

# A comparative Study of the Behavioral Effects of the Locus Coeruleus and the Dorsal Noradrenergic Bundle Lesions in the Rat

MARC VERLEYE AND FRANÇOIS BERNET\*

*Laboratoire de Physiologie Neuromusculaire, \*Laboratoire de Neurobiologie Fonctionnelle, L.A. No. 308 C.N.R.S. Université des Sciences et Techniques de Lille, F 59655 Villeneuve d'Ascq Cedex, France*

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VERLEYE, M. AND F. BERNET. *A comparative study of the behavioral effects of the locus coeruleus and the dorsal noradrenergic bundle lesions in the rat.* PHARMACOL BIOCHEM BEHAV 21(3)357-363, 1984.—The effects of bilateral lesions of the dorsal noradrenergic bundle (DB) or of the locus coeruleus (LC) on the rat's behavior in different anxiogenic behavioral situations have been studied. The DB rats defecate less but ambulate more than shams in the open-field (O.F.); these data suggest a decrease in the reactivity of these animals to novelty. Furthermore, the LC rats have a behavior identical to that of shams in the O.F. We note moreover that the DB rats do not habituate to a novel stimulus. During the Henderson test, the behavioral inhibition of all lesioned animals seems to be less important than that of shams. These results are discussed in relation to existing hypotheses of the DB function. The lesions of DB induce a decrease in the noradrenaline (NA) cortical level and in the catecholamines level in hypothalamus. The lesions of LC produce 30% loss of forebrain NA. These results reveal a discrepancy between the effects induced by the lesioning of DB fibers and those produced by lesions of LC, which originate in the DB. The presence of non-noradrenergic elements, fibers of passage, which do not travel with the DB fibers or terminals in the LC region may be elements for interpretation.

Locus coeruleus      Dorsal noradrenergic bundle      Open-field      Conditioned emotional response  
Brain catecholamines and serotonin      Electrolytic lesions

THE ascending noradrenergic fibers which have as their origin the locus coeruleus (LC) travel along the dorsal noradrenergic bundle (DB), innervate wide areas of the brain such as the neocortex, hippocampus, septal area and amygdala. Behavioral functions of these projections have created several controverted theories. The two major hypotheses, about the behavioral functions of DB are those developed by Gray [18,19] and those held by Mason and Iversen [35]. According to Gray [18,19], DB mediates the behavioral effects of stimuli associated with the omission of reward, with punishment and novel stimuli. According to Mason and Iversen [35], DB plays a role in selective attention. Several pieces of research, like that of Anlezark *et al.* [2] and that of Owen *et al.* [40] have tried to determine which of these two hypotheses is able to predict the effects of DB lesions in the mediation of different behavioral patterns which lead to, for example, novelty. Now, Crawley *et al.* [12] have emphasized the difficulty of interpreting the effects of lesion and stimulation paradigms of LC. This is because this major noradrenergic nucleus is localized in the pontic region [31] characterized by an anatomical complexity which complicates the interpretation of the physiological and behavioral modifications observed. Moreover, some research reveals a discrepancy

between the effects induced by the lesioning of ascending noradrenergic DB fibers and the effects produced by the lesions of LC, which originate in the DB. The experiments of Clark *et al.* [11] and of Sessions *et al.* [45] in the copulatory behavior in male rats illustrate this. Clark [10] states that his results cannot be compared to those of Session *et al.* [45], because he considers it would have been difficult for these authors to avoid disrupting the passage fibers when placing LC lesions.

The purpose of this present investigation is to test the two hypotheses concerning the role of DB in the mediation of a rat's behavior in two anxiogenic situations: open-field test, which makes it possible to evaluate the reactivity and the habituation of the rat to novelty and a conditioned emotional response (Henderson Test); in this test, the animal's behavior has been examined following the administration of an intense and unavoidable electric shock. Likewise, we have checked to see if the lesions of LC, induced neurovegetative and behavioral modifications identical to those produced by the destruction of ascending DB fibers, originating in the LC. The brain concentrations of noradrenaline (NA), dopamine (DA) and of serotonin (5-HT) have been measured after DB and LC lesioning.

## METHOD

*Animals*

Sixty male Sprague Dawley rats, weighing 250–330 g are used. Animals are housed in propylene cage (43×43×20 cm) (5 per cage), given food and water ad lib and maintained on 12 hr light and 12 hr dark cycle (light 7.00 hr–19.00 hr).

*Operations*

The rats are anaesthetized with equithesine (0.4 ml/100 g, IP) and mounted in a stereotaxic apparatus (Precision Cinematographique). The bilateral electrolytic lesions of DB and LC are made by passing a direct current (intensity: 1.5 mA for 10 sec), through a bipolar electrode (0.25 mm tip diameter). The skull is level between bregma and lambda and the lambda serves for reference. The dorsoventral coordinates are measured from the top of the level skull. The stereotaxic coordinates of the LC have been determined both from trials with histological controls, like those utilized by Crow *et al.* [13] and according to the coordinates of the common stereotaxic atlas [15,26]. The stereotaxic coordinates for lesioning DB are derived from Ritter and Stein [43], according to the coordinates of the König and Klippel atlas [26].

	Antero-posterior	Medio-lateral	Dorso-ventral
DB rats (n=15)	+ 0.5 mm	1.4 mm	6.5 mm
LC rats (n=15)	-2.6 mm	1.1 mm	7.6 mm

The control rats (15 LC rats and 15 DB rats) are subjected to a sham operation in which the electrode is lowered to the same coordinates but no current is applied. After the operation, the animals return to their home cage until the first trial in the open-field.

*Behavioral Methods*

*Open-field behavior.* Ten days after lesions, the rat's behavior is observed in a circular arena or open-field the detailed description of which has been already fixed [5], with nevertheless a modification concerning the subdivision of the arena's floor into 19 equivalent sectors (12 "peripheral" and 7 "central"). Each animal is placed in the center of the arena and its behavioral activity is recorded continually over a 3 minutes period. Three analogous tests are carried out over three consecutive days, between 8.00 hr and 12.00 hr with a view to limiting the effect of possible nycthemeral variations.

Four parameters are in this way recorded. (a) Defecation, measured by the number of fecal boli excreted. (b) The central and the peripheral horizontal activity, in arbitrary units (each unit corresponds to the crossing from the sector where the animal is placed, to the next sector). (c) Number of times the animal stood up on its hind legs (rearing). (d) The time (in seconds) spent in washing itself (grooming).

*Henderson test.* This test described by Henderson [21] and modified by Jaffard [24] is carried out in a rectangular cage of Plexiglas (75×25×32 cm), the floor of which is electrified. The cage is crossed by two photoelectric beams

which make it possible to measure the locomotor activity of the animal.

This test consists of four experimental sessions:

(1) Each animal is placed gently in the experimental area, where it is free to walk about for a 30 sec period. The animals used are called "Non stimulated" (NS).

(2) Twenty four hours later, the animal is again placed in the area for a 3 mn period. Three parameters are recorded during this period. (a) The latency of movement expressed in seconds, or the time to go over the distance from the point where the animal was placed to the furthest infra-red beam. (b) The number of fecal boli excreted during the 3 mn period. (c) The ambulation, recorded by the number of times the photocell beams are broken.

(3) Twenty four hours after this second passage, the animal is again placed in the area for a 30 seconds period, but this time, an electric shock of 1.5 mA lasting 1 sec is delivered to the cage floor after the 30 seconds. These animals, henceforth are called "stimulated" (S).

(4) Then, twenty four hours later, the animals are again tested for a 3 min period and the three described parameters (cf. 2) are recorded.

*Biochemical and Histological Methods*

At the completion of the experiment, the rats are sacrificed by decapitation. Their brains are quickly removed and dissected:

(a) in the DB rats, into four parts according to the method of Glowinski and Iversen [17]: the cerebral cortex, hypothalamus, mesencephalon and medulla oblongata; (b) in the LC rats, into two parts by precollicular section (caudally to hypothalamus): the forebrain (cerebral cortex + hippocampus + hypothalamus) and the brainstem with cerebellum. The four brain parts of DB rats and the forebrain of LC rats are frozen in isopentane (-160°C). Following this rapid freezing (15 sec), the parts are then stored in a refrigerator (-20°C) until the extraction and fluorimetric determination of brain amines [23].

A histological control is carried out on the forebrain of DB rats and on the brainstem part of LC rats to ascertain the exact location and the extent of lesions. After fixation in 10% Formalin and freezing of brain, the 50 µm sections are stained with Cresyl violet.

*Statistics*

Statistical analysis are performed using the Student's *t*-test for independant and paired groups and the *r* of Bravais-Pearson.

## RESULTS

*Open-Field Behavior*

The results are shown in Fig. 1.

The DB rats defecate less than the Shams ( $p < 0.05$ ) but ambulate more both in the central sectors ( $p < 0.001$ ) and in the peripheral sectors of the O.F. ( $p < 0.05$ ). Ambulation and defecation are unchanged in rats with LC lesions. The number of rearings and the time of washing in lesioned rats is identical to that of sham rats.

The evolution of the total ambulatory activity for the three trials in the O.F. is shown in Fig. 2.

The repetitive presentation of the novel stimuli induces in the shams and LC rats a significant decrease in the ambulation for each trial. On the other hand, the decreased ambula-

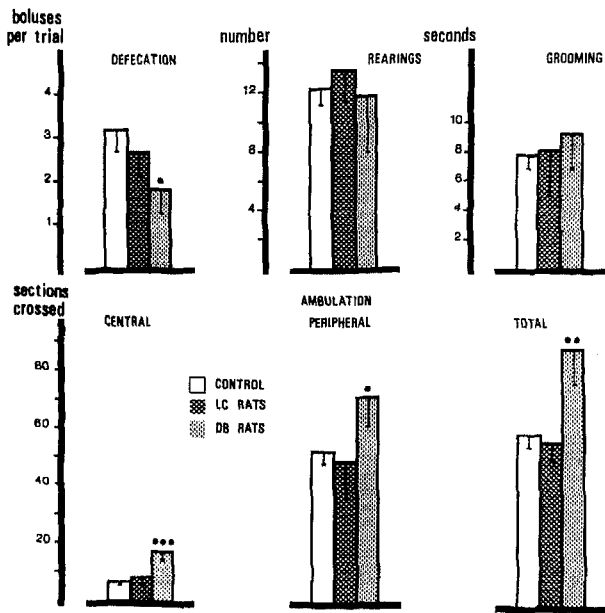


FIG. 1. Mean values of the principal parameters in the O.F. test in sham rats (n=23) and lesioned rats (DB, n=10; LC, n=7). Each value indicates the mean of three passages in the O.F.; each value is indicated with S.E.M. in one direction. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  between sham and lesioned rats.

tion recorded for each trial is not statistically significant in the DB rats.

**Henderson Test**

The results of behavioral scores measured during the second and the fourth passage are shown in Fig. 3.

**NS animals.** The number of fecal boli excreted by lesioned animals is identical to that of shams. Ambulation in DB rats is greater than that of shams ( $p < 0.001$ ). Movement latency is unchanged in lesioned rats.

**S animals.** The lesioned S rats defecate less than S shams, however, the difference between sham rats and DB rats is statistically significant ( $p < 0.05$ ). The lesioned S rats ambulate more than S shams but only the LC rats ambulate more than the S shams to any significant level ( $p < 0.05$ ). Movement latency in lesioned S rats differs by only a little to that of S shams.

**Comparison of NS animals and S animals.** The S shams compared with NS shams have an increased defecation ( $p < 0.01$ ), a longer movement latency ( $p < 0.001$ ) and a decreased ambulation ( $p < 0.001$ ).

The lesioned S rats ambulate significantly less than the lesioned NS rats ( $p < 0.001$ ). On the other hand, defecation of lesioned S animals does not differ to that of lesioned NS animals. The lesioned S animals wait longer before ambulating than the NS animals but only the difference in DB rats between NS animals and S animals is significant ( $p < 0.01$ ).

**Biochemical Results**

Following lesions of DB, catecholamine concentrations decreased in some parts of the brain (Table 1).

In this way, bilateral lesions of DB decreased both the cortical and hypothalamic noradrenaline level ( $p < 0.01$ ;  $p < 0.05$ ).

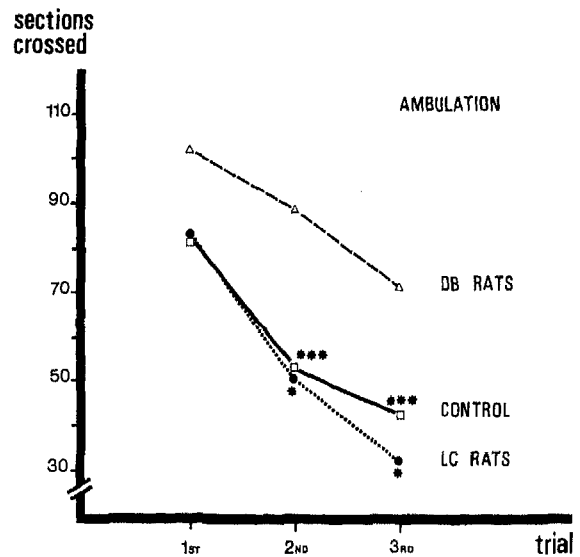


FIG. 2. Evolution of the mean value of the horizontal activity during three passages in the O.F. in sham rats (n=23) and lesioned rats (DB, n=10; LC, n=7). \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  compared with the value recorded during the first passage.

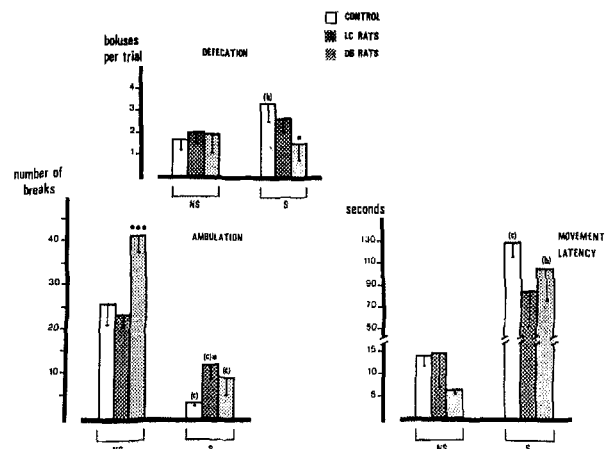


FIG. 3. Mean values of parameters in the HENDERSON test (Shams, n=23; DB rats, n=10; LC rats, n=7), after passage without electric shock (NS) and after passage with electric shock (S). Each value is indicated with S.E.M. in one direction. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  between sham and lesioned rats. (a)  $p < 0.05$ ; (b)  $p < 0.01$ ; (c)  $p < 0.001$  between NS and S animals in the same group.

These lesions induced a significant decrease in the dopamine level in hypothalamus ( $p < 0.05$ ) but the 5-HT level in the brain is unchanged.

The bilateral lesions of LC decreased the NA level in the forebrain ( $p < 0.01$ ) (Table 2). The NA depletion is approximately 30% compared to shams. The levels of DA and 5-HT in the forebrain were unchanged.

**Histological Results**

Because it is impossible to carry out both a biochemical

TABLE 1  
NORADRENALINE, DOPAMINE AND SEROTONIN CONCENTRATION IN DIFFERENT BRAIN REGIONS

Brain region	Experimental group	NA	DA	5-HT
Medulla-oblongata	Sham (n=9)	540 ± 37	45 ± 15	864 ± 34
	DB lesion (n=9)	480 ± 45	22 ± 10	898 ± 132
Mesencephalon	Sham (n=8)	460 ± 47	285 ± 55	999 ± 85
	DB lesion (n=9)	374 ± 35	252 ± 103	802 ± 50
Hypothalamus	Sham (n=9)	2,165 ± 326	535 ± 152	1,526 ± 320
	DB lesion (n=8)	1,283 ± 132*	123 ± 67*	969 ± 96
Cerebral cortex	Sham (n=9)	252 ± 14	633 ± 87	565 ± 42
	DB lesion (n=9)	164 ± 26†	606 ± 69	525 ± 67

The amine concentrations are expressed in ng·g<sup>-1</sup> wet weight (M ± SEM).

\**p*<0.05 and †*p*<0.01 with respect to shams.

dosage and a histological control in the same DB rat, we have examined first of all the location of mesencephalic lesions, in a rat whose O.F. behavior is representative of the group and secondly in the animals whose behavior after lesions is identical to that of shams.

Figure 4 shows the lesions which agree with the regions described by Ungerstedt [48], involving the DB fibers.

In four DB rats, the ventrolateral region of the periaqueductal gray matter was lesioned.

The conclusions from the histological control of LC lesions agree with the biochemical results (Fig. 5). In five LC rats, the lesions were not accurately positioned in the LC.

These nine animals (4 DB rats and 5 LC rats) are excluded from the statistical analysis of the results.

#### DISCUSSION

The novel stimulus represented by O.F. situation (open space, brightly lit) evokes a pattern of neurovegetative (defecation, miction), endocrine (increase in plasma corticosterone level) and somatomotric (ambulation, rearings) responses [16,20].

In general, the animals characterized by a hyperreactivity to novelty or neophobia, present a high defecation and a low locomotor activity during the O.F. test [50].

Our results indicate that the lesions of DB induce an increase in ambulation in the peripheral and central sectors in the O.F. and a decrease in fecal boli excreted in the lesioned animals. The existence of a significant negative correlation,  $r(10) = -0.68$ ,  $p < 0.05$ , that is revealed by the calculation of *r* Bravais Pearson, between defecation and ambulation, supports the hypothesis, according to which the DB rats became less reactive in the O.F. The fact that the lesions have an effect more upon the ambulation than rearings underlines a difference as to their behavioral signification. Lát [30], then Soubrie and Boissier [46] suggest that the rearings could reflect the wakefulness level of the animal.

On the other hand, we observe that the lesions of LC do not induce behavioral modifications in lesioned animals in the O.F.

During the Henderson test, the behavioral patterns of shams after the administration of an intense and unavoidable electric shock are characterized by a decrease in ambulatory activity, an increase in the movement latency and in defecation. The behavioral inhibition which represents the immobility reaction or 'freezing' conditioned by the intense elec-

TABLE 2  
NORADRENALINE, DOPAMINE AND SEROTONIN CONCENTRATIONS IN THE FOREBRAIN IN SHAMS AND LC RATS

Amine	Animals	
	Sham (n=9)	Lesioned (n=7)
NA	436 ± 34	304 ± 14*
DA	804 ± 81	717 ± 57
5-HT	702 ± 41	759 ± 53

The amine concentrations are expressed in ng·g<sup>-1</sup> wet weight (M ± SEM).

\**p*<0.01 with respect to shams.

tric shock [22] is a prominent component of the rat's defensive behavior repertory [3,42]. The hyporeactivity of DB rats in the O.F. is interpreted during the Henderson test in "stimulated" animals by a defecation significantly less than that of shams ( $p < 0.05$ ) and identical to that of "non stimulated" animals.

On the other hand, ambulation values (locomotion, latency to move) of "stimulated" DB rats do not differ to those of "stimulated" shams. The somatic-motor inhibition of these animals induced by an aversive stimulus is not changed by the lesions of DB. In the same way, the LC rats seem less inhibited than shams by the electric shock, delivered the day before, since they ambulate more and with a shorter movement latency. The tendency to an increase in defecation in the "stimulated" situation, associated with a decreased inhibition of the ambulation probably can be attributed to an emotional conditioned response which is less significant than that of shams. Therefore, the DB rats are hyporeactive to novelty, whereas the LC rats have a behavior identical to that of shams in the O.F. After an aversive stimulus, a smaller behavioral inhibition characterizes the lesioned rats. Now, O.F. situation and Henderson test are situations which differ to their "anxiogenic" character. The nature of the "stressful" stimulus, its intensity, its duration are multiple variables differently acting upon central circuits and consequently influencing the neurovegetative and behavioral responses of animals [1, 37, 47]. Furthermore, we

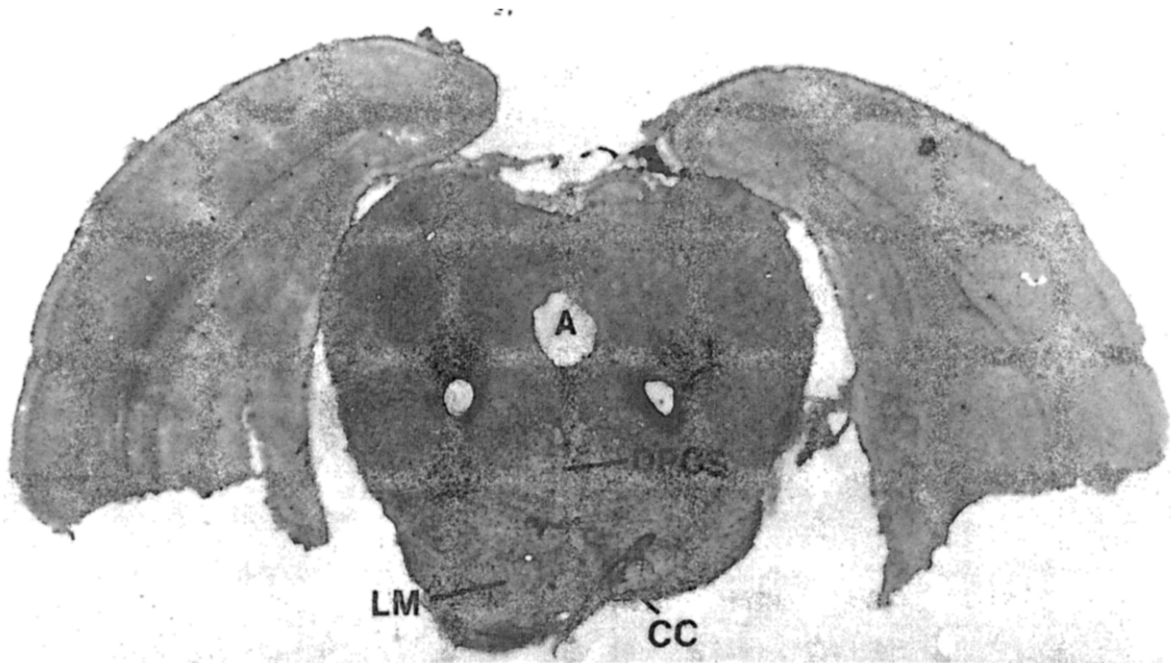


FIG. 4. Mesencephalic frontal section showing bilateral lesions of the dorsal noradrenergic bundle. A: cerebral aqueduc; DPCS: decussation superior cerebellar peduncle; L: lesions of DB; LM: medial lemniscus; CC: corpus cerebri.

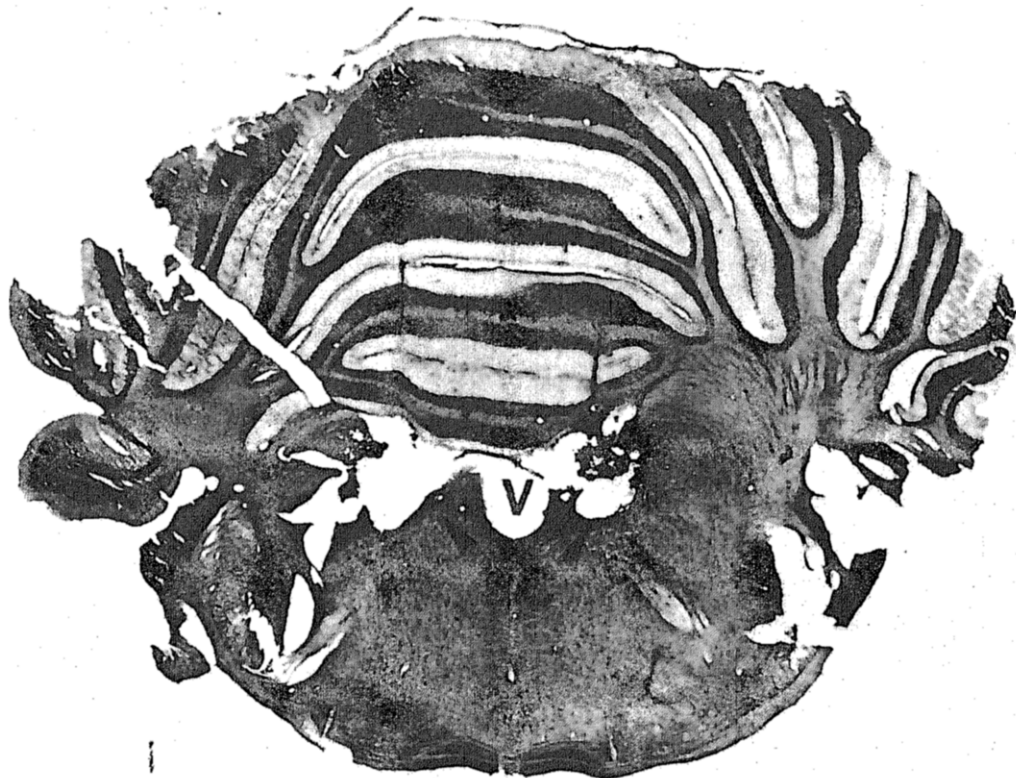


FIG. 5. Frontal section showing bilateral lesions involving the LC.

note that the repetitive presentation of a novel stimulus, during the three passages in the O.F., brings out the habituation of shams and LC rats to this situation: a phenomenon which does not appear in the DB rats. Thus, the absence of habituation in DB rats would be linked, according to several authors, to attentional deficits [34, 35, 36]. Although, Mason and Iversen [35] suggest that the DB plays a predominant role in selective attention, our results do not allow us to decide in favour of this hypothesis or that developed by Gray [18,19], who supports the hypothesis according to which the DB mediates the behavioral effects induced by "anxiogenic" stimuli.

The decrease in the cortical NA level, after bilateral lesions of DB agrees with that observed by many authors, who report that all the areas of cortical cortex receive large projections of DB fibers, which originate in the locus coeruleus [33,48]. The decrease in the hypothalamic NA level in these same animals, already observed by Cuello *et al.* [14]; Roizen *et al.* [44]; Mason and Fibiger [34] may be due to lesions of the ascending fibers of the intermediate noradrenergic bundle, which are situated ventrally in relation to fibers of DB in mesencephalon [33]. Olson and Fuxe [39] show that the sub-coeruleus nucleus and the anterior portion of the locus coeruleus which creates this pathway, project mostly to hypothalamus. Nevertheless, other results from histochemical fluorescence and autoradiographic methods reveal the projection of DB fibers, from the LC on the supra-optic nucleus of hypothalamus [25,32].

Many assumptions can explain the decrease in the hypothalamic DA level following lesions of DB.

A possible mediation between the intrahypothalamic dopaminergic pathways the presence of which has been revealed by Björklund *et al.* [6]; Björklund *et al.* [7] and the collaterals of the ascending noradrenergic fibers of DB, could explain the secondary or indirect effects of noradrenergic bundle lesionings on the functioning of these dopaminergic neurons [8].

In the same way, the lesions of dopaminergic fibers, which originate in area A8 and A9 (substantia nigra) and travel with the ascending noradrenergic fibers, can explain the DA depletion in the hypothalamus, a region which receives projections of the DB. This hypothesis is supported by the data, recently contested by Milon and McRae-

Degueurce [38], of Versteeg *et al.* [49], who have revealed that 25% of catecholaminergic neurons, which originate in LC are dopaminergic.

The hypothesis of the united lesions of area A8 and A9 (substantia nigra) in the mesencephalic region is excluded given the choice of stereotaxic coordinates used to reach the ascending fibers of DB [43].

The use of 6-OHDA would have provoked without doubt more effective and more specific lesions of noradrenergic neurons but the spread of 6-OHDA which is difficult to check, would have prevented a selective lesion of the DB. It is for this reason, that electrolytic lesions were used in this experiment.

The significant brain NA depletion in the LC rats represents a low value compared to that reported by Koob *et al.* [27]. This difference is probably due to the choice of the brain parts in which the catecholamines determination has been carried out. The selection of smaller parts with high NA content such as the hippocampus or the cerebral cortex would allow observation of a more important depletion. On the other hand, our results are like that from Kostowski *et al.* [29] who used the same part of the brain.

The differences between the effects due to lesions of DB and those induced by lesions of LC appear mainly due to novelty (O.F.). Only the lesions of DB decrease nephobia. The anatomic complexity of the LC can be an element for interpretation. Indeed, the electrolytic destruction of LC has reached non-noradrenergic elements, or noradrenergic efferent pathways from cerebellum, spinal cord, raphe nuclei and from the ventral tegmental area (Area A10), which interact with the coeruleus neurons [12, 38, 41]. The research of Kostowski [28] reported the existence of interactions between the dorsal nucleus of raphe and the LC in regulating the ambulatory behavior in the rat. Furthermore, Clark [10] suggests that the destruction of the region of the LC can reach neurons whose projections do not travel with the DB. These neurons can be enkephalinergic [4,9] or dopaminergic [49].

In conclusion, it is hoped that these results will prevent investigators from coming to too hasty conclusions and confusing the effects induced by lesions of LC with the effects induced by lesions of DB whereas in fact the latter originate from this former.

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