# A NEW METHOD OF EVALUATING ANXIETY STATES AND PHOBIAS IN RATS

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The study of the pathogenetic mechanisms of anxiety states and phobias and the creation of experimental models of these conditions and the development of effective therapeutic correction have come up against the problem of objectivity of testing these states and their changes in experimental animals [8].

The emotional reactivity of animals (including that characterized at the level of anxiety and fear) is known to vary depending on the functional state of the emotiogenic substrate exposed to the influence of various physical and zoosocial factors [1], whereas the appearance of a specific response and its intensity on repeated presentation of the stimulus are stochastic in character [5]. To assess adequately emotion in animals, traditionally attention is paid to the character of the specific response to a series of ethologically adequate test stimuli, inducing the appearance of basic emotional states of fear, anxiety, and aggression. This complex approach to the evaluation of emotions in laboratory rats has been applied in a widely known text, based on rank scale of intensity of aggressive-defensive response reactions in animals in various ethologically adequate situations [6]. However, there is as yet no complex method of effective and selective evaluation of anxiety states and phobias in rats for use in laboratory practice.

The aim of this investigation was to develop a new multiparametric method of assessing anxiety states and phobias in rats, enabling a comprehensive description to be given of the individual level of anxiety in animals and changes in that level during repeated testing with a range of behavioral reactions and a battery of ethologically adequate tests, based on creation of emotiogenic situations.

## EXPERIMENTAL METHOD

Experiments were carried out on 200 male Wistar albino rats weighing 250-300 g. The animals were kept under standard animal house conditions, with 5-7 rats to a cage, and with natural alternation of light and darkness and with free access to food and to drink. The animals were tested in batches of 4-6 rats. At the end of each test, the laboratory equipment was deodorized.

The choice of tests for the formation of a ranking scale for assessment of anxiety states and phobias in rats was based on two fundamental situations in which rats demonstrate responses connected with the appearance of fear and anxiety [9]: 1) coming up against an unfamiliar inanimate object or unfamiliar situation (tests I-V) and 2) the action of the experimenter's hand (tests VI-IX) (see the scale for assessment of anxiety states and phobias in rats).

The order of the investigation of the rats was always the same: from I to IX. Single limits of the change in intensity of the response were established for all tests included in the composition of the scale: from 0 to 3 points (discrete ranking with intervals of 1 point). A high point rating corresponded to a more marked response of the animal and, consequently, a higher level of anxiety or phobia.

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TABLE 1. Scale for Assessing (in points) Anxiety States and Phobias in Rats

I. Latent period (t) of stepping down
- 0—0<t≤30
- 1—30<t≤60
- 2—60<t≤180
- 3 – has not stepped down after 180 sec

II. Latent period (t) of passage through hole
- 0—0<t≤30
- 1—30<t≤60
- 2—60<t≤180
- 3 – has not passed through after 180 sec

III. Latent period (t) of emerging from "house"
- 0—0<t≤15
- 1—15<t≤30
- 2—30<t≤180
- 3 – has not emerged after 180 sec

IV. Latent period (t) of leaving center of "open field"
- 0—0<t≤15
- 1—15<t≤30
- 2—30<t≤60
- 3 – has not left after 60 sec

V. Retreating-1 (in "open field" situation spontaneously or in response to a sudden change of illumination)
- 0 – absence
- 1 – 0-0.5 square
- 2 – 0.5-2 squares
- 3 – more than 2 squares

VI. Retreating-2 (to action of experimenter's hand)
- 0 – absent in response to stroking and approaching with the hand
- 1 – arises in response to stroking
- 2 – arises in response to approaching with the hand
- 3 – still present after action of stimulus ceases

VII. Immobility
- 0 – absent on stroking and approaching with the hand
- 1 – observed during stroking
- 2 – observed during approaching with the hand
- 3 – preserved after action of stimulus had ceased

VIII. Vocalization
- 0 – absent on stroking and approaching with the hand
- 1 – arises during stroking
- 2 – arises during approaching with the hand
- 3 – preserved after action of stimulus ceases

IX. Pressing back the ears
- 0 – absent during stroking and approaching with the hand
- 1 – observed during stroking
- 2 – observed during approaching with the hand
- 3 – preserved after action of stimulus had ceased

**Legend.** If during testing the animal showed spontaneous reactions of VII, VIII, and IX, an additional 3 points is added to their rating for each one manifested; if during performance of tests II and III the animals exhibit vacillation (for explanation, see in text), an additional 0.5 point is added to the rating.

## EXPERIMENTAL RESULTS

**Test I** – determination of the latent period of stepping down from a height (the "step-down" test). This was used to assess instinctive defensive behavior in rats [7]. The rat was placed on the top platform of a parallelepiped (transparent plastic, 20 × 14 × 14 cm), resting on a brightly lit exposed white surface, and measurement of time

began from that moment. The time taken by the rat to step down from the parallelepiped was recorded. The stepping down was considered to have ended when all four limbs of the animal rested on the floor. If the animal did not step down from the height in 180 sec, the action was considered to be incomplete and testing ended.

Performance of the action by the animal in this test was assessed by a higher number of points, the later the action was performed: performance of the action during the experimentally set period of time was assessed at O points (see the scale in Table 1) and 50-70% of the animals in the population performed the action within those limits; nonperformance of the corresponding action by the animals was rated at 3 points.

**Test II** – determination of the latent period of passing through a hole. When the intensity of illumination was that of the room, the animal was placed in one of two compartments of a chamber (25 × 23 × 24 cm each) made of black plastic, with a floor made of a grating of metal rods, and at the moment the time during which the rat passed through the hole (7 × 10 cm) in the partition separating the compartments, into the other compartment of the chamber. The passage was considered to be complete it the animals crossed the line of the partition with all four limbs. If the animal did not pass through in 180 sec, the action was taken to be not performed and the test was ended.

Performance of the action by the animal in this case was assessed in the same way as in Test I. If the animal vacillated during performance of the test (peering into the hole or beginning to pass through it but not completing the passage) the assessment for performance of the action was increased by 0.5 point.

**Test III** – determination of the latent period of emerging from a "house." The animal was placed in a cage made of transparent plastic (16 × 12 × 15 cm), or "house," the door was closed, and the "house" was quickly placed in the center of an "open field." Conditions of illumination were the same as in Test I. The door was opened 15 sec after the "house" had been placed in the field. The time after which the rat emerged from the "house" was recorded. Emerging was considered to be complete if the animal crossed the line of the door with all four limbs. If the animal did not come out of the "house" in 180 sec, the action was considered to be not performed and the test was ended.

Performance of the action by the animal in this test was assessed in the same way as in Test I. If the animals exhibited vacillation when coming out of the "house" (peering from the "house" or not completing the emergence, the assessment for performance of the action was increased by 0.5 point.

In Tests I-III the rat was returned from the experimental situation to the cage, not earlier than 20 sec after performance of the corresponding action, or after the time of testing had elapsed in the case of nonperformance of the action. The intervals between these tests were 15-20 min.

Investigation by all the remaining tests (IV-IX) took place under "open field" conditions. The diameter of the field, divided into squares with a 20-cm side, was 120 cm, and the height of the walls was 28 cm. Incandescent lamps (6 lamps each of 60 W) were arranged in a circle 60 cm in diameter at a height of 80 cm from the field circle. In the course of the investigation of the animal for rating purposes the rat was placed in the field once.

**Test IV** – determination of the latent period of leaving the center of the "open field." It is used to assess the appearance of an anxiety response, often linked with diminution of motor activity in rats [10]. The rat was placed in the center of the field and at that moment the time during which the animal left the four central squares was recorded. Leaving the squares was considered to be complete if the animal crossed the conventional boundary between the four central squares and the rest. If the animal did not leave the center of the field after 60 sec the action was regarded as incomplete, but observation on the animal still continued (see Tests V-IX). Performance of the action by the animal in this case was assessed in the same way as in Test I.

**Test V** – assessment of the onset of a "reversal" spontaneously and/or in a response to a sudden change in the intensity of illumination in the "open field" (reversal-1). The results of previous experiments [2, 3] show that in rats with pathologically enhanced anxiety and fear spontaneous reversal and reversal caused by an external stimulus are observed more often than in normal animals, and this may be an indication of a corresponding emotional state of the animals. Testing was carried out as follows. The intensity of illumination in the field was abruptly changed 180 sec after the animal had been placed in it: instead of the usual 6 incandescent lamps (with a total power of 360 W) one lamp with a red glass was lit for 60 sec (power 40 W), after which the original level of illumination was restored. Observations on the animal conducted for 300 sec, starting from the time of placing the rat in the center of the "open field," the total distance (in squares) which the animal retreated spontaneously and/or in response to the sudden change of brightness, was determined.

For every increase in distance which the animal retreated during the testing time there was a corresponding increase in the point rating.

Immediately after the end of Test V in the "open field" situation a change was made to assessment of the animal's response to handling by the experimenter: bringing the hands close and stroking (see Test VI. IX). Testing was carried out by the experimenter gradually putting his hands close to the animal from the front, so that the rat could see the hand. Bringing the hand close and stroking the animal were carried out 2 or 3 times in succession.

**Test VI** – estimation of the onset of a retreating response to handling by the experimenter (reversal-2). The presence or absence of a reversal response to bringing the hand close or stroking, and also preservation of the reversal response (if it had appeared) after the action of the stimuli had ceased.

Absence of the above response to bringing the experimenter's hands closer and/or stroking was assessed at 0 points at a minimum. The appearance of a response to a contact stimulus (stroking) was assessed by fewer points than the response to a distant stimulus (bringing the hand closer) (1 and 2 points respectively). Preservation of this response (if it had arisen to bringing the experimenter's hands closer and/or stroking) after the end of action of the stimuli was assessed at 3 points at the maximum.

**Test VII** – assessment of appearance of an "immobility" response to the action of the experimenter's hand. The presence or absence of an immobility response was recorded (the animal froze in a strained posture, on extended limbs, or pressed tightly against the floor, sometimes with the ears pressed tightly back and/or with the eyes closed) in response to bringing the experimenter's hands closer and/or stroking, and also preservation of the immobility response (if it had appeared) after the end of the action of the stimuli. The appearance of an immobility response in this test was rated in the same way as in Test VI.

**Test VIII** – assessment of the onset of vocalization in response to handling. The presence or absence of vocalization was recorded in response to bringing the hands closer and/or stroking, and also preservation of vocalization (if it had appeared) after cessation of the action of the stimuli. The appearance of vocalization in this test was rated in the same way as in Test VI.

**Text IX** – assessment of onset of a response of pressing the ears back to handling by the experimenter. The presence or absence of a response of pressing back the ears to bringing the hand closer and/or stroking, and also preservation of the response of pressing back the ears (if it had appeared) after the end of the action of the stimuli were recorded.

Appearance of the response of pressing the ears back was rated in this in the same way as in Test VI.

If in different stages of the investigation the rats exhibited spontaneous immobility, vocalization, and pressing back their ears (response in the absence of handling by the experimenter), three points were added to the assessment of onset of the corresponding responses in Tests VII, VIII, and IX, since in normal rats, under the experimental conditions described above, these responses virtually did not appear.

To characterize the level of anxiety and phobia in the rats as whole, the index of the total number of points reflecting the results of the full investigation, according to the suggested scale, was used. This index for normal animals averaged $6.15 \pm 0.29$ points ($n = 126$).

Special investigations showed that during frequently repeated investigations of the rats at intervals of 4-7 days their level of anxiety and phobia was unchanged, i.e., habituation to the test procedure did not develop with these intervals.

Values of each of the 9 parameters (in points) used in the scale during repeated investigations of the animals varied considerably from time to time, but variation of the total number of points was significantly less: coefficients of variation of the estimate [4] in individual tests and of the total rating amounted to 80-240 and 40% respectively. Thus the more stable parameter of the total number of points which we used to assess the level of anxiety and phobia of the rats on a scale increases the reliability and accuracy of representation of the animals' state of anxiety and phobia.

Special experiments using the scale suggested above showed that a single injection of metrazol, an anxiogenic drug, into rats (in a subconvulsant dose of 10 mg/kg) and of sodium lactate (in a dose of 600 mg/kg) led to a considerable increase in the level of anxiety and phobia in the animals, whereas a single injection of the well-known anxiolytic seduxen (in doses of 0.1 and 0.6 mg/kg) did not change it. Preliminary injection of seduxen (0.6 mg/kg) prevented the anxiogenic effect of metrazol. These results are evidence of the sensitivity of the method for detecting anxiogenic and anxiolytic effects.

The new multiparametric method of assessing states of anxiety and phobia in rats, which we have suggested, is simple and is readily available in laboratory practice, it requires no complicated equipment, and yields rapid results.

As our experience of the use of the combined scale shows, this method offers new prospects for the study of the pathogenetic mechanisms of anxiety states and phobias, and also for the preclinical substantiation of the appropriate drugs to correct states of this kind.

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# OPPOSITE EFFECTS OF ADAPTATION TO CONTINUOUS AND INTERMITTENT HYPOXIA ON ANTIOXIDATIVE ENZYMES


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Preliminary adaptation to intermittent hypoxia under pressure chamber conditions has a powerful protective effect on the heart and, in particular, it significantly limits ischemic and reperfusion-induced arrhythmias as well as the size of ischemic and adrenergic necrotic lesions [5, 13, 15]. If the antiarrhythmic action of adaptation to intermittent hypoxia is compared with that of adaptation to continuous hypoxia at medium altitudes, both forms of adaptation are found to have a protective effect against ischemic arrhythmias caused by ligation of the coronary artery. Meanwhile their effect on reperfusion arrhythmias arising after removal of the occlusion were found to be opposite in kind: adaptation to intermittent hypoxia protected against reperfusion arrhythmias and abolished fibrillation of the heart and mortality among the animals whereas adaptation to continuous hypoxia, on the other hand, sharply potentiated arrhythmias and increased mortality among the animals [4]. In an attempt to explain the causes of this paradoxical result, attention was paid to the fact that activation of free-radical oxidation plays an important role in


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