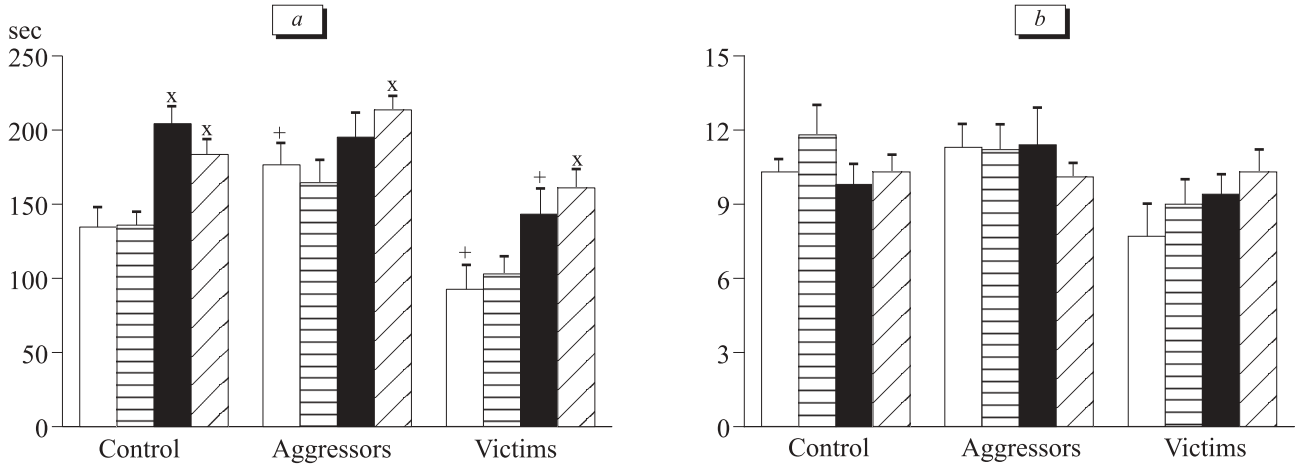

Fig. 1. Effects of AOAA (10 mg/kg) on behavioral reaction of CBA/Lac males with different psychoemotional status to an unknown male and a receptive female in the wall test. Here and in Fig. 2: *a*) time spent near the wall; *b*) number of approaches. Light bars: reaction to a male in animals injected with saline; horizontal hatching: reaction to a male in animals injected with AOAA; dark bars: reaction to a female in animals injected with saline; cross hatching: reaction to a female in animals injected with the drug. $p<0.05$ *vs.* *animals injected with saline; +control animals injected with saline; xreaction to a female after the same treatment.

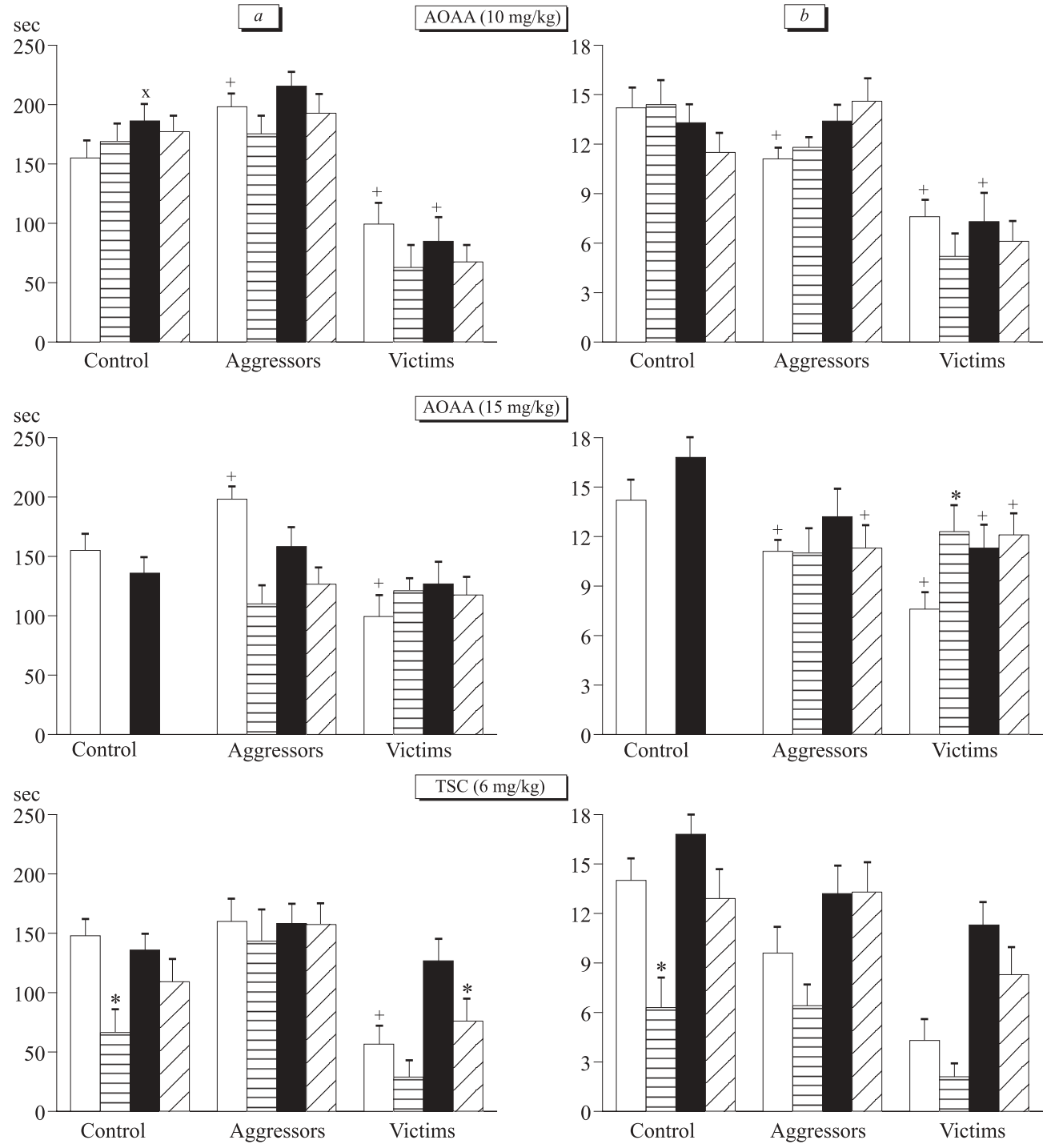

Fig. 2. Effects of GABAergic drugs on behavioral reactions of C57Bl/6J mice with different psychoemotional status to an unknown male and receptive female.

parison with animals injected with saline. In victims, the injection of AOAA restored the time spent near the wall separating them from an unknown male and a receptive female, this indicating anxiolytic effect of the preparation. In aggressors, the time spent near the wall with a male behind it decreased after AOAA injection to the control level (saline injection), which was a characteristic indicator of reduced aggressive motivation of these animals [5], while the time of reaction to a female was prolonged in comparison with the reaction to a male in aggressors injected with AOAA. On the whole, the behavior of aggressors injected with AOAA did not differ from that of intact mice, which

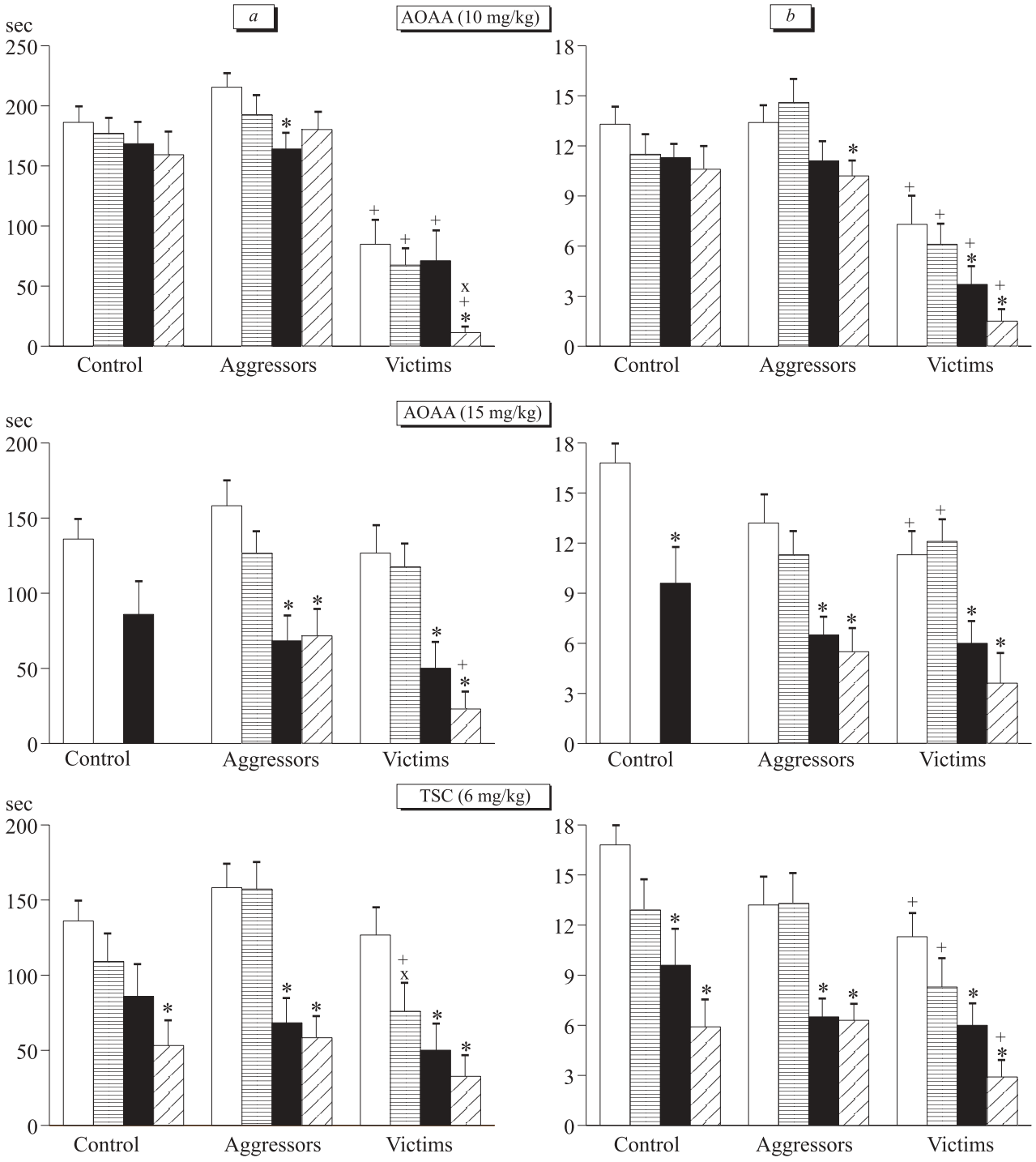

Fig. 3. Effects of GABAergic drugs on sexual motivation stability of C57Bl/6J males with differently psychoemotional status. *a*) time spent near the wall; *b*) number of approaches to the wall. Light bars: session 1 with saline injection; horizontal hatching: session 1 with the drug; dark bars: last session with saline; cross hatching: last session with the drug. $p<0.05$ *vs.* *the parameter in session 1; +controls injected with saline at the same time; x the parameter in animals injected with saline at the same time.

is in line with the concept on reduced tone of the GABAergic system in aggressive animals [7]. Hence, slight modifications of GABA level in the CNS did not change the behavior of intact males, but had an opposite effect on social contacts of aggressors and victims (reducing in the former and restoring in the latter). On the other hand, a uniform slight stimulatory effect of AOAA on the primary sexual motivation of males was observed in both groups.

The time spent by intact and anxious C57Bl/6J males near the wall with a receptive female behind it varied: did not differ from the time of sensory

contact with the male or was longer. For aggressors the time of reaction to a male was longer or the same as in the controls, while the duration of reaction to a female did not differ from the duration of contacts with an unknown male. Similarity of behavioral reactions of mice of different strains presumably indicates their universal pattern: intact and submissive males prefer contacts with females, while aggressors are liable to male-male contacts.

Injection of AOAA (10 mg/kg) to C57Bl/6J males did not change the parameters of reactions to unknown male and receptive female in comparison with animals of the same psychoemotional status injected with saline (Fig. 2). In intact males injected with AOAA, the duration of reaction to a female decrease to a level recorded for the reaction to an unknown male. No appreciable shifts were detected in the group of submissive animals. In aggressors, the duration of reaction to an unknown male decreased to a control level after injection of AOAA. Hence, the drug in this dose had different effects on the CBA/Lac and C57Bl/6J males. A common result was reduction of aggressive motivation in aggressive animals of both strains after AOAA injection. No resocializing effect of GABAergic preparation in victims and no stimulation of sexual motivation of animals with different psychoemotional status by the drug were detected.

Injection of AOAA in a higher dose (15 mg/kg) to anxious males restored the duration of contacts with unknown males to the level recorded in control mice injected with saline and increased the number of approaches to the wall; the duration of behavioral reaction to a female did not change (Fig. 2). In aggressive males treated with AOAA, the duration of contact with unknown male decreased significantly in comparison with aggressors injected with saline; the number of approaches to the wall in contact with the female decreased. The behavioral reactions to male and female virtually did not differ in aggressors and victims injected with AOAA.

Hence, AOAA exhibited a genotype-dependent effect on animal behavior. Submissive C57Bl/6J males with genotypically low level of GABA needed a higher dose for attaining the anxiolytic effect than submissive CBA/Lac males. AOAA did not stimulate the primary sexual motivation in C57Bl/6J mice.

The effect of AOAA on behavior depends on the initial psychoemotional status of animals. The drug effect on intact animals was minimum; its effects on social interactions of aggressors and victims of both strains were opposite: it reduced aggressive motivation of aggressors and exhibited an anxiolytic effect in submissive animals, prolonging the duration of social contacts to the control level.

Injection of TSC (6 mg/kg) to intact C57Bl/6J males decreased in the duration and number of contacts with unknown males and did not change the parameters of contacts with females (Fig. 2). The behavioral reactions of submissive males to unknown males did not change, but the duration of contact with females decreased. The drug did not modify the behavior of aggressive males in both tests. Hence, TSC exhibited an anxiogenic effect depending on the psychoemotional status of animals and manifesting by reduced number and duration of social contacts in intact males and by reduced reaction to a receptive female in submissive males. The absence of parallelism in the drug effects on the social and sexual behavioral reactions indicates an intricate relationship between elevated anxiety and sexual motivation. It seems that their neurochemical regulation is not always unidirectional. Moreover, the anxiolytic effect of AOAA in anxious CBA/Lac males was paralleled by activation of behavioral reaction to the female, while in submissive C57Bl/6J males anxiolysis did not modulate the primary sexual motivation. Presumably, a higher dose of the drug is needed for attaining an appreciable effect on the sexual motivation of C57Bl/6J males. In aggressive mice with reduced content of GABA in the CNS its further reduction after injection of TSC virtually did not change their social and sexual interactions.

Intact males exhibited the same time in the first and last sessions of the sexual motivation stability test, spending the same time near the wall in reaction to a receptive female, which indicates stability of their sexual motivation. In submissive males the duration of reaction to the female was reduced from the first session of the test in comparison with control males, or the reduction was observed in the course of the test; the number of approaches to the wall also decreased. In aggressive males the duration of contacts with the female decreased in the last session of the test in comparison with session 1. The results indicate instability of sexual motivation of males taking part in long-lasting social conflicts [1,2].

In control males injected with AOAA in a dose of 10 mg/kg, the duration and number of approaches to the wall did not change during the last session in comparison with the first (Fig. 3). In submissive males the duration of reaction to the female decreased significantly during the last session in comparison with the first one. In aggressive males injected with AOAA, the duration of contact with females during the last session did not differ from that in the first one, though the number of approaches decreased.

Injection of AOAA in a higher dose (15 mg/kg) decreased in the duration of contact with the female during the last session of the test in submissive animals, while the parameters of behavioral reaction of aggressors did not change (Fig. 3). Hence, AOAA in the studied doses did not modulate the stability of sexual motivation in intact males, but promoted its exhaustion in submissive animals. In aggressive animals, the low dose of the drug corrected exhaustion of sexual motivation, but this effect ceased with increasing the dose.

Injection of TSC in a dose of 6 mg/kg to intact males decreased in the duration of reaction to the female during the last session of the test in comparison with the first one and did not change the duration of contact with the female during the last session of the test in both aggressors and victims (Fig. 3). Hence, the stability of sexual motivation of males is regulated by GABA over time, GABA effect depending on the psychoemotional status of animals. In intact males disorders in sexual motivation stability are most probable in case of GABA level reduction in the brain, but not its elevation. On the other hand, the decrease in GABA content was inessential for sexual motivation disorders characteristic of aggressive and submissive males, while accumulation of the mediator augmented these disorders, at least in anxious animals.

These experiments gave new data on the functioning of the mechanism of GABAergic regulation of behavioral response depending on the genotypical and phenotypical characteristics of animals. The first results were obtained due to thorough selection of doses, as a double increase of AOAA dose could have a sedative effect (reduction of all motivations), while the increase in TSC dose could lead to convulsive states. It seems that intact mice need a certain level of the amino acid in the brain for the appetent phase of sexual behavior, because changes in this parameter disturb the primary reaction to a receptive female or stability of this reaction with time. Drugs with opposite effects on the neurochemistry have unambiguous negative effect on animal behavior if they destabilize the CNS processes [4]. Presumably, optimally tunned GABAergic and neurochemical mechanisms coupled with them, represent a type of vitally important stationary processes, destabilization of which leads to behavioral disorders. AOAA treatment of submissive animals augmented the intrinsic disorders of sexual motivation.

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