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Muricide After Serotonin Depleting Lesions of Midbrain Raphé Nuclei[1]

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GRANT, L. D., D. V. COSCINA, S. P. GROSSMAN AND D. X. FREEDMAN. *Muricide after serotonin depleting lesions of midbrain raphé nuclei.* PHARMAC. BIOCHEM. BEHAV. 1(1) 77–80, 1973.–Thirty days after dorsal and median raphé lesions, when the forebrain content of serotonin (5-HT) was approximately 70 per cent below normal values, lesioned rats showed increased frequency and decreased latency for lethal attacks on mice. Additional observations of muricide on Days 2, 5, 8, and 30 after lesions revealed that frequency and latency of killing increased and decreased, respectively, over time. Since the time-course of this muricide paralleled a progressive reduction in forebrain 5-HT, these data suggest that this neurohumor normally participates in mechanisms exerting inhibitory control over mouse killing.

Muricide Serotonin Midbrain raphé nuclei

RECENT evidence suggests that serotonin (5-hydroxytryptamine or 5-HT) may inhibit some types of aggressive behaviors. For example, p-chlorophenylalanine (PCPA), a potent inhibitor of 5-HT synthesis [7], not only increases mouse killing in rats [12], but also potentiates the facilitatory effects on killing which are produced in that species by removal of the olfactory bulbs [5]. This drug induced potentiation of muricide after bulbectomy seems to be due to the selective effects of PCPA on 5-HT, since injections of pargyline or 5-hydroxytryptophan which increase 5-HT concentrations block the muricide induced by olfactory injury [5].

The effects of these compounds on attack behavior have been ascribed to their direct actions on serotonin dependent portions of the central nervous system (CNS). It is clear, however, that each of these agents also affects peripheral 5-HT stores, and the possible contribution of peripheral mechanisms is unknown. The present experiments were undertaken in order to provide more conclusive evidence for a central serotonergic control of muricide. The dorsal and median raphé nuclei of the midbrain have been shown [1] to be the origin of most serotonin containing fibers in the rat forebrain. If central serotonergic mechanisms are involved in the control of mouse killing, destruction of these nuclei should modify this behavior, and the effect should have a time course which parallels the gradual disappearance of 5-HT from the forebrain [10].

EXPERIMENT 1

METHOD

Animals

Twenty male Sprague-Dawley (Holtzman) rats weighing 325–375 g at surgery were used in the first experiment. None had previously been exposed to mice. The animals were housed singly in metal cages (9 1/2 x 7 x 7 in.) with wire mesh floors and fronts. Lab chow and water were available ad lib throughout the experiment.

Procedures

Electrolytic lesions were made in the midbrain raphé nuclei of 10 animals as follows: With the incisor bar located 2.5 mm below the intra-aural line, a stainless steel electrode, insulated except for the cross section of the tip, was stereotaxically inserted into the brain such that its tip terminated in the dorsal raphé nucleus (A = 0.8 mm; L = 0.0 mm; H = 6.4 mm below dura). A 1 mA anodal direct current was passed for 10 sec between this electrode and a metal clamp attached to the tail. The electrode was then withdrawn and reinserted such that its tip terminated in the median raphé nucleus (AP = 1.2; L = 0.0; H = 8.5 below

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dura). A 1 mA anodal current was then passed for 10 sec. The remaining 10 animals served as sham operated controls. These rats were subjected to the same surgical procedures as the experimental animals except that the electrode was lowered only into cortex and no current was passed.

The mouse killing behavior of all animals was assessed 30 days after surgery at a time when the lesions should produce maximal 5-HT depletion in the forebrain [10]. A single adult male mouse was placed in the home cage of each rat for 60 min between 2:00–4:00 p.m. The rats and mice were observed throughout this period, and the latency for each lethal attack was recorded. Dead mice and survivors were removed at the end of the test session.

After behavioral testing, the rats were decapitated and their brains removed and dissected into forebrain and hindbrain portions by a cut from the quadrigemina dorsally to the caudal border of the mammillary bodies ventrally. Hindbrains were fixed in formalin and later processed for histological examination of lesion sites, using 40 μ frozen sections stained with cresyl violet. Forebrain portions were immediately analyzed fluorometrically for serotonin and its major CNS metabolite, 5-hydroxyindole acetic acid (5-HIAA). 5-HT was analyzed by a modification [11] of the Bogdanski *et al.* [2] method for serotonin, with the 5-HT being reacted with ninhydrin to increase the sensitivity of the method [13]. The method of Udenfriend *et al.* [14] was used for 5-HIAA estimation.

RESULTS

Nine of 10 rats with raphé lesions killed mice 30 days after surgery, whereas only 3 of 10 sham operated controls did so. This difference was statistically reliable ($p<0.05$, chi square). A comparison of the latency to kill of the killers of each group showed that lesioned rats killed with a mean latency of 12.2 min whereas the control animals killed after 32.7 min on the average ($p<0.02$, Mann-Whitney U). The topography of the killing behavior of rats with raphé lesions was similar to that exhibited by control animals, i.e., the mouse was typically grasped by the head and lower back from above and was then bitten along the cervical region of the spinal cord in stereotypic fashion normally displayed by natural killer rats. Raphé lesions thus appear to produce more permanent and more natural changes in mouse killing than lesions in forebrain structures such as the septum [9] which receive some of the serotonin containing projections from the raphé region.

Histological examination showed that the lesions were about 1 mm in dia. and destroyed nearly the entire dorsal and median raphé nuclei as well as some central gray tissue surrounding the dorsal raphé nucleus. Biochemical assays revealed that the lesions caused a 70 per cent reduction in forebrain 5-HT and an 84 per cent fall in 5-HIAA.

EXPERIMENT 2

METHOD

Procedures

The second experiment was conducted to assess the time course of increased aggressiveness toward mice after raphé lesions in relation to the time course of forebrain 5-HT depletion. Using the procedures described above, rats (325–375 g) were tested for muricide on two consecutive days prior to surgery. Fifteen rats then received lesions in the median and dorsal raphé nuclei and 9 additional animals were subjected to comparable surgical trauma but received no lesions. All rats were retested 2, 5, 8, and 30 days after surgery. The time course for the effects of our lesions on forebrain serotonin was determined by assays made of the brains of additional animals which were killed at various intervals after surgery (see Fig. 2).

RESULTS

Representative histological results are shown in Fig. 1. The results of the behavioral tests are summarized in Table 1. One rat of each group killed during both preoperative tests. The animal with raphé lesions which had killed prior to surgery failed to kill on the second day after surgery (possibly because of motor deficits) but did so again at every test thereafter. The other experimental rats also did not attack mice during the first postoperative test. By the fifth day, 5 of them killed mice, and by Day 8, the number of killers increased to 7 ($p<0.05$, chi square). On the final test, conducted 30 days after surgery, 8 experimental animals killed mice. The gradual increase in aggressiveness after the lesions was also reflected in the decline of the mean latencies of the attack responses. No statistically significant increase in killing occurred among the control animals in repeated postoperative tests.

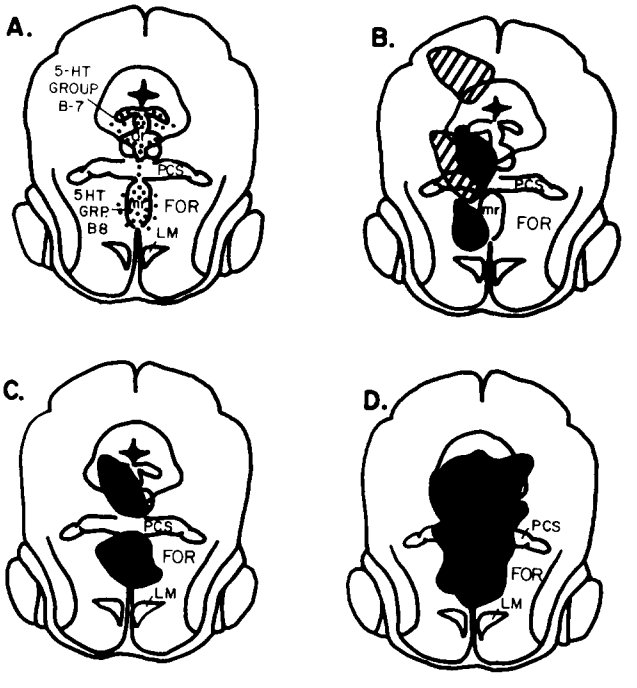

FIG. 1. Representative histological results from Experiment 2. (a) Target sites for lesions (serotonin cell groups B7 and B8 of Dahlstrom and Fuxe [4] in the dorsal and median raphé nuclei); (b) Two lesions which caused only 15–30 per cent reductions in forebrain 5-HT and 5-HIAA and did not induce mouse killing; (c) Area of destruction of smallest lesion effective in inducing muricide; (d) Area of damage by most extensive lesion that induced muricide. Abbreviations: dr = dorsal raphé nucleus; mr = median raphé nucleus; FOR = reticular formation; LM = medial lemniscus; PCS = superior cerebellar peduncle.

TABLE 1

DEVELOPMENT OF INCREASED AGGRESSIVENESS TO MICE BY RATS AFTER MIDBRAIN RAPHÉ LESIONS*

Animals	Days After Operation				
	Pretest	2 Days	5 Days	8 Days	30 Days
Operated Control Animals:					
No. Killers/No. Tested	1/9	2/9	3/9	2/9	2/9
Mean Latency to Kill	57 min	47 min	40 min	47 min	47 min
Raphé Lesion Animals:					
No. Killers/No. Tested	1/15	0/15	5/15	7/15†	8/15†
Mean Latency to Kill	56 min	60 min	42 min	37 min‡	30 min‡

*Rats were tested for mouse killing by placing a single mouse in each rat's home cage for 1 hour.

†Compared with pretest for same group, difference is significant at $p<0.05$ by chi square test for correlated proportions.

‡Compared with latency scores for control animals, difference is significant at $p<0.001$ by Mann-Whitney U Test.

Biochemical assays conducted 35 days after surgery showed an overall decrease of 58 per cent and 52 per cent respectively in forebrain 5-HT and 5-HIAA. When the assay data from two experimental animals that did not kill mice on any postoperative test and were found to have only slight damage to the raphé nuclei were dropped from the analysis, 5-HT and 5-HIAA were depleted 70 per cent and 63 per cent respectively. The time course of 5-HT and 5-HIAA depletion after raphé lesions was established in additional animals (see Fig. 2).

A comparison of our behavioral and biochemical data suggests that the increase in muricide after midbrain raphé lesions developed in parallel with a concomitant reduction in forebrain serotonin. It is possible that this correlation may be fortuitous and may reflect merely a gradual increase in the ability of the experimental animals to display effective aggressive behavior as lesion induced motor deficits diminish. However, several animals with severe and persisting motor impairments killed mice as early as 5 days after surgery while others which displayed only mild motor deficits from the outset did not kill until Day 8 or 30. It is possible that the increased muricide could be due to a depletion of other amines, e.g., norepinephrine (NE) or dopamine (DA), but this interpretation is unlikely since we [9] as well as others [8] have found that lesions such as ours that are restricted to the raphé nuclei typically produce little or no reduction in brain catecholamines. Lesions which do extend into the lateral aspects of the brainstem at this level do affect NE or DA, but we have not observed an increase in muricide in rats with lesions lateral to the raphé.

Reports [6] of heightened reactivity to pain and general irritability after lesions that reduce central serotonin levels suggest that the observed increase in intraspecies aggressiveness might be only one manifestation of a general syndrome. Our animals displayed increased motor activity, hyperreactivity to painful electric shock, and some resistance to handling, but the time course of these effects did not duplicate the gradual development of the mouse killing response. Motor hyperactivity was observed immediately after surgery and persisted undiminished throughout the 30

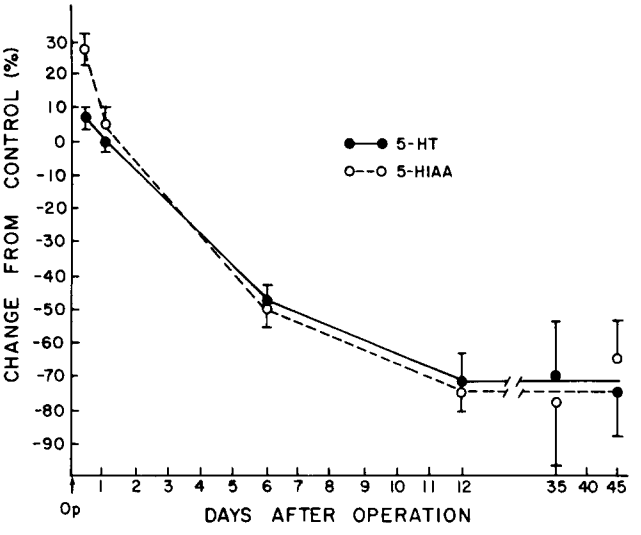

FIG. 2. Per cent change from control levels of forebrain serotonin (5-HT) and its main CNS metabolite, 5-hydroxyindole acetic acid (5-HIAA), after midbrain lesions destroying the dorsal and median raphé nuclei. Each point is based on data from 4–6 lesioned animals and 3–6 controls. Means ± S.E. for all control values: 5-HT = 687 ± 19; 5-HIAA = 316 ± 12. S. E. values shown above for each point are in terms of the per cent of the corresponding mean.

days of the experiment. Resistance to handling was greatest immediately after surgery and diminished over time as the animals appeared to habituate to capture. Decreased thresholds for a jump response to electric shock were seen immediately after surgery. These thresholds returned to normal values between days 5–7 but then fell again about 10–12 days after the lesion. This curious biphasic pattern also describes some other behavioral effects of raphé lesions [3].

Our observations indicate that serotonergic pathways originating in the midbrain raphé nuclei may exert inhibitory control over mouse-killing responses in the rat.

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