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Differential Role of Serotonin and Noradrenaline on Anxiety Reduction After Ejaculation in the Rat

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SALDÍVAR, A., C. RÍOS AND A. FERNÁNDEZ-GUASTI. *Differential role of serotonin and noradrenaline on anxiety reduction after ejaculation in the rat.* PHARMACOL BIOCHEM BEHAV **38**(4) 807–812, 1991.—As previously reported, a reduction in anxiety after ejaculation was observed. In a previous report it was demonstrated that the GABA-benzodiazepine system is involved in the mediation of this reduction in anxiety. The anxiety levels were measured using a defensive burying model. This work was performed to elucidate the serotonin and noradrenaline participation in the mediation of this phenomenon. Two experiments were made. In the first experiment the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT, 10 μg/10 μl) was intracerebroventricularly injected. Five days after its administration the behavioral tests were performed. In the second experiment, the noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4, 50 mg/kg × 2) was IP administered. The neurochemical data reveal a drastic reduction in various brain areas respective monoamine levels after these treatments. The lesion produced by 5,7-DHT was able to reverse the reduction in anxiety in copulating males, but produced no changes in noncopulating animals. This finding supports the idea that the serotonergic system is involved in the reduction of anxiety observed after ejaculation. The results of the DSP4 experiment suggest that there is not a direct participation of the noradrenergic system in the anxiety reduction observed after ejaculation.

Anxiety reduction after ejaculation 5,7-DHT DSP4 Serotonin Noradrenaline Burying behavior

RECENTLY we reported a reduction in anxiety after ejaculation in the rat (11). Additionally, we have found that the administration of GABAergic and benzodiazepinic antagonists inhibited the reduction in anxiety after ejaculation leading to the conclusion that the GABA-BZ system is involved in this effect (14). Besides GABA, the participation of serotonin (5-HT) and noradrenaline (NA) in the mediation of both anxiety and masculine sexual behavior has been reported. Thus several studies suggest an inhibitory role of 5-HT in the control of masculine sexual behavior (3,28) and anxiety (5, 17, 22). The role of NA in these processes seems to be less clear, however, it is suggested that this neurotransmission is increased during anxiety states (1, 24, 32). Finally, the facilitatory role of the noradrenergic system in the neural control of male sexual behavior has also been suggested (3, 26, 29). Since 5-HT and NA participate in the control of copulatory behavior and in the mediation of anxiety, in the present experiments we analyzed whether these neurotransmitter systems are involved in the reduction of anxiety found after ejaculation. As in the previous study (11), the burying behavior paradigm was chosen to measure the anxiety levels. In view of the large number of postsynaptic receptor types described for 5-HT (31) and NA (25), in the present experiments the role of these neurotransmitter systems was studied by lesioning the terminals using the selective neurotoxins 5,7-DHT (4) and DSP4 (21).

GENERAL METHOD

Animals

Male Wistar rats (250–300 g) were used. The animals had free access to Purina rat chow and water all over the study and were maintained in a 12:12-h light-dark controlled room (lights off from 1000 to 2200 h). Animals were individually housed for at least three days before the experiment. The experiments were made two h after initiating the dark phase.

Drugs

The following drugs were used in these experiments: 5,7-dihydroxytryptamine (5,7-DHT), Sigma, St. Louis, MO; desipramine, Sigma, St. Louis, MO; 2-chloroethyl-N-ethyl-2 bromobenzylamine

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TABLE 1

MONOAMINE ASSAYS (ng/g TISSUE) ON THE BRAIN STEM, HIPPOCAMPUS, HYPOTHALAMUS AND FRONTAL CORTEX OF 5,7-DHT AND ASCORBIC ACID 20%-TREATED RATS

	Groups	Serotonin	5HIAA	Noradrenaline
Brain Stem	Control	311 ± 22	224 ± 24	540 ± 75
	5,7-DHT	117 ± 36‡(38)	108 ± 32†(48)	650 ± 61^{ns}(120)
Hippocampus	Control	239 ± 63	223 ± 33	476 ± 115
	5,7-DHT	104 ± 33*(44)	125 ± 19†(56)	525 ± 99^{ns}(110)
Hypothalamus	Control	635 ± 125	361 ± 71	1712 ± 199
	5,7-DHT	203 ± 59‡(32)	162 ± 32†(45)	1576 ± 195^{ns}(92)
Cortex	Control	457 ± 76	414 ± 53	524 ± 51
	5,7-DHT	108 ± 17‡(24)	108 ± 16‡(26)	758 ± 205^{ns}(144)

Student *t*-test, ns: nonsignificant; *$p \leq 0.05$; †$p \leq 0.02$; ‡$p \leq 0.002$.

5,7-DHT (10 μg/10 μl ICV) was injected 60 min after desipramine (25 mg/kg, IP). Values are expressed as means ± S.E. Values in parentheses indicate percentage of control.

(DSP4); Astra Läkmedel AB, Sweden; and zimelidine hydrochloride, Astra, Läkmedel, AB, Sweden. 5,7-DHT was dissolved in 20% ascorbic acid; desipramine and zimelidine in physiological saline and DSP4 in distilled water.

Anxiety Test

The defensive burying behavior paradigm was used (40). As previously described (11), this technique can be useful to study anxiety under pharmacological manipulations (39) and different physiological status (11). This paradigm consists of an acrylic cage measuring 27 × 23 × 16 cm with the floor covered with sawdust. An energized prod emerges from one of the walls of the cage. After the animal receives the electric shock (0.3 mA) it displays the burying behavior that consists of a series of movements oriented to cover the prod with the fine sawdust. The time from the shock to the behavior display was defined as the burying behavior latency. The animals were observed during a ten-minute test and the cumulative burying behavior recorded. The increase in burying behavior latency and a decrease in the cumulative burying behavior is interpreted as a reduction in anxiety levels (40).

Sexual Behavior Observations

Male sexual behavior tests were made with females brought into sexual receptivity by the administration of estrogen (estradiol valerianate 5 μg/ rat, at 0 h) followed by progesterone (1 mg/rat 44 h). Sexual behavior tests were made 4 h after progesterone injection. Male rats were placed in a cylindrical cage two min before the receptive/proceptive female was introduced. The sexual behavior parameters recorded were: intromission and ejaculation latencies, number of mounts and intromissions preceding ejaculation and postejaculatory interval. For description of each behavioral parameter see Fernández-Guasti et al. (11). The sexual behavior parameters were recorded during two consecutive series of copulation.

Biochemical Assay

Noradrenaline, serotonin and 5-hydroxyindolacetic acid (5-HIAA) were analyzed by HPLC with electrochemical detection following the technique described by Saligaut et al. (34) with minor modifications.

The animals were sacrificed by decapitation. The brain was removed, placed on a cold plate and the hippocampus (Hc), hypothalamus (Ht), frontal cortex (Cx), and brain stem (BS) dissected according to the method of Iversen and Glowinski (20). After thawing, cerebral structures were added with an antioxidant solution containing 0.1% w/v $Na_2S_2O_5$ in 0.05 M perchloric acid, 0.3 ml for Ht, 0.7 ml for Hc, 1.0 ml for Cx and 0.7 ml for BS. The tissue was then homogenized by sonication and centrifuged at 8000 × g for 10 min, the supernatant transferred to a polypropylene tube with cap and stored frozen until analysis. All samples were filtered on millipore filters (0.22 μm) prior to injection into the HPLC system. The HPLC apparatus consisted of a Perkin-Elmer series 3B liquid chromatograph with a 20 μl sample loop. Monoamines signal was monitored with a Metrohm amperometric detector using an oxidation potential of 0.8 V vs. Ag/AgCl reference electrode at 5 nA of sensitivity scale. Peaks were integrated with a Sigma 10 chromatography data station. Mobile phase consisted of 0.03 M phosphate buffer (pH 3.5) containing 0.08% w/v sodium octyl sulphate, 0.03% EDTA and 15% methanol. The column was an Alltech C-18 reversed phase (100 × 4.6 mm, 3 μm of average particle size), and the flow rate was 1.4 ml/min. The results shown in Tables 1 and 2 were expressed as ng of monoamine/g fresh tissue.

Statistical Analysis

The sexual, burying and spontaneous ambulatory behavior data were statistically analyzed by help of the Kruskal-Wallis analysis of variance followed by the Mann-Whitney U-test (36). The neurochemical data were compared using the Student's *t*-test (37).

TABLE 2

MONOAMINE ASSAYS (ng/g TISSUE) ON THE HIPPOCAMPUS AND HYPOTHALAMUS OF DSP4- AND DISTILLED-WATER TREATED RATS

	Groups	Noradrenaline	Serotonin
Hippocampus	Control	352 ± 77.3	122 ± 51
	DSP4	51 ± 51†(14)	161 ± 46^{ns}(131)
Hypothalamus	Control	952 ± 209	193 ± 66
	DSP4	477 ± 100*(50)	159 ± 34^{ns}(83)

Student *t*-test, ns: nonsignificant; *$p \leq 0.05$; †$p \leq 0.002$.

DSP4 (2 × 50 mg/kg, IP) was injected 60 min after zimelide (2 × 20 mg/kg, IP). Values are expressed as means ± S.E. Values in parentheses indicate percentage of control.

The motor coordination data were statistically analyzed by the Wilcoxon *t*-test (36).

Experiment 1: Effect of the neurotoxin 5,7-DHT on the reduction in anxiety induced by ejaculation. In order to lesion the serotonergic fibers the neurotoxin 5,7-DHT was intraventricularly injected as follows: the noradrenergic reuptake inhibitor, desipramine (25 mg/kg IP) was injected one hour before 5,7-DHT (10 μg/10 μl ICV) administration. The control group received desipramine or saline one hour before 5,7-DHT vehicle, consisting of 10 μl ascorbic acid 20%. The animals were anesthetized with pentobarbital (35 mg/kg IP) and mounted in a stereotaxic instrument with the intraaural plane in a horizontal position (parallel horizontal lines between the intraaural plane and the incisor bars). The bregma and lambda lines identified. The coordinates to inject into the right lateral ventricle were: 6.06 mm anterior to lambda, 1.4 mm right to the middle line and 3.9 mm deep (23). These coordinates were confirmed by histological methods. The drug was administered at a rate of 2 μl per minute and the needle stayed in the ventricle 5 min after injection.

Five days after the neurotoxin injection the experiment was made. The three main groups included in this experiment were: lesioned (desipramine + 5,7-DHT), nonlesioned (desipramine + ascorbic acid), and sham operated (saline + ascorbic acid). These groups were additionally divided into two groups: control (tested for anxiety without sexual behavior) and experimentals (tested for anxiety immediately after two ejaculations). After the experiments the animals were sacrificed and the monoamines levels determined as previously described.

Experiment 2: Effect of noradrenergic neurotoxin DSP4 on the reduction in anxiety induced by ejaculation. In order to lesion the locus coeruleus noradrenergic fibers, the neurotoxin DSP4 was systemically (IP) injected as follows: the neurotoxin DSP4 (50 mg/kg) was administered one hour after the serotonergic reuptake inhibitor, zimelidine hydrochloride (20 mg/kg). The control groups received zimelidine or saline one hour before the DSP4 vehicle, 2 ml/kg of distilled water. One week later the treatment was repeated and the behavioral test performed three weeks after the second treatment. As in Experiment 1 three main groups were included: lesioned (zimelidine + DSP4), nonlesioned (zimelidine + distilled water), and control (saline + distilled water). These groups were additionally divided into two groups: control (tested for anxiety without sexual behavior) and experimentals (tested for anxiety immediately after two ejaculations). After the experiments the animals were sacrificed and the monoamine levels determined as previously described. Additionally, in this experiment, motor coordination and spontaneous ambulatory behavior tests were performed. The motor coordination was made as previously described (12). Briefly, the animals were trained to walk in a rotarod (7 cm diameter, 11 rpm) for three consecutive days. The number of falls during a 5-min period was counted. The data of the third test were compared to those obtained in the second test.

The spontaneous ambulatory behavior test was made as previously described (13). The motor activity was recorded in a box measuring 43 × 36 × 19 cm that was placed over a sensitive plaque (48 × 40 cm) of an activity meter (Stoelting Co., Chicago, IL) connected to a counter (Stoelting Co., Chicago, IL). The animal was placed in the cage and the number of counts were recorded after a 10-min period.

RESULTS

Experiment 1: Effect of the Neurotoxin 5,7-DHT on the Reduction in Anxiety Induced by Ejaculation

The biochemical assay results are shown in Table 1. There were no statistically significant changes in brain noradrenaline levels both in experimental and control groups (lesioned and nonlesioned), while a statistically significant decrease of 5-HT and its metabolite 5-HIAA levels was found in the lesioned group compared with the nonlesioned.

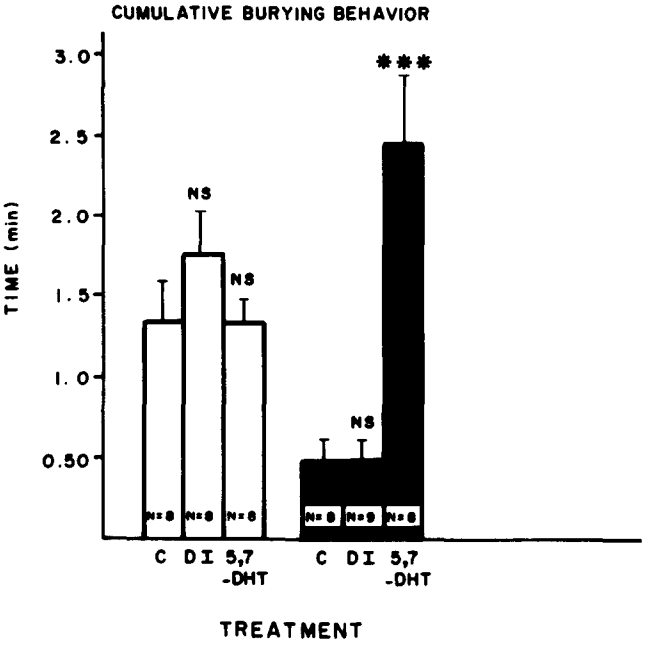

FIG. 1. Effect of the serotonergic neurotoxin 5,7-DHT on the reduction in burying behavior observed after ejaculation. Figure shows mean ± S.E. of the cumulative burying behavior during a 10-min test. (C) control; (DI) desipramine; (5,7-DHT) 5,7-dihydroxytryptamine groups. The clear bars represent the burying behavior levels of animals tested after the pharmacological treatment but which did not achieve sexual behavior (control groups). The dark bars represent the burying behavior levels of the treated animals tested after ejaculation. Kruskal-Wallis analysis of variance for all groups: $H(5) = 27.3105$, $p < 0.001$; for the control group, $H(2) = 2.3897$, nonsignificant; for the experimental groups, $H(2) = 15.7375$, $p < 0.001$. Mann-Whitney U-test versus their respective control, $^{***}p < 0.002$.

The effect of the neurotoxin on the reduction in anxiety after ejaculation is shown in Fig. 1. As previously described (11), ejaculation produced a consistent reduction in burying behavior (both sham-operated vehicle-treated groups). As may be observed in Fig. 1, the treatment with desipramine plus 5,7-DHT vehicle had no effect either in control (without sexual behavior) or in experimental (tested after two ejaculations) groups. The group of 5,7-DHT-lesioned animals, tested for anxiety without sexual activity, did not show statistically significant differences as compared both with the control or desipramine-treated groups. Most remarkable, the lesion of the serotonergic terminals by 5,7-DHT was highly effective in preventing the reduction in anxiety consistently observed after ejaculation. Thus the lesioned group reveals a highly statistically significant difference as compared with the controls and desipramine groups. Moreover, treatment with this neurotoxin not only prevented the reduction in anxiety after ejaculation, but also resulted in an increase in anxiety levels as compared with the sham-operated noncopulating animals. In this experiment, the burying behavior latency was not modified [data not shown; Kruskal-Wallis analysis of variance, $H(5) = 5.1397$, nonsignificant].

No treatment besides 5,7-DHT modified any parameter of masculine sexual behavior. As previously reported (3,29), the le-

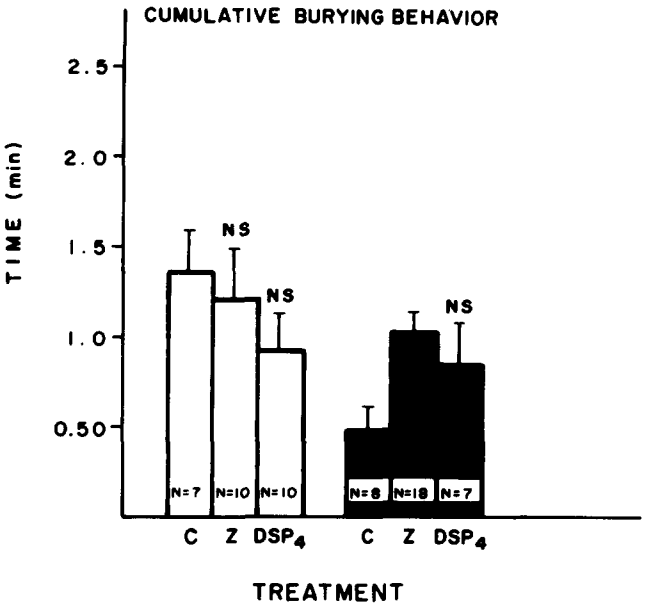

FIG. 2. Effect of the noradrenergic neurotoxin DSP4 on the reduction in burying behavior observed after ejaculation. Figure shows mean ± S.E. of the cumulative burying behavior during a 10-min test. (C) control; (Z) zimelidine; (DSP4) DSP4 groups. The clear bars represent the burying behavior levels of animals tested after the pharmacological treatment which did not achieve sexual behavior (control groups). The dark bars represent the burying behavior levels of the treated animals tested after sexual behavior. Kruskal-Wallis analysis of variance for all groups: $H(5) = 8.2271$, nonsignificant.

sion caused with 5,7-DHT facilitates the performance of sexual behavior by reducing the number of intromissions preceding ejaculation (Table 3).

Experiment 2: Effect of noradrenergic neurotoxin DSP4 on the reduction in anxiety induced by ejaculation. The biochemical data of this experiment are shown in Table 2. A statistically significant reduction in noradrenaline levels in hippocampus and hypothalamus was found. No changes in 5-HT were detected.

As described in Experiment 1, a reduction in anxiety levels evidenced as a reduction in the cumulative burying behavior was observed after ejaculation (control groups with and without sexual activity, Fig. 2). By contrast with previous experiments, no differences in the cumulative burying behavior values were found. However, zimelidine slightly blocked the reduction in anxiety induced by ejaculation. From these data it was concluded that the noradrenergic lesion was not effective in preventing the reduction in anxiety produced by ejaculation. The performance of sexual behavior was unaffected by this treatment (Table 3).

No differences in burying behavior latency were found between all groups involved in this experiment [data not shown, Kruskal-Wallis analysis of variance, $H(5) = 2.1675$, nonsignificant]. The motor coordination data revealed no statistically significant differences (median values for the second and third tests, respectively 1 and 0; Wilcoxon *t*-test, nonsignificant). However, the general spontaneous ambulatory behavior was significantly diminished in DSP4-treated animals (mean counts per 10 min ± S.E. for zimelidine control 412.4 ± 30.2 and for experimentals, 314.6 ± 29.4; Mann-Whitney U-test, $p < 0.05$).

DISCUSSION

As previously reported (11), in this study we observed a consistent reduction in anxiety measured in the burying behavior paradigm associated to the period following ejaculation. The lesion of the serotonergic fibers, by 5,7-DHT, produced a significant increase in cumulative burying behavior, leading to the conclusion that this neurotoxin effectively reverses the reduction in anxiety observed after ejaculation. Conversely, DSP4, despite its ability to diminish the noradrenaline levels in discrete brain regions, did not block the anxiety reduction after ejaculation.

TABLE 3

EFFECT OF THE NEUROTOXINS 5,7-DHT AND DSP4 ON RAT MASCULINE SEXUAL BEHAVIOR DURING TWO CONSECUTIVE EJACULATORY SERIES

	IL	NM	NI	EL	PEI	NM	NI	EL
Desipramine	0.20	6	10	9.79	6.67	2	5	4.25
5,7-DHT	0.30	8	8*	8.38	5.62	3	4	3.70
Zimelidine	0.68	2	10	6.35	5.89	4	6	2.95
DSP4	0.27	3	12	7.13	6.21	2	4	2.43

The table shows median values. IL, intromission latency; NM, number of mounts; NI, number of intromissions; EL, ejaculation latency; and PEI, postejaculatory interval. The comparisons were made with the respective control: desipramine with 5,7-DHT group; zimelidine with DSP4 group. Mann-Whitney U-test; *$p \leq 0.05$.

The physiological role of the serotonergic system in the mediation of anxiety remains controversial. Thus, while some authors have found an anxiogenic role of this amine (19,33), others have shown an anxiolytic action. In the latter line of thought an antiaversive effect of increased serotonergic transmission has been proposed (18,27). Additionally, the antagonists metergoline and ketanserin blocked the effect of serotonin and its agonists, but per se did not show proconflict activity. These data have been interpreted as a phasic, rather than a tonic, mechanism of serotonin antiaversive action (18).

In relation to the sexual behavior, the inhibitory role of endogenous serotonin seems very clear (3, 12, 26, 28). Thus the serotonergic fibers lesion (by 5,7-DHT administration) results in a facilitation of copulation reflected as a reduction in the length of the PEI and in a decrease of postejaculatory ultrasonic vocalizations (39). These data suggest that there is an increase in the serotonergic transmission after ejaculation. Direct experimental evidence supporting this idea is given by Ahlenius et al. (2) and Mas et al. (30) who have found an increase in 5-HT turnover in discrete brain regions involved in the control of masculine sexual behavior. Interestingly, present data showed that treatment with 5,7-DHT did not alter the anxiety levels in noncopulating animals but effectively blocked the reduction in anxiety observed after ejaculation. This observation, together with aforementioned data, led us to the proposition that ejaculation is triggering a serotonergic mechanism, which in addition to being involved in the regulation of masculine sexual behavior, participates in the reduction of anxiety associated to mating. Furthermore, the finding showing a differential effect of the serotonergic lesion in copulating and noncopulating animals strengthens the idea of a phasic control of serotonin in the mediation of anxiety processes. Needless to mention, further research should be made to fully confirm this idea.

From present data it is not possible to discriminate on which kind of serotonergic receptor subtypes the neurotransmitter is acting to produce its putative postejaculatory anxiolytic activity. Recently, we have proposed that the inhibitory action of this amine on masculine sexual behavior is mediated through the stimulation of the 5-HT_{1B} receptor subtype (12). However, on pharmacological basis (6,13), most likely the anxiolytic actions of endogenous

5-HT are mediated via the 5-HT_{1A} receptor. Nevertheless, other serotonergic compounds with anxiolytic properties like 5-HT_2 and 5-HT_3 antagonists have been reported leading to the consideration of these receptor subtypes in the mediation of the anxiolytic action of serotonin (5,22). Further experiments designed to block the postejaculatory anxiolytic effect of 5-HT with specific antagonists should be undertaken to clarify this point.

The role of noradrenaline in the mediation of anxiety remains controversial. Thus some authors have suggested that an increase in the noradrenergic transmission resulted in anxiogenic processes (1, 24, 32), and that the interference of the noradrenergic transmission by beta blockers (7,9) or by alpha presynaptic agonists (38) produce anxiolytic and sedative responses. However, a second group has failed to find alterations in anxiety by adrenergic blocking agents (16,35). The effects of lesioning the locus coeruleus, either electrolytically or neurotoxically, on anxiety is confusing. Thus some authors (8, 15, 42) have failed to find any effect of the neurotoxic or electrolytic lesion of the locus coeruleus on anxiety, while Velley et al. (41) have reported an increased neophobia (inferred as a reduction in ambulation in a novel situation) in the open-field test after lesioning this area. This last result, interpreted as an anxiogenic action, could be explained on the basis of present findings showing that treatment with DSP4 produces a decrease in spontaneous ambulatory behavior. All these results considered together would suggest that the locus coeruleus is not involved in the regulation of anxiety.

The noradrenergic control of masculine sexual behavior seems to involve primarily arousal processes (26). In line with this idea, McIntosh and Barfield (29) have shown that electrolytic lesions of the locus coeruleus produce a significant increase in the length of the postejaculatory interval. However, present findings and previous results (10) showed that the DSP4 locus coeruleus lesion does not affect the length of the postejaculatory interval. Whether the different results could be interpreted on the basis of the different techniques used to lesion the locus coeruleus remains a matter of speculation.

In closing, the results given in these series of experiments led us to the conclusion that ejaculation activates a serotonergic mechanism that in turn produces a reduction in anxiety. The noradrenergic system does not seem to participate in this phenomenon.

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