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# BRIEF COMMUNICATION

# Raphe Lesions in Cats: Forebrain Serotonin Avoidance Behavior[1]

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LORENS, S. A. *Raphe lesions in cats: forebrain serotonin and avoidance behavior.* PHARMAC. BIOCHEM. BEHAV. 1(4) 487–490, 1973.–Lesions in the central superior nucleus of Bechterew (also termed the median raphe nucleus) in cats resulted in a 35% reduction in forebrain serotonin (5-HT) concentration and in more rapid acquisition of a shuttlebox conditioned avoidance response. The data were discussed in terms of the locus and size of the lesion as well as the associated fall in brain 5-HT. The results support the hypothesis that the raphe nuclei and central 5-HT play an important role in mediating behaviors controlled by painful stimuli. It appears, furthermore, that the raphe system serves a similar function in both rats and cats.

Raphe lesions  Forebrain serotonin  Avoidance behavior

IN PREVIOUS studies [3, 5] we have shown that simultaneous ablation of the dorsal and median (called the central superior nucleus of Bechterew in the cat; [1,8]) raphe nuclei in the rat produces: (1) transient elevations in water intake; (2) faster acquistion of a shuttlebox (or 2-way) conditioned avoidance response, as well as; (3) reductions in the telencephalic concentrations of both 5-hydroxytryptamine (75–85%) and norepinephrine (27–30%). The effect on norepinephrine was ascribed to paramedial reticular damage. Lesions in the dorsal and median raphe nuclei, however, failed to affect responding for rewarding lateral hypothalamic stimulation or activity level as measured by wheel-running. It was concluded that the raphe system plays an important role in central serotonin (5-HT) metabolism and in the mediation of behaviors controlled by negative reinforcement.

The purpose of the present study was to determine whether raphe lesion induced changes in 5-HT would facilitate avoidance learning in another species, the cat.

## METHOD

### *Animals*

Twelve adult domesticated male cats weighing 3.3–5.8 kg at the time of surgery were used. The animals were housed individually in Acme stainless steel cages (32 in. high x 18 in. wide x 24 in. deep) which contained 2 wooden platforms (1 x 16 x 6 in.), one in the rear wall 8 in. above the floor, and one in the front wall 16 in. above the floor. The cats were given 8 oz. of commercial food daily. Water was available ad lib.

### *Surgery and Histology*

The cats were anaesthetized with 35 mg/kg IP sodium pentobarbital. Lesions were produced sterotactically (a Kopf instrument was employed) in 6 cats by passing 4 ma d. c. for 1 min between an intracranial cathode and rectal anode. The cathode was a 24 ga. stainless steel wire insulated with Epoxylite excepting 2.5 mm at its tip. The electrode was angled 46° caudal to the coronal plane and preset to terminate at A 0.5, V 6.00, L. 0.0 [1]. The animals also received 150,000 units of procaine penicillin IM. Operated control cats (n = 6) were treated in the same manner as the lesion animals, except that the electrode was not lowered into the brain.

At the end of the experiment, the cats (excepting one lesion and 2 control animals) were anaesthetized with 40 mg/kg IP sodium pentobarbital and their brains removed. The forebrain was dissected away at the di-mesencephalic junction and assayed for 5-HT. The remaining portion of the brainstem of the lesion animals was placed in 10% Formalin for at least 3 weeks then used for histological analysis of the lesions. 5-HT was determined by a solvent extraction procedure [2] and fluorescence assay [7].

[1] The majority of this work was completed while the author was at the Department of Psychiatry, University of Iowa. The author wishes to express his gratitude to Dr. Sara A. Millard for conducting the 5-HT assay, to Mr. Ralph Nurnberg for his assistance throughout the course of the experiment, and to Ms. Aase Larsen for preparing the histological material.

The brainstems of the lesion cats were embedded in paraffin. Coronal sections were cut at 30 $\mu$. Every tenth section was saved and stained by the cresylecht violet or thionine technique.

*Apparatus*

Avoidance conditioning was carried out in a shuttlebox containing 2 compartments (23 3/4 in. long x 18 1/2 in. wide x 17 1/2 in. high) separated by a V-shaped stainless steel barrier. The apex of the V was 3 in. above the grid floor while the arms of the V opened to 16 in. at the ceiling of the box. The grid floor was composed of 1/4 in. dia. aluminum rods spaced 5/8 in. center-to-center. The interior of the chamber was painted white, except for a one-way mirror (12 1/2 in. high and 41 in. long) in the front of the box. Each compartment was illuminated by a 25 W fluorescent light. The unconditioned stimulus (UCS) was a constant current shock of 1.0 ma and 0.5 sec duration delivered as previously described [4]. The conditional stimulus (CS) was the sound produced by a conventional door buzzer located in the ceiling of the apparatus over the barrier.

*Procedure*

The animals were handled almost daily beginning two weeks preoperatively and throughout the remainder of the experiment. Body weights were obtained weekly.

Avoidance training was initiated 9 weeks postoperatively. Each cat was permitted 5 min on the first training day in order to explore the apparatus prior to the first CS presentation. On subsequent days training began 1 min after the animal was placed in the box. The CS-UCS interval was 5 sec. The CS was terminated when the cat crossed the barrier. Shock was delivered only when the animal was motionless or moving in a direction away from the barrier. Intertrial crossings were recorded but not punished. Each animal was given 10 trials/day until criterion (18 avoidance responses made in the last 20 trials) was reached.

Two weeks after the completion of avoidance training, the cats were anaesthetized and their brains removed for 5-HT and lesion analysis.

## RESULTS

*General Observations*

Upon recovery from the anaesthetic the lesion animals appeared groggy and were aphagic and immobile for up to five days. The animals sat in a relaxed, wakeful posture with eyes closed. No evidence of hyperactivity was observed. One week postoperatively all cats were eating and drinking normally. No gross neurological, emotional, or other behavioral disturbances were apparent. The lesion cats lost weight during the first postoperative week, however, by the time avoidance training commenced no group difference was found.

*Conditioned Avoidance Behavior (CAR)*

As seen in Table 1, the lesion cats required significantly fewer trials to reach the criterion for the establishment of a CAR. The two groups, however, did not differ significantly with respect to the number of errors committed (raphe 32 ± 6; control 54 ± 9) or the number of shocks (raphe 110 ± 42; control 199 ± 72) received prior to reaching CAR criterion. In addition, the two groups did not differ in terms of the mean number of barrier crossings made during the first 5 min in the apparatus (raphe 3 ± 2; control 3 ± 1) or in terms of the mean number of intertrial crossings made throughout the remainder of testing (raphe 12 ± 6; control 14 ± 6).

TABLE 1

EFFECT OF LESIONS IN THE CENTRAL SUPERIOR NUCLEUS (RAPHE) ON FOREBRAIN SEROTONIN (5-HT) CONCENTRATION AND THE NUMBER OF TRIALS NEEDED TO ACQUIRE THE CONDITIONED AVOIDANCE RESPONSE (CAR)

| Group | N | CAR | N | 5-HT ($\mu$g/g) |
|---|---|---|---|---|
| Control | 6 | 82 ± 11* | 4 | 0.52 ± 0.06 |
| Raphe | 6 | 53 ± 6† | 5 | 0.34 ± 0.03† |

*Mean ± standard error
†$p<0.05$ as determined by student's test, two-tailed

*5-HT and Lesion Analysis*

The lesions (Fig. 1) were well localized and destroyed at least 50% of the central superior nucleus of Bechterew. The medial aspect of the adjacent reticular formation, including the dorsomedial portion of the pontine tegmental reticular nucleus, was invaded in all lesion animals. In addition, the lesions produced a significant 35% reduction in forebrain 5-HT concentration (Table 1).

## DISCUSSION

Lesions in the central superior nucleus of Bechterew (called by some authors the median raphe nucleus, particularly in the rat) in cats lowered forebrain 5-HT concentration and facilitated the acquisition of a shuttlebox CAR. These data correspond to findings following similar lesions in rats [5] and support the hypothesis that 5-HT plays an important role in mediating behaviors controlled by negative reinforcement. In addition, they suggest that the raphe nuclei serve a similar function in both rats and cats.

It should be noted that the lesion cats evidenced superior CAR performance only in terms of the number of trials required to reach criterion. Although the raphe lesion cats tended to receive fewer shocks and make less errors prior to reaching criterion, the group differences were not significant. In addition, the lesion cats did not differ from control animals with respect to the number of barrier crossings made either during their first 5 min in the box or during the intertrial interval throughout the course of conditioning. These results differ from our previous finding in rats [5] in the following respects: (1) the raphe lesion rats not only required fewer trials to reach CAR criterion, but made significantly fewer errors; and (2) the raphe lesion rats made a significantly greater number of spontaneous barrier crossings both prior to the first CS presentation and during the balance of the conditioning procedure. Thus, the performance of the lesion cats differs from that of the lesion rats.

However, in our first study [5] with rats, the lesions not

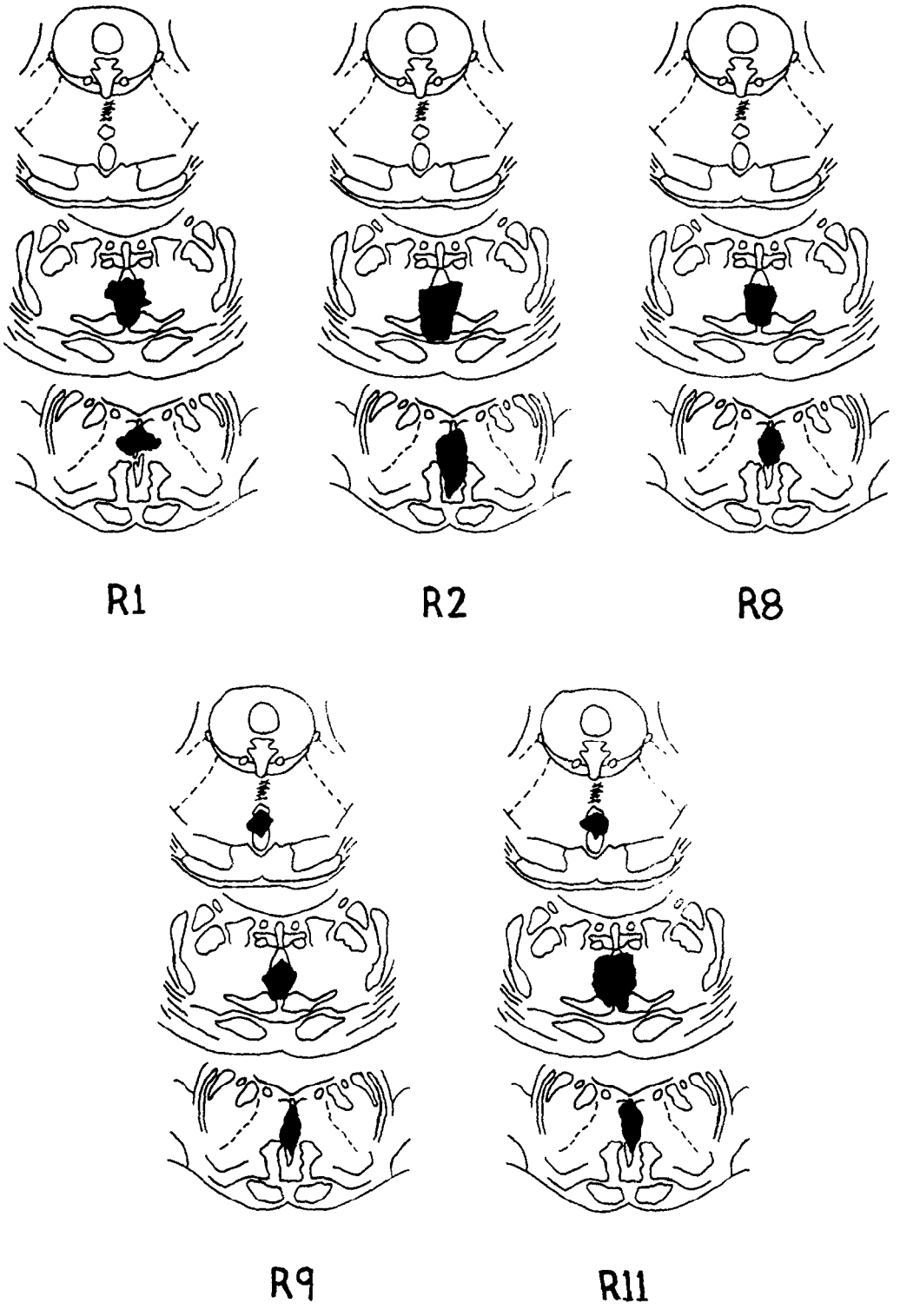


FIG. 1. Locus and extent of lesions (blackened area) in 5 of the 6 cats (R1, etc.) used in this study.

only destroyed the median raphe nucleus, but the dorsal raphe nucleus as well. This resulted in an 85% reduction in telencephalic 5-HT concentration. In the present study the lesions were restricted to the central superior (median raphe) nucleus and resulted in only a 35% fall in forebrain 5-HT concentration. Unfortunately, the telencephalic 5-HT concentration was not determined in this study, restricting generalization. However, the percent 5-HT fall in forebrain should be similar to that in telencephalon alone. It appears, therefore, that the more rapid acquisition of a shuttlebox CAR by animals with lesions in the raphe nuclei depends on the size and locus of the lesions as well as the associated reduction in central 5-HT concentration. This hypothesis is supported by our recent findings [6] that lesions confined to the median raphe nucleus of rats lower the telencephalic concentration of 5-HT by only 30% and fail to affect two-way CAR performance.

## REFERENCES


1. Berman, A. L. *The Brain Stem of the Cat: A Cytoarchitectonic Atlas with Sterotaxic Coordinates.* Madison: Univ. of Wisconsin Press, 1968.
2. Gal, E. M. and S. A. Millard. The relevance of changes in cerebral 5-hydroxytryptamine to reserpine-produced sedation. *Biochem. Pharmac.* **17**: 2193–2203, 1968.
3. Lorens, S. A. Effect of lesions in the raphe system on self-stimulation in the rat. *Physiol. Behav.* **7**: 815–818, 1971.
4. Lorens, S. A., J. P. Sorensen and J. A. Harvey. Lesions in the nuclei accumbens septi of the rat: Behavioral and neurochemical effects. *J. comp. physiol. Psychol.* **75**: 284–290, 1970.
5. Lorens, S. A., J. P. Sorensen and L. M. Yunger. Behavioral and neurochemical effects of lesions in the raphe system of the rat. *J. comp. physiol. Psychol.* **77**: 48–52, 1971.
6. Lorens, S. A. and L. M. Yunger. Morphine analgesia, avoidance and consummatory behaviors following lesions in the raphe system of the rat. Paper presented to the Third Scandinavian Meeting on Physiological Psychology and Psychophysiology, Copenhagen, May, 1973.
7. Maickel, R. P., R. H. Cox, Jr., J. Gaillant and F. P. Miller. A method for determination of serotonin and norepinephrine in discrete areas of the rat brain. *Int. J. Neuropharmac.* **7**: 275–281, 1968.
8. Taber, E., A. Brodal and G. Walberg. The raphe nuclei of the brain stem in the cat. I: Normal topography and cytoarchitecture and general discussion. *J. comp. Neurol.* **114**: 161–187, 1960.