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Behavior of Rats in an Open Field Test as a Prognostic Indicator of Corticosterone Levels Before and After Stress

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Studies were performed to assess the role of behavioral indicators in an open field test in predicting the blood corticosterone level in stress. The most reliable indicators of behavior in the open field, reflecting a high probability of a significant increase in the corticosterone concentration after 3-h restraint, were a short latent period of first movement and a low level of motor activity. The probability of high corticosterone in normal "unstressed" conditions is reflected by a low level of motor activity and, conversely, a long latent period for the excursion to the central squares of the field.

Keywords: emotional stress, corticosterone, open field.

Resistance to the negative actions of stress in individuals in human and animal populations differs significantly [11] and depends mainly on certain genetic factors and stresses experienced at different periods of life [8]. Koplik et al. [17] showed that rats which are highly active in an open field were distinguished by significant resistance to unfavorable stress-inducing factors, while low-activity rats, in contrast, had low resistance [1]. Stress resistance depends on the levels of various hormones, neurotransmitters, and neuropeptides in the brain [2, 4]. From the point of view of Anokhin's functional systems theory, stress produces mismatch in harmonic interactions between different functional systems in the body [3]. The systems mechanisms of the body's vital activity on exposure to stress are to a significant extent defined by the personal characteristics, which vary significantly between different individuals, of the sympathoadrenal and hypothalamo-hypophyseal-adrenal mechanisms of regulation, and particularly the plasma corticosterone level [9]. Thus, the aim of the present work was to seek correlations between the characteristics of animals' behavior in an open field test and blood corticosterone levels before and after restraint stress.

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Methods

Experiments were performed using 20 male Wistar rats weighing 280–320 g. Animals were kept with free access to food and water. Experiments were performed in compliance with the requirements of decree No. 267 of the Ministry of Health of the Russian Federation (June 19, 2003) and the "Regulations for studies using experimental animals" (Anokhin Research Institute of Normal Physiology, Protocol No. 1 of September 3, 2005).

First, a complex evaluation of the rats' behavior in the open field test was performed in 3-min trials. While the animal was in the illuminated circle, the latent periods of the first movement and first excursion to the central squares of the field were measured, as were the total numbers of peripheral and central sectors crossed and the duration of grooming. The first blood sample was then collected from the tail vein under brief ether anesthesia. Plasma was separated by centrifugation (3000 rpm for 3 min) and stored for one month at –80°C. After two days, the animals were placed in a conflict situation, which generates emotional stress. This was modeled by placing the animal in a special tight-fitting cage (restraint) for 3 h. The effectiveness of this stress method is confirmed by published data [14].

A repeat blood sample was collected on decapitation after stress. Plasma corticosterone levels in controls and after stress were assayed by enzyme immunoassay (EIA) us-

TABLE 1. Indicators of Motor Activity in Rats with High and Low Corticosterone Levels

Indicators	High pre-stress corticosterone	Low pre-stress corticosterone	High post-stress corticosterone	Low post-stress corticosterone
Baseline corticosterone level, ng/ml	40.8 ± 3.8	18.9 ± 2.4	65.5 ± 4.8	32.5 ± 2.0**
LP of first movement, sec	4.0 ± 1.3	3.5 ± 0.7	2 ± 0.4	5 ± 1.1*
LP of excursion to center, sec	34.9 ± 8.7*	15.3 ± 3.5	21.5 ± 7.3	28.3 ± 6.4
Number of squares crosses at the periphery of the field	39.6 ± 5.4	36.3 ± 2.1	32.5 ± 4.5	41.8 ± 3*
Grooming, sec	17.4 ± 4.4	16.8 ± 4.7	21.9 ± 4.1	13.6 ± 4.5

*$p < 0.00001$ compared with animals with high corticosterone levels; **$p < 0.05$ compared with animals with high corticosterone levels; LP – latent period.

ing an IDS Corticosterone EIA test system (UK) and a Multiskan EX EIA reader (Thermo Fisher Scientific Inc., USA) following the manufacturer's instructions. Data were analyzed statistically in Statistica 6.0. Hypotheses regarding differences in independent cohorts were tested using nonparametric statistical methods, the Mann and Whitney U test, and the Spearman correlation coefficient.

Results

Stress is known to elicit a significant increase in the plasma corticosterone level in rats [9]. The mean corticosterone concentration in 20 animals in standard conditions was 29.9 ± 3.4 ng/ml, while the post-stress level showed a significant ($p < 0.05$) increase, to 47.3 ± 4.5 ng/ml. However, analysis of individual values showed that 3-h restraint stress produced directly opposite changes in six of the 20 animals, i.e., a decrease in the corticosterone level. Comparison of the corticosterone level in individuals in normal conditions and after restraint showed that there was no correlation between these two measurements (the Spearman correlation coefficient was –0.14).

The interaction of behavioral measures in the open field with post-restraint corticosterone levels was studied by dividing the animals into two groups depending on the initial corticosterone level. Analysis of behavioral indicators showed that rats with low corticosterone concentrations had shorter (15.3 ± 11.2 sec) latent periods of making excursions to the center of the open field ($p < 0.05$) than animals with higher corticosterone levels (34.9 ± 26.1 sec). The number of squares crossed at the center of the open field and the number of objects investigated were higher (not statistically significantly) in animals with lower post-stress corticosterone levels.

We then divided the animals into two more groups, this time on the basis of post-stress corticosterone levels. Two groups were identified with significant differences in the post-stress hormone level. The first group of animals (nine rats) was characterized by high plasma corticosterone levels after stress (65.5 ± 4.8 ng/ml) and the second had lower concentrations (32.5 ± 2.0 ng/ml, 11 rats). Thus, the groups of rats identified here differed significantly in terms of the corticosterone level ($p < 0.00001$).

Animals with low corticosterone levels after restraint were characterized by significantly longer latent periods of first movement (5 ± 1.1 sec) in preliminary testing in the open field ($p < 0.05$) as compared with 2 ± 0.4 sec in rats with higher post-stress corticosterone (see Table 1). Animals with low corticosterone levels showed a tendency to a longer latent period of excursion to the central squares of the open field (28.3 ± 6.4 sec) than rats with a high hormone concentration (21.5 ± 7.3 sec). The numbers of open field squares crossed by these two groups of rats were also different. In the group with low corticosterone levels, this value was 41.8 ± 3.0 squares in the peripheral part of the field, compared with a significantly smaller value of 32.4 ± 4.5 squares in the group with a high hormone level ($p < 0.05$). The activity of rats with low corticosterone levels was also greater at the center of the open field. This group of rats investigated more objects in the open field (these differences, however, did not reach statistical significance). Grooming duration showed a statistically insignificant increase in individuals of the high-corticosterone group (21.9 ± 4.1 sec) over that in animals with a low hormone level (13.6 ± 4.5 sec).

Discussion

Thus, 3-h restraint evoked different changes in the plasma corticosterone level in different individuals in a population: while the mean hormone concentration in these conditions increased significantly, the corticosterone level in 30% of the rats decreased. No correlation was seen between the corticosterone level in animals after stress and its level in the same rats before restraint. Thus, the pre-stress corticosterone level did not predict a high or low post-restraint corticosterone concentration. These points can also be explained by the marked rhythmic oscillations in the levels of this hormone (not considered in the present studies) over the course of the day. During these so-called ultradian wave-like rhythms, the plasma corticosterone level is known to change several-fold, such that this is the main contributor to measurements of corticosterone levels in standard unstressed conditions. Many reports of studies addressing ultradian hormone rhythms have been published [13]. The basal corticosterone level can be obtained more precisely by taking more than one measurement, though this is not always possible in real conditions [4].

Previous studies have not addressed the relationship between the plasma corticosterone level and behavioral characteristics in rats [12]. We found that parameters of behavior in the open field test can be used to predict corticosterone levels before and after stress-inducing restraint. A short latent period of excursion to the central sectors and a high level of movement activity (more squares crossed in the field) are predictive indicators for a low basal corticosterone level and minor emotional tension at rest [4]. However, restraint stress in some members of this group of animals provoked secretion of significant quantities of this hormone, exceeding the mean population level. Predictive criteria for a low corticosterone level after 3-h restraint were, conversely, the duration of the freezing period before the first movement, a long latent period of excursion to the center of the open field, and a high level of movement activity.

It follows from published data that a prolonged latent period of first movement on testing in the open field is a criterion for fear and uncertainty: long freezing and strong fear are typical of animals with high corticosterone levels [10]. However, a longer latent period in our experiments was typical of animals with low post-restraint corticosterone concentrations. We therefore believe, in contrast to the usual view, that short latent periods of first movement and of excursion to the center of the field are predictive indicators of significant emotional tension and a high post-psychoemotional stress corticosterone level. A short latent period of excursion to the center of the field provides a prognostic reflection of a low corticosterone level only before stress.

The levels of movement and investigative activity in the open field are inversely proportional to animals' levels of tension and fear [5]. Entirely consistent with this, the movement and investigative activity of the rats in our tests were higher in individuals with low corticosterone levels before and after stress, i.e., in those less inclined to emotional overload. In some studies, grooming is interpreted as a sign of a comfort state [7], while other reports, conversely, regard it as a sign of increased tension [6]. However, short-term (so-called "incomplete grooming"), which we generally recorded, is a "displacement activity" and an index of stress-induced tension. Grooming was longer-lasting in animals with a high corticosterone level.

Thus, these experiments showed that the plasma corticosterone level before stress did not correlate with the concentration of this hormone after 3-h restraint. The probability of a significant increase in the corticosterone concentration after 3-h restraint was high in animals with short latent periods of first movement and low levels of movement activity. A high corticosterone level in standard "nonstressed" conditions was seen in rats with a low level of movement activity and, conversely, a long latent period for excursion to the central squares of the field.

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