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METHODS

Modeling of Focal Seizures with Automatisms in Rats with Pendulum Movements

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Phenotypical study was carried out in rats with pendulum movements. The animals exhibited a high level of abortive seizures in response to audiogenic stimuli and longer postictal catalepsy in comparison with those in Wistar population. Seizure severity positively correlated with the duration of poststimulus catalepsy (r=0.90). High aggressiveness towards humans, the absence of BP elevation in stress, lower body weights, and lower weights of the kidneys and spleen in PM rats are considered concomitant traits. Correlations were detected between startle-1 and BP in rats with pendulum movements (r=0.70) and between startle-10 and BP in narcotized Wistar rats (r=-0.0.71). The newly described signs in rats with pendulum movements did not contradict the signs of the focal seizure model with typical automatisms in humans.

Key Words: *pendulum movements; seizure; blood pressure; heart and kidney weights*

Selection rats with high amplitude of pendulum movements (PM) can serve as a model of focal seizure epilepsy. In PM rats, the forepart of the body swings in the lateral direction at rest, the amplitude of PM increasing in stress (Fig. 1). Audiogenic stimulation induces seizures in 90% PM rats, in contrast to just 25-30% in the initial Wistar rat population [2]. This increase is determined by higher incidence of abortive seizures that do not eventuate in clonic-tonic convulsions, *i.e*. are not generalized. The models generalized convulsion are Krushinsly—Molodkina rats [9], GEPR [5], and WAR [4] rats. In PM rats, audiogenic stimulation provokes chaotic running and stereotyped forced jumps never leading to lethal outcomes. By the common behavioral picture, abortive seizures resemble focal seizures with typical automatisms. The content of inhibitory transmitter taurine (2-aminoethansulfonic acid) in the hippocampus of PM rats is reduced [1], which indicates possible epileptiform focus in this brain structure. Selection line of rats with 95% incidence of pendulum hyperkinesis has never been described.

We analyze the relationship between direct selection for more intense PM and high level of convulsive activity and the related stress-dependent characteristics in rats of the studied line.

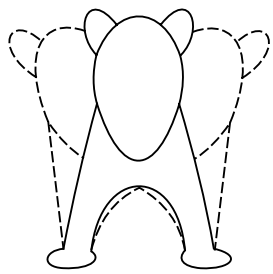

Fig. 1. Scheme of pendulum movements.

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MATERIALS AND METHODS

The study was carried out on PM rats (n=20; selective breeding for 45 generations) and Wistar rats (n=16). The animals were kept in a vivarium (Institute of Cytology and Genetics) with free access to water and food. The conditions of handling and protocols of the experiments were approved by Bioethics Committee of the Institute of Cytology and Genetics, all manipulations on animals were carried out in accordance with the recommendations of the European Parliament and EC Council (Directive 2010/63/EU, September 22, 2010).

The number of animals in the litter at birth and the number of females without pregnancy were recorded.

Audiogenic epilepsy testing was carried out in a plexiglas box with plastic bottom, 50×50×50 cm, with an electric bell (110 dB, 60 Hz) fixed inside the box. Acoustic stimulation lasted <1 min. The reaction was scored as follows: 0 — no motor excitation; 1 — abortive seizure; 2 — clonic tonic seizures. The duration of postictal catalepsy (sec) was recorded from the moment of the acoustic stimulus switch-off until the first head movement.

The results of the "glove" test (animal reaction to humans) were evaluated in points from -4 to 4: -4 to -1 for aggressiveness (negative reaction) and 1 to 4 for emotionally positive reactions [8].

Cataleptic freezing was tested as follows: the rat was placed into the corner of the cage and raised vertically (with a bar) by the muzzle, after which the duration (sec) of passive retention of this forced vertical posture was measured.

The "nervous" reaction was evaluated in parallel with the catalepsy testing. If the rat startled when touched with a stick or ran or dashed around the box, the reaction was evaluated as 1 point; calm reaction to the stick, or sniffing or gnawing the stick was scored as 0.

The acoustic startle response was also studied in the Startle Response device (TSE Equipment) — a sound-proof box with a sensitive platform, onto which a restriction cage was placed. After 3-min adaptation (white noise, 65 dB), ten acoustic signals were presented (white noise, 40 msec, 115 dB) at 15 sec intervals. The platform fluctuations were recorded automatically, the digital values of pressure on the platform were shown on the monitor.

BP was measured by the sphygmographic method (recording of caudal artery wall fluctuations under ether narcosis). One week after measurement of BP at rest, conscious rats were placed in tight tubes (under conditions of limited mobility) and BP was measured in 15 and 30 min.

The animals were sacrificed using a guillotine under ether narcosis. The body weight and weights of the heart, kidneys, adrenals, and spleen were measured on electronic scales (SK/SK-D/SK-WP).

The results were statistically processed using Statistica 10.0 software. The differences in the behavioral and physiological parameters (freezing, "nervousness", startle amplitude, BP, seizure severity, duration of catalepsy, weights of organs) were assessed using one-way ANOVA and LSD test; $M\pm m$ were calculated for each parameter. Analysis of correlations was carried out using nonparametric Spearman's test.

RESULTS

The incidence of abortive seizures in response to acoustic stimulation differed in rats selected for high PM hyperkinesis. Seizures in PM rats were almost 4-fold more incident than in Wistar rats (Table 1). Abortive seizures manifested in chaotic running and forced jumping. The incidence of jumps reached one per second. The excitation abated after ten or more

TABLE 1. Emotiogenic Parameters, Birth Rate, and Weights of Organs in Wistar and PM Rats ($M\pm m$)

Parameter	Wistar (n=8)	PM (n=10)
Audiogenic seizures, score	0.38±0.38	1.50±0.34*
Postictal catalepsy, sec	113.4±35.8	228.3±36.6*
Aggressiveness, score	1.3±0.08	-0.33±0.33****
Cataleptic freezing, sec	1.13±0.74	5.6±2.7
Nervousness, score	0.0±0.0	0.2±0.13
Startle-1/body weight	1.83±0.34	2.00±0.34
Startle-2/body weight	1.56±0.26	1.78±0.28
Startle-3/body weight	1.39±0.23	2.20±0.29
Startle-4/body weight	1.12±0.21	1.44±0.26
Startle-5/body weight	1.16±0.22	1.54±0.31
Startle-6/body weight	1.14±0.26	1.64±0.34
Startle-7/body weight	1.36±0.36	1.42±0.24
Startle-8/body weight	1.43±0.30	1.27±0.19
Startle-9/body weight	1.38±0.33	0.97±0.12
Startle-10/body weight	1.53±0.36	1.20±0.18
BP under narcosis, mm Hg	128.6±4.8	125.4±4.1
BP in 15 мин, mm Hg	146.9±3.6	126.9±4.6***
BP in 30 min, mm Hg	155.0±5.1	133.0±6.8**
Number of pups per litter	9.1±1.1	4.8±0.7***
Body weight, g	460.9±12.2	392.7±12.9***
Heart weight, g	1.54±0.06	1.40±0.06
Kidney weight, g	3.45±0.09	3.00±0.12***
Adrenal weight, mg	68.1±3.1	59.9±3.5
Spleen weight, g	1.31±0.04	1.13±0.06*

Note. *p<0.05, **p<0.03, ***p<0.01, ****p<0.001 (LSD test).

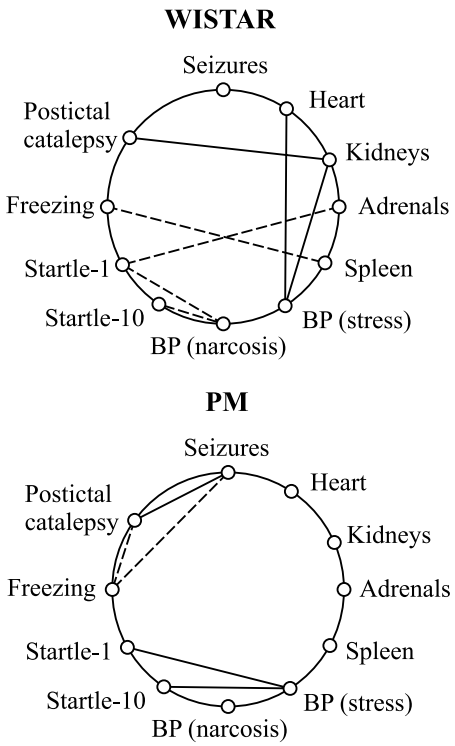

Fig. 2. Coefficients of correlation (*r*) between emotiogenic parameters and weights of organs in Wistar and PM rats. Solid line: positive correlation; punctate line: negative correlation.

acts of this kind and was not followed by clonic tonic seizures in the overwhelming majority of cases, but eventuated in long-lasting postictal catalepsy (Table 1). Analysis of correlations showed a high significant relationship between the severity of seizures and duration and intensity of catalepsy (r=0.90; Fig. 2). A similar relationship between the intensity of seizures and subsequent catalepsy was previously demonstrated for Krushinsky—Molodkina rats [3]. A negative correlation (r=-0.80) between the intensity of seizures and duration of cataleptic freezing in the home cage seemed to be unexpected in PM rats (Fig. 2).

In the "glove" test, PM rats demonstrated high aggressiveness towards human (Table 1). Presumably, the aggressive reactions are the signs associated with liability to seizures in animals and humans [7,10].

No differences between animal lines were observed for the parameter "freezing in home cage". However, the duration of catalepsy in PM rats was 5-fold longer than in Wistar rats, though the difference was insignificant due to high error of the mean. This fact could be explained by interspecies intermittent aggression, typical of rodents with seizures and described previously [7]. Aggressive reactions in these animals were paralleled by passive freezing reactions to humans.

Wistar and PM rats did not differ by the startle response and nervousness. Short startle reaction (milliseconds) in the startle box and the intensity of the acoustic signal (the rods of the cage touched with a piece of metal) were identical.

A peculiarity of PM rats was BP reaction to long restriction in a tight tube: BP increased in Wistar rats, but remained unchanged in PM rats (Table 1). In Wistar rats, positive correlations between the heart and kidney weights (r=0.73 and r=0.85) and BP in stress were revealed (Fig. 2). Enlargement of organs of the cardiovascular system (heart and kidneys) under conditions of long-lasting stress is a natural reaction. The absence of BP elevation in stress indicated specific reaction of PM rats with seizures [6].

Birth rates and number of females without pregnancy among PM rats were higher than in Wistar rats, which was characteristic of psychosomatic disorders in humans [7].

Positive correlations between startle-1 (in response to the first unexpected stimulus) and BP after long stay in the tube (r=0.70) were observed in PM rats (Fig. 2). Negative correlation between startle-10 (stereotypical reaction) and BP under narcosis (r=-0.71) was detected in Wistar rats. Correlative associations between stress-dependent reaction and weights of organs (heart, adrenals, kidneys, and spleen) were detected in Wistar rats (Fig. 2). However, not a single significant correlation between organ weights and behavioral parameters was detected in PM rats. It seems that long selection for high PM hyperkinesis manifested by destabilization of the systems of the organism liable to epileptic seizures.

Presumably, these signs in PM rats can serve as markers of focal epileptiform states and do not contradict the signs of the model of focal seizures with typical automatisms.

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