

# Behavior of Rats with High and Low Levels of Freezing in Defensive Situations and on Selection of Food Reinforcement

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Behavior in rats with different levels of freezing in a classical defensive conditioned reflex was compared on acquisition of conditioned passive and active avoidance reflexes and on selection of food reinforcement of different values. Rats with prolonged freezing acquired the passive avoidance reflex better and retained it longer during extinction than animals with shorter freezing. At the same time, the active avoidance reflex in the shuttle box was acquired more easily by animals with short periods of freezing. Rats with prolonged freezing preferred a delayed and more valuable reinforcement in the model of choosing food reinforcements of different values (low level of impulsivity), while animals with short freezing preferred the low-value reinforcement without a delay (high level of impulsivity). The thresholds of pain sensitivity were no different in rats of the different groups. Thus, rats with prolonged freezing demonstrated a passive behavioral strategy in defensive situations and a low level of impulsivity, while rats with short freezing used an active behavioral strategy and showed a high level of impulsivity.

**Keywords:** classical defensive reflex, passive avoidance reflex, active avoidance reflex, pain sensitivity threshold, selection of food reinforcement of different values (delay discounting).

Acquisition of a classical defensive conditioned reflex (fear conditioning) is currently widely used in studies of memory, learning mechanisms, and emotionality. Many studies have revealed high levels of individual variability in the manifestations of fear, such that in some animals the proportion of time spent in freezing on testing of the reflex exceeds 90%, while in others it can be less than 30% [9, 14, 17, 31]. Extinction of the reflex is hindered in animals with high levels of freezing [10, 11]. Some studies have suggested that animals with high levels of freezing have more strongly fixed memories of fear [14] or greater plasticity in the lateral nucleus of the amygdala [18]. Other authors have indicated high reactivity to fear [25] or different manifestations of fear [32], without linking differences in fear with memory. Experiments with local injections of dopaminergic and serotonergic receptor ligands into the amygdala showed different sensitivities to these substances in animals

with different levels of freezing [10, 11]. The question of the mechanisms inducing the different manifestation of the fear reaction in response to the danger signal is far from resolved. It is unclear whether these animals differ in learning ability, pain sensitivity, or predisposition to acquiring other defensive reflexes (active and passive avoidance), i.e., to different behavioral strategies. It is not known how the different ability to prolonged freezing in defensive situations correlates with the level of impulsivity in these rats as determined in a feeding situation in terms of the preference for reinforcements of different values and delays in receipt (the food reinforcement selection method, or delay discounting) [4–6]. A previous comparison did not give clear results [7].

On the basis of these questions, the present study was undertaken with the aim of addressing additional phenotypic features in rats with different levels of freezing, which might aid understanding the causes of such individual-group features of behavior. The study tasks were: 1) to identify groups of rats with different levels of freezing after acquisition of a classical defensive conditioned reflex; 2) to compare pain thresholds in these groups of rats (thresholds of

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flinching and jumping); 3) to compare the acquisition and extinction of a passive avoidance reflex in a dark-light box in these groups of rats; 4) to study the dynamics of acquisition of an active avoidance reflex in a shuttle box; to analyze the levels of impulsivity of these groups of rats in terms of their preferences for a low-value immediate or a valuable delayed reinforcement in a delay discount model.

**Methods.** *Objects studied.* Experiments were performed in chronic conditions using 46 male Wistar rats weighing 330–400 g obtained from the Stolbovaya branch of the Scientific Center for Biomedical Technologies, Russian Federal Medical-Biological Agency. Animals were kept in the animal house with a standard 12-h light regime with free access to water and standard feed in cages containing 5–6 rats. Experiments were performed in compliance with the humanitarian principles laid out in the directives of the European Community (2010/63/EU) and the positions of the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, relating to work using experimental animals.

*Assessment of pain sensitivity thresholds.* Experiments were performed in chamber No. 1 (Startle and Fear Combined System, PanLab Harvard Apparatus, Spain, 2000). The inner chamber, of size 24 × 24 × 24 cm, in which the animal was placed, was mounted on four sensors to record the rat's movements. The chamber floor was a grid used for delivery of increasing electric currents (0, 0.025, 0.05, and so on with a 0.05-mA step to 0.35 mA) with interstimulus intervals of 90–120 sec and stimulus duration 0.5 sec. Rats' responses to shocks were determined visually and from mechanogram traces. The strength of rats' responses was assessed and clearer identification of freezing reactions was obtained by analyzing mechanograms at 5-sec intervals before, during, and 50 sec after the stimulus. Thresholds of flinching and jumping were determined. After threshold experiments, fear in response to the context of the chamber was extinguished for two days by placing the animal in the chamber for 10 min. In all subsequent experiments in this chamber, the context was significantly altered: the floor was covered with sawdust and the walls were papered.

*Acquisition and extinction of passive avoidance reflex.* At the second stage of the studies, rats were trained to a passive avoidance reflex in a new light-dark chamber, No. 2, in which the light sector was of size 27 × 27 × 27 cm and the dark sector was of size 24 × 24 × 24 cm. The floor of the dark sector was a grid for delivery of electric shocks; the sectors were connected by an opening of size 7 × 5 cm which could be closed by lowering a door. The light sector illumination was 270–300 Lx and the dark sector illumination was 2–12 Lx. At the beginning of the experiment, the rat was placed in the light sector for familiarization with the apparatus, and the door was closed. After 30 sec, the door was opened and then, over a period of 180 sec, the latent period of entry of the rat into the dark sector was determined, along with the times spent in each sector and the numbers of

boluses and urinations. After two such control experiments, the passive avoidance reflex was acquired and after the rat entered the dark sector the door was closed and the floor of the chamber was used to deliver a shock of 0.8 mA for 3 sec. Acquisition of the reflex was tested after 24 h; after opening the door, the rat was given the opportunity to enter the dark sector for 180 sec. The latent period of transfer into the dark sector was measured, along with the times spent in the dark and light sectors, the numbers of rearings, grooming acts, boluses, and urinations, and the number of glances (glances with the head entering the dark sector and glances with placing of one or more paws into the dark sector were counted separately). Retention of the passive avoidance reflex was then tested at 7 and 14 days. Finally, forced extinction of conditioned reflex fear to the context of the dark sector of the chamber was then performed by placing the rat in the dark sector for 10–15 min in two experiments.

*Acquisition, testing, and extinction of conditioned reflex fear.* In the third stage, rats were trained to a classical defensive reflex in chamber No. 1 (Startle and Fear Combined System, PanLab Harvard, Spain, 2000). Training after a 120-sec chamber familiarization period consisted of presentation of three combinations of a sound (30 sec, 80 dB, 2000 Hz) and electrocutaneous stimulation of the paws via the grid floor (2 sec, 0.8 mA, delayed 28 sec from the start of the sound) with interstimulus intervals of 40–60 sec. Conditioned reflex fear was tested 24 h after training (Test 1). After a 120-sec chamber exploration period, a sound (120 sec, 80 dB, 2000 Hz) was presented, which was followed by a 120-sec period without stimuli (aftereffect). Freezing times in Test 1 were used to divide rats into groups with different freezing levels.

Then (after 2–3 days), conditioned reflex fear was extinguished in three experiments; a 120-sec period of exploring the chamber was followed by 10 isolated presentation of sound stimuli (30 sec, 80 dB, 2000 Hz) without electrocutaneous stimulation with 20-sec intersignal intervals. Retention of the reflex was tested 24 h after each extinction experiment (Tests 2, 3, and 4, respectively).

*Acquisition of active avoidance reflex.* At the fourth stage, animals were trained to an active avoidance reflex in a new shuttle box, No. 3, consisting of two identical sectors each of size 25 × 21 × 18 cm connected by an opening of size 6 × 6 cm. The floor of both sectors was a grid for delivery of shocks. The conditioned stimulus was a sound at 60 dB lasting up to 30 sec, the delay to the unconditioned stimulus (shock) was 3 sec, the current was selected individually for each rat and ranged from 0.35 to 0.6 mA. When rats transferred to the other sector in response to activation of the sound (avoidance reaction) or in response to the current (escape response), all stimuli were terminated. Ten stimulus combinations were delivered in each experiment. The latent periods of transfers of rats to the other sector were measured from the start of the sound and the numbers of avoidance, escape, and missed reactions were counted, along with the

numbers of intersignal transitions between sectors and the numbers of boluses and urinations.

*Determination of levels of impulsivity using the "right to choose food reinforcement value" method (delay discounting).* A total of 22 rats were used; food motivation was maintained in the animal house by limiting the available food such that the rats' body weight was approximately 80% of that of an animal with free access to food. The experimental apparatus consisted of a rectangular chamber of size 30 × 60 × 30 cm (chamber No. 4). A feeder was mounted in the middle of the front wall of the chamber, and access to the feeder was blocked by a mobile transparent plastic screen. Extendable pedals were positioned each side of the feeder. Signal lamps were positioned in the feeder and above each pedal. Rats were initially trained in the experimental apparatus to press a pedal to obtain food, which consisted of standard 45-mg pellets (food granules) (Bio-Serv, USA). Training was continued until the rat pressed the two pedals with equal probabilities. The animal was then given the opportunity to select either a low-value immediate reinforcement or a valuable but delayed food reinforcement. The scheme of the experimental test was as follows. After triggering of the experimental program, the general illumination lamp in the chamber was switched on and the lamp in the feeder was switched on at the same time. The rat was given the opportunity to move the screen with its nose for 10 sec, to obtain access to the feeder. Movement of the screen led to extension of both pedals, which remained extended for 10 sec. When the pedal on the right was pressed, the rat received one pellet immediately and when the pedal on the left was pressed the rat received four pellets with a 5-sec delay. Each rat completed 25 trials in each daily experiment. Training times for rats to achieve stability in pedal selection for receipt of reinforcement corresponding to the preferred strategy took 1–2 months. After completion of experiments and a period of increased feeding and recovery of initial body weight, these rats underwent all four stages of the study using acquired defensive reflexes.

In each experiment, the numbers of pedal presses to obtain the low-value immediate reinforcement ( $k_1$ ) and the number of pedal presses to obtain the more valuable but delayed reinforcement ( $k_2$ ) were determined, along with the number of missed screen movement reactions and the number of missed pedal-pressing reactions. The coefficient of impulsivity was calculated as  $K = k_2/(k_1 + k_2)$ .

*Statistical analysis.* Results were processed statistically using the standard program Statistica 8.0. Groups of rats were compared by ANOVA/MANOVA in the multifactorial analysis (Factorial ANOVA) and unifactorial analysis (One-way ANOVA) modules. Post hoc analysis was with the Fisher LSD. Differences were regarded as statistically significant at  $p < 0.05$  and tendencies were identified at  $0.05 \leq p \leq 0.1$ . The *Basic Statistics* module was used to plot histograms of the distribution of the number of rats vs. freezing time in Test 1. In addition, Spearman correlation

coefficients between the percentage freezing time in response to sound in Test 1 and the coefficient of impulsivity (Spearman rank Order Correlations, Nonparametrics module of Statistica) were calculated.

**Results.** *Identification and comparison of groups of rats depending on freezing time in Test 1 after acquisition of a classical defensive reflex.* Figure 1, A shows the distribution of rats depending on the mean percentage freezing time in Test 1 in the intervals before and during exposure to sound. Based on the nature of distributions, three groups of rats were identified: "low-" (1), "intermediate-" (2), and "high-freezing" (3) animals. Group 1 consisted of animals with freezing times of less than 57% (13 rats) and group 3 consisted of those with freezing times of greater than 90% (12 rats). Group 2 contained 21 rats with freezing times of greater than 57% and less than 90%. Comparison of freezing times in Test 1 using Factorial ANOVA identified a significant influence for the Group factor ( $F_{2,129} = 76.553, p < 0.001$ ). Comparison of freezing times at different time intervals in the experiment (Fig. 1, B) by post hoc analysis showed that both during the period before and during and after exposure to sound, the percentage freezing time was significantly greater in rats of group 3 than that in group 1. Rats of group 2 occupied an intermediate position and differed from the extreme groups in the intervals before and during the sound. Rats of group 3 had a greater duration of episodes of freezing in Test 1 as compared with animals of groups 1 and 2 (Fig. 1, C). There were no between-group differences in the numbers of boluses and urinations (Fig. 1, C).

Between-group differences were seen at the training stage for the Group factor,  $F_{2,301} = 13.613, p < 0.001$  (Fig. 1, D). Comparison of freezing times at different time intervals in the experiment (Fig. 1, D) with post hoc analysis showed that initially, freezing times in the periods before and during the first sound did not differ between the three groups of rats; differences appeared after use of the first electric shock (interval ISI1), when rats of group 3 showed significantly greater freezing than animals of group 1. Comparison of freezing on exposure to sound before the combination (Fig. 1, D) and after training (Fig. 1, B) showed significant differences in all groups of rats, i.e., training was successful in all animals.

*Comparison of pain sensitivity and reactivity of rats to electric currents.* The fact that differences between groups of rats on training were seen after the first shock suggested that rats had different pain sensitivities. In the flinch-jump method for determining pain sensitivity, the main indicators are the thresholds for flinching and jumping. The average flinch threshold in all rats was  $0.049 \pm 0.004$  mA and the threshold for jumps was  $0.227 \pm 0.013$  mA. It follows from Fig. 2, A, B that the thresholds for flinching and jumping were not different in rats of groups 1–3 (one-way ANOVA, Group factor,  $F_{2,34} = 0.051, p = 0.95$  and  $F_{2,28} = 1.51, p = 0.239$ , respectively), which is evidence for identical pain sensitivities in rats of different groups.

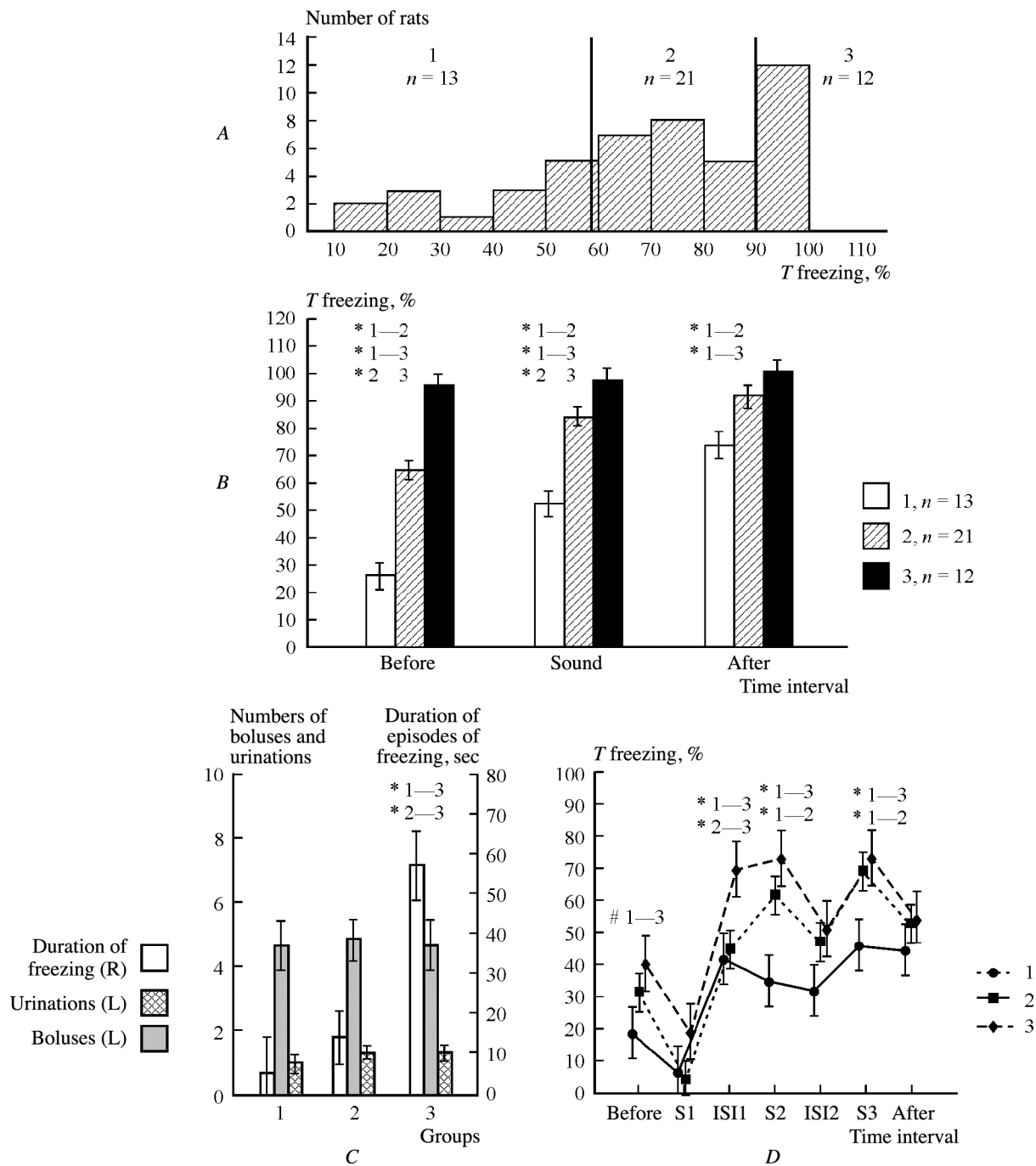


Fig. 1. Identification and comparison of groups of low- (1), intermediate- (2), and high- (3) freezing rats on acquisition and testing of a classical defensive conditioned reflex. *A*) Distribution of rats depending on percentage freezing time in Test 1 after training. The abscissa shows the number of rats; the ordinate shows the percentage freezing time. *B*) Percentage freezing times in rats before (Before), during (Sound) and after (After) exposure to sound in Test 1. *C*) Numbers of boluses and urinations and mean duration of freezing episodes in Test 1. *D*) Percentage freezing time during training. The abscissa shows time intervals. Before – before combinations started; S1–3 – during exposure to sounds 1–3; ISI1,2 – during the first and second intersignal intervals; After – after combinations. *n* is the number of rats in the group. \*Statistically significant differences between groups, numbers alongside are group ID numbers, #tendency. Data are shown as mean ± error of the mean.

Analysis of freezing times in rats in the 50 sec after use of low-strength shocks (from 0 to 0.25 mA) showed (Fig. 2, C) that freezing time in rats of group 3 was somewhat greater (at the level of a tendency) than in rats of group 1 at

the same current strength (Factorial ANOVA, post hoc analysis). Thus, when stimulated with low-strength current, rats of group 3 preferred to react by freezing more frequently than rats of group 1.

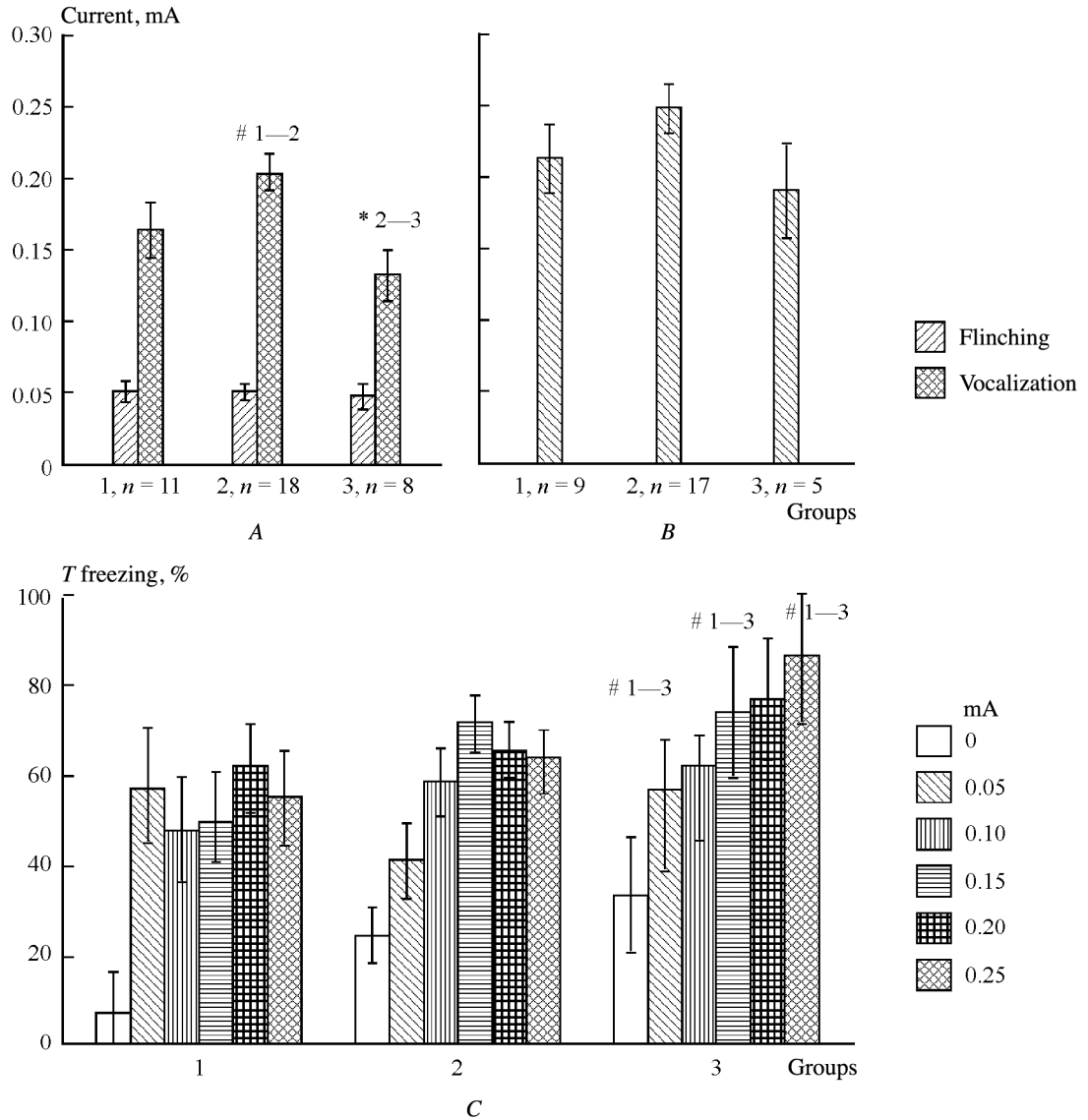


Fig. 2. Occurrence of flinching, jumping, and freezing on exposure to shocks of increasing strength in rats of groups 1, 2, and 3. A) Threshold of flinching; B) threshold of jumping. The ordinates in A and B show current strength, mA; the abscissa shows groups of rats. C) Percentage freezing time in rats during the 50 sec after exposure to currents of increasing strength from 0 to 0.25 mA. #Tendencies to differences between groups, numbers alongside give group ID numbers. For further details see caption to Fig. 1.

Acquisition and extinction of a conditioned passive avoidance reflex (CPAR). Analysis of the rats' behavior during the acquisition and extinction of the CPAR with Factorial ANOVA identified the influences of the Group factor on the probability of entering the dark sector ( $F_{2,419} = 4.50, p = 0.012$ ), latency of entry ( $F_{2,418} = 4.42, p = 0.013$ ), the number of boluses ( $F_{2,379} = 4.64, p = 0.010$ ), and the number of glances with placing of the paws ( $F_{2,301} = 10.38, p < 0.000$ ). Post hoc analysis showed that before acquisition of the reflex, three control placings in the chamber did not reveal any differences in the behavior of rats of different groups and all animals entered the dark sector with short latency (Fig. 3, A, B). The largest differences in the behavior of rats of different groups were seen on days 1 and 2 after acquisition. The

probability of entering the dark sector was significantly greater and the latency of entry was shorter in rats of group 1 than those of group 3 (Fig. 3, A, B). Overall, 46% of the rats of group 1, 29% of those of group 2, and only 8% of those of group 3 entered the dark sectors on days 1–2 (Fig. 3, E), the difference between groups 1 and 3 being significant ( $\chi^2, p = 0.035$  ( $2 \times 2$  Table, Nonparametric Statistic)). These data provide evidence that acquisition of the CPAR was more successful in rats of group 3 than those of group 1.

Analysis of the number of boluses during the seven days after training showed (Fig. 3, C) that the number was greater in rats of group 3 than in rats of group 1 on days 1–4, which appears to be evidence of greater emotional tension in rats of group 3. Rats of group 1 performed more glances

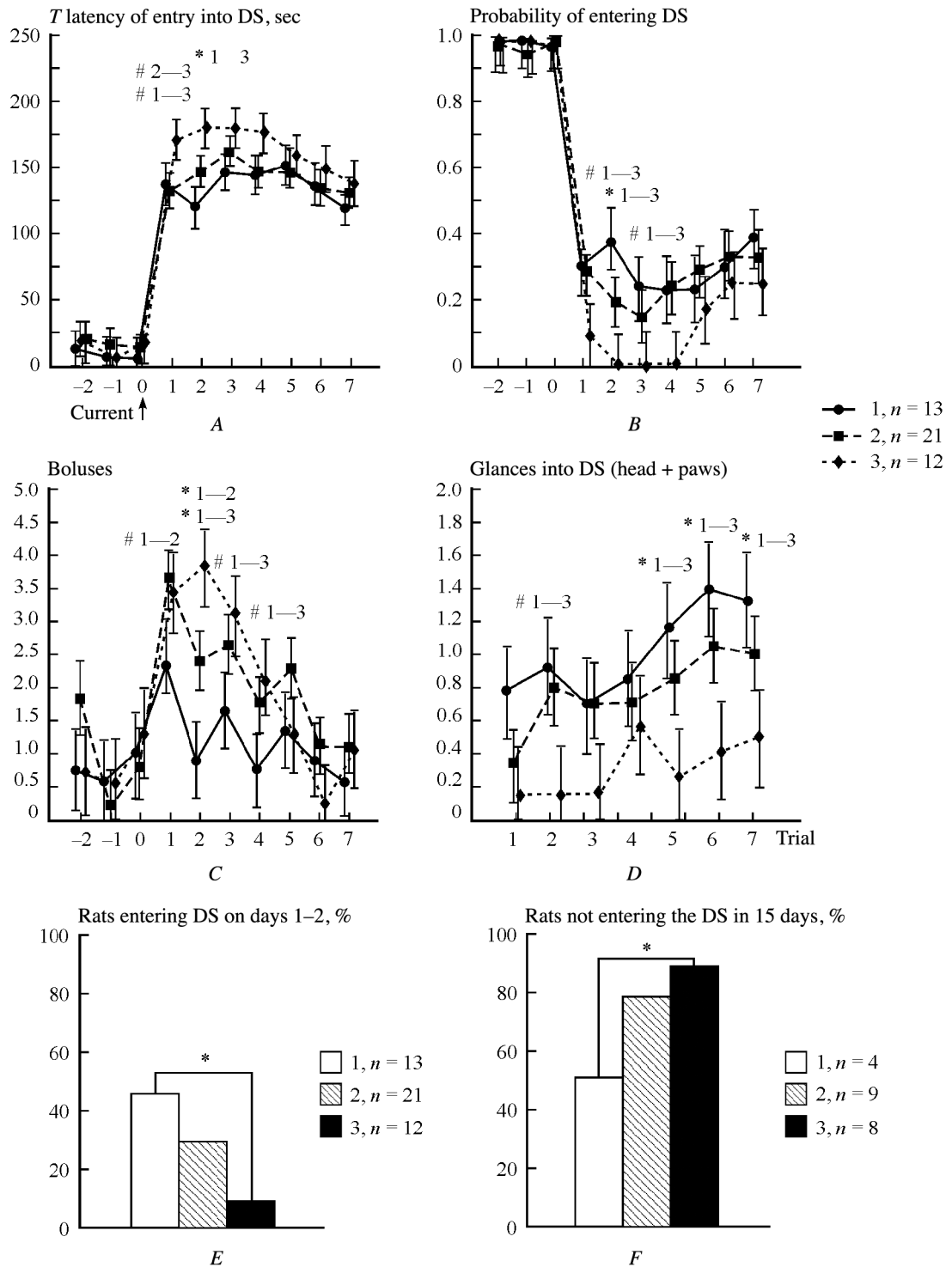


Fig. 3. Acquisition and extinction of a passive avoidance reflex in rats of groups 1, 2, and 3. A) Latency of entry into the dark sector of the chamber; B) probability of entry into the dark sector; C) number of boluses; D) number of glances into the dark sector with paw placing. Abscissas: A-D) trial Nos. (-2 and -1 are before acquisition of the reflex, 0 is on the day of acquisition, 1-7 are after acquisition); E) percentage of rats entering the dark sector in trials 1-2 and after training; F) percentage of rats not entering the dark sector even once during the 15 days after training. For further details see caption to Fig. 1.

with positioning of the forepaws on the floor of the dark chamber (Fig. 3, D).

Analysis of the dynamics of CPAR extinction over the 15 days after acquisition was performed using a cohort of

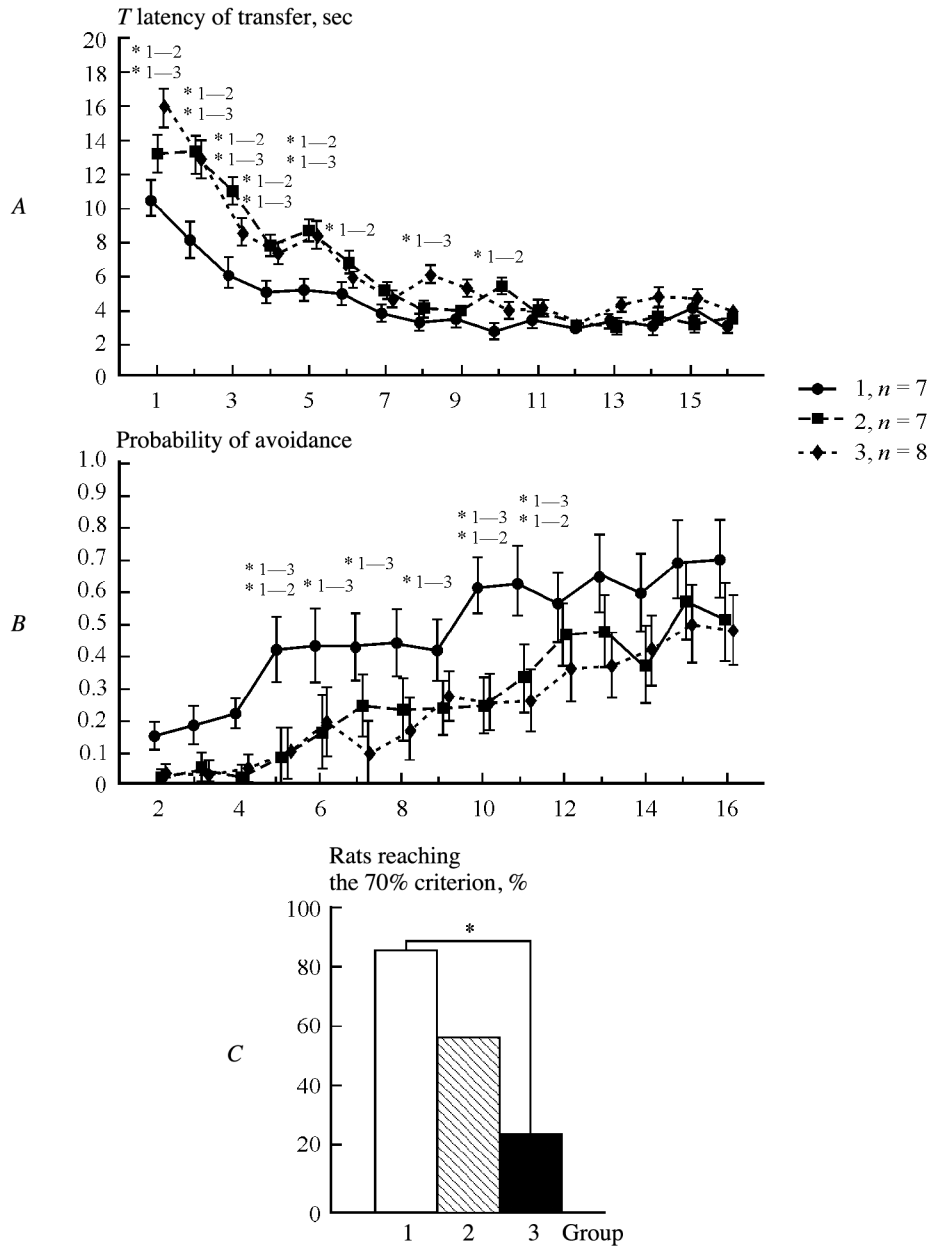


Fig. 4. Acquisition of active avoidance reflex in rats of groups 1, 2, and 3. A) Latency of transfers into the other sector after activation of the sound, sec; B) probability of avoidance reactions (transfers to the other sector before activation of the current). The abscissa on A and B show trial Nos.; C) percentage of rats reaching the criterion of 70% avoidance reactions per experiment. For further details see caption to Fig. 1.

21 rats which successfully acquired the reflex (did not enter the dark sector on days 1–2). A total of 50% of the rats of group 1 did not enter the dark sector even once during the whole of the extinction period, compared with 78% of group 2 rats and 89% of group 1 rats (Fig. 3, F). Differences between rats of groups 1 and 3 were statistically significant ( $2 \times 2$  Table, Nonparametric Statistics), i.e., resistance to extinction was greater in rats of group 3 than those of group 1.

Thus, rats of group 3, as compared with animals of group 1, not only acquired the CPAR better, but also extinguished the reflex worse.

*Acquisition of an active avoidance reflex.* Comparison of the dynamics of the acquisition of the active avoidance reaction demonstrated a Group factor influence on the latency of transfer to the other sector of the chamber ( $F_{2,217} = 13.16, p < 0.001$ , Factorial ANOVA). Comparison by post hoc analysis of the latency of transfer of the rats from one sector to the other (Fig. 4, A) showed that latency in rats of group 1 was shorter than that in animals of groups 2 and 3 at the beginning of training, experiments 1–6, also in experiments 8–10, while by the end of training there were no significant differences.

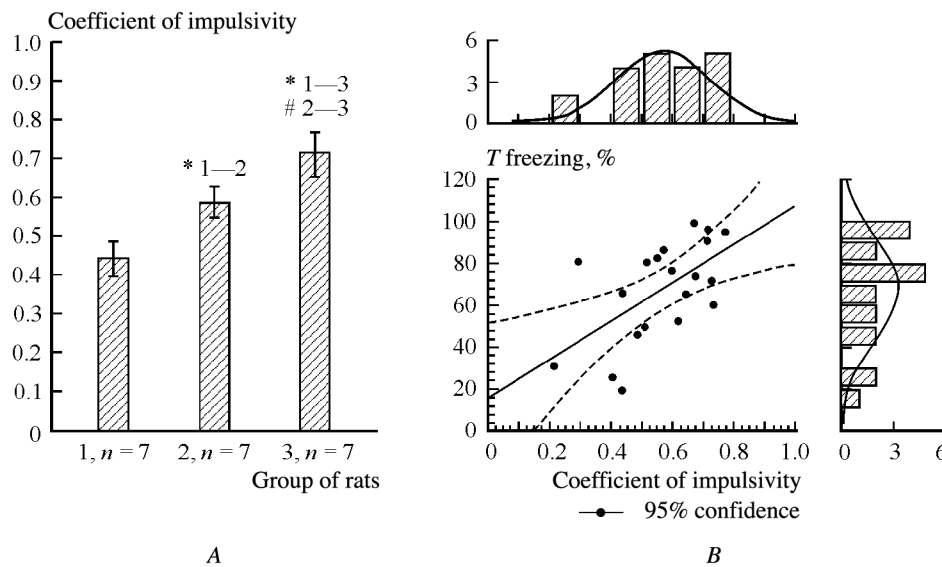


Fig. 5. Comparison of freezing levels in Test 1 after acquisition of the classical defensive reflex and coefficient of impulsivity in a delay discounting model. A) the average coefficient of impulsivity in rats of groups 1-3; B) correlation matrix between impulsivity coefficient and percentage of freezing time.

Separate analysis of escape and avoidance reactions showed that Group factor influenced the probability of avoidance ( $F_{2,19} = 3.04, p = 0.072$ ) and the probability of escape ( $F_{2,19} = 3.11, p = 0.068$ ), in both cases at the tendency level. Overall, the latency of escape was significantly ( $p = 0.033$ ) shorter in group 1 (5.72 sec) than group 3 (6.58 sec), while the latency of avoidance was shorter ( $p = 0.030$ ) in rats of group 1 (2.02 sec) than rats of group 2 (2.27 sec).

Avoidance reactions appeared from the second experiment (Fig. 4, B). Post hoc analysis showed that the probability of avoidance was significantly greater from experiments 5–8 and in experiments 10–11 in rats of group 1 than those of group 3, while the probability of avoidance animals of group 2 was no different from that of rats of group 3.

Analysis of learning success (Fig. 4, C) showed that in group 1, 86% of rat achieved the criterion of acquisition of active avoidance (70% avoidance reactions per experiment), and this occurred during experiments 4–6 in 57% of these animals. In group 2, 57% of rats reached the criterion, compared with only 25% in group 3, this occurring only by training days 10–14. The difference between the percentages of trained rats in groups 1 and 3 was significant ( $\chi^2, p = 0.019$  ( $2 \times 2$  Table, Nonparametric statistic)).

Thus, the probability of avoidance reactions in rats of group 1 at the beginning of training was greater, the latency of transfers was shorter, and acquisition of avoidance reactions was faster and more successful than in rats of group 3.

*Selection of food reinforcements of different values.* Correlation analysis between the coefficients of impulsivity obtained in the delay discounting model and the percentage of time spent freezing in Test 1 after acquisition of the classical defensive reflex identified a significant positive correlation (correlation coefficient  $r = 0.592, p < 0.05$ , Fig. 5, B),

i.e., as the duration of freezing increased, the preference for the valuable but delayed reinforcement also increased. Analysis of groups of rats showed (post hoc analysis, one-way ANOVA, Fig. 5, A) that rats of group 1 had a low coefficient of impulsivity (0.44), i.e., preferred the low-value reinforcement without a delay. Animals of group 3 had a significantly greater coefficient of impulsivity (0.72) and preferred the valuable reinforcement with a delay. Rats of group 2 occupied an intermediate position.

Thus, a relationship was seen between the level of freezing in rats in the defensive situation and the level of impulsivity on choosing food reinforcements of different value.

**Discussion.** In the present studies, three presentations of the combination of the conditioned and unconditioned stimuli were delivered for acquisition of a classical defensive reflex, rather than five, as in our previous studies [9–11]. This was to decrease the strength of the reflex and facilitate its subsequent extinction. In the present study, as after use of five combinations, the rats showed a large spread in the percentage freezing time in the post-training test. The cohort was large enough to identify three groups: “low-,” “intermediate-,” and “high-freezing” rats. The percentage freezing times in all groups of rats after training in response to sound was significantly greater than before training, i.e., all animals acquired the reflex. These groups of rats also differed in terms of the duration of freezing episodes in Test 1, while there were no differences in the numbers of boluses and urinations. These data lead to the conclusion that both the low- and high-freezing rats successfully acquired conditioned reflex fear, though the manifestation of fear in the form of freezing could differ between groups.

The fact that between-group differences arose on acquisition of the reflex after the first shock led to the sugges-



tion that the different groups of rats had different levels of pain sensitivity. However, analysis of flinch and jump thresholds did not reveal any significant differences between groups, which contradicted this suggestion. Previous studies also did not find any correlation between the level of freezing in conditioned reflex fear to context and pain sensitivity in rats determined in the two tests, flinch-jump and tailflick [27].

The literature contains contradictory data on pain thresholds in rats belonging to different strains and groups. Rats with high (KHA strain) and low (KLA strain) rates of acquisition of active avoidance also showed no differences in pain sensitivity thresholds on delivery of shocks through the grid floor, with analysis of flinch and paw-placing thresholds [3]. Rats of the Roman strain with high and low rates of acquisition of active avoidance showed differences in the thresholds of paw-stepping and jumping [29]. Rats with high and low exploratory activity in the open field (HRA and LRA) also showed no difference in pain sensitivity in the hotplate and tailflick tests [16]. However, a number of studies dividing rats into groups depending on the pain response threshold found differences in the manifestations of conditioned defensive reflexes and behavior in aversive situations. In conditioned reflex fear to context, low-sensitivity rats with high thresholds mounted greater reactions in the form of ultrasound vocalization, while high-sensitivity rats with low thresholds displayed greater freezing reactions [26]. Rats with low (strain H) and high (strain B) arousal thresholds on electrical stimulation of the hindlimb showed differences in behavior in the open field [13] and on acquisition of emotional resonance and active avoidance [1].

It is interesting to note that analysis of freezing times in 50-sec intervals after low-strength shocks (from 0 to 0.25 mA) in our work showed a tendency to a greater freezing time in high-freezing rats than low-freezing rats, i.e., the action of a weak current already started to show the preferential nature of responding in rats of different groups.

Analysis of the success of acquisition of the conditioned passive avoidance reflex in the dark-light box and the dynamics of its extinction showed significant differences between groups of rats. High-freezing rats acquired the reflex more successfully than low-freezing animals. Analysis of the extinction dynamics of the reflex over a period of 14 days in successfully training rats showed longer persistence of the reflex in high-freezing animals than low-freezing. It would appear that the faster extinction of the passive avoidance reflex in low-freezing rats promoted glancing into the dark sector with placing of the forepaws. Freezing as a preferred form of reaction to a dangerous situation in high-freezing rats probably occurred on being placed in the light sector after training, suppressed the exploratory motivation, and prevented entry into the dark sector.

On acquisition of active avoidance in the shuttle box, significant differences between groups of rats arose at the beginning of training. In high-freezing rats, the latency of

transfer to the other sector was greater and the probability of avoidance was lower than in low-freezing animals. The percentage of rats achieving the 70% avoidance reaction criterion was greater in the low-freezing group than the high-freezing group. In the situation of active avoidance, the preferred reaction to the danger signal in the form of freezing prevented acquisition of the reflex in high-freezing rats.

Thus, the dominance of active (runs, jumps) or passive (freezing) defensive reactions to danger signals in low- and high-freezing rats, respectively, led to different levels of success in acquisition of the active and passive avoidance reflexes. It is important to note similarity in the behavioral strategies in these groups of Wistar rats in all the conditioned defensive reflexes studied, i.e., with real danger; rats with high levels of freezing demonstrated a passive behavioral strategy, while animals with low freezing levels, conversely, used an active behavioral strategy. In contrast to the conditions of defensive reflexes in a potential threat, behavior of individual rats in tests for anxiety varied depending on the type of test [9].

Selection of the type of defensive behavior is known to depend on the nature and vicinity of the threatening stimuli, i.e., whether they are potential, remote, or close [15, 30]. In addition, there are individual features in the selection of the preferred reaction. The literature contains developments of the concepts of active and passive behavioral strategies (coping styles) in aversive situations [2, 20–22, 33]. Animals with the passive behavioral strategy have increased levels of activation of the hypothalamo-hypophyseal-adrenal system and a correspondingly elevated blood corticosterone level as compared with individuals with the active strategy [20, 33]. A special population of cells in the central nucleus of the amygdala, termed type I cells, which are characterized by depolarization after action potential generation, play an important role in switching of the behavioral response from freezing to active behavior [21]. Inhibition of these cells leads to decreases in freezing and increases in the cortical arousal reaction, which is cholinergic in nature. Animals preferring different behavioral strategies also differed in terms of resistance to neuroticization and stress [2, 8, 12, 24], sensitivity to alcohol [28] and narcotics [19], and predisposition to immunopathological diseases [23, 34]. The active and passive behavioral strategies in aversive situations give the animal certain survival advantages in different environmental situations [2, 24]. Thus, for example, individuals with the active behavioral strategy have advantages in social conflicts with increases in population size, while those with the passive strategy have advantages in unfavorable environmental changes when population size decreases.

Our study demonstrated a positive correlation between the duration of freezing in a defensive situation and the ability to select a valuable food reinforcement with a delay, i.e., there was a link between the rats' behavioral strategy in defensive and feeding situations. It can be suggested that the ability to delay active motor reactions is a universal proper-

ty of the nervous system and is apparent in both defensive and feeding situations. Previous studies have demonstrated links between behavioral impulsivity in feeding situations and anxiety in rats in the elevated plus maze [7], the open field and the light-dark box [5], and behavior in an emotional resonance model [4]. Comparison of freezing times in response to sound in the classical defensive reflex did not previously reveal any difference in rats with different levels of impulsivity [7]; between-group differences were seen only in response to presentation of context. In this work, acquisition occurred with a single combination, and self-controlled animals could learn worse, as their signal memory was less apparent than that in impulse rats [6].

This study answers a number of the questions posed in the introduction. Conditioned reflex fear was acquired both in low- and high-freezing rats on development of a classical defensive reflex, though its manifestations were different – some preferred active motor reactions, while others preferred freezing. The dominant behavioral strategy in response to danger signals was clearly apparent on acquisition of the active and passive avoidance reflexes. The ability or inability to carry out prolonged freezing was also reflected on selection of food reinforcements of different values with different delays.

**Conclusions.** Rats with high levels of freezing in a classical defensive reflex, in contrast to animals with low levels of freezing, were more successful in acquiring a passive avoidance reflex and retained it longer during extinction.

Rats with low levels of freezing acquired the active avoidance reflex in the shuttle box more quickly than animals with prolonged freezing.

Thresholds for the flinch and jump pain reactions on delivery of shocks via the grid floor were no different in rats with different levels of freezing.

Animals with high levels of freezing preferred a delayed but more valuable reinforcement in the delay discounting model (low level of impulsivity), while low-freezing rats preferred the low-value reward without a delay (high level of impulsivity).

Rats with high levels of freezing demonstrated the passive behavioral strategy in defensive situations and low levels of impulsivity in selecting food reinforcement, while rats with low levels of freezing adhered to an active behavioral strategy and showed high levels of impulsivity.

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