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Effect of electrolytic and neurotoxic lesions of the median raphe nucleus on anxiety and stress

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Abstract

To study the role played by 5-HT mechanisms of the MRN, behavioural and physiological parameters were presently measured in rats having either electrolytic or 5,7-dihydroxytryptamine (5,7-DHT) lesion of the MRN made 7 days before testing. Half the animals were submitted to 2-h restraint 24 h before the test. In the elevated plus-maze, the electrolytic lesion increased the percentage of open-arm entries and of time spent on open arms — an anxiolytic effect — in both restrained and nonrestrained rats. The neurotoxic lesion had a similar effect, but only on restrained rats. Restraint had anxiogenic effect. The electrolytic lesion increased transitions between the light and dark compartments and the time spent in the bright compartment of the light–dark box in both restrained and nonrestrained rats. The neurotoxic lesion only increased bright time in restrained rats. The incidence, number and size of gastric ulcers were increased by either the electrolytic or the neurotoxic lesion in both restrained and nonrestrained animals. Both types of lesion depleted 5-HT in the hippocampus in restrained and nonrestrained rats. Restraint increased 5-HT levels. These results implicate 5-HT mechanisms of the median raphe nucleus in the regulation of anxiety and in the genesis of gastric stress ulcers.

Keywords: Median raphe nucleus; 5-HT; Anxiety; Gastric ulcers; Stress

1. Introduction

Serotonergic neurons originating in the raphe nuclei have been implicated in several behavioural and physiological functions (Soubrié, 1986; Villar, 1994). Lesion of the MRN and/or hippocampus has been shown to result in behavioural disinhibition (Andrade et al., 1999; Briley et al., 1990; File and Deakin, 1980; File et al., 1979; Gray and McNaughton, 1983; Jacobs and Cohen, 1976; Jacobs et al., 1974; Srebro and Lorens, 1975). Drugs that deplete serotonin (5-HT) or decrease of the firing rate of serotonergic neurons cause a similar effect (Andrews et al., 1994; Carli and Samanin, 1988; Carli et al., 1989; Chopin and Briley, 1987; De Almeida et al., 1998; File et al., 1996; Schreiber and De Vry, 1993a,b). As a consequence, the behavioural disinhibition observed after MRN lesion has been attributed to impairment of the serotonergic pathway that originates in the MRN and innervates the hippocampus (Andrews et al., 1994; Avanzi et al., 1998; De Almeida et al., 1998; File et al., 1996; Schreiber and De Vry, 1993a,b).

Behavioural inhibition has been related to anxiety (Gray, 1982, 1987). In this regard, Graeff and Silveira Filho (1978) have shown that electrical stimulation of the MRN induces behavioural inhibition, defecation, crouching, micturition, piloerection and teeth chattering. These changes are characteristic of the rat emotional reaction to conditioned aversive stimuli, usually referred to as conditioned emotional response or CER. Complementary results have been reported, showing that microinjection of the 5-HT_{1A} receptor agonist 8-OH-DPAT inside the MRN, which inhibits firing of 5-HT neurons, had anxiolytic-like effects measured in several animal models of anxiety (Andrews et al., 1994; Carli and Samanin, 1988; De Almeida et al., 1998; Schreiber and De Vry, 1993a,b). Together, these results are suggestive that 5-HT containing neurons of the MRN regulate anxiety. However,

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largely negative results have been reported following neurotoxic lesion of the MRN in the rat social interaction test (File et al., 1979) and in the elevated plus-maze (Thomas et al., 2000).

Based on clinical and experimental evidence, Deakin and coworkers have hypothesized that the 5-HT MRN-hippocampal pathway underlies adaptation to chronic, unavoidable stress, failure of this mechanism resulting in depressive disorder (Deakin and Graeff, 1991; Graeff et al., 1996). In a similar vein, Hoshino and Sugizaki (1986) have suggested that 5-HT mechanisms in the MRN exert a protective function against acute stress. The latter view is based on experimental results showing that electrolytic lesion of the MRN markedly increased gastric ulcers induced by fasting in rats, while decreasing 5-HT concentration in the midbrain, cortex and hippocampus.

To further explore the role played by 5-HT mechanisms of the MRN in stress, behavioural and physiological parameters related to stress were presently measured in rats having either electrolytic or 5,7-dihydroxytryptamine (5,7-DHT) lesion of the MRN made 7 days before testing. In contrast to the widespread neuronal destruction caused by the electrolytic lesion, the neurotoxin 5,7-DHT has been shown to selectively damage 5-HT-containing neurons (Stewart et al., 1978; Thomas et al., 2000). Behaviour was evaluated in two widely used animal models of anxiety, the elevated plus-maze (Pellow et al., 1985) and the light–dark transition test (Brioni and Orsingher, 1988). The physiological parameters were the occurrence, number and size of gastric ulcers (Hoshino and Sugizaki, 1986). Body weight loss and death rate were also recorded. In addition to the effect of the lesions, per se, their interaction with restraint (Kenett and Joseph, 1981), a stress-inducing manipulation, was presently evaluated. The depletion of 5-HT concentration in the hippocampus determined by the two types of MRN lesion was measured through a high-performance liquid chromatography assay (HPLC).

2. Materials and methods

2.1. General

2.1.1. Animals

Male *Wistar* rats weighing 200 g at the beginning of the experiments were used. They were housed in polypropylene cages with sawdust floor, five animals per cage, having free access to food and water during 7 days until the surgery. Room temperature was kept at $21 \pm 1^{\circ}C$. Lights were on at 07:00 h and off at 19:00 h. The animals were manipulated three times a week to clean the cages.

2.1.2. Electrolytic lesion

Rats were anesthetized with sodium thiopental (50 mg/kg, Ip) associated with local anesthesia (2% xylocaine with vasoconstrictor) and fastened to a stereotaxic instrument (David-Kopf, USA). A unipolar electrode of stainless steel, 0.12 mm in diameter, isolated with enamel, except for 0.5 mm from the tip, was inserted into the brain through a hole drilled in the skull at an angle of 20° with the vertical plane to avoid the sagittal sinus. The following coordinates from bregma were used: posterior = − 7.8 mm, lateral = 2.9 mm, deep = 9.0 mm. Electrolytic lesions were made with a lesion-producing device (Ugo Basile, Italy). A current of 3 mA was applied for 10 s. Soon after the lesion, the electrode was removed, the hole in the bone was closed with acrylic cement, and the skin was sutured with cotton thread.

2.1.3. Neurotoxic lesion

A 13-mm long stainless steel needle with 0.6 mm of external diameter and 0.3 mm of internal diameter was implanted, as described for the brain electrode. A volume of 1 μl of a solution containing 8 μg of 5,7-DHT (Sigma, USA) dissolved in saline with 0.2% ascorbic acid was microinjected into the MRN during 1 min by means of a computer-controlled Hamilton (EUA) microsyringe. Sham-injected animals received the same volume of vehicle. To protect noradrenergic neurons, animals were injected intraperitoneally with 25 mg/kg of desipramine (Sigma) diluted in distilled water 60 min before the intracerebral injection of 5,7-DHT.

2.1.4. Restraint

On the sixth day after the surgery, rats were immobilized for 2 h inside a metal cage measuring $19.5 \times 7 \times 4$ cm, according to the procedure described by Guimarães et al. (1993). Following restraint, each rat remained for 24 h, individually housed, in a polypropylene box of $28 \times 17 \times 13$ cm, having water and food ad libitum until the behavioural evaluation. The animals, no-restraint, stayed in group until the behavioural evaluation.

2.1.5. Histology

At the end of the experiments, rats were sacrificed under deep ether anesthesia. The brain was perfused through the heart with 10% formaline solution before being removed for histological analysis.

The procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behaviour Guidelines for Care and Use of Laboratory Animals, which are in compliance with international laws and policies. All efforts were made to minimise animal suffering.

2.2. Behavioural measures

2.2.1. Elevated plus-maze

2.2.1.1. Apparatus. The elevated plus-maze was made of wood and had two open arms (50×10 cm), perpendicular to two closed arms of equal dimensions, surrounded by

40-cm walls. The apparatus was elevated 50 cm from the floor (Pellow et al., 1985). To avoid falls, a 3-mm wooden rim surrounded the open arms. Illumination was provided by two 60-W fluorescent bulbs placed above the center of the maze to avoid shade in any of the arms. Luminosity at the level of the maze arms was 190 lux.

2.2.1.2. Procedure. Experimental sessions were conducted between 14:00 and 17:00 h, 7 days after the surgery. The rat was placed at the center of the plus-maze heading an enclosed arm and allowed to explore the environment for 5 min. Before the next rat, the apparatus was cleaned with 20% ethanol. The experimenter stayed outside the room, and the behaviour of the rat was recorded on videotape, which was later analysed using standardised software (Noldus Observer, Netherlands).

Two kinds of measures were taken, namely the traditional indexes of exploration, as well as frequency and duration of selected behavioural categories. For the former measures, the data were expressed as percentage of entries (with the four paws) into and time spent on the open arms in relation to total number of entries and time, respectively, in both open and closed arms. The total number of entries into the closed arms was also recorded.

The following behavioural categories were measured: *rearing* — raising the front paws from the floor; *head-dipping* — looking down from the edge of an open arm; *scanning* — head over the edge of an open arm looking at any other direction; *open-arm end exploration* — when the animal reaches the extremity of an open arm, that is opponent to center or closed arms; *stretch attending* — walking forward with stretched body and the abdomen near the floor; *flat back approach* — stretching the body forward and retracting back without locomotion; *closed-arm return* — putting the head and front paws outside a closed arm and then back; *peeping out* — putting the head out of a closed arm; *immobility* — absence of any movement, except for breathing; and *grooming* — licking the fur, beginning by the muzzle, going to the ears and down to the rest of the body.

2.2.1.3. Data analysis. Data were analysed by a three-factor ANOVA. The factors being the presence of lesion, restraint and lesion method (Electrolytic × Neurotoxic). In case of significant or nearly significant interactions involving presence of lesion, post-hoc comparisons between lesion and sham-operated animals were performed using Newman–Keuls test. A value of $P<.05$ was considered significant.

2.2.2. Light–dark box

2.2.2.1. Apparatus. The light–dark box described by Santucci et al. (1994) was used. A wooden box measuring $80 \times 40 \times 20$ cm was divided into two compartments of equal size. The bright compartment had a transparent glass ceiling, and the lateral walls and floor were painted white. Black lines divided the floor into nine 13.3×13.3 cm^2. The dark compartment had a wooden ceiling and its inside was painted black. A door of 10×8 cm in the partition wall allowed the rat to go from one compartment to the other (transition). Two 60-W fluorescent bulbs placed above the bright compartment provided illumination (190 lux).

2.2.2.2. Procedure. The experimental session lasted 10 min and was conducted between 14:00 and 18:00 h inside a sound-attenuated room. Each naive rat was placed at the center of the bright compartment with the head towards the door. The experimenter was outside the room, and the behaviour of the rat was recorded on videotape, which was later analysed by an observer unaware of the experimental treatment with the help of a computation program (Observer, Noldus, Holland). After each rat, the apparatus was cleaned with a flannel damped in 20% ethanol solution.

The following parameters were measured: total number of transitions between the light and dark compartments, number of transition attempts from the dark to the bright compartment (the animal places the head out of a dark side without crossing to the light side), number of line crossings between adjacent squares in the bright compartment and total time spent in the bright compartment. From the last two measures, the number of crossings per minute was calculated.

2.2.2.3. Data analysis. Data were analysed by a three-factor ANOVA and post-hoc Newman–Keuls test (as in Experiment 1). The level of significance was $P<.05$.

2.3. Physiological measures

2.3.1. Gastric ulcers

2.3.1.1. Procedure. Before brain perfusion (see above), the stomach was removed. The large curvature was incised to expose the mucous membrane, that was inspected both macroscopically and through a magnifying glass (Citoval 2, Carl Zeiss, Jena, Germany). Two trained observers assessed incidence, location (glandular portion), number and size (length) of gastric ulcers.

The rats were weighted before the surgery and after the end of the experiments. The weight loss was the difference between the last and the first weight. The death rate was also calculated.

2.3.1.2. Data analysis. Incidence of gastric ulcers, as well as lethality, was analysed through the χ^2 test. For analysis of number and size of gastric ulcers, as well as of body weight, a three-factor ANOVA and post-hoc Newman–Keuls test were performed (as in Experiment 1). The level of significance was $P<.05$.

2.3.2. Hippocampal 5-HT

2.3.2.1. Procedure. Naive rats, submitted to electrolytic or neurotoxic lesions and sham animals, restrained or nonrestrained, were decapitated between 8:00 and 10:00 h, and their brain was removed and dissected on ice. The hippocampus was separated and placed in Eppendorf vials containing 200 μl of precooled (−40°C) 2% trichloroacetic acid solution (TCA) and stored in a freezer at −70°C. The assay of 5-HT was performed using HPLC as described by Huang and Kissinger (1996). A volume of 2 ml of 0.33 M NaH_2PO_4 (pH 6) solution containing 0.5 mM EDTA was added to the homogenate and vortexed. This suspension was then centrifuged for 5 min at 14,000 rpm. A volume of 200 μl of the supernatant was filtered through a 0.45-μm Nylon-66 membrane. A volume of 50 μl of the supernatant was injected into the chromatography column (Liquid Chromatographic ODS-C 183 mm). All procedures were undertaken at room temperature (21°C). The results were expressed in nanograms per milligram of protein.

2.3.2.2. Data analysis. The data were submitted to a three-factor ANOVA and post-hoc Newman–Keuls test (as in Experiment 1). The level of significance was $P<.05$.

3. Results

3.1. Electrolytic lesion

The lesioned area included the MRN and adjacent tissue going from anterior to posterior midbrain. A typical lesion is illustrated in Fig. 1.

3.1.1. Elevated plus-maze

3.1.1.1. Traditional indexes. The changes in anxiety indexes are illustrated in Fig. 2.

The percentage of open-arm entries was significantly affected by lesion [$F(1,82)=4.00$, $P=.049$], type of lesion [$F(1,82)=9.83$, $P=.002$] and restraint [$F(1,82)=30,90$, $P<.001$]. There was a nearly significant interaction between lesion and restraint [$F(1,82)=3.22$, $P=.076$]. Post-hoc comparisons showed a nearly significant increase in open-arm entries caused by the electrolytic lesion in nonrestrained rats ($P=.056$), and a significant increase caused by the neurotoxic lesion in restrained animals ($P<.01$). Restraint generally decreased the percentage of open-arm entries ($P<.01$).

The percentage of time spent on open arms was also significantly changed by lesion [$F(1,82)=34.05$, $P<.001$], type of lesion [$F(1,82)=54.36$, $P<.001$] and restraint [$F(1,82)=32.02$, $P<.001$]. There were significant interactions between type of lesion and restraint [$F(1,82)=7.42$, $P=.008$] and between type of lesion and lesion [$F(1,82)=40.56$, $P<.001$]. Post-hoc analysis revealed significant lengthening of permanence on open arms after the electrolytic lesion in both nonrestrained ($P<.001$) and restrained ($P<.01$) animals. In contrast, the neurotoxic lesion had opposed effects as a function of restraint — a tendency to shorten the time on open arms in nonrestrained rats ($P=.059$) while lengthening the same measure in restrained rats ($P=.043$). Restraint generally decreased the percentage of time on open arms ($P<.05$).

Therefore, the electrolytic lesion of the MRN had an anxiolytic effect regardless of restraint, while the neurotoxic lesion had an anxiolytic effect only in rats previously submitted to restraint. Restraint had an anxiogenic effect on both indexes.

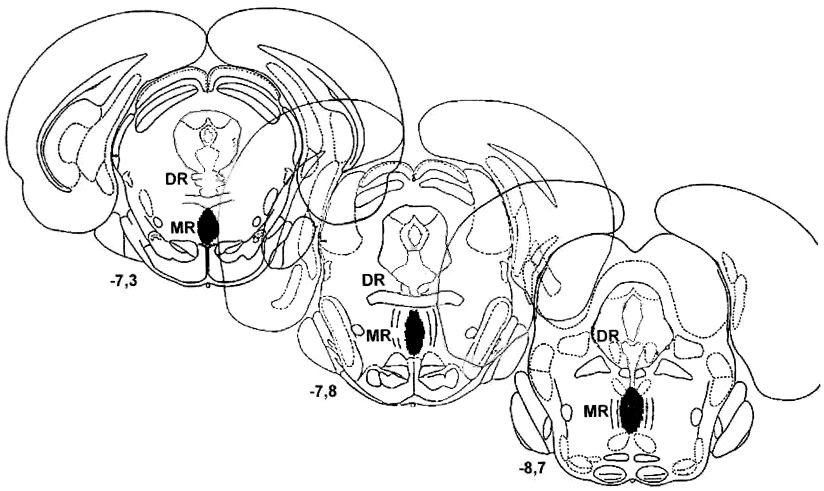

Fig. 1. Representative electrolytic lesion of the median raphe nucleus (shaded areas), projected on a section of the Paxinos and Watson (1990) rat brain atlas. DR = dorsal raphe nucleus, MR = median raphe nucleus.

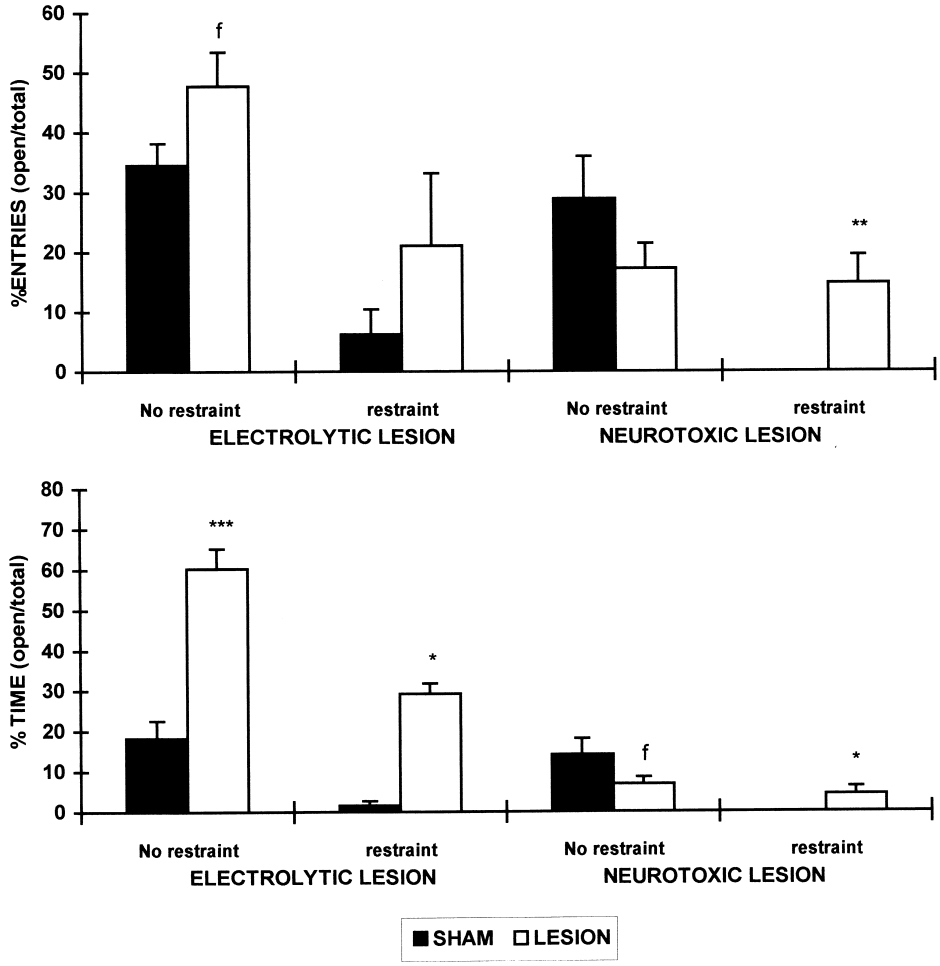

Fig. 2. Changes of anxiety indexes in the elevated plus-maze caused by median raphe nucleus lesion and restraint. Columns represent mean and bars the S.E.M. $N=10-18$. Electrolytic or neurotoxic (5,7-DHT) lesion were made 7 days before testing. Restraint was made 24 h before the test. * $P<.05$, ** $P<.01$, *** $P<.001$, borderline, differences between sham and lesion animals (Newman–Keuls test).

Table 1 shows the effects on closed-arm entries, regarded as an index of locomotion. The number of entries into closed arms was significantly affected by restraint [$F(1,82)=14.30$, $P<.001$], and there were nearly significant effects of lesion [$F(1,82)=2.99$, $P=.087$] and type of lesion [$F(1,82)=3.81$, $P=.054$]. No significant interaction among these factors was detected. Overall, restraint decreased locomotion. The electrolytic lesion tended to increase locomotion, especially in nonrestrained rats, while the neurotoxic lesion was ineffective.

3.1.1.2. Behavioural categories. The obtained results are summarised in Tables 2 and 3.

3.1.2. Rearing

Rearing was generally affected by restraint [$F(1,84)=4.59$, $P=.035$] and type of lesion [$F(1,84)=7.27$, $P=.008$], but not by lesion [$F(1,84)=1.21$, $P=.273$]. There were significant interactions between type of lesion and restraint [$F(1,84)=4.94$, $P=.030$] as well as restraint and lesion [$F(1,84)=6.07$, $P=.016$]. Post-hoc analysis evidenced that

Table 1
Effect of lesion of the median raphe nucleus and of restraint on number of closed-arm entries in the elevated plus-maze

	Electrolytic lesion				Neurotoxic lesion			
Group	*n*	No restraint	*n*	Restraint	*n*	No restraint	*n*	Restraint
Sham	11	6.18 ± 0.74	10	3.30 ± 0.62	10	5.40 ± 0.89	11	2.45 ± 0.47
Lesion	14	10.43 ± 1.93	4	4.00 ± 1.91	18	5.17 ± 0.91	12	3.92 ± 0.75

Figures represent mean ± S.E.M. of 4–18 rats.

Table 2
Effect of lesion of the median raphe nucleus and of restraint on occurrence of behavioural categories recorded during 5-min exploration of the elevated plus-maze

		Electrolytic lesion		Neurotoxic lesion	
Behavioural category	Group	No restraint	Restraint	No restraint	Restraint
Rearing	Sham	12.25 ± 0.88	6.21 ± 0.49	12.80 ± 1.54	9.82 ± 0.92
	Lesion	8.85 ± 1.16*	6.33 ± 1.20	9.22 ± 1.10*f*	12.36 ± 1.40
Grooming	Sham	4.33 ± 0.88	4.00 ± 0.68	4.70 ± 0.67	5.64 ± 0.96
	Lesion	2.08 ± 0.61*	0.67 ± 0.33*	5.28 ± 0.60	5.73 ± 1.39
Flat back approach	Sham	0.42 ± 0.15	2.00 ± 0.47	0.60 ± 0.22	2.55 ± 0.37
	Lesion	0.08 ± 0.08	1.00 ± 1.00	0.39 ± 0.14	1.73 ± 0.54
Closed arm return	Sham	1.42 ± 0.36	2.21 ± 0.28	3.90 ± 0.75	2.55 ± 0.68
	Lesion	0.08 ± 0.08	0.00 ± 0.00	3.33 ± 0.71	2.36 ± 0.85
Peeping out	Sham	3.42 ± 0.50	2.57 ± 0.44	3.60 ± 0.56	2.27 ± 0.41
	Lesion	0.77 ± 0.28***	0.33 ± 0.33*	3.78 ± 0.62	4.18 ± 0.82*f*
Head dipping	Sham	1.50 ± 0.47	0.29 ± 0.16	0.60 ± 0.27	0.18 ± 0.12
	Lesion	7.38 ± 1.48**	6.67 ± 2.67***	1.94 ± 0.55*	1.45 ± 0.39**
Scanning	Sham	3.42 ± 0.81	0.50 ± 0.31	3.50 ± 0.87	0.00 ± 0.00
	Lesion	15.46 ± 1.37***	5.67 ± 2.67***	2.06 ± 0.43	1.36 ± 0.59*
Open arm end exploration	Sham	2.08 ± 0.60	0.07 ± 0.07	1.80 ± 0.55	0.00 ± 0.00
	Lesion	5.69 ± 0.97**	2.33 ± 1.20***	0.67 ± 0.26*	0.27 ± 0.14*f*

Figures represent mean ± S.E.M. of 4–14 rats; differences between sham and lesion animals (Newman–Keuls test).
* $P<.05$.
** $P<.01$.
*** $P<.001$.
f = Borderline difference.

the electrolytic lesion significantly decreased rearing in nonrestrained rats ($P<.05$), and that a similar though non-significant trend ($P=.067$) occurred after the neurotoxic lesion. Restraint generally decreased rearing ($P<.05$).

On duration of rearing, there was no overall effect of either lesion [$F(1,84)=1.62$, $P=.206$], type of lesion [$F(1,84)=0.51$, $P=.477$] or restraint [$F(1,84)=0.16$, $P=.690$], but there was a significant interaction between restraint and lesion [$F(1,84)=13.96$, $P<.001$]. Post-hoc comparisons evidenced that electrolytic lesion significantly shortened rearing in nonrestrained rats ($P<.01$). Restraint generally shortened rearing duration ($P<.05$).

3.1.3. Grooming

There was an overall effect of type of lesion on the occurrence of grooming [$F(1,84)=12.93$, $P<.001$], but

Table 3
Effect of lesion of the median raphe nucleus and of restraint on duration (s) of behavioural categories recorded during 5-min exploration of the elevated plus-maze

		Electrolytic lesion		Neurotoxic lesion	
Behavioural category	Group	No restraint	Restraint	No restraint	Restraint
Rearing	Sham	41.75 ± 5.80	24.00 ± 3.66	37.50 ± 5.28	28.64 ± 2.22
	Lesion	18.69 ± 2.47**	34.67 ± 8.21	28.33 ± 3.85	33.82 ± 3.74
Grooming	Sham	37.67 ± 10.14	65.86 ± 10.16	26.10 ± 3.98	53.09 ± 0.89
	Lesion	11.92 ± 3.10*	6.33 ± 5.36*	32.17 ± 5.31	44.91 ± 11.24
Flat back approach	Sham	2.42 ± 1.71	8.50 ± 2.35	1.70 ± 0.73	12.36 ± 2.67
	Lesion	0.15 ± 0.15	3.33 ± 3.33	1.39 ± 0.53	5.82 ± 1.96*
Closed arm return	Sham	12.00 ± 2.80	20.36 ± 3.45	18.40 ± 3.10	14.91 ± 3.69
	Lesion	0.15 ± 0.15***	0.00 ± 0.00*	18.67 ± 4.51	9.91 ± 3.67
Peeping out	Sham	17.25 ± 3.86	22.14 ± 4.42	15.10 ± 2.57	12.82 ± 1.89
	Lesion	1.23 ± 0.50***	0.33 ± 0.33*	15.61 ± 2.49	21.00 ± 3.66*f*
Head dipping	Sham	3.25 ± 1.45	0.79 ± 0.45	1.30 ± 0.88	0.18 ± 0.12
	Lesion	20.54 ± 3.37***	38.33 ± 5.70***	4.11 ± 1.21	4.09 ± 1.45*
Scanning	Sham	14.92 ± 4.19	2.64 ± 1.71	20.30 ± 4.65	0.00 ± 0.00
	Lesion	75.69 ± 6.05***	19.00 ± 7.64**	11.28 ± 2.76*f*	6.45 ± 2.98*
Open arm end exploration	Sham	12.25 ± 3.43	0.36 ± 15.07	9.30 ± 3.44	0.00 ± 0.00
	Lesion	33.62 ± 7.04*	29.33 ± 5.07***	2.56 ± 1.17*	1.36 ± 0.85

Figures represent mean ± S.E.M. of 4–14 rats; borderline difference, differences between sham and lesion animals (Newman–Keuls test).
* $P<.05$.
** $P<.01$.
*** $P<.001$.
f = Borderline difference.

no influence of either lesion [$F(1,84)=2.76$, $P=.100$] or restraint [$F(1,84)=0.00$, $P=.993$]. There was a significant interaction between type of lesion and lesion [$F(1,84)=4.60$, $P=.035$]. Post-hoc comparisons showed that grooming frequency was significantly decreased ($P<.05$) by the electrolytic lesion in both restrained and nonrestrained rats, whereas the neurotoxic lesion was ineffective.

Concerning duration, there was an overall effect of lesion [$F(1,84)=10.01$, $P=.002$] and restraint [$F(1,84)=5.09$, $P=.026$], but no effect of type of lesion [$F(1,84)=1.56$, $P=.215$]. There was a significant interaction between type of lesion and lesion [$F(1,84)=9.06$, $P=.003$]. Further group comparisons showed that only the electrolytic lesion significantly shortened ($P<.05$) the duration of grooming in either restrained or nonrestrained animals. Restraint generally lengthened grooming duration.

3.1.4. Flat back approach

The frequency of flat back approach was affected by lesion [$F(1,84)=4.86$, $P=.030$] and restraint [$F(1,84)=29.05$, $P<.001$], but was not influenced by the type of lesion [$F(1,84)=2.71$, $P=.103$]. No interaction among the factors was detected. As shown in Table 2, MRN lesion decreased while restraint increased the occurrence of this behavioural category ($P<.01$).

Similarly, duration of flat back approach was affected by lesion [$F(1,84)=6.77$, $P=.011$] and restraint [$F(1,84)=19.68$, $P<.001$], but not by type of lesion [$F(1,84)=1.56$, $P=.214$]. Also, no significant interaction among factors occurred. It may be seen in Table 3 that MRN lesion shortened the duration of this behaviour whereas restraint lengthened the same duration ($P<.05$).

3.1.5. Closed-arm return

Occurrence of this behavioural category was generally affected by lesion [$F(1,84)=4.70$, $P=.033$] and type of lesion [$F(1,84)=18.07$, $P<.001$], but not by restraint [$F(1,84)=0.65$, $P=.421$]. No significant interaction among factors was found. It may be seen in Table 2 that the electrolytic lesion markedly decreased the frequency of closed-arm returns while the neurotoxic lesion was ineffective.

Also, on duration, there were overall effects of lesion [$F(1,84)=9.56$, $P=.003$] and type of lesion [$F(1,84)=6.05$, $P=.016$], but no effect of restraint [$F(1,84)=0.11$, $P=.736$]. There was a significant interaction between lesion and type of lesion [$F(1,84)=5.29$, $P=.024$]. Table 3 shows that the electrolytic lesion shortened the duration of this category in nonrestrained ($P<.001$), as well in as restrained rats ($P<.05$).

3.1.6. Peeping out

Occurrence of this behaviour was changed by type of lesion [$F(1,84)=13.19$, $P<.001$], but not affected by either lesion [$F(1,84)=2.27$, $P=.135$] or restraint [$F(1,84)=1.41$, $P=.238$]. There was a significant interaction between type of lesion and lesion [$F(1,84)=14.11$, $P<.001$]. Table 2 shows that peeping out was decreased by the electrolytic lesion in both nonrestrained ($P<.001$) and restrained ($P<.05$) rats. The neurotoxic lesion tended to increase peeping out in restrained rats ($P<.05$), but was ineffective on nonrestrained animals.

The duration of peeping out was influenced by lesion [$F(1,84)=7.97$, $P=.005$] and type of lesion [$F(1,84)=5.21$, $P=.025$], but not by restraint [$F(1,84)=0.47$, $P=.493$]. There was a significant interaction between type of lesion and lesion [$F(1,84)=20.32$, $P<.001$]. The figures of Table 3 show a significant decrease in duration of peeing out caused by the electrolytic lesion in either nonrestrained ($P<.001$) and restrained ($P<.05$) animals. In contrast, there was a nearly significant trend for the neurotoxic lesion to prolong peeping out in restrained rats ($P=.06$).

3.1.7. Head dipping

The frequency of head dipping was generally affected by lesion [$F(1,84)=36.07$, $P<.001$] and type of lesion [$F(1,84)=21.25$, $P<.001$], but there was no effect of restraint [$F(1,84)=2.76$, $P=.100$]. A significant interaction between lesion and type of lesion [$F(1,84)=14.00$, $P<.001$] was detected. Table 2 shows that the electrolytic lesion increased the frequency of head dipping in nonrestrained ($P<.01$) as well as restrained ($P<.001$) rats. The neurotoxic lesion increased the same behaviour in restrained rats ($P<.01$), and there was a trend to the same direction in nonrestrained animals ($P=.09$).

The duration of this behaviour was changed by lesion [$F(1,84)=116.37$, $P<.001$], type of lesion [$F(1,84)=87.00$, $P<.001$] and restraint [$F(1,84)=6.19$, $P=.015$]. There were significant interactions between lesion and type of lesion [$F(1,84)=71.10$, $P<.001$], type of lesion and restraint [$F(1,84)=8.33$, $P=.005$] as well as lesion and restraint [$F(1,84)=14.01$, $P<.001$]. The figures in Table 3 show that restraint decreased the duration of head dipping. This measure was increased by electrolytic lesion in nonrestrained ($P<.001$) and restrained ($P<.001$) rats. The neurotoxic lesion significantly prolonged head dipping in restrained rats ($P<.05$).

3.1.8. Scanning

The occurrence of scanning was generally affected by lesion [$F(1,84)=50.63$, $P<.001$], type of lesion [$F(1,84)=56.34$, $P<.001$] and restraint [$F(1,84)=38.96$, $P<.001$]. There were interactions between lesion and type of lesion [$F(1,84)=51.54$, $P<.001$] as well as type of lesion and restraint [$F(1,84)=8.71$, $P=.004$]. It may be seen in Table 2 that restraint decreased scanning behaviour. Post-hoc comparisons showed that electrolytic lesion increased scanning in both nonrestrained ($P<.001$) and

restrained ($P<.001$) rats. The neurotoxic lesion had the same effect, but only in restrained animals ($P<.05$).

The duration of scanning was influenced by lesion [$F(1,84)=36.55$, $P<.001$], type of lesion [$F(1,84)=36.20$, $P<.001$] and restraint [$F(1,84)=58.19$, $P<.001$]. Interactions occurred between lesion type and lesion [$F(1,84)=41.75$, $P<.001$], type of lesion and restraint [$F(1,84)=12.63$, $P<.001$] as well as restraint and lesion [$F(1,84)=5.50$, $P=.021$]. The figures in Table 3 show that restraint shortened the duration of scanning. Further comparisons revealed that electrolytic lesion prolonged scanning in either nonrestrained ($P<.001$) or restrained ($P<.01$) animals. The neurotoxic lesion enhanced scanning duration in restrained animals, but tended to shorten the same duration in nonrestrained rats ($P=.08$).

3.1.9. Open-arm end exploration

The occurrence of this behavioural category was affected by lesion [$F(1,84)=9.23$, $P=.003$], type of lesion [$F(1,84)=20.37$, $P<.001$] and restraint [$F(1,84)=21.05$, $P<.001$]. There was a significant interaction between lesion and type of lesion [$F(1,84)=16.67$, $P<.001$]. The figures in Table 2 show that restraint decreased the occurrence of arm end exploration. Post-hoc comparisons showed significant increases in the occurrence of this category in both nonrestrained ($P<.01$) and restrained ($P<.001$) rats. With the electrolytic lesion, there was a tendency to the same direction in restrained rats ($P=.067$), but an opposite effect of the neurotoxic lesion in nonrestrained rats ($P<.05$).

The duration of end exploration was influenced by lesion [$F(1,84)=15.38$, $P<.001$], type of lesion [$F(1,84)=29.57$, $P<.001$] and restraint [$F(1,84)=5.41$, $P=.022$]. A significant interaction between lesion and type of lesion also occurred [$F(1,84)=23.63$, $P<.001$]. It may be seen in Table 3 that restraint decreased the duration of end exploration. Further comparisons showed that the electrolytic lesion lengthened the duration of this behaviour in both nonrestrained ($P<.05$) and restrained ($P<.001$) rats, whereas the neurotoxic lesion shortened end exploration in nonrestrained rats ($P<.05$).

In summary, behavioural categories positively correlated with anxiety, such as flat back approach, closed-arm return and peeping out, were reduced by electrolytic lesion in both restrained and nonrestrained rats. In addition, the same lesion increased behavioural categories inversely correlated with anxiety, such as head dipping, scanning and open-arm end exploration. The effect of the neurotoxic lesion was less clear. Behavioural categories directly correlated with anxiety were not significantly affected by this type of lesion, while increases in head dipping and scanning reached significance only in restrained rats; even decreases occurred in nonrestrained animals following the neurotoxic lesion of the MRN. Restraint itself decreased most of the behavioural categories inversely correlated with anxiety indicating an anxiogenic effect. Nevertheless, the behavioural categories directly correlated with anxiety were not significantly affected by this manipulation.

The frequency of stretch attending and immobility was very low. As a consequence, the results with these behavioural categories were not analysed.

3.1.10. Light–dark box

The number of transitions between the light and dark compartments of the experimental box was changed by lesion [$F(1,75)=15.98$, $P<.001$], but there was no effect of either type of lesion [$F(1,75)=0.002$, $P=.959$] or restraint [$F(1,75)=2.28$, $P=.135$]. A significant interaction between lesion and type of lesion [$F(1,75)=10.70$, $P=.002$] occurred. Fig. 3 shows that the electrolytic lesion increased transitions in both nonrestrained ($P<.01$) and restrained ($P<.01$) animals, while the neurotoxic lesion was ineffective.

Transition attempts were affected by type of lesion [$F(1,75)=25.92$, $P<.001$], but there was no effect of lesion [$F(1,75)=0.01$, $P=.918$] or restraint [$F(1,75)=0.07$, $P=.787$]. There was a significant interaction between lesion and type of lesion [$F(1,75)=10.75$, $P<.001$]. Further comparisons showed that the electrolytic lesion decreased transition attempts in both nonrestrained ($P<.01$) and restrained ($P<.05$) rats, whereas the neurotoxic lesion tended to enhance the same behaviour in restrained ($P=.053$) rats only (Fig. 3).

The time spent on the bright side of the box was affected by lesion [$F(1,75)=55.63$, $P<.001$] and type of lesion [$F(1,75)=26.68$, $P<.001$], but there was no effect of restraint [$F(1,75)=0.015$, $P=.904$]. There was a significant interaction between type of lesion and lesion [$F(1,75)=38.38$, $P<.001$], and a nearly significant interaction between restraint and lesion [$F(1,75)=3.93$, $P=.051$]. Further comparisons revealed that the time in the bright compartment was significantly lengthened by the electrolytic lesion in either nonrestrained ($P<.001$) or restrained ($P<.001$) rats, and by the neurotoxic lesion in restrained rats ($P<.05$), alone (Fig. 3).

In summary, the electrolytic lesion had a neat anxiolytic effect on both nonrestrained and restrained rats, evidenced by the three above indexes of anxiety. However, the picture is less clear with the neurotoxic lesion, since the latter had an anxiolytic effect on time spent in the bright side of the light–dark box in restrained rats, alone. In these animals, neurotoxic lesion even tended to have an anxiogenic effect on transition attempts. In contrast with the above anxiogenic effect in the elevated plus-maze, restraint, per se, had no effect on anxiety indexes in the light–dark box.

The results on locomotion are summarised in Table 4. Neither lesion nor restraint affected locomotion, measured by the number of line crossings while the rat was in the

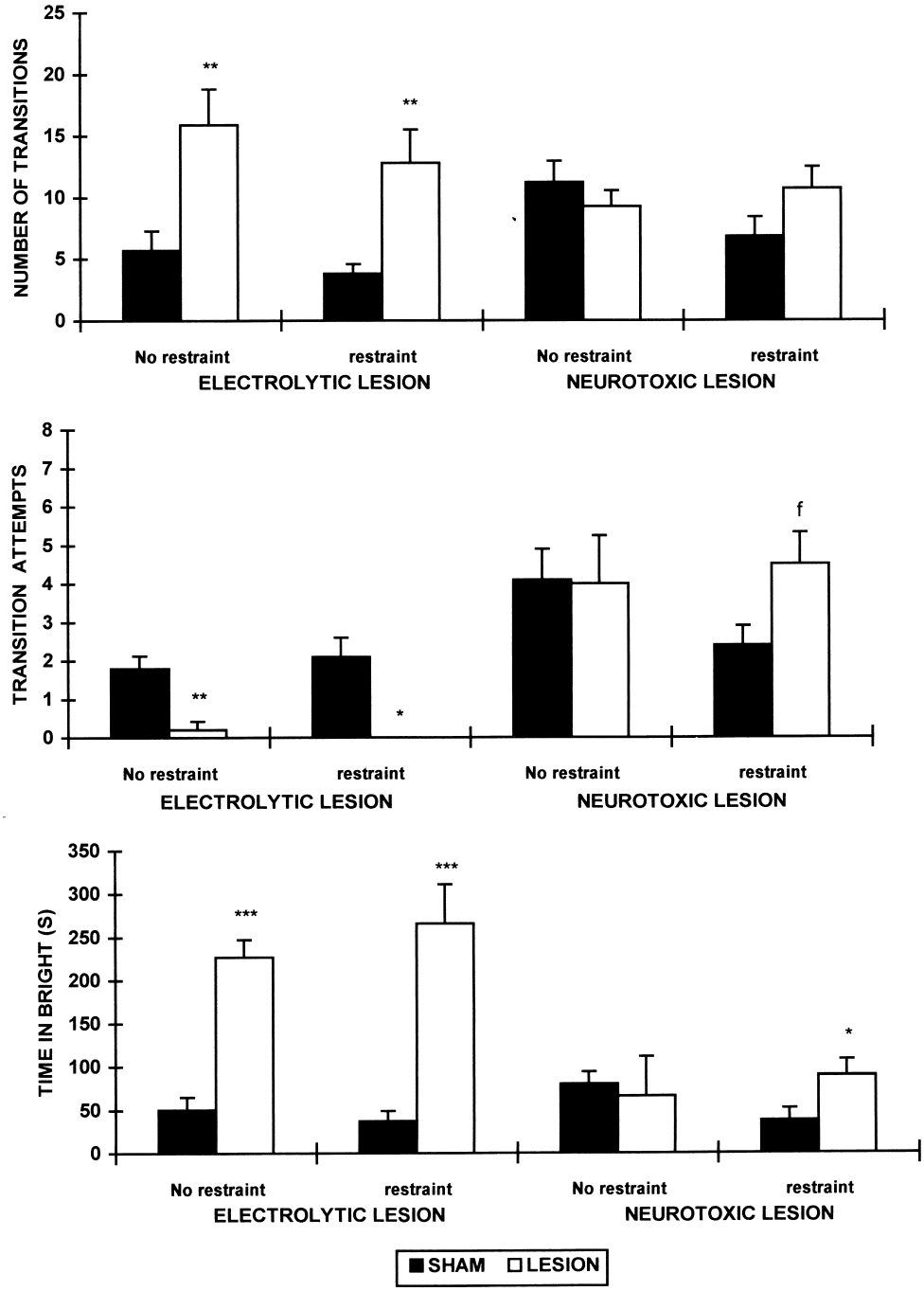

Fig. 3. Changes of anxiety indexes in the light–dark box caused by median raphe nucleus lesion and restraint. $N = 10–18$. Further specifications as in the legend of Fig. 2.

bright compartment of the experimental box. The ANOVA evidenced no overall effect of lesion [$F(1,75) = 0.00$, $P = .968$], type of lesion [$F(1,75) = 0.81$, $P = .369$] or restraint [$F(1,75) = 1.40$, $P = .240$].

Table 4
Effect of lesion of the median raphe nucleus and of restraint on locomotion (line crossings/s) in the bright compartment of light–dark box

	Electrolytic lesion				Neurotoxic lesion			
Group	*n*	No restraint	*n*	Restraint	*n*	No restraint	*n*	Restraint
Sham	10	0.28 ± 0.06	10	0.46 ± 0.08	10	0.49 ± 0.02	10	0.47 ± 0.04
Lesion	9	0.45 ± 0.10	10	0.54 ± 0.13	12	0.60 ± 0.16	12	0.38 ± 0.03

Figures represent mean ± S.E.M. of 9–12 rats.

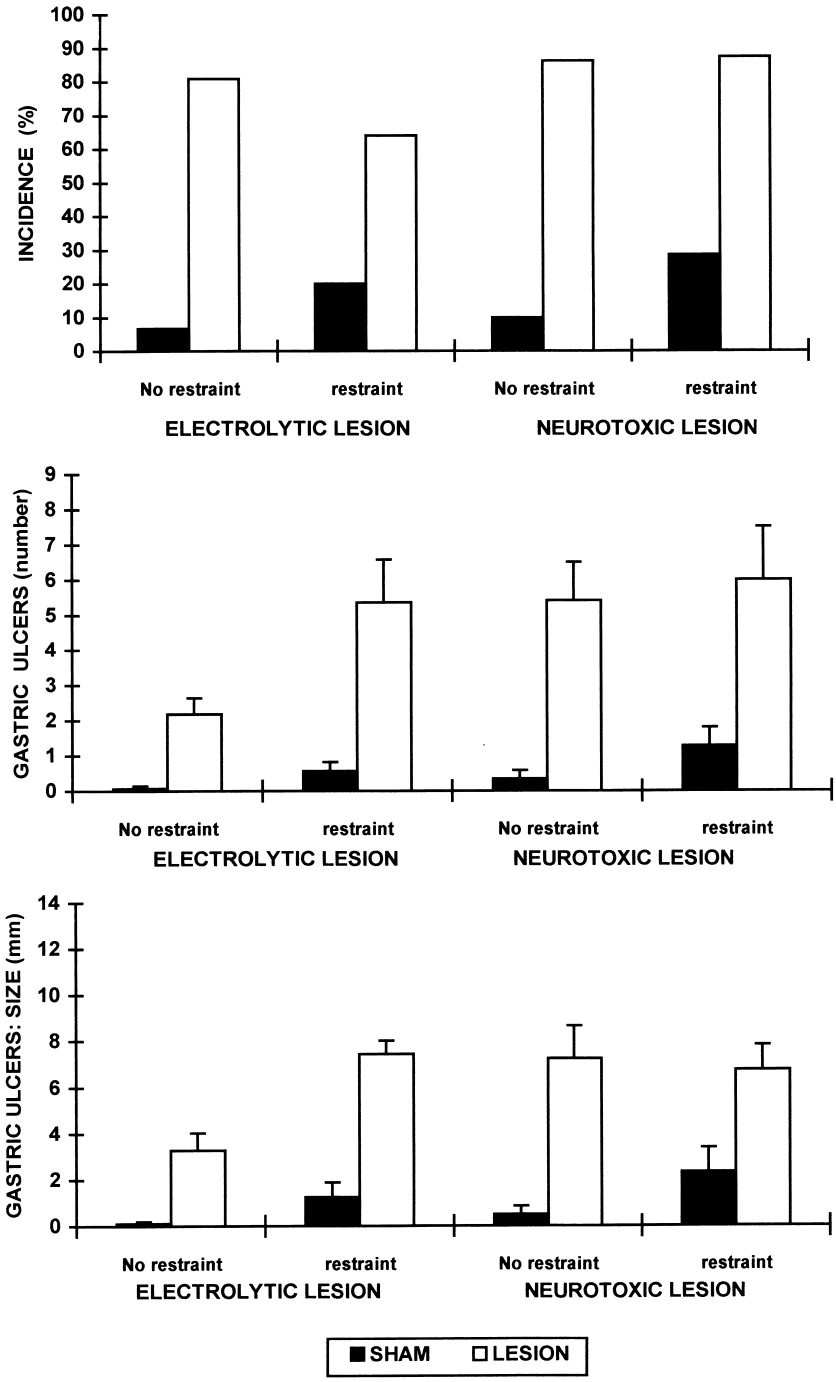

Fig. 4. Effect of lesion of the median raphe nucleus and restraint on gastric ulcers. Columns represent mean and bars the S.E.M. $N = 15-29$. Further specifications as in the legend of Fig. 2.

3.1.11. Gastric ulcers

The results are shown in Fig. 4.

The electrolytic lesion increased the incidence of ulcers in both nonrestrained ($\chi^2 = 19.31$, $P < .001$) and restrained ($\chi^2 = 8.21$, $P = .004$) rats; neurotoxic lesion had a similar effect in nonrestrained ($\chi^2 = 19.85$, $P < .001$) and restrained animals ($\chi^2 = 9.76$, $P = .002$).

Regarding the number of gastric ulcers, ANOVA showed an overall effect of lesion [$F(1,172) = 32.46$, $P < .001$], as well as nearly significant trends to an effect of either type of lesion [$F(1,172) = 2.76$, $P = .098$] and restraint [$F(1,172) = 3.13$, $P = .079$]. There was no significant interaction among the factors. It may be seen in Fig. 4 that both electrolytic and neurotoxic lesions markedly increased the number of gastric ulcers. This effect tended to be larger with the neurotoxic lesion, and restraint tended to generally increase the number of ulcers.

Table 5
Loss of body weight (g) after surgery

	Electrolytic lesion				Neurotoxic lesion			
Group	*n*	No restraint	*n*	Restraint	*n*	No restraint	*n*	Restraint
Sham	18	23.89 ± 6.04	20	13.45 ± 6.04	12	21.71 ± 3.29	11	15.33 ± 2.99
Lesion	13	43.92 ± 9.33***	15	52.53 ± 9.36***	22	16.40 ± 2.51	17	11.79 ± 3.04

Figures represent mean ± S.E.M. of 11–22 rats; differences between sham and lesion animals (Newman–Keuls test).
*** $P < .001$.

The size of gastric ulcers was affected by lesion [$F(1,172) = 24.79$, $P < .001$], but not by type of lesion [$F(1,172) = 1.35$, $P = .247$] or restraint [$F(1,172) = 2.61$, $P = .108$]. No interaction among the factors occurred. Fig. 4 shows that electrolytic and neurotoxic lesions increased the size of gastric ulcers in both nonrestrained and restrained animals.

3.1.12. Body weight loss

The results are summarised in Table 5.

There were overall effects of lesion [$F(1,164) = 102.00$, $P < .001$], type of lesion [$F(1,164) = 77.52$, $P < .001$] and restraint [$F(1,164) = 4.52$, $P = .035$]. A significant interaction between type of lesion and lesion [$F(1,164) = 78.25$, $P < .001$] occurred. Post-hoc comparisons evidenced that the electrolytic lesion caused weight loss in both nonrestrained ($P < .001$) and restrained ($P < .001$) rats, whereas the neurotoxic lesion was ineffective.

3.1.13. Lethality

As it may be seen in Table 6, the electrolytic lesion increased the death rate in nonrestrained ($\chi^2 = 3.94$, $P = .047$) and in restrained ($\chi^2 = 9.81$, $P = .002$) rats.

3.1.14. Hippocampal 5-HT

The results are summarised in Table 7.

The concentration of 5-HT in the hippocampus was affected by lesion [$F(1,32) = 53.78$, $P < .001$] and restraint [$F(1,32) = 7.82$, $P = .009$]. The overall effect of type of lesion was nearly significant [$F(1,32) = 3.28$, $P = .080$]. There were significant interactions between type of lesion and restraint [$F(1,32) = 7.05$, $P = .012$] as well as between lesion and restraint [$F(1,32) = 7.41$, $P = .010$]. Lesion generally decreased whereas restraint increased 5-HT level. Post-hoc comparisons showed that the electrolytic lesion caused a marked depletion of 5-HT concentration in both nonrestrained ($P < .05$) and restrained ($P < .01$) rats. The neurotoxic lesion also caused significant decreases in 5-HT level in both nonrestrained ($P < .05$) and restrained ($P < .001$) rats.

4. Discussion

The present results show that electrolytic lesion of the MRN increased (only in no-restraint animals) the number of entries into the closed arm of the elevated plus-maze, a measure that is taken as an index of locomotion (Cruz et al., 1994; Fernandes and File, 1996; File, 1992; Rodgers et al., 1997). Nevertheless, another widely used index of locomotion, the number of line crossings in the bright side of the light–dark box, was not affected. The neurotoxic lesion was ineffective on both measures of locomotion. These results agree with published results showing that electrolytic, but not neurotoxic, lesion of the MNR increases locomotor activity (Albinsson et al., 1996; Asin and Figiber, 1983; Gerson and Baldessarini, 1980; Hilegaart, 1990; Hilegaart and Hjorth, 1989; Jacobs et al., 1973, 1974; Wirtshafter and Asin, 1982; Wirtshafter and McWilliams, 1987; Wirtshafter et al., 1989). This evidence is usually interpreted as an indication that 5-HT systems originating in the MRN are not involved in motor regulation (Wirtshafter and McWilliams, 1987; Wirtshafter et al., 1989). This notion is not undisputed, however, since microinjection of 8-OH-DPAT into the MRN has been shown to increase locomotion (Hilegaart, 1990; Hilegaart and Hjorth, 1989). As this drug stimulates autosomic 5-HT_{1A} receptors that inhibit neuron firing (Andrews et al., 1994; Bonvento et al., 1992; Mongeau et al., 1997; Schreiber and De Vry,

Table 6
Death rate after surgery (%)

Experimental	Electrolytic lesion		Neurotoxic lesion	
group	No restraint	Restraint	No restraint	Restraint
Sham	10.80	0.00	0.00	0.00
Lesion	30.51*	36.84**	0.00	0.00

Figures represent mean ± S.E.M. of 25–59 rats; differences between sham and lesion animals (χ^2 test).
* $P < .05$.
** $P < .01$.

Table 7
Concentration of 5-HT in the hippocampus in nanograms per milligram of protein

Experimental	Electrolytic lesion		Neurotoxic lesion	
group	No restraint	Restraint	No restraint	Restraint
Sham	1.57 ± 0.43	1.70 ± 0.29	0.90 ± 0.11	3.11 ± 0.64
Lesion	0.10 ± 0.04*	0.03 ± 0.02**	0.42 ± 0.10*	0.52 ± 0.11*

Figures represent mean ± S.E.M. of five rats per group; differences between sham and lesion animals (Newman–Keuls test).
* $P < .05$.
** $P < .01$.

1993a,b), enhanced locomotion may be due to release from inhibition mediated by 5-HT.

The present results further show that the electrolytic lesion had a stronger anxiolytic effect than the neurotoxic lesion. In the elevated plus-maze, the electrolytic lesion enhanced the percentage of open-arm entries and of time spent on the open arms in both restrained and nonrestrained rats, while the neurotoxic lesion was effective only in previously restrained animals. In addition, the electrolytic lesion reduced behavioural categories directly related to anxiety (flat back approach, closed-arm return and peeping out) as well as increased behavioural categories inversely correlated with anxiety (head dipping, scanning and open-arm end exploration). The neurotoxic lesion did not significantly change behavioural categories directly correlated with anxiety and increased head dipping and scanning in restrained rats, only. Even decreases in categories inversely related to anxiety occurred in nonrestrained animals following neurotoxic lesion of the MRN. In the light–dark box, as well, the electrolytic lesion had a neat anxiolytic effect on both nonrestrained and restrained rats. This was evidenced by increased light–dark transitions, prolonged time spent in the bright side and less transition attempts. In contrast, the neurotoxic lesion had mixed effects. It lengthened the time spent in the bright side of the apparatus in restrained rats, having thus an anxiolytic effect, but tended to increase transition attempts in the same experimental group, an anxiogenic effect.

In two reported studies in which 5,7-DHT was microinjected into the MRN, no change in anxiety has been observed in the social interaction test (File et al., 1979), and, in the elevated plus-maze (Thomas et al., 2000), only a small, though significant, increase in the time spent on the open arm — indicative of anxiolysis — occurred on the fifth day after the lesion. The ineffectiveness of brain 5,7-DHT lesions on phenomena likely to be mediated by 5-HT has been attributed to compensatory mechanisms that occur along the 3 weeks following injection (Patel et al., 1996; Thomas et al., 2000), and impaired function has been reported to progressively recover after the 14th day from the lesion (Fischette et al., 1987; Haring, 1991). Nevertheless, in the study by Thomas et al. (2000), the release of 5-HT in the extracellular pace of the hippocampus caused by exposure to the elevated plus-maze was abolished 8–10 days after 5,7-DHT lesion of the MRN, and that caused by fenfluramine injection markedly decreased. These results indicate that the functional capacity of the MRN-hippocampal pathway was severely impaired by the lesion at this moment, which was very close to the seven-day interval between lesion and test used in the present study.

This greater efficacy of the electrolytic lesion of the MNR on anxiety may due to destruction of nonserotonergic in addition to serotonergic neurons. Nevertheless, the degree of impairment of 5-HT systems may also be an important factor, since the present results have shown that the electrolytic lesion markedly depleted hippocampal 5-HT in both nonrestrained and restrained rats, while the neurotoxic lesion had a similar effect in restrained rats, but caused less 5-HT depletion in nonrestrained animals. Therefore, the conclusion may be drawn that 5-HT neurons of the MRN regulate anxiety, at least in part. Accordingly, reported results show that microinjection into the MNR of 5-HT_{1A} receptor agonists had an anxiolytic-like effect in several animal models of anxiety (Andrews et al., 1994; Carli et al., 1989; Carli and Samanin, 1988; De Almeida et al., 1998; File et al., 1996; Schreiber and De Vry, 1993a,b). As complementary evidence, electrical stimulation of the MRN has been shown to suppress lever-pressing behaviour in rats, an anxiogenic-like effect that was counteracted by inhibiting 5-HT synthesis with PCPA (Graeff and Silveira Filho, 1978).

Immobilization for 2 h, performed 24 h before testing, had an anxiogenic effect in the elevated plus-maze, indicated by both the classical anxiety indexes and by behavioural categories inversely related to anxiety. This finding agrees with previously reported results (Albonetti and Farabollini, 1992, 1993; Guimarães et al., 1993; Padovan and Guimarães, 1993; Titze de Almeida et al., 1994). Nevertheless, the same manipulation failed to increase anxiety in the light–dark box. This may due to either low sensitivity or test selectivity. In regard to the last possibility, reported results indicate that certain drugs and/or brain lesions are effective on certain animal models of anxiety, but not in others (File and Gonzalez, 1996; Graeff et al., 1996; Griebel, 1995; Handley et al., 1993; McBlane et al., 1992; McNaughton, 1993; Rodgers et al., 1997). In addition to directly affecting anxiety measures, restraint interacted with the neurotoxic lesion, unveiling its anxiolytic effect (see above). This finding gives support to the suggestion that destruction of 5-HT neurons in the MRN removes a brain mechanism that limits the consequences of stress (Deakin and Graeff, 1991; Graeff et al., 1996).

The present results showing that electrolytic lesion of the MRN increased the incidence of gastric ulcers, as well as their number and size generally agree with previously reported evidence (Hoshino and Sugizaki, 1986). These ulcers are localised in the glandular portion of the stomach, thus, being related to stress (Paré, 1972). Because in the results of Hoshino and Sugizaki (1986), fasting was a necessary condition for the occurrence of ulcers, these authors suggested that MRN lesion potentiated stress by removing an inhibitory mechanism. Furthermore, since MRN lesion led to 5-HT depletion in several brain areas, Hoshino and Sugizaki (1986) inferred that this stress-dampening mechanism involved 5-HT neurons. The last suggestion is supported by the present results showing that the neurochemical lesion was at least as effective as the electrolytic lesion for producing gastric ulcers. At variance with the work by Hoshino and Sugizaki (1986), however, fasting was not presently needed to evidence the ulcerogenic effect of the MRN lesions. This discrepancy may nevertheless be due a ceiling effect of the present lesions. Notice, in this regard,

that a nonsignificant trend for restraint to increase the number of gastric ulcers occurred.

It is widely accepted that anxiety is a behavioural symptom of stress (Graeff et al., 1996; McNaughton, 1993). Yet, in the present study, rats having a high incidence of stress ulcers show lessened anxiety in the two animal models used. Moreover, the electrolytic lesion of the MRN, which caused more gastric ulceration, weight loss and death, had a stronger anxiolytic effect than the neurotoxic lesion (see above). At first sight, these results cast doubt on the reliability of the animal models used for assessing anxiety. However, the anxiety indexes in these models detect short-lived emotional changes. In contrast, MRN lesion seems to generate a long-lasting state of stress, under which the test challenges might be perceived as less frightening than usual. Anyway, the seeming discrepancy between physiological and behavioural measures shown by the present results highlight the importance of studies that combine both types of measure for a deeper understanding of the mechanisms underlying stress and anxiety.

In conclusion, the present results showing that both electrolytic and neurotoxic lesions of the MRN induce gastric stress ulcers clearly implicate the 5-HT pathway ascending from this nucleus to the hippocampus in their generation. Because the anxiolytic effect of the neurotoxic lesion was less marked than that of the electrolytic lesion, only partial regulation of anxiety by the same 5-HT pathway may be suggested. Finally, the lack of change in locomotion following the neurotoxic lesion tends to rule out the MRN-hipoccampal 5-HT pathway in motor regulation.

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