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Open Field Activity and Avoidance Behavior Following Serotonin Depletion: A Comparison of the Effects of Parachlorophenylalanine and Electrolytic Midbrain Raphe Lesions

CHRISTER KÖHLER[1] AND STANLEY A. LORENS[2]

Institute of Psychology and Department of Pharmacology, University of Bergen, Bergen, Norway

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KÖHLER, C. AND S. A. LORENS. *Open field activity and avoidance behavior following serotonin depletion: a comparison of the effects of parachlorophenylalanine and electrolytic midbrain raphe lesions.* PHARMAC. BIOCHEM. BEHAV. 8(3) 223–233, 1978. – Three experiments were performed in order to compare the behavioral effects of electrolytic destruction of the dorsal and median mesencephalic raphe nuclei (MR lesion) and parachlorophenylalanine (pCPA; 300 mg/kg, IP) administration. Forebrain 5-hydroxytryptamine (5–HT) was measured in all animals following completion of behavioral testing. In the first experiment open field behavior (one 50 min session) and two-way (shuttle) conditioned avoidance acquisition (50 massed trials) were examined 68–72 hr after vehicle or pCPA administration in rats which had received control operations or MR lesions two weeks earlier. Only the MR lesion and the MR lesion + pCPA groups evidenced increased open field activity and facilitated two-way avoidance learning. Although the reduction in forebrain 5–HT of the pCPA group (85%) was greater than in the MR lesion group (55%), the pCPA treated animals did not differ from the control group. In the second experiment animals were tested in the open field 24, 48 or 72 hr after pCPA treatment to determine its effects on activity level as a function of the time after injection. No differences between the vehicle and pCPA groups, however, were found. In the third experiment, the effects of pCPA (72 hr postinjection) on the acquisition of an unsignalled one-way avoidance response was examined. MR lesion rats tested in the same apparatus and with the same procedure repeatedly have been shown to be impaired in this task. The pCPA and vehicle animals, however, did not differ. Reductions in 5–HT following electrolytic MR lesions and pCPA administration, thus, produce different behavioral effects. MR lesions, but not pCPA treatment, result in (1) increased activity in a novel environment, (2) facilitated two-way conditioned avoidance learning, and (3) impaired acquisition of an unsignalled one-way avoidance response. These data support earlier studies suggesting that the behavioral effects of electrolytic MR lesions are not due primarily to their disruption of ascending 5-HT pathways. The role of 5–HT in avoidance conditioning and the regulation of activity level, furthermore, remains to be elucidated.

5–Hydroxytryptamine Midbrain raphe Parachlorophenylalanine Open field activity Conditioned avoidance acquisition

SEVERAL methods have become available to study the behavioral effects of central 5-hydroxytryptamine (5–HT, serotonin) depletion. Most prominently these include: (1) electrolytic midbrain raphe lesions [12, 17, 21, 26, 38]; (2) systemic administration of the tryptophan hydroxylase inhibitor, parachlorophenylalanine (pCPA) [18, 19, 41, 43]; or (3) of the neurotoxic agent, 4-chloroamphetamine (pCA) [10, 20, 33, 36, 42]; and (4) the central injection of the neurotoxic hydroxylated tryptamines, 5,6- and 5,7-dihydroxytryptamine (5,6–DHT; 5,7–DHT) [2, 4, 25].

Recently it has become apparent that these methods can produce different behavioral effects. This is due in part to variations in the testing procedures employed. For example, one week following pCA administration, acquistion of a conditioned shuttle avoidance response can be facilitated [42] or severely impaired [33] depending on the paradigm and apparatus used. Comparison of different methods of 5–HT depletion on the same behavioral measure also can produce different results. Thus, pCPA, but not electrolytic raphe lesions or intrabrainstem 5,7–DHT administration, increased pain sensitivity as measured by the flinch-jump technique [13,14]. These data suggest that although central 5–HT depletion may be important for the appearance of

[1] Department of Neuropharmacology, Astra Läkemedel AB, S–151 85 Södertälje, Sweden.
[2] Reprint requests to S. A. Lorens, Department of Pharmacology – Bldg. 135, Stritch School of Medicine, Loyola University, Maywood, IL 60153.

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certain behavioral changes, differences in the mode of action and specificity of the different methods of depleting 5–HT are critical in determining their behavioral effects [23].

We have suggested recently that the enhanced open field activity and impaired one-way avoidance learning of animals with electrolytic midbrain raphe lesions are not due exclusively to their interruption of ascending 5–HT pathways [25,38] and could well be due to damage of adjacent tegmental nuclei and/or their projections [24]. Intrabrainstem 5,7–DHT administration, thus, reduced regional forebrain 5–HT to as great (or greater) extent as electrocoagulation of the mesencephalic raphe nuclei, but did not affect open field locomotion or one-way avoidance conditioning [25].

In the present study we have compared the effects of pCPA and electrolytic midbrain raphe lesions on open field activity and conditioned avoidance acquisition tested with identical procedures and in the same apparatus. A similarity in the behavioral effects of the two treatments would support the hypothesis that the behavioral changes are related to a disruption of 5–HT metabolism and a resultant reduction in central 5–HT level. The observation that pCPA, in contrast to midbrain raphe electrocoagulation, failed to affect open field behavior and conditioned avoidance learning does not support this hypothesis, and provides further evidence for the view that the behavioral effects of electrolytic midbrain raphe lesions are due to their disruption of non–5–HT pathways [23, 24, 25, 38].

EXPERIMENT 1

Electrolytic destruction of both the dorsal and median mesencephalic raphe nuclei consistently has been observed to facilitate the acquisition of a two-way (shuttle) conditioned avoidance response [26, 27, 38]. Selective lesions in the dorsal or median raphe nucleus, however, are not effective [27,38]. To our knowledge, the effects of pCPA on two-way avoidance learning have not been examined, except in a recent study by Stoff, Wyatt, and Gillin [40]. In that study, pCPA (150 mg/kg) 24 hr postinjection did not affect two-way avoidance acquisition in comparison to controls. Thus, one of the objectives of the present experiment was to compare the effects of pCPA and electrolytic raphe lesions on two-way avoidance conditioning.

Electrocoagulation of the midbrain raphe has been reported to enhance running wheel, stabilimeter cage, and open field activity [12, 15, 17, 26, 38]. Increased activity is seen after selective lesions of the median but not of the dorsal raphe nucleus [12, 15, 17, 38], and is evidenced immediately after placement of the lesion animals in the test situation. Higher activity levels also have been observed in pCPA treated animals, but not until 30 min after placement in a novel environment, or beginning 24 hr after injection if the animals reside in the test chamber [9, 16, 28, 35]. In contrast, several authors have reported no change or decreased locomotor activity after pCPA [5,41] or pCA [20,42]. The second objective of this experiment, therefore, was to compare the behavior of pCPA treated and electrolytic midbrain raphe lesion rats in an open field during a 50 min session.

METHOD

Animals

The animals were 33 male albino rats (Møll-Wistar) weighing 291–321 g at the time of surgery. The animals were housed individually in cages located in a temperature (22 ± 1°C) and illumination (12 hr dark-light cycle) controlled room. Food and water were available ad lib. All testing was carried out during the light phase (08:00–20:00).

Surgical, Biochemical, and Histological Procedures

Electrolytic lesions (n = 15) were produced under sodium pentobarbital anaesthesia (50 mg/kg, IP) in the dorsal and median raphe nuclei as detailed previously [38]. In brief 2 mA DC was passed for 10–15 sec between an intracerebral cathode and an anode clipped to the wound margin. Operated control rats (n = 18) underwent the same procedure except that an electrode was not lowered intracranially.

At the end of the experiment the animals were sacrificed by cervical dislocation. The brains were rapidly removed, and the forebrain separated from the brainstem by means of a dorsoventral knife cut which passed just rostral to the superior colliculus and just caudal to the mammillary body. The forebrain was frozen in dry ice and subsequently assayed for 5–HT as previously described [38]. The brainstem was placed in Formalin for histological analysis as detailed previously [38].

Drug

Parachlorophenylalanine (pCPA; Pfizer) was suspended in 2.0% Tween–80 in distilled water to a final concentration of 60 mg/ml.

Apparatus

All testing was conducted in a sound attenuated room immediately adjoining the animal quarters. A background white noise (55 dB) was provided by the ventilation fan.

Activity measures were obtained in an open-field measuring 100 cm × 100 cm × 40 cm high. The floor was painted white and divided by black stripes into 25 squares (20 cm × 20 cm). A 15 W lamp was located 100 cm above the center of the field.

Avoidance conditioning was conducted in a shuttle-box consisting of two equal compartments (50 cm × 24 cm × 30 cm) separated by a 7 cm high hurdle (see ref. [38] for additional details). The conditional stimulus (CS) was a 75 dB white noise delivered through loudspeakers located in the end walls. Constant current (0.5 mA) shock (US) could be delivered by Grason-Stadler equipment to either compartment.

Procedure

Beginning 19–21 days postoperatively the animals were handled daily (4–5 min/day). On Days 21–23 the animals received either pCPA (300 mg/kg) or vehicle (5 ml/kg, 2.0% Tween–80 in distilled water) intraperitoneally. Thus, four groups were obtained: (1) animals receiving control operations plus vehicle injections (vehicle group; n = 8); (2) control operations plus pCPA (pCPA group; n = 10); (3) raphe lesions plus vehicle injections (MR group; n = 10); and (4) raphe lesions plus pCPA (MR + pCPA group; n = 5).

Behavioral measures were obtained during the third day (68–72 hr) postinjection.

Open field testing was conducted between 08:00–14:00 and began by placing the rat in the center of the field. The number of squares entered, rearings (rat stands on hind limbs, sniffing), and grooming responses (cleaning body surface with forepaws and/or mouth) emitted per 5 min was scored for 50 min. During the first 5 min of the session these behaviors were tallied for each min. The latency to the first grooming response and the number of fecal boli also were noted.

Acquisition of a two-way (shuttle) avoidance response was examined between 14:00–18:00. Following placement in the apparatus the rat was allowed to adapt for 3 min. Subsequently, 50 conditioning trials (intertrial interval = 30 sec) were given. If the rat did not cross to the other compartment within 5 sec after onset of the CS, the US was administered until the animal made an escape response. The CS was turned off when the rat crossed the barrier to the opposite compartment. If the rat failed to escape, the CS and shock were terminated after 30 sec. Intertrial barrier crossings were scored but not punished. Escape and avoidance latencies were measured with a stop-watch. Fecal boli were counted after the session.

Two hr following completion of avoidance testing each animal was sacrificed and its brain obtained for histological and biochemical analysis.

RESULTS

General Observations

All animals gained weight postoperatively and appeared healthy. All pCPA treated rats lost weight (12–21 g) from the day of injection to the time of sacrifice. However, no significant between group differences in body weight were found, either at the time of injection or the time of sacrifice.

Lesion Analysis

The lesions were confined to the midline and destroyed 20–75% of the dorsal and median raphe nuclei (Fig. 1). The dorsal raphe lesions reached the level of the trochlear nucleus in 6 rats and the oculomotor nucleus in 2 animals. The dorsal raphe nucleus was damaged bilaterally in all rats, although the lesions were assymetrical in 6 animals. The median raphe lesions were primarily unilateral in 5 animals and spared the rostral one-fourth of the median raphe nucleus in 9 rats. The medial longitudinal fasciculus, Gudden's tegmental nuclei, the pontine raphe nucleus, and the B6 5–HT cell group were damaged bi- or unilaterally in most of the animals.

Forebrain 5–HT

As seen in Table 1, the MR lesions produced a 55%

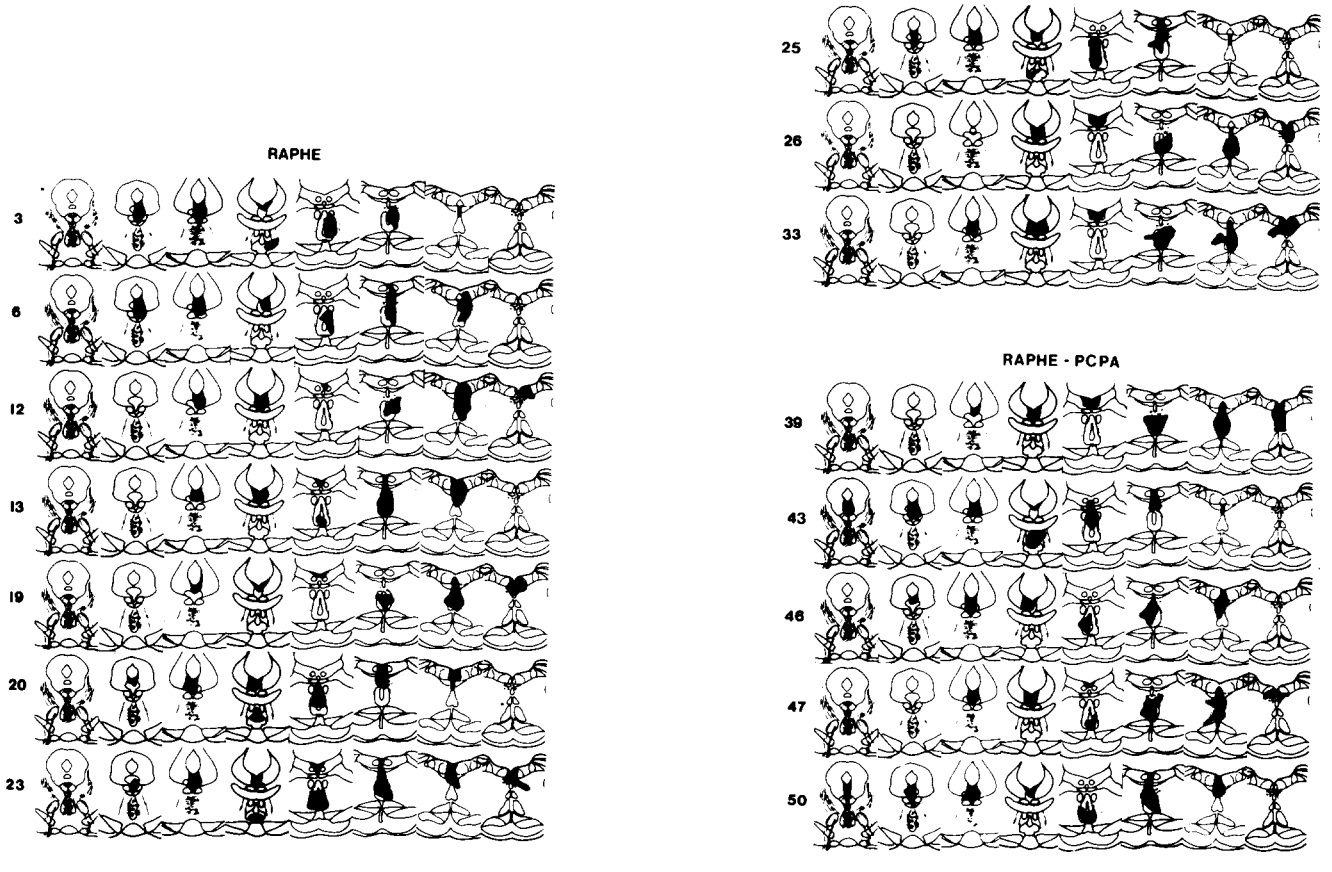

FIG. 1. Reproductions of raphe lesions (blackened area) on 8 coronal planes (from 38), each separated by about 0.6 mm. Numbers identify individual animals.

TABLE 1

MEAN (± SD) FOREBRAIN 5-HYDROXYTRYPTAMINE (5-HT) CONCENTRATIONS (NG/G) 24–26 DAYS AFTER ELECTROLYTIC MIDBRAIN RAPHE LESIONS (RAPHE) OR CONTROL OPERATIONS (CONTROL) AND 73 HR AFTER PARACHLOROPHENYLALANINE (300 MG/KG, IP) OR VEHICLE INJECTIONS

Group	n	5-HT	% Change
Control + Vehicle	8	272 ± 51	–
Control + pCPA	10	42 ± 11	85†
Raphe + Vehicle	10	122 ± 46	55*
Raphe + pCPA	5	40 ± 54	85†

*Significantly lower than control + vehicle group ($p<0.01$, t-test, two-tailed)

†Significantly lower than both the control + vehicle ($p<0.0001$, t-test) and raphe + vehicle ($p<0.01$, t-test) groups

decrease in forebrain 5–HT. The pCPA and MR + pCPA groups, which themselves did not differ, showed 85% reductions in forebrain 5–HT which were significantly greater than in both the MR lesion and control groups.

Open Field Behavior

Squares entered. An overall two-way analysis of variance (ANOVA) for squares crossed per 5 min during the 50 min session revealed significant treatment ($F(3.29) = 8.475$, $p<0.005$), time ($F(9.261) = 35.119$, $p<0.0001$), and interaction ($F(27.261) = 3.311$, $p<0.0001$) effects. Individual between group comparisons showed that the MR ($F(1.16) = 17.691$, $p<0.0009$) and the MR + pCPA ($F(1.11) = 9.286$, $p<0.01$) groups, but not the pCPA treated group, were more active than the control group (Fig. 2). Furthermore, the MR ($F(1.18) = 17.079$, $p<0.0009$) as well as the MR + pCPA ($F(1.13) = 10.176$, $p<0.007$) groups also crossed more squares than the pCPA group. The MR and the MR + pCPA groups did not differ.

Overall ANOVA (min by min) of the squares entered during the first 5 min in the open field also yielded significant treatment ($F(3.31) = 12.208$, $p<0.0001$), time ($F(4.124) = 5.758$, $p<0.005$), and interaction ($F(12.124 = 2.616$, $p<0.004$) effects. Individual analysis showed that the MR ($F(1.18) = 13.218$, $p<0.002$) and MR + pCPA ($F(1.13) = 8.714$, $p<0.01$) groups, but not the pCPA group, crossed significantly more squares than the control rats. The MR lesion and MR + pCPA groups, furthermore, also crossed more squares than the pCPA group ($p<0.03$). In addition, the MR + pCPA animals were more active, ($F(1.13) = 21.557$, $p<0.007$) than the MR group. Only the MR group was significantly more active ($t = 2.19$, $df = 18$, $p<0.05$) than controls during the first minute of exposure to the open field (Fig. 2, inset). No other between group differences were found. None of the groups showed a reduction in activity during the first 5 min period. However, in contrast to all other groups, the control rats evidenced an increase ($t = 2.44$, $df = 9$, $p<0.05$) in locomotor activity during this period.

The pCPA treated rats evidenced a significant within group decrease ($t = 2.79$, $df = 9$, $p<0.05$) in activity as measured by comparing the first and third 5 min block. Within 25 min, however, all animals significantly ($p<0.05$, t-test) decreased their locomotor activity. Compared with the MR + pCPA group, the MR group was significantly ($t = 3.51$, $df = 9$, $p<0.01$) less active during the last 25 min of the session. During this period neither the MR nor the MR + pCPA groups reached the same level of activity as the control or pCPA treated rats (see Fig. 2).

The MR and pCPA groups, furthermore, exhibited more crossings in the center of the field, as compared to the controls (both $p<0.05$). No other group differences were observed.

Rearing. An overall ANOVA of rearings emitted during each 5 min of the 50 min session did not reveal a significant treatment effect. During the first 5 min in the open field the pCPA ($t = 3.54$, $df = 9$, $p<0.01$) and MR ($t = 2.27$, $df = 9$, $p<0.05$) groups significantly increased their rearing activity. Within 25 min, however, all groups, except the MR + pCPA group, had significantly (all $p<0.01$, t-test) decreased their rearing activity.

Other measures. No significant group differences were observed with regard to the time to leave the center of the field, the latency to the first grooming response, or the number of fecal boli dropped during the session.

Summary. The pCPA treated animals did not differ from the control group in terms of open field locomotor and rearing activity, except that, like the MR group, they crossed more squares in the center of the field. The MR and MR + pCPA groups were hyperactive in terms of locomotor but not rearing activity. All animals, except the MR + pCPA group, significantly decreased their locomotor and rearing activity over the 50 min session, suggesting an additive effect of the two treatments on habituation.

Two-way Active Avoidance Acquisition

An overall ANOVA of avoidance acquisition based on five blocks of 10 trials, revealed significant treatment ($F(42.9) = 4.939$, $p<0.007$), time ($F(4.116) = 147.162$, $p<0.0001$) and treatment × time interaction ($F(12.116) = 2.698$, $p<0.003$) effects. Individual between group comparisons showed that the MR ($F(1.16) = 10.973$, $p<0.005$) and MR + pCPA ($F(1.11) = 5.113$, $p<0.04$), but not the pCPA group, differed from the controls (Fig. 3). Furthermore, the MR ($F(1.18) = 9.675$, $p<0.006$) and the MR + pCPA ($F(1.13) = 4.699$, $p<0.05$) groups made significantly more avoidance responses than the pCPA group. The MR and MR + pCPA groups, however, did not differ significantly.

The MR and the MR + pCPA groups made significantly more avoidance responses within the first 10 trials compared to the control group (all $p< 0.01$, t-test). During the second block of 10 trials the MR group made significantly more avoidance responses compared to both the control and the pCPA treated groups ($p<0.01$, t-test). During the same period the MR + pCPA group made significantly more avoidance responses than the control ($t = 2.87$, $df = 11$, $p<0.01$) group.

The groups did not differ with regard to the number of barrier crossings made during the 3 min adaptation period immediately preceding the first conditioning trial. After the first shock, however, the MR ($t = 4.59$, $df = 16$, $p<0.001$) and the MR + pCPA ($t = 3.98$, $df = 11$, $p<0.01$) groups made significantly more intertrial crossings during the

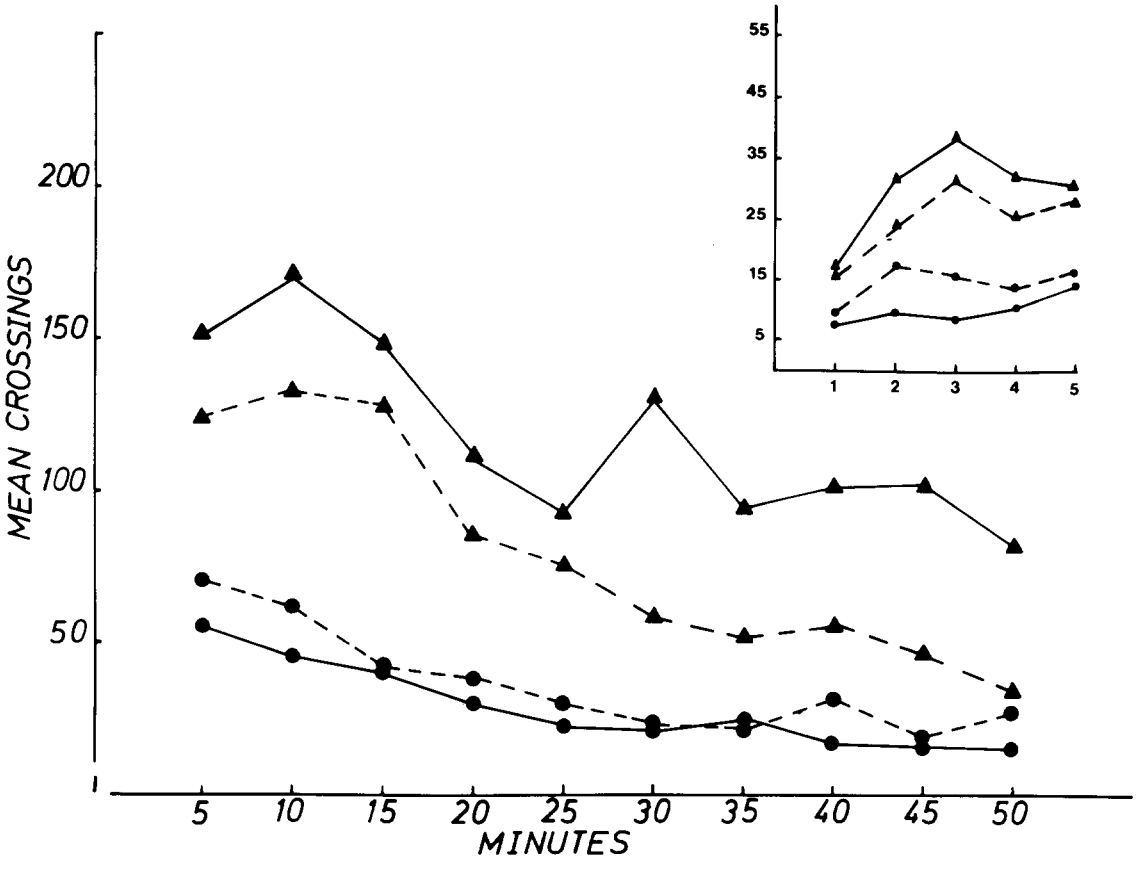

FIG. 2. Group mean number of squares entered per 5 min during open field testing 3 days after vehicle or pCPA (300 mg/kg) administration in rats receiving control or midbrain raphe lesions two weeks earlier. Both the raphe lesion plus vehicle (▲– – –▲; n = 10) and lesion plus pCPA (▲———▲; n = 5) groups showed a significantly higher level of activity compared to the control plus vehicle (●———●; n = 8) and control plus pCPA (●– – –●; n = 10) groups. Inset shows mean squares entered per min for each group during the first 5 min of the session.

remainder of the session than the control group. The pCPA treated group made significantly less intertrial barrier crossings compared to both the MR (t = 4.48, df = 18, $p<0.001$) and the MR + pCPA (t = 3.41, df = 13, $p<0.01$) groups, but did not differ from the control group. No group differences were observed with regard to number of fecal boli dropped during the session.

Summary. The pCPA treated animals did not evidence any differences from the control group during the acquisition of the two-way active avoidance task. The MR lesion animals, as well as the MR + pCPA treated rats, made more avoidance responses than either the control or pCPA group, especially during the initial 20 conditioning trials. Subsequent to the first trial, the MR lesion and MR + pCPA groups also showed a significantly higher number of intertrial crossings than either of the other groups.

DISCUSSION

The above results indicate that pCPA treatment does not produce the increased open field activity or facilitated acquisition of a two-way conditioned avoidance response seen after electrolytic dorsal plus median midbrain raphe lesions. The pCPA data are in agreement with the reports of Tenen [41], Brody [5] and Stoff *et al.* [40]. Along with the demonstration [25] that intrabrainstem 5,7–DHT administration does not affect open field activity or one-way avoidance acquisition, in spite of dramatic reductions in 5–HT throughout the forebrain, the present experiment supports the view that the behavioral effects of electrocoagulation of the mesencephalic raphe nuclei are not due primarily to a disruption of ascending 5–HT projections.

Differences in the effects of pCPA and MR lesions on shuttlebox avoidance could be due to their differential effects on pain sensitivity. pCPA, but not MR lesions, has been reported to produce hyperalagesia as measured by the flinch jump and hot plate tests [13,14]. Increasing the intensity of the unconditioned stimulus (shock) leads to slower learning of a two-way avoidance response [2,22]. On the other hand, pCPA did not alter the effect of MR lesions on either two-way avoidance learning or open field locomotion, suggesting that the peripheral effects of pCPA are not the major factor underlying the differential behavioral effects of pCPA and MR lesions.

EXPERIMENT 2

The lack of an effect of pCPA on open field activity disagrees in part with the findings of Pirch [35] and Fibiger

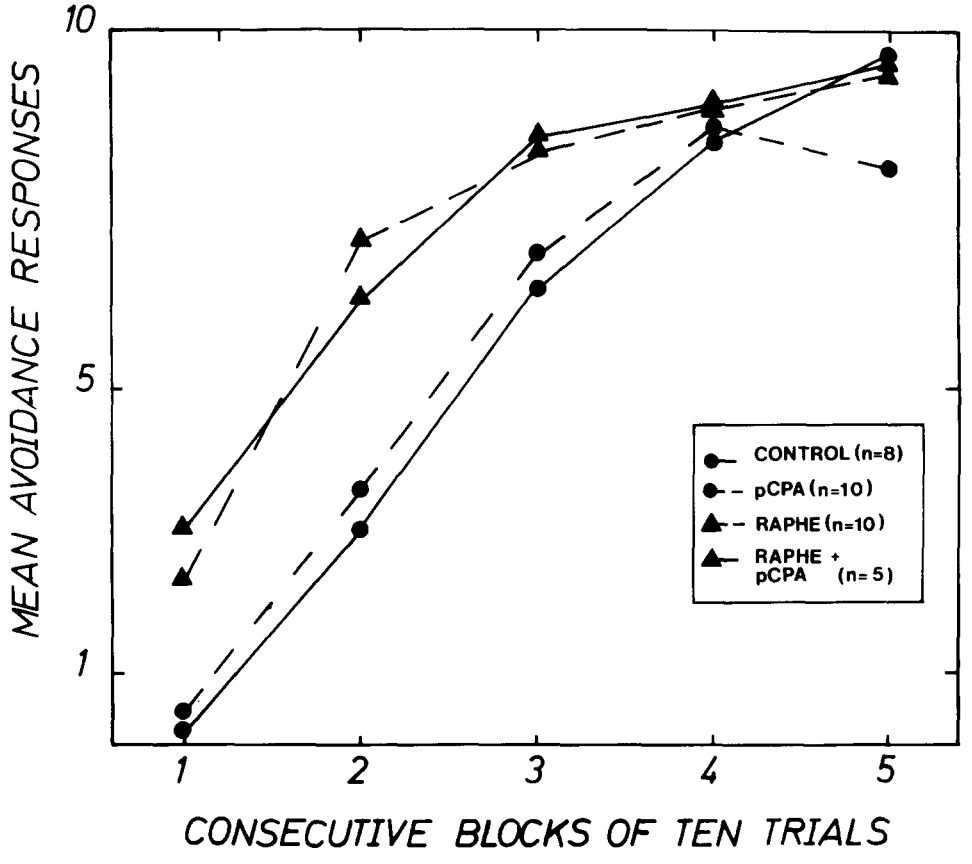

FIG. 3. Effects of pCPA (300 mg/kg) and raphe lesions on two-way (shuttle) conditioned avoidance acquisition. Each rat was given a total of 50 massed trials. Group means are shown for each block of 10 trials. Both the raphe and raphe + pCPA groups showed more rapid acquisition than the other two groups.

and Campbell [9]. These workers reported that their pCPA treated animals maintained a higher level of activity than controls, beginning about 30 min after the animals had been placed in the test situation. Pirch [35] and Fibiger and Campbell [9], however, tested their animals 24 and 48 hr after injection, respectively, whereas the animals in Experiment 1, as well as in the studies of Tenen [41] and Brody [5], were observed 72 hr after pCPA administration.

pCPA inhibits both phenylalanine and tyrosine hydroxylase in addition to tryptophan hydroxylase [18, 19, 29]. Thus, after a single injection (300–400 mg/kg), there is an increase in brainstem phenylalanine (maximally 48 hr postinjection; see ref. [18]), and a decrease in norepinephrine (NE) synthesis and concentration [19, 30, 34]. The maximal degree of cerebral tryptophan hydroxylase inhibition, furthermore, has been reported to occur 48 hr postinjection [11]. In addition, Miller *et al.* [30] have shown that the effect of pCPA (400 mg/kg) on both NE and 5–HT levels varies as a function of the brain area assayed and the time after injection. We, therefore, decided to investigate the effects of pCPA on open field activity at three different times after injection (24, 48 and 72 hr).

METHOD

Animals

Experimentally naive adult male Møll-Wistar rats weighing 285–315 g at the time of injection were used. The rats were housed individually as in Experiment 1.

Procedure

The animals were handled 4–5 min daily beinning 3 days prior to the injection of pCPA (300 mg/kg; suspended in 2.0% Tween 80 in distilled water) or vehicle (5 ml/kg). Starting 24, 48 or 72 hr after injection the animals were tested in the open field for 50 min using the same apparatus and procedure as described above (Experiment 1). Six vehicle injected rats were used, 2 being tested at each of the postinjection intervals. Since these rats did not differ in any ascertainable way, their results have been combined. One hr after completion of testing the animals were sacrificed and their forebrain 5–HT levels determined as in Experiment 1.

RESULTS

Body Weight and Forebrain 5–HT

In comparison to the vehicle group, a significant (all $p<0.001$, t-test) reduction in body weight (9–23 g) was observed in the pCPA groups 24, 48 and 72 hr after injection.

Forebrain 5–HT was greatly reduced in the pCPA treated animals (Table 2). The forebrain 5–HT content of the pCPA–48 hr ($t = 5.66$, $df = 10$, $p<0.001$) and pCPA–

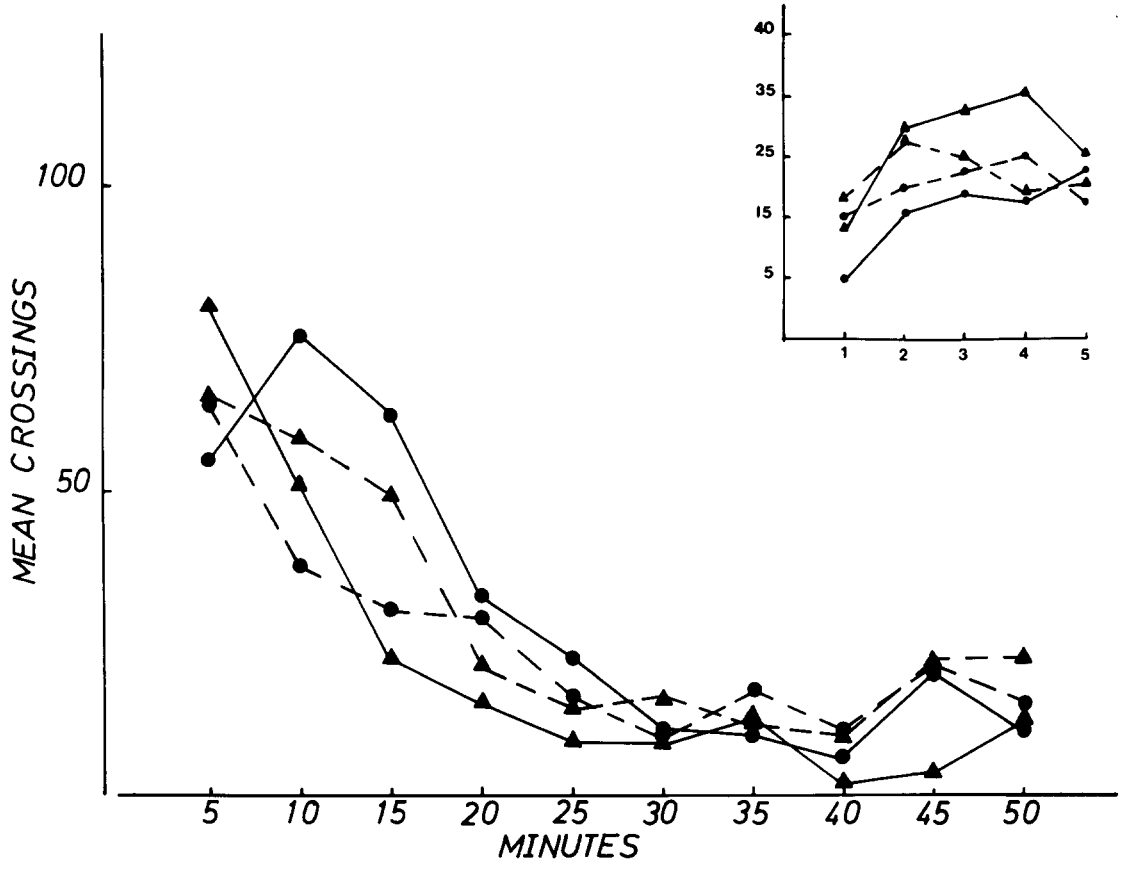

FIG. 4. Effects of pCPA (300 mg/kg) on open field activity 24 (●----●; n = 6), 48 (▲——▲; n = 6), and 72 hr (▲----▲; n = 6) after injection. No differences were found in comparison to the vehicle-control group (●——●; n = 6). Inset and other detail as in Fig. 2.

TABLE 2

GROUP MEAN (±SD) FOREBRAIN 5-HYDROXYTRYPTAMINE (5-HT) CONCENTRATION (NG/G) AT THREE DIFFERENT TIMES (HOURS) AFTER PCPA (300 MG/KG, IP) ADMINISTRATION

Group	Hours	n	5-HT	% Change
Vehicle		6	287 ± 34	
pCPA	24	6	51 ± 12	−82*
pCPA	48	6	22 ± 5	−92†
pCPA	72	5	18 ± 7	−94†

*Significantly ($p<0.0001$, t-test) less than control group; students t-test, two-tailed

†Significantly less than both control ($p<0.0001$, t-test) and pCPA−24 hr ($p<0.0001$, t-test) groups

72 hr (t = 5.72, df = 10, $p<0.001$) groups, furthermore, were significantly less than that of the pCPA − 24 hr group.

Open Field Behavior

Crossings. An overall ANOVA for squares crossed per 5 min during the 50 min session revealed significant time ($F(9.180) = 31.074$, $p<0.0001$) and interaction ($F(27.180) = 1.873$, $p<0.0087$) effects. No significant treatment effect was observed.

Overall (minute by minute) ANOVA of the first 5 min did not indicate any significant effects. Furthermore, no group differences were observed with regard to crossings made during the first minute.

Individual within-group analysis demonstrated that only the control group changed its activity over the first 5 min, showing a significant (t = 3.03, df = 6, $p<0.05$) increase in the number of squares entered. Only the pCPA−24 hr (t = 5.17, df = 5, $p<0.01$) and pCPA−72 hr (t = 3.37, df = 4, $p<0.05$) groups evidenced a significant decrease in activity within the first 15 min. Within 25 min, however, all animals (all $p<0.05$; t-test) had habituated their locomotor activity. No group differences were observed with regard to center squares crossed during the session.

Rearings. Overall ANOVA of the number of rearings emitted over the 30 min period showed significant treatment ($F(3.20) = 6.993$, $p<0.0024$) and time ($F(9.180) = 28.209$, $p<0.0001$) effects. Individual between group com-

parisons revealed significantly lower rearing activity in the pCPA–24 ($F(1.11) = 6.515$, $p<0.0257$) and pCPA–72 hr ($F(1.11) = 8.828$, $p<0.0138$) as compared to the control group.

Analysis (minute by minute) of rearings within the first 5 min did not reveal any reliable effects. No significant change could be detected between the first and the fifth min in any of the groups. The pCPA–24 hr and the pCPA–72 hr groups did evidence a decrease from the first to the second five min period (both $p<0.05$, t-test). Due to a lack of a similar effect in the control group, the pCPA 24 hr and 72 hr animals made less rearings than the controls within the second 5 min period. Within the first 15 min all but the control group significantly (all $p<0.05$, t-test) reduced their rearing activity. After 30 min the rearing activity of the control group also was significantly ($t = 2.47$, $df = 5$, $p<0.05$) lower compared to the first five min.

Other measures. No group differences were observed with regard to the latency to the first grooming response, the latency to leave the center square, and the number of fecal boli dropped during the sessions

Summary. Regardless of the time after injection, the pCPA treated animals did not differ from controls with respect to the number of squares crossed. The pCPA–24 and the pCPA-72 groups, however, did make significantly less rearings compared to the control group. This difference was due to a rapid decrease in rearing activity in the pCPA treated groups within the first 10 min, a phenomenon not observed in the control group.

DISCUSSION

The results of this experiment confirm and extend those of Experiment 1 as well as those of Tenen [41] and Brody [5]. Thus, regardless of the time after injection (24–72 hr), pCPA does not produce the open field hyperactivity seen after electrolytic midbrain raphe lesions. The lack of effect of pCPA on open field locomotion, furthermore, does not seem to be due to variations in phenylalanine [5] or NE levels. It should be noted, however, that mesencephalic raphe lesions do not affect regional forebrain catecholamine levels [23].

The absence of an effect of pCPA (24–72 hr post-injection) on open field activity is in apparent disagreement with the reports of Pirch [35] and Fibiger and Campbell [9]. These workers found that pCPA treated animals were more active in an open field beginning 30 min after the start of the session. It should be emphasized, however, that their pCPA and control animals did not differ during the first 30 min of testing. Fibiger and Campbell (see Fig. 5 in ref. [9]), furthermore, showed that during the first 30 min both the control and pCPA animals demonstrated rapid habituation (within-session reduction of locomotion). Subsequently, whereas the control rats became virtually inactive 1 1/2 hr into the session, the pCPA animals remained active. Pirch [35], likewise, reported that the mean activity of his pCPA animals was significantly higher than control during the last 2 1/2 hr of the session. It is possible that if we had observed our animals for a longer period, 2 hr for example, a difference may have appeared. However, as can be seen in Fig. 2 and 4, the pCPA and control animals showed very little activity after 25 min in the open field. It seems more likely that differences in procedure account for the discrepancies. Thus, Fibiger and Campbell [9] tested their rats in a darkened, small wire mesh cage (35 cm in diameter and 45 cm high), activity being measured by interruption of one of two photocell beams. Pirch's [35] rats were tested in a 7.6 cm wide circular runway (outer circumference 48 cm), closure of one of four microswitches equispaced on the floor being used to count activity (the lighting conditions were unspecified, although the description suggests that the apparatus was dark). In contrast, our animals were tested in an illuminated and larger apparatus (100 cm × 100 cm × 40 cm high), activity measures being recorded by direct experimenter observation. Other than extraneous stimuli, such as rapid or sudden changes in lighting and background noise [5,41], it appears that the ambient level of illumination and time of day, and, perhaps, apparatus size, are critical variables. Thus, pCPA treated animals residing in stabilimeter cages or running wheels, are quantitatively more active than controls during the dark phase of the diurnal cycle than during the light (daytime) period (see Fig. 3 in ref. [9]).

EXPERIMENT 3

We consistently have found that rats with electrolytic midbrain raphe lesions are deficient in the acquisition and forced extinction of an unsignaled one-way conditioned avoidance response [14, 25, 38]. If this effect is related to reduced forebrain 5–HT, then we would expect pCPA treated rats to exhibit a similar impairment. The purpose of this experiment, thus, was to determine the effect of pCPA on one-way avoidance learning.

METHOD

Animals

Twelve experimentally naive Møll-Wistar rats weighing 290–310 g at the time of injection were used. The animals were housed individually in conditions indentical to those described in Experiment 1.

Procedure

The animals were handled 4–5 min daily beginning 3 days prior to injection. Acquisition of an unsignaled one-way avoidance response was studied beginning 72 hr after injection of pCPA (300 mg/kg, IP; n = 6) or vehicle (5 ml/kg; n = 6), prepared in the same manner as in Experiment 1. The apparatus, conditions, and procedure employed were identical to those detailed previously [38]. In brief, the rat was placed in the apparatus (the same as used in Experiment 1), facing the end wall of the shock-compartment. After 5 sec, continuous shock was administered until the animal crossed to the other (safe) compartment. The rat was left undisturbed in the safe-compartment for 25 sec. If, during this period, it crossed to the shock-compartment, shock was delivered until it returned to the safe-compartment. If the animal stayed in the safe-compartment for 25 sec, it was removed and placed on a waiting stand for 5 sec prior to the start of the next trial. An avoidance response was recorded if the animal crossed the hurdle within 5 sec after being placed in the shock-compartment. Avoidance and escape latencies were obtained with a stop-watch. The rats were run until they reached criterion (10 consecutive avoidance responses). One hour later, they were sacrificed and their forebrains removed for 5–HT analysis as in Experiment 1.

RESULTS

Body Weight

Although a significant (t = 2.66, df = 5, $p<0.05$) reduction in body weight (11–24 g) was observed 72 hr after injection in the pCPA group, the animals appeared healthy and the weights of the vehicle and pCPA treated groups did not differ significantly.

Forebrain 5–HT

pCPA treatment resulted in a significant (t = 11.58, df = 10, $p<0.0001$) 90% reduction in forebrain 5–HT (mean ± SD, ng/g, wet weight: control, 219 ± 42; pCPA, 21 ± 5).

One-way Avoidance Acquisition

The two groups did not differ on any of the following measures: trials to reach the acquisition criterion (pCPA: M ± SD = 4.5 ± 2.1; control: 7.8 ± 6.0); trials to make the first avoidance response (pCPA: 3.5 ± 2.4; control: 4.3 ± 2.5); and, the number of shocks received during the session (pCPA: 4.0 ± 2.9; control: 5.0 ± 2.8). None of the rats returned to the shock-compartment during the 25 sec immediately following an escape or avoidance response. The pCPA treated rats, however, did evidence a significantly (t = 2.33, df = 10, $p<0.05$) shorter escape latency on the first trial. Escape and avoidance latencies on subsequent trials did not differ.

DISCUSSION

pCPA failed to affect the acquisition of an unsignaled one-way avoidance response. This is in striking contrast to our repeated observation of deficits in electrolytic mesencephalic raphe lesion rats tested in the same apparatus with an identical procedure [14, 25, 38].

pCPA treated rats have been reported to acquire a signaled one-way avoidance response, such as pole climbing [37] or jumping onto a platform [5,41], more rapidly than control animals. All of these studies employed a conditional stimulus (CS), such as a 5 sec tone preceding US onset. Raphe lesion animals, to our knowledge, have not been tested using this paradigm. The facilitation of signaled one-way avoidance acquisition in pCPA treated rats may be due to their lowered jump threshold or increased reactivity to electric shock [13,41], as signaled one-way avoidance learning is facilitated at higher shock intensities [31,37]. Tenen [41], in fact, demonstrated that pCPA facilitated one-way avoidance at a low (0.35 mA; a value below the control jump threshold) but not at a high (0.95 mA) shock intensity. Raphe lesion rats are not hypersensitive to electric shock as measured by the flinch-jump or hot plate methods [13,14].

Both pCPA treated [6] and raphe lesion [7] rats evidence sensitization (increased startle response amplitude beginning on the second trial) to loud noise. pCPA treated rats also appear hypersensitive to other exteroceptive stimuli and have been reported to acquire a brightness discrimination more rapidly than controls [32,39]. The absence of a CS in the paradigm employed in the present study, thus, may account for the normal, as opposed to facilitated, one-way avoidance acquisition of our pCPA treated animals. It should be noted, furthermore, that the pCPA animals in the present study showed a significantly shorter escape latency on the first trial, suggesting increased sensitivity to the electric shock.

GENERAL DISCUSSION

Three major differences between the effects of electrolytic midbrain raphe lesions and pCPA (300 mg/kg, IP) administration were demonstrated: (1) electrocoagulation of the mesencephalic dorsal and median raphe nuclei, unlike pCPA, facilitated the acquisition of a two-way (shuttle) conditioned avoidance response; (2) electrolytic midbrain raphe lesions, unlike pCPA, produced increased activity in a novel environment (open field); and, (3) electrolytic destruction of the midbrain raphe, but not pCPA, impairs the acquisition of an unsignaled one-way conditioned avoidance response.

In addition to the above observations, pCPA, but not electrolytic midbrain raphe lesions or intrabrainstem 5,7–DHT, has been reported to produce hyperalgesia [13,14]. Both pCPA and intrabrainstem 5,7–DHT, furthermore, produce much greater falls in forebrain 5–HT than mesencephalic raphe damage. It appears, therefore, that these behavioral effects of midbrain raphe destruction are not related in any simple way to interruption of ascending 5–HT pathways and the resulting reduction in forebrain 5–HT level.

There are several possible reasons for the differences in the behavioral effects of pCPA and midbrain raphe lesions. One concerns the peripheral effects of pCPA on 5–HT and its effects on norepinephrine (NE) and phenyalanine synthesis [18, 19, 30]. Electrolytic midbrain raphe lesions do not affect regional forebrain NE or spinal 5–HT concentrations [23]. However, it appears unlikely that these biochemical differences can account for the differential behavioral effects, as pCPA did not block or modify the effects of electrolytic raphe lesions (Experiment 1) on open field activity or two-way avoidance acquisition. pCPA, on the other hand, does not appear to affect tryptophan hydroxylase in the midbrain raphe perikarya to as great extent as in terminal regions [1,8]. Raphe lesions obviously destroy a large number of these perikarya. Thus, the two procedures could produce additive effects, as was suggested by the open field behavior of the MR + pCPA group in Experiment 1. Electrolytic raphe lesions also are nonspecific in that they destroy non–5–HT containing perikarya and fibers [23,24]. Importantly, intrabrainstem 5,7–DHT, which greatly depletes forebrain 5–HT without affecting catecholamine levels, does not affect pain sensitivity, open field activity, or one-way avoidance behavior [14,25]. Thus, it appears that the behavioral effects of electrolytic midbrain raphe lesions are due to the destruction of non–5–HT perikarya and fibers, such as those forming Gudden's tegmental nuclei [24]. Our most recent evidence (to be published) supports this hypothesis, as lesions caudal to the mesencephalic raphe can facilitate two-way avoidance learning without affecting regional forebrain 5–HT.

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