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Lesion of the Median Raphe Nucleus: A Combined Behavioral and Microdialysis Study in Rats

HEIKE THOMAS,* HEIDRUN FINK,† REINHARD SOHR* AND MECHTHILD VOITS*[1]

*Institute of Pharmacology and Toxicology, Medical Faculty (Charité) of the Humboldt University at Berlin, 10098 Berlin, Germany; and †Institute of Pharmacology and Toxicology, School of Veterinary Medicine, Fu Berlin, Koserstr. 20, 14195 Berlin, Germany

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THOMAS, H., H. FINK, R. SOHR AND M. VOITS. *Lesion of the median raphe nucleus: A combined behavioral and microdialysis study in rats.* PHARMACOL BIOCHEM BEHAV **65**(1) 15–21, 2000.—The purpose of the present study was to investigate the behavioral consequences and the neurochemical correlates of a 5,7-dihydroxytryptamine (5,7-DHT) lesion of the median raphe nucleus (MRN) in rats. Anxiety-related behavior was assessed in the elevated plus maze test on days 5, 14, and 21 after lesioning. In general, behavior of MRN-lesioned rats was unchanged when compared with sham-lesioned or untreated controls. Neurochemically, microinjection of 5,7-DHT into the MRN resulted in 87.5% depletion of hippocampal 5-HT content. Using the in vivo microdialysis technique, the exposure of 5,7-DHT–lesioned rats to the elevated plus-maze failed to increase extracellular 5-HT release (94%) in the hippocampus, as shown in sham-lesioned (150%) or untreated controls (194%). Moreover, application of fenfluramine (10 mg/kg, IP) evoked a 10-fold increase in hippocampal extracellular 5-HT levels in sham-lesioned animals, whereas in 5,7-DHT lesioned rats 5-HT was only slightly increased. The results demonstrate, that a marked reduction of 5-HT release from the MRN is not necessarily accompanied by anxiolytic-like behavior. © 1999 Elsevier Science Inc.

5,7-Dihydroxytryptamine Elevated plus-maze Hippocampus Median raphe nucleus Microdialysis Rat Serotonin

ENHANCED serotonin (5-HT) function is thought to cause anxiety and its diminution to cause anxiolysis (9,19). However, intracerebroventricular (ICV) application of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) led to anxiolytic-like behavior in some (5,24,30) but not in other (6,22,29) animal models of anxiety. Variable results were also obtained with 5,7-DHT lesions of the dorsal raphe nucleus (DRN), i.e., no changes in the Geller-Seifter conflict test (33) and the elevated plus maze test (8), but anxiolysis in the social interaction test (10). The median raphe nucleus (MRN) is also a main origin for ascending serotonergic projections to brain structures, such as the hippocampus, which are important for the state of emotion (3). However, as far as we are aware, the only study to have examined the effects of a selective 5,7-DHT destruction of the MRN did not show anxiolytic-like behavior, as assessed by the social interaction test (10). On the other hand, acute administration of the 5-HT_{1A} agonist 8-OH-DPAT into the MRN, which leads to decreased firing of 5-HT neurons, causes anxiolysis in different animal models (1,7,28).

Moreover, less is known about the neurochemical background, which underlies the behavior observed after neuronal destruction. In general, behavioral changes were simply related to the lack of 5-HT activity in the 5,7-DHT–lesioned animals. However, it was reported that compensatory mechanisms occur within 3 weeks after 5,7-DHT lesioning (4,11, 18,20,36,37). Behavioral studies performed during this period have reported inconsistent findings. One possible explanation for this may be that the stage of the regenerative process influences anxiety-related behavior in 5,7-DHT–lesioned rats.

[1]Requests for reprints should be addressed to Dr. Mechthild Voits, Institute of Pharmacology and Toxicology, Medical Faculty (Charité) of the Humboldt University at Berlin, D-10098 Berlin, Germany.

The purpose of the present study was to investigate both the behavioral and neurochemical consequences of a 5,7-DHT lesion of the MRN. Anxiety-related behavior was assessed in the elevated plus maze test at different times after lesioning. This test is generally accepted as a reliable method to measure "anxiety" in rodents (13,25). In a second part of our study, the behavioral testing was combined with neurochemical analysis of 5-HT release using the in vivo microdialysis technique. MRN lesioned rats were placed on the elevated plus maze and extracellular 5-HT levels in the hippocampus were assessed and compared with those of sham-lesioned and untreated controls. In previous studies, it has been shown that exposure of rodents to an aversive environment was accompanied by an increase of extracellular 5-HT levels in different brain regions (23,34,35).

In a third part of the study, the hippocampal 5-HT release of rats with a destruction of the MRN was measured after stimulation by the 5-HT releaser and uptake blocker fenfluramine, given peripherally, again, using the in vivo microdialysis technique.

METHOD

Animals

Male Sprague–Dawley rats weighing 150–180 g (Tierzucht Schönwalde GmbH, Germany) were housed under standard conditions (22 ± 2°C room temperature) with an artificial 12 L: 12 D cycle (lights on 0600–1800 h). They had free access to standard rat laboratory diet (Altromin 1326) and tap water. The experimental protocol was approved by the Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit Berlin, No. G 0390/95.

Drugs

Desipramine (Research Biochemicals International, MA), 5,7-dihydroxytryptamine (5,7-DHT, Research Biochemicals International, Natick, MA), and *d,l*-fenfluramine (Sigma, St. Louis, MO) were freshly dissolved in 0.9% saline. All other chemicals were obtained from Merck, Darmstadt, Germany. Artificial CSF consisted of 125 mM NaCl, 2.5 mM KCl, 27 mM $NaHCO_3$, 0.5 mM NaH_2PO_4 H_2O, 2.4 mM Na_2HPO_4 $2H_2O$, 0.5 mM Na_2SO_4, 1.0 mM $MgCl_2$ $6H_2O$, 1 mM $CaCl_2$ $2H_2O$, adjusted to pH 7.4.

5,7-DHT Lesion

Rats were pretreated with desipramine (25 mg/kg, IP, 30 min prior to lesion) to prevent destruction of catecholaminergic terminals, and anesthetized with sodium-pentobarbitone (50 mg/kg, IP). 5,7-DHT (10 μg in 0.5 μl over 4 min) was injected stereotaxically into the median raphe nucleus. The coordinates were A = 0.35, L = 4.60 from interaural zero, V = 8.08 with an angle of 31° (15). Sham-lesioned rats received equal volume of saline. According to the experiment, rats were observed after surgery for 5 to 21 days.

Behavioral Testing

The elevated plus maze consisted of two open arms (43 × 17 cm) and two enclosed arms (43 × 17 × 25 cm). The arms extended from a central platform (17 × 17 cm). The apparatus was raised to a height of 60 cm by a single central support. The maze was placed in a sound-attenuated chamber. A video camera was suspended above the elevated plus maze and connected with a computer-automated tracking system (VideoTrack) to record and analyze the behavior.

The rats were placed in the center of the plus maze and observed for 10 or 20 min. Time spent on the open arms and number of entries into the open arms served as measures of anxiety. Locomotor activity was controlled by the total number of entries into all four arms.

Microdialysis Procedure

For microdialysis experiments, separate groups of rats were placed in a stereotaxic frame immediately after lesioning and a microdialysis guide cannula (CMA 12, Sweden) was implanted in the ventral hippocampus. The coordinates were (AP = 5.8 mm, L = 4.6 mm from bregma, and 4.5 mm from the skull surface (21). The guide cannula was fixed to the skull with stainless steel screws and cold curing resin (Technovit, Kulzer, Germany). Rats were allowed to recover from surgery for 8–10 days. During this time the rats were kept individually in cylindrical cages (35 cm diameter, 40 cm side) which served as home cages during the microdialysis experiments.

The microdialysis probe was inserted through the guide cannula at 1400 h the day before the experiment. The microdialysis membranes (CMA 12) were 3 mm long, with an outer diameter of 0.5 mm and a 20,000 molecular weight cutoff. According to our in vitro calibration test, the relative recovery was about 25% for 5-HT. Overnight perfusion with artificial CSF was performed with a flow rate of 0.7 μl/min. On the day of the experiment, the flow rate was increased to 1 μl/min allowing stable levels for 5-HT to be reached approximately within 2 h thereafter. This flow rate allowed the collection of 20 μl samples every 20 min into microvials. To each sample a preload of 5 μl 0.1 M perchloric acid was added, resulting in a total sampling volume of 25 μl.

Dialysates were analyzed by reverse-phase high-performance liquid chromatography with electrochemical detection (HPLC-ECD). The detection limit was 0.25 fmol/5 μl. Samples were injected directly into a valve with a 5 μl-loop (Rheodyne, USA). The sample was separated by a 100 mm (SepStik microbore column MF-8494, 3 μm, i.d. 1 mm; BAS, USA) column. The mobile phase contained 0.15 mM NaH_2PO_4, 1 mM EDTA, 0.23 mM OSA, 4% isopropanol at pH 3.7. Mobile phase was delivered by a LC-10AD pump (Shimadzu Europe, Germany) with an external pulse dampener at flow rate of approximately 50 μl/min. Serotonin was oxidized at 0.650 V (UniJet CC5 electrochemical cell, BAS, USA, and DECADE electrochemical detector, Antec, The Netherlands).

Experimental Design

Experiment I: elevated plus maze test at different times after surgery. At 5, 14, and 21 days after surgery 5,7-DHT–lesioned, sham-lesioned, and untreated rats were placed on the elevated plus maze and observed for 10 min (divided in two time bins of 5 min). Behavioral data were recorded and analyzed.

Experiment II: 5-HT release during exposition to the elevated plus maze. In 5,7-DHT–lesioned, sham-lesioned, and untreated animals four 20 min baseline samples were collected in the home cage 8–10 days after surgery. Thereafter, the rats were placed on the elevated plus maze for 20 min and behavioral data and a dialysis-sample were collected. The rats were then returned to the home cage and microdialysis sampling was continued for further 120 min.

Experiment III: Stimulation of 5-HT release by fenfluramine. Baseline samples were collected in 5,7-DHT–lesioned and sham-lesioned animals as described above. Thereafter, fenflu-

ramine (10 mg/kg, IP) was injected, and 20-min samples were collected for further 140 min. Rats remained in their home cages during the whole experiment.

Histology

At the end of the behavioral and microdialysis experiments the correct placements of the microinjection cannula (Fig. 1) and microdialysis probe were verified histologically by an observer unaware of the experimental data. Animals with a placement of the cannula tip or microdialysis probe out of the target area were excluded from statistical analysis.

5-HT Content

The neurochemical success of the lesion was controlled by analyzing 5-HT content. Separate groups of 5,7-DHT–lesioned and sham-lesioned rats were sacrificed by decapitation 8–10 days after surgery. The brains were quickly removed, immediately frozen, and stored in fluid nitrogen. For the assay brains were cut in 1-mm slices and two punches (diameter 1 mm) were taken (2.0 ± 0.08 mg) from the ventral hippocampal area, the anterior cingulate cortex and the nucleus accumbens. We chose these structures because they are known to be involved in anxiety-related behavior (32,35). Further, the hippocampus and cingulate cortex are significantly innervated by the MRN (3). The samples were homogenized with 600 μl 0.1 M perchloric acid (PCA) and centrifuged (14,000 rpm, 10 min, +5°C). The supernatant was analyzed for 5-HT (nM) using HPLCECD.

Statistics

In the elevated plus maze test the number of entries and time spent on the open arms were expressed as percent of total entries or total time, respectively. In the microdialysis experiments, data from three dialysates before exposure to the elevated plus maze or treatment with fenfluramine were averaged for each animal, and the mean regarded as baseline and

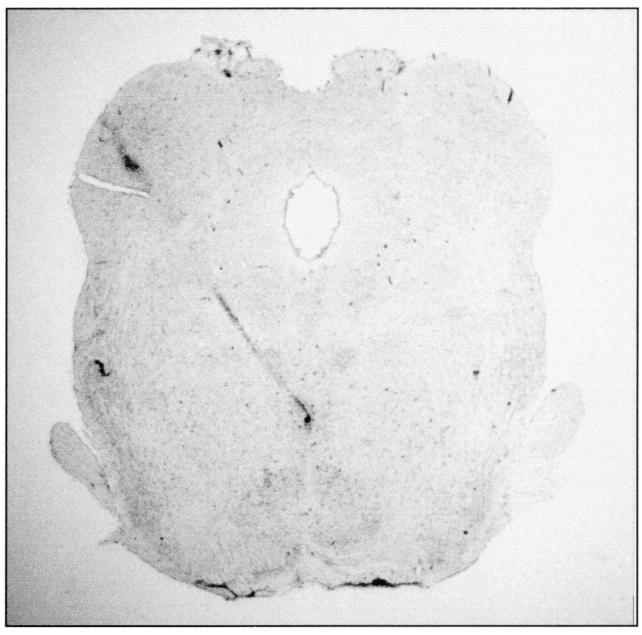

FIG. 1. Example of histological verification of the cannula placement for microinjection of 5,7-DHT into the median raphe nucleus. Coordinates were A= 0.35, L = 4.60 from an interaural zero, V = 8.08 with an angle of 31°.

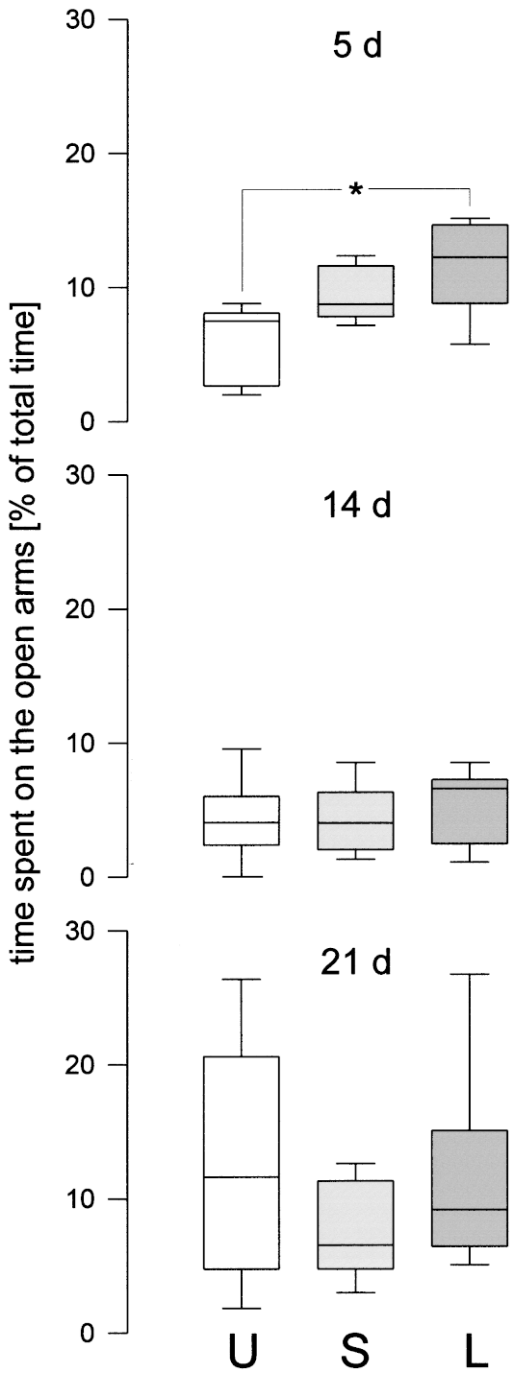

FIG. 2. Percent time spent on the open arms (median, 25./75. percentile and 10/90 percent extremes) of the elevated plus maze (10 min) in 5,7-DHT–lesioned rats (L) compared with sham-lesioned (S) and untreated controls (U) on days 5, 14, and 21 after surgery (number of animals see Table 1). Differences between the groups are based upon Kruskal–Wallis ANOVA on ranks followed by Dunn's method, 5,7-DHT lesioned vs. untreated, $*p < 0.05$.

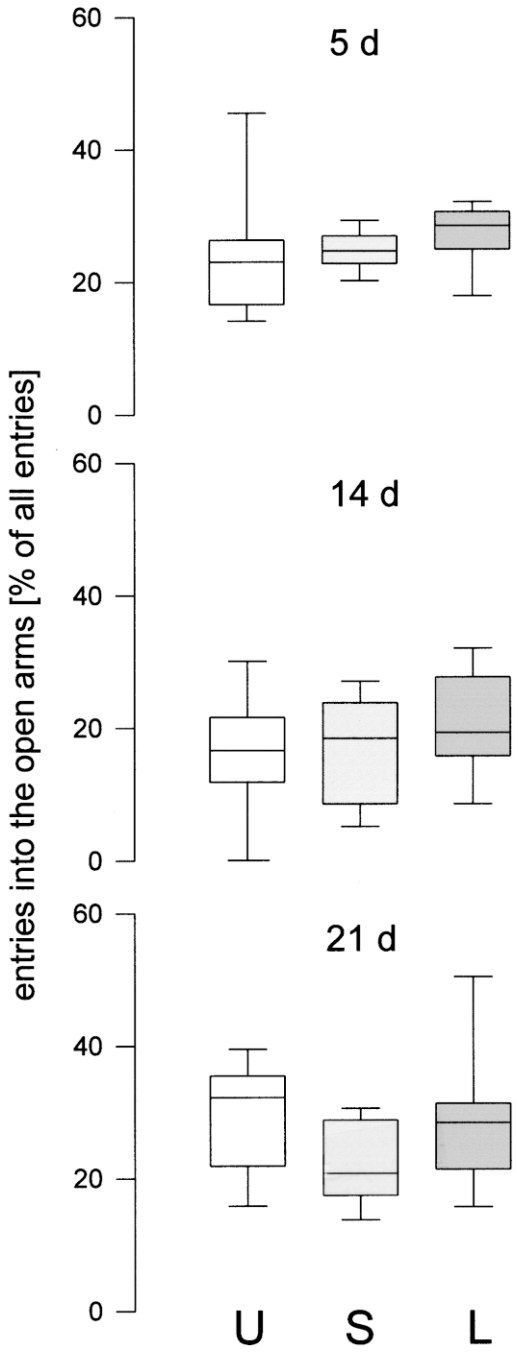

FIG. 3. Percent entries into the open arms (median ± 25./75. percentile and 10/90 percent extremes) of the elevated plus maze (10 min) in 5,7-DHT–lesioned rats (L) compared with sham-lesioned (S) and untreated controls (U) on days 5, 14 , and 21 after surgery (number of animals see Table 1).

set as 100%. All other individual data were calculated as percent of this individual baseline.

All behavioral and neurochemical data were analyzed using Kruskal–Wallis ANOVA on ranks followed by Dunn's method. Mann–Whitney's rank sum test was used where appropriate. Data were presented as medians with the 25 and 75 percentile (med., 25/75). A probability level of $p < 0.05$ was regarded as significant.

TABLE 1
NUMBER OF TOTAL ARM ENTRIES IN THE ELEVATED PLUS MAZE

Days After Surgery	Untreated	Sham-Lesioned	5, 7-DHT–Lesioned
5	23 (12/29)	31 (25/37)	35 (28/36)
	$n = 9$	$n = 6$	$n = 9$
14	10 (8/23)	18 (17/22)	18 (14/22)
	$n = 11$	$n = 9$	$n = 8$
21	25 (21/33)	24 (19/28)	22 (19/27)
	$n = 13$	$n = 11$	$n = 10$

Data obtained during 10 min of test are expressed as median (25./75. percentile) of 5, 7-DHT–lesioned rats, sham–lesioned, and untreated controls on days 5, 14, and 21 after surgery.

RESULTS

Elevated Plus Maze Test at Different Times After Surgery

In the first experiment, the behavior of 5,7-DHT–lesioned rats was overall not different from sham-lesioned and untreated rats tested in the elevated plus maze. Neither percent time spent on the open arms nor percent entries into the open arms were changed in 5,7-DHT–lesioned rats on days 5, 14, and 21 after surgery when compared with sham-lesioned rats (Figs. 2 and 3). However, 5 days after surgery 5,7-DHT–lesioned rats spent significantly more time on the open arms compared with untreated controls ($H = 9.68$, $p = 0.0079$). There were no group differences in locomotor activity indicated by total arm entries at any time after surgery (Table 1). Dividing the observation time into two time bins (2×5 min) did not reveal differences in the behavior (data not shown).

5-HT Release During Exposition to the Elevated Plus Maze

In Experiment II, hippocampal extracellular 5-HT levels increased significantly in the first sample after placing the animal on the elevated plus maze to 194 (180/214)% of baseline in untreated rats ($H = 22.2$, $p = 0.0083$) and to 150 (133/173)% of baseline in sham-lesioned rats ($H = 23.2$, $p = 0.0058$) (Fig. 4). In contrast, in rats with a 5,7-DHT lesion, the extracellular 5-HT levels were not affected by exposure of the animal to the elevated plus maze (94, 76/107% of baseline) (Fig. 4). The hippocampal 5-HT levels in the dialysis sample obtained during the exposure to the elevated plus maze of 5,7-DHT–lesioned rats differed significantly from sham-lesioned and unlesioned controls ($H = 18.1$, $p = 0.0001$).

Similar to the first experiment, 5,7-DHT–lesioned, sham-lesioned, and untreated rats exhibited no differences in percentage of time spent on and number of entries into the open arms during the 20 min of exposure to the elevated plus maze test (Table 2). However, locomotor activity measured by the number of total entries during the 20-min test was higher in 5,7-DHT–lesioned and sham-lesioned animals compared with untreated controls ($H = 9.8$, $p = 0.0075$) (Table 2). Overall, behavioral data during microdialysis revealed similar to those obtained in the elevated plus maze test in the first part of the study.

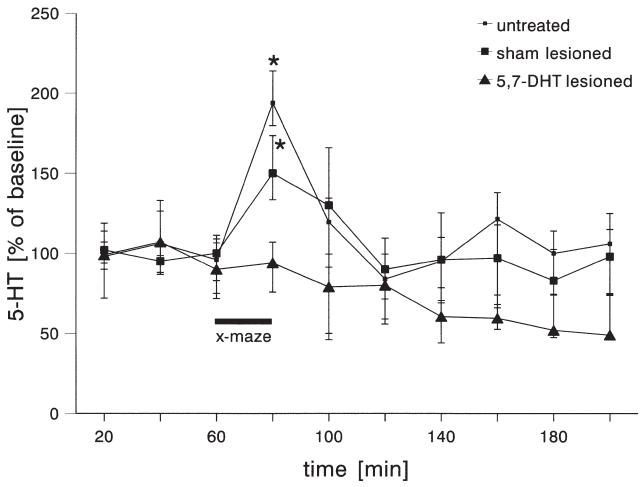

FIG. 4. Effect of exposure to the elevated plus maze (20 min) on hippocampal dialysate 5-HT (median ± 25./75. percentile) in 5,7-DHT–lesioned (n = 9), sham-lesioned rats (n = 9), and untreated controls (n = 7). Average extracellular 5-HT baseline levels were approximately 2 fmol/20 μl dialysate and consistent across the groups. Increases in extracellular 5-HT levels were analyzed by Kruskal–Wallis ANOVA on ranks followed by Dunn's method, $^*p < 0.05$.

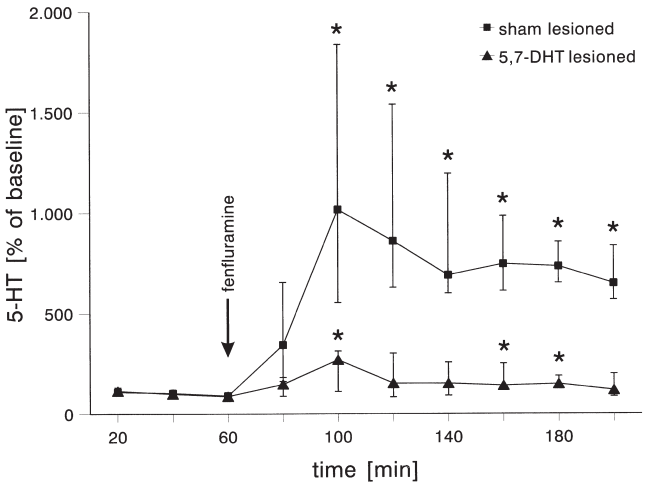

FIG. 5. Effect of fenfluramine (10 mg/kg, IP) on hippocampal dialysate 5-HT (median ± 25./75. percentile) of 5,7-DHT–lesioned (n = 7) and sham–lesioned rats (n = 9). Average extracellular 5-HT baseline levels were approximately 2 fmol/20 μl dialysate and consistent across the groups. Increases in extracellular 5-HT were analyzed by Kruskal–Wallis ANOVA on ranks followed by Dunn's method, $^*p < 0.05$.

Stimulation of 5-HT Release by Fenfluramine

In the third experiment, administration of 10 mg/kg fenfluramine (IP) to sham-lesioned rats led to a significant, long-lasting increase of hippocampal 5-HT release (H = 60.4, $p <$ 0.0001) with a maximum of 1017 (553/1837)% of baseline in the second 20-min sample after treatment (Fig. 5). In contrast, in 5,7-DHT–lesioned rats fenfluramine increased extracellular 5-HT only slightly (H = 18.0, p = 0.035) to a maximum of 269 (110/313)% of baseline in the second 20-min sample after treatment (Fig. 5). Comparison of the maximum 5-HT levels (40 min after treatment) in 5,7-DHT–lesioned and sham-lesioned rats revealed again a significant difference with p = 0.0043 (Mann–Whitney rank sum test, T = 32.0).

5-HT Content

In 5,7-DHT lesioned rats (n = 8) 5-HT tissue content was significantly reduced by 87.5% in the hippocampus (p = 0.00015, T = 100), 44.3% in the anterior cingulate cortex (p = 0.028, T = 89.0), and 52.4% in the nucleus accumbens (p = 0.003, T = 95.0) when compared with sham-lesioned controls (n = 8) (Table 3).

TABLE 2

BEHAVIOR IN THE ELEVATED PLUS MAZE DURING MICRODIALYSIS EXPERIMENTS

Parameter	Untreated (n = 7)	Sham-Lesioned (n = 9)	5, 7-DHT-Lesioned (n = 9)
Time spent on the open arms (% of total time)	2.4 (2.2/6.7)	7.5 (7.0/8.4)	5.5 (3.0/9.7)
Number of entries into the open arms (% of total entries)	22 (15/31)	29 (27/30)	29 (24/36)
Number of total entries	21 (10/26)	31 (29/39)*	31 (23/46)

Behavioral data obtained during 20 min of test are expressed as median (25./75. percentile) of 5,7-DHT–lesioned rats, sham-lesioned, and untreated controls. Differences between the groups are based upon Kruskal–Wallis ANOVA on ranks followed by Dunn's method, 5,7-DHT–lesioned and sham–lesioned vs. untreated controls, $^*p <$ 0.05.

DISCUSSION

The experiments reported here are based on the hypothesis that a reduced 5-HT release is accompanied by an anxiolytic-like behavior (19). The consequences of a neurotoxic destruction of 5-HT neurons of a single raphe nucleus were investigated behaviorally and neurochemically. The MRN was chosen, because it is a main origin of ascending 5-HT pathways innervating brain structures responsible for emo-

TABLE 3

5-HT CONTENT

	Sham-Lesioned (n = 8)	5,7–DHT-Lesioned (n = 8)
Ventral hippocampus	12 (11/13)	1.5 (0.85/2.9)*
Anterior cingulate cortex	7.9 (6.4/8.1)	4.4 (3.2/6.4)*
Nucleus accumbens	21 (15/29)	10 (5.2/12)*

Data obtained in three brain areas are expressed as median (25./75. percentile) of 5–HT concentration in supernatant PCA (nM) of 5,7–DHT–lesioned and sham–lesioned rats (for further explanations, see the Method section). Differences between the groups are based upon Mann–Whitney rank sum test, $^*p < 0.05$.

tional output (3), and only few studies exist concerning behavioral changes due to a loss of MRN input. However, in the present study we failed to demonstrate behavioral differences between rats with a neurotoxic lesion of the MRN compared with sham-lesioned or unlesioned controls. Our results are in accordance with findings of File et al. (10), who could not show an effect of a MRN lesion on social interaction in rats. To our knowledge, this is the only study investigating the impact of 5,7-DHT lesions of the MRN on anxiety-related behavior of rats. Both findings are not consistent with the view that a reduced 5-HT activity is important for anxiolysis. In contrast, acute microinjection of the 5-HT_{1A} receptor agonist 8-OH-DPAT, which reduces the firing rate of serotonergic neurons, into the MRN, resulted in an anxiolytic effect, as shown in the social interaction test, the two-compartment exploratory box, and the conditioned suppression of drinking (1,7). Also, the results of studies investigating the effects of "intraraphe" or ICV administration of neurotoxins in conditioned (24,29,30) as well as unconditioned (5,6,22) anxiety tests are inconsistent, independent of the used paradigm. We assume that the time of testing after lesion may influence the behavioral results because in neurochemical studies compensatory mechanisms have been shown to occur even within 3 weeks after lesioning. For example, it was reported that in surviving 5-HT neurons following 5,7-DHT lesion the activity and mRNA of tryptophan hydroxylase are increased (4,31), and a homotypic collateral sprouting of intrinsic neurons has been observed that may functionally restore the defect (36,37). Moreover, postlesion plastic properties of 5-HT receptors in several brain regions have been proposed as adaptive changes (11,18,20). Therefore, in the present study, the behavioral tests were performed at three times after lesion to consider compensatory mechanisms.

Overall, no marked changes in anxiety-related behavior were seen within the 3-week period after destruction of the MRN, although the hippocampal 5-HT content was reduced by 87.5% in the MRN-lesioned rats.

We assumed that the dorsal raphe nucleus (DRN) or other parts of the raphe system may compensate for the diminished serotonergic activity in the terminal structures, and that this may lead to the unchanged behavior. To examine this idea, in vivo method of brain microdialysis was used to monitor central 5-HT release in the home cage and during exposure to the elevated plus maze. We focused on the ventral hippocampus because this brain area has been emphasized to be involved in anxiety-related behavior (19) and likewise gets inputs mainly from the MRN but also from the DRN (3). Previous in vivo microdialysis studies have shown that exposure of animals to an aversive environment was accompanied by an increase of extracellular 5-HT levels in different brain regions including the hippocampus (23,34,35).

As expected, a significant increase in hippocampal 5-HT levels was observed in sham-lesioned and untreated rats, whereas in MRN-lesioned rats the hippocampal 5-HT levels were not affected by exposure to the maze. Thus, results provide evidence that the DRN does not compensate for the deficiency in serotonergic activity in MRN-lesioned rats directly by an enhancement of hippocampal 5-HT release.

In the third part of the study we tested whether in MRN-lesioned rats an increase of hippocampal 5-HT can be pharmacologically induced at all. In sham-lesioned rats fenfluramine evoked a 10-fold enhancement in hippocampal 5-HT release. In contrast MRN-lesioned rats exhibited only a slight 5-HT increase. This additionally underlines the discrepancy between the severity of the damage of the 5-HT system (87.5% reduction in the 5-HT content), the loss of function (no increase in extracellular 5-HT following stimulation), and the absence of behavioral changes in MRN-lesioned animals. This is in accordance with other microdialysis studies showing that in rats that received 5,7-DHT into the DRN or ICV, the 5-HT release was reduced but not totally abolished after stimulation with KCl or fenfluramine. Basal 5-HT release was not altered (14,26). The authors assumed that compensatory mechanisms may enable surviving 5-HT terminals to maintain basal 5-HT levels but those mechanisms are not sufficient to allow the damaged system to respond to pharmacological challenge. The present study supports the above-mentioned idea that the loss of MRN innervation in the hippocampus is not compensated for by an input of the DRN to this structure. On the one hand, it could be that differences in the projection sites of the MRN and DRN are responsible for distinct functional relevance of these nuclei. It seems likely that other brain regions that receive a predominant 5-HT input from the DRN, for example, the periaqueductal gray or the amygdala, contribute substantially to expression of anxiety-related behavior (12,19). There may also be other transmission systems involved in compensating for the defective 5-HT system. For example, the dopaminergic and the noradrenergic systems, which are known to interact closely with the 5-HT system, are possible candidates (16,27). It could be argued that while a partial defect in the 5-HT system may not be sufficient to unsettle anxiety-related behavior, a further defect in this or another transmission system may evoke behavioral changes. This is supported by findings that profound behavioral deficits were shown in rats with an unspecific electrolytic lesion of the MRN but not with a 5,7-DHT lesion (2,17). These conclusions open a wide field for further investigations.

In summary, this is the first study investigating behavior in combination with neurochemical analysis in 5,7-DHT raphe-lesioned rats. The results show that a MRN lesion does not affect anxiety-related behavior within 3 weeks after surgery, as assessed in the elevated plus maze test. In contrast to control rats, the MRN-lesioned rats failed to show an increase in hippocampal 5-HT levels when exposed to an aversive environment. In addition, fenfluramine induced a marked increase of hippocampal 5-HT release in controls to more than 1000%, whereas it was only weak in MRN-lesioned rats. In conclusion, the data suggest that impaired function of a part of the 5-HT system is not necessarily accompanied by anxiolytic-like behavior.

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