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Aggressive Rats: Some Properties of Learning, Memory and of the Limbic System

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NOVÁKOVÁ, V., V. FLANDERA AND W. SANDRITTER. *Aggressive rats: some properties of learning, memory and of the limbic system.* PHARMAC. BIOCHEM. BEHAV. 2(6) 729–733, 1974. – Rats – stable mouse-killers – elaborate an acoustic conditioned reflex, enforced by the drinking of water, more slowly than non-killer rats. The re-elaboration of the conditioned reflex after a three months' pause was also slower in killer rats. In this case it was necessary to repeat the connection between the conditioned and unconditioned stimulus the same number of times as during the elaboration of the reflex in non-killer rats. When the killer rats were allowed a free choice between a mouse, an estrous female rat, water and food after a 24 hr period of hunger and thirst, they first killed the mouse then their second and third reactions were feeding and drinking. Their reaction to the female was only random. Non-killers took food and water in the first, second and third place. Their reaction to the female was also an exception. The total content of ribosomal RNA, measured cytophotometrically in individual cells of certain structures of the limbic system, was different in the two groups of rats. Mousekillers had a higher content of RNA in the pyramidal cells of the dorsal hippocampus and neurons of the central amygdaloid nucleus, and a lower content in the neurons of the medial and lateral hypothalamus and in septal neurons. No differences in the RNA content were observed in the neurons of the frontal pole of the cerebral cortex. The differences were apparent only in neurons, not in interneurons or glia.

Mouse-killer Conditioning Drives Limbic system RNA in brain cells

ONE trait of the behavior pattern of some laboratory rats is the killing of an animal of another species. Rats that kill a mouse placed in their chamber are considered to be killers. Rats that never kill mice under the same conditions are called non-killers. Muricide activity can be experimentally evoked in non-killers by performing lesions or by the stimulation of certain brain structures. On the other hand, the interspecies aggression of the killer-rat can be transiently inhibited or even lastingly abolished by the same kind of brain manipulations.

According to Karli [11], the posterior region of the lateral hypothalamus plays the most important role in the mechanism of the development of aggression in rats. After the destruction of this region performed symmetrically on both sides, killers stop killing. The stimulation of the region leads to muricide reactions in non-killers. Muricide activity is also evoked by the stimulation of the medial hypothalamus [29]. After bilateral destruction of the nuclei of the medial hypothalamus, aggressiveness towards mice appears in 30% of initially non-killer rats. If other regions of the hypothalamus are stimulated, this behavior does not occur. Karli assumes that the neural substrate for aggression in rats is localised in the hypothalamus [11]. The medial hypothalamus exerts an inhibitory influence on the lateral hypothalamus. According to Panksepp [21] muricide activity is more intensive after bilateral destruction of the medial hypothalamus and is less intensive after bilateral destruction of the lateral hypothalamus. The lateral hypothalamus facilitates muricide activity by non-specific (motoric) influence. The medial hypothalamus inhibits the activity of the lateral hypothalamus. Panksepp [22] aroused aggressive behavior in rats by stimulating these regions of the hypothalamus lying above the optic tract, around the fornix and on the lateral part of the ventromedial nucleus. Paxinos [23] observed no change in the muricide activity after having surgically separated the region of the medial hypothalamus from regions of the lateral hypothalamus.

Other parts of the brain participating in the occurrence of aggressive behavior in rats can facilitate or inhibit the centrum in the lateral hypothalamus [12]. Facilitation is elicited by the central amygdaloid nucleus from which diffuse fibres lead underneath the pallidum and into the hypothalamus [9,26]. The destruction of the central amygdaloid nucleus or the removal of afference from this region results in inhibition of muricide activity for a shorter or longer period, depending on the localisation of the lesion. Cutting the stria terminalis does not have this effect. The lateral

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hypothalamus is facilitated directly and not through the medial hypothalamus [13, 27, 30]. According to the results of Karli [10], the septum is indifferent. However according to Miczek *et al.* [19] and Miley *et al.* [20] septal lesions are efficient in non-killers having little or no prior experience with mice. Certain rhinencephalic structures inhibit muricide activity. Bilateral lesions of tractus olfactorius lateralis, gyrus olfactorius lateralis and cortex pyriformis provoked muricide activity in rats that were initially non-killers [12]. The inhibitory function of the dorsomedial nucleus of the thalamus was proved by an experiment in which the bilateral lesion of this structure brought about muricide reactions in the majority of non-killers [1,2]. The stimulation of the dorsal hippocampus before presenting a mouse to the killer rat prevented the appearance of a muricide reaction [28].

Aggressiveness in the behavior pattern of certain rats is not the only property in which killers differ from non-killers. Hudgens *et al.* [6] found that mice that are dominant in a fight have difficulties with elaborating a conditioned defensive reaction. Under conditions of an open-field test, aggressive individuals behave differently from the non-aggressive ones [31]. Feeding behavior – hoarding – differs in rat-fighters and non-fighters [25].

The first aim of our work was to compare some characteristics of the behavior of killers and non-killers. We investigated the intensity of drives in a free choice between a mouse, an estrous female rat, water and food after 24 hr of hunger and thirst. Apart from that, we determined the rate at which rats elaborated a conditioned reflex enforced by drinking of water, and also the stability of the memory trace. In the second part of our work we wanted to characterize cytologically those structures of the limbic system which were found to be important in the development of the muricide reaction.

METHOD

Animals

The experiment was performed on 10 stable mouse-killers and 10 non-killers. Ninety-day-old male rats of the Wistar strain were used. Stable killers were rats that fulfilled conditions of the so-called short-term and long-term criteria. Under conditions of the short-term criterion, the rat in the experimental chamber was successively presented with five mice, each new one immediately after the previous mouse had been killed. The longest presentation was 60 min. Under conditions of the long-term criterion the rat was presented with mice for four days. If the rat kills each mouse, it is classified as a stable mouse-killer. If the rat never kills a mouse, it is taken as a non-killer. In saturation testing, the killers kill an average of 31.3 mice [3].

Procedure

In the first experiment we investigated the intensity of certain drives. The rats were left without food or water for 24 hr. We then registered the sequence of free-choice reactions of rats to a mouse, an estrous female rat, food and water. The rats were placed in the experimental chamber 1 hr before the beginning of the experiment in order to adapt and to eliminate the orientation reaction to the new environment. The results were evaluated by calculating the percentage of reactions to individual stimuli from the first to the fourth sequence. The percentage of reactions of killers and non-killers was compared by χ^2.

In the second experiment we elaborated a classical conditioned reflex. The sound of a damped electric bell was applied as a conditioned stimulus, the drinking of water was the unconditioned stimulus. The experiment was performed simultaneously with the whole group of rats. The rats were kept in individual chambers with wire mesh floors that served as the first electrode. The second electrode was in the vessel containing drinking water. The rats were thirsty for 23 hr before the beginning of the experiment. They were allowed to drink in the experiment only during the ringing of the bell. In the intervals between the stimuli, the drinking vessel was electrically charged. The conditioned reflex was considered to be elaborated when the rat reacted correctly to the signal in 100% of the cases during one experimental session. The rate at which the conditioned reflex was elaborated was evaluated by the number of times the conditioned and unconditioned stimuli had to be applied simultaneously in order to attain the criterion. After the conditioned reflex had been elaborated, the rats were left at rest for 3 months. After this period the stability of the memory trace was estimated. The conditioned reflex was re-elaborated so as to reach the criterion again. During the 7 days preceding the experiment, the rats were adapted to experimental conditions by drinking 1 hr a day in the experimental chamber. They learned to drink their daily ration of water during these periods. The apparatus was a modified version of that described by Hunt *et al.* [7]. The results were statistically evaluated by the Student's t test.

In the third experiment, we measured the total RNA content in individual cells by means of an integration microdensitometer (Barr and Stroud, Glasgow). The rats were decapitated without narcosis, the brain was immediately excised, and the septum, dorsal hippocampus and the frontal pole of the cerebral cortex were separated. The remaining brain tissue was cut up into 80 μ thick slices in a cryostat. Using a preparation microscope, the central amygdaloid nucleus and the medial and lateral hypothalamus were prepared. The histological slices, the whole septum, and 2 mm of the tissue from the dorsal hippocampus and frontal cortex were then crushed [23]. The preparations were fixed for 12 hr in Carnoy's liquid. DNA was extracted by DNAse (1 mg per 1 ml of a 0.003 M solution of $MgSO_4 \times 7\ H_2O$). The preparations were then stained with gallocyanine-chromalum [13]. The measurements were performed on whole undamaged neurons, interneurons and glia at a wave length of 560 nm, condensor nA – 0.3, objective 100 ×, the size of the measuring point was 0.7 μ in this magnification. In each region, we measured 10 cells per rat, or 10 interneurons and glial cells which, in this preparation, cannot be distinguished from interneurons. The results were evaluated by Student's t test. Mean values for each given structure were calculated from ten measurements in each rat.

RESULTS

Succession of reactions to mouse, estrous female, food and water. All killers first killed the mouse. Non-killers immediately started feeding or drinking. Both killers and non-killers reacted to food and water in the second and third sequence. Sixty percent of killers and 70% of non-killers did not react to the estrous female during the given period.

Elaboration and re-elaboration of conditioned reflex. In order to elaborate an acoustic conditioned reflex reinforced by the drinking of water, killers need more connections between the conditioned and unconditioned stimuli than non-killers ($t = 2.4$, $p<0.05$). After a three months' pause, in the first session the killers had 11% correct reactions to the conditioned stimulus and non-killers 15%. The difference is not statistically significant ($t = 0.05$). The re-elaboration of the conditioned reflex was slower in killers than in non-killers ($t = 3.6$, $p<0.003$). The killers needed as many connections between the conditioned and unconditioned stimuli for the re-elaboration of the conditioned reflex as non-killers for the elaboration. The difference in the rate at which killers re-elaborate and non-killers elaborate the conditioned reflex is not statistically significant ($t = 1.2$).

Total content of ribosomal RNA in cells of certain regions of the limbic system and the frontal cerebral cortex. Killers have a higher content of ribosomal RNA than non-killers in the pyramidal cells of the dorsal hippocampus and in neurons of the central amygdaloid nucleus (hippocampus; $t = 2.47$, $p<0.05$; amygdaloid nucleus; $t = 2.37$, $p<0.05$). The total RNA content in septal neurons is lower in killers than in non-killers ($t = 2.48$, $p<0.05$). The neurons of the medial and lateral hypothalamus did not form two different populations according to their total content of RNA and we therefore evaluated them as one region. In killers, the RNA content of neurons from these regions was lower than in non-killers ($t = 2.37$, $p<0.05$). The interneurons and glial cells of the septum and dorsal hippocampus had the same total RNA content in killers and non-killers (septum; $t = 0.4$, hippocampus; $t = 0.9$). The RNA content of neurons from the frontal cortex of killers did not differ from that of non-killers ($t = 1.24$).

TABLE 1

THE RATE OF THE ELABORATION AND OF THE REELABORATION OF A CONDITIONED REFLEX IN KILLERS AND NON-KILLERS. THE NUMBERS ARE AVERAGE VALUES OF THE NUMBER OF CONNECTIONS OF THE CONDITIONED AND UNCONDITIONED STIMULI NECESSARY FOR REACHING THE CRITERION, AND STANDARD ERRORS OF THE MEANS.

	Killers	Non-Killers	p*	t†
Elaboration	220.0 ± 9.1	153.0 ± 15.0	0.05	2.4
Reelaboration	127.0 ± 20.3	50.0 ± 5.5	0.003	3.6

*Level of significance †Student's t test

DISCUSSION

The killing of an animal of a different species is a dominant trait in the behavior pattern of killer rats. If a sexually inexperienced, hungry and thirsty killer is allowed to choose between the killing of a mouse, an estrous female, food and water, its first reaction is the killing of the mouse. Afterwards, it feeds and drinks. Aggressiveness towards animals of their own species is also more pronounced in killer rats than in rats that are not spontaneously aggressive towards animals of another species [4].

TABLE 2

TOTAL RNA CONTENT OF NEURONS OF SOME BRAIN STRUCTURES IN KILLERS AND NON-KILLERS. THE NUMBERS ARE AVERAGE VALUES OF TOTAL RNA CONTENT AND STANDARD ERRORS OF THE MEANS.

Structures	Killers	Non-Killers	p*	t†
frontal cortex	113.6 ± 3.5	108.5 ± 2.2	n.s.	1.24
hippocampus pyramidal cells	143.8 ± 3.8	127.0 ± 5.6	0.05	2.47
amygdala ncl centralis	133.5 ± 6.5	116.7 ± 4.8	0.05	2.37
hypothalamus med. + lat.	101.4 ± 2.4	114.9 ± 4.3	0.05	2.37
septum	106.4 ± 3.2	119.8 ± 4.3	0.05	2.48

*Level of significance †Student's t test

TABLE 3

TOTAL RNA CONTENT OF INTERNEURONS AND GLIA SEPTAL AND HIPPOCAMPAL CELLS IN KILLERS AND NON-KILLERS. THE NUMBERS ARE AVERAGE VALUES OF TOTAL RNA CONTENT AND STANDARD ERRORS OF THE MEANS.

Structures	Killers	Non-Killers	p*	t†
septum	38.9 ± 0.8	38.5 ± 0.6	n.s.	0.4
hippocampus	42.3 ± 1.8	40.3 ± 1.5	n.s.	0.9

*Level of significance †Student's t test

Aggressiveness towards another species is not the only type of behavior characteristic of killers. In experiments, these animals are slower in mastering a food stereotype that requires more complicated differentiation. They forget more quickly a reaction they had already learned. This could be explained by a weaker drive for drinking. However, this explanation does not apply in our case. Golda *et al.* [4] found in experiments allowing free choice between water and saline that killers of the same origin as our experimental rats took more fluids than non-killers. It is more probable that killers are less capable of learning and remembering. This is typical of rats which are classified by Lát [15,16] as the so-called excitable type. Judging by results obtained under conditions of the studying of exploratory behavior the majority of our killers placed themselves on the right-hand side of the Gaussian distribution. They therefore belong to the excitable type [18]. This is also connected with the fact that killers, like all excitable rats, are

less able to habituate exploratory behavior [15,16]. Rats of the excitable type have a drive for NaCl [17]. We observed this property in our killers when they had a free choice between water and NaCl [4]. Thus, all the facts support the assumption that killers are excitable rats.

In exploratory behavior tests, killers have a different type of motoric activity than non-killers. Killers have greater horizontal activity, they orient themselves by means of tracks on the ground. Non-killers have greater vertical activity, they have a tendency to escape from a closed space [18]. This is probably why killers have a higher threshold of the leaping escape reaction to an electric shock than non-killers [4].

Under conditions of free choice between a mouse, an estrous female rat, food and water, neither the killers nor the non-killers reacted to the female. The experiments were performed with animals that had been hungry and thirsty and this fact reinforced the drive for food and water before the sex instinct. In the normal life of sexually inexperienced male rats, killers of the same origin and breeding conditions as our rats, showed pre-copulation activity in 81% cases, whereas non-killers only in 29% [5]. The number of males that passed from precopulation activity to copulation did not differ in killers and non-killers.

We do not know the direct significance of the differences found in the total RNA content in neurons of some structures. But if the quality and quantity of the cellular RNA are indicative of the intensity of the function of cells and structures of the brain [8], then a high activity of neurons of central amygdala and hippocampus and a low activity of neurons of septum and hypothalamus is typical of killers. Rat killers have a lowered capability to adapt to a new environment and to solve more complicated situations in life. It may even lead to maladaptation, as it is apparent from the fact that the attacking, eventually the killing of an animal of a different species which currently occurs in nature during the defense of territory, is in the case of rats connected with the loss of the ability to differentiate an intruder from the young of their own species.

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