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Novelty-Oriented Behavior in the Rat After Selective Damage of Locus Coeruleus Projections by DSP-4, A New Noradrenergic Neurotoxin

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DELINI-STULA, A., E. MOGILNICKA, C. HUNN AND D. J. DOOLEY. *Novelty-oriented behavior in the rat after selective damage of locus coeruleus projections by DSP-4, a new noradrenergic neurotoxin.* PHARMACOL BIOCHEM BEHAV 20(4) 613–618, 1984.—Open-field behavior and reactions to a novel object (white-colored cube) or a familiar object (drinking bottle) were investigated in rats treated with DSP-4 N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine, a new noradrenergic neurotoxin which selectively damages locus coeruleus projections. Altered behavior in the open-field and in the presence of the novel object (white cube) was observed in DSP-4 rats. This was reflected in decreased exploration-oriented locomotor responses and in longer latencies to approach the novel cube. Also, there was a trend towards fewer center entries and a shorter duration of object exploration. Although these behavioral responses of DSP-4 rats were indicative of enhanced neophobia, other measures of emotionality, such as grooming and defecation, were either unchanged or slightly decreased. Moreover, when the familiar drinking bottle was present in the open-field, water-deprived DSP-4 rats showed no change in any measure of fear including the latency to the first approach and lick, and the duration of the licking episodes. The results of this study suggest that noradrenergic neurons of the locus coeruleus are involved in the regulation of certain, but not all, novelty-oriented responses in the rat. Explorative behavior in the novel environment seems to be particularly dependent on central noradrenaline.

DSP-4 Neurotoxin Noradrenaline Locus coeruleus Open-field Novelty Neophobia Behavior Exploration Rat

THE exact role of central noradrenergic systems in fear-motivated behavior is still a matter of controversy. Studies using stumptail macaque monkeys, in which the locus coeruleus (LC) was electrolytically destroyed or electrically stimulated, indicate facilitating effects of central noradrenaline (NA) on fear and anxiety reactions [8, 19, 20]. In contrast, several investigations using rats have yielded discrepant results concerning the effects of central NA depletion on such reactions [2, 6, 12, 13, 14]. These discrepancies are probably due to different paradigms which were used to generate fear-motivated responses in the rat. Alternatively, NA may have no effect on manifestations of anxiety in the rat.

Recently, Archer and colleagues [2] showed that rats treated with DSP-4 (N-(2-chlorethyl)-N-ethyl-2-bromobenzylamine) had no significant neophobic response to a novel saccharin solution unless this solution was paired with a novel drinking bottle. DSP-4 is a new and highly selective noradrenergic neurotoxin [3, 4, 11] which, after intraperitoneal injection to rodents, markedly reduces NA in CNS regions innervated by the LC (e.g., neocortex, hippocampal formation, cerebellum, spinal cord). This central effect of DSP-4 is long-lasting, whereas peripheral NA depletion by DSP-4 is transitory with recovery occurring several days after injection [9]. These features make the neurotoxin a suitable tool for investigating the role of central NA in behavior.

In view of the existing controversies about the role of NA in neuphobia, and the fact that DSP-4 appears to offer a better and more selective tool for investigation than 6-hydroxydopamine (6-OHDA), we decided to test the behavior of DSP-4 rats in an unfamiliar environment such as the open-field. It is generally assumed that behavioral responses in the open-field, namely ambulation, rearing, grooming, and defecation, reflect a fear-motivated state of the rat encountering novelty. The open-field testing was performed under 3 different conditions: (a) open-field presentation was the sole novel stimulus, (b) open-field presentation

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was paired with the presentation of a novel object (white-colored cube), and (c) open-field presentation was paired with the presentation of a familiar object (drinking bottle).

METHOD

Subjects

Male albino rats (Tif:RAIf (SPF), Tierfarm Sisseln, Sisseln, Switzerland), initially weighing 220–240 g, were caged in groups of 5 in an air-conditioned laboratory room (T=20°C) under 12-hr light/dark cycles. Unless otherwise stated, the animals had free access to food and water. All experiments were performed during the light phase of the cycle.

DSP-4 Treatment

The preparation of animals was as previously described [3,4]. Briefly, they were injected with CGP 6085 A, a selective and potent 5-hydroxytryptamine-uptake inhibitor [23], at a dose of 2.7 mg/kg (10 μmol/kg) IP 30 min before 63 mg/kg (200 μmol/kg) IP of DSP-4 or 0.9% saline. The control and DSP-4 rats were then used in experiments 10 days later between 08.00 and 12.00 hr. At 10 days after DSP-4 injection, results of previous neurochemical experiments [3,4] indicate substantial reductions of NA (<25% of control NA remaining) in neocrotex, hippocampal formation, cerebellum, and spinal cord. Central NA depletion in the DSP-4 was confirmed after experiments by the absence of the post-decapitation reflex [3,4].

Apparatus

A grey-painted square box (65×65×48 cm) served as the open-field device. The floor was divided into 9 squares by black plastic adhesive strips. In Experiment 1, the open-field was diffusely illuminated by two 75 W bulbs suspended above the two opposite corners of the open-field. In Experiments 2 and 3, the center of the open-field was illuminated by a 75 W bulb suspended directly above it. During all experiments the laboratory room was dark.

In Experiment 2, a white-colored cube (6×6×6 cm) as a novel object was placed in the center of the open-field. In Experiment 3, instead of the cube, a familiar drinking bottle was suspended in the open-field center in such a way as to allow rats easy access and undisturbed drinking.

Experiment 1

Ten days after DSP-4 injection individual rats were gently placed in the open-field. Locomotor activity (ambulation and rearing), grooming, and defecation were recorded over 12 min in 3 successive time-blocks of 4 min each. Ambulation was defined as the number of crossings of the squares (all 4 paws in a square), rearing as the frequency of stand-up reactions, and grooming as the time (sec) spent grooming any part of the body. Mean values for each time-block and mean cumulative total values (±S.E.M.) were calculated for each group (n=14 per group).

Experiment 2

The second experiment was performed in essentially the same way as the first except that additional parameters were also measured: (a) latency (sec) to first approach the object, (b) duration (sec) of object exploration, and (c) the number of entries into the center of the open-field. The total observation time in this experiment was 8 min (two successive time-blocks of 4 min each). This observation time was selected on the basis of prelimnary and other experiments which indicated rapid and similar habituation responses of control and DSP-4 rats; no further information was gained by longer open-field exposures.

Mean values for each time-block and mean cumulative total values (±S.E.M.) were calculated for each group (n=38–39 per group).

Experiment 3

Prior to testing in the open-field, some control and DSP-4 rats were individually caged in order to determine their total 24 hr water intake, and water intake during only 2 hr of daily access (over successive days 1, 2, and 3) at times corresponding to open-field sessions. Twenty-two hours before the actual testing began, these and other rats were deprived of water. The following parameters were recorded every min during 8 min of open-field exposure: (a) the latency (sec) to first approach the drinking-bottle, (b) the latency (sec) to first lick the nipple after the first approach, and (c) the total duration (sec) of licking (drinking) episodes.

In addition, ambulation, rearing, grooming, and defecation were recorded as in previous experiments. Mean cumulative total values (±S.E.M.) were calculated for each parameter and for each group (n=18 per group).

Data Analysis

Statistical significance of the mean total values for each parameter was analyzed using either the Kruskal-Wallis test or the Mann-Whitney test. The level of significance was calculated using normal two-tail approximation or chi 2 distribution with 1 degree of freedom.

RESULTS

Experiment 1

Before the start of the open-field test, visual inspection revealed no particular changes in gross behavior between control and DSP-4 rats. Also, body weights of animals from the two groups were similar. In the open-field, however, differences in the behavior of control and DSP-4 rats were evident as presented in Fig. 1 and Table 1. The locomotor activity (ambulation and rearing) of DSP-4 rats was diminished compared to that of control animals; this lowered activity was present at each successive time-block. However, the sustained decrease of behavior from the first to the last time-block was similar to that shown by control rats, indicating that habituation was not impaired. Comparison of mean cumulative total values indicated that control and DSP-4 rats significantly differed both in respect to the number of crossed squares ($T=11.9$, $p<0.001$, Kurskal-Wallis) and number of rearing episodes ($T=11.2$, $p<0.001$) exhibited during the 12-min observation period. DSP-4 rats spent slightly, but significantly, less time grooming (−27%, $T=5.49$, $p<0.01$) than control rats, but the rate of defecation in the two groups was similar.

Experiment 2

In the presence of the novel object, 17% of control rats failed to approach, explore, or enter into the center of the open-field during the 8-min observation period (cut-off time of 480 sec). In comparison, about 40% of DSP-4 rats did not

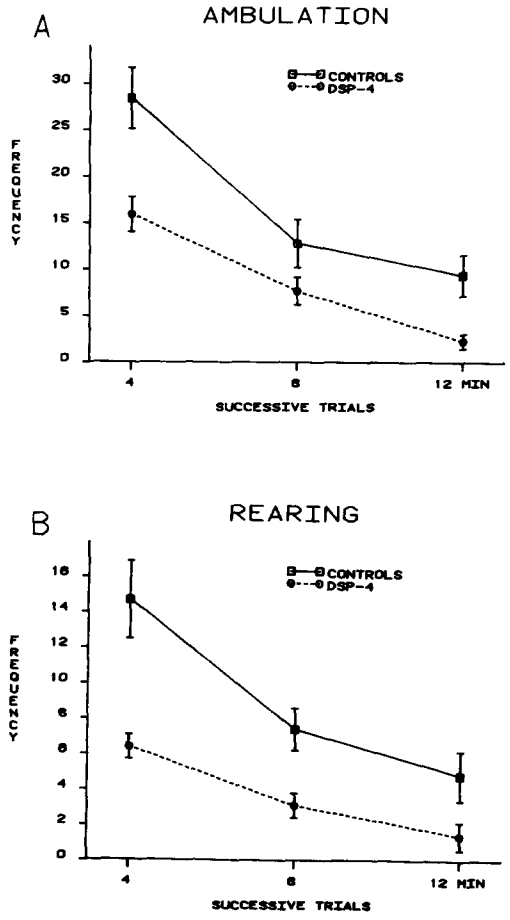

FIG. 1. Exploration-oriented behavior of control and DSP-4 rats in an unfamiliar open-field. Parameters are ambulation (A) and rearing (B). Each point on the curves represents the mean frequency (±S.E.M., n=14) of crossed squares or rears recorded in three successive 4-min trials of a 12-min open-field session.

show any object-oriented response. The difference between the two groups in respect to the latency to the first approach (Fig. 2A) proved to be significant ($p=0.03$, Mann-Whitney). There appeared to be a trend towards a lower duration of object exploration in DSP-4 rats (Fig. 2B); however, this difference failed to reach the 5% significance level ($p=0.78$, Mann-Whitney). There was also no statistically significant difference either in the initial (4 min) or total number of center entries exhibited by control and DSP-4 rats (mean± S.E.M. for total number: 4.0±0.5 and 2.3±0.5, respectively; $p=0.96$, Mann-Whitney). Mean cumulative total values of ambulation, rearing, grooming, and defecation are given in Table 1. As seen in the Table, the results are consistent with the observations of Experiment 1; namely, exploratory activity of DSP-4 rats was significantly lower than that of control rats. Grooming activity and defecation rate were similar for both groups.

Experiment 3

The results of this experiment are presented in Fig. 3 and Table 1. In the presence of the drinking bottle, one control rat and one DSP-4 rat failed to approach it during the 8 min. The time which elapsed between the placement of the rat in the open-field and its first approach to the drinking bottle (i.e., latency to the first approach) was almost the same for both groups ($p=0.34$, Mann-Whitney). Moreover, there was no clear difference ($p=0.11$, Mann-Whitney) between the groups in respect to the time interval between the first approach and the first lick (i.e., latency to the first lick). There was a trend for DSP-4 rats to spend a longer time drinking than control animals, although this difference did not reach statistical significance ($p=0.08$, Mann-Whitney). Water consumption of DSP-4 rats (Table 2), either over 24 hr or during the limited period of 2 hr/day, was similar to that of control rats. Also, weights of control and DSP-4 animals were not significantly different.

DISCUSSION

The selective neurotoxic effect of DSP-4 on LC projections resulting in remarkable decrease of NA content in

TABLE 1

FREQUENCY OF OPEN-FIELD BEHAVIORS IN CONTROL AND DSP-4 RATS

	Cumulative Total Values				
Treatment	Ambulation	Rearing	Grooming	Defecation	N
Experiment 1					
Controls	51 ± 6.2	27 ± 3.6	146 ± 12.9	4.5 ± 0.5	14
DSP-4	26 ± 2.7‡	11 ± 1.6‡	106 ± 8.1†	4.0 ± 0.7	14
Experiment 2					
Controls	38 ± 2.9	18 ± 1.5	31 ± 2.9	3.5 ± 0.4	39
DSP-4	28 ± 3.0*	10 ± 0.9*	30 ± 3.9	3.0 ± 0.3	38
Experiment 3					
Controls	43 ± 4.2	18 ± 1.4	26 ± 4.6	1.8 ± 0.6	18
DSP-4	35 ± 3.5	16 ± 1.9	11 ± 2.3	0.7 ± 0.4	18

Open-field exposure in Experiment 1 was for 12 min; Experiments 2 and 3, 8 min. Measures of the parameters are as defined in the text. Values given are mean ± S.E.M. Asterisks indicate a significant difference (*$p<0.05$, †$p<0.01$, ‡$p<0.001$; Kruskal-Wallis and Mann Whitney).

TABLE 2
WATER INTAKE IN CONTROL AND DSP-4 RATS

Treatment	Water intake (ml/2 hr) Day 1	Day 2	Day 3	N	Water intake (ml/24 hr)	N
Controls	16.2 ± 3.5 (275 ± 2)	21.4 ± 2.0 (277 ± 2)	21.2 ± 1.1 (282 ± 2)	5	36.0 ± 1.9 (235 ± 5)	5
DSP-4	18.2 ± 4.8 (273 ± 15)	22.4 ± 2.8 (276 ± 15)	20.8 ± 3.1 (279 ± 15)	5	42.0 ± 2.3 (251 ± 10)	5

Body weights are in parentheses. Values given are mean ± S.D. There was no significant difference from the control treatment.

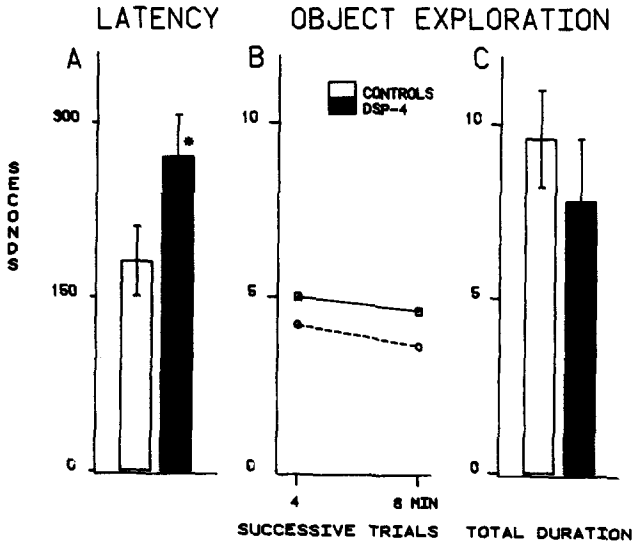

FIG. 2. Exploration-oriented behavior of control and DSP-4 rats in an unfamiliar open-field. Parameters are latency to first approach novel object (A) and duration of exploration of the novel object (B and C). In (A), the columns represent the mean latency (±S.E.M., n=38–39) to first approach the object. An asterisk in (A) indicates a significant difference (*$p<0.05$, Mann-Whitney) from the control treatment. In (B), each point on the curves represents the mean duration of object exploration recorded in two successive 4-min trials of an 8-min open-field session. In (C) the columns represent the mean cumulative total values (±S.E.M., n=38–39) across these 4-min trials.

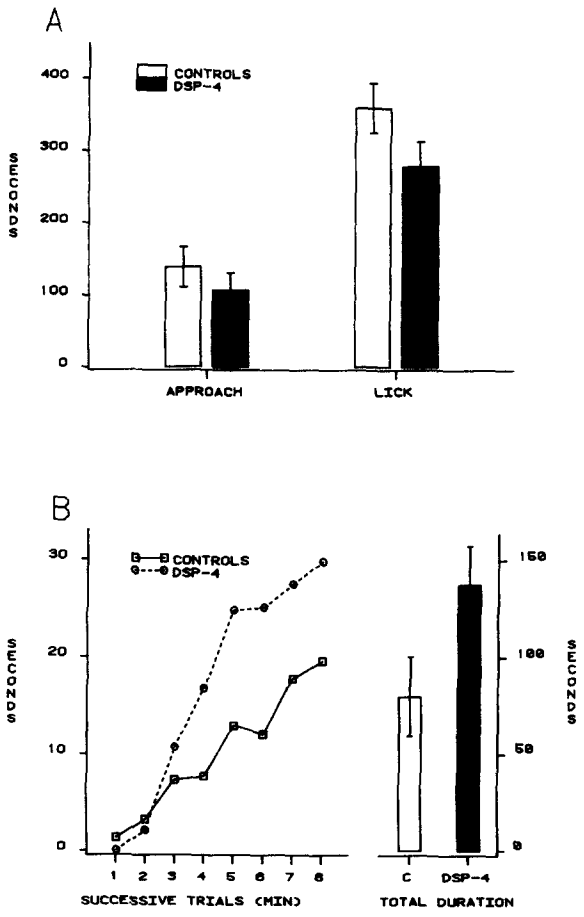

FIG. 3. Drinking behavior of control and DSP-4 rats in an unfamiliar open-field. Parameters are latencies to first approach the drinking bottle and to first lick of the nipple (A), and duration of the licking episodes (B). In (A), the columns represent the mean latencies (±S.E.M.) to the first approach and to the first lick. In (B), each point on the curves represents the mean duration of licking episodes recorded in eight successive 1-min trials of an 8-min open-field session. The columns represent the mean cumulative total values (±S.E.M., n=18) across these 1-min trials.

neocortex (83%), hippocampus (89%), cerebellum (81%) and spinal cord (77%) was recently confirmed by Dooley *et al.* [3]. The authors also showed that after DSP-4 treatment NA levels in the hypothalamus and brain stem decrease to only 31–34% and dopamine and serotonin concentrations, in the presence of selective 5-HT-uptake inhibitor CGP 6085, do not change at all. Moreover, DSP-4 produced no alterations in the levels of various amino-acids or acetylcholine in the rat brain (R. Bernasconi, personal communication). Therefore, it is reasonable to assume that observed changes in

behavioral responses of DSP-4 rats exposed to novelty, relate to the damage of LC projections with consequent NA depletion.

The consistent finding in our study is the suppression of exploration-oriented locomotor responses, including ambulation, rearing, and object approach, in DSP-4 rats exposed to novelty (open-field) or novelty paired with another novelty. This could represent a generalized decrease of motor activity or an inability of DSP-4 animals to initiate certain behaviors. However, such explanations are difficult to reconcile with results from other studies, including our own, which indicate that locomotion of DSP-4 rats, as measured by other techniques, is not usually inhibited [1, 2, 4, 21]. Moreover, DSP-4 rats acquire positively reinforced tasks at a normal rate [20], and in the present study, initiate drinking behavior with a latency similar to that of control animals.

The specific suppression of ambulation, rearing, and approach behavior in response to novelty, or novel object in an unfamiliar environment, may be related to an enhanced fear-induced "behavioral inhibition" as defined by Gray *et al.* [7]. Inhibition of ongoing behavior has been suggested to be directly associated with anxiety-inducing stimuli; this inhibition is typically blocked by anxiolytic drugs. REM-sleep deprivation, which exerts antineophobic effects in rats [17], has been found to reverse the behavioral deficits of DSP-4 rats in the open field [16]; this finding could be compatible with the notion that such behavioral deficits reflect an enhanced neophobia. From our results, but contrasting with those of Archer *et al.* [2], it appears that enanced neophobia i.e., decrease of exploratory-oriented response after LC damage, occurs under conditions of novelty or novelty paired with another novelty. However, behavioral responses controlled by strong motivational incentives, such as water deprivation, do not seem to be inhibited either in their magnitude or initiation after LC damage. In fact, it should be noted that in this respect our findings seem to support the hypothesised role of NA in "selective attention" processes, although such role has been contested by some most recent findings [18].

An interesting observation in our study is the dissociation of the effects of DSP-4 on exploratory responses in novelty situations, and on responses related to emotionality such as grooming and defecation. Grooming activity was variable under different experimental conditions, whereas defecation was unchanged. This observation is somewhat similar to that reported by Martin-Iverson and his colleagues [12]. They found enhanced neophobic reactions to a novel environement and novel food in rats with bilateral 6-OHDA lesions of the dorsal noradrenergic bundle (DNB). This neophobia was, however, not associated with enhanced corticosterone secretion; levels of corticosterone, an index of emotionality, were similar to those of control rats. In our study corticosterone levels were not measured, but no change in the circadian rhythm of this hormone was observed in DSP-4 rats by comparison to controls in other experiments (Hausler, personal communication). Also, under stress conditions there was in this respect no difference between DSP-4 and control rats [5]. The rather small decrease in hypothalamic NA concentrations produced by DSP-4 (30%) and 6-OHDA (in the Martin-Iverson's study) did not appear, therefore, to functionally alter the hypothalamo-pituitary axis [3,11]. This explanation may possibly also account for the lack of change in defecation rate in DSP-4 rats.

In view of the diverse methods of measuring of fear in rats with lesions of LC neurons, it is difficult to compare our findings with those reported by other researchers. For instance, Mason *et al.* [14], found that some, but not all, measures of neophobia are increased in rats with 6-OHDA lesions of DNB. This finding would be in general agreement with the results of our study. In his experiments, lesioned rats placed in a novel environement showed longer latencies to approach and consume novel food; however, in a familiar cage, they failed to show enhanced neophobia when a novel object was presented. In disagreement with our results are his observations that DNB-lesioned rats exhibit increased exploration and a lack of habituation. In all our experiments, DSP-4 rats habituated to novelty as evidenced by the sustained decrease of exploration-oriented behavior from one-time block to the next during the test session. Also, the shapes of the habituation curves for control and DSP-4 rats were essentially identical, irrespective of the magnitude of the initial response. Several possible explanations for this difference between 6-OHDA and DSP-4 rats are (A) the testing paradigm used, (B) the NA depletion in cerebellum and spinal cord after DSP-4 (which does not occur with the DNB lesion), and/or (C) the marked reduction of NA in the hypothalamus (ca 74%) after DNB lesion in the study of Mason. The latter point is of especial interest: a lesion of the ventral noradrenergic bundle, which causes a marked loss of only hypothalamic NA, increases the freqquency and duration of exploratory behavior in the open-field [10,15].

In conclusion, although the results of this study do not clarify the role of LC in neophobic behavior, they favor the assumption that LC noradrenergic neurons are implicated in the control of some, but not all, novelty-oriented responses in the rat. Apparently, explorative behaviors are the ones subjected to an enhanced behavioral inhibition during exposure to novelty; these responses seem to be under the positive control of LC noradrenergic neurons.

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