## CATECHOLAMINES IN BRAIN STRUCTURES OF RATS DISTINGUISHED BY PERFORMANCE IN THE OPEN FIELD TEST

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The open field test, including responses which characterize motor activity and vegetative functions (defecation), is widely used to study the emotional reactivity of small animals. Many investigations have been devoted to analysis of the role of the brain catecholamines (CA) in behavioral reactions, but no investigations have been undertaken to compare emotional reactivity with the CA level in individual CA-synthesizing nuclei and groups of brain neurons.

To study the role of brain CA in the organization of emotional behavior, CA concentration levels were studied in the principal CA-synthesizing brain nuclei in animals distinguished by their behavior in the open field.

## EXPERIMENTAL METHOD

Male Wistar rats weighing 300-400 g were tested [5] in an open field for 4 successive days, for 2 min each day, between 8 a.m. and 12:30 p.m. The experiments were carried out in a dark, soundproofed room. A 100-W lamp was placed above the center of the arena at a height of 40 cm. The number of times the animals crossed the boundaries between squares (crossing) stood up on their hind limbs (rearing), performed combing, washing and licking movements (grooming), and the number of fecal pellets were counted.

The animals were decapitated 2 days after the end of the experiment between 8:30 and 9:30 a.m. The brain was removed and frozen with dry ice on cryostat stages. The following nuclei and groups of neurons were taken from 300 sections by the punching method [8]: region  $A_1$  (a group of adrenalin-synthesizing neurons located in the dorsal part of the lateral medullary reticular nucleus), region  $A_2$ , locus coeruleus, nucleus subcoeruleus, substantia nigra, area ventralis tegmenti, nucleus arcuatus, nucleus periventricularis.

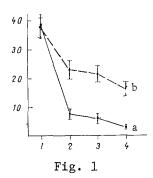
The concentrations of adrenalin, noradrenalin, and dopamine in each nucleus were determined by a radioenzyme method [9, 10].

## EXPERIMENTAL RESULTS

Changes in the test parameters in the course of the experiment (from the 1st through the 4th day) were not equivalent. The sharpest change took place in the number of crossings and the number of rearings. Both indices fell from the 1st to the 4th day of the experiment inclusive. Significant individual differences were found in the motor activity of the experimental animals, on the basis of which they could be divided into two groups, with 10 rats in each group (Figs. 1 and 2). The number of crossings and rearings by the rats of group 1 on the 2nd, 3rd, and 4th days of the experiment was significantly less than for the animals of group 2.

Increased emotional reactivity is connected with low motor activity and increased defecation [1, 2, 6, 7, 11]. However, there is evidence that this negative correlation is confined

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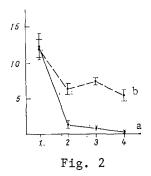


Fig. 1. Number of crossings of square boundaries by rats of groups 1 (a) and 2 (b). Abscissa, days of experiment; ordinate, number of crossings.

Fig. 2. Number of rearings by rats of groups 1 (a) and 2 (b). Abscissa, days of experiment; ordinate, number of rearings.

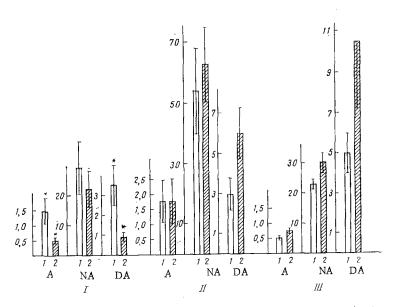


Fig. 3. Concentration of catecholamines (in pmoles/mg) in region  $A_1$  (I), locus coeruleus (II), and arcuate nucleus (III) in groups of animals distinguished by behavior in the open field test. A) adrenalin, NA) noradrenalin, DA) dopamine. Unshaded column — animals of group 1, shaded column — animals of group 2.

to the indices mentioned above [3, 4]. In the present experiment the number of fecal pellets did not correlate with the behavioral manifestations: No difference could be found between the value of this index in the two groups of animals. This result may be attributable to some degree to specific differences in the animals used: In Wistar rats and related lines, the level of defecation is independent of emotionality measured by means of other tests [3].

Grooming movements changed in a fundamentally different way in the animals of the two groups. In the animals of group 1 the number of movements was virtually the same on the 2nd, 3rd, and 4th days of the experiment as in rats of group 2. In the course of the experiment, in the animals of group 2 grooming, expressed numerically, remained virtually unchanged. In the rats of group 1 there was a significant increase in this parameter on the 2nd, 3rd, and 4th days of the experiment compared with the 1st day.

The adrenalin and dopamine levels in region  $A_1$  were significantly lower in the animals of group 2 than in the rats of group 1 (Fig. 3). The noradrenalin concentration remained virtually the same in the animals of both groups.

A marked tendency was found for the dopamine level in the locus coeruleus in the animals of group 2 to be higher than in the rats of group 1 (by 100%). The adrenalin and noradrenalin concentrations were practically the same in the animals of both groups.

In the arcuate nucleus the tendency for the dopamine level to be higher in the rats of group 2 than in those of group 1 was just as strong as in the locus coeruleus. In the other nuclei studied (region  $A_2$ , n. subcoeruleus, substantia nigra, area ventralis tegmenti, n. periventricularis) the CA concentration was practically the same in all animals. In region  $A_2$ , for example, the adrenalin level in the rats of group 1 was 0.277  $\pm$  0.071 pmole/mg, in the rats of group 2 0.284  $\pm$  0.06 pmole/mg; the noradrenalin level was 31.714  $\pm$  4.036 and 29.562  $\pm$  4.895 pmoles/mg and the dopamine level 2.267  $\pm$  0.458 and 1.835  $\pm$  0.029 pmoles/mg respectively.

It can be concluded from data in the literature [1, 2, 6, 7, 11] that rats of group 1, whose motor activity was sharply depressed in the course of the experiment, are emotionally more reactive than rats of group 2, whose motor activity was significantly higher. The results showed significant differences in the CA level in the animals of the two groups distinguished on the basis of the open field test. The rats of group 1 (emotionally reactive) were characterized by a significantly higher adrenalin and dopamine level in region A<sub>1</sub> and a lower dopamine level in locus coeruleus and the arcuate nucleus. The variability of the original CA level in individual nuclei is to a certain degree comparable with reactions in the open field, which means that the control animals could be grouped in accordance with differences in the original CA concentration in their brain nuclei.

The sharpest differences between the groups of animals were found in the adrenalin and dopamine levels in region A<sub>1</sub>; this evidently points to a significant role of neurons in this region in the formation of emotional behavior. It is considered [12] that increased adrenalin synthesis in the brain promotes the formation of restless behavior. In the present experiments rats of group 1 were characterized by a significantly higher adrenalin and dopamine level in region A<sub>1</sub>, i.e., it may be supposed, by more intensive adrenalin synthesis. The rats of group 1 also differed from those of group 2 in the sharper decline in their motor activity. If "restless behavior" in the investigation cited above corresponds to "increased emotionality," it can be concluded that the results of the present investigation are in harmony with the view expressed above.

Since significant differences in the test parameters in animals of the two groups also were found the locus coeruleus and the arcuate nucleus, it can be concluded that the character of responses of Wistar rats in the open field is determined by interaction between central CA in which, besides the adrenalin-synthesizing neurons of region  $A_1$ , noradrenalin-synthesizing neurons of the locus coeruleus and dopamine-synthesizing cells of the arcuate nucleus also participate.

In conclusion the selective participation of the different CA-synthesizing brain nuclei in the formation of emotional reactivity must be emphasized: Of the two adrenalin-synthesizing regions studied, significant differences in catecholamine concentrations were found in the animals of the two groups in region A, but their level in region A<sub>2</sub> was virtually identical; of the two noradrenalin-synthesizing nuclei there was a significant difference between concentrations in the locus coeruleus but virtually no difference in n. subcoeruleus. Nuclei containing cell bodies of four brain dopamine-synthesizing systems were chosen — nigrostriatal, mesolimbic, tuberoinfundibular, and incertohypothalamic. Only in the arcuate nucleus, a component of the tuberoinfundibular system, were significant differences found between groups of animals differing in their responses in the open field. It can be postulated that among the factors determining this result are the close connection of neurons of the arcuate nucleus with the pituitary gland and their direct participation in the regulation of endocrine functions.

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