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Lesions in Gudden's Tegmental Nuclei Produce Behavioral and 5-HT Effects Similar to those After Raphe Lesions[1]

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LORENS, S. A., C. KÖHLER AND H. C. GULDBERG. *Lesions in Gudden's tegmental nuclei produce behavioral and 5-HT effects similar to those after raphe lesions.* PHARMAC. BIOCHEM. BEHAV. 3(4) 653–659, 1975. – Lesions largely restricted to the dorsal and ventral tegmental nuclei of Gudden (GTN) produced several effects similar to those seen after midbrain raphe lesions. GTN lesions significantly reduced the 5-hydroxytryptamine (5-HT) concentration of the diencephalon (31 percent), hippocampus (59 percent), and remaining portion of the telencephalon (29 percent). Striatal 5-HT, however, was not affected. GTN lesions enhanced activity in an enclosed field and facilitated two-way avoidance acquisition. Pain sensitivity as measured by the flinch-jump method was not affected. These results suggest that the GTN may be the origin of ascending 5-HT fibers and may be involved in the regulation of activity level and the adaptation of an animal to aversive situations. Thus, some of the behavioral and 5-HT effects of lesions in the midbrain raphe nuclei may be due to their involvement of the GTN and associated pathways.

5-Hydroxytryptamine Avoidance learning Activity Pain sensitivity Midbrain raphe
Tegmental nuclei of Gudden

LESIONS in the dorsal or median raphe nucleus produce different behavioral effects. Lesions in the median but not in the dorsal raphe nucleus enhance activity level [14, 17, 26] and retard the forced extinction of a one-way avoidance response [26]. Both lesions impair one-way avoidance acquisition, but the effects of the median lesion are of a greater magnitude [12,26]. Lesions which destroy both midbrain raphe nuclei produce effects similar to those seen after lesions in the median nucleus alone, but unlike median raphe lesions, also facilitate two-way (shuttle) avoidance acquisition [21, 22, 26].

Although the dorsal raphe nucleus appears to contribute a greater number of serotonergic (5-HT) fibers to the forebrain than the median raphe nucleus [12, 20, 26], lesions restricted to the dorsal nucleus have affected few of the behavioral parameters studied [14,26]. The median raphe nucleus may be the principal source of hippocampal 5-HT [14,20]. Thus, the behavioral effects of median lesions may be related to their associated reduction in hippocampal 5-HT. On the other hand, facilitated two-way avoidance acquisition has been observed after combined (dorsal plus median nucleus) but not selective midbrain raphe lesions [22,26], suggesting that destruction of hippocampal 5-HT fibers alone is not sufficient to produce this behavioral change.

The tegmental nuclei of Gudden (GTN) are known to send fibers lateral, to, and through the median raphe nucleus. These fibers enter the tegmentopeduncular tract and mamillary peduncle and terminate in the interpeduncular nucleus and mamillary body, respectively. Some continue in the medial forebrain bundle to the lateral hypothalamus, septum, and perhaps other telencephalic structures [2, 5, 6, 10, 23, 25]. In addition, the dorsal tegmental nucleus contributes a large number of fibers to the dorsal longitudinal fasciculus [23].

Although the nature of the transmitter(s) used by these neurons is unknown, some may be serotonergic. There appear to be 5-HT containing perikarya in the ventral tegmental (medialis profundus) nucleus of the squirrel monkey [8,13] (Hubbard, personal communication, 1974). Recent diagrams (Fig. 3a of Björklund *et al.* [4]; Fig. 1 of Jonsson *et al.* [16]) depicting brainstem 5-HT containing cells in the rat suggest an overlap between the distribution of brainstem indoleamine fluorescing neurons and the anatomical position of the ventral nucleus of Gudden. The dorsal tegmental nucleus also contains 5-HT, but it is not clear whether this is located in terminals, fibers of passage, or perikarya [24]. However, indoleamine fluorophores were not seen in the dorsal tegmental nucleus of the squirrel monkey [8,13] (Hubbard, personal communication, 1974). Lesions in the

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GTN of the cat, however, have been reported to reduce telencephalic 5-HT [15].

The median and combined midbrain raphe lesions reported to affect behavior and forebrain 5-HT also damaged the GTN and associated fibers [12, 14, 17, 21, 22, 26]. The GTN, furthermore, may be the origin of ascending 5-HT fibers [13, 15, 24]. Therefore, we have examined the effects of GTN lesions on locomotor activity, exploration, two-way avoidance acquisition, pain sensitivity, and regional forebrain 5-HT.

METHOD

Animals

Thirteen male Wistar albino rats (bred in the Department of Pharmacology, University of Bergen) weighing 260–307 g at the time of surgery were used. The animals were housed individually in a temperature (22° ± 1°C) and illumination (12 hr. light-dark cycle) controlled room, and had food and water available ad lib.

Surgical, Histological, and Biochemical Procedures

Electrolytic lesions were produced under pentobarbital (50 mg/kg IP) anaesthesia by passing 2 mA for 5 sec through an intracranial cathode (0.25 mm tungsten wire insulated with Epoxylite except for 0.5 mm at its tip) and an anode clipped to the wound margin. The cathode was inserted through the cerebellum at an angle of 47° to the vertical plane. A Kopf stereotaxic instrument was used with the incisor bar set 3.2 mm above the inter-aural line. Lesions were produced bilaterally in both the dorsal and ventral tegmental nuclei of Gudden (N = 7). Coordinates were based on measurements taken from the midline skull suture 1.0 mm rostral to lambda: 8.0 mm caudal; 0.5 mm lateral; and, 10.5 mm (dorsal nucleus) and 11.7 mm (ventral nucleus) ventral. Operated control animals (N = 6) were treated in the same manner as the lesion rats, except that an electrode was not lowered intracranially.

At the end of the experiment the animals were decapitated and their brains rapidly removed. The brain was dissected on a glass plate covering a tray of dry ice. The diencephalon, hippocampus, striatum, and remaining portion of the telencephalon were obtained as previously described [20]. Each sample was wrapped in aluminium foil, frozen in dry ice, and stored at −20°C for no more than 2 weeks. The brain samples were assayed for 5-HT according to the method of Bertler [3] with the modification of Ahtee *et al.* [1]. Recovery of 5-HT added to homogenates was consistently about 60 percent. Results have not been corrected for recovery.

The brainstems were placed in 10 percent Formalin for at least 1 week. Frozen sections were cut at 30 μm. Every tenth section was saved and stained by the thionine technique. Lesion cavitation and glial scarring was determined microscopically and plotted on diagrams drawn from normal material.

Apparatus

Activity measures were obtained in an enclosed 50 X 50 X 50 cm chamber. The front and rear walls were made of clear Plexiglas while the ceiling and remaining walls consisted of a dark green metal. The floor was painted grey and divided into nine squares of equal size by thin white lines. A green painted board containing 16 equidistantly placed holes (3 cm in diameter) could be exposed by removing the floor. The bottom of the apparatus was 3 cm below the hole-board. Illumination was provided by a 15W bulb located just outside the rear Plexiglas wall.

Avoidance testing was conducted in a shuttlebox with two identical compartments (50 X 24 X 30 cm high) separated by a 7 cm high hurdle. The front wall was made of clear Perspex while the ceiling and remaining walls were constructed of white Perspex. The grid floor was composed of stainless steel rods (0.3 cm in diameter) spaced 1.0 cm center-to-center. Illumination was provided by a 25 W frosted lamp mounted outside each rear wall. The unconditioned stimulus (UCS) was a constant current (0.5 mA) shock generated by Grason-Stadler equipment. The conditional stimulus (CS) was a 75 dB white noise (background noise = 55 dB) delivered simultaneously through 4 ohm speakers located in each of the end walls just below the ceiling.

Flinch and jump thresholds were obtained in a Grason-Stadler (Mod. E3125 – A100) chamber (19 X 29 X 23 cm high) contained within a sound attenuating cubicle. The ventilation fan provided background white noise. Grason-Stadler equipment was used to deliver scrambled 0.2 sec shocks 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.

Procedure

Body weight was measured at the time of surgery and just before sacrifice. The animals were handled daily beginning on the tenth postoperative day.

Activity was measured 6 min/day for 4 consecutive days (postoperative Days 14–17). The number of squares entered and of rearings (rat stands on hindlimbs with head up, sniffing) were obtained for each minute. The latency for the first grooming response and the number of fecal boli dropped in each session also were recorded. On the third and fourth days, the floor was removed exposing the hole-board, and the number of head-dips (rat inserts snout into hole) counted per 3 min.

Two-way (shuttle) avoidance conditioning was conducted between postoperative Days 19–23. Five daily sessions of 20 trials were given to all animals. On Day 1 the first training trial began 5 min after the rat was placed in the apparatus. On subsequent days testing began 1 min after the rat entered the apparatus. The inter-trial interval was 30 sec. The CS was terminated when the rat crossed the hurdle. The UCS was administered if the animal failed to make an avoidance response within 5 sec after CS onset. Inter-trial barrier crossings were recorded but not punished.

Flinch and jump thresholds were obtained on postoperative Days 26–27 in the same manner as previously described [12]. Shocks were given manually at 15–45 sec intervals, but only when the rat was motionless and had at least 3 paws on the grid. The intensity of the first shock was 0.05 mA and was increased stepwise until a flinch (a startle response with one foot typically leaving the floor) occurred. 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. Subsequently, the shock intensity was increased stepwise until a jump (both rear paws left the grid simultaneously, or the rat vigorously stepped with all four feet)

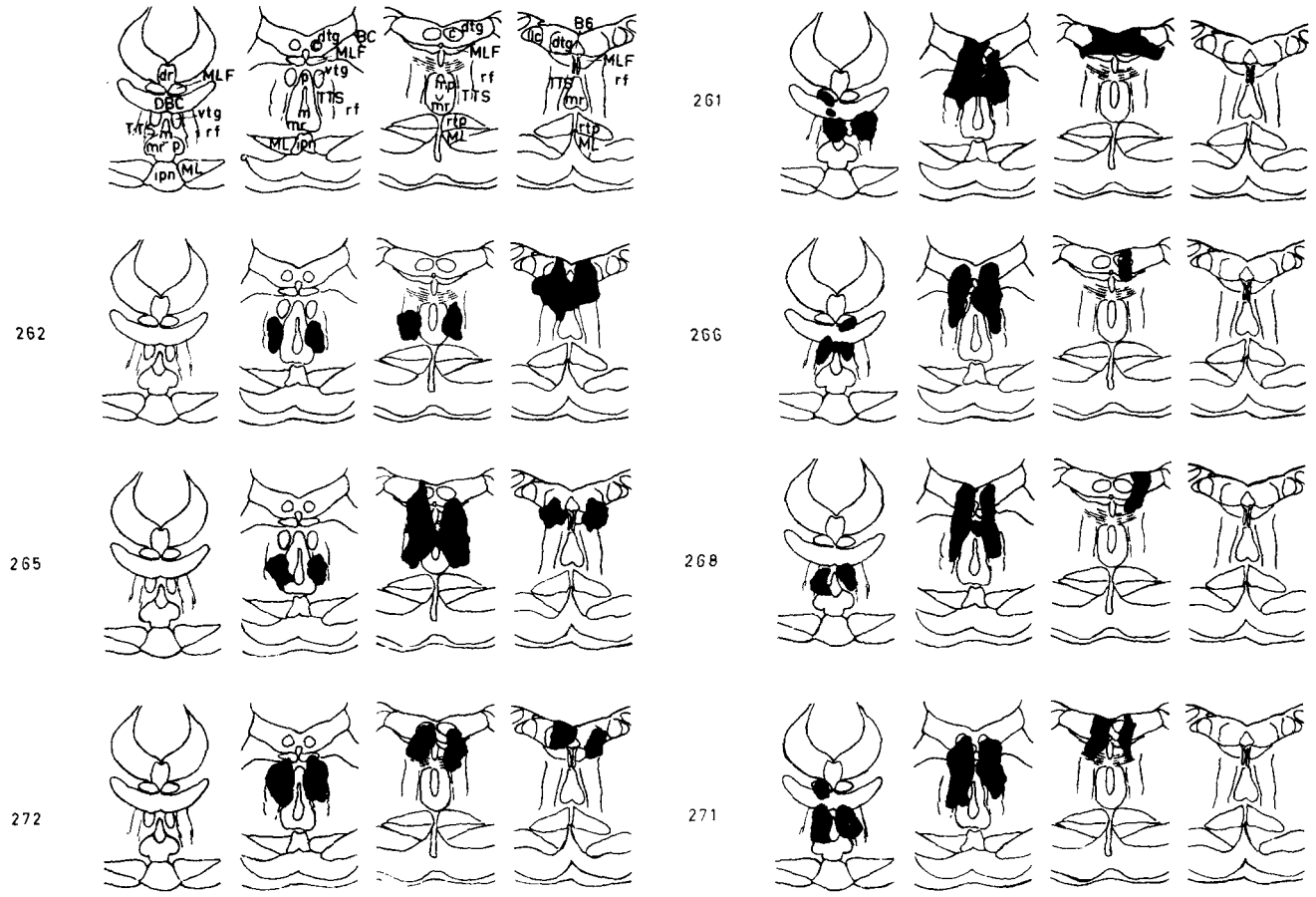

FIG. 1. Reconstruction of damage (blackened area) in the GTN lesion rats. Numbers identify individual animals. Abbreviations: c: dorsal tegmental nucleus, pars centralis [23], dr: dorsal raphe nucleus, dtg: dorsal tegmental nucleus of Gudden, ipn: interpeduncular nucleus, lc: locus coeruleus, m: median raphe nucleus, medium-sized cell part [28], mr: median raphe (or, central superior) nucleus, p: median raphe nucleus, small-sized cell region [28], rf: reticular formation, rtp: pontine tegmental reticular nucleus, vtg: ventral (or, deep) tegmental nucleus of Gudden, B6: cell group B6 [7]; caudal extension of dorsal raphe nucleus [28], DBC: decussation of brachium conjunctivum, ML: median lemniscus, MLF: medial longitudinal fasciculus, TTS: tecto-spinal tract.

was seen. The shock intensity then was raised and lowered stepwise in the same manner as for flinch determination until 8 jumps were obtained. Median thresholds were calculated by interpolation.

The animals were sacrificed on the 32nd day postoperatively between 10:00–13:00, and the histological and biochemical analyses performed.

RESULTS

General Observations

All animals survived the surgery and appeared healthy. No hyper-irritability (sham rage) was observed.

The lesion (GTN) rats (N = 7) weighed 7–10 percent less than the control animals (N = 6) both at the time of surgery (GTN rats: Mean ± S.D. = 276 ± 11 g; controls: 297 ± 12 g; $t = 3.189$, $p<0.01$) and sacrifice (GTN: 299 ± 12; controls: 334 ± 19; $t = 3.999$, $p<0.01$). All rats gained weight postoperatively. However, the controls gained (36 ± 10 g) significantly ($t = 2.539$, $p<0.05$) more weight than the GTN lesion animals (23 ± 10 g).

Lesion Analysis

The lesions damaged both the dorsal (pars centralis and ventromedialis [23]) and ventral tegmental nuclei of Gudden (Fig. 1), except in two rats (No. 262 and 265). The lesions in these animals (as well as in No. 272) were centered more posteriorly, but would be expected to interrupt a number of fibers leaving the GTN. The lesions in these rats, furthermore, extended caudally into the pars posterior [23] of the dorsal tegmental nucleus. The dorsal raphe nucleus was completely spared in all lesion animals, and only minor damage to the dorso-lateral border of the median raphe nucleus was observed. The medial longitudinal fasciculi and tectospinal tracts, however, were damaged bilaterally in all lesion animals.

5-HT

As seen in Table 1, the GTN lesion rats showed significant reductions in regional forebrain (except striatum) 5-HT. The more rostrally placed lesions (No. 261, 266, 268, and 271; Fig. 1), furthermore, produced a significantly (t =

TABLE 1

REGIONAL 5-HYDROXYTRYPTAMINE (ng/g) CONCENTRATIONS (MEAN ± STANDARD DEVIATION)

Region	Groups		
	Control (N = 6)	Gudden Lesion (N = 7)	Percent Change
Striatum	281 ± 26	310 ± 71	+10
Hippocampus	263 ± 87	108 ± 69	−59*
Remaining Telencephalon	320 ± 65	227 ± 41	−29*
Diencephalon	399 ± 81	274 ± 18	−31*

*$p<0.01$, student's two-tailed t-test

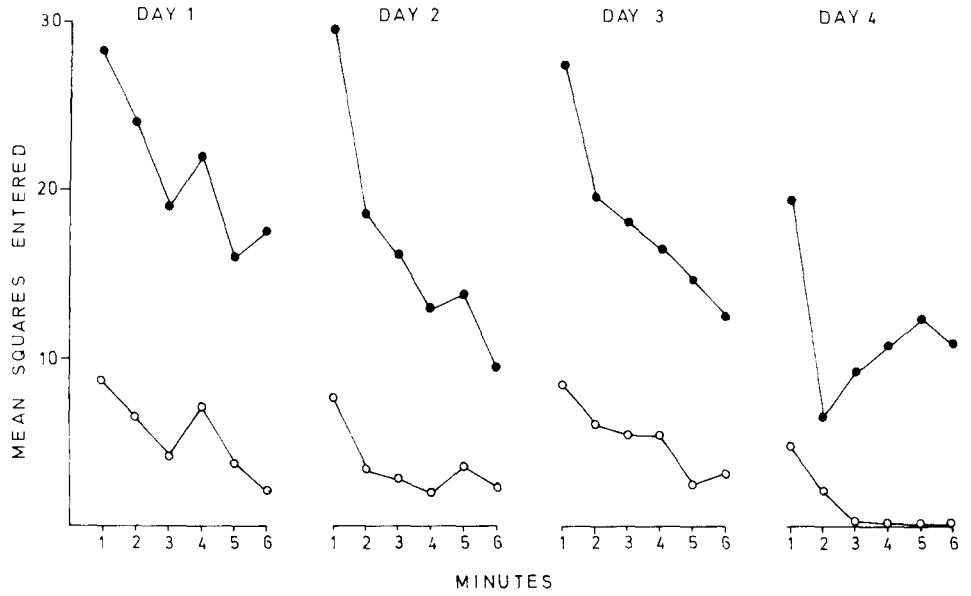

FIG. 2. Locomotor activity per min. of the GTN lesion (●——●) and control (○——○) animals on each of the four testing days. It should be noted that on days 3 and 4 the hole-board was exposed.

3.034, $df = 5$, $p<0.05$) greater fall (76 percent) in hippocampal 5-HT than the more caudally placed lesions (36 percent). Otherwise, the 5-HT effects of these lesions were quite similar.

Activity

Analysis of variance (ANOVA) of the number of squares entered (Fig. 2) revealed significant lesion, Fs (1,11) = 5.51 – 14.14; $p<0.04$, and time, Fs (5,55) = 3.33 – 5.25, $p<0.01$, effects within each of the 4 days. The lesion X time interaction was not significant on any day. Thus, the lesion rats were hyperactive, but, like the controls, evidenced within-day habituation.

In contrast to the GTN rats, the controls entered significantly less squares on Day 2 than Day 1 ($t = 2.634$, $df = 5$, $p<0.05$) and on Day 4 than Day 3 ($t = 3.395$, $df = 5$, $p<0.02$). Thus, the GTN animals, unlike the controls, did not show across-day habituation of locomotor activity. However, ignoring the change in testing conditions on Day 3, the number of squares entered by both groups was lower on Day 4 than on Day 1 (GTN: $t = 3.568$, $p<0.02$; controls: $t = 5.464$, $p<0.01$).

ANOVA did not reveal group differences in the amount of rearing. Both groups, furthermore, showed a significant (GTN: $t = 4.377$, $p<0.01$; controls: $t = 2.630$, $p<0.05$) across day reduction in rearings, but only from Day 1 (GTN: 18 ± 7; controls: 16 ± 3) to Day 2 (GTN: ± 6; controls: 9 ± 6).

The groups did not differ either in the number of fecal boli dropped or in the latency of the first grooming response on any day. On the other hand, in contrast to the

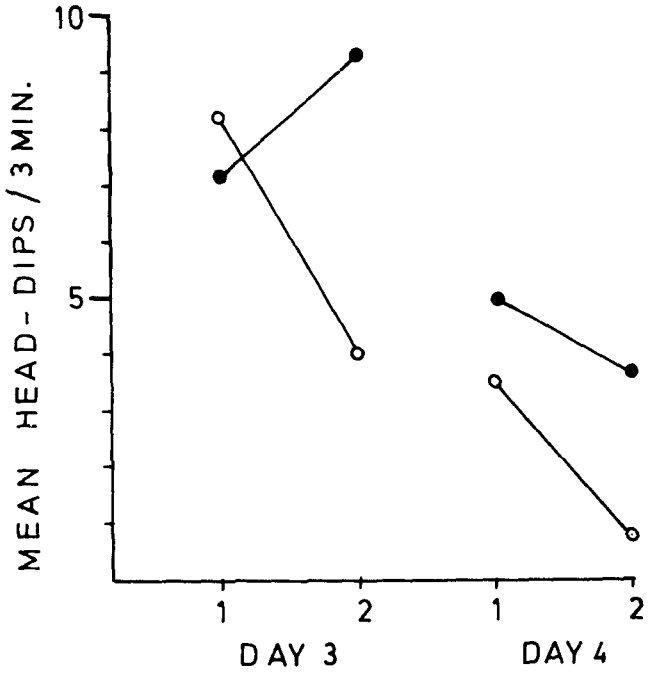

FIG. 3. Exploration of hole-board during the first and second three min. of the two daily sessions. Although the two groups did not differ significantly in the total number of head-dips during any of the 3 min. periods or per day, the GTN (●——●) animals did not show, unlike the controls (○——○), significant reductions in head-dips either within or across days.

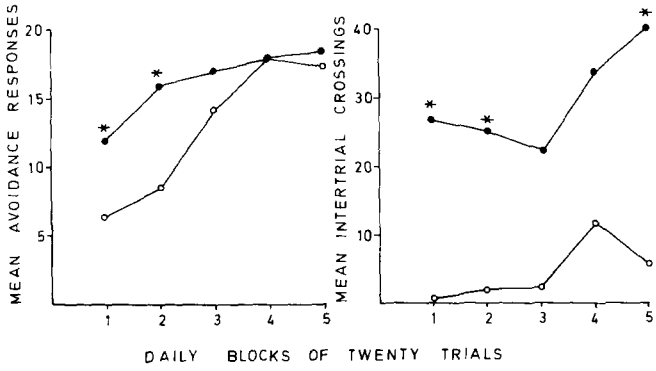

FIG. 4. Performance during two-way avoidance training of the GTN (●——●) and control (○——○) animals. Asterisks indicate significant differences between groups.

controls, the GTN rats showed a significant ($t = 4.347$, $df = 6$, $p<0.01$) increase in defecation from Day 2 (M ± S.D. = 0.6 ± 0.8) to Day 3 (3.6 ± 2.1), suggesting an enhanced emotional response to environmental change.

The two groups were similar in the total number of head dips emitted on Days 3 and 4. However, as seen in Fig. 3, the pattern of this exploratory behavior differed. Thus, unlike the GTN animals, the controls evidenced both across ($t = 5.329$, $df = 5$, $p<0.01$) and within day (Day 3: $t = 3.666$, $p<0.02$; Day 4: t 2.697, $p<0.05$) habituation of this response.

Avoidance Conditioning

As seen in Fig. 4, the animals with GTN lesions made significantly more avoidance responses (CARs) than the controls on both the first ($t = 3.078$, $p<0.02$) and second ($t = 3.302$, $p<0.01$) days of testing. The lesion animals thus made significantly more CARs in 100 trials than the controls (GTN: 81 ± 10; controls: 65 ± 9; $t = 3.207$, $p<0.01$). The lesion rats also required fewer trials to make their first CAR (GTN: 2 ± 2; controls: 10 ± 5; $t = 3.623$; $p<0.01$). The groups did not differ in the latency of their first escape response (GTN: 1.9 ± 1.8 sec; controls: 2.1 ± 1.5 sec).

The lesion rats clearly were more active in the avoidance apparatus than the controls. During their first 5 min in the box, the GTN animals made significantly more barrier crossings than the controls (GTN: 21 ± 10; controls: 6 ± 3; N_1, $N_2 = 6.7$, $U = 3$; $p = 0.004$). This hyperactivity persisted throughout training (Fig. 4), averaging one inter-trial response per 15 sec on Day 5. Interestingly, during the 30 sec inter-trial period following the first shock presentation and escape response none of the control rats spontaneously recrossed the barrier, whereas all but one (No. 262) of the GTN animals did (GTN group: M ± S.D. = 2.4 ± 1.7).

Pain Sensitivity

The two groups showed no significant differences in either their flinch (GTN: mean = 0.05 mA, range 0.04–0.13 mA; controls: mean = 0.06 mA, range 0.05–0.06 mA) or jump (GTN: mean = 0.30 mA, range 0.25–0.40 mA; controls: mean = 0.29 mA, range 0.23–0.46 mA) thresholds.

DISCUSSION

Lesions damaging the tegmental nuclei of Gudden and their ascending fibers reduced forebrain (except striatum) 5-HT, increased activity level, and facilitated two-way avoidance acquisition. Pain sensitivity, as measured by the flinch-jump method, was not affected. These data suggest that some of the effects of midbrain raphe lesions may be due to damage of the tegmental nuclei of Gudden (GTN) and their ascending projections.

It is clear from several studies that the GTN send fibers around, to, and through the median raphe nucleus [2, 5, 23, 25]. Thus, lesions restricted to the median raphe nucleus will interrupt the GTN fibers which form the tegmentopeduncular tract. Median raphe lesions which extend beyond the nucleus laterally will affect GTN fibers which ascend in the mamillary peduncle. At present, unfortunately, the trajectory of median raphe efferents within the brainstem is unknown.

The GTN lesions produced regional 5-HT falls remarkably similar to those recently reported after lesions in the median raphe nucleus [14,20]. It should be noted, however, that the median raphe lesions in those studies extended into the GTN. Most likely the ventral [13] (and possibly the dorsal [24]) tegmental nucleus includes 5-HT containing perikarya. Jalowiec *et al.* [15], furthermore, have reported that regional forebrain 5-HT is reduced (except in the hypothalamus, striatum, and basal forebrain area) after lesions in the region of the GTN of the cat. On the other hand, Fig. 2 from Fuxe and Jonsson [9] suggests that 5-HT fibers are concentrated near the rostral pole of the ventral tegmental nucleus (at the level of the decussation of the brachium conjunctivum). At present, there-

fore, it is not possible to say whether the regional reductions in 5-HT in the present study are due exclusively to interruption of fibers leaving the median raphe nucleus, the GTN, or more caudal areas.

The hyperactivity (squares traversed in either an open or enclosed field) seen after GTN lesions appears similar to that observed after median and combined raphe lesions [26]. In addition, similar to raphe lesion animals [26], the rearing scores of the GTN animals did not differ from control, nor did the latency to the first grooming response or the number of fecal boli dropped (presumed indicators of emotionality). Also, the incidence of head dips (a presumed index of exploration in a novel condition) did not differ.

The GTN animals showed within day habituation of their locomotor activity, but reductions in activity were not seen across Days 1 to 2 and 3 to 4. The level of activity on Day 4, however, was lower than on Day 1. The GTN animals also failed to evidence habituation (either within or across days) of the head-dip response, and showed, furthermore, an increased amount of defecation (compared to the previous day) when first exposed to the change in apparatus (presentation of hole-board) on Day 3. These results suggest that the increased locomotion of GTN animals is accompanied by subtle changes in the emotional response to novel stimuli, and in the habituation of exploratory behavior.

The GTN lesions clearly produced a superior performance during the two-way avoidance learning. Lesions in both the dorsal and median raphe nuclei have been required to produce a similar effect in the rat. Selective lesions in the midbrain raphe have not resulted in facilitated two-way avoidance acquisition [22,26]. The dorsal raphe nucleus was not damaged in the present study. In addition, the striatal 5-HT concentration was normal [20]. These data suggest that the previously reported facilitated two-way CAR learning after large midbrain raphe lesions [21, 22, 26] may be due to involvement of the GTN.

The GTN group evidenced an extraordinarily high incidence of inter-trial responses. Such a high level of anticipatory responding would be expected to increase the probability that a CAR would occur by chance. Observation of the GTN animals, however, indicated that they oriented and responded promptly to the CS. The superior performance of the GTN rats, nevertheless, may be due to an alteration of their unconditioned response to shock, namely a reduction in shock induced freezing, as well as an increased locomotor response to a novel situation.

There are two reports that median raphe nucleus lesions facilitate avoidance behavior. Steranka and Barrett [27] found that median raphe lesions facilitated Y-maze avoidance (shuttle) learning (a light was on continuously in the safe compartment, and inter-trial responses were punished). Unfortunately, however, the extent of their lesions was not described. Thus, it is possible that their lesions extended into the GTN. Lorens [19] found that central superior (median raphe) nucleus lesions facilitated two-way avoidance learning in cats. However, this facilitation was seen only in the number of trials to criterion and not in terms of the number of errors. The median raphe lesion cats, furthermore, did not differ from controls in terms of the number of intertrial barrier crossings, nor in the level of home cage activity. Thus, median raphe lesions in cats appear to produce behavioral effects quite distinct from those of GTN and median raphe lesions in rats.

Finally, the GTN lesions did not affect pain sensitivity as measured by the flinch-jump method. This is in contrast to the report of Lints and Harvey [18] who found that lesions in the "dorsomedial tegmentum" which involved the tegmental nuclei of Gudden (as well as the median raphe nucleus) produced a 49 percent fall in brain 5-HT and reduced the jump threshold. The nature of this discrepancy is not clear, but could be due to differences in lesion locus and/or the testing procedure. However, the role of the midbrain raphe nuclei and adjacent anatomical areas as well as central 5-HT in the response to painful stimuli remains a matter of controversy [11,12]. It is clear, nevertheless, that a large reduction in forebrain 5-HT by itself is not a sufficient condition to enhance pain sensitivity [12].

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