Pharmacology Biochemistry & Behavior, Vol. 19, pp. 121–130, 1983.

T-Maze Learning, Spontaneous Activity and Food Intake Recovery Following Systemic Administration of the Noradrenaline Neurotoxin, DSP4.

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Received 17 June 1982

ARCHER, T., A. K. MOHAMMED, S. B. ROSS AND U. SÖDERBERG. *T-maze learning, spontaneous activity and food intake recovery following systemic administration of the noradrenaline neurotoxin, DSP4.* PHARMACOL BIOCHEM BEHAV **19**(1)121–130, 1983.—Following systemic administration of the noradrenaline (NA) neurotoxin, DSP4 (50 mg/kg), rats were found to be retarded in the rate at which they acquired the "right-turn" running response in a modified T-maze choice situation, as measured by the total number of errors per session and median latency to reach the goal box. Desipramine (DMI, 20 mg/kg), injected 30 min before DSP4 blocked the acquisition retardation. DSP4 was found to have a short-lasting effect upon spontaneous motor activity, while food and water intake recovery was complete within 7 days of the injection. Both the NA-accumulation data and endogenous NA concentrations indicated profound NA, but not 5-hydroxytryptamine (5-HT) and dopamine (DA), depletions in the cortex, hippocampus and cerebellum. These data seem to confirm the role of the locus coeruleus-noradrenaline (LC-NA) system in an instrumental learning situation.

Motor activity Noradrenaline neurotoxin DSP4 Spontaneous activity

THE influence of the putative neurotransmitter noradrenaline (NA) upon learning and memory has for some time now stimulated much theoretical debate [8, 9, 12, 21, 24, 34] and empirical endeavour [3, 13, 17, 28]. As a result of demonstrations that electrodes placed in the area of the locus coeruleus (LC) support intracranial electrical self-stimulation [10,11], it was proposed that NA-containing neurons originating in the LC in effect function as a reinforcement system [12,21]. Subsequent empirical support was provided in positively- [3] and negatively-reinforced [13] learning situations. Anlezark, Crow and Greenaway [3] found drastically prolonged running latencies for food reward in an L-shaped runway following bilateral LC lesions, a finding that was essentially replicated by Sessions, Kant and Koob [44] though Amaral and Foss [2] did not do so. Microinjections of 6-hydroxydopamine (6-OHDA) into the fibres of the dorsal ascending noradrenergic bundle (DB) produce a much greater depletion of NA in forebrain areas, without the hazardous problem of nonspecific damage accompanying the electrolytic LC lesion [29,31]. Several investigations into the role of forebrain NA in instrumental reward learning using the 6-OHDA DB procedure have invariably shown negative results [28, 38, 39]. However, Mason and Fibiger [26] did find that 6-OHDA DB lesions that produced 95% NA depletions in the cortex and hippocampus caused an impairment in the acquisition of a spatial alternation task in the T-maze situation. In spite of this the general concensus seems to be that the relative role of central NA in the acquisition of these tasks in the rat is largely redundant.

The pharmacological action of the haloalkylamine N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP4) involves a selective neurotoxic effect upon peripheral and central NA neurons [18–20, 40, 42]. In both rats and mice it has been found that DSP4 produces a drastic, but highly transient, reduction of endogenous NA concentrations and ^{3}H-NA accumulation in iris and atrium tissues; this reduction dissipated within 7 days [20]. The central NA depletion on the other hand was found to be of a much longer-lasting, almost permanent, nature in several forebrain areas [20]. Fluorescence histochemical analysis of mouse brain following systemic DSP4 by Jonsson, Hallman, Ponzio and Ross [20] demonstrate the disappearance of catecholamine terminals in the cortex, hippocampus, spinal cord and cerebellum, but was not observed in the striatum. Jonsson *et al.* [20] found no evidence of alterations to dopamine (DA) and serotonin (5-HT) neurons. DSP4 crosses the blood-brain barrier and through a single intraperitoneal or intravenous injection exerts its effect upon NA terminals in the several brain regions innervated by the LC [40,42]; cell bodies remain unaffected. Thus, DSP4 offers a novel and useful pharmacological tool for studying the functional role of NA terminals innervated by the LC in learning processes. Recent experi-

ments in which DSP4 (50 mg/kg) was administered systemically to rats seven days before the testing of one- and two-way avoidance acquisition indicated impairments of task performance [5, 6, 37]. In view of the avoidance deficits produced by DSP4 treatment, it is of direct interest to investigate whether the central NA-depletions caused by this neurotoxin could also result in some alteration of the acquisition of a positively reinforced task.

In order to investigate the role of central NA pathways in maze learning we chose a modified T-maze apparatus which offers the rat a choice situation at once a more complicated and difficult task for the animal than either a straight or L-shaped runway. In addition, the use of a T-maze allows by definition the computation of errors by each rat, a measure that provides a valuable complement to the measurement of running speed latencies avoiding as it does the problem of alterations to general activity following pharmacological manipulations. A correct trial with the present maze required a "right-turn" running response over a length roughly equal to that of Anlezark *et al.* [3] but slightly shorter than of Sessions *et al.* [44]. In both the T-Maze-learning experiments described below DSP4 (50 mg/kg, IP) was injected at least 14 days prior to the onset of the maze learning task. In the second experiment, a group injected with the selective NA uptake inhibitor desipramine (DMI, 20 mg/kg, IP), 30 min before DSP4, was included as the pharmacological control for the DSP4 condition.

METHOD

Animals

Male Sprague-Dawley rats (AB Anticimex, Sollentuna, Sweden) weighing 200–250 g on arrival at the laboratory, were used in all the experiments. Rats were allowed at least 3 weeks adaptation to the laboratory milieu with an ad lib food (Lab. chow R3, Ewos, Södertälje, Sweden) and water regime.

NA Depletion

Rats were randomly divided into different groups within each experiment and treated with either the catecholamine-specific NA neurotoxin DSP4 (50 mg/kg, intraperitoneally, IP), the selective NA uptake inhibitor, DMI (20 mg/kg, IP) 30 min prior to DSP4 (50 mg/kg), or distilled water (5 ml/kg). Behavioural testing was initiated at least 10 days following these treatments. DSP4 was dissolved in distilled water, DMI in 0.9% saline.

Experimental Design

Following injections, spontaneous activity measurement was carried out in a modified hole-board in Experiment 1. The rats were habituated to the start box 18 days after the injection. The acquisition of the running response for food reward in the modified T-maze (see below) was initiated 19 days after the DSP4 administration. In the second experiment, the habituation to the start box and the first acquisition session occured 13 and 14 days after DSP4 injections, respectively. One month after the completion of the behavioural studies the rats in Experiment 2 were sacrificed and their hippocampal regions analyzed for NA and 5-HT uptake.

Procedure

T-maze learning procedure. T-maze acquisition was carried out in the modified T-maze apparatus during daylight hours between 1000 and 1500 hours. Figure 1 presents the modified T-maze used in the learning experiments. Acquisition trials were initiated 19 and 14 days after the DSP4 treatment in Experiments 1 and 2 respectively, and were in each case, preceded by a 5-min habituation trial for each rat on the day before the first acquisition session. For the habituation trial each rat was placed in the start box with the door closed and allowed to remain there for 5 min. All the acquisition trials, five of which were presented during each session, were identical. Each rat was weighed prior to each session, placed in the start box and the door was raised as soon as the rat had oriented towards it. Each animal's progress towards the goal box was carefully observed in the mirror (see Fig. 1) and each error recorded. Errors are defined as follows: (a) an about-turn after release from the start box but before reaching point B, (b) a left-turn at point B and, if the rat had made a left-turn and was returning to point B after an about-turn at the left-arm barrier, a right-turn (which would take the rat back to the start box) was also scored as an error, obviously an about-turn at point B (taking the rat back to the left arm barrier) was also registered as an error, (c) an about-turn after the rat had passed point B, following a correct right turn, but had not reached the goal box would take the animal

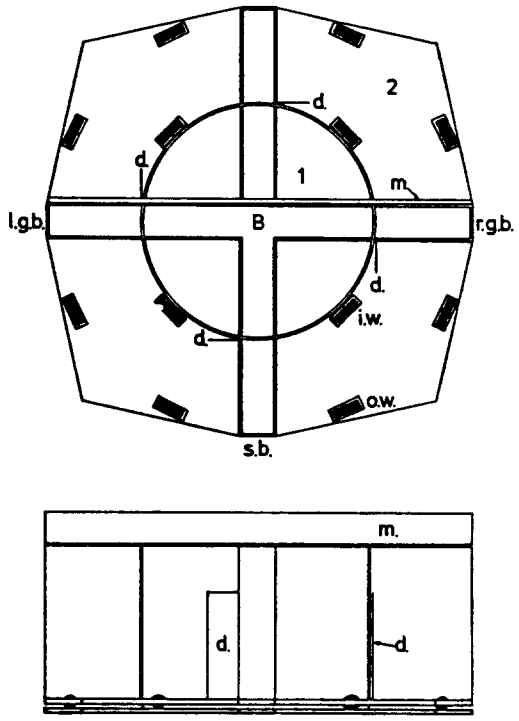

FIG. 1. Top and front view of the modified T-maze used for studying the acquisition of a right-turn running response in Experiments 1 and 2. In both the experiments rats were placed in the start box (s.b.) with the door (d) to the left goal box (l.g.b.) closed and that to the right goal box (r.g.b.) open. Each rat's reflection in the mirror (m) was monitored until the animal had entered the right goal box and the door closed behind it. The right goal box was then rotated clockwise, on the inner (i.w.) and outer (o.w.) wheels, until it reached the start box position. "B" signifies the left/right choice point, "1" the fixed part of the maze and "2" the rotating part.

TABLE 1

THE CHRONOLOGICAL DETAILS OF THE PROCEDURES FOR EXPERIMENTS 1 AND 2

Experiment 1			
Schedule	Treatment and Tests	Schedule	Treatment and Tests
Day 0	DSP4/distilled water	Day 0	DSP4/DMI+DSP4/ distilled water
Day 12	Hole-Board Test 1		
Day 19	Habituation	Day 14	Habituation
Days 20–29	T-Maze: Sessions 1–10	Days 15–19	T-Maze: Sessions 1–5
Day 32	Hole-Board Test 2		
Days 39 and 40	Food intake Tests		
Day 57	Hole-Board Test 3		

back to B, if the rat continued past point B, either straight or making a left-turn (back to start box), this was also scored as an error. Latency to enter goal box was measured manually from the time each rat had left the start box till that time the animal's body (excepting its tail) had entered the goal box. The number of trials to the first error-free trial as well as the number of trials to a criterion of five consecutive error-free trials were also computed. On entering the goal box each rat was presented with a shallow white plastic food cup (located on the floor towards the back of the goal box) containing a food pellet and the goal box door closed. Each rat was allowed a 40-sec undisturbed period to consume the pellet after which the goal box was rotated in a clockwise direction until it reached the position of start box. After another 15 sec the start box (formerly goal box) door was opened and the next trial started. On completion of the fifth trial of that particular session, each rat was replaced in its home cage and given its daily food ration (2–4 pellets weighing 8–14 g) depending on its particular body weight prior to the start of the T-maze learning session. The next session was started 24 hours later and proceeded in an identical manner.

Biochemical analysis. Following the behavioural studies in Experiment 2, the rats were sacrificed and brain homogenates were studied for uptake of ^{3}H-NA and ^{14}C-5-HT according to the modified double labelling of the Snyder and Coyle [47] technique [41]. Rat hippocampal regions were homogenized in 0.25 M sucrose (1:10 w/v) with an all-glass Potter-Elvehjem homogenizer. Homogenates were incubated at 37°C for 4 min in 11 μmol glucose, 0.1 μmol pargyline, 2.2 μmol ascorbic acid and 0.26 μmol ethylendinitrilotetraacetic acid disodium salt (EDTA) in 1.8 ml of Krebs-Henseleit buffer, pH 7.4. Immediately after incubation the tubes were chilled in an ice-bath and centrifuged at 20,000 g for 20 min at 0°C. The pellets and tubes were washed twice with 5 ml of ice-chilled 0.9% saline. The pellet was dissolved in 1.0 ml of Soluene-350 at room temperature; 10 ml of the scintillation solution (Permablend III, Packard) was added and the radioactivity was measured in two separate channels in a liquid scintillation spectrometer as previously described [43]. Endogenous amine concentrations of the cortex-hippocampus, cerebellum and limbic structures, from DSP4, DMI+DSP4 and control rats were analysed in a separate experiment 36 days after the treatments. Following decapitation the brain regions were rapidly dissected on ice and stored at −70°C until analysis. After homogenization and centrifugation the homogenates were purified on a strongly acidic cation-exchange column (Dowex SOW-X-4) (for details see [7]). After elution NA, DA and 5-HT were analysed by fluorimetry (Amino-Bowman spectrofluorometer).

Statistical analysis. Pairwise comparisons between groups were made with the Mann-Whitney U-test [45] in both experiments. The 5% level of significance was maintained throughout.

EXPERIMENT 1

PROCEDURE

Two groups of rats, DSP4 (n=12) and control (n=8), were injected with DSP4 (50 mg/kg, IP) and distilled water (5 ml/kg IP), respectively. The chronological details of procedure for Experiments 1 and 2 are presented in Table 1.

Eleven days after the injections (Day 12) all the rats were given a hole-board activity test (Test 1) whereby each rat was placed in the centre of a modified hole-board (100×100 cm) which was divided into 16 squares (25×25 cm) and contained 16 holes (diameter 3.5 cm) according to the design of File *et al.* [15,16]. Ambulation, i.e., the number of squares entered, rearing responses and head-dips, defined by the submergence of the rat's head into one of the holes, up to at least eye-level, as well as fecal boli, were all recorded. Immediately after Test 1, all the rats were placed on a deprivation schedule whereby they were maintained at 80% of their normal body weight. On Day 19 (i.e., 18 days after treatment) each rat was habituated to the T-maze apparatus for a 5-min period. The first acquisition session was initiated on Day 20 using the procedure outlined above (T-maze Learning), each rat being placed in the start box until it oriented toward the door which was then opened and the animal allowed to run to the right-hand goal box (see Fig. 1) to collect a food pellet. Latency to enter the goal box and the number of errors (see above) made on the way were recorded for each trial. Once the rat had entered the goal box the door was closed behind it and this signalled the end of that particular trial after which the goal box was rotated around so that is was placed again

TABLE 2

THE MEDIAN NUMBER OF TRIALS TO THE FIRST ERROR-FREE TRIAL AND THE MEDIAN NUMBER OF TRIALS TO A CRITERION OF FIVE CONSECUTIVE ERROR-FREE TRIALS BY THE DSP4 (n=12) AND CONTROL (n=8) GROUPS IN EXPERIMENT 1 AND THE DSP 4 (n=10), DMI + DSP4 (n=9) AND CONTROL (n=10) GROUPS IN EXPERIMENT

	No. of trials to first errorless trial	No. of trials to 5 consecutive correct trials
Experiment 1:		
DSP 4	1.5	34.5*
Q	±1.0	±4.0
Control	2.0	20.0
Q	±2.0	±8.0
Experiment 2:		
DSP 4	1.0	19.5
Q	±1.0	±5.25
DMI+DSP4	1.5	18.0
	±1.0	±4.0
Control	2.0	15.5
Q	±1.0	±5

Values are expressed as medians, ± quartiles (Q).
*Significantly different from control, two-tailed Mann-Whitney U-test, $p<0.02$.

in the position of start box and 15 sec later the next trial was started. Five trials were presented during each session (one per day) and between each session the T-maze apparatus was carefully cleaned. Ten such acquisition sessions were presented on Days 20 to 29 after which the animals were allowed an ad lib food regime. On Day 32, a second hole-board activity test (Test 2) was given identical to the first. Food intake was measured during two 24-hour food intake tests on Day 39 and 40. On Day 57, a third hole-board activity test (Test 3) was presented.

Sixteen rats were studied in a separate experiment. The effect of DSP4 (50 mg/kg) on food and water intake was measured pre-injection (Day 0) and for 7 days post-injection (Days 1–7); body weights were measured concomitantly. The control rats received distilled water. These data can be found in Table 4.

RESULTS AND DISCUSSION

The total number of errors per session and the mean latency to reach the goal box per trial was calculated for each rat for each acquisition session. Pairwise differences between the DSP4 and control groups for both the T-maze sessions and the activity tests were tested for with the Mann-Whitney U-test [45].

DSP4-treated rats were markedly retarded in the rate at which they acquired the right-turn running response. Figure 2 presents the median number of errors/session and the median average latency to enter goal box/trial during the acquisition session for DSP4 and control rats. Thus, the DSP4 rats made both significantly more errors, on Days 20, 21, 22, 23, 25 and 26 (acquisition sessions 1, 2, 3, 4, 6 and 7), and had significantly longer latencies to reach the goal box, on Days 20, 22, 23 and 25 (acquisition sessions 1, 3, 4 and 6), than the control rats (Mann-Whitney U-tests, $U\geq 17$, $p<0.05$). There was no difference between the DSP4 and control group with regard to the number of trials required to attain the first error-less trial but the DSP4 group required considerably more trials to reach a criterion of five consecutive error-less trials. Table 2 presents the median number of trials to the first error-free trial as well as the median number of trials to reach a criterion of five consecutive error-free trials. Mann-Whitney U-tests indicated no difference between the DSP4 and control groups with regard to trials to the first error-less trial ($U=44$, $p>0.1$) whereas there was a significant difference for the number of trials required for the criterion of five consecutive error-less trial to be reached ($U=10$, $p<0.02$). This result suggests that while DSP4-

TABLE 3

AMBULATION, REARING AND HEAD-DIPS FOLLOWING DSP4 TREATMENT

	Test 1		Test 2		Test 3	
	DSP4	Cont.	DSP4	Cont.	DSP4	Cont.
Ambulation	65.5	127.0	156.5	152.0	93.0	92.5
Rearing	8.0	17.5	28.5	19.0	12.5*	7.5
Head-dips	18.5	27.5	27.5	19.5	13.0	14.0
F. Boli.	2.0	2.0	0	0	0	0

Two groups of rats were administered either DSP4 (50 mg/kg IP) or distilled water (5 ml/kg IP) 11 days before the first activity test. Each rat was placed in the modified hole-board and ambulations, rearings and head-dips were scored during each 15-min test session. Tests 2 and 3 were carried out 32 and 57 days post-treatment, respectively. (See text for details).
Values are expressed as medians.
*Significantly differed from control, two-tailed Mann-Whitney U-test, $p<0.05$.

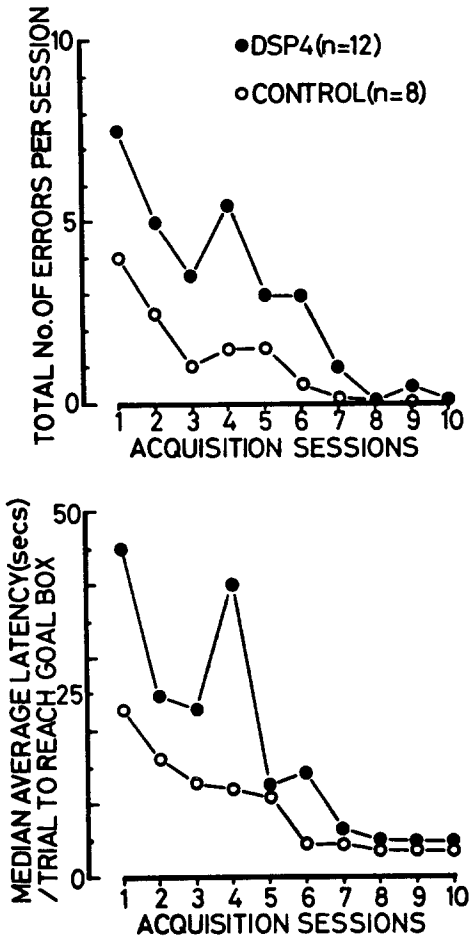

FIG. 2. The effect of DSP4 (50 mg/kg, IP) on the acquisition of a right-turn running response in a modified T-maze. Median total number of errors per session and median average latency (sec) per trial to reach the goal box by DSP4 and control rats over ten acquisition sessions. Each session consisted of five reinforced trials.

treated apparently can make an error-free trial at least as easily as control rats they fail to utilize this information as effectively as the control rats.

Table 3 presents ambulation, rearing, head-dips and fecal boli data for DSP4 and control groups during the activity and exploration tests (Tests 1–3 on Days 12, 32 and 57, respectively). Although the DSP4-treated rats did evidence fewer ambulation, rearing and head-dip counts during Test 1 than the controls, this difference was not significant. By Test 2 it is evident that the DSP4 group was if anything slightly more active although the difference in this direction only reached significance for the number of rearings on the third and final test (see Table 3). No differences in the median number of fecal boli by DSP4-treated and control rats was obtained. The findings of the T-maze acquisition learning study, when taken in conjunction with the three activity tests suggests a clear retardation in the ability DSP4-treated rats to acquire the right-turn running response for food reward. It is unlikely that this effect could be the result of motor incapacity, in spite of the slight activity discrepancy on Test 1, since Test 2 occurred shortly after the acquisition phase. In addition, an activity deficit may possibly explain the longer latency to reach the goal box but hardly the increased number of errors. However, in order to test the possibility that some motor defect may have interfered with the DSP4 rat's ability to perform the running response a Spearman rank correlation [46] comparing ambulation counts from Test 1 and average latencies to reach the goal box from Day 20 (first acquisition session) for each rat in the DSP4 group was carried out. The rank order correlation between ambulation counts and running response latencies was 0.244 and this was not significant, $t=(10)\ 0.796$, $p \geq 0.4$. Thus, DSP4 rats that were poor ambulators were not necessarily slow performers on the T-maze task, and vice versa.

The two food intake tests were performed in order to control for possible motivational changes as a result of the treatment. No differences between DSP4 and control rats were evidenced during the two food intake tests on Days 39 and 40. Table 4 presents food intake data for Experiment 1 and a separate experiment in which food and water intake, as well as body weight, were monitored following a single DSP4 injection.

EXPERIMENT 2

Recent findings (Spyraki and Fibiger, [48]) indicated no effects of DSP4 treatment on either Y- or L-maze learning. The purpose of Experiment 2 was to replicate the procedure of Experiment 1 and also to investigate whether prior DMI, a selective NA uptake inhibitor treatment would provide an effective protection against the behavioural and NA depleting effects of DSP4.

PROCEDURE

Thirty-two male Sprague-Dawley rats were randomly assigned to three groups: DSP4 (n=12), DMI+DSP4 (n=10) and control (n=10) that were injected DSP4 (50 mg/kg, IP), DMI (20 mg/kg, IP 30 min prior to DSP4 50 mg/kg IP) and distilled water (5 ml/kg), respectively, on a single occasion. One rat in the DSP4 group and one if the DMI+DSP4 died while another rat in the DSP4 group showed an incomplete recovery from the gastrointestinal distress. Thus, for experimental purposes n=10 for the DSP4 group and n=9 for the DMI+DSP4 group.

T-Maze Learning

Three days after injections the rats were placed on the deprivation schedule by which their body weights were reduced by 20%. The same general procedure as for the previous T-maze experiments was followed with certain exceptions. On Day 14, 13 days after DSP4, DMI+DSP4 and distilled water injections, each rat was given the 5-min habituation to the T-maze start-box, as described above. The first acquisition session was initiated on the next day and the same procedure as in Experiment 1 was maintained. Each rat was placed in the start box and as soon as it had oriented towards the maze the door was opened and the progress of the rat to the right-hand goal-box was observed. Five daily trials were presented on each of five consecutive acquisition sessions. Experiment 2 differed from the previous experiment with respect to the number of acquisition sessions (5 and not 10); this was so because the groups studied reached an asymptotic level of performance at an early stage. One month after the final acquisition session, the rats were decapitated and ^{3}H-NA and ^{14}C-5-HT uptake in the hippocampus was measured.

TABLE 4

FOOD INTAKE BY DSP4 AND CONTROL RATS ON DAYS 39 AND 40, AFTER THE ACQUISITION SESSIONS IN EXPERIMENT 1; FOOD INTAKE RECOVERY IN THE SEPARATE EXPERIMENTS

Experiment 1: Food intake (Median g/24 hours)

	Day: 39	40
DSP4	29.0	25.0
Control	30.5	27.0

Separate Experiment Day:		0	1	2	3	4	5	6	7
					Food intake, g/24 hr				
DSP4	mean	24.5	11.9*	13.5*	19.1	25.1	24.1	24.75	22.5
	±S.E.M.	0.65	3.97	3.74	1.72	1.16	1.78	0.77	0.50
Control		25.75	25.75	25.6	22.0	29.0	26.75	26.1	22.25
		0.59	0.49	0.84	0.90	0.78	0.75	0.66	0.77
					Water intake, ml/24 hr				
DSP4		30.75	15.1*	21.0*	28.1	30.25	30.6	33.0	33.1
		1.11	4.67	3.94	1.49	1.13	1.52	1.90	1.47
Control		31.1	32.9	32.0	29.9	34.0	35.4	33.6	35.5
		1.87	1.76	1.18	1.16	1.45	1.34	1.28	1.19
					Mean body weight, g				
DSP4		242	237	233*	239*	255*	261*	263*	269
		1.6	4.2	6.5	6.5	5.8	5.9	5.1	6.5
Control		242.5	255	256	258	275	281	282.5	284
		2.5	2.8	2.5	3.0	3.1	3.1	3.3	3.2

The rats were on an ad lib food and water regime during this period. Food and water intake recovery and body weight measurements for DSP4 (50 mg/kg IP) and control rats in a separate experiment (see text for details).

*Two-tailed t-test, $t(112) \geq 2.78$, $p \leq 0.01$.

Split-plot ANOVA [22] indicated significant Groups × Days interaction $F(7,98)=8.08$, 7.52, and 7.08, $p \leq 0.01$, for food intake, water intake and body weight, respectively.

Food Intake Test

All the rats in the DSP4, DMI+DSP4 and control groups were given a food intake test, 2–3 hours after the habituation to the T-maze, on Day 14, by which the time taken by each rat to consume one pellet (4.0 g) was recorded.

RESULTS AND DISCUSSION

The DSP4-treated rats showed a retarded acquisition of the T-maze task as measured by both the number of errors and the average latency to reach goal box per session; this result confirms that of Experiment 1.

Experiment 2 also indicated that the T-maze learning retardation was completely blocked by the prior administration of DMI (20 mg/kg), a result that implies the selective involvement of NA. Figure 3 presents the total number of errors per session and the median average latency to reach the goal box by the DSP4, DMI+DSP4 and control groups during the five acquisition sessions. Mann-Whitney U-tests indicated significant DSP4 vs. control and DSP4 vs.

TABLE 5

ACCUMULATION OF ^{3}H-NA (5×10^{-8} M) AND ^{14}C-5-HT (5×10^{-8} M) IN HOMOGENATES OF HIPPOCAMPUS FROM DSP4 AND DMI+DSP4 TREATED RATS FROM EXPERIMENT 2

		Amine accumulation			
		^{3}H-NA		^{14}C-5-HT	
Experiment 3:	n	pmol/g/5 min	% of control	pmol/g/5 min	% of control
Control	10	192 ± 24		359.0 ± 23	
DSP4	10	14.5 ± 7*	7	303.5 ± 32	84
DMI+DSP4	9	175.0 ± 23	91	384.5 ± 58	107

Values are expressed as medians ± quartiles.

*Significantly different from control and DMI+DSP4 groups, Mann-Whitney U-test $p \leq 0.001$.

The control-DSP4 difference for ^{14}C-5-HT was not significant ($U=24$, $p \geq 0.2$).

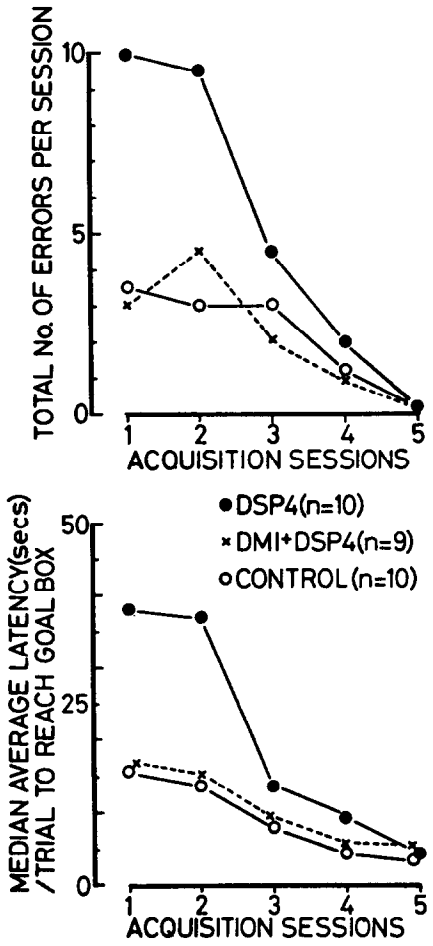

FIG. 3. The acquisition of T-maze learning by DSP4, DMI+DSP4 and control groups in the second experiment. Median total number of errors per session and median average latency (sec) per trial to reach the goal box by DSP4, DMI+DSP4 and control rats over five acquisition sessions.

TABLE 6

THE AMINE CONTENT OF BRAIN REGIONS OF RATS TREATED IN A SEPARATE EXPERIMENT

	DSP4	DMI+DSP4 (ng/g)	Control
		Cerebellum	
NA	8.0*§	25.5†	47.5
n	8	8	8
%	(17)	(60)	
5-HT	46.5	44.0	59.0
n	8	8	8
%	(79)	(75)	
		Cortex-hippocampus	
NA	21.0*‡	110.5*	181.0
n	6	7	8
%	(11)	(61)	
5-HT	196.0	166.5†	223.0
n	8	8	7
%	(88)	(74)	
		Olfactory tubercles + Nucleus Accumbens	
NA	223.0*§	279.0†	381.0
n	6	7	7
%	(59)	(73)	
DA	1438.0†	1375.0	1278.0
n	8	8	8
%	(113)	(108)	
5-HT	359.0	348.0†	415.5
n	8	8	8
%	(87)	(84)	

Mann-Whitney U-tests: *significantly different from controls, $p \leq 0.001$; †$p \leq 0.01$; ‡significantly different from DMI+DSP 4 group, $p \leq 0.001$; §$p \leq 0.01$.

The indicated values are median NA, 5-HT and DA concentrations expressed as nanograms amine per g of tissue. DSP4 (50 mg/kg) was injected IP, DMI (20 mg/kg) was injected 30 min prior to the DSP4 injection. The rats in this experiment were decapitated 36 days after treatment. Percent values of control are expressed in parentheses.

DMI+DSP4 differences for the error data during sessions 1 and 2. For the latency data the DSP4-control difference was significant during sessions 1–4 while the DSP4-DMI+DSP4 difference was significant during sessions 1–3. The median total number of errors throughout the acquisition phase was computed and were as follows: DSP4=27; DMI+DSP4=13; and control=12 errors. The DSP4 vs. DMI+DSP4, control differences were significant. The median average latencies to reach goal box over all sessions was also computed: DSP4=19.3; DMI+DSP4=13.5; and control=9.8 sec. The DSP4 vs. DMI+DSP4, control differences were also significant. There were no differences between the DSP4, DMI+DSP4 and control groups for the number of trials required to reach the first error-free trial and for the number of trials required to attain the criterion of five consecutive error-free trials (see Table 2). Kruskal Wallis ANOVAs were not significant (H=0.3, NDF=2, $p<0.8$, and H=3.2, NDF=2, $p<0.1$, respectively). This result confirms that of Experiment 1 for the number of trials to reach the first errorless trial but is not the result obtained in Experiment 1 as regards the criterion data.

Food Intake Test

There were no differences in the median latencies of DSP4, DMI+DSP4 and control groups to consume one pellet weighing 4.0 g on Day 14. Thus, the median latencies were: DSP4=7.2; DMI+DSP4=7.5; and control=7.6 min.

Biochemical Analyses

The rats in Experiment 2 were sacrificed one month after the acquisition sessions and NA and 5-HT uptake measured. DSP4 (50 mg/kg) caused a profound reduction in the accumulation of ^{3}H-NA in the hippocampus (7% of control values) but had only a slight affect on ^{14}C-5-HT accumulation (84%). DMI+DSP4 provided near total protection of ^{3}H-NA uptake (91%), as shown in Table 5. Thus, the uptake data provide reliable evidence for the selective involvement of NA depletion, i.e., a correlation with T-maze acquisitive performance in Experiment 2.

In a separate experiment rats were sacrificed 36 days after DSP4 (50 mg/kg, IP), DMI (20 mg/kg, IP) 30 min prior to DSP4 (50 mg/kg), and distilled water (controls). Table 6

presents the amine content of the cerebellum, cortex-hippocampus and limbic structures. It will be noticed that DSP4 caused marked NA, but not DA, depletions in the cortex-hippocampus and cerebellum and a lesser NA depletion in the olfactory tubercles + nucleus accumbens region. There was also some slight 5-HT depletion in all three regions; this was not blocked at all with prior DMI treatment. It will be noticed that protection of NA depletion afforded by DMI (20 mg/kg) was only partial in all three regions and this also confirms our earlier findings [4,37].

GENERAL DISCUSSION

The long-term effect of DSP4 (50 mg/kg) upon spontaneous activity, and the acquisition of a right-turn running response was studied in the above experiments. It was found that DSP4 caused several effects upon rats' behaviour including: (1) a small, transient (though non-significant) decrease in spontaneous activity which had been overcome three weeks after the DSP4 treatment, (2) a significant retardation of the rate of acquisition of the T-maze right-turn running response, as measured both by the total number of errors per session and by the average latency to reach the goal box; (3) the prior administration of DMI (20 mg/kg) 30 min before DSP4 was found to antagonize the retardation of the acquisition of the T-maze task; (4) the maze learning deficit as a result of DSP4 may be correlated with 75–90% NA depletions in the cortex, hippocampus and cerebellum; DMI antagonized these depletions to varying degrees. The T-maze learning retardation is not readily explained on the basis of alterations in motivation for at least three reasons. First, there was a recovery of food and water intake by the 7th day after DSP4 injection and a lack of any difference in the food intake tests (see Table 3). Second, no differences between the DSP4, DMI+DSP4 and control groups for latency to consume one pellet were evidenced (Experiment 2). Third, NA-depletions are not generally accompanied by any lasting decrease in food intake [1].

The DSP4-treated rats made much fewer ambulation, rearing and head-dip responses than the control animals during the first 15-min test session in the modified hole-board; due to the very large within-group variations, however, this effect did not reach significance. Despite the non-significance of this result, we have in other contexts (e.g., macron activity boxes, Archer *et al.* in press) found that DSP4 rats were temporarily less active than control animals. The transient activity deficit of the DSP4 rats was significant in one test but not in the other tests. Thus, it was of considerable importance to ascertain whether or not the debilitating effects of DSP4 on activity could have accounted for the subsequently longer latencies by these rats (Experiment 1) to reach the goal box. However, this suggestion was rendered unlikely as a result of the very low correlation between the number of ambulation counts during the first test and the average latency during the first acquisition session ($r=0.244$); note that this correlation was low in spite of the very high within-group (DSP4) variation (median ambulation=65.6, quartile=46.5; median latency=45.3, quartile=11.3). The second and third hole-board activity tests demonstrated that the DSP4 rats were at least as active as controls. The general tendency of these activity data underline the transient nature of the probable reduction in general activity after DSP4 administration.

Taken together, the results of the T-maze experiments offer evidence that DSP4 administration at least two weeks prior to the onset of the acquisition sessions impeded acquisition of the right-turn running response, and to some extent may confirm the 6-OHDA DB induced impairment in the spatial alteration T-maze task [26]. This conclusion is underlined by the significant effects obtained both with the total number of errors per session and with the average latency to enter goal box measures. Prior administration of DMI (20 mg/kg), 30 min before DSP4 (50 mg/kg), blocked the increase in the number of errors and latency to reach goal box as a result of DSP4 in Experiment 2. The ^{3}H-NA accumulation data (see Table 5) indicate an almost complete protection by DMI (91%) but note also that endogenous NA concentrations in the cerebellum and cortex-hippocampus regions following the single DMI+DSP4 treatment (see Table 6) were only 60 and 61 percent, respectively, of the control values; there were however, marked differences between the DMI+DSP4 and DSP4 for both the cerebellum (60 and 17 percent, respectively) and the cortex-hippocampus (61 and 11, respectively). The net result of DSP4 may resemble that of the electrolytic locus coeruleus lesion which has often resulted in marked acquisition deficit [3,44] although a marked impairment has been obtained with 6-OHDA DB lesions [26]. The pattern of NA depletion in different brain regions seems to vary widely. Thus, the 6-OHDA DB [30] lesion causes notable decreases in NA concentrations of the hypothalamus (70±10% decrease) and limbic system (85–55%) while the effect of DSP4 upon those regions is much lesser (hypothalamus: 30±15%; limbic system: 41%). Systemic DSP4 and DB 6-OHDA interventions resemble each other with regard to the near total NA depletion in the cortex and hippocampus. The latter has no effect, however, upon NA concentrations in the cerebellum and spinal cord [30], whereas DSP4 treatment invariably causes 70–90% reductions in those regions [18,20] which agrees well with the investigations on projections from the LC [35,36]. Another possible discrepancy may be the problem of sprouting in NA neurons [14,23]. Thus, systemic DSP4 seems to offer a NA lesion that differs in extent from the DB lesion and in forebrain magnitude from the bilateral electrolytic lesion (with the added advantage of lack of nonspecific damage); the data from the experiments presented here generally seem to correlate well with this comparison. Neither the food intake data (Table 4) nor previous investigations [1] offer any evidence that destruction of the NA-LC system caused any lasting motivational alterations.

In spite of the plausibility of the present findings for the theoretical positions of Crow [10,12] and Kety [21], a note of caution should be emphasised. The results of the runway studies by Anlezark *et al.* [3] and Sessions *et al.* [44] were found not to be accompanied by any general learning deficit. In the Amaral and Foss [2] study, the longer running speeds of LC lesioned rats in a straight runway were possibly occasioned by the low general activity of those rats; nor was the Mason and Fibiger [26] finding of an impaired T-maze spatial alternation acquisition following 6-OHDA DB lesions interpreted in terms of any general learning deficit. DSP4 rats, too, seem only to be impaired or retarded on particular tasks, generally ones involving a potentially stressful experience and/or a choice situation. Thus, it may not be a coincidence that DSP4 rats are found to perform notably worse in two-way active avoidance and T-maze acquisition, slightly worse than controls on one-way active avoidance and as well as controls in taste-aversion learning, passive avoidance and the straight forward escape-from-water-maze task. Much observational data suggest that the DSP4 rats' reaction to the

handling, injection and temperature measurement procedures is more pronounced than the control rats. It is possible therefore that the impaired acquisition of DSP4-treated rats in two-way avoidance and T-maze tasks is a consequence not only of NA-depletion, but mediated partly by a concomitant alteration of the pituitary-adrenal axis regulating the balance of stress hormones.

In conclusion, the experiments described above studied the functional role of NA in behaviour following systemic injections of DSP4. DSP4 treatment resulted in a retardation of the acquisition of a right-turn running response in the T-maze. This may be relevant to the postulated role of the LC-system in memory and attention [26, 27, 32, 33, 49, 50]. Recent evidence from our laboratory [6] renders unlikely the possibility that peripheral NA depletions could have caused the retardation of T-maze acquisition. Thus, two groups were injected, systemically, with either DSP4 (50 mg/kg, once) or 6-OHDA (30 mg/kg, twice) 7 and 7 and 5 days, respectively before the avoidance test, in which the DSP4, but not the 6-OHDA, group was found to be impaired. NA uptake in the left heart atrium was measured 14 days after treatment for the DSP4 group and 21 days after in the case of the 6-OHDA group. It was found that while NA accumulation by the heart tissue of the DSP4 rats was similar to the controls 14 days after treatment, that of the 6-OHDA rats was only 60% of control values, 21 days after treatment. Thus, the acquisition deficit could hardly have been the result of any peripheral NA-depletion. Additionally, T-maze acquisition was initiated at least 14 days after DSP4 administration in the present study. The present data add to the existing evidence, e.g., from the taste and environment neophobia [4, 25, 31] and active avoidance procedures [6,37], indicating that DSP4 exerts an influence upon central NA pathways that may be at odds, functionally, with that exerted by the DB lesion.

ACKNOWLEDGEMENTS

The excellent technical assistance of Göran Johansson, who also drew the figures, is very much appreciated. The present research would not have been accomplished without the encouragement and support of Per Olov Svärd. Valuable criticism was offered by Christer Köhler.

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