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## OPEN FIELD BEHAVIOR OF RATS AFTER BILATERAL COAGULATION OF THE LOCUS COERULEUS

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It was shown previously that emotional stress in animals causes increased permeability of the blood-brain barrier, and injury to the intracerebral vessels in the mesencephalic reticular formation, with resulting death of neurons. It has been postulated that it is destruction of the mesencephalic reticular formation that is largely responsible for behavioral changes caused by emotional stress [1, 2, 5]. Since information has been obtained that the locus coeruleus participates in the regulation of functions of the blood-brain barrier [2, 8-9], it might be supposed that the injuries to blood vessels observed in the mesencephalic reticular formation are also the result of dysfunction of the locus coeruleus induced by emotional stress.

The aim of this investigation was to test this hypothesis by assessing behavioral changes developing after bilateral injury to the locus coeruleus, using the open field test. Unlike workers who studied this problem previously [3, 4], we modernized the method used for the open field test to some extent by introducing into it a sudden stress stimulus in order to simulate the "start reflex," known in neurology as a protective response linked with the function of the tegmentum mesencephali.

## EXPERIMENTAL METHOD

Experiments were carried out on 33 male Wistar rats weighing 150-320 g. The animals were tested once in the course of 5 min by the modified open field test. The arena, measuring 60 × 60 cm, was divided into 16 squares, each side of which measured 20 cm. Above the center of the arena hung a 40-W lamp at a height of 80 cm. The test was carried out in a dark, sound-proofed room.

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TABLE 1. Averaged Data of Behavioral Parameters of Rats in Open Field Test in Background Period and after Coagulation of Locus Coeruleus ( $M \pm m$ )

Parameters, group of animals	Testing	Crossing boundaries between squares	Crossing more than one square	Visits to center of arena	Attempts to get away	Vertical postures	Fixed postures	Sniffing at objects	Sniffing at other rats	Defecation	Urination	Body weight, g
1- ( $n=12$ )	Background	50.8 $\pm$ 5.7	13.5 $\pm$ 1.6	2.3 $\pm$ 0.6	4.5 $\pm$ 1.2	13.1 $\pm$ 1.3	26	5	5.6 $\pm$ 1.2	13	5	278.4 $\pm$ 5.5
2- Mock operation, $n=14$	Repeated	55.8 $\pm$ 6.4	13.9 $\pm$ 1.3	4.8 $\pm$ 2.1	2.6 $\pm$ 0.9	10.6 $\pm$ 1.9	6*	14*	3.4 $\pm$ 1.0	16*	5	277.6 $\pm$ 8.6
3- (Control, $n=7$ )	Background	63.2 $\pm$ 4.2	15.1 $\pm$ 1.3	4.7 $\pm$ 1.0	3.1 $\pm$ 0.8	14.5 $\pm$ 1.0	21	13	4.4 $\pm$ 0.9	28	10	286.0 $\pm$ 5.9
	Repeated	44.0 $\pm$ 4.9**	13.1 $\pm$ 1.6	2.1 $\pm$ 0.5*	2.8 $\pm$ 0.8	16.9 $\pm$ 1.7	16*	20*	6.1 $\pm$ 0.9	24	8	303.4 $\pm$ 5.2*
	Background	54.7 $\pm$ 6.3	15.0 $\pm$ 1.9	2.7 $\pm$ 1.1	3.9 $\pm$ 1.8	11.4 $\pm$ 1.4	7	6	2.4 $\pm$ 0.9	9	1	270.7 $\pm$ 8.4
	Repeated	55.0 $\pm$ 5.4*	7.4 $\pm$ 1.7*	0*	2.1 $\pm$ 0.9	7.0 $\pm$ 1.8	6	10	4.7 $\pm$ 1.5	12	4	294.1 $\pm$ 4.4*

Legend. Values given for parameters of fixed posture, sniffing at objects, defecation, and urination are in the form of their arithmetic sum. \* $p < 0.05$ .

The following parameters were studied: the number of times the animals crossed the boundaries of the squares, the number of crossings involving more than one square, the number of visits to the center of the arena, the number of attempts to go out, vertical postures, fixed postures, sniffing at objects, sniffing at other rats, and also the number of defecations and urinations. After testing for 5 min the animals' response was tested to a short-term negative procedure, namely a sharp tap of the hand in the center of the arena and the response to handling.

The next day some animals (group 1) were subjected to coagulation of the locus coeruleus. This procedure was done bilaterally with an anodal current of 2 mA for 7 sec with a monopolar electrode. The coordinates of the coagulation points were determined from the atlas [6]: AP 8.8, L 1.1, H 6.6 mm. The location of the lesion was verified in histological sections. In rats undergoing the mock operation (group 2) a channel was made at H = 5.6 mm. Animals of the third group constituted the animal-house control.

Degeneration of the terminal branches of the locus coeruleus could be assumed to have taken place 6 days after its destruction, and the animals of all three groups were then retested.

The results were subjected to statistical analysis on the Mikro-2000 computer by Student's test and also by a nonparametric chi-square method.

#### EXPERIMENTAL RESULTS

During testing in the open field, similar levels of behavioral parameters were obtained in the background investigations of the animals (Table 1). Sniffing at objects, fixed postures, defecations, and urinations were observed only in single cases and not in all the animals.

In response to a short-term negative procedure, namely a sharp tap in the center of the arena, the animals in the background observations responded in the same way: by a sudden jump (82.9% of individuals), accompanied by a vertical defensive posture and an attempt to jump out of the arena. The response took the form of demonstration of a fixed posture, accompanied by defecation or urination, in only 17.1% of animals. After the negative procedure the rats avoided the experimenter's hand, kept at a certain distance from it, or assumed a defensive posture when the experimenter attempted to move his hand nearer. In response to handling they attempted to get away, and these attempts were accompanied by strong contraction of the body muscles and by loud vocalization.

On the 6th day after coagulation of the locus coeruleus the body weight of the animals of group 1 remained unchanged compared with the background measurement, whereas in rats of groups 2 and 3 there was a significant increase of body weight by 6 and 8.6% respectively. The mean number of crossings from one square to another by the animals of group 1 remained at its previous level with only a small tendency to increase, whereas in rats of groups 2 and 3 it was considerably reduced. The same pattern also was observed for visiting the center of the arena: this remained at its previous level in the rats of group 1, the number of visits was reduced by 2.2 times in group 2, and in the animals of group 3 there were no visits to the center of the arena during retesting.

Statistical analysis of the results by the nonparametric chi-square method showed that parameters such as sniffing at objects, fixation, and defecation changed significantly in

the animals of group 1 compared with the background. Whereas in the background test sniffing behavior was observed in one of 12 animals, after coagulation of the locus coeruleus it was observed in one of seven. Conversely, fixation behavior, observed in 11 animals, was found in only three. The fixation became short in duration and episodic in character, whereas in the background period it lasted a considerable time. In group 2, statistical analysis of the data by the chi-square test showed that only the parameters of sniffing at objects and fixation showed significant changes, and in group 3 no significant changes whatsoever were found (Table 1).

Parameters such as the number of crossings involving more than one square, the number of attempts to get away, vertical postures, and sniffing other rats remained unchanged in the animals of all three groups.

In animals with coagulation of the locus coeruleus the response to the negative procedure (a sharp tap with the hand) ceased to be adequate in character. In response to this procedure there was absolutely no negative emotional manifestation such as was observed previously: in response to the sharp tap the rats turned their head toward the hand and, without any visible signs of fear, examined it, sniffed at it, tested it with their teeth, and attempted to get up on the hand. The response of keeping a distance also was not present: touching the animal's body was not accompanied by an avoidance reaction or vocalization. In response to handling of the animals they made no attempt to get away from the hand, and unlike in the background observations, the animal's body was relaxed.

The most characteristic feature of the rats' behavior in the area after coagulation of the locus coeruleus was a rapid, externally unoriented, stereotyped motor activity. The animals moved with their limbs straight, with their abdomen off the ground, the tail unnaturally raised, and it went into a semicircle at the end. In some cases the limbs were so placed that walking became "duck-like" in character.

In the animals of groups 2 and 3 responses to the short-term tapping and handling, and also the features of locomotion remained unchanged compared with the background observations.

Thus animals surviving 6 days after coagulation of the locus coeruleus showed significant behavioral differences compared with animals undergoing mock operations and the animal-house control. Coagulation of the locus coeruleus led to a sudden change in the response to a sudden sharp tap: the animals made no attempt to jump out or run away and did not adopt a fixed posture. The number of ambulations across the boundaries between the squares and the number of visits to the center of the arena were not reduced, as in the control groups, but remained at the background level. The characteristics of locomotion were changed: the rats moved with their limbs straight and their tails curled into a hook. To this must be added the absence of any significant increase in body weight. This last feature was observed previously after damage to the locus coeruleus [8]. So far as the remaining changes are concerned, it is extremely difficult to interpret them. However, it can be tentatively suggested that reversal of the response to a sudden tap or to handling, and also the time course of several of the behavioral parameters used in the open field test are based on a disturbance of the adaptive nature of the animals' behavior as a result of coagulation of the locus coeruleus.

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