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Social Interactions in Rats: Behavioral and Neurochemical Alterations in DSP-4-Treated Rats

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ZAGRODZKA, J., M. WIECZOREK AND A. ROMANIUK. *Social interactions in rats: Behavioral and neurochemical alterations in DSP-4-treated rats.* PHARMACOL BIOCHEM BEHAV **49**(3) 541–548, 1994.—Noradrenergic neurotoxin DSP-4, preceded by zimelidine to prevent serotonin depletion, was administered IP to rats behaving in a defensive-submissive manner in a resident–intruder paradigm. Computer-based ethological analysis revealed the decrease of frequency and duration of defensive episodes and marked increase of offensive aggression. This might suggest an increase of aggressiveness and therefore support the notion of an inhibitory role of the noradrenergic system in aggressive behavior independently of the model used. Dramatically changed attitude toward the partner might also result from fear reduction or inadequate responsiveness to environmental factors because DSP-4-treated rats explored more than controls in stressogenic, highly illuminated area. HPLC analysis showed significant reduction of noradrenaline (NA) concentration in amygdala, hypothalamus, hippocampus, and frontal cortex. Simultaneously there occurred a considerable decrease in dopamine (DA) and serotonin (5-HT), and their metabolite levels. This suggests an attenuated activity of the DA and 5-HT systems that we consider as an effect secondary to NA depletion, which reflects the functional interactions between DA, 5-HT, and NA systems.

DSP-4 Locus coeruleus Noradrenaline Monoaminergic interactions Ethological analysis Offense Defense Photo-phobic test Rat

THE RESEARCH over the past three decades indicates that multiple neurotransmitters, remaining probably in mutual interactions, are involved in the control of aggressive behavior. Some findings in this respect are remarkably consistent, some others are not, which is not surprising considering the complexity of the phenomenon labeled aggression and the multitude of various experimental approaches used.

Conflicting data concern the role of central noradrenergic system in the modulation of aggression. There are reports suggesting excitatory noradrenaline (NA) control of various forms of aggressive behavior (12,13,43), lack of significant NA involvement (40), as well as inhibitory influence over affective and predatory aggression (11,14,28,31,33,44). The notion that the central noradrenergic system exerts an inhibitory control over aggressive behavior has been founded mostly upon the pharmacological experiments on rats with the use of shock (SIF)- and apomorphine-induced fighting (AIF) paradigms. Both models, although producing behavioral display similar to the one observed in affective aggression, suffer from a variety of disadvantages. First of all, they do not reflect naturally occurring aggressive behavior in its full complexity [see also Rodgers (39)]. Moreover, shock-induced fighting is related to individual pain reactivity and organism's reaction to stress. The latter is particularly important if the involvement of NA is considered because noradrenergic neurons of locus coeruleus change their activity on presentation of stressful stimuli (17,21,34,36). Stimulation of DA receptors with apomorphine alters dopaminergic transmission and therefore it might affect monoaminergic interactions; moreover, the "responsiveness" to this procedure varies among individuals. It should also be emphasized that shock-induced fighting as well as apomorphine-induced fighting are the models of defensive behavior (5). Ethoexperimental research has differentiated two categories of affective aggression: offensive and defen-

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sive, depending on motivation, behavioral pattern, and the neural control involved (1,6,7).

In light of existing controversies about the role of NA system in aggressive behavior, we decided to test the "inhibitory hypothesis" using a relatively natural model of rat's agonistic behavior. Social interactions, with special attention paid to defensive and offensive behaviors, were observed in an intruder–resident paradigm and then submitted to ethological analysis. Neurotoxin DSP-4 [*N*-(2-chlorethyl)-*N*-ethyl-2-bromobenzylamine] was used to destroy noradrenergic neurons in a controllable way. DSP-4 is known as a better and more selective NA neurotoxin than 6-OHDA. It easily crosses the blood–brain barrier after peripheral administration, and produces a marked and long-lasting depletion of NA in brain regions innervated by locus coeruleus (22).

The responsiveness to stressful stimuli was investigated as well, because some electrophysiological and behavioral findings suggest noradrenergic involvement in modulation of the reactivity to novel and stressful stimuli rather than in transmission of specific information (15,16,,221,34).

It is known that DSP-4 injected IP in the dose used produces considerable depletion of NA in the neocortex, hippocampus, amygdala, and hypothalamus in rats (10). In the present experiment, we examinated possible changes not only at the level of NA, but also dopamine (DA), serotonin (5-HT), and their metabolites to analyze possible interactions between noradrenergic system activity and activity of other monoaminergic systems in correlation with behavioral alterations.

METHOD

Subjects

The experiments were performed on male, hooded rats weighing 230–260 g bred in Nencki Institute Animal House. Intruder rats submitted later to DSP-4 treatment (n = 14) were kept in colony and were introduced into the home cage of their partners during social encounters. Resident rats (n = 14) consisted of weight-matched conspecifics housed individually. Biochemical analysis was performed in DSP-4-treated rats and intact controls (n = 8) of the same age, bred in the same conditions. Animals from this group were also used as controls in photo-phobic test.

The animals were maintained on a 12 L : 12 D cycle with food and water ad lib. The behavioral recordings took place between 0900 and 1600 h.

Social Interactions Test

Animals from each pair were confronted in resident's home cage one time before the DSP-4 treatment. The second social encounter took place 3 weeks later (i.e., 12 days after the treatment) because previous neurochemical experiments indicate substantial NA reduction at 10 days after DSP-4 injection (10).

The encounters were recorded for 10 min on video tape. Ethological analysis of both partners' behavior using a computer-based method for encoding and analyzing social and nonsocial activities in terms of their frequency and duration (System Activities & Reader by Peter Donat, 1992, Institute of Pharmacology, Praque) was performed. The following behavioral events [according to Vergnes et al. (46)] were observed:

1. nonsocial activities: i.e., cage exploration; self-grooming; immobile posture
2. social approach: i.e., partner investigation; crawl under/over; allogrooming
3. offense: i.e., attack; offensive sideways; offensive upright
4. defense: i.e., defensive upright; defensive sideways or lateral defense; on the back (submissive posture).

Muricide Testing

An alive white mouse was placed into a rat's cage for 10 min and rats killing their prey within that period were designated as killers. The killers were tested six times before the injection; the mean killing latency was established on the basis of last three experimental sessions. Thirteen days after the injection (DSP-4 or saline), the killing reaction was assessed again in rats previously selected as killers as well as in nonkillers.

Photo-Phobic Open Field Test

Fourteen days after DSP-4 injection, animals (treated and nontreated group of controls) were tested in photo-phobic, forced open field.

A black painted square (100 × 100 cm) with a floor divided into 25 squares served as the open field area. Nine squares in the center of the field were illuminated by a 200-W bulb suspended directly above it. Peripheries of the open field as well as the laboratory room were dark. Ambulation and the total time spent in the center and/or in peripheral areas were recorded on video tape during 6 min.

Neurotoxin Injections

Intruders (i.e., rats housed in the colony confronted one time with the partner in its home cage) were injected with DSP-4 (ASTRA, Sweden) at a dose of 60 mg/kg in a volume of 2 ml, IP. Residents were treated with 0.9% saline in a 2-ml volume, IP. To prevent serotonin depletion, 45 min before DSP-4 injection all animals, including residents, were treated with 5-HT uptake inhibitor zimelidine (Lundbeck, Dennmark) 10 mg/kg, IP.

Biochemical Analysis

The concentrations of NA, DA, 5-HT, DOPAC, HVA, and 5-HIAA in the hypothalamus (HPT), hippocampus (HIP), amygdala (AMY), and frontal cortex (CTX) were determinated using high performance liquid chromatography with electrochemical detection (HPLC-ED).

After the completion of behavioral experiments (17 days after IP DSP-4 or IP 0.9% NaCl treatments), all rats were killed by decapitation; their brains were quickly removed, placed on dry ice, and dissected into relevant blocks according to the stereotaxic atlas (23). HPT, HIP, AMY, and CTX were separated and kept frozen at −70°C until analysis. Each frozen brain region was weighted and homogenized in 1000 μl 0.1 M perchlorid acid containing 0.4 mM sodium metabisulphite. After centrifugation at 10,000 × g for 25 min at 4°C, the supernatants were filtered through a 0.22-μm filter (Sigma) and then 20 μl of filtrate was injected onto the HPLC system.

The HPLC system consisted of a delivery pump model HPP 5001 (Laboratorni Pristroje, Praque), a sample injector model 7125 (Rheodyne, Berkeley), and an analytical column ODS2 C18, 4.6 × 250 mm, particle size 5 μm (Hewlett-Packard) protected by a quard column ODS2 C18, 2.1 × 20 mm, particle size 5 μm (Hewlett-Packard). A programmable electrochemical detector model HP 1049A (Hewlett-Packard) with glassy carbon working electrode was used at a voltage setting of +0.65 V vs. an Ag/AgCl reference electrode. The

detector response was plotted and measured using a chromato-integrator (Esoft, Łódź). The concentrations of monoamines and related metabolites in each sample were calculated from integrated chromatographic peak area and are expressed as ng/g of wet tissue.

The mobile phase was 0.15 M sodium dihydrogen phosphate, 0.10 mM EDTA, 0.5 mM octanesulfonic acid, 12–14% methanol (v/v) and 5 mM lithium chloride. The pH was adjusted to 3.4 with phosphoric acid. A column temperature of 25°C and a flow rate of 1.4 ml/min (32 MPa) were used.

All chemicals were obtained from the Sigma Chemical Co. except for methanol, which was obtained from Serva.

Statistics

The data were elaborated statistically with the two-dimensional ANOVA followed by the Newman-Keuls test (biochemical data) or Duncan test (behavioral data). Additionally, for some open field data, Mann-Whitney test was used.

RESULTS

Social Interactions

Ethological analysis allowed an evaluation of various nonsocial and social activities including the episodes of offense and defense in residents as well as intruder rats before and after DSP-4 treatment. During the first encounter, significant differences between residents and intruders in the frequency, $F(4, 14) = 28.670$, $p < 0.001$, and duration, $F(4,104) = 16.360$, $p < 0.001$, of particular behavioral categories were observed. Generally, both partners spent most of their time on nonsocial activities, exploring the cage, self-grooming, or simply sitting motionless; however, in terms of frequency, nonsocial activity was higher in residents ($p < 0.001$). In addition, social approach, particularly partner investigation, was more pronounced in residents ($p < 0.05$). As was expected, the occurrence and duration of offensive postures were statistically higher in residents ($p < 0.001$), whereas the frequency and duration of defensive episodes were more pronounced in intruders ($p < 0.001$) (Table 1, Fig. 1).

Three weeks after the first encounter, all the intruders

TABLE 1

BEHAVIORAL EVENTS DURING FIRST (INTACT RATS) AND SECOND (DSP-4-TREATED INTRUDER) ENCOUNTER

	Residents		Intruders	
	Incidence	Duration (s)	Incidence	Duration (s)
		First Encounter		
Exploration	19.3 ± 1.0*	276.0 ± 20.4	13.6 ± 1.4	279.9 ± 32.1
Grooming	5.4 ± 0.6	51.1 ± 6.7	4.6 ± 1.1	53.7 ± 13.5
Immobile	0.7 ± 0.2	9.3 ± 4.3	1.1 ± 0.3	27.2 ± 11.6
Investigation	10.3 ± 0.9†	71.7 ± 4.8	6.5 ± 0.9	28.6 ± 3.9
Crawl o/u	0.5 ± 0.2	2.5 ± 1.4	0.1 ± 0.1	0.3 ± 0.3
Allogrooming	0.6 ± 0.2	4.2 ± 2.2	1.0 ± 0.4	6.9 ± 3.7
Attack	1.3 ± 0.3*	4.7 ± 2.2*	0.5 ± 0.2	4.5 ± 1.8
Lat. offence	5.9 ± 0.8	116.5 ± 19.2	0.2 ± 0.1	1.2 ± 0.8
Off upright	11.0 ± 0.9	39.8 ± 5.4	0.7 ± 0.2	7.7 ± 3.7
Def. upright	0.2 ± 0.2	1.5 ± 1.5	4.5 ± 0.7*	105.3 ± 32.2*
Lat. defence	0.6 ± 0.2	2.9 ± 1.2	4.6 ± 0.4	66.5 ± 11.0
On the back	0.0	0.0	0.8 ± 0.3	21.5 ± 7.6
		Second Encounter		
Exploration	18.2 ± 1.3	285.8 ± 21.4	17.9 ± 1.4	263.5 ± 21.5
Grooming	3.2 ± 0.9	37.5 ± 8.3	6.5 ± 0.9	157.4 ± 25.6
Immobile	1.7 ± 0.3	36.1 ± 20.6	2.2 ± 0.4	41.2 ± 8.6
Investigation	21.0 ± 5.5	96.1 ± 15.5	5.5 ± 0.9	21.7 ± 3.3
Crawl o/u	3.3 ± 0.9	15.6 ± 4.6	0.3 ± 0.1	1.0 ± 0.5
Allogrooming	1.5 ± 0.5	10.3 ± 3.5	0.0	0.0
Attack	0.6 ± 0.2	3.1 ± 1.5	1.2 ± 0.4*	7.0 ± 3.1*
Lat. offence	1.6 ± 0.6	6.6 ± 1.5	5.2 ± 1.2	43.5 ± 11.8
Off upright	2.2 ± 0.7	5.5 ± 2.9	9.2 ± 1.2	66.7 ± 11.2
Def. upright	3.1 ± 0.5	20.5 ± 5.1	0.7 ± 0.2*	3.7 ± 1.5*
Lat. defence	5.8 ± 0.9	39.2 ± 6.9	0.8 ± 0.2	4.2 ± 1.3
On the back	1.0 ± 0.2	27.1 ± 10.0	0.0	0.0

Values are mean ± SEM.
*$p < 0.001$, significantly different, Duncan's test.
†$p < 0.05$, significantly different, Duncan's test.

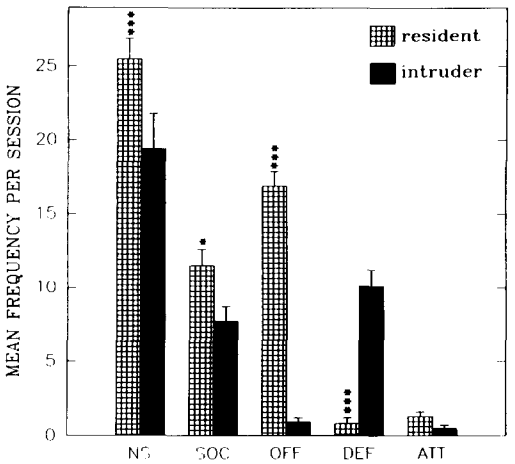

FIG. 1. Mean incidence per session of all behavioral categories observed before DSP-4 administered to intruder rats. NS: nonsocial behavior, SOC: social behavior, OFF: offense, DEF: defense, ATT: attack. $*p < 0.05$, $***p < 0.001$.

treated with DSP-4 10 days earlier were opposed again to their partners (residents) in the residents' home cage for a 10-min session. ANOVA demonstrated significant differences between the groups in the frequency, $F(4, 104) = 19.580$, $p < 0.001$, and duration, $F(4, 104) = 21.550$, $p < 0.001$, of particular behavioral categories. The Duncan test showed that the relationships between rats changed dramatically—a kind of role shift was observed. Offensive episodes, such as offensive sideways, offensive upright postures, and attacks, were clearly increased in previously submissive DSP-4-treated rats, in comparison with their behavior during the pretreatment period as well as in comparison with the current performance of residents ($p < 0.001$). In consequence, defensive postures were displayed less in intruders than in residents in terms of frequency ($p < 0.001$) and duration ($p < 0.001$) (Table 1, Fig. 2). It is of interest that DSP-4 rats displayed increased, but

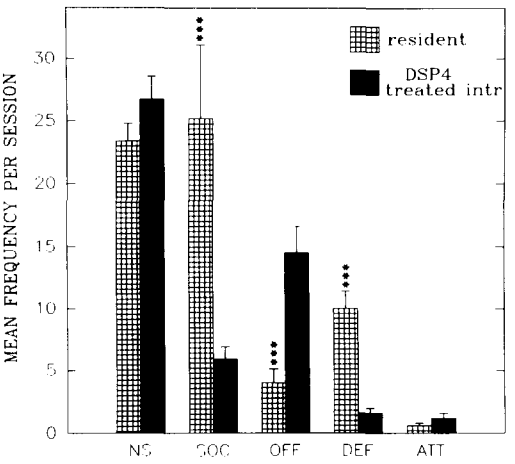

FIG. 2. Mean incidence per session of all behavioral categories observed after DSP-4 injection to intruder rats. NS: nonsocial behavior, SOC: social behavior, OFF: offense, DEF: defense, ATT: attack. $***p < 0.001$.

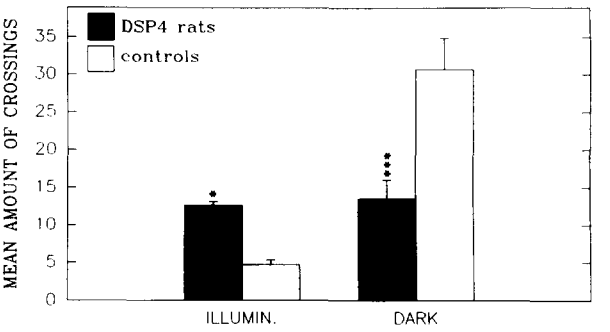

FIG. 3. Mean amount of crossings in the illuminated and dark areas of the open field in DSP-4-treated rats and control rats. $*p < 0.5$, $***p < 0.001$.

statistically nonsignificant, nonsocial activity; mean incidence of exploration episodes increased, but mean total duration of exploration remained the same. Mean incidence of self-grooming increased slightly, but mean total duration of self-grooming had reached a very high level.

Muricide Behavior

During the pretreatment period, 6 out of 14 intruders were selected as spontaneous killers, with the mean latency of killing ranking from 7 min to 2 min. DSP-4 injection did not affect muricide behavior in any way. The latency of killing remained the same in killers. The neurotoxin had no muricide-inducing ability in nonkillers.

Photo-Phobic Open Field Test

ANOVA showed that there is a statistically significant difference between the groups (DSP-4 vs. control) in locomotor activity (ambulation) in the highly illuminated center of the open field and dark areas, $F(1, 20) = 26.590$, $p < 0.001$. Locomotor activity of control animals was significantly less in the center ($p < 0.001$), whereas in the DSP-4 group locomotor activity in the center was not statistically different from that in dark areas. However, it was augmented in comparison with the illuminated area activity of the control group ($p < 0.05$) (Fig. 3).

Mann-Whitney test showed that there was no differences between groups in total amount of crossings in the open field as a whole (in illuminated and dark areas together) (Fig. 3).

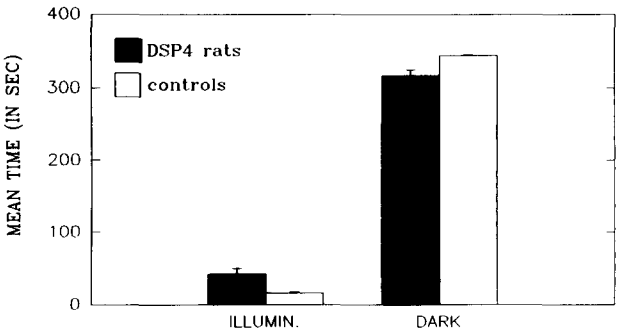

FIG. 4. Mean time (in seconds) spend by DSP-4 rats and controls in illuminated and dark areas of the open field.

TABLE 2

REGIONAL BRAIN CONCENTRATIONS OF NA, DA, 5-HT, DOPAC, HVA, AND 5-HIAA IN DSP-4-TREATED RATS

Group	Brain Region	Monoamine and Metabolite Content in ng/g Wet Tissue					
		NA	DA	5-HT	DOPAC	HVA	5-HIAA
1. Control (n = 8)		364.7 ± 83.7	73.4 ± 25.1	29.9 ± 10.2	105.5 ± 18.6	95.9 ± 23.5	287.5 ± 30.4
2. DSP-4 (n = 14)	HTP	30.1 ± 6.8	39.1 ± 6.5	14.0 ± 2.3	275.9 ± 48.6	32.7 ± 5.1	227.7 ± 62.0
		$p < 0.001$	NS	NS	$p < 0.01$	$p < 0.01$	NS
1. Control (n = 8)		65.6 ± 14.7	27.3 ± 4.3	14.4 ± 1.3	173.0 ± 14.6	8.8 ± 1.9	137.7 ± 31.5
2. DSP-4 (n = 14)	HIP	14.3 ± 2.5	15.2 ± 2.4	3.6 ± 0.6	27.0 ± 2.7	6.2 ± 1.3	88.0 ± 16.5
		$p < 0.001$	$p < 0.05$	$p < 0.001$	$p < 0.001$	NS	NS
1. Control (n = 8)		516.6 ± 85.1	130.4 ± 24.1	11.8 ± 3.1	374.1 ± 70.6	199.2 ± 17.5	597.1 ± 117.6
2. DSP-4 (n = 14)	AMY	37.1 ± 9.1	31.5 ± 5.7	5.7 ± 1.1	153.1 ± 34.8	47.9 ± 11.5	162.3 ± 16.7
		$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$
1. Control (n = 8)		43.8 ± 5.4	10.1 ± 1.0	9.5 ± 0.9	167.3 ± 27.4	74.6 ± 21.9	196.3 ± 32.5
2. SAP-4 (n = 14)	CTX	10.2 ± 1.9	33.6 ± 8.2	5.7 ± 1.0	38.9 ± 5.0	13.8 ± 1.5	147.9 ± 21.3
		$p < 0.001$	$p < 0.01$	NS	$p < 0.001$	$p < 0.001$	NS

Values are mean ± SEM.
Statistical significance: Newman-Keuls test.

Animals from both groups spent more time in the dark area; DSP-4 rats spent more time than controls in the illuminated center of the field (Fig. 4). Time : amount of crossings ratio was statistically different in illuminated vs. dark areas, $F(1, 20) = 23.080$, $p < 0.001$, as well as in groups, $F(1, 20) = 4.770$, $p = 3.9E\text{-}002$. However, there was no interaction between groups and different parts of the open field.

Biochemical Results

Regional brain concentrations of monoamines and their metabolites and ratios of metabolites to their parent amines in group 1 (control) and group 2 (DSP-4 treated) are presented in detail in Tables 2 and 3.

NA

In DSP-4-treated animals a marked decrease of NA level occurred: in HPT by 91.8%, in HIP by 78.3%, in AMY by 92.9%, and in CTX by 76.8%. ANOVA demonstrated significant differences between the groups in the content of NA, $F(1, 20) = 126.860$, $p < 0.001$. Some further and more detailed analysis by means of Newman-Keuls test showed that the level of NA was lower in group 2 than in group 1 in all brain regions ($p < 0.001$). Moreover, ANOVA demonstrated that there were significant differences between particular brain regions for NA, $F(3, 60) = 16.610$, $p < 0.001$.

DA, DOPAC, HVA, DOPAC : DA Ratio and HVA : DA Ratio

ANOVA demonstrated significant differences between the groups in the contents of DA, $F(1, 20) = 17.740$, $p < 0.001$; DOPAC, $F(1, 20) = 9.20$, $p < 0.001$; HVA, $F(1, 20) = 66.230$, $p < 0.001$; DOPAC : DA ratio, $F(1,20) = 14.730$, $p < 0.01$; and HVA : DA ratio, $F(1, 20) = 14.920$, $p < 0.001$. Newman-Keuls test showed that the DA level was lower

TABLE 3

RATIOS OF METABOLITES TO THEIR PARENT AMINES IN REGIONAL BRAIN AREAS IN DSP-4-TREATED RATS

Group	Brain Region	DOPAC : DA	HVA : DA	5-HIAA : 5-HT
1. Control (n = 8)		0.28 ± 0.16	0.18 ± 0.12	1.07 ± 0.10
2. DSP-4 (n = 14)	HTP	0.86 ± 0.06	0.10 ± 0.05	1.15 ± 0.09
		$p < 0.001$	NS	NS
1. Control (n = 8)		0.82 ± 0.08	0.54 ± 0.13	0.90 ± 0.08
2. DSP-4 (n = 14)	HIP	0.27 ± 0.08	0.30 ± 0.08	1.37 ± 0.08
		$p < 0.001$	NS	NS
1. Control (n = 8)		0.44 ± 0.07	0.25 ± 0.03	1.74 ± 0.03
2. DSP-4 (n = 14)	AMY	0.62 ± 0.08	0.11 ± 0.09	1.53 ± 0.10
		NS	NS	NS
1. Control (n = 8)		1.20 ± 0.05	0.73 ± 0.16	1.29 ± 0.10
2. DSP-4 (n = 14)	CTX	0.15 ± 0.09	0.28 ± 0.09	1.16 ± 0.04
		$p < 0.001$	$p < 0.001$	NS

Statistical significance: Newman-Keuls test.

in HIP ($p < 0.05$) and AMY ($p < 0.001$) and higher in CTX ($p < 0.01$) in group 2 vs. group 1. The DOPAC level was lower in HIP ($p < 0.001$), AMY ($p < 0.001$), and CTX ($p < 0.001$) and higher in HPT ($p < 0.001$) in group 2 vs. group 1. The HVA level was lower in HPT ($p < 0.01$), AMY ($p < 0.001$), and CTX ($p < 0.001$) in group 2 vs. group 1. The DOPAC : DA ratio was higher in HPT ($p < 0.001$) and lower in HIP ($p < 0.001$) and CTX ($p < 0.001$) in group 2 vs. group 1. Moreover, ANOVA demonstrated that there were significant differences between particular brain regions for DA, $F(3, 60) = 8.980$, $p < 0.001$; for DOPAC, $F(3, 60) = 12.260$, $p < 0.001$; for HVA, $F(3, 60) = 26.740$, $p < 0.001$; for DOPAC : DA ratio, $F(3, 60) = 3.90$, $p < 0.002$, and for HVA : DA ratio, $F(3, 60) = 7.090$, $p < 0.001$.

5-HT, 5-HIAA, and 5-HIAA : 5-HT Ratio

ANOVA demonstrated significant differences between the groups in the contents of 5-HT, $F(1, 20) = 17.490$, $p < 0.001$, and 5-HIAA, $F(1, 20) = 14.380$, $p < 0.01$. Newman-Keuls test showed that the level of 5-HT was lower in HIP ($p < 0.001$) and AMY ($p < 0.05$) in group 2 vs. group 1. The 5-HIAA level was lower in AMY ($p < 0.001$) in group 2 vs. group 1. No significant differences occurred in the 5-HIAA : 5-HT ratio. Moreover, ANOVA demonstrated that there were significant differences between particular brain regions for 5-HT, $F(3, 60) = 6.620$, $p < 0.001$; for 5-HIAA, $F(3, 60) = 9.820$, $p < 0.001$; and for 5-HIAA : 5-HT ratio, $F(3, 60) = 11.190$, $p < 0.001$.

Between brain regions and groups a significant interaction occurred: for NA, $F(3, 60) = 21.629$, $p < 0.001$; for DA, $F(3, 60) = 10.810$, $p < 0.001$; for DOPAC, $F(3, 60) = 12.640$, $p < 0.001$; for HVA, $F(3, 60) = 15.370$, $p < 0.001$; for 5-HIAA, $F(3, 60) = 9.290$, $p < 0.001$; for DOPAC : DA ratio, $F(3, 60) = 35.510$, $p < 0.001$; and for HVA : DA ratio, $F(3, 60) = 15.360$, $p < 0.001$.

DISCUSSION

Contrary to our expectations based on the previous reports indicating the increase of defensive aggression after the destruction of noradrenergic system (11,28,33,44), detailed ethological analysis of social interactions after DSP-4 administered to intruder rats revealed a decrease of previously exhibited defensive postures and a marked increase of offensive aggression. Intruders, clearly defensive during the first encounter, not only displayed more offensive acts, but also succeeded in eliciting defensive postures in residents, which underline the offensive character of their attitude toward the opponent. This is in agreement with the reports of increased aggression in 6-OHDA-lesioned rats observed in social interaction test and in the situation of competition for water (9,14). Lack of the detailed analysis did not allow authors to determine precisely the category of the behavior they observed. They described it as the form of agonistic behaviors "resembling those of offensive aggression" (14).

Facilitation of SIF or AIF after destruction of noradrenergic neurons by 6-OHDA or, more recently, DSP-4, might be model-specific and might not reflect the increase of defense. There still remains the unanswered question to what extent fighting evoked by aversive or dopaminergic stimulation and defensive behavior displayed in nature belongs to the same category in terms of physiological and biochemical mechanisms. Increase of SIF, even if its behavioral manifestation is very similar to the defensive reaction observed in natural conditions, might be due to the alterations in pain sensitivity. It is known that DSP-4 injection into amygdala in rats produces an increased sensitivity to pain in a hot plate test (33).

The increase of offensive postures observed in our experiments might suggest an increase of aggressiveness and would therefore support the notion of an inhibitory role of noradrenergic system in aggressive behavior. The results of other tests performed on the same animals indicate, however, that some other explanations for the dramatically changed attitude toward the partner should be taken into consideration. It has been proposed that locus coeruleus system is involved in fear and anxiety mechanisms and its destruction produces fear reduction (20,27,35). The problem is clearly controversial though, because there are also findings that disagree with the fear reduction hypothesis (9,14,26). In our experiment, DSP-4-treated rats spent more time in the stressogenic, highly illuminated center of the open field than did the controls. That might indicate the decrease of fear, leading to the increased tendency to attack in a social situation. But an alternative explanation that involves inadequate responsiveness to environmental factors is possible as well. In fact, this is supported partly by the same evidence that supports the fear reduction hypothesis—our rats behave offensively because of fear reduction or because they do not recognize the partner as a dominant, as a danger. They explore in photo-phobic test more than controls because they are not afraid of the light or they do not perceive it as an aversive stimulus. The latter would be in agreement with Jacobs et al.'s suggestion (21) that the noradrenergic system acts to enhance an organism's reactivity to environmental stimuli (i.e., it is involved in processing of sensory information). Redmond and Huang (35) suggest that a control of anxiety and fear is only a part of the function of the locus coeruleus noradrenergic system. Inability to react properly, according to the biological importance of the environmental cues after noradrenergic depletion, is in agreement with the concept of a locus coeruleus as an alarm system that acts as a novelty or danger detector or an attention focuser. Its role, according to Redmond and Huang (35), is associated with improved chances for survival. Foote et al. (16), on the basis of their electrophysiological experiments, hypothesize that LC participates in inducing, throughout many brain regions, the alert state that makes a precondition for subsequent appropriate orienting. The "cautionary" function of the LC system is supported by the recent work of Hajos and Engberg (18), who showed that DSP-4 treatment in rats significantly impairs escape reactions from noxious heat. The results reported by Cornwell-Jones (8) indicate that this hypothetical function of LC is not limited to aversive or threatening situations only. It has been shown that some characteristic female odors attracted sexually experienced control males, but not DSP-4-treated rats. In our experiments on cats, we found that DSP-4 destruction of LC did not produce alterations in well-established dominance-submissive order during predatory competition, except the situation when the stressful stimuli was presented to the pairs of cats. Dominant animals with an intact LC, apparently disturbed by the noise, did not show any interest in the prey; therefore, previously submissive, DSP-4-treated cats became dominants in competition (Kubiak and Zagrodzka, in preparation).

The biochemical results demonstrate that DSP-4 treatment significantly reduced NA concentration in all investigated structures. Simultaneously there occurred a considerable decrease in dopamine and serotonin and their metabolite levels, and significant decrease in the HVA : DA and DOPAC : DA ratios. Also, a decrease in 5-HT and 5-HIAA content has been observed. This speaks for the attenuated activity of the dopaminergic and serotoninergic systems. DSP-4 is a highly

selective neurotoxin for central NA neurons. It has been found that it does not have any cytotoxic effects on DA neurons (19). Its minor neurotoxic effect on 5-HT nerve terminals can be selectively autoponized by 5-HT uptake blocker what has been done in our experiment by zimlidine pretreatment. Therefore, we consider the diminished activity of DA and 5-HT systems as an effect secondary to NA depletion that reflects the functional interactions between DA, 5-HT, and NA systems. It is well known that the noradrenergic system anatomically and functionally remains in mutual and multidirectional relationships with other neurotransmitter systems in the brain (24,25). It is suggested that noradrenergic neurons have a tonic facilitatory action on DA and 5-HT neurons (2,3,37,38); therefore, a decrease in DA and 5-HT turnover might be due to the loss of a facilitating input from locus coeruleus. There are studies indicating the involvement of DA and 5-HT in aggressive behavior. Although the role of dopamine as excitatory or inhibitory seems to be controversial (4,29,32,43), the results concerning serotonin involvement are rather consistent. It appears that different kinds of aggression may be increased as a consequence of central 5-HT depletion (30,41,42,45). Mouse-killing behavior, as has been clearly demonstrated by many authors, is facilitated after 5-HT depletion (30). Some studies also point to the inhibitory role of dopamine in muricide behavior (4). According to our present data as well as other results (24), mouse killing is not affected by damage to the locus coeruleus projection. This fact indicates that the behavioral outcome of NA depletion and direct 5-HT or DA depletion is not parallel, but the similarity in the direction of changes seems to relfect noradrenaline–serotonin–dopamine interactions in the modulation of emotional behavior. A growing amount of evidence indicates that each form of aggression involves a complex, multitransmitter constellation and, to establish the neurochemical profile of the behavior, the balance among the transmitters should be systematically studied.

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REFERENCES

1. Adams, D. B. Brain mechanisms for offense, defense and submission. Behav. Brain Sci.2:204–241; 1979.
2. Amaral, D. G.; Sinnamon, H. M. The locus coeruleus: Neurobiology of a central noradrenergic nucleus. Prog. Neurobiol. 9:147–196; 1977.
3. Anden, N. E.; Grabowska, M. Pharmacological evidence for a stimulation of dopamine neurons by noradrenaline neurons in the brain. Eur. J. Pharmacol. 39:275–282; 1976.
4. Baggio, G., Ferrari, F. Role of brain dopaminergic mechanisms in rodent aggressive behavior: Influence of (±)N-n-propylforapomorphine on three experimental models. Psychopharmacology (Berlin) 70:63–68; 1980.
5. Blanchard, C. D.; Blanchard, R. J. Aggressive behavior in the rat. Behav. Biol. 21:197–224; 1977.
6. Blanchard, R. J.; Blanchard, C. D. Ethoexperimental approaches to the biology of emotion. Annu. Rev. Psychol. 39:43–68; 1988.
7. Brain, P. F. Differentiating types of attack and defense in rodents. In: Brain, P. F.; Benton, D., eds. Multidisciplinary approaches to aggression research. New York: Elsevier; 1981: 53–77.
8. Cornwell-Jones, C. A. DSP4, a noradrenergic neurotoxin, impairs male rats attraction to specific odors. Behav. Neural Biol. 50:1–15; 1988.
9. Crow, T. J.; Deakin, J. F. W.; File, S. E.; Longden A.; Wendlandt, S. The locus coeruleus noradrenergic system—evidence against a role in attention, habituation, anxiety and motor activity. Brain Res. 155:249–261; 1978.
10. Dooley, D. J.; Bittiger, H.; Hauser, K. L.; Bischoff, S. F.; Waldmeier, P. C. Alteration of central alpha-2 and beta-adrenergic receptors in the rat after DSP-4, a selective noradrenergic neurotoxin. Neuroscience 9:889–898; 1983.
11. Eichelman, B. S.; Thoa, N. B.; Ng, K. Y. Facilitated aggression in the rat following 6-hydroxydopamine administration. Physiol. Behav. 8:1–3; 1972.
12. Eison, M. S.; Stark, A. D.; Ellison, G. Opposed effects of locus coeruleus and substantia nigra lesions on social behavior in rat colonies. Pharmacol. Biochem. Behav. 7:87–90; 1977.
13. Ellison, G. Monoamine neurotoxins: Selective and delayed effects on behavior in colonies of laboratory rats. Brain Res. 103:81–92; 1976.
14. File, S. F.; Deakin, J. F. W.; Longden, A.; Crow, T. J. An investigation of the role of locus coeruleus in axiety and agonistic behavior. Brain Res. 169:411–420; 1979.
15. Foote, S. L.; Bloom, F. E.; Aston-Jones, G. Nucleus locus coeruleus: New evidence of anatomical and physiological specificity. Physiol. Rev. 69:844–914; 1983.
16. Foote, S. L.; Berridge, C. W.; Adams, L. M.; Pineda, J. A. Electrophysiological evidence for the involvement of the locus coeruleus in alerting, orienting and attending. In: Barnes, C. D.; Pompeiano, O., eds. Progress in brain research. vol. 88. New York: Elsevier Science Publishers; 1991:521–532.
17. Grant, S. J.; Redmond, D. E. Neuronal activity of the locus coeruelus in awake macaca arctoides. Exp. Neurol. 84:701–708; 1984.
18. Hajos, M.; Engberg, G. Impaired escape reaction from noxious and nonnoxious heat in rats treated with the selective noraderenegic neurotoxin DSP-4. Pharmacol. Biochem. Behav. 39:809–811; 1991.
19. Hallman, H. Neurochemical studies on centtral monoamine neurons: Consequences of selective toxic lesions. Stockholm: Kongl. Carolinska Medico Chirurgiska Institutet; 1984:22–31.
20. Huang, Y. H.; Redmond, D. R.; Snyder, D. R.; Maas, J. W. In vivo location and destruction of the locus coreuleus in the stumptail macaque (*Macaca artoides*). Brain Res. 100:91–97; 1975.
21. Jacobs, B. L.; Abercrombie, E. D.; Fornal, C. A.; Levine, E. S.; Morilak, D. A.; Stafford, I. L. Single-unit and physiological analyses of brain norephrine function in behaving animals. In: Barnes, C. D.; Pompeiano, O., eds. Progress in brain research. vol. 88. New York: Elsevier Science Publishers; 1991:159–165.
22. Jonsson, G.; Hallman, H.; Ponzio, F.; Ross, S.B. DSP-4/N-2-chloroethyl-N-ethyl-2 bromobenzylamine/—a useful denervation tool for central and peripheral noradrenaline neurons. Eur. J. Pharmacol. 72:173–188; 1981.
23. König, J. F.; Klippel, R. A. The rat brain: A stereotaxic atlas of the forebrain and the lower part of the brain stem. Baltimore: Williams and Wilkins; 1963.
24. Kostowski, W.; Członkowski, A.; Jerlicz, M.; Bidziński, A.; Hauptman, M. Effect of locus coeruleus lesions on aggressive behavior in rats. Physiol. Behav. 21:695–699; 1978.
25. Maeda, T.; Kojima, V.; Avai, R.; Fujimiya, H.; Komura, H.; Kitahama, K.; Geffard, M. Monoaminergic interaction in the central nervous system: A morphological analysis in the locus coeruleus of the rat. Comp. Biochem. Physiol. [C] 98:193–202; 1991.
26. Martin-Iverson, M. T.; Pisa, M.; Chan, E.; Fibiger, H. C. Enhanced neophobia but normal plasma corticosterone levels in rats with dorsal noradrenergic bundle lesions. Pharmacol. Biochem. Behav. 17:639–643; 1982.

27. Mason, S. T.; Roberts, D. C. S.; Fibiger, H. C. Noradrenaline and neophobia. Physiol. Behav. 21:353-361; 1978.
28. Mogilnicka, E.; Dooley, D. J.; Boissard, C. G.; Delini-Stula, A. Facilitation of shock-induced fighting in the rat after DSP-4, a selective noradrenergic neurotoxin. Pharmacol. Biochem. Behav. 18:625-628; 1983.
29. Nakamura, K.; Nakamura, K. Behavioral and neurochemical changes following administration of 6-hydroxydopamine into the ventral tegmental area of the midbrain. Jpn. J. Pharmacol. 26: 269-273; 1976.
30. Pucilowski, O.; Kostowski, W. Aggressive behaviour and the central serotoninergic systems. Behav. Brain Res. 9:33-48; 1983.
31. Pucilowski, O.; Kozak, W.; Valzelli, L. Effect of 6-0HDA injections into the locus coeruleus on apomorphine-induced aggression. Pharmacol. Biochem. Behav. 25:773-775; 1986.
32. Pucilowski, O.; Valzelli, L. Chemical lesions of the nucleus accumbens septi in rats: Effects on muricide and apomorphine induced aggression. Behav. Brain Res. 19:171-179; 1986.
33. Pucilowski, O.; Trzaskowska, E.; Kostowski, W.; Valzelli, L. Norephinephrine-mediated suppression of apomorphine-induced aggression and locomotor activity in the rat amygdala. Pharmacol. Biochem. Behav. 26:217-222; 1987.
34. Rasmussen, K.; Morilak, D. A.; Jacobs, B. L. Single unit activity of locus coeruleus neurons in the freely moving cat: I. During naturalistic behaviors and in response to simple and complex stimuli. Brain Res. 371:324-334; 1986.
35. Redmond, D. E.; Huang, Y. H., II. New evidence for a locus coeruleus-norepinephrine connection with anxiety. Life Sci. 25: 2149-2162; 1979.
36. Reiner, P. B. Correlational analysis of central neuronal activity and sympathetic tone in behaving cats. Brain Res. 378:86-96; 1986.
37. Reinhard, J. F.; Roth, R. H. Noradrenergic modulation of serotonin synthesis and metabolism. II. Inhibition by clonidine in vivo. J. Pharmacol. Exp. Ther. 221:541-546; 1982
38. Reisine, T. D.; Chesselet, M. F.; Lubetzki, C.; Cheramy, A.; Glowinski, J. A role for striatal beta-adrenergic receptors in the regulation of dopamine release. Brain Res. 241:123-130; 1982.
39. Rodgers, R. J. Drugs, aggression and behavioral methods. In: Brain, P. F.; Benton, D., eds. Multidisciplinary approaches to aggression research. Amsterdam: Elsevier/North-Holland Biomedical Press; 1981:325-340.
40. Romaniuk, A.; Gołębiewski, H. Adrenergic modulation of the hypothalamic cholinergic control of emotional-defensive behavior in the cat. Acta Neurobiol. Exp. 39:313-326; 1979.
41. Romaniuk, A.; Strzelczuk, M.; Wieczorek, M. Serotonin depletion with p-chlorophenylalanine in the cat: Effects on carbachol-induced defensive behavior and regional brain amine content. Acta Neurobiol. Exp. 49:130-140; 1989.
42. Sewell, R. G.; Gallus, J. A.; Gault, F. P.; Cleary, J. P. p-Chlorophenylalanine effects on schock-induced attack and pressing responses in rats. Pharmacol. Biochem. Behav. 17:945-950; 1982.
43. Sheard, M. H. Shock-induced fighting (SIF): Psychopharmacological studies. Aggress. Behav. 7:41-49; 1981.
44. Shibata, S.; Watanabe, S.; Liou, S. Y.; Ueki, S. Effects of adrenergic blockers on the muricide by desimipramine and noradrenaline injected into the amygdala in olfactory bulbectomized rats. Pharmacol. Biochem. Behav. 18:203-207; 1983.
45. Vergnes, M. Serotoninergic inhibition of mouse-killing behavior in the rat: Localization of the brain structures involved. Aggress. Behav. 8:208-211; 1982.
46. Vergnes, M.; Depaulis, A.; Boehrer, A. Parachlorophenylalanine-induced serotonin depletion increases offensive but not defensive aggression in male rats. Physiol. Behav. 36:653-658; 1986.