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Behavior, Adrenocortical Activity, and Brain Monoamines in Norway Rats Selected for Reduced Aggressiveness Towards Man

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NAUMENKO, E. V., N. K. POPOVA, E. M. NIKULINA, N. N. DYGALO, G. T. SHISHKINA, P. M. BORODIN AND A. L. MARKEL. *Behavior, adrenocortical activity, and brain monoamines in Norway rats selected for reduced aggressiveness towards man.* PHARMACOL BIOCHEM BEHAV **33**(1) 85–91, 1989.—Wild Norway rats were selected over 20 generations for reduced aggressivness towards man. Selection for this characteristic was accompanied by many physiological changes. Although neophobia was significantly inhibited, and irritable aggression reduced by selection, no changes were revealed in mouse-killing behavior or in intermale aggression. The mean level of 5-hydroxyindole acetic acid in the hypothalamus as well as serotonin (5-HT) content in the hypothalamus, the midbrain and the cortices was higher in the 'domesticated' than in aggressive rats. Mean hypothalamic norepinephrine (NE) level also tended to be higher in the 'domesticated' animals. The resting corticosterone level and the response of the hypothalamic-pituitary-adrenocortical axis to an emotional stressor or intracerebroventricularly administered 5-HT or NE were decreased in domesticated rats compared to their aggressive counterparts. It is suggested that the diminution of the hypothalamic-pituitary-adrenocortical function as a result of selection for domesticated types of behavior depends, at least partly, on changes in brain monoaminergic systems.

Aggressiveness Domestication Serotonin Norepinephrine Adrenocortical system Emotional stress

IT has been hypothesized (3) that domestication involved unconscious selection of wild animals for reduced aggressive reactivity towards handlers. It has also been assumed (5) that the first stages of domestication in all animal species are also associated with permanent exposure to stress caused by direct contact with man. Therefore, selection for the domesticated type of behavior may also involve selection for resistance to stress induced by captivity. That is probably why alterations are found not only in reactivity to man (4) but also in pituitary-adrenocortical function (16) in domesticated silver foxes (*Vulpes fulvus* Desm.) as compared with nondomesticated counterparts. Changes in adrenocortical function as well as in aggressive behavior may involve brain monoamines (15). Aggressive behavior, is not, however, a unitary construct (14), and various types of aggressiveness are controlled by different monoaminergic mechanisms (9, 13, 20–22). Therefore, the question remains as to what types of aggressive behavior are changed during the domestication of animals.

Attempts have been made to study the influence of domestication by comparing wild and laboratory rats. It has been found that the neophobia, i.e., escape from novel stimuli which is very characteristic of wild rats, is attenuated in laboratory animals (1,8). However, as the various strains of laboratory rats also result from some mutations, it can never be known precisely what degree of behavioral complexity is due to prolonged selection for the 'tame' phenotype, and what is the result of mutation. Thus, the influence of domestication can be more precisely evaluated by comparison of wild rats with rats selected specifically for reduced aggressiveness towards man.

The present study examined changes in emotional and aggressive behavior, in pituitary-adrenocortical function, and in serotonergic and noradrenergic brain mechanisms in Norway rats selected through 20 generations (S_{20}) both for reduced and for high aggressive reactivity with respect to man.

METHOD

Animals

Selection for reactivity towards man was started on the second generation of Norway rats trapped near Novosibirsk in 1972. A glove test (7) was used for evaluation of aggressiveness towards man in initial and in each successive generation in both males and females at the age of 3 months. A hand was introduced into the

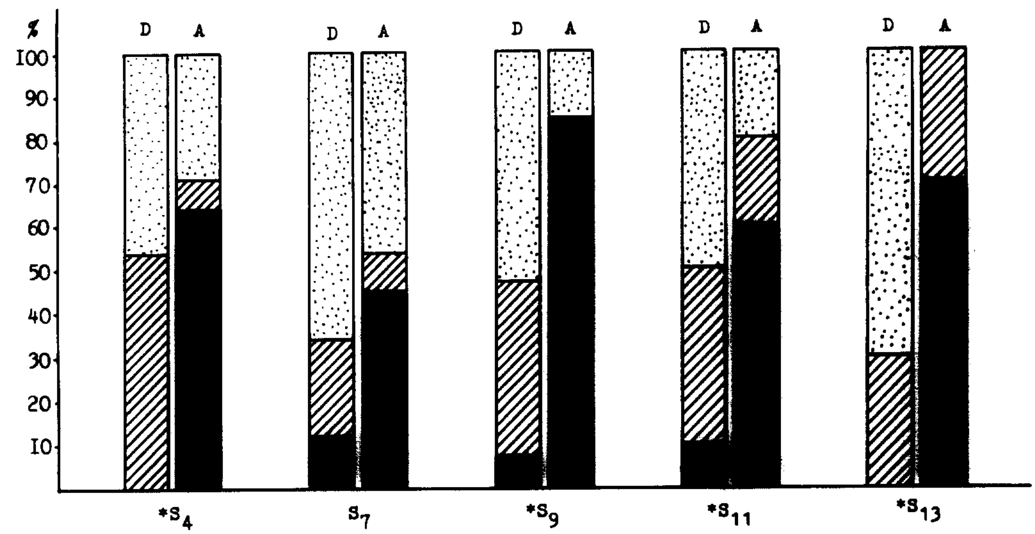

FIG. 1. Reactivity to novel object for Norway rats selected for behavior towards man. D = domesticated rats, A = aggressive rats. Dotted columns = positive reactions. Solid columns = negative reaction. Striped columns = no reaction. *Indicates $p<0.001$.

home cage and moved around the cage until the rat could be pinned against the back wall. The expression of savageness was rated on a 5-point rating scale where: 0—lack of defensive reaction to handling; 1—defensive reactions (motor excitement, vocalization) appeared only during handling; 2—the animal bit the glove and vocalized after the contact with the hand near the back wall of the cage; 3—the rat actively attacked the glove in the front part of the cage; and 4—where "rage" attacks occurred at the front door of cage in response to the appearance of the glove in front of the closed cage.

On the basis of the glove test, two founder groups were selected from the initial population: "domesticated" rats with a weak expression of defensive reactions towards man (scores 0 and 1), and "aggressive" rats (score 4). These groups were selected in each succeeding generation for low (scores 0–1) and high (score 4) reactivity, with the coefficient of selection being about 10–15 percent in both stocks. The stocks were maintained using a system which maximally avoided inbreeding. After 20 generations of selection, the coefficient of inbreeding is not higher than 0.13.

The offspring of each generation used in the experiments were removed from their mothers to group cages (4–5 same-sex rats per 50 × 33 × 20 cm cage) after weaning at 30 days of age, and were 4 to 6 months old at the beginning of the experiments. No fewer than 10 males with a body weight of 300–350 g for both the domesticated and aggressive groups were used in each experiment. Rats were placed in individual cages (50 × 33 × 20 cm) a week before behavioral testing.

Procedure

Besides the glove test, rats were tested for different kinds of aggression in the following order: Predatory, intermale, and irritable aggression. Each type of aggression was assessed once a day for three days in rats which had not been deprived of food and water prior to testing.

For the study of predatory aggression (2), an adult albino male mouse weighing 18–20 g was put into each subject's home cage for 40 min, and the animals killing their mice were designated as "killers." The mouse was removed from the cage immediately after it had been killed.

To study irritable aggression (10) pairs of aggressive or domesticated rats were placed into a cage (25 × 25 × 30 cm), and electric shock of 2.5 mA was delivered for 2 sec every 6 sec to the floor of the cage. An experimental session consisted of 100 foot shocks within 10 min. A fighting episode was recorded when the rats standing face-to-face on their hind limbs sparred and bit each other.

Intermale aggression was studied by putting a pair of aggressive or domesticated rats into an unfamiliar clean cage (7). The number of attacks and aggressive postures (upright and threatening) was registered during 40 min.

In order to test neophobia (1), rats were placed on 4 consecutive days in an open-field device (70 × 140 cm) and left undisturbed for a 10-min habituation period. At the end of this period on the 4th day, a 4 × 5 × 2 cm piece of plastic was put in front of the rat, and its reaction to this novel object was evaluated during a 2-min period. Five types of responses were rated: 1—aggressive reaction (vocalization, attack and biting of the plastic); 2—escape reaction; 3—lack of any reaction to the plastic; 4—exploration of the plastic at a distance (visual examination and sniffing without attempts to avoid); 5—direct contact with the plastic (touch and play with the plastic). The first two reactions were evaluated as "negative," and the last two as "positive."

All behavioral tests were carried out between 09:00 and 11:00 hr on domesticated and aggressive rats of the same generation. Three days after behavioral testing of the individually-housed rats, subjects were decapitated between 09:00 and 10:00 hr. The brains were quickly removed in the cold and dissected into three parts: hypothalamus, midbrain, and anterior portion of the right hemisphere. The brain tissues were analyzed fluorimetrically for norepinephrine (NE), serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) content (11).

The resting corticosteroid level in the peripheral blood or the level of corticosteroids in the blood after emotional stress induced by 1 hr restriction was determined fluorimetrically (23). Emotional stress was induced by individually placing the animal into restraining cylindrical wire-mesh cages (d—6 cm, 1—22 cm) for 60 min.

In addition, in S_{19}, hypothalamic-pituitary-adrenocortical function was examined 1 hr after a systemic stress produced by cutting the tip of the tail under light ether anesthesia or 1 hr after intraventricular NE or 5-HT injection. For this injection, a steel cannula was implanted into the lateral ventricle of the brain 3–4

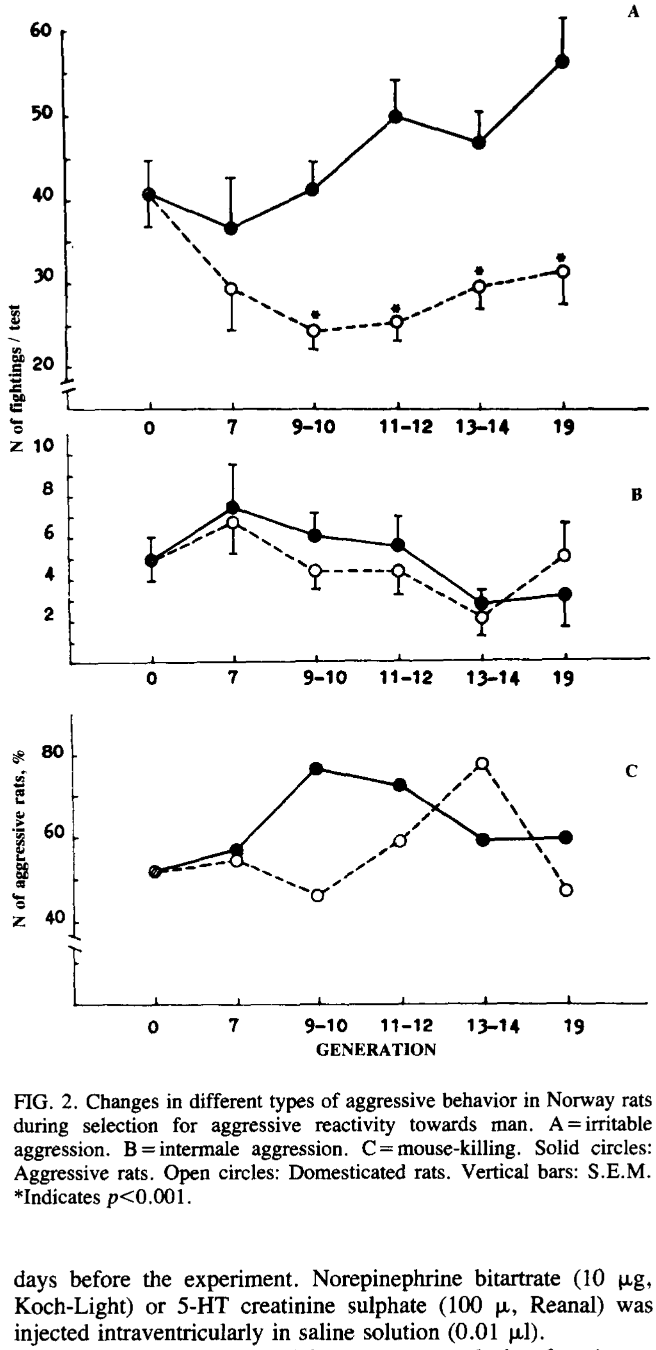

FIG. 2. Changes in different types of aggressive behavior in Norway rats during selection for aggressive reactivity towards man. A = irritable aggression. B = intermale aggression. C = mouse-killing. Solid circles: Aggressive rats. Open circles: Domesticated rats. Vertical bars: S.E.M. *Indicates $p<0.001$.

days before the experiment. Norepinephrine bitartrate (10 μg, Koch-Light) or 5-HT creatinine sulphate (100 μ, Reanal) was injected intraventricularly in saline solution (0.01 μl).

The data were evaluated by two-way analysis of variance, two-tailed Student's t-test, chi square analysis, or sign test.

RESULTS

The neophobia study showed that 64 percent of aggressive rats displayed aggressive or escape reactions to a novel object by the fourth generation (S_4), while no domesticated animals of the same generation showed negative responses to novelty (Fig. 1). Concomitantly, the percentage of animals with positive reactions (exploration of the new object or playing with it) was almost twice as high in the domesticated as in the aggressive rats. Although no aggressive reactions were found in subsequent generations of domesticated animals, a few animals continued to show escape reactions to novelty. The most clear-cut difference in neophobia was revealed in rats of S_{13} (Fig. 1): No positive reactions were found among the aggressive rats, and 70 percent of these rats displayed negative responses (aggressive or escape reactions) to novelty. At the same time, 70 percent of the domesticated rats demonstrated positive reactions (exploration at distance or playing with the plastic) and not a single rat showed neophobia.

A significant difference in irritable aggression occurred between domesticated and aggressive rats in response to electric foot shock (Fig. 2). The number of fights decreased markedly in the group of tame rats. The distinction was found to be consistent and clearly manifested in subsequent generations. At the same time, the number of spontaneous intermale fights did not change during selection for domesticated behavior in respect to man. No single generation of selected animals showed any difference in spontaneous intermale aggression between the domesticated and aggressive ones.

Although some fluctuations in percentage of "killers" occurred from generation to generation in the mouse killing test, no significant difference was found between domesticated and aggressive rats.

Selection for reactivity towards man was followed by a marked change in the brain 5-HT metabolism of the domesticated animals. Analysis of variance revealed that the mean level of 5-HIAA in the hypothalamus, $F(1,71)=7.05$, $p<0.01$, as well as the 5-HT content in the hypothalamus, $F(1,72)=11.61$, $p<0.001$, in the midbrain, $F(1,74)=4.83$, $p<0.02$, and in the cortical hemisphere, $F(1,74)=10.28$, $p<0.002$, of the domesticated rats was higher than in aggressive counterparts. A comparison of these two groups of animals in each generation showed that in S_{15-16} 5-HIAA content appeared to be significantly higher in the hypothalamus of the domesticated rats than in the aggressive animals. This difference was maintained in subsequent generations. Increases not only of 5-HIAA, but of 5-HT itself were found (Fig. 3) in the cortical hemisphere in S_{15-16}, and in the hypothalamus and midbrain in the last generation studied.

By contrast to the serotonergic system, differences in brain NE content were inconsistent. The mean NE level in the hypothalamus tended to be higher in the domesticated animals, $F(1,72)=3.69$, $p<0.055$, but this difference was significant only in S_{15-16}. Concomitantly, NE content in the midbrain and in the cortical hemisphere (except in S_{19}) of the domesticated and of the aggressive rats did not differ significantly, $F(1,70)=0.06$, $p>0.05$, and $F(1,74)=3.04$, $p>0.05$, respectively (Fig. 4).

The resting corticosterone level in the peripheral blood plasma tended to be lower in the domesticated than in the aggressive animals. Although this difference was significant only in S_{20} (Fig. 5), the sign test revealed in all the generations studied a lower mean corticosterone level in the domesticated rats as compared with aggressive animals ($p<0.05$). It was found that the adrenocoritcal response to stress induced by 1 hr restriction was significantly decreased in the domesticated rats after S_{13} (Fig. 6). In contrast to emotional stress, the stress of tail cutting, as revealed in rats of the S_{19}, was followed by equal increases of the corticosterone level in the blood of both the domesticated and the aggressive groups (41.3 ± 6.5 μg% and 56.6 ± 4.3 μg%, respectively).

An intracerebroventricular injection of NE or 5-HT was accompanied by a clear-cut increase of the corticosterone level in the peripheral blood of domesticated as well as aggressive animals. However, 1 hr after injection of NE or 5-HT into the lateral ventricle of the brain, the blood corticosterone level was markedly higher in the aggressive rats than in their domesticated counterparts (Fig. 7).

DISCUSSION

A comparison of the behavior of the domesticated and the

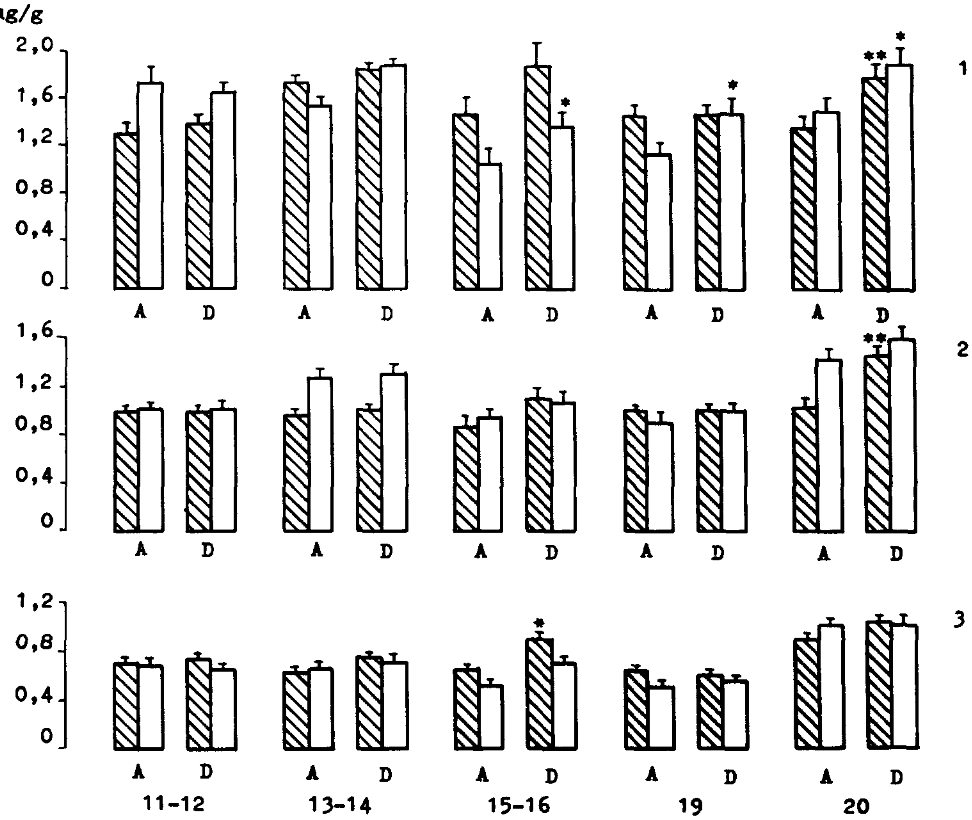

FIG. 3. Concentrations of 5-HT and 5-HIAA in brains of domesticated and aggressive Norway rats during selection for aggressive reactivity to man. A = aggressive rats. D = domesticated rats. 1 = hypothalamus. 2 = midbrain. 3 = hemisphere. Striped columns: 5-HT. Open columns: 5-HIAA. *Indicates $p<0.05$; **indicates $p<0.001$.

aggressive Norway rats revealed considerable changes which occurred in the course of twenty generations of selection for tameness in response to man. The most marked of these were the loss of neophobia and the reduction of irritable aggression.

Neophobia disappeared in domesticated rats by S_4, giving way to exploratory behavior in subsequent generations of tame rats, while neophobia was maintained at a relatively high level during all generations studied, in the aggressive rats. Concomitantly, a clear-cut diminution of irritable aggression occurred in the domesticated group. It may be assumed that the decrease of irritability is an adaptive response of wild rats to captivity and to permanent contact with man. Although it has been shown that albino rats, used by some authors as a model of domestication (8,12), killed mice less frequently than did wild Norway rats (12), in our experiments the domesticated Norway rats expressed as much mouse-killing behavior as the aggressive animals. Finally, the study of intermale aggression did not reveal any difference between domesticated and aggressive rats. These data suggest that the mechanisms of control of intermale as well as predatory aggression are not altered by selection for domesticated behavior in Norway rats. This finding coincides with numerous data (9, 14, 20) demonstrating differences in the control of various types of aggressive behavior.

Some marked alterations in brain biogenic amines were also found during domestication, with increases in 5-HIAA in the hypothalamus and an increase of 5-HT in all brain regions studied in the domesticated rats.

These data support previous results on silver foxes: Levels of 5-HT and 5-HIAA in hypothalamus, midbrain, and hippocampus

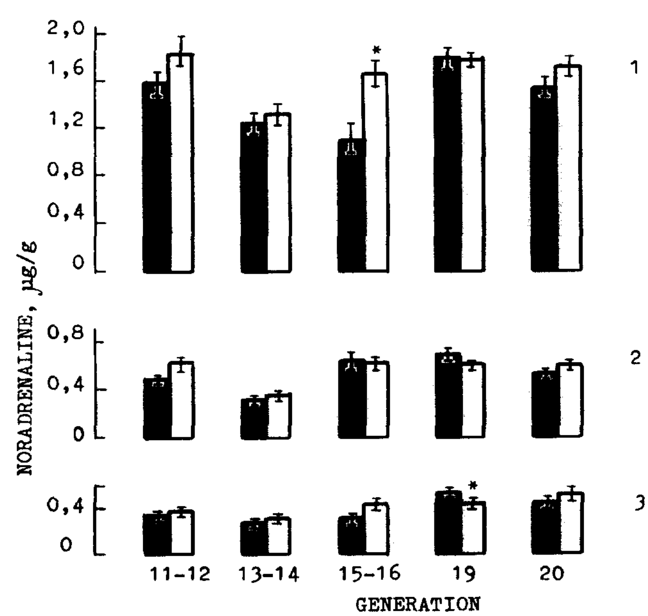

FIG. 4. Concentrations of NE in brains of domesticated and aggressive Norway rats during selection for aggressive reactivity towards man. 1 = hypothalamus. 2 = midbrain. 3 = cortical hemisphere. Solid columns: Aggressive rats. Open columns: Domesticated rats. Vertical bars: S.E.M. *Indicates $p<0.05$.

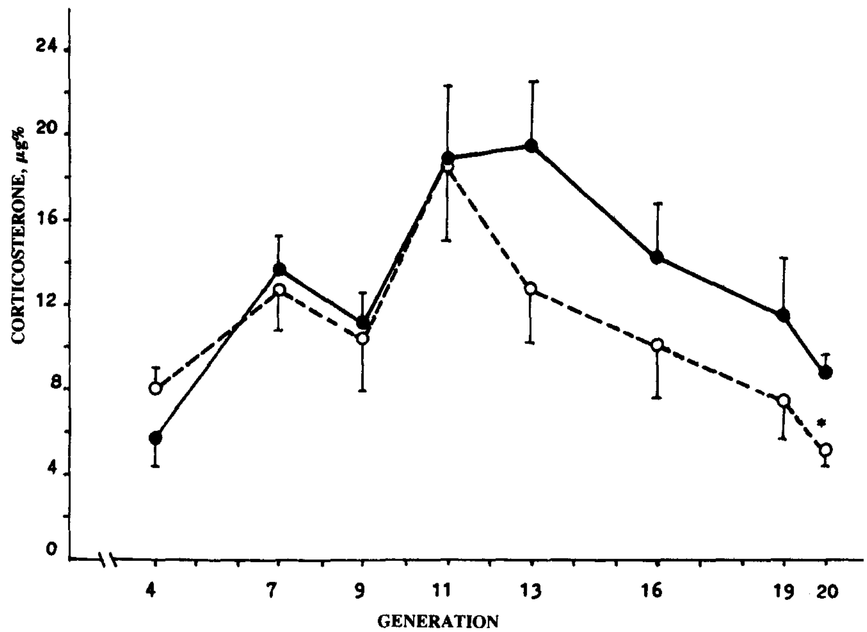

FIG. 5. Resting corticosterone levels in the peripheral blood of demesticated and aggressive Norway rats during selection for aggressive reactivity towards man. Solid circles: Aggressive rats. Open circles: Domesticated rats. Vertical bars: S.E.M. *Indicates $p<0.05$.

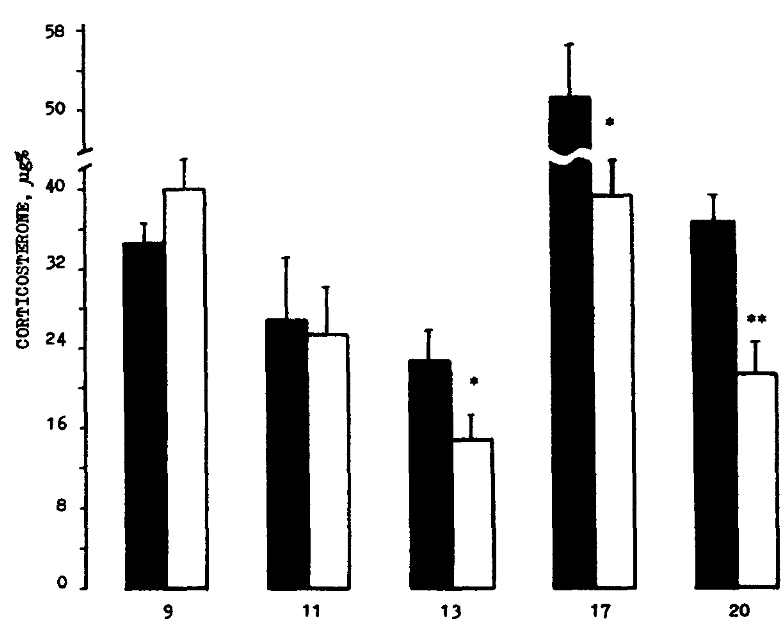

FIG. 6. Levels of corticosterone in peripheral blood produced by emotional stress in domesticated and aggressive Norway rats during selection for aggressive reactivity towards man. Solid columns: Aggressive rats. Open columns: Domesticated rats. Vertical bars: S.E.M. *Indicates $p<0.05$; **indicates $p<0.001$.

were significantly higher in domesticated than in nondomesticated silver foxes (19).

Since brain 5-HT seems to play an inhibitory role with reference to predatory as well as intermale aggression (9,20), it is somewhat puzzling that these behaviors were unaffected by selection for reactivity towards man. It remains a possibility that, despite the regional 5-HT differences obtained here, whole brain serotonergic levels were not altered by selection. Alternatively, one cannot rule out the possibility that changes in other neurotransmitter systems such as acetylcholine activating predatory

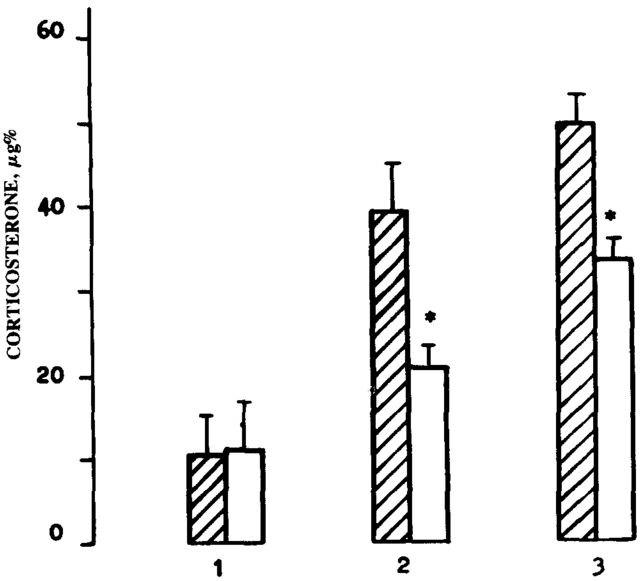

FIG. 7. Levels of corticosterone in peripheral blood produced by NE or 5-HT injection into the lateral ventricle in domesticated and aggressive Norway rats of S_{19}. 1 = saline. 2 = NE. 3 = 5-HT. Striped columns: Aggressive rats. Open columns: Domesticated rats. Vertical bars: S.E.M. *Indicates $p<0.001$.

aggression (24) or dopamine stimulating intermale aggression (6) might have countered the inhibitory effect of increased 5-HT in tame rats.

Selection for reactivity towards man was also followed by some changes in the noradrenergic system. Although the alterations of NE level appeared to be inconsistent, it was nevertheless found in the experiments with intracerebroventricular NE injection that the sensitivity of the brain tissue of the domesticated rats to NE was changed.

It has been stressed that domestication is not a mere training of animals to live with man. It represents an artificial selection which is followed by a gene-linked reorganization of animal behavior to counter the stress of an unusual environment. It has been demonstrated that selection for reactivity to man rearranges both central and peripheral mechanisms of the neuroendocrine control of ontogeny (16). In the present study domestication involved a diminution of both the pituitary-adrenocortical response to emotional stress and basal corticosterone levels in the peripheral blood plasma. These results correspond precisely with those obtained earlier on the silver foxes (16).

In contrast to emotional stress, the pituitary-adrenocortical response to a systemic stressor (tail cut) did not reveal any distinction between domesticated and aggressive rat groups. This suggests that a specific central mechanism involving control of the hypothalamic-pituitary-adrenocortical response to emotional stress is altered in domestication. The present results suggest that both brain 5-HT and NE play a part. This view agrees with previous results indicating that, in albino rats, there is a significant positive intergroup correlation between the responses of the hypothalamic-pituitary-adrenocortical system to NE injection into the lateral ventricle and to an emotional stressor (17). Similar interrelations were found between 5-HT metabolism in the brain, reactivity towards man, and the response of the adrenocortical system to an emotional stressor in silver foxes (18).

On the basis of research on domesticated and nondomesticated silver foxes, a hypothesis has been put forward suggesting that selection for reactivity towards man is able to modify the function of the hypothalamic-pituitary-adrenocortical system through changes in brain neurochemical mechanisms (18). The present results confirm this idea and suggest that changes in serotonergic and noradrenergic brain mechanisms may be involved in this effect.

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