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Response to Electric Shock in Rats: Effects of Selective Midbrain Raphe Lesions[1]

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HOLE, K. AND S. A. LORENS. *Response to electric shock in rats: Effects of selective midbrain raphe lesions.* PHARMAC. BIOCHEM. BEHAV. 3(1) 95–102, 1975. – The forebrain serotonin (5-HT) concentrations of rats with lesions in the median (M; n = 5), dorsal (D; n = 5), and both (DM; n = 6) midbrain raphe nuclei were, respectively, 22, 48 and 70% lower than in control animals (n = 10). The lesion and control groups, however, did not evidence differences in pain sensitivity as measured by the flinch-jump technique. On the other hand, of the animals tested, those with M (n = 3) and DM (n = 4) lesions required more trials than controls (n = 6) to acquire a one-way avoidance response. D lesion rats (n = 2) did not differ from controls in one-way avoidance learning, except in terms of prolonged escape latencies during the first three trials. The previously reported increased sensitivity to painful stimuli subsequent to medial forebrain bundle lesions or para-chlorophenylalanine administration, therefore, does not appear to be due exclusively to disruption of ascending 5-HT fibers originating in the dorsal and median raphe nuclei. The effects of midbrain raphe lesions on avoidance learning, furthermore, depend on lesion locus, and are not due to either hypo- or hyperalgesia.

5-Hydroxytryptamine Midbrain raphe nuclei One-way avoidance learning Pain sensitivity
5,7-Dihydroxytryptamine p-Chlorophenylalanine

THERE have been several reports that reductions in brain 5-hydroxytryptamine (5-HT) following para-chlorophenylalanine (p-CPA) administration or lesions in the medial forebrain bundle (MFB) result in increased sensitivity to painful stimuli [9, 10, 11, 15, 16, 31, 32]. Administration of the 5-HT precursor, 5-hydroxytryptophan, furthermore, has been observed to reverse the lesion and p-CPA induced changes in pain sensitivity [9, 15, 31, 32].

It is generally agreed that forebrain 5-HT has its primary origin in the midbrain B7-9 cell groups [6]. These cells give rise to fibers which ascend in the MFB to innervate a variety of forebrain structures [2, 4, 6, 23, 24]. Lesions damaging both the dorsal (B7) and median (B8) raphe nuclei, furthermore, have been reported to reduce forebrain 5-HT, 5-hydroxyindole acetic acid, and tryptophan hydroxylase by up to 80% [3, 5, 7, 13, 14, 19, 20, 27, 29].

Few studies have examined the effect of midbrain raphe lesions on pain sensitivity, although stimulation of this region has been reported to induce analgesia [21]. Bläsig *et al.* [3] have reported a significant decrease in the current threshold to elicit vocalization by electrical stimulation of the tail 8–15 days after median raphe lesions in rats. In their discussion section, Grant *et al.* [7] cite unpublished data showing that large raphe lesions produce lowered jump thresholds immediately and 10–12 days (but not 5–7 days) after surgery. Pepeu *et al.* [26], on the other hand, did not observe changes in jump threshold "at least seven days" after median raphe lesions in their rats. Likewise, Harvey *et al.* [10] failed to find changes in jump thresholds after dorsal plus median raphe lesions which reduced telencephalic 5-HT by 82%. Response (repetitive lifting of the hind limbs, or paw lick) latencies after placement on a hot plate and tail flick latency to radiant heat also have not been altered following midbrain raphe lesions [5, 20, 27]. In contrast, MFB lesions have been reported to shorten paw lick latencies [11]. Thus, the effect of raphe lesions on pain reactivity is far from clear.

Recently we [18] demonstrated that lesions restricted to the dorsal or median raphe nucleus produced differential effects on regional forebrain 5-HT. Only lesions in the dorsal raphe nucleus led to reductions in striatal 5-HT concentration (54%) whereas only median raphe lesions affected hippocampal 5-HT content (62%). The lowered 5-HT concentrations of the remaining portion of the telencephalon (50%) and of the diencephalon (24%) after dorsal raphe lesions, furthermore, were twice as great as after

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median raphe lesions. Spinal (cervical-thoracic region) 5-HT was not affected.

In another study [29], we also have found that lesions restricted to the dorsal or median raphe nucleus produce different behavioral effects. Lesions in the median but not in the dorsal raphe nucleus resulted in increased open field activity and retarded forced extinction of a one-way avoidance response. Both lesions affected one-way avoidance acquisition, but the effects of the median lesion were of a greater magnitude. Lesions simultaneously damaging both the dorsal and the median raphe nucleus produced effects similar to those after lesions in the median nucleus alone, but, unlike selective raphe lesions, also facilitated two-way (shuttlebox) avoidance acquisition [19, 20, 29].

In the present study we have examined, therefore, the effects of selective as well as combined dorsal and median raphe lesions, p-CPA, and intra-brainstem administered 5,7-dihydroxytryptamine on pain sensitivity as measured by the flinch-jump technique. Furthermore, one-way avoidance acquisition was studied in a few animals.

METHOD

Animals

Fifty-two male albino rats (Möll-Wistar) weighing 310–360 g at time of surgery (or p-CPA treatment) were used. The animals were housed individually in conventional rat cages and had food and water available ad lib.

Surgery

Electrolytic lesions were produced stereotactically in either the dorsal (n = 10), median (n = 10), or both (n = 10) midbrain raphe nuclei. The procedure employed was the same as previously described [18]. In brief, 2 mA d.c. was passed through an intracranial cathode and an anode clipped to the wound margin. The cathode was a 0.25 mm dia. tungsten wire insulated with Epoxylite except for 0.5 mm at its tip, and was inserted into the brain through the cerebellum at an angle 47° to the vertical plane. Control rats (n = 10) were treated in the same manner as the lesion rats, except that an electrode was not lowered intracranially.

In 4 rats 5 μg of 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT) was injected in the vicinity of both the dorsal and the median raphe nucleus by means of a 10 μl Hamilton syringe (701-N) mounted on a Kopf microinjection (No. 1209) attachment. The stereotactic approach described above was used, but in order to reduce nonspecific damage to the target areas, the needle tip was lowered to a point 0.5–1.0 mm above each ventral lesion coordinate. The 5,7-DHT solution (5 μl) was injected at a rate approximating 1.5 μl/min. The needle was left in situ for 1 min after completion of each injection.

Histological and Biochemical Analyses

At the completion of the experiment the animals were sacrificed by decapitation and their brains rapidly removed. Each brain was dissected on a cold glass plate which covered a tray containing dry ice. The pineal gland, optic nerve, and olfactory tract (at the level of the frontal pole) were removed and discarded. The forebrain was freed by a dorso–ventral section made rostral to the superior colliculi and caudal to the mammillary body. Each forebrain was wrapped in aluminum foil, frozen in dry ice, stored at −20°C for no more than 3 weeks, then assayed for 5-HT by the method of Ahtee *et al.* [1].

The brainstems of the lesion and 5,7-DHT injected animals were kept in 10% Formalin for at least one week. Frozen sections then were cut at 30 μ and every tenth section saved and stained by the thionine technique. Lesion cavitation and glial scarring were determined microscopically and plotted on pre-prepared diagrams.

Drugs

The 5,7-DHT solution (pH = 3.1) was prepared by dissolving 5,7-dihydroxytryptamine creatinine sulfate (kindly supplied by Dr. A. Manian, N.I.M.H., Bethesda, Md., U.S.A.) in 0.9% NaCl containing 0.2% ascorbic acid such that a concentration of 1 μg/μl was obtained.

Each 300 mg of D,L-p-chlorophenylalanine (p-CPA; Chas. Pfizer and Co.) was suspended in 7.5 ml 0.5% polyoxyethylene-23-lauryl ether (BRIJ-35; Pierce Chemical Co.) in 0.9% saline.

Apparatus

Flinch-jump tests were conducted in a Grason-Stadler (Model E3125-A100) chamber (19 × 29 × 23 cm) contained within a sound-attenuating cubicle (Model E3125AA-3). Background white noise was provided by the ventilation fan. Scrambled shocks of 0.2 sec duration were delivered via a Grason-Stadler (Model E1004GS) shock generator to the grid floor at the following intensities: 0.05, 0.06, 0.08, 0.10, 0.13, 0.16, 0.20, 0.25, 0.30, 0.40, 0.50, 0.60, and 0.80 mA.

One-way avoidance training was conducted in a shuttlebox containing two identical compartments (50 × 24 × 40 cm) separated by a hurdle 7 cm high. The ceiling, side and back walls were constructed of white Perspex. The front wall was made of clear Perspex. The grid floor consisted of 0.3 cm diameter stainless steel rods separated by 1.0 cm. The apparatus was illuminated by two 20 cm frosted 25 W lamps placed immediately above each ceiling. Grason-Stadler equipment was used to deliver scrambled constant-current (0.5 mA) shock.

Procedure

Flinch-jump thresholds were obtained for the lesion, 5,7-DHT injected, and control rats 18–21 days postoperatively. In an additional 8 animals, testing was conducted 72 hr after p-CPA (300 mg/kg) or vehicle (7.5 ml/kg 0.5% BRIJ-35) administration intraperitoneally. Testing was conducted during the day (8:00–12:00) and began 2 min after the animal entered the apparatus. Shocks were given manually at 15–45 sec intervals, but only when the rat was not moving and had at least 3 paws on the grid floor. The intensity of the first shock was 0.05 mA and was increased by one step until a flinch was observed. A flinch was defined as a crouch or startle response with one front paw typically leaving the floor. The shock intensity was lowered by one step whenever a flinch was scored and increased by one step when a flinch was not observed until 8 flinches were obtained. The shock intensity then was increased stepwise until a jump was observed. A jump was defined as a response in which either both rear paws left the grid simultaneously or the rat vigorously and repetitively stepped with all four feet. The shock intensity was lowered stepwise until a jump no longer was elicited, and then was increased until a jump occurred. Testing continued until 8 jumps had been observed. In addition, the occurence of

post-shock running [9,11] was recorded. Median flinch and jump thresholds for each rat were calculated by interpolation using the current intensities at which each of the responses were obtained.

Six animals were selected at random from each of the lesion and control groups and one-way avoidance conditioning conducted, as previously described [29], on the thirty-fifth postoperative day. Each rat was placed in a waiting box for 1 min, then into the shock compartment such that it faced the end wall. After 5 sec had elapsed continuous shock (0.5 mA) was administered through the grid floor until the animal crossed the hurdle into the safe compartment. The rat was left undisturbed in the safe compartment for 25 sec, then removed to the waiting box for 5 sec prior to the start of the next trial. If the rat returned to the shock compartment before 25 sec had elapsed, he was given continuous shock until recrossing to the safe compartment. If an animal failed to escape the shock within 20 sec, the shock was discontinued, and the rat was immediately removed from the apparatus and placed in the waiting box for 25 sec. Response latencies were recorded with a stop watch. Training continued until 10 consecutive avoidance responses (crossing into the safe compartment within 5 sec) were obtained.

On the thirty-ninth postoperative day the animals were sacrificed and their brains used in the biochemical and histological analyses. The 5 p-CPA and 3 vehicle treated animals, on the other hand, were killed 2 hr following flinch-jump testing.

Statistical Analysis

Analysis of the flinch-jump data was conducted by means of the Mann-Whitney U test, two-tailed, with the confidence limit set at the 5% level. Other statements of statistical significance are based on Student's t test, two-tailed, $p<0.05$.

RESULTS

All animals survived the surgery and appeared healthy. At the time of testing no significant differences in group body weights were found.

Histology

Five animals from the median, 5 from the dorsal, and 4 from the combined raphe lesion groups were eliminated from the study prior to analysis of the behavioral or biochemical data because the lesions in these animals either were unilateral or did not damage the target nuclei. The accepted lesions are shown schematically in Figs. 1 and 2.

Median raphe lesions. These lesions ablated 30–65% of the median raphe nucleus (Fig. 1). In Rat R-41 the lesion was confined to the median raphe nucleus. In the remaining 4 animals the lesion extended into the medial longitudinal fasciculus, ventral tegmental nucleus of Gudden, and the tecto-spinal tract. The lesion invaded the decussation of the superior cerebellar peduncle only in one rat (R-14).

Dorsal raphe lesions. These lesions destroyed the rostral 30–60% of the dorsal raphe nucleus (Fig. 1). The ventromedial aspect of the periacqueductal grey, the trochlear nucleus, and medial longitudinal fasciculus were damaged either uni- or bilaterally in all five animals.

Dorsal plus median raphe lesions. The lesions in this group were not uniform (Fig. 2). The lesions ablated at least 30% (virtually 100% in Rat R-1) of the dorsal raphe nucleus. In 4 rats the caudal part of the oculomotor nuclei was damaged. In one rat (R-40) the rostral and intermediate linear nuclei were invaded. The lesions also destroyed 20–60% of the median raphe nucleus and extended rostrally to the level of the decussation of the brachium conjunctivum, except in 3 animals (R-17, R-21, and R-40). The median longitudinal fasciculus, tegmental nuclei of Gudden, and tecto-spinal tract were all subtotally invaded. The decussation of the brachium conjunctivum was damaged in only 2 animals (R-1 and R-33).

5,7-DHT injected animals. Injections of 5 μg (in 5 μl of vehicle) of 5,7-DHT into the vicinity of both the dorsal and median raphe nuclei produced areas of cell loss (cavitation) well-demarcated by surrounding and infiltrating glial proliferation (Fig. 1). Similar effects of intracerebrally injected 5,7-DHT have been described by Björklund *et al.* [4]. The area damaged, however, was not as extensive as that seen subsequent to the electrolytically produced lesions described above. The medial longitudinal fasciculus, decussation of the brachium conjunctivum, and region immediately dorsal to the caudal one-half of the interpeduncular nucleus were damaged in three rats. The dorsal and median raphe nuclei were only slightly affected.

Although cell counts were not performed and cell shrinkage was not thoroughly examined, the cells outside the area of glial scarring appeared normal (as in the case of the electrolytic lesions). The large and small-sized cells making up the dorsal and median raphe nuclei thus appeared unchanged (within the range of light microscopy) after serotonin depleting intramidbrain injections of 5,7-DHT. However, because of the location of the lesion damage in these animals (Fig. 1), the mechanical or nonspecific (see Myers [25]) effects of the injections cannot be discriminated from their neurotoxic effect with respect to the induced fall in forebrain 5-HT. The low forebrain 5-HT concentration of R-52 (37 ng/g) and the relatively high forebrain 5-HT content of R-57 (135 ng/g) could be explained by the rostral extent of the lesions (see Lorens and Guldberg [18]) as well as by the most effective site of injection and uptake (see Björklund *et al.* [4]).

Biochemical Analysis

As seen in Table 1, all lesion and drug treatments produced significant reductions in forebrain 5-HT. The 22, 48 and 70% reductions in forebrain 5-HT subsequent to median, dorsal, and combined raphe lesions, furthermore, differed significantly from each other. It should be mentioned, in addition, that we recently have found (unpublished data) that regional forebrain norepinephrine and striatal dopamine concentrations were not affected by similarly placed lesions and 5,7-DHT injections.

Flinch-Jump Thresholds

Only the p-CPA treated animals showed a significant (n_1, n_2 = 3,5; U = 0; p = 0.036) reduction (40%, versus the vehicle injected group) in jump threshold (Table 1). Flinch threshold was not significantly affected. Thus, significant reductions in forebrain 5-HT subsequent to midbrain raphe electrolytic lesions or 5,7-DHT injections did not result in increased sensitivity to electric shock as reflected in lowered flinch-jump thresholds. Furthermore, there was no evidence of postshock running.

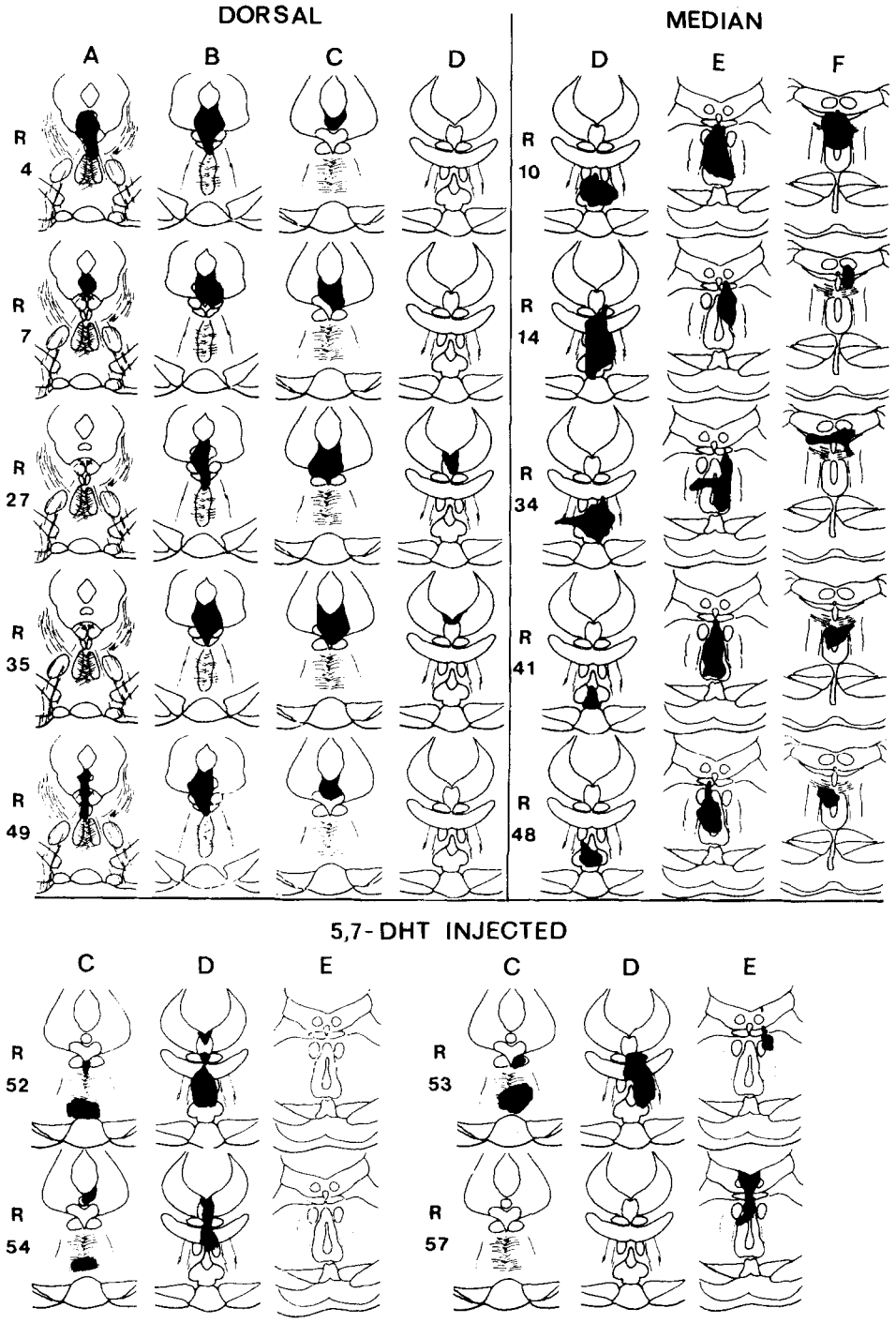

FIG. 1. Reconstruction of damage (blackened area) in the dorsal raphe lesion, median raphe lesion, and 5,7-DHT injected groups. Numbers identify individual animals. Letters refer to coronal plane (see Fig. 2).

One-way Avoidance Acquisition

Figure 3 shows that both the M (n = 3) and DM (n = 4) lesion animals required significantly more trials (these animals also committed more errors and received more shocks) to reach criterion than the control group (n = 6). This retardation of one-way avoidance acquisition was clearly more severe in the combined (DM) lesion rats. Analysis of response latencies showed a similar picture. The only deficit in the D (n = 2) raphe lesion group was a prolonged escape latency during the first three trials.

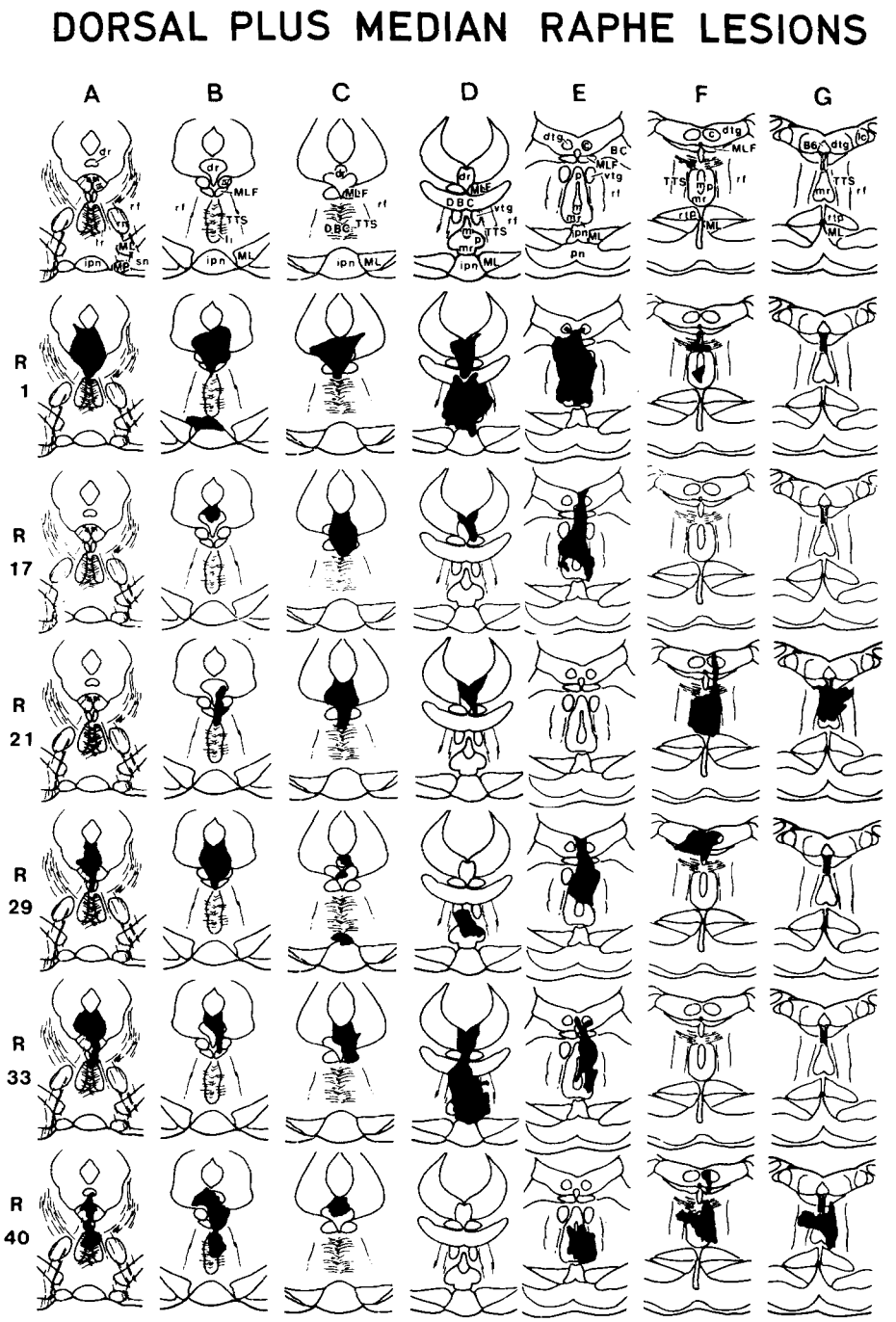

FIG. 2. Reproductions of dorsal plus median raphe lesions (blackened area) on 7 coronal planes each separated by approximately 0.6 mm. Numbers identify individual animals. Abbreviations: c: dorsal tegmental nucleus, pars centralis; dr: dorsal raphe nucleus; dtg: dorsal tegmental nucleus of Gudden; ipn: interpeduncular nucleus; lc: locus coeruleus; li: intermediate linear nucleus; lr: rostral linear nucleus; m: median raphe nucleus, medium-sized cell part [30]; mr: median raphe (or, central superior) nucleus; p: median raphe nucleus, small-sized cell region [30]; pn: basal pontine nuclei; rn: red nucleus; rf: reticular formation; rtp: pontine tegmental reticular nucleus; sn: substantia nigra; vtg: ventral (or, deep) tegmental nucleus of Gudden; B6: cell group B6 of Dahlström and Fuxe [6]; caudal extension of dorsal raphe nucleus [30]; BC: brachium conjunctivum (superior cerebellar peduncle); DBC: decussation of brachium conjunctivum; III: oculomotor nucleus; IV: trochlear nucleus; ML: medial lemnisucs; MP: mammillary peduncle; MLF: medial longitudinal fasciculus; TTS: tecto-spinal tract.

TABLE 1

MEAN (± STANDARD DEVIATION) FOREBRAIN 5-HYDROXYTRYPTAMINE (5-HT) CONCENTRATION (ng/g) AND FLINCH-JUMP THRESHOLDS (mA)

Group	n	5-HT	%	Flinch	Jump
Control	10	323 ± 42		0.11 ± 0.02	0.33 ± 0.11
Dorsal	5	167 ± 18†	−48	0.11 ± 0.02	0.40 ± 0.08
Median	5	250 ± 11*	−22	0.11 ± 0.03	0.40 ± 0.11
Dorsal + Median	6	95 ± 44‡	−70	0.12 ± 0.14	0.40 ± 0.14
5–7 DHT	4	78 ± 42‡	−75	0.10 ± 0.04	0.42 ± 0.04
Vehicle	3	285 ± 20		0.10 ± 0.03	0.43 ± 0.04
PCPA	5	30 ± 7§	−89	0.07 ± 0.03	0.26 ± 0.06§

*Significantly lower than control group.
†Significantly lower than control and median lesion groups.
‡Significantly lower than the control group and the median and dorsal lesion groups.
§Significantly lower than vehicle group.

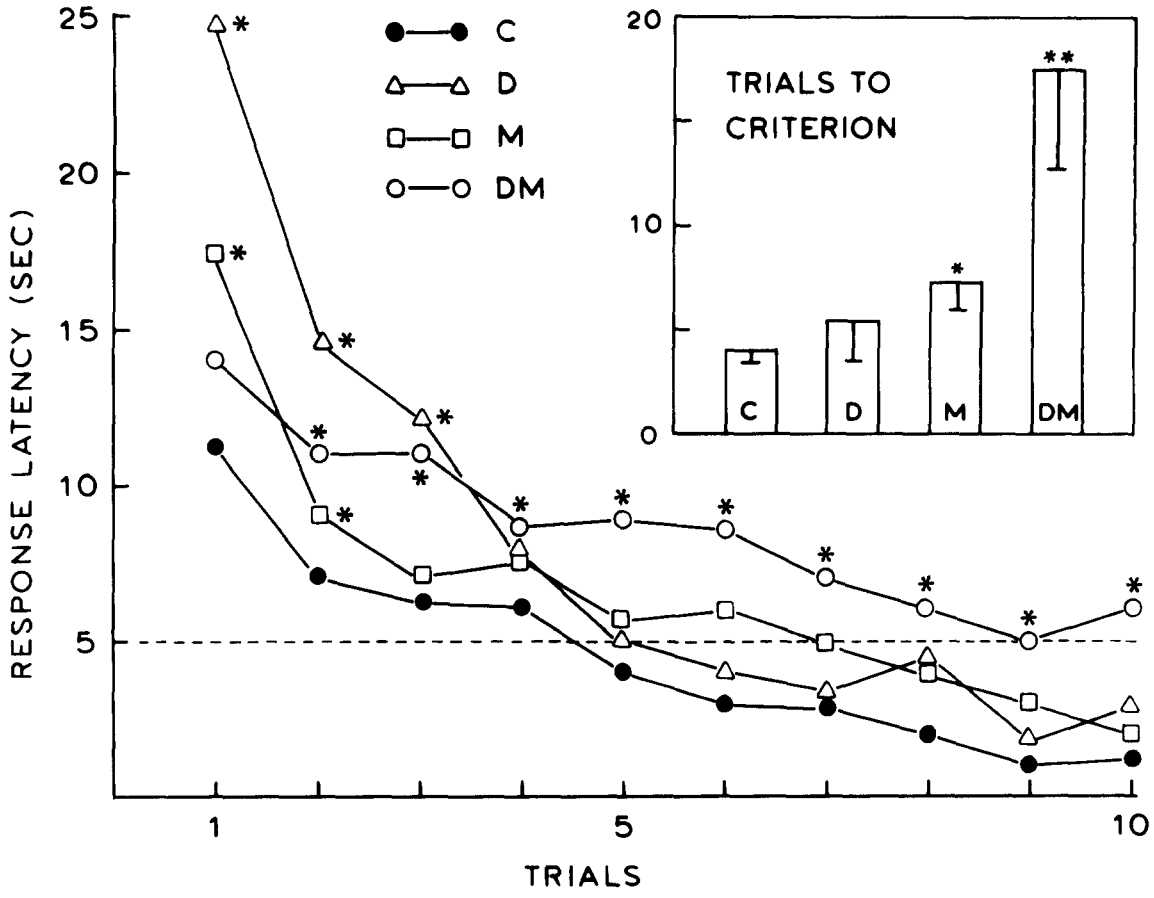

FIG. 3. Mean response (escape or avoidance) latencies during first ten trials and mean (± standard deviation) trials to criterion (insert) during one-way avoidance performance of control (C; n = 6), dorsal (D; n = 2), median (M; n = 3), and dorsal plus median (DM; n = 4) raphe lesion animals. Single asterisk indicates a significant difference from the control group; double asterisk indicates significant difference from all 3 other groups.

Although the group n's were small (the results from 4 D, 3 M, and 2 DM rats were excluded because of inappropriate lesions; see above), these data, nevertheless, largely replicate those of a previous study [29].

DISCUSSION

Lesions in the dorsal (D), median (M), or both (DM) midbrain raphe nuclei produced significant reductions in forebrain 5-HT, but failed to affect shock sensitivity as measured by the flinch-jump technique. On the other hand, the midbrain raphe lesions significantly affected the acquisition of a one-way avoidance response. The M and DM lesion animals required significantly more trials to reach the one-way avoidance criterion. The DM lesion rats, furthermore, were the most severely affected. The D lesion animals did not differ from the group except in terms of prolonged escape latencies during the first three trials. These results are consistent with those of a previous study [29] and suggest, furthermore, that the effects of midbrain raphe lesions on avoidance learning [17, 19, 20, 29] are not due to either hypo- or hyperalgesia.

The lack of effect of lesions in the brainstem 5-HT B7 (dorsal raphe nucleus), B8 (median raphe nucleus), or both cell groups [6] on jump threshold is in agreement with the reports of Harvey *et al.* [10] and Pepeu *et al.* [26]. In addition, it supports the observations of other studies [5, 20, 27] which did not find changes in pain sensitivity after raphe lesions as measured by the tail flick and hot plate techniques.

Harvey and Lints [9] have argued that MFB lesion induced hyperalgesia is secondary to reductions (77%) in the telencephalic concentration of 5-HT. This conclusion is based largely on their observed 0.8 correlation between jump threshold and telencephalic 5-HT concentration, as well as on the observation that p-CPA injections in MFB lesion animals produced additional reductions in brainstem and diencephalic 5-HT, but did not further lower the jump threshold.

We have found that 70–75% reductions in forebrain (including telencephalon) 5-HT following midbrain raphe electrolytic lesions or 5,7-DHT injections did not change jump thresholds. Two rats had forebrain 5-HT concentrations of 37 (R-52, after 5,7-DHT) and 32 (R-1, after DM lesions) ng/g (both within the p-CPA treatment range, 21–39 ng/g) but demonstrated jump thresholds (0.42 and 0.33 mA, respectively) within normal range. Thus, a relationship between forebrain (including telencephalon) 5-HT concentration and pain sensitivity is not supported by our data, nor that referred to above [5, 10, 20, 26, 27].

Harvey *et al.* [10] recently have reported that ventral central grey lesions (including dorsal raphe nucleus damage) resulted in a 46% decrease in telencephalic 5-HT but produced hypoalgesia (longer paw lick latency after placement on a hot plate) during both light and dark hours. Ventral central grey plus dorsal and median raphe nuclei lesions also produced hypoalgesia (but only during the dark hours) in spite of a 66% decrease in telencephalic 5-HT. Earlier Melzack *et al.* [22], using a one-way hurdle jump escape task, suggested that the central tegmental fasciculus (lesions in which produced hyper-responsiveness but not escape deficits) and the central grey (lesions restricted to which produced deficits in escape behavior) "have opposing roles in the total afferent pain process" such that an "additional lesion in the central tegmental fasciculus somehow 'cancels out' the effects of a satisfactory central grey lesion" (p. 365). Citing Melzack *et al.* [22], Harvey *et al.* [10] suggested that their 5-HT depleting raphe lesions failed to produce hyperalgesia because the additional central grey damage may have counteracted this effect.

The lesions reported in the present study (Figs. 1 and 2) did not involve the central grey (except for the dorsal raphe nucleus in the D and DM lesion groups). Our dorsal raphe (D) lesion group, furthermore, did not evidence hypoalgesia (a higher jump threshold), as might be expected if the central grey had been seriously invaded. In addition, our median raphe (M) lesion and 5,7-DHT injected rats did not sustain central grey damage, but, in spite of significant reductions in forebrain 5-HT, showed normal flinch and jump thresholds. It seems clear, therefore, that a reduction in the brain concentration of 5-HT is not a sufficient condition for hyperalgesia. Whether such a reduction is necessary for the appearance of hyperalgesia, however, remains to be determined.

It should be noted that lesions reported to produce lowered jump thresholds not only affect 5-HT concentration but norepinephrine (NE) as well [9, 16, 23]. Bläsig *et al.* [3], for example, have argued that the catecholamines play an important role in the mediation of nociceptive stimuli. Thus, one possibility is that in order for 5-HT depleting lesions to affect pain sensitivity, they must simultaneously reduce NE concentration as well. Unfortunately, forebrain NE concentration was not measured in the present study. However, after similar lesions in the rat we [18] did not find any changes in regional NE content. The effectiveness of 5-hydroxytryptophan in reversing MFB lesion or p-CPA induced hyperalgesia [9, 15, 31, 32], furthermore, may be due to its uptake not only by intact 5-HT neurons but by catecholamine neurons as well [25].

The now well established property of p-CPA to increase pain sensitivity cannot be ascribed unequivocally to its central effects. Furthermore, in addition to its inhibitory effect on tryptophan hydroxylase, p-CPA also inhibits transport into the brain of some amino acids [8], inhibits tyrosine hydroxylase, and reduces, although temporarily, catecholamines throughout the organism [12].

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