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Effects of Long-term Psychological Stress on Sexual Behavior and Brain Catecholamine Levels

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ABSTRACT: The effects of long-term psychological stress on sexual behavior and brain catecholamines were investigated in rats. Stress was applied using the communication box developed by Ogawa and Kuwabara (1996), and a psychological stress group ($n = 12$), a physical stress group ($n = 5$), and a control group ($n = 5$) were established. Stress was applied for 1 hour every day for 10 consecutive weeks. Sexual behavior was observed before the start of exposure to stress and 2, 4, 6, 8, and 10 weeks thereafter. The results showed that long-term psychological stress impaired the sexual behavior of male rats. Long-term psychological stress decreased the concentrations of catecholamine and its metabolites in the brain, especially in the medial preoptic area (MPOA). Thus, we hypothesized that low catecholamine neurotransmission in the brain results in impairment of male rat sexual behavior. We then tried to restore the impaired sexual behavior by administration of a cerebral-activating drug, indeloxazine hydrochloride. The administration of indeloxazine hydrochloride for a 3-week period restored the sexual behavior that had been impaired by long-term psychological stress. These present results suggest that impairment of neurotransmission in the central nervous system could be a cause of sexual dysfunction, and activation of neurotransmission may result in restoration of impaired male sexual behavior.

Key words: Psychological stress, sexual behavior, Catecholamines.

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It is believed that various forms of stress, especially psychological stress, cause a decrease in sexual function. There have been several reports on studies of the relationships between stress and sexual behavior employing stress-generating methods, such as restraint, electrical shock, hanging, etc. These stress-generating methods, however, included physical elements in addition to psychological elements (Caggiura and Eibergen, 1969; Caggiura and Vlahoulis, 1974; Menendez-Patterson et al, 1978). Accordingly, we wanted to investigate the effect of psychological stress alone on male sexual behavior. Therefore, we used the communication box, developed by Ogawa and Kuwabara (1966), that is capable of generating psychological stress and excluding physical elements. Using this box, we exposed male rats to long-term psychological stress and studied the changes in their sexual behavior. We then measured catecholamine concentrations in the brain, because catecholamine neurons in the brain respond to stress and are related to regulation of sexual behavior. The brain areas investigated, including the medial preoptic area (MPOA), the amygdala, the substantial nigra, the striatum, the cortex, and the nucleus accumens, are considered to regulate male sexual behavior. These areas are also related to the regulation of emotion. The psychological stress generated by the communication box is transmitted as an emotional stress in responder rats (Ogawa and Kawabara, 1966). Therefore, there is a possibility that monoamine neurons in the areas that mediate male sexual behavior are affected by long-term psychological stress.

Furthermore, we studied the possibility of using a cerebral activating drug as an effective therapeutic agent for treating male sexual dysfunction caused by stress. Therefore, we investigated the facilitative effect of indeloxazine hydrochloride on the sexual behavior of male rats exposed to long-term psychological stress. This drug stimulates monoamine neurons in the brain and is widely used in medical treatment (Yoneyama et al, 1993). Indeloxazine has some cerebral activating properties. One is an action that increases ATP and glucose utilization in the brain. A second is its action as a monoamine re-uptake inhibitor. By these actions indeloxazine has a facilitative effect on monoamine neurotransmission (Yamamoto and Shimizu, 1987a, b; Yoneyama et al, 1993). These behavioral changes contribute to action activating neurotransmission of indeloxazine. In these studies the dose of indeloxazine employed was 10 mg/kg body weight, the same as the dose in these experiments.

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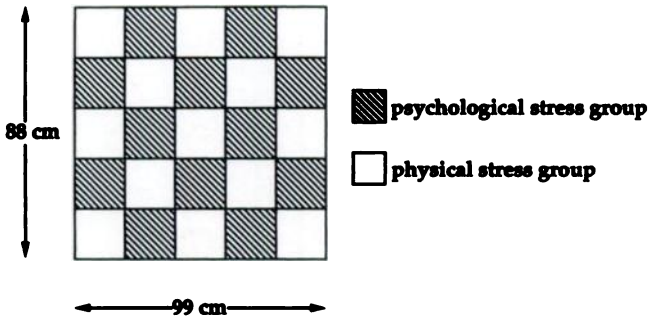

FIG. 1. Communication box: a floor plan of the communication box. Physical stress group: rats in open compartments received electrical foot shock from a floor grid. Psychological stress group: rats in shaded compartments were prevented from receiving foot shock by a plastic plate on the floor, but they were exposed to the response of the neighboring rats receiving the foot shock.

Materials and Methods

Experiment I

Experimental Animals and Housing Conditions—Male and female Wistar-line rats, 12 weeks of age at the start of stress loading, were employed. The animal cages were made of clear plastic, 40 × 25 × 25 cm in size. Wood chips were used as bedding. Four rats of the same sex and same experimental group were housed per cage. The animal room was maintained at a constant temperature of 24°C, 12-hour light/12-hour dark schedule (time of illumination: from 7 AM to 7 PM). Except during tests, food and water were freely available to the animals.

Experimental Equipment and Animal Groups—Experimental equipment: we used a communication box, as modified by Tanaka et al (Iimori et al, 1982; Tanaka et al, 1991). As shown in Figure 1, the communication box was a clear plastic (acrylate) box, 99 × 88 × 50 cm in size, that was divided into 25 compartments using the same clear plastic board. The floor grid was made of stainless-steel rods, each with a diameter of 3 mm, and the distance between rods was 13 mm. Electric current was passed through the rods to apply electrical shock at desired time intervals.

Animal groups: the following three male animal groups were established. 1) Physical stress group (n = 5). At 30-second intervals, a 3-mA electric current was applied for 5 seconds to the floor grid of the open compartments in Figure 1; this electrical shock was repeated for 1 hour. The rats showed squealing, jumping, and other reactions in response to the periodical electrical shock. The 13 rats were placed in open compartments, as shown in Figure 1. Some rats were replaced by new rats of the same age during the 10-week stress-loading period, because they exhibited protective behavior against the electrical shock. These rats turned over in their compartments, and their back fur prevented electrical shock. They did not show any squealing and jumping, and therefore could not be used to examine the role of physical stress. Finally, only five rats received electrical shock throughout the 10-week period, and we evaluated the data for these five rats. The rats were rotated daily through all the compartments used for physical stress loading. 2) Psychological stress group (n = 12). The animals in the psychological stress group were placed in the shadowed compartments shown in Figure 1. A plastic board was placed over the floor grid in these compartments. Therefore, the animals in these compartments were not exposed to electrical shock, but they were exposed to psychological stress consisting of the squealing, jumping, and odor, generated by the neighboring animals in the physical stress group for 1 hour every day. The rats were rotated daily through the compartments used for psychological stress loading. 3) Control group (n = 5). The animals in the control group were placed in the communication box for 1 hour every day without being exposed to electrical shock or the reactions of neighboring animals. The rats in the control group were kept in the animal room during the stress loading and were completely separated from the reactions of the physical stress group.

Experimental Schedule—1) Stress application: stress loading using the communication box was performed for 1 hour every day for 10 consecutive weeks. 2) Observation of sexual behavior: each animal was observed for sexual behavior before the start of stress loading and after 2, 4, 6, 8, and 10 weeks of stress loading. In the observations performed before stress loading, each animal was observed for two sessions after being permitted to copulate several times. The mean of the data from the two sessions was employed as the baseline value. On the day of observation of sexual behavior, the animal was stress loaded at least 3–4 hours before the sexual behavioral test. 3) Measurement of increment rate of body weight: we measured the body weights of the rats of each group every 2 weeks for a 10-week period. We calculated a ratio of the incremental changes in body weight (body weight/initial body weight) in each stress group and control group. 4) Measurement of brain catecholamines, the weight of bilateral adrenal glands, and serum testosterone levels: on the day when the 10-week stress loading was finished, the rats in each group were sacrificed by decapitation, following release in the animal cage for 2 hours after the final stress loading. We then measured the brain catecholamine levels described below.

To further measure the intensity of stress after 10-week stress loading, we also removed the bilateral adrenal glands and calculated the weight of the glands per body weight.

To evaluate the effects of long-term psychological stress or physical stress on the serum testosterone levels, we measured serum testosterone after 10-week stress loading in each stress group and the control group as well. Testosterone was assayed by radioimmunoassay (RIA) (total testosterone kit TKTT1; Diagnostic Products Corp., Los Angeles, California).

Observation of Sexual Behavior—1) Method of observation: sexual behavior was observed as follows. Each male rat was placed in a plastic observation cage, 60 × 50 × 40 cm in size, and allowed to become accustomed to the environment for 10 minutes. Then a female was gently introduced into the cage, and observation of the sexual behavior was begun. The female had been administered estradiol benzoate intramuscularly (i.m.) at a daily dosage of 5 mg beginning 5 days before mating to induce estrus, and only females exhibiting lordosis were employed in the study.

On the day of the sexual behavioral test, the stress loading was finished at least 3 hours before the test. 2) Observation parameters: each sexual behavioral test was 30 minutes long, and the frequencies of mounts with or without intromission and ejaculations were recorded. We evaluated male rat sexual be-

havior using the following three parameters. a) Mount rate. Mount rate was calculated as the mean number of mounts per minute during the observation period except for the postejaculatory interval. The postejaculatory interval was measured as the time from ejaculation to the next mount with or without intromission. b) Intromission latency. Intromission latency was measured as the time to the first intromission from the start of the test. c) Ejaculation latency. Ejaculation latency was measured as the time from the first mount to the first ejaculation.

Measurement of Brain Catecholamines—After 10 weeks of stress loading, we measured the levels of brain catecholamines and metabolites by high-performance liquid chromatography (HPLC). The male rats were killed by decapitation. The whole brain was rapidly removed, placed on dry ice, and sectioned into 0.5-mm slices. We punched out the MPOA, substantia nigra, amygdala, striatum, nucleus accumbens, and cortex, according to the atlas of Paxinos and Watson (1986), on the dry ice. The wet weight of each tissue was measured, and it was then homogenized in 1 ml of 0.2 M $HClO_4$ buffer containing 0.2 mM ethylenediamine tetraacetic acid (EDTA). The homogenates were centrifuged at 10,000 × *g* for 15 minutes. The supernatant was analyzed for amine content by using an HPLC electrochemical detector (HPLC-ECD). The chromatographic system was an HPLC-ECD 100 (EICOM, Kyoto, Japan). It consisted of an MA-50DS reverse-phase column (4.6 mm i.d. × 250 mm) and a WE-3G graphite electrode, set at +0.75 V vs. an Ag/AgCl reference electrode. Samples were eluted with 0.1 M sodium acetate and 0.1 M citric acid at a pH of 3.9. The mobile phase was pumped at 1 ml/minute. Sample catecholamine and metabolite concentrations were estimated from the peak heights by comparison with injections of known amounts of pure standards (Sigma Chemical Co., St. Louis, Missouri). Levels of catecholamine and its metabolites, which were dopamine (DA), the DA metabolite 5,6-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and noradrenaline (NA), were measured together in each sample.

Data Analysis—The changes in sexual behavior and body weight in each group were analyzed by repeated measures analysis of variance (ANOVA) and post hoc test. The differences in concentrations of brain catecholamines, serum testosterone levels, and weights of adrenal glands among stress-loading groups and the control group were analyzed by the Mann-Whitney *U*-test and Kruskal-Wallis test.

Experiment II

In experiment II, we investigated whether a cerebral activator could restore sexual behavior affected by long-term psychological stress. We used indeloxazine as a cerebral metabolism enhancer (Yamamoto and Shimizu, 1987a).

Experimental Animals and Housing Conditions—The male and female Wistar rats used were 12 weeks of age at the time of initiation of stress loading. Housing conditions were the same as in experiment I.

Drug—The drug used in this experiment was indeloxazine hydrochloride ([±]-2-[(inden-7-yloxy) methyl] morpholine hydrochloride; YM-08054), at 10 mg/kg body weight.

Animal Groups—Rats were divided randomly into an indeloxazine-treated group and a vehicle-treated group before starting stress loading. 1) Indeloxazine-treated group: the rats were administered 10 mg/kg indeloxazine intraperitoneally (i.p.) 30 minutes before stress loading. Indeloxazine was administered daily for 3 weeks from the 7th week in the stress-loading period. Indeloxazine was not administered to the rats on test days to prevent the short-term action of indeloxazine from facilitating their sexual behavior. 2) Vehicle-treated group: the rats were administered 0.5 ml of saline i.p. 30 minutes before stress loading. Saline was administered daily for 3 weeks from the 7th week in the stress loading period, but it was not administered on the test days.

Experimental Schedule—1) Stress application: the rats in the two treated groups were exposed to psychological stress for 1 hour per day for 10 weeks using a communication box, just as in experiment I. On the days of sexual behavioral tests, the stress loading was finished at least 3 hours before the test. 2) Observation of sexual behavior: sexual behavior tests were performed for 30 min periods before starting stress loading and at 2, 4, 6, 8, and 10 weeks after starting stress loading.

Observation of Sexual Behavior—Sexual behavior was evaluated using two parameters: mount rate and ejaculation latency. However, some rats did not exhibit ejaculation within the 30-minute test period. Those rats that did not exhibit ejaculation were defined as suffering from a loss of ejaculation.

In experiment II, we measured the sexual behavior of rats only in the psychological stress group.

Data Analysis—Analysis of the data on sexual behavior was carried out using repeated measures ANOVA. The incidences of loss of ejaculation within 30 minutes in the indeloxazine-treated group and the vehicle-treated group were compared by chi-square analysis.

Results

Experiment I

Changes in Body Weight, Weight of Adrenal Glands, and Serum Testosterone Levels—1) Body weight: there were no significant differences in body weight before stress loading among the psychological stress, physical stress, and control groups (Kruskal-Wallis test). There were no significant differences in the rate of change in body weight during the experiment among the three groups (repeated measures ANOVA, $F[22,88] = 1.735$, $P = 0.1065$). 2) Weight of adrenal glands (Table 1): in the control group, the weight of bilateral adrenal glands per body weight was $0.67 \pm 0.05 \times 10^{-4}$ (mean ± standard deviation [SD]). In the psychological stress group and the physical stress group, the weight of bilateral adrenal glands per body weight was $1.05 \pm 0.19 \times 10^{-4}$ weight and $1.00 \pm 0.08 \times 10^{-4}$, respectively. The weight of the adrenal glands in both stress groups was significantly heavier than that in the control group (psychological vs. control, $P = 0.0034$; physical vs. control, $P = 0.0045$; Mann-Whitney *U*-test). 3) The level of serum total testosterone (Table 1): in the psychological stress group, the level of serum total tes-

Table 1. *Effects of psychological and physical stress on adrenal gland weight and serum total testosterone*

	Adrenal glands (×10⁻⁴/body weight)	Testosterone (ng/ml)
Psychological	1.05 ± 0.19*	2.18 ± 2.11
Physical	0.99 ± 0.08*	2.75 ± 3.45
Control	0.67 ± 0.05	1.92 ± 1.06

Values are means ± SD. The weights of adrenal glands in both stress groups were significantly heavier than those of the control group.

* $P < 0.01$ (Mann-Whitney *U*-test). There were no significant differences between both the stress groups and the control group for serum total testosterone (Mann-Whitney *U*-test).

tosterone was 2.18 ± 2.12 ng/ml (mean ± SD). In the physical stress group, it was 2.75 ± 3.45 ng/ml, and in the control group, 1.92 ± 1.06 ng/ml. There were no significant differences between both the stress groups and the control group (Mann-Whitney *U*-test).

Effect on Sexual Behavior—The sexual behavior of the male rats was analyzed on the basis of the three observation parameters. The two stress groups were compared with the control group to determine statistical differences (Table 2). 1) Mount rate: in the control group, no significant change in the mount rate was seen throughout the 10-week period (by one-way repeated measures ANOVA). There were no significant differences in the interaction between the mount rate and the time course between the physical stress group and the control group (by two-way repeated measures ANOVA, $F[5,40] = 1.951, P = 0.1072$). Between the psychological stress group and the control group, there were significant differences in the interaction between the mount rate and the time course ($F[5,75] = 5.682, P = 0.0002$). In the psychological stress group, the values for the mount rates in the 8th and 10th weeks after starting stress loading were significantly decreased in comparison with the value before starting stress loading ($P < 0.001$). 2) Intromission latency: in the control group, no significant changes in the intromission latency were observed throughout the 10-week period (by one-way repeated measures ANOVA). As for the effect of the time course on intromission latency, there were no significant differences between the physiological stress group and the control group (by two-way repeated measures ANOVA, $F[5,40] = 0.837, P = 0.5313$). Between the psychological stress group and the control group, there were significant differences in the effect of the time course on intromission latency ($F[5,75] = 3.692, P = 0.0048$). In the psychological stress group, the value for intromission latency in the 4th week after starting stress loading was significantly shortened ($P < 0.05$), and the value for the 10th week was significantly prolonged in comparison with the value before starting stress loading ($P < 0.001$). 3) Ejaculation latency: in the control group, there were no significant changes in ejaculation latency throughout the 10 week period (by one-way repeated measures ANOVA). As for the effect of the time course on ejaculation latency, there were no significant differences between the physiological stress group and the control group (by two-way repeated measures ANOVA, $F[5,40] = 1.032, P = 0.4121$). Between the psychological stress group and the control group, there were significant differences in the effect of the time

Table 2. *Effects of psychological and physical stress on male rat sexual behavior*

	Pre-stress	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
			Mount (times/minute)			
Psychological*	2.21 ± 0.43	1.91 ± 0.46	2.67 ± 0.82	1.72 ± 0.53	1.58 ± 0.27†	1.36 ± 0.29†
Physical	2.03 ± 0.28	2.47 ± 0.51	2.68 ± 1.05	3.81 ± 1.75	3.32 ± 0.65	2.94 ± 0.64
Control	2.08 ± 0.44	2.08 ± 0.31	2.18 ± 0.47	2.24 ± 0.24	2.38 ± 0.33	2.36 ± 0.41
			Intromission latency (minutes)			
Psychological‡	1.19 ± 0.78	1.52 ± 2.13	0.64 ± 0.42†	1.69 ± 1.31	1.27 ± 0.90	3.64 ± 2.25†
Physical	1.28 ± 0.75	1.42 ± 1.04	1.04 ± 0.68	0.93 ± 0.51	0.64 ± 0.40	0.57 ± 0.48
Control	2.54 ± 1.52	1.58 ± 0.75	1.52 ± 0.75	1.13 ± 0.67	1.44 ± 0.82	1.30 ± 0.87
			Ejaculation latency (minutes)			
Psychological§	10.77 ± 4.54	9.16 ± 4.14	6.05 ± 2.69†	14.18 ± 7.69	15.88 ± 8.09	20.61 ± 7.64†
Physical	11.17 ± 3.41	7.91 ± 4.18	9.78 ± 4.83	5.46 ± 2.78	8.36 ± 4.14	5.41 ± 3.88
Control	16.05 ± 7.89	12.12 ± 3.62	10.2 ± 4.52	10.08 ± 4.04	9.53 ± 2.41	11.17 ± 3.02

Values are means ± SD.

* Between the psychological stress group and control group, there were significant differences in the interaction between the mount rate and time course ($F[5,75] = 5.682, P = 0.0002$).

† There were significant differences in comparison with the value for pre-stress loading.

‡ Between the psychological stress group and the control group, there were significant differences in the effect of the time course in intromission latency ($F[5,75] = 3.692, P = 0.0048$).

§ Between the psychological stress group and the control group, there were significant differences in the effect of the time course on ejaculation latency analyzed by repeated-measures ANOVA ($F[5,75] = 4.770, P = 0.0008$).

Table 3. *Effects of psychological and physical stress on the concentration of catecholamines in the brain*

	DA	HVA	DOPAC	NA
		MPOA		
Psychological	261.2 ± 200.1*	127.9 ± 30.2*	227.1 ± 87.8*	655.2 ± 404.4*
Physical	430.3 ± 228.4	231.5 ± 108.5	396.1 ± 251.4	1,617.9 ± 1,144.0
Control	470.9 ± 192.1	209.7 ± 72.8	403.9 ± 146.6	1,131.7 ± 374.8
		Cortex		
Psychological	10.8 ± 4.4	15.0 ± 6.6	14.3 ± 4.4	117.4 ± 71.0
Physical	14.1 ± 7.1	13.7 ± 6.2	12.2 ± 6.2	127.4 ± 60.2
Control	11.0 ± 5.8	18.1 ± 8.2	16.3 ± 9.1	182.9 ± 76.0
		Amygdala		
Psychological	20.2 ± 14.9*	25.9 ± 12.7*	27.6 ± 15.3	340.9 ± 173.6
Physical	72.6 ± 79.1	38.4 ± 21.0	37.8 ± 19.5	330.2 ± 102.3
Control	97.8 ± 78.4	50.2 ± 14.9	53.5 ± 36.3	506.4 ± 250.1
		Nigra substantia		
Psychological	101.5 ± 57.0*	38.4 ± 19.1*	58.3 ± 37.6	324.6 ± 133.1
Physical	155.8 ± 48.8	70.1 ± 10.3	77.5 ± 17.9	760.6 ± 271.1
Control	179.8 ± 65.4	66.5 ± 19.1	104.1 ± 64.5	410.1 ± 200.0
		Striatum		
Psychological	1,545.8 ± 1,099.0	307.3 ± 249.9*	832.5 ± 678.3	376.4 ± 242.6
Physical	1,441.3 ± 527.8	342.3 ± 300.8	585.4 ± 229.1	483.1 ± 180.9
Control	1,728.8 ± 1,080.0	510.2 ± 230.1	1,140.9 ± 735.8	608.3 ± 205.7
		Nucleus accumbens		
Psychological	331.4 ± 211.3	172.9 ± 95.2*	395.5 ± 328.2	562.5 ± 467.7
Physical	691.4 ± 280.3	345.5 ± 176.6	813.3 ± 352.2*	588.1 ± 332.0
Control	634.4 ± 553.5	300.9 ± 212.7	460.1 ± 370.8	602.9 ± 417.3

Abbreviations: DA, dopamine; HVA, homovanillic acid; DOPAC, 5,6-dihydroxyphenylacetic acid; NA, noradrenaline; MPOA, medial preoptic area. Values are means ± SD. All measurements are in ng/g tissue. Levels of significance indicate the mean significant difference from the control mean.
* $P < 0.05$ (analyzed by Mann-Whitney *U*-test).

course on ejaculation latency (F [5,75] = 4.770, P = 0.0008). In the psychological stress group, the value for ejaculation latency in the 4th week after starting stress loading was significantly shortened ($P < 0.01$), and the value for the 10th week after starting stress loading was significantly prolonged in comparison with the value before starting stress loading. These results suggested that long-term psychological stress impaired the sexual behavior of male rats ($P < 0.001$).

Effect on Brain Catecholamines (Table 3)—In the MPOA, the amounts of DA, NA, DOPAC, and HVA were significantly decreased in the psychological stress group compared to the control group ($P < 0.05$). In the substantia nigra and amygdala, concentrations of DA and HVA significantly decreased ($P < 0.05$), and in the striatum and nucleus accumbens, only the concentration of HVA significantly decreased, compared with the control group ($P < 0.05$). In the cortex, however, there was no difference between the psychological stress group and the control group. In the physical stress group, DOPAC was significantly increased only in the nucleus accumbens ($P < 0.05$). These results indicated low production and release of catecholamines in the critical area of the brain mediating sexual behavior after long-term psychological stress (by Mann-Whitney *U*-test).

Experiment II

There were no significant differences between the mount rates in the indeloxazine-treated group and vehicle-treated group until the 6th week ($F[3,42] = 0.099$, $P = 0.9602$). However, there were significant differences between the indeloxazine-treated group and vehicle-treated group in the interaction between the mount rate and the time course throughout the 10-week stress-loading period ($F[5,70]$ = 2.577, $P = 0.0338$). The indeloxazine-treated group had a higher mount rate than the vehicle-treated group from the 8th week onward (Table 4).

There were no significant differences in the incidence of loss of ejaculation until the 8th week. At the 10th week, however, significant differences existed between the indeloxazine-treated group and vehicle-treated group by chi-square analysis ($P < 0.01$) (Table 5). These experimental results suggested that administration of indeloxazine restored the sexual behavior that had been impaired by long-term psychological stress.

Table 4. *Effect of indeloxazine on the mount rate*

	Pre-stress	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
			Mount rate (times/minute)			
Indeloxazine	1.29 ± 0.71	1.28 ± 0.50	1.21 ± 0.76	0.30 ± 0.30	0.79 ± 0.26	0.80 ± 0.64
Vehicle	1.41 ± 0.52	1.46 ± 0.47	1.43 ± 0.45	0.36 ± 0.40	0.40 ± 0.33	0.14 ± 0.20

Indeloxazine was administered from the 7th week. There were no significant differences in the mount rate between the indeloxazine-treated group and vehicle-treated group until the 6th week ($F[3,42] = 0.099$, $P = 0.9602$). However, there were significant differences between the indeloxazine-treated group and saline group in the interaction between mount rate and time course throughout the 10-week stress loading period ($F[5,70] = 2.577$, $P = 0.0338$; repeated measures ANOVA).

Discussion

We investigated the relationship between psychological stress and sexual behavior in rats using a communication box (Ogawa and Kuwabara, 1966; Iimori et al, 1982), which is capable of creating psychological stress. The stress-loading method using a communication box exposes rats to emotional stimulation (psychological stress) by forced exposure to the squealing, jumping, and odor, generated by surrounding animals. Several previous studies concerning stress and male sexual behavior have been reported. In most of those studies, physical stress loading was employed (Caggiura and Eibergen, 1969; Caggiura and Vlahoulis, 1974; Menendez-Patterson et al, 1978). Therefore, we think that stress loading using a communication box, which produces non-physical stress, is a very interesting approach.

Under the stress conditions, there is some possibility of corticoid effects on sexual behavior. In this study, we did not measure corticoids because we could not collect adequate blood samples. However, hypertrophied adrenal glands were recognized in both stress groups. Therefore, it is possible that hypercortisolism existed in both stress groups.

Generally, corticosteroids affect neuronal excitability (Joëls and de Kloet, 1989). As regards cathecholamine neurons, it has been reported that corticosteroids enhance the expression of brain dopamine receptors (Biron et al, 1992). Therefore, the hypercortisolism induced by acute stress could activate cathecholamine neurons in some brain areas. These changes might induce acceleration of sexual behavior.

On the other hand, the hypothalamic–pituitary–adrenal axis, activated by stress, which brings about the hypercortisolism, affects the sex hormone level. The endocrinological state is a very important factor affecting sexual behavior (Meisel and Sachs, 1994). It has been reported that neurons containing corticotropin-releasing hormone (CRH) have a direct synaptic connection to luteinizing hormone-releasing hormone (LHRH)-secreting cells (MacLusky et al, 1988) and seem to decrease LHRH secretion (Nicolarakis et al, 1986). Thus, it is possible that serum testosterone was decreased by a diminution of the luteinizing hormone (LH) level. Such hormonal change might reduce male sexual behavior.

However, there was no difference in adrenal weight and serum testosterone levels between the two stress groups in this study. Thus the decline of sexual behavior in the psychological stress group could not be explained only by the activation of the hypothalamic–pituitary–adrenal axis, although this axis might make some contribution to the changes in sexual behavior in both stress groups. We hypothesized the existence of other causes for long-term psychologically reduced sexual behavior in male rats.

It is widely known that stress increases catecholamine turnover (Dunn, 1988; Rossetti et al, 1990). Iimori et al (1982) investigated the character of repeatedly loaded psychological stress for cathecholamine neurons with the communication box. They reported that repeated psychological stress activated catecholamine metabolism more intensively than repeated physical stress. They concluded that psychological stress does not work strongly as an acute stress, but with repeated loading the intensity of psychological stress grows larger and larger (Iimori et al, 1982).

Table 5. *Effect of indeloxazine on the incidence of loss of ejaculation*

	Pre-stress	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks*
			Incidence of loss of ejaculation % (n)			
Indeloxazine-treated group	0	0	12.5 (1)	50 (4)	0	0
Vehicle-treated group	0	0	0	50 (4)	25 (2)	75 (6)

The incidence of loss of ejaculation means the rate of rats that did not show any ejaculation within the 30-minute observation period in each treatment group. Therefore "0" indicates that all rats of the treatment group showed ejaculation. Indeloxazine was administered from the 7th week. There was no significant difference between the indeloxazine-treated group and vehicle-treated group until the 8th week.

* There were significant differences at the 10th week ($P < 0.01$ by chi-square analysis).

We suspected that strong stress conditions, induced by this type of psychological stress, might be related to temporary acceleration of sexual behavior due to activation of cathecholamine neurotransmission. However, it is surmised that an intensely stressful state depresses the metabolism of brain catecholamines. It has been reported that loading forced-running stress on rats, which creates a very stressful state, results in decreases in both the metabolic turnover in NA-releasing nerve ends and the fluorescent intensity of DA in the DA-containing tissue of the median eminence–infundibulum (Kitayama, 1983). These data showed that the metabolic turnover of catecholamine neurons was decreased due to severely stressful conditions. Thus we speculated that intense stress conditions produced by long-term psychological stress might be related to the decrease of sexual behavior due to a decline of cathecholamine contents.

In our study, in psychologically stressed rats, catecholamines and their metabolites did not decrease equally in all areas of the brain. In the MPOA, located in the hypothalamus, the contents of all catecholamines decreased significantly compared with control rats. In the amygdala, the DA and HVA concentrations decreased. Decreased content of catecholamines and their metabolites might indicate a low neurotransmission level (Lavielle et al, 1979). These results suggested that, in the MPOA and the amygdala, a decline in catecholamine neurotransmission was induced by the long-term psychological stress.

The psychological stress generated by the communication box is transmitted as an emotional stress in responder rats (Ogawa and Kuwabara, 1966; Iimori et al, 1982). This psychological stress increases NA release selectively in the hypothalamus and the amygdala (Tsuda et al, 1986; Tanaka et al, 1990). These two brain areas regulate the emotional state. The amygdala, especially, is the most important area for such regulation. The hypothalamus, which includes the MPOA, also has functions for generation and regulation of emotion (Bishop et al, 1963). In our results, the areas involved in regulation of emotion were those mainly affected by long-term psychological stress.

These areas are simultaneously important areas for the regulation of male sexual behavior. There is widespread agreement that the MPOA is perhaps the single most critical area of the brain mediating male sexual behavior (Brackett et al, 1986; Horio et al, 1986; Marsuury, 1971). Various kinds of information on sexual stimulation, including sex hormonal information, are concentrated in the MPOA. The MPOA sends commands regarding sexual behavior. It has been reported that lesions of the MPOA in male rats eliminate male sexual behavior, whereas, conversely, electrical stimulation of the MPOA accelerates it. It is known that the amygdala plays an important role in relaying scent information, which is essential for the manifestation of sexual behavior in male rats. Efferents from the MPOA course through the median forebrain bundle and terminate in the substantia nigra. Bilateral lesions of the dorsolateral tegmentum eliminate male sexual behavior, and it has been reported that the striatum and nucleus accumbens play roles in the motivation of sexual behavior (Mogenson, 1981).

It is known that, among the various monoamines in the brain, dopamine plays an especially important role in promoting male sexual behavior. DA neurons in the MPOA originate from the incerto-hypothalamic dopamine neuron system (Anders et al, 1975), which is composed of DA cell groups (A11, A13, A14) located in the zona incerta and around the third ventricle. The fibers run mainly to the medial hypothalamus. Hull et al (1986) reported that intra-MPOA infusion of apomorphine accelerates male sexual behavior. This finding shows that DA neurons in the MPOA play an important role in mediating such behavior.

Therefore, there was a possibility that a decrease of catecholamine content, especially DA in the brain area mediating sexual behavior, might have reduced the male rat sexual behavior.

In experiment II, administration of indeloxazine prevented the decline in sexual behavior induced by long-term psychological stress. Indeloxazine was introduced as a cerebral metabolism enhancer (Yamamoto and Shimizu, 1987a) and has broad effects on neurotransmission and metabolism in the brain.

There are at least two possible mechanisms by which indeloxazine affects the restoration of sexual behavior. One is its contribution to increments of ATP and glucose utilization. Increments of ATP and glucose utilization activate the neuronal metabolism. The second mechanism is the action of indeloxazine that increases the monoamine content in the brain. By such pharmacological actions, Yamamoto and Shimizu (1987b) found that indeloxazine enhances the acquisition of learned behavior and has anti-anoxic actions. In their experiments, the dose of indeloxazine was 10 mg/kg, the same as the dose used in this study. Therefore, in this study as well, neurons suffering from long-term psychological stress might have regained their activity through the same pharmacological action of indeloxazine. We suspect that such restoration of neurotransmission might contribute to the acceleration of sexual behavior.

Indeloxazine also acts as an inhibitor of monoamine re-uptake. It prevents uptake of monoamine in the synaptic cleft, and extracellular DA is increased. The increment of extracellular DA may facilitate dopaminergic neurotransmission. We confirmed that general administration of indeloxazine increased the extracellular DA level and activated copulatory behavior. However, in this study, indeloxazine was not administered on the days

when sexual behavior tests were performed. Therefore, its action as a monoamine re-uptake inhibitor might not have affected the sexual behavior of the rats.

It has not been reported that indeloxazine affects the endocrinological situation. It is, however, reported that indeloxazine does not affect spontaneous movement (Yamamoto and Shimizu, 1987a). Therefore, we suspect that indeloxazine may contribute to normalization of sexual behavior by activating the deteriorating catecholamine neurons of male rats suffering from long-term psychological stress.

Conclusions

Long-term psychological stress, which was generated using a communication box, impaired the sexual behavior of male rats. The long-term psychological stress decreased the content of catecholamine and its metabolites in the brain. Thus, we hypothesize that low catecholamine neurotransmission in the brain results in impairment of the sexual behavior of male rats. The administration of indeloxazine hydrochloride for a 3-week period restored the sexual behavior that had been impaired by long-term psychological stress. These present results suggest that impairment of neurotransmission in the central nervous system could bring about sexual dysfunction, and that activation of neurotransmission may result in restoration of impaired male sexual behavior.

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