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Effect of the Septal Lesion on the Non-Learned Responses of the Rat

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Effect of septal lesions has been studied in rats to find if they alter aspects of its SSDR. Results may be summarized as follows: 1. Animals with the septal syndrome tried to attack only when about to be caught, while they showed exaggerate freezing and submissive responses once caught. 2. A shock associated situation caused an increase in the freezing behavior and a decrease in the number of boluses excreted. 3. The septal animals never left walls of the open field, and did not explored its internal areas, showing an increase in thigmotaxis. 4. They showed an increase in the emergence time. The results are interpreted as due to an enhancement of the species-specific defence reactions.

In previous experiments it was shown (25) that the lesion of the postcommissural part of the septum resulted in an increase in the exploratory behavior in the open field which decreased rapidly; a decrease in the number of defecations in this test and a decrease in the time leaving a dark environment for exploration. In the shuttle box a permanent increase in the intertrial activity was found. The results were interpreted as showing two aspects: on the one hand the postcommissural septal lesion decreased the emotivity of the animals, on the other hand the fact that animals showed an adaptation in the open field exploratory behavior and failed to reduce their activity in the shuttle box was interpreted as due to some alteration of the species-specific defence reactions as defined by BOLLES (2).

It was not the first time that changes in behavior observed following septal lesions were interpreted as due to alterations of species-specific defensive reactions.

CAPLAN (5) said the species-specific defence reactions are enhanced following septal damage, when quoting an experiment by DUNCAN (10) in which the effects of temporary septal disfunction in freezing behavior were studied.

There are some contradictory reports on the effect of septal lesions on the emotivity of animals. Certain authors found an increase in the emotional reactivity (3, 7, 8, 24), others reported a decrease in the autonomic reactivity which may be interpreted as a decrease in emotivity (6, 10, 16, 17), others attributed the changes in behavior following septal damage as due to a decrease in fear (18, 21, 27), others said that no changes in emotional behavior occurred (4, 22).

The object of this study was *a*) to test the hypothesis of a possible alteration of the species-specific defence reactions following precommissural lesions and *b*) since the reports on emotionality are contradictory, to study the emotional changes after lesions.

Since what brought us to the hypothesis that the postcommissural septal damage might alter the species-specific defensive reactions was the different evolution of the spontaneous activity in the open field and in the shuttle box, we attempted to submit the animals to a series of tests in which the emotional and exploratory behavior was put into play in different degrees and in which some of the spontaneous responses of rats to potentially dangerous situations could be observed. These tests were: a test of a conditioned emotional response (defecations) to an electric shock (C.E.R.); an open field test; an emergence test; a test of exploratory behavior in an enclosed maze; a test in an activity box and the study of inter-trial activity during the acquisition of a two-way active avoidance conditioning in the shuttle box.

Materials and Methods

Animals. The experiments started with a group of 17 naïve Wistar strain male rats, weighing 320 g. Animals were individually housed with food and water *ad libitum.* The lighting regime was 12 h light-12 h dark. Animals were divided into two groups according to the number of boluses in the C.E.R.; average values were 4.0 and 4.3 for the control and experimental groups respectively. One of the groups was submitted to the septal lesion (experimental group) and the other was given a sham operation (control group). One of the animals of the experimental group and one of the control group died during the operation. The groups were reduced to 8 experimental and 7 control animals.

Apparatus and Tests. All tests were carried out individually. Apparatus were located in a sound proofed room provided with a noise of 50 db. All tests were performed before and after the operations.

C.E.R. This test was carried out in a transparent plastic box measuring 20 × 20 × 40 cm provided with an electric grid connected to a programmable shock generator. On the first preoperative day of test, animals were put into the box and after 2 min habituation three 75 V. A.C. current shock of 0.7 s of duration were delivered. On the following two days animals were tested for 5 min in the box, but no more shocks were delivered and defecations and freezing times were recorded. Defecations were considered as an emotional response (C.E.R.). Freezing time was defined as the total immobility of the animals, including the absence of vibrissae movement. Freezing was only taken into account if it was longer than 30 sec. Only after 30 sec of cessation of vibrissae movement did freezing time begin to count.

This test was repeated twice before surgery and twice after it. The first postoperative test was on the 6th day after surgery.

Open field. A circular open field one metre in diametre, fully described previously (25) was used. The field was divided into 12 external (close to the wall) and 6 internal areas of equivalent dimensions. The behavior of the rat was observed for 3 min and the total exploration (the total number of areas entered), the exploration of the internal areas (total number of internal areas entered), and defecations were recorded. This test was repeated 4 times before surgery and 4 times after it. The first post-operative day of test was the 8th.

Emergence test. It was carried out in a conventional run-way 1 meter long with a completely darkened start box and a mildly lighted passage-way and end box. The duration of the test was 2 min. *a*) The time that animals took to abandon the start box to explore the passage-way of the apparatus (emergence time), *b*) the distance of the run-way explored and *c*) the number of arrivals at the end box and the 10 cm closest to it were recorded. This test was performed 4 times before surgery and 4 times after it. The first postoperative test was on the 15th day.

Exploration in the maze. A closed maze similar to that described by BORST and DELACOUR (1) but without blind alleys was used. The number of doors crossed by rats in a 5 min test period was recorded. The number of times the animals recrossed the same door it had just explored was also recorded. This test was carried out once before surgery and twice after it. The first postoperative day of test was the 20th after lesions were made.

Activity test. It was carried out in a box measuring 12 × 34 × 14 cm provided with an electric cell situated in the middle of the longest wall, 5.5 cm from the floor. The cell was connected to an electromechanical counter which recorded the number of times the animals crossed the light beam in a 5 min test period. This test was carried out once before and twice after surgery. The first postoperative day of test was the 23th.

Two-way active avoidance conditioning. The apparatus was a conventional shuttle box as described in a previous report (25). The CS was a light provided by two 20 W bulbs located on the wall of the box. The UCS consisted of a 45 V AC current applied to the grid. The maximum duration of the CS was 5 s and the maximum duration of the shock was 3 s. The inter-trial interval was 30 s and 10 daily trials were given. Avoidance responses (the number of times animals crossed the barrier during the 5 s CS) the inter-trial activity (number of times animals crossed the barrier during the inter-trial interval), latencies of the avoidance responses and defecations were recorded.

The whole test was carried out after surgery and the first training day was the 28th after it.

Surgical procedure. It was performed under Nembutal anaesthesia at a dose of 45 mg/kg, using a 1 % Nembutal solution. 0.2 ml of atropine sulphate at 1 per thousand was added to 10 ml of this solution.

Bilateral lesions were produced using a Grass radiofrequency lesion maker (LM4). The emplacement of the electrodes was performed on a stereotaxic apparatus. The coordinates for the lesions were 2 mm anterior to bregma, 0.5 mm lateral to the middle line and 5.0 mm ventral to the surface of the dura. Control animals were submitted to an operation identical to that of the experimental group, including the penetration of the electrode, and excluding only the coagulation itself.

Histology. Once the behavioral tests were anaesthetized with an overdose of nembutal and perfused with a 10 % formalin solution, abscissions were made by freezing, and stained by the Nissl method with thionin, for further study. Figure 1 shows an example of the lesions obtained.

Statistical method. Only non-parametric statistical tests were used. The results were treated with the Mann and Whitney U test and the Wilcoxon test (14). Significance threshold was only considered in the two-tailed form.

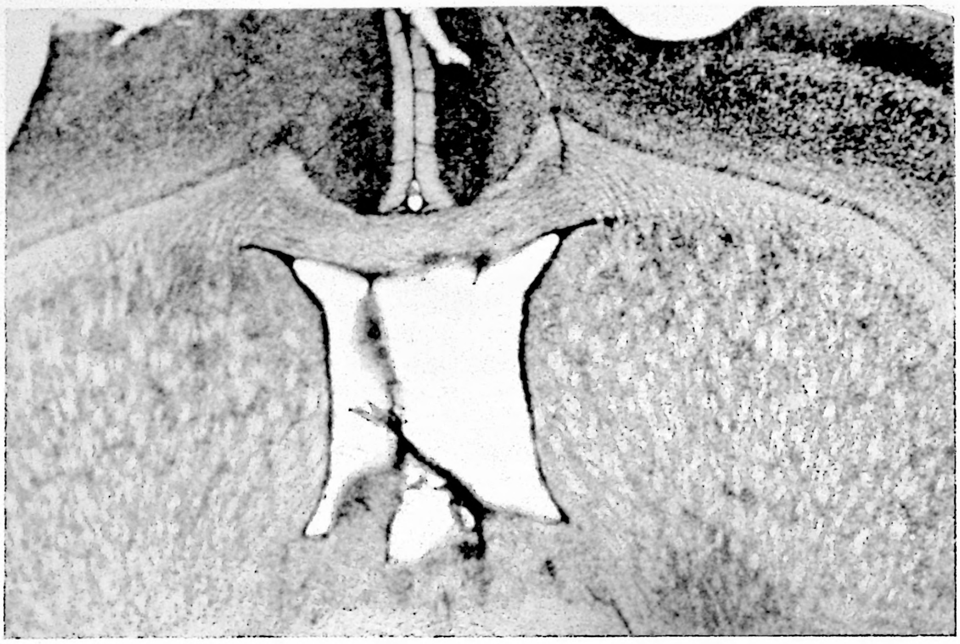

Fig. 1. *An example of the septal lesions.*
Surrounding structures, as the anterior commissure are not damaged.

Results

Histological results. In all animals medial and lateral septal nuclei were extensively destroyed and the nuclei of the diagonal band of Broca was damaged in its upper part. The more posterior part of posterior hippocampus was destroyed in three animals (two of them presented hyperreactivity, but not the third). Lesions extended caudally to the triangular nucleus which was slightly damaged also in three rats. Nuclei interstitialis stria terminalis was lightly injured, but the damage inflicted never affected more than 30 % of that structure. In some cases a lesion was made, although of minor importance in the corpus callosum and in the anterior commissure. In no case did the lesion extended caudally to the columns of the fornix or the fimbria.

Results on hyperreactivity. Three out of eight lesioned animals showed septal hyperreactivity. The reactions observed were those described by many authors: difficulty in handling, increased startle reactions to sound and tactile stimuli, attack with biting when captured and the typical motion like a rabbit, when animals went out of their cages during the hyperreactivity tests. Nevertheless, the same animals that bite fiercely when captured became calm once in the hand and stopped the attemps to bite.

As a response to repeated blowing close to the nose, and to sonorous stimulation (taps with a stick on a wooden blackboard 10 cm from the animal) a submissive, nearly supine posture was observed. This posture was accompanied by a very loud scream. Then, a mild touch with the stick caused a fierce attack with biting. This response seemed similar to that observed in a rat that has been submitted in a fight.

Of the remaining septal rats, two were more docile to manage than controls and three were similar to them.

Results on the C.E.R. After septal lesions, experimental animals showed an important decrease of defecations when compared with control animals and when compared with their own preoperative defecation rate in the box where they had been shocked. The decrease of defecations in aversive situations is usually interpreted as due to a decrease in the emotional reactivity of the rats. Since control and experimental animals were very homogeneous in the preoperatory test, the decrease of defecations suggested a decrease in the emotivity level.

Table I presents the results. Differences are significant at the level of $p < 0.005$ on the first day ($U = 3.8$, $N = 8$, $N' = 7$) and $p < 0.01$ for the second day ($U = 5$).

The animals of the septal group showed an increase in the freezing time when compared with controls. The freezing time registered was 16.2 min for the experimental animals and 3 min for the control ones. Differences were significant at the level of $p < 0.005$ ($U = 2.22$).

Results in the open field. After lesions, septal animals showed an important decrease in their total exploration of the open field when comparing their preoperative and postoperative performance, and when comparing the postoperative performance between septal and control groups. The average of areas entered of the two last days of preoperative tests were 30.8 and 39.1 for the experimental and control groups respectively. After lesion, the average of areas explored by the experimental group was 8.0 and for the control one 21.3. For the whole duration of the postoperative test the diference between pre and postoperative performance of the experimental group is significant at the threshold of $p < 0.01$ (Wilcoxon test $T = 0$). The U test reveals a significant difference at the level of $p < 0.01$ between control and experimental animals for the three last days of postoperative test. Table II shows the evolution of the postoperative exploration of both groups during the postoperative tests.

The exploration of the internal areas of the field was 0 in each of the animals of the experimental group during the four days of the postoperative test. It was not the case for the animals of the control group. The mean of internal areas entered was 0 for the experimental group and 6.7 for the control one. The difference is

Table I. *Mean of defecations in the C.E.R.* Mean of defecations presented by experimental and control animals before surgery and after it.

	Pre-op.	Post-operative 1st day	Post-operative 2nd day
Exper. (N = 8)	4.3	0.75****	1.2***
Control (N = 7)	4.0	4.0	4.8

**** $p < 0.005$ (both tail).
*** $p < 0.01$ (both tail).

Table II. *Exploration of the open field.* *a*) Mean of total areas explored by experimental and control animals during the postoperatory test. *** $P < 0.01$ (both tail). *b*) Total number of internal areas explored by experimental and control animals during the postoperatory test. *** $P < 0.01$, **** $P < 0.001$.

Days	1	2	3	4
a) Total exploration of the open field				
Exper. (N=8)	11.2	8.3***	5.6***	7.1***
Control (N=7)	21.4	21.6	20.3	22.0
b) Exploration of the internal area of the open field				
Exper. (N=8)	0	0***	0****	0****
Control (N=7)	4	13	16	14

significant at the level of $p<0.005$ (U=0).

The mean of defecations registered during the four days of postoperative test was 8.1 for the experimental group and 9.4 for the control one. The difference is not significant.

Results in the emergence test. The emergence time was calculated taking into account only the rats that effectively left the start box in order to explore in the two min provided for doing so. Three of the experimental animals failed to leave the start box in the different test sessions. All the control rats left to explore in the time provided. The mean time of departure was 39.5 sec for the experimental animals and 12.6 for the control rats. The difference is significant at the level of $p < 0.05$ (U = 9).

As far as the distance run by the experimental and control group is concerned, lesioned animals ran less than control did (70.1 cm for the experimental and 110 for the control rats), but the difference is not significant (U = 15).

Experimental animals explored the parts of the runway far from the start box less than control ones and it was observed that control animals increasingly explored the end box of the apparatus as the test was repeated whereas the exploration remained constant for the experimental animals (table III). The difference for the three last days is significant at the level of $p < 0.05$ and $p < 0.02$ (both tail). The difference in defecations in this test was not significant.

Results in the exploration of the maze. Animals with septal lesions passed through the doors significantly less than controls. The mean of exploration was 34.7 for the former and 63.6 for the latter. The difference is significant at the level of $p<0.05$ (U = 8). Nevertheless the direct observation of the animals showed that experimental animals when engaged into an alley tended to explore it repeatedly without crossing the doors. This lead us to assume that this result might reflect not a lack of exploration but an alteration of the pattern of exploration.

The number of times animals crossed the door two consecutive times was recorded. Experimental animals repeated the exploration in 27 % of the crossings, control ones did so in 22 % of them (table IV).

Results in the activity box. This test was the only one in which the death of the two animals quoted above led to lack of homogeneity between groups. In the preoperative test experimental animals crossed the beam of the photoelectric cell 31.2 times, and control ones 57.0 After the operation experimental animals crossed 40.6 times and control ones 40.0. The Wilcoxon test applied between preoperative and postoperative tests of each group showed a significant increase for the experimental animals (T = 1 $p < 0.02$ N = 7). The same test did not reveal an increase in their postoperative activity, for the control group (T = 3).

Table III. *Exploration of the end box of the run-way.*
Mean of times animals of experimental and control group entered the end box of the run-way and the 10 cm closest to it.

Days	1	2	3	4
Exper. (N = 8)	10	10*	11**	11*
Control (N = 7)	9	18	21	27

** $p < 0.02$ (both tail).
* $p < 0.05$ (both tail).

Table IV. *Results on the maze.*
Mean of exploration of the maze by experimental and control animals and the % of times animals explored the same door in two or more consecutive sequences. Postoperatory tests.

	Exploration	Reiteration %
Exper. (N = 8)	37.7*	27
Control (N = 7)	63.6	22

* $p < 0.05$ (both tail).

Results in the shuttle-box. This test started 28 days after the completion of the operation. No differences between experimental and control groups were found in the acquisition of the avoidance response. Both groups reached more than 60 per cent of avoidance responses on the 3rd day of test.

The number of defecation was smaller in the experimental than in the control group (3.4 and 4.8 respectively) but the differences were not significant.

Inter-trial activity was greater in the experimental than in the control group (8.7 inter-trial crossing for the experimental animals and 5.1 for the controls) but the difference is not significant.

Discussion

The effects of the lesion of the pre-commissural part of the fornix may be summarized as follows: a septal syndrome in 3 out of 8 animals; a significant decrease of defecations produced in a shock-associated situation and an increase of freezing time in this test; a decrease in the exploratory activity in the open field as well as in the emergence test and in the exploration of an enclosed maze; an increase (when compared with the pre-operative activity level) in a small activity box; and a slight increase of the inter-trial activity in the shuttle box. We must point to a total inhibition of the exploration of the internal areas of the open field and an increase in the time that subjects delayed in exploring the passage-way of a run-way and the tendency of septal animals to stay near the start box of the apparatus in this test.

The set of observations on hyperre-activity suggested to us that there might be two components in the septal syndrome. One component would be an exaggerated defence reaction: when septal animals accidentally became free, they did not attack until they were close to being caught, and when air was blown close to their nose, submissive postures were observed. The other component would be an exaggerated freezing response once in the hand, which made animals stop their attemps to bite. BRADY and NAUTA (3) observed that the reaction of septal rats to the approach of an object differed in two ways from that observed in normal rats: when approached slowly the normal exploration was replaced by freezing, and rapidly approaching objects were vigorously attacked.

In our opinion the hyperactivity reported before, the increase of freezing in the shock-associated situation, the decrease in the exploration in the open field as well as in other tests in which some sort of exploratory behavior was involved, as in the emergence and maze tests, was not due to any increase in the emotivity level (fear reactions) of septal animals because they are not concomitant with an increase in the defecations in a C.E.R. situation and no increase of defecation is found in any of the tests used.

It is also difficult to attribute the decrease of the exploratory behavior to a decrease in the arousal in the septal animals. A decrease in the level of arousal would be accompanied by a deficit in the acquisition of any type of conditioned responses. In fact several authors (12, 13, 15, 19, 20) found a facilitation in the acquisition of two-way avoidance responses and the deficit observed in the acquisition of passive avoidance responses is never attributed to any decrease in the arousal.

Results presented here may not be attributed to an alteration of the motoricity. Direct observations of the animals never led us to suspect such an alteration. Other authors who made specific tests for motoricity such as EICHELMAN (11) reported that even though motor latencies in a paw test were longer for septal than for control animals, fighting behavior, which requires a good coordination, was increased after septal lesions.

The fact that in a previous report (25) an increase in the open field activity on the first postoperative day was found may be explained, in part, by the different location of the lesions. In that experiment only the postcommissural septum was lesioned, and in the present report the main lesions were carried out in the precommissural septum. THOMAS and THOMAS (26) reported that the activity of animals with septal lesions was very variable and suggested that it may be related to the amount of damage suffered in the area of the colums of the fornix. This may suggest that there could be two or more neural mechanisms which regulated the spontaneous activity, one of them linked to the postcommissural septum and colums of the fornix, a lesion of which would produce an increase in the activity, and another linked to the precommissural part of the septum, since a general decrease in the activity is found by several authors (6, 9, 23) after precommissural septal lesions.

From the behavioral point of view, the spontaneous activity tests employed in the present study put into play some of the innate behavioral responses of the rat in a new situation. The main responses considered here were: reaction to handling, freezing responses in a highly dangerous and unavoidable situation, thigmotaxis and the tendency to stay in closed dark and known areas. The effect of septal lesions, which increased freezing time, decreased the exploration of the open field and made animals never move away from the walls of the apparatus during four days in all animals, delaying the time of departure in the emergence test and decreasing the exploration of the areas of the run-way far from the start box, etc., suggested to us that the effect of septal lesion may be related, as suggested by CAPLAN (5) to an enhancement of the specific behavioral responses linked to the preservation of the rat as an individual.

Resumen

Se estudia el efecto de la lesión del septum precomisural para probar la hipótesis que mantiene que estas lesiones pueden alterar algunos aspectos de las reacciones defensivas específicas. Se observa que, cuando se produce el síndrome septal, se pueden distinguir dos componentes: *a*) una fuerte reacción de ataque sólo cuando el animal está a punto de ser cogido, y *b*) una vez capturado desarrolla fuertes respuestas de sumisión y de inmovilización. Un incremento de las reacciones de inmovilización en una situación asociada al choque eléctrico, con un descenso en el número de defecaciones excretadas. Los animales septales no separan nunca su cuerpo de las paredes del campo abierto y no exploran las áreas internas de este aparato, mostrando un gran incremento en su tigmotaxis. Muestran un incremento en el test de emergencia. El conjunto de resultados confirma la hipótesis.

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