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Social Interactions, Brain Monoamines, and GABA Alterations in MFB-Lesioned Cats[1]

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KROTEWICZ, M. AND A. ROMANIUK. *Social interactions, brain monoamines, and GABA alterations in MFB-lesioned cats.* PHARMACOL BIOCHEM BEHAV **60**(2) 533–538, 1998.—The effects of denervation of central noradrenergic system on the interpartner relationships of adult cats were examined in a predatory test in the competitive situation for paired animals. Direct administration of the noradrenaline neurotoxin, N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP-4 12 μg) into the medial forebrain bundle (MFB) of submissive cats changed previously established dominant–submissive relationship. Biochemical analysis demonstrated a significant reduction of noradrenaline (NA) concentration in the hypothalamus (AH), amygdala (AM), hippocampus (HC), and frontal cortex (CTX), and elevation of NA content in the midbrain central gray matter (CG) in MFB-lesioned cats. Simultaneously, DSP-4–induced lesions exerted significant decrease of 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) content in AH, CG, HC and CTX, and increased GABA level in AH, CG, AM, and HC. These results suggest that a coincident decrease of NA metabolism and increase of GABA metabolism led to fear drive reduction. © 1998 Elsevier Science Inc.

Predatory test Dominance and submission DSP-4 MFB Brain monoamines GABA HPLC Cat

DOMINANT–SUBMISSIVE relationships of paired animals have been often regarded as equivalent to social organization (28), and are useful in the studies of social behavior if a variety of social interactions can be predicted in them (26). Social behavior is of relevance to several problems in psychiatry, i.e., depression, autism, or schizophrenia (16). The neurobiology of social behavior provides a useful framework for developing animal models potentially relevant to psychiatric disorders. The social hierarchy established during interactions between cats in a predatory competition test enable to observe specific behavior of dominant and submissive animals in seminatural conditions (7).

Our previous work has demonstrated increased concentration of noradrenaline (NA) in the hypothalamic region of submissive animals (19). In addition, the pharmacological studies revealed that the most pronounced changes in dominant–submissive interactions were exerted by imipramine (35). Imipramine, a tricyclic antidepressant, acting mainly by inhibition of NA uptake (21), elicited a predatory competition in the established social hierarchy leading to predatory domination by submissive animals (34). On the other hand, 6-hydroxydopamine (6-OHDA)-induced lesion of the dorsal noradrenergic bundle in rats failed to change a response in an operant conflict test (18). Moreover, rats with lesions of cortical noradrenaline neurons did not alter their response to social interactions in an unfamiliar situation (5). The neurotoxin 6-OHDA induces denervation of catecholamine neurons, both noradrenergic and dopaminergic (12). Another NA neurotoxin, N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP-4), has been shown to exert a neurotoxic effect preferably on central noradrenaline neurons without influences on dopaminergic neurons, but in cats it also destroyed serotonergic neurons (9,10,14). Moreover, DSP-4 after peripheral administration easily crosses the blood–brain barrier and produces preferably depletion of NA neurons in the brain regions innervated by the locus coeruleus (13,14).

Lesioning of central noradrenergic neurons with DSP-4 has influenced the social interactions in rats (4,36). Submissive rats with DSP-4–induced destruction of noradrenergic system displayed more offensive acts and succeeded in eliciting defensive postures in dominants (36). Similar effects were observed after DSP-4 administration by Cornwell et al. (4). The authors of both articles suggested that NA depletion reduced sensitivity to stress or even elicited fear reduction.

The administration of DSP-4 into the locus coeruleus of submissive cats did not change the dominance status in cats

[1]The procedure used to kill the animals in this study is not allowed in U.K. or U.S.A.

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(20). It should be mentioned that the locus coeruleus fibers innervate only several hypothalamic centers, including most medial part of the parvocellular division of paraventricular nucleus, vasopressin neurons in the supraoptic nucleus, and dorsomedial nucleus (8,24). It is noteworthy that the entire hypothalamus, especially the dorsomedial, periventricular, infundibular, supraoptic and paraventricular nuclei, and the internal layer of the median eminence are supplied with fibers of ventral noradrenergic pathway, ascending to the diencephalon in the median forebrain bundle (24). Because significant changes in NA concentration have been observed in the hypothalamic regions of submissive animals (19), we sought to examine the effects of NA depletion by DSP-4 administration into the medial forebrain bundle (MFB) of submissive animals on dominant–submissive relationships of paired cats in a predatory test in the competitive situation. Moreover, the present investigation was undertaken to answer the question of whether and what interactions exist between the monoaminergic and GABAergic systems activity after these lesions.

METHOD

Animals

The study was performed on 14 adult cats of either sex (4 males and 10 females) weighing 3.0 ± 0.5 kg. The animals were obtained from our own unlicensed breeding, and housed individually in 100 × 90 × 60 cm wire mesh cages in an animal room with controlled temperature (22 ± 2°C) and 12 L:12 D reversed cycle. Food (cereal with meat and milk) and tap water were available continuously.

Experimental Procedure

At the beginning of the study all cats were individually tested on their predatory abilities. This test was intended to eliminate animals that did not prey spontaneously. The tests were performed in an experimental chamber (180 × 180 × 120 cm), in which the animals were able to freely move, jump, and catch a mouse. The animal, after 24-h food deprivation, was placed in the experimental chamber for 5 min. After this time a freely moving white mouse (b.wt. 25–30 g) was dropped into the chamber through a port in the upper wall. Three such tests were performed during one session, i.e., three mice were dropped, one at a time, each after consumption of the previous mouse. Each cat participated in three sessions. In the course of these experiments three times were measured, namely the time in which the cat actually bit a mouse to death (latency of effective attack), the time in which it started devouring the dead mouse, and the time in which the cat devoured the mouse (consumption time).

Each test was conducted between 1000 and 1200 h.

Predatory Test in a Competitive Situation for Paired Animals (PC Test)

The cats selected in the previous test were paired. The base of pairs selection was the same sex, approximate weight and latency of effective attack. The paired animals were introduced at the same time into the experimental chamber for 5 min following 24-h food deprivation, and then a freely moving white mouse was dropped into the chamber through a port in the upper wall. Three such tests were performed during one session, i.e., three mice were dropped, one at a time, each after consumption of the previous mouse. The tests were videotaped for subsequent analysis of interpartner relationships of the cats, and only the pairs with marked dominance of one of the paired cats were used in the experiment. In these pairs the established dominance was stable during three successive sessions, i.e., a dominant cat always caught, killed, and ate each mouse, while a submissive cat never preyed for a mouse in the presence of its partner. Three following experimental sessions consisting of three tests each were carried out after establishing the dominance in all pairs used and then cannulae into the MFB were bilaterally implanted. The experimental session was carried out following surgery, i.e., implantation of cannulae, to verify and consolidate the established hierarchy and then all the cats were treated with zimelidine, and 40 min later DSP-4 was administered into the MFB of submissive animals, whereas dominants received injection of vehicle.

Effects of injections on interpartner relationships were tested twice after 10 and 12 days in the PC test because various authors mentioned different times of maximal drop in NA concentration after DSP-4 administration (9,10,13). An analysis of interpartner relationships observed after DSP-4 injections revealed new behavioral categories. The submissive animals completely reversed the hierarchy (RH) or made efforts to take the mouse already caught by a dominant cat (ETM) or at least made attempts to come near the snout of a dominant animal (ACNS). Even if the hierarchy was not completely reversed the submissive cats fought against their partners for getting a good position to attack a mouse (FAP).

Surgery and Animal Handling

The animals were anaesthetised with a hexobarbital (90 mg/kg, IP). Two cannulae were chronically bilaterally implanted in the MFB using stereotaxic techniques. The following coordinates, obtained from the atlas of Snider and Niemer's (29), were used: A = 9.0, L = 3.0, H = −4.0. The guide stainless steel cannulas (1.0 mm outer and 0.6 mm inner diameter) were fixed to the skull with self-polymerising methacrylate resin (Duracryl Special, Spofa, Prague). The outer openings of the cannulae were plugged with cotton swabs soaked with 0.9% saline solution and sealed with wax. The chronically implanted cannulae served as guides for an injection cannula (0.5 mm outer and 0.2 mm inner diameter) directly connected to a microinjector (E. Zimmermann, Leipzig). After surgery, cats were allowed to recover for 10 days prior to behavioral testing. During this period animals were handled and familiarized with the injection procedure.

On the 11th day following the surgery, cats were injected bilaterally into the MFB with distilled water as vehicle (dominant cats) or 12 μg of DSP-4 (N-2-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride, RBI, Natick, MA) per one site, diluted in vehicle before injection (submissive cats). Neurotoxin or vehicle solutions were injected into the MFB manually in a volume of 2 μl at a rate of about 0.1 μl/s. All animals received zimelidine, dissolved in 0.9% saline (Zimelidine dihydrochloride, RBI, Natick, MA) in the dose of 10 mg/kg, intraperitoneally, 40 min before DSP-4. Two following experimental sessions consisting of three PC tests were performed on the 10th and 12th day after the injection. The behavior of animals was videotaped and analyzed.

Biochemical Analysis

The concentrations of noradrenaline (NA), dopamine (DA), 5-hydroxytryptamine (5-HT), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic (5-HIAA), and GABA were measured in the selected brain regions using

high-performance liquid chromatography with electrochemical detection (HPLC-ED).

Sample Preparation

The animals were killed by decapitation, 24 h after the end of the third test during the second session following DSP-4 or vehicle administration (Note: The procedure used to kill the animals in this study is not allowed in the UK or the USA.) The brains were quickly removed and selected regions, i.e., the anterior hypothalamus (AH), midbrain central gray matter (CG), hippocampus (HC), amygdala (AM), and the prefrontal cortex (CTX) were separated [for details, see (27)] and kept frozen at −70°C until analyzed. The injection sites were verified according to the atlas of Snider and Niemer's (29) during dissection of the brain tissue. The selected tissues were weighed and homogenized with an ultrasonic cell disruptor (Vibracell 72434, 50 W, Bioblock, Illkrich-Cedex) in 1 ml 0.1 M perchloric acid containing 0.4 mM sodium metabisulphite. The samples were then centrifuged at 10,000 × g for 25 min at 4°C, and the supernatants were filtered through a 0.22-μm filter (Sigma), and 20 μl filtrates were injected into the HPLC system.

Chromatographic and Detection Conditions

A delivery pump Model HP 1050 (Hewlett-Packard) was used with a sample injector Model 7125 (Rheodyne, Berkeley, CA). The analytical column ODS 2 (250 × 4.6 mm), particle size 5 μm (Hewlett-Packard) protected by guard column ODS 2 (20 × 2.1 mm), particle size 5 μm (Hewlett-Packard). The electrochemical detector model HP 1049 A (Hewlett-Packard) with glassy carbon working electrode was used at a voltage setting of +0.65 V for monoamines and their metabolites, and +0.60 V for GABA, vs. an Ag/AgCl reference electrode. The chromatographic peaks were integrated using a chromatointegrator (Esoft, Tódź). The quantification of monoamines and their related metabolites concentrations in each sample were calculated from the integrated chromatographic peak area and expressed as ng/g wet tissue. The concentration of GABA was calculated as the monoamines and expressed as μg/g wet tissue.

Monoamines and Their Metabolites Determination

The mobile phase comprised a 0.15 M sodium dihydrogen phosphate, 0.1 mM EDTA, 0.5 mM sodium octanesulphonic acid, 10% methanol (v/v), and 5 mM lithium chloride. The mobile phase was adjusted to pH 3.4 with phosphoric acid, filtered through a 0.22 μm filter (Sigma), and degassed with helium. The analytical column was operated at a flow rate 1.4 ml/min and at the column temperature 30°C.

GABA Determination

The mobile phase for GABA determination was 0.1 M sodium acetate buffer with 0.1 mM EDTA and 5 mM lithium chloride in 25% (v/v) methanol. The mobile phase was adjusted to pH 5.5 with acetic acid, filtered through a 0.22 μm filter (Sigma), and degassed with helium. The examined amino acid was eluted with the linear methanol gradient from 25 to 75% in 15 min, 75% of methanol in next 2 min, and from 75 to 25% in following 6 min [a modified method of Arias et al. (2)]. Just before the injection into the HPLC system, GABA was derivatizied with *o*-phtalaldehyde-thiol (OPT-thiol) reagent for 2 min (15).

The separations were carried out at a flow rate of 1.3 ml/min and at the column temperature 34°C.

Chemicals

Methanol was purchased from Serva (Heidelberg). Other chemicals for HPLC were purchased from Sigma Chemical Co. (St. Louis, MO).

All experimental procedures were in agreement with the European Communities Council Directive of 25 November 1986 (86/609/EEC).

Statistics

The results were analyzed by the two-way ANOVA followed by the a priori test.

RESULTS

The Effects of DSP-4 Administration Into the MFB of Submissive Cats

Behavioral data. Once the hierarchy in the paired cats was established, the dominant cat was taking the initiative to attack a mouse in the middle of the chamber close to the place of preying. When the mouse was let into the chamber it was immediately caught by the cat, killed, and devoured. At the same time the submissive cat was sitting motionless in the corner of the chamber and was observing its partner's behavior. The administration of DSP-4 into the MFB of submissive cat completely changed its behavior and revealed new behavioral categories that were not observed before injections. The cat first passive and submissive in relation to its partner undertook competition and fought for dominance. This led to a reversal of hierarchy (RH) or to a significant weakening of the position of the dominant cat. Even if the hierarchy was not completely reversed the cats earlier submissive fought against their partners for getting a good position to attack a mouse (FAP), made attempts to come near the snout of a dominant animal (ACNS), and even made efforts to take the mouse (ETM) that was already the partner's prey. Behavioral incidents were differentiated and counted in each pair of animals according to the above-mentioned behavioral categories and then summed up for every pair in each behavioral category separately. Quantitative data for each behavioral category were calculated as percentage of the total number of tests (seven pair × three tests in one session) for 10 and 12 days after administration of DSP-4 into the MFB of previously submissive animals. Behavioral results are shown in Fig. 1.

Biochemical data. Regional brain concentrations of monoamines and their metabolites after the vehicle (group 1, dominant cats) and DSP-4 (group 2, submissive cats) injections into the MFB are presented in Table 1.

In group 2, a marked decrease of NA level occurred in AH by 36.5%, in AM by 42.5%, in HC by 61.9%, in CTX by 37.4%, and increase in CG by 104.6%. Additionally, in group 2 a marked decrease of MHPG level occurred in AH by 60.2%, in CG by 69.4%, in AM by 53.9%, in HC by 88.6%, and in CTX by 85.0%. ANOVA demonstrated significant differences between the groups in the contents of NA, $F(1, 12) = 10.02$, $p < 0.01$, and of MHPG, $F(1, 12) = 13.38$, $p < 0.01$, and a priori test showed that the level of NA was lower in AH ($p < 0.05$), in AM ($p < 0.05$), in HC ($p < 0.001$), in CTX ($p < 0.05$), and higher in CG ($p < 0.01$) in group 2 vs. group 1. The MHPG level was lower in AH ($p < 0.05$), in CG ($p < 0.05$), in HC ($p < 0.001$), and in CTX ($p < 0.01$) in group 2 vs. group 1.

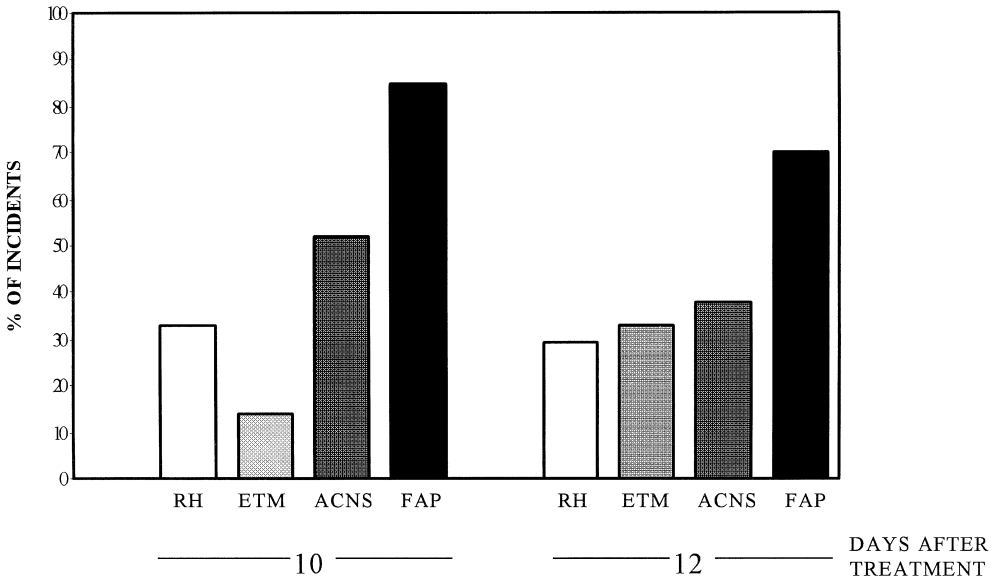

FIG. 1. Histogram expressed as percentage of the total number of incidents of behavioral categories (seven cats × three tests per session = 100%) observed in previously submissive cats on the 10th and 12th day following the injection of DSP-4 into the MFB. RH—reversal of hierarchy; ETM—efforts to take the mouse already caught by a dominant cat; ACNS—attempts to come near the snout of a dominant animal; FAP—fight for taking a better position in relation to the place in which mouse is dropped. Each datum represents the mean of seven pairs of cats.

No significant differences were observed between the groups in the contents of DA, DOPAC, HVA, 5-HT, and 5-HIAA

Regional brain concentrations of GABA after vehicle (group 1, dominant cats) and DSP-4 (group 2, submissive cats) injections into the MFB are presented in Table 2.

In group 2, GABA content was increased in AH by 68.3%, in CG by 72.6% , in AM by 88.7%, in HC by 223.2%, and in CTX by 41.1%. ANOVA demonstrated significant differences between the groups in the content of GABA, $F(1, 12) = 134.71$, $p < 0.001$, and a priori test showed that the level of

TABLE 1

REGIONAL BRAIN DISTRIBUTION OF NA, DA, 5-HT, MHPG, DOPAC; HVA, AND 5-HIAA AFTER DSP-4 ADMINISTRATION INTO THE MFB OF SUBMISSIVE CATS

Experimental Group	Brain Region	Monoamine and Metabolite Content in ng/g Wet Tissue						
		NA	DA	5-HT	MHPG	DOPAC	HVA	5-HIAA
1. Sham		2001.2 ± 146.7	1667.1 ± 297.5	1264.7 ± 123.3	104.6 ± 23.1	205.1 ± 59.3	700.3 ± 121.8	352.2 ± 70.8
2. DSP-4	AH	1270.8 ± 139.7	1608.0 ± 358.4	1201.0 ± 98.4	41.6 ± 11.7	220.2 ± 55.4	745.9 ± 125.2	344.4 ± 40.5
		$p < 0.05$	NS	NS	$p < 0.05$	NS	NS	NS
1. Sham		723.6 ± 90.7	190.7 ± 32.8	1968.7 ± 433.5	162.0 ± 44.8	76.8 ± 1 4.7	266.6 ± 50.6	682.0 ± 146.2
2. DSP-4	CG	1480.8 ± 112.0	165.5 ± 29.0	2415.7 ± 316.3	49.6 ± 14.9	79.9 ± 9.2	262.7 ± 37.9	606.8 ± 144.4
		$p < 0.01$	NS	NS	$p < 0.05$	NS	NS	NS
1. Sham		775.8 ± 139.2	312.0 ± 55.9	1145.5 ± 232.3	142.9 ± 46.6	113.0 ± 14.2	232.7 ± 37.9	228.0 ± 43.1
2. DSP-4	AM	446.3 ± 50.7	315.0 ± 32.4	1113.0 ± 199.9	65.9 ± 27.0	112.7 ± 32.7	248.8 ± 38.2	284.4 ± 65.8
		$p < 0.05$	NS	NS	NS	NS	NS	NS
1. Sham		512.9 ± 132.3	152.6 ± 30.4	662.9 ± 113.9	132.9 ± 47.6	65.1 ± 19.7	91.3 ± 16.8	165.2 ± 38.9
2. DSP-4	HC	195.7 ± 45.0	169.6 ± 28.1	761.9 ± 148.6	15.2 ± 5.6	62.3 ± 10.1	76.8 ± 10.09	195.1 ± 38.5
		$p < 0.001$	NS	NS	$p < 0.001$	NS	NS	NS
1. Sham		479.9 ± 52.9	116.6 ± 13.7	376.1 ± 77.3	120.4 ± 47.2	46.2 ± 6.0	45.2 ± 5.8	86.8 ± 21.4
2. DSP-4	CTX	300.9 ± 52.9	121.5 ± 15.1	401.9 ± 59.8	18.1 ± 6.2	47.9 ± 9.8	41.3 ± 4.5	78.0 ± 13.0
		$p < 0.05$	NS	NS	$p < 0.01$	NS	NS	NS

Values are means ± SEM; $n=7$ for each group.
Statistical significance: *a priori* test.

TABLE 2
REGIONAL BRAIN DISTRIBUTION OF GABA AFTER DSP-4 ADMINISTRATION INTO THE MFB OF SUBMISSIVE CATS

Experimental Group	Brain Region	GABA Content in μg/g Wet Tissue
1. Sham		254.6 ± 24.1
2. DSP 4	AH	428.6 ± 47.0
		$p < 0.001$
1. Sham		521.6 ± 91.7
2. DSP4	CG	900.4 ± 48.2
		$p < 0.01$
1. Sham		256.5 ± 38.0
2. DSP4	AM	484.2 ± 67.8
		$p < 0.01$
1.Sham		159.3 ± 25.3
2. DSP4	HC	514.9 ± 51.2
		$p < 0.001$
1. Sham		196.9 ± 19.5
2. DSP4	CTX	277.8 ± 29.6
		NS

Values are means ± SEM; $n = 7$ for each group. Statistical significance: *a priori* test.

GABA was higher in AH ($p < 0.001$), in CG ($p < 0.01$), in AM ($p < 0.01$), and in HC ($p < 0.001$) in group 2 vs. group 1.

DISCUSSION

The use of a noradrenergic neurotoxin, DSP-4, in the present investigation let us analyze the role of noradrenergic system in social behavior of cats. The administration of DSP-4 into the MFB of submissive animals produced marked effects on the dominant–submissive relationships. The DSP-4–treated animals changed previously established hierarchy. Simultaneously, DSP-4 decreased NA concentrations in all the brain regions examined in the submissive animals with exception of the midbrain central gray matter. Additionally, DSP-4 administration was responsible for a significant reduction of MHPG concentration in AH, CG, HC, and in CTX of submissive cats. At the same time the GABA level increased in AH, CG, AM, and in HC.

On the one hand, the neurotoxin, DSP-4 was demonstrated to exert a specific action on the noradrenergic neurons (11). On the other hand, several studies reported changes in 5-HT concentration after administration of DSP-4 (16,32). This effect may be prevented by a pretreatment with the 5-HT uptake blocker zimelidine (13). In the present study, 5-HT neurons were protected by premedication with zimelidine and were not affected by DSP-4 administration. Simultaneously, we found out that centrally administered DSP-4 depleted NA concentration in the hypothalamus of the submissive animals. Other authors demonstrated that peripherally administered DSP-4 produced a greater depletion of NA in the extrahypothalamic regions, such as frontal cortex and hippocampus, than in the hypothalamus (22), which would support the selectivity of DSP-4 for locus coeruleus neurons. Moreover, in the same study it was demonstrated that intracerebroventricular administration of 6-OHDA was more effective within the hypothalamus then DSP-4 (22). The results of our study revealed that intra-MFB administration of DSP-4 not only decreased hypothalamic NA concentration but also depleted MHPG level and increased GABA concentration in the same brain regions. It may suggest interactions between noradrenergic and GABAergic systems, the more so as analogical coincidental depletion of NA or MHPG concentration and elevation of GABA level was noted not only in the hypothalamus but also in the other brain regions (midbrain central gray matter, amygdala, and hippocampus). It would appear that mutual relations of the NA and GABA systems were likely to be answerable for noradrenergic mechanism involved in the regulation of anxiety.

The results of our previous investigation indicated that dominant and submissive cats differed from each other in the hypothalamic NA concentration. The submissive position in the cat hierarchical scheme was accompanied by elevation of the hypothalamic NA level (19). The elevation of NA was also demonstrated in response to fear and anxiety (1,25). Moreover, it was postulated that the elevated brain noradrenergic neuronal activity was closely related to the provocation of negative emotions, i.e., stress or anxiety (3,30,31). Other studies demonstrated that the compounds responsible for increasing the central noradrenergic neurotransmission might induce anxiety responses in humans (6). Neurotoxic lesions of NA neurons exerted by DSP-4 were a supplement to the investigations relating to the noradrenergic system role in the regulation of anxiety. In another study the authors demonstrated a decrease of fear leading to the increasing tendency of the social contact in DSP-4–treated rats (36). Moreover, the animals administered DSP-4 spent more time in the stressogenic, highly illuminated center of the open field (36). Additionally, in an earlier study it was demonstrated that the noradrenergic system lesions also decreased anxiety in rats tested in the open-field test (33). The above-mentioned results are in agreement with our data. We may hypothesise that animals treated with DSP-4 changed their behavior through decreasing anxiety.

In conclusion, the obtained data are likely to confirm the hypothesis that a submissive position in the social hierarchy is due to anxiety that is induced by the presence of a dominant animal. The administration of DSP-4 into the MFB of submissive cats significantly reduced NA and MHPG in the key emotional brain structures (hypothalamus, amygdala, hippocampus) and simultaneously there occurred a significant increase in GABA level. The behavioral consequence of these neurochemical changes is a fear reduction in previously submissive cats and partially reversed dominant–submissive relationships.

Such an interpretation is fully supported by numerous studies demonstrating that anxiolytic drugs (for instance: benzodiazepines) may evoke the effects by intensification of GABAergic transmission (17,23,30). Our investigations reveal a very close interaction between the noradrenergic and GABAergic systems in central regulation of fear drive.

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