Anxiety and Predisposition to Audiogenic Epilepsy in Rats of Different Genotypes

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We studied plus-maze behavior of inbred Krushinskii–Molodkina, Wistar, and black-hooded rats (originating from the Long-Evans outbred strain) differing by predisposition to audiogenic seizures. The severity of audiogenic seizures partially correlated with anxiety and negatively correlated with the total level of locomotor activity in the elevated plus-maze. The anxiety parameters in Krushinskii–Molodkina rats were evaluated after injection of anticonvulsant levetiracetam and anxiolytic afobazol. Levetiracetam and afobazol somewhat stimulated locomotor activity.

Key Words: *anxiety; locomotion; elevated plus-maze; audiogenic epilepsy; Krushinskii—Molodkina rats*

A total of 25-30% patients with epilepsy exhibit high anxiety and predisposition to depressions [7]. Hence, evaluation of anxiety levels in animals, which serve as laboratory models for studies of epilepsy mechanisms, is essential for detecting the relationship between levels of convulsive readiness and anxiety.

One of approaches to evaluation of the genetic component of predisposition to epilepsy is the study of audiogenic epilepsy in rodents (*e. g.* rats) [2,4]. Krushinskii–Molodkina (KM) rat strain is widely used as a model of human convulsive states [4]. In audiogenic seizure (AS), excitation arises in the upper compartments of the brain stem [2,4]. Presumably, genetically determined structural and functional abnormalities of brain stem structures are essential for the level of anxiety in AS-prone rats. This hypothesis is supported by the data on high anxiety level in WAG/Rij rats exhibiting AS [11]; behavioral changes are also characteristic of WAR (Wistar audiogenic rats) [5].

Anxiety in KM rats in comparison with other strains has never been evaluated before. Possible changes in anxiety during modulation of seizure readiness with anticonvulsant levetiracetam and anxiolytic afobazol have never been studied.

We studied the level of anxiety of Wistar and KM rats in the elevated plus-maze (EPM) test [8]. The behavior of an animal placed into new settings (in our case in EPM) is determined by exploratory motivation and inherent fear of open space. The dependence of anxiety in EPM and AS intensity on animal age was analyzed.

Hence, we analyzed the parameters of predisposition to audiogenic epilepsy and anxious behavior in rats of different genetic groups.

MATERIALS AND METHODS

The study was carried out on 83 male rats: inbred KM (M. V. Lomonosov University) aged 2.5 months (n=8) and 6 months (n=30), outbred Wistar (Institute of Pathophysiology) aged 2.5 months (n=9) and 6 months (n=21), and 4-month-old black-hooded rats (n=15) of the 34th generation of inbred mating, selected by predisposition to audiogenic epilepsy, from a small population of Long-Evans rats (Institute of Pharmacology). Animal body weight was 250-300 g.

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Wistar rats were divided into 2 groups exhibiting and not exhibiting AS (audiogenic AW, and nonaudiogenic NW, respectively). The rats were kept under standard laboratory conditions with free access to water and food. Experimental procedures were carried out in accordance with the regulations of the European Commission Directive No. 86/309/EEC of November 24, 1986.

Levetiracetam (UCB; 80 mg/kg) and afobazol (Farmstandart; 5 mg/kg) were injected intraperitoneally 60 min before the experiment. The substances were diluted to working concentrations with distilled water. Controls were injected with similar volumes of distilled water. Each animal was tested once.

Predisposition to audiogenic epilepsy was scored: 0: no reaction; 1: motor stimulation (clonic running); 2: clonic convulsions; 3: clonic tonic convulsions; and 4: tonic convulsions [2].

The black plastic maze had 50×10 cm arms, 38 cm walls, and 10×10 cm central area and was 73 cm elevated above the floor. Testing was carried out over 3 min starting from the moment when the rat was placed into the EPM center. The number of visits to open arms, time spend there, number of peeping off and visits to closed arms, transitions from open to dark and from dark to open arms, *etc.*, were recorded (Table 1).

The results were processed by standard Statistica 6.0 software using the nonparametric Mann–Whitney test. The differences between the samples were evaluated by Student's *t* test using Fisher φ accessory variable.

RESULTS

The intensity of AS in rats of different genotypes is summed up in Table 2. KM rats exhibited the highest AS intensity score (4 points) and the shortest AS latency. In Wistar-AW rats, the mean seizure intensity score was 1.8. They responded to the sound by only "clonic running" and sometimes by clonic but not tonic convulsions. NW rats exhibited no reaction to acoustic stimulus over 90 seconds (0). The mean AS score in AW and black-hooded rats was significantly lower than in KM rats. No age-specific differences by this sign were detected in Wistar or KM rats in our sample. Young KM and Wistar rats differed significantly ($p<0.001$) by AS intensity (higher in KM rats) and latency (longer in Wistar). No differences were recorded in two-wave reactions (indicator of low intensity of AS) in KM rats, in contrast to Wistar rats which exhibited these differences (data not presented).

The results of EPM testing are presented in Table 1. No appreciable differences in the parameters were detected between 2.5-month-old AW and NW rats, but AW spent less time in open arms than NW rats.

Just few age-associated differences in the behavior of Wistar rats in EPM were found. The number of grooming episodes was significantly lower and that of visits to the EPM open arms slightly higher in the group of 6-month-old Wistar rats in comparison with younger animals. Age-associated differences were observed in KM rats. A significantly lesser number of visits to the open arms and shorter time spent in the center were registered in 6-month-old *vs.* 2.5-month-old rats. Younger rats exhibited significantly greater numbers of peeping out, grooming, and rearing episodes. These facts indicated higher level of anxiety of 6-month-old KM rats. Significant differences in the behavior of young KM compared to Wistar rats consisted in a greater number of rearing episodes (7.33 *vs.* 2.88, $p<0.05$) and longer time spent in the center (Table 1; $p<0.05$).

The behavior of 6-month-old KM rats in the EPM test differed significantly ($p<0.01$) from those in both groups of Wistar rats: the numbers of their visits to open and closed arms (*vs.* NW rats) were significantly lower ($p<0.05$). The time spent by KM rats in the open

TABLE 1. Anxiety (EPM Test) in Rats of Three Genotypes

Group	*n*	Visits to open arms	Visits to closed arms	Time spent in open arms, %	Time in the center, %	Peeping out
KM, 2.5 months	8	1.38±0.46	1.38±0.53	22.16±0.14	39.44±0.17	4.00±1.36
KM, 6 months	11	0.36±0.20^{+}	1.00±0.19	10.51±0.09	12.07±0.10^{+}	1.09±0.91
AW, 2.5 months	3	1.00±0.57	1.33±0.32	5.93±0.13	5.00±0.12	3.67±2.33
NW, 2.5 months	6	1.17±0.40	1.33±0.42	22.60±0.17	3.10±0.07*	3.33±1.52
AW, 6 months	12	1.75±0.37**	1.92±0.45	43.10±0.14*	5.32±0.06	5.75±1.40*
NW, 6 months	9	1.78±0.34**	1.78±0.22*	23.39±0.14	9.13±0.09	4.22±1.00*
Black-hooded, 4 months	14	2.00±0.49**	2.36±0.44*	25.80±0.11	17.30±0.10	4.64±1.27*

Note. *$p<0.05$, **$p<0.01$ compared to KM rats; $^{+}p<0.05$ compared to 2.5-month-old KM rats.

arms was less than that of NW rats (slightly) and AW rats (significantly). The number of peeping out episodes was significantly (p<0.05) less in the KM *vs.* both Wistar groups. These data indicate higher anxiety of KM rats (Table 1).

The AS score of black-hooded rats was somewhat higher in comparison with AW (Table 2) and the level of exploratory activity in EPM was also higher: many visits to the open (2.00±0.49) and closed arms (2.36±0.44), this differing significantly from KM rats, and many rearing episodes (14.36±2.14), this differing significantly from KM and Wistar rats.

In KM rats, injection of levetiracetam reducing AS intensity [1] increased the number of visits to closed arms and reduced the time spent in the center. The number of peeping down from the maze and rearing episodes virtually did not change. This effect could be interpreted as a slight increase in locomotor activity paralleled by reduction of AS level.

Injection of anxiolytic afobazol to KM rats slightly increased the number of visits to closed arms (1.00 *vs.* 1.44), this indirectly attested to an increase in their locomotor activity. Afobazol produced a moderate anxiolytic effect: the rats exhibited slightly more peeping out (1.09 *vs.* 1.56) and rearing (5.73 *vs.* 8.22) episodes, which could be interpreted as reduction of anxiety. Reduction of anxiety after afobazol injection was also seen from reduced number of defecation acts (1.55 *vs.* 0.44).

Experiments demonstrated high anxiety of KM rats selected by high predisposition to audiogenic epilepsy. These rats demonstrated lower number of visits to the open EPM arms and spent less time there. High anxiety of KM rats seemed to be also responsible for the lower number of peeping out episodes in the open EPM arms. Since KM rats had the highest AS score in comparison with other genetic groups of rats used in the study, it was just logical to suggest a positive correlation between anxiety level and predisposition to AS. This hypothesis was confirmed by direct evaluation of coefficient of correlation between the values characterizing AS and anxiety level. AS intensity correlated significantly with the number of visits to open arms (r=-0.45, p<0.05) in KM and black-hooded rats. No correlation of this kind was observed in the Wistar rat group (r=0.05). One more indirect evidence of positive correlation between AS intensity and level of anxiety was slow progress (by the age of 6 months) of the "adult" anxiety level in KM rats and slight age-associated differences in this sign manifestation in Wistar NW rats (in the subgroup without AS in response to audiostimulus) which had no differences in AS levels. A previous study had shown the formation of audiogenic seizure of the maximum severity by the age of 3.5-4.0 months as a rule [3]. Unfortunately, we have no data on the age of formation of these signs in black-hooded rats. Importantly, black-hooded rats exhibiting higher AS activity than AW rats (Table 2) also exhibited better exploratory activity (number of rearing episodes and movements in EPM). Black-hooded rats, passing the first steps of selection by high AS intensity, originated from Long-Evans strain. Therefore, the strain-specific differences (Long-Evans/Wistar) could be responsible for higher exploratory activity of these animals; for example, their pigmented eyes (higher vision acuity) determined better spatial orientation. Despite better exploratory activity of these animals, they exhibited (similarly as KM rats) a positive correlation between anxiety and AS.

The association of high anxiety and high predisposition to AS described for rats of these genotypes can be essential for the analysis of the epilepsy pathogenesis in humans. It has been shown that the efficiency of anxiolytics reducing animal anxiety largely depended on its initial level [6,10]. The genetically determined anxiety level of KM rats was presumably so high that injections of levetiracetam and afobazol led to just slight stimulation of locomotion without appreciably reduc-

TABLE 2. Intensity of Audiogenic Seizure in Rats of Three Genotypes

Group	*n*	Seizure latency, sec	Seizure intensity, score
KM, 2.5 months	8	1.75±0.16	3.88±0.08
KM, 6 months	11	1.40±0.14	3.98±0.02
AW, 2.5 months	3	16.67±5.61***	1.67±0.33***
NW, 2.5 months	6	0	0
AW, 6 months	12	14.20±1.83***	1.80±0.13***
NW, 6 months	9	0	0
Black-hooded, 4 months	15	16.40±4.43***	2.07±0.19***

Note. *p<0.05, **p<0.01, ***p<0.001 compared to KM rats.

ing anxiety. However, a trend to reduction of anxiety after injection of antiepileptic levetiracetam could be a manifestation of the above association between anxiety and high convulsive readiness. A possible mechanism of high anxiety manifestation in rats selected by predisposition to AS could be AS-associated shifts in brain levels of monoamines and other neurotransmitter amino acids demonstrated previously [9].

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