

Some Features of Learning in the Morris Water Test in Rats Selected for Responses to Humans

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UDC 612.821:591.513:577.175.52

Translated from Zhurnal Vysshei Nervnoi Deyatel'nosti imeni I. P. Pavlova, Vol. 57., No. 3, pp. 344–351, May–June, 2007. Original article selected June 21, 2006, accepted December 25, 2006.

The characteristics of learning in the Morris water test were studied in gray rats subjected to prolonged selection for elimination (the tame strain) and enhanced (the aggressive strain) aggressivity towards humans. Blood corticosterone levels at different stages of learning were also estimated. Tame rats learned to locate the invisible platform better than aggressive rats. The time spent seeking the platform by aggressive rats increased because they spent more time at the periphery of the basin. The duration of vertical investigative activity while on the platform was greater in tame rats than in aggressive rats. Fixation of the memory trace was demonstrated by the observation that rats of both strains spent more time in the sector in which the platform had been located during the training period. Rats of the two strains showed essentially no difference in terms of the time spent seeking the platform when it was placed in the opposite sector. After one day of training, blood corticosterone was significantly lower in tame than in aggressive rats. On subsequent training days, hormone levels in tame animals increased and were no different from those in aggressive rats. It is suggested that decreased emotionality and stress reactivity facilitated the learning process in tame rats in the Morris water test.

KEY WORDS: tame and aggressive gray rats, learning in the Morris water test, blood corticosterone levels.

Behavioral adaptation is particularly characteristic of most species of animals which have been successfully domesticated [26]. There is no doubt that the leading role in this process is played by experience acquired during learning, so the process of domestication might be expected to be associated with higher levels of learning ability. This, according to Thorpe [27], may also be facilitated by an increase in the role of the seeking phase of the behavioral act, which becomes more independent of stage completion in domesticated animals. However, the ecological situation applying at a given point in time is more complex and variable for animals in natural populations than in the usual conditions of captivity. It is therefore not surprising that the ability to solve extrapolation tasks in some domesticated and laboratory animal species is lower than that in members

of the same species living in natural conditions [2]. As regards learning ability in these and other members of the species, there are less abundant data obtained by comparative analysis. There are small numbers of such studies in wild and laboratory rats, which showed that laboratory rats had greater ability to solve various tasks than wild rats, which demonstrated stereotypical movement reactions and marked neophobia [15–17].

There is no doubt that at the early stages of domestication, humans and the anthropogenic environment were stress factors for animals, and selection for domestication was, in essence, selection for weakening of the emotional reactions of fear and aggression to these factors, so it is logical to suggest that learning ability, tested in stressful situations, will be greater in domesticated animals than the initial wild strains. Some support for this may be provided by data on the correlational relationship between learning ability in a shuttle box and the degree of domestication of one species subjected to experimental domestication – the silver

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fox [1]. However, this does not resolve the question of the ability of domesticated animals to learn in other situations.

Studies over many years at the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences have involved selection for domestication and aggressivity in another system – wild gray rats [10]. The present study addresses comparative analysis of learning ability in the Morris spatial water test in gray rats subjected to prolonged selection for the elimination and enhancement of aggressive behavior towards humans.

METHODS

Experiments were performed on adult male gray rats (*Rattus norvegicus*) of the 64th generation of selection for elimination of defensive reactions to humans (the tame strain) and enhancement of aggressive behavior towards humans (the aggressive strain). Each experimental group consisted of 12–14 animals. Animals were kept in standard cages in groups of four per cage with a natural photoperiod and free access to food and water. All experiments were performed from 14:00 to 18:00.

Spatial learning ability was studied in the Morris water test [21], where the animal's task was to learn to find a platform hidden beneath the water surface.

Apparatus: A round swimming basin of diameter 150 cm and height 54 cm was filled with milk-tinted water to a depth of 37 cm. The temperature of the water in the basin was maintained at 24–26°C. The basin was arbitrarily divided into four equal sectors. The invisible platform (34 cm high and 10 cm in diameter) was placed in the center of one of these sectors. A metal grid was placed above the platform for the animal to mount on reaching it. Maps were drawn on the walls closest to the basin which, when the apparatus was placed in the room, could serve as spatial orientation markers.

Learning ability was studied in two experiments.

Procedure of experiment 1. The training of each animal consisted of four daily trials over a period of seven days. The animal was placed in water from the same point in all trials, and was allowed to swim for 70 sec. Regardless of whether the animal found the platform itself or was directed to it, it remained on the platform for 20 sec. The animal was then removed from the basin, dried, and placed in the transfer cage and, after 10–12 min, the training procedure was repeated. Fixation of the memory trace was tested on day 8, in the absence of the platform.

Procedure of experiment 2. During training, the animals was placed into the basin from four sequential equidistant points, changing the start point (first placing in basin) in random order; the same sequence of placing in the basin was used for all animals. Otherwise, the procedure used in experiment 2 was the same as that of experiment 1. After training, the platform was moved to the opposite sector on day 8 and

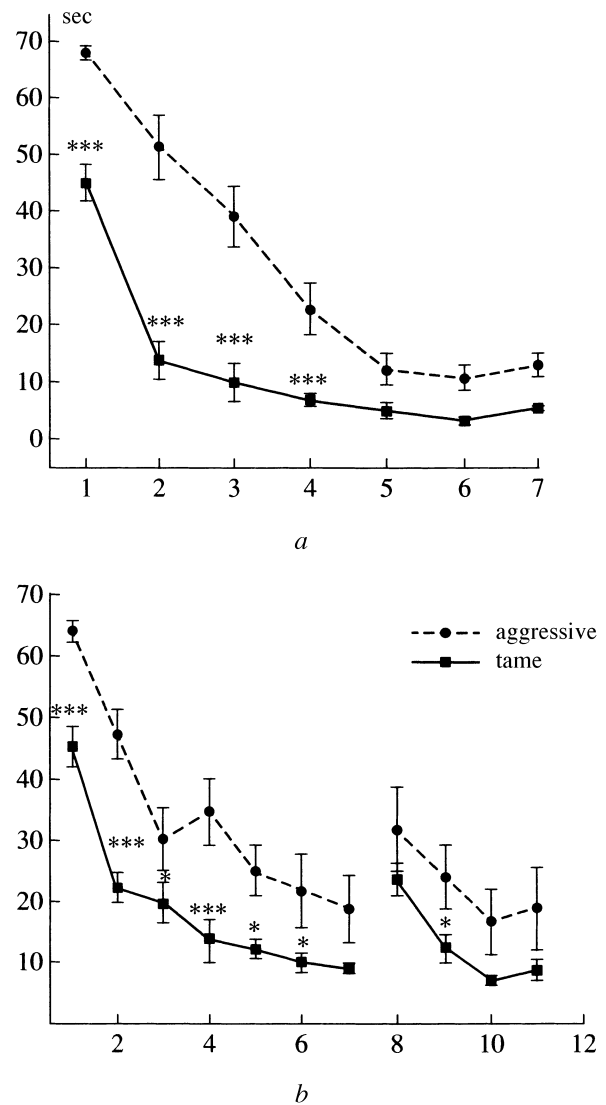


Fig. 1. Platform seeking times in tame and aggressive rats: a) experiment 1; b) experiment 2. The abscissa shows training days 1–7 and retraining on days 8–11. * $p < 0.05$; *** $p < 0.001$ compared with aggressive rats (comparison of group means, Neuman–Keuls test).

the training procedure was repeated for a further four days, allowing assessment of the animals' relearning ability.

All experiments were recorded using a video camera located beneath the basin, and were subsequently processed using programs developed by ourselves for behavioral data [6]. The following parameters were assessed: platform seeking time (if the rat failed to find it, this was taken as 70 sec), the time spent swimming close to the basin wall (within 15 cm), the times spent in each sector on testing for memory trace fixation, and the time spent rearing on the hindpaws while the rats were on the platform.

Peripheral blood corticosterone levels were estimated by competitive protein binding assay as modified by Tinni-

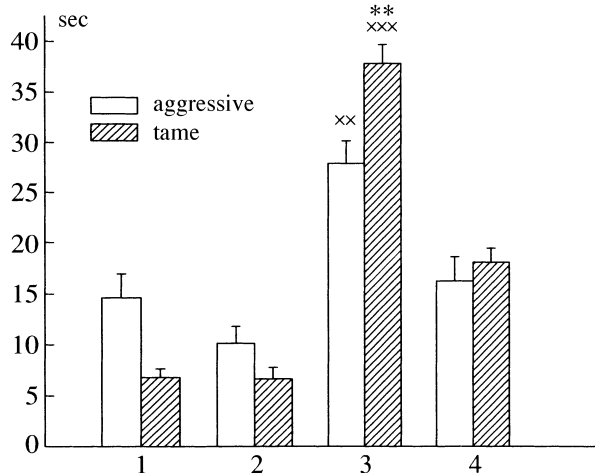


Fig. 2. Durations of swimming in tame and aggressive rats in each sector of the basin in the absence of the platform. The platform was located in sector 3 during training. The abscissa shows the sectors of the basin. $**p < 0.01$ compared with aggressive rats; $**p < 0.01$, $xxxp < 0.001$ compared with the other sectors (comparison of group means, Neuman–Keuls test).

kov and Bazhan [9]. Blood was collected from the tail vein. Corticosterone levels were estimated during training using samples collected immediately after training and on days 1, 2, and 7 from rats in the second experiment. Baseline corticosterone levels were estimated one week before training started.

Data, except for measures of vertical movement activity on the platform, were analyzed statistically by unifactorial dispersion analysis for repeat measurements, where the independent factor was the genotype, with subsequent comparison of group means using the Neuman–Keuls test. The significance of differences between tame and aggressive rats during swimming at the periphery and vertical rearing activity on the platform was evaluated using the Kruskal–Wallis non-parametric ANOVA statistic.

RESULTS

Experiment 1. Dispersion analysis revealed a significant influence for the genotype factor ($F_{1,21} = 53.15$, $p < 0.001$) on the platform seeking time and changes in this parameter during the learning process ($F_{6,126} = 92.24$, $p < 0.001$), along with a significant interaction between the dynamics of seeking time and genotype ($F_{6,126} = 9.88$, $p < 0.001$). As shown in Fig. 1, *a*, tame rats found the platform as early as day 1; from day 2, there was a significant reduction in seeking time, which changed little on subsequent training days. On day 1, virtually all aggressive rats were unable to find the platform and showed a gradual reduction in seeking time over the following three days, such that on day 5 they achieved the values seen in tame rats

on day 2, though the level remained higher than in tame rats to the end of training.

During testing of memory trace fixation, the time spent swimming in the third sector, from which the platform was removed, was significantly greater than the swimming time in the other sectors in both tame and aggressive rats (Fig. 2). However, tame rats more frequently entered this sector and spent longer periods of time in it than aggressive rats.

Experiment 2. Dispersion analysis of the platform seeking time revealed the same features as in experiment 1. Tame rats learned to find the platform more quickly than aggressive rats ($F_{1,20} = 17.37$, $p < 0.001$); the seeking time decreased significantly in rats of both strains during training ($F_{6,120} = 55.98$, $p < 0.001$), though changes in platform seeking time in aggressive rats were less steep than those in tame rats ($F_{6,120} = 2.49$, $p < 0.05$; Fig. 1, *b*). Tame rats differed from aggressive rats in that they spent significantly less time swimming at the periphery of the basin (Fig. 3, *a*). As shown in Fig. 3, *a*, aggressive animals remained in the edge zone of the basin in virtually all four tests on training day 1. The behavior of tame and aggressive rats also differed in terms of the time spent on the platform. Thus, the durations of vertical motor activity on the platform on virtually all training days were greater in tame rats than in aggressive rats (Fig. 3, *b*).

On retraining, when the platform was moved to the opposite sector of the basin, animals of both strains successfully coped with finding the platform in its new location on day 1. Genotype differences in the platform seeking time were borderline significant ($F_{1,20} = 4.02$, $p = 0.06$); this measure was significantly lower in tame rats than in aggressive only on retraining day 2 (Fig. 1, *b*).

The basal peripheral blood corticosterone level in tame rats was significantly lower than in aggressive rats (Fig. 4). On training day 1, there were significant increases in hormone levels in animals of both strains, though the corticosterone level in tame rats was significantly lower than that in aggressive rats. On training days 2 and 7, corticosterone levels in tame rats reached values characteristic of aggressive rats.

Comparison of platform seeking times during training in rats of the two groups, using different training procedures, showed that when rats entered the water from different points around the basin, tame rats showed an increase in the platform seeking time as compared with the platform seeking time when rats learned by the simpler scheme, i.e., when they entered the water from a single point around the basin ($F_{1,22} = 9.26$, $p < 0.01$); no such differences were seen in aggressive rats ($F_{1,22} = 0.06$, $p > 0.05$).

DISCUSSION

The results obtained here show that tame and aggressive strains created by prolonged selection of wild rats for

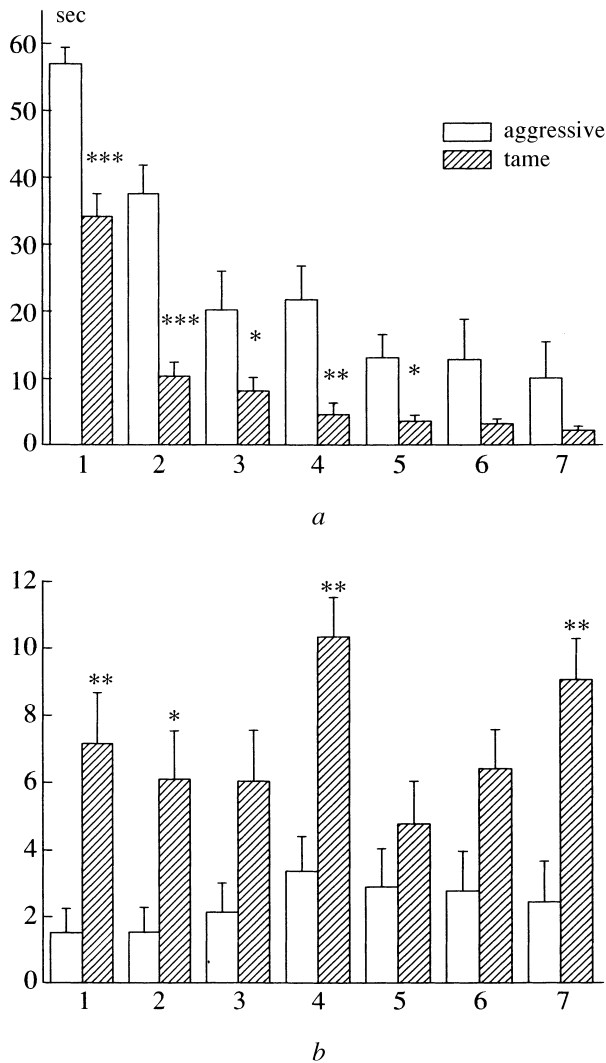


Fig. 3. Time spent swimming at the periphery of the basin (up to 15 cm from the walls, *a*) and duration of vertical investigative activity while on the platform (*b*) in tame and aggressive rats. The abscissa shows training days 1–7. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with aggressive rats (Kruskal–Wallis ANOVA).

elimination and enhancement of defensive reactions to humans were able to learn in the Morris water test. Previous studies used comparison of learning ability in rats selected for the absence of aggressivity towards humans with laboratory rats. Thus, tame rats were shown to be more successful than laboratory Wistar rats in learning a passive avoidance skill [3]. Gray rats of the Lyapunovaya breed, i.e., selected for the absence of defensive reactions to humans, also had greater learning ability than laboratory rats of the same Wistar strain in terms of the rate of acquiring an active avoidance skill [12]. In our studies, comparison of tame and aggressive rats showed that tame males were more successful than aggressive males in coping with finding the plat-

form hidden under the water in experimental conditions with different training procedures, and that from training day 2 their swimming started to acquire a purposeful character, while this was seen in aggressive animals only on training day 5. The fact that aggressive rats differ from tame rats in that they have greater emotionality and stress reactivity in the open field test is well known [5, 24]; they also have higher withdrawal reflex amplitudes [8] and stronger hypophyseal-adrenal axis reactions to acute and chronic emotional stress [11]. During training in the Morris water test, a procedure which is undoubtedly aversive in nature, it appears that aggressive animals experience stronger emotional stress than tame animals. As a result, aggressive rats, particularly at the early stage of training, spend significantly longer times swimming at the periphery of the basin, which appears to hinder the formation of the spatial concept of platform location. Furthermore, while tame rats showed an increase in the platform seeking time when the task in the Morris water test becomes more complex (experiments 1 and 2), no such difference was seen in aggressive rats, which is presumably evidence that the water test situation has a strong action on aggressive rats. Evidence supporting the notion that tame rats experience less stress than aggressive rats may also be provided by data showing longer periods of investigative activity in tame rats on being placed on the platform. Tame rats reaching the platform take up vertical postures, actively investigating the environment, which was hardly seen in aggressive animals, which sat and pressed themselves onto the platform. Nonetheless, both tame and aggressive rats remembered the position of the platform, as the test for memory trace fixation after completion of training, when the platform was removed from the basin, showed that animals of both strains spent significantly longer periods swimming in the sector in which it had previously been located. However, this time was significantly shorter in aggressive rats than in tame rats, perhaps because of their greater stress reactions to the changing situation. It is also possible that tame rats, because of their more successful learning as compared with aggressive rats, remembered the position of the platform better. Similar relationships between sensitivity to the aversive actions of the water test and learning ability in the Morris water test have previously been demonstrated in rats selected for low (KHA) and high (KLA) abilities to acquire an active avoidance reflex [7].

The existence of an interaction between emotionality, the corticosterone reaction to stress, and learning ability in the Morris water maze has been demonstrated in mice of different strains [18]. Thus, BALB mice, characterized by high stress reactivity, showed extremely low levels of spatial learning ability [18]. At the same time, there is a significant amount of data providing evidence of a close relationship between learning ability and corticosteroid levels; both very low and high blood hormone levels had negative effects on learning ability, while moderate increases could

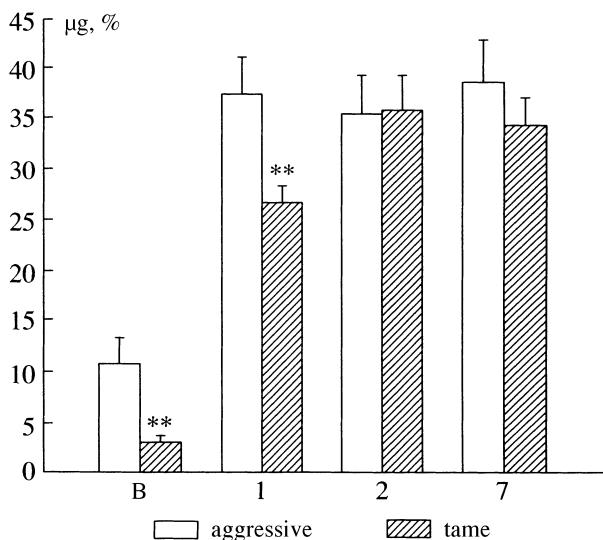


Fig. 4. Blood corticosterone levels in tame and aggressive rats at different stages of training. B is the baseline level; 1, 2, and 7 are training days. $***p < 0.01$ compared with aggressive rats (comparison of group means, Neuman-Keuls test).

facilitate learning [13, 14]. Our results showed that plasma corticosterone levels on training day 1 were significantly lower in tame rats than in aggressive rats, while platform seeking time in these animals was very significant. However, on training days 2, and 7, when virtually all tame animals could solve the task, hormone levels increased to the values seen in aggressive animals. This suggests that the Morris water test is a stress situation, including emotional stress (contextual novelty) and systemic stress (physical loading, temperature). Regulation of the activity of the hypophyseal-adrenal axis in these types of stress is known to differ [25]. It is possible that on training day 1, animals were predominated by emotional stress to novelty such that the corticosterone level in tame rats was lower than that in aggressive rats, which is consistent with our previous data showing decreased hypophyseal-adrenal axis reactions to emotional stress in tame rats [11]. It is possible that on subsequent training days, activation of the hypophyseal-adrenal axis was due mainly to the physiological adaptation of the animals (maintenance of muscle activity and body temperature) to the physical components of the complex action of the water test.

The mechanisms mediating the effects of stress on the formation of memory are widely studied [28]. In investigations of the processes of spatial learning and memory, most attention is paid to the role of glucocorticoid receptors in the hippocampus [20]. The authors suggested that blockade or deficiency of hippocampal receptors may lead to impairments in selective attention and sensory integration, which degrades the solution of spatial tasks. Thus, the result of chronic stress in male rats was detection of a reduction in

the number of hippocampal receptors and degradation of learning in Morris water test; in females, with an elevated level of receptors, learning was no different from that in controls, though memory for the location of the platform was more fixed [19]. Mice with knockout of the glucocorticoid receptor gene also showed deficiency in the formation of spatial memory [22]. Administration of the glucocorticoid receptor antagonist RU38486 at the start of training degraded task solution in the Morris water maze in male rats, regardless of the dose used, while constant administration of antagonist at high dose facilitated learning [23]. In tame rats, as demonstrated previously, the density of glucocorticoid receptors in the hippocampus was greater than in aggressive rats [4], which is evidently also important for the initial learning process.

Of particular interest are data obtained during assessment of the ability to locate the platform when it was moved to the opposite sector, when the spatial concept of the position of the platform was formed on the basis of previous experience. As shown by the data, tame and aggressive rats in this situation showed virtually no difference in the time taken to solve the task of finding the platform ($p = 0.06$), though the platform seeking time remained greater in aggressive than in tame animals. Aggressive rats are evidently already adapted to the conditions of the water test, such that changes in the position of the platform did not have a strong influence, which contributed to retraining success. Thus, it can be suggested that the deficiency in learning ability in the Morris water test in aggressive rats may be determined primarily by increased reactivity to the test situation. The decreased stress response to novelty at the start of the learning process in tame rats, as compared with aggressive rats, facilitates acquisition of the spatial concept of the platform position from the first steps.

CONCLUSIONS

1. Tame rats learned in the Morris water test more successfully than aggressive rats. The time taken to seek the invisible platform was significantly shorter in tame rats than in aggressive rats. The increased seeking time in aggressive rats was linked to the fact that they spent longer periods of time at the periphery of the basin, especially at the early stages of learning.

2. Aggressive rats, like tame rats, remembered the location of the platform, as when it was absent the day after training, they showed a preference for the sector in which the platform had previously been located. However, tame rats differed from aggressive rats in terms of a longer time spent in this sector.

3. The blood corticosterone level in tame rats was significantly lower than that in aggressive rats on training day 1 but was no different from that in aggressive rats on training days 2 and 7.

4. Tame and aggressive rats were not significantly different on retraining, though aggressive rats preserved the tendency to an increased platform seeking time.

The authors would like to express their deep thanks to R. V. Kozhemyakina for assistance in performing these experiments.

This study was supported by the Russian Foundation for Basic Research (Grant No. 05-04-48378).

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