PHARMACOLOGY AND TOXICOLOGY

Effect of Short-Term and Chronic Caffeine Intake on Rats with Various Anxiety Level

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Effect of one-day and chronic (3 week) drinking of 0.1% caffeine as a single source of fluid on anxiety in rats with different anxiety level (either genetically determined or individually acquired) was studied. When housed in groups, Fisher-344 rats had a significantly higher anxiety level than WAG/G rats. When the rats were housed individually, their anxiety gradually increased. The effect of short-term and chronic intake of caffeine in high doses depended on both genetically determined sensitivity to this agent and environmental factors (social isolation). It was concluded that chronic caffeine drinking does not induce persistent emotional changes similar to drug abuse, because caffeine withdrawal produced little effect on animal anxiety.

Key Words: *caffeine; emotionality; inbred rats; social isolation*

Caffeine is a psychoactive substance that is present in coffee, tea, cacao, many soft drinks, and other foodstuffs. Therefore this substance is regularly consumed in appreciable amounts in various social strata. Adult Americans consume 3 mg caffeine per kg body weight per day, mainly with coffee [2]. American children consume 0.5-1.8 mg/kg/day caffeine mostly with soft drinks and chocolate [5]. We found no data on caffeine consumption in Russia.

High doses of caffeine produce a number of pharmacological effects from somatoautonomic stimulation to modification of the higher psychic functions in humans [3,6]. One of the best-studied effects of caffeine is modulation of anxiety [9,11,12]. However, published data on chronic effects of caffeine are not numerous, and their results are contradictory. Chronic caffeine intake on the one hand enhances anxiety [12], but on the other hand, leads to tolerance, which manifests in reduced anxiogenic effect [4,7,8]. At the same time, cessation of chronic intraperitoneal injection of caffeine pronouncedly enhanced anxiety in rats [4].

Our aim was to study the effect of one-day and 3-week regular consumption of 0.1% caffeine solution as a single source of fluid on rats with initially different level of anxiety (genetically determined or individually acquired).

MATERIALS AND METHODS

Experiments were carried out on male WAG/G and F-334 rats (48 rats of each strain). A half of each group was housed in groups of 8 animals per cage (40×30×15 cm). Others were placed in individual cages (25×10×20 cm) immediately after arrival. The rats received food (standard combined fodder) and water ad libitum and were maintained under conditions of 12:12 h light-dark cycle.

Water in drinking bowls was replaced with 0.1% caffeine solution after 1 week (16 rats of each

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strain housed in groups or individually) or on experimental day 27 (other 16 rats of each strain).

On experimental days 7 and 28, anxiety was tested in a chamber (35×16×21 cm) consisting of dark and light compartments separated with a wall with a hole. The rats were placed in the light compartment. The latency of the first transition to the dark compartment and the total time spent in the light compartment over 5 min were measured.

After 3 days, all rats were tested in the Vogel conflict test. Before the test, the rats were subjected to 72-hour drinking deprivation. Then the rats were placed in an experimental chamber with drinking water. Every tenth approach to water bowl was punished with an electric shock (0.5 mA). For each rat the number of punishments for 5 min was calculated.

The results were analyzed statistically using Student's *t* test and three-factor analysis of variances.

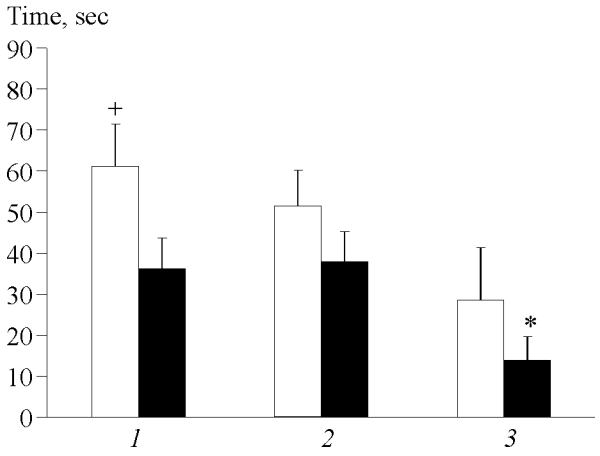

Fig. 1. Time spent in the light compartment by WAG/G (light bars) and Fisher-344 rats (solid bars) in the dark/light compartment test. *1*) group housing; *2*) individual housing during 1 week; *3*) individual housing during 4 weeks. Here and on Fig. 2: $p<0.05$ *compared to grouped housing; $^{+}$compared to F-344 rats.

RESULTS

F-344 rats housed in groups demonstrated significantly higher anxiety in the dark/light compartment test compared to WAG/G rats, which agrees with our previous data [1,10]. The time spent in the light compartment was 36.14±7.78 and 61.14±9.90 sec, respectively. In individually housed rats, anxiety gradually increased: the time spent in the light compartment tended to decrease with increasing the duration of individual housing. On experimental day 28, these changes in F-344 rats became significant (Fig. 1).

Evaluation of anxiety in the Vogel test also revealed differences between these strains. F-344 rats demonstrated higher anxiety than WAG/G rats (WAG/G rats made a greater number of approaches to the drinking bowl irrespective of housing conditions, Fig. 2). Anxiety of WAG/G rats revealed in the Vogel test increased when the rats were housed individually. Factor analysis showed that higher an-

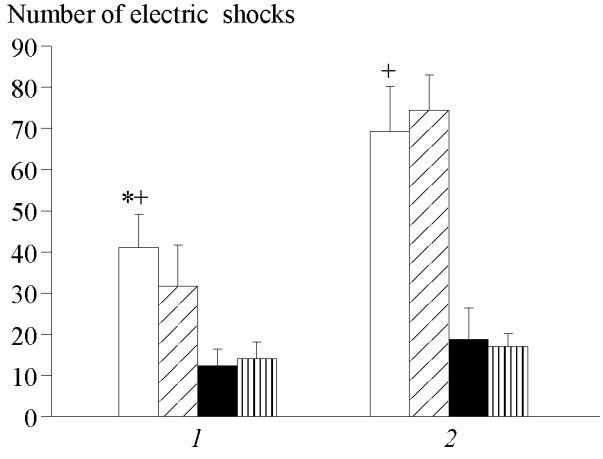

Fig. 2. Number of electric shocks in the Vogel test in WAG/G and Fisher-344 rats housed individually (*1*) or in groups (*2*). Here and in Fig. 3: light bars and oblique hatching correspond to WAG/G rats receiving water and 0.1% caffeine, respectively; solid bars and vertical hatching correspond to F-344 rats receiving water and 0.1% caffeine, respectively.

TABLE 1. Water and 0.1% Caffeine Consumption in Rats (ml/day/rat, *M*±*m*)

Series		WAG/G		F-344	
		water	caffeine	water	caffeine
Individual housing					
	day 1	31.2±1.8	30.0±1.1	39.1±3.0	30.6±3.1
	day 21	27.2±1.2	26.6±1.2	30.8±2.1	29.1±2.4
Group housing					
	day 1	27.2	18.6	25.7	21.4
	day 21	21.5±1.8	31.0±1.3	31.5±2.8	33.3±1.2

Note. *Measurement was performed for the whole group and the arithmetic mean was calculated.

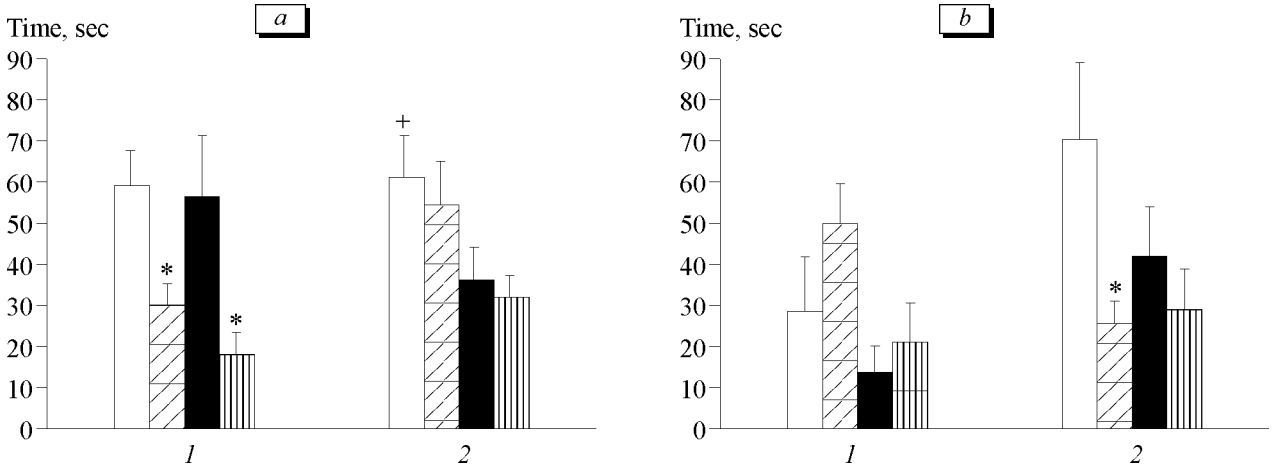

Fig. 3. Time spent by WAG/G and Fisher-344 rats in the light compartment in the dark/light compartment test. The rats housed individually (*1*) or in groups (*2*) received caffeine for 1 day (*a*) or 3 weeks (*b*). $p<0.05$ *compared to water; +compared to F-344 rats.

xiety in the Vogel test was determined by genetic factors (F=37.426, p<0.001) and social isolation (F=1 0.035, p=0.003), while in the dark/light compartment test the leading role was played by genetic factors (F=5.899, p=0.019).

Water consumption did not depend on rat genotype and housing conditions. Drinking of caffeine solution was also similar in various rat groups (30.00± 1.23 ml caffeine solution per rat, *i.e.* 122.80±5.93 mg caffeine per kg body weight per day, Table 1). This dose of caffeine is high enough to produce an anxiogenic effect [4,12].

Drinking 0.1% caffeine for 1 day produced no significant effects on the anxiety level in rats housed in groups. When the rats were housed individually, caffeine increased anxiety in both strains, but this increase was more pronounced in F-344 rats (Fig. 3, *a*). Factor analysis revealed the main contribution of genetic peculiarities (F=5.899, p=0.019) and caffeine drinking (F=9.368, p=0.003) to the genesis of anxiety. In addition, it revealed significant interrelationship between social isolation and caffeine drinking (F= 4.895, p=0.031).

Drinking 0.1% caffeine for 3 weeks had no effect on anxiety in individually housed rats of both strains, and in F-344 rats housed in groups. The time spent in the light compartment was shortened only in WAG/G rats housed in groups (Fig. 3, *b*).

Seventy-two hours after caffeine withdrawal (after 3 weeks treatment), there were no changes in anxiety level in rats of both strains irrespective of housing conditions (Fig. 2).

Our data show that the effect of short-term and chronic of intake caffeine in high doses depends on both genetically determined sensitivity to this substance and environmental factor (social isolation). In low-anxious rats kept under comfortable conditions, anxiety increased only after chronic caffeine intake, while in high-anxious rats (in particular, in individually housed rats) the effect of short-term caffeine intake was more pronounced. Thus, long-term caffeine drinking led to adaptation to its anxiogenic effect in high-anxious and to sensitization in low-anxious rats. In contrast to intraperitoneal injection of caffeine [4], chronic caffeine drinking induced no persistent changes in emotionality similar to physical dependence, because caffeine withdrawal had no significant effect on animals anxiety.

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