Pharmacology Biochemistry & Behavior, Vol. 16, pp. 197–202, 1982. Printed in the U.S.A.

The Effect of DSP-4 on Some Positively Reinforced Operant Behaviors in the Rat

CHRISTINA SPYRAKI, GORDON W. ARBUTHNOTT AND H. C. FIBIGER[1]

Division of Neurological Sciences, Department of Psychiatry
University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Received 10 August 1981

SPYRAKI, C., G. W. ARBUTHNOTT AND H. C. FIBIGER. *The effect of DSP-4 on some positively reinforced operant behaviors in the rat.* PHARMAC. BIOCHEM. BEHAV. **16**(2) 197–202, 1982.—The effects of a new neurotoxin for noradrenergic (NA) neurons, DSP-4, on the acquisition, retention and extinction of positively reinforced operant responses were studied in rats. The acquisition of an L-shaped runway task for food reward, of a simultaneous light-dark discrimination in a Y-maze task and of a lever press response for food was not affected in DSP-4 treated animals. No deficit in retention behavior was found in the Y-maze task after DSP-4 treatment. The DSP-4 treated animals showed increased resistance to extinction in the L-shaped runway. However, the extinction of the lever press response for food (CRF) was unaffected in the same animals. In accordance with previous results, DSP-4 resulted in a widespread depletion of NA in cortex, hippocampus, cerebellum and spinal cord. One week after the DSP-4 administration, histochemical studies at the light microscopic level indicated that the cell bodies of the locus coeruleus remained intact in DSP-4 treated animals. Desipramine pretreatment provided only partial protection from the neurotoxic effects of DSP-4. The results are discussed in relation to the mechanism by which DSP-4 produces the long-term depletion of central NA and with reference to the role of central NA neurons in behavior.

DSP-4 Learning Noradrenaline

THE role of the neurotransmitter noradrenaline (NA) in learning has been the focus of considerable speculation in recent years. On the basis of theoretical considerations, both Crow [3] and Kety [7] have independently hypothesized that NA-containing neurons originating in the nucleus locus coeruleus (LC) and innervating the cortex [9] could be crucially involved in the learning of positively reinforced tasks. Although the subsequent findings of Anlezark *et al.* [2] provided evidence for this postulate, it has not been supported by a considerable body of subsequent research [1, 18, 21]. It is now generally agreed that rats depleted of NA do not suffer from a general learning impairment since they are not deficient in acquiring a wide variety of appetitive and aversive tasks.

The model of the bilateral lesions of the dorsal NA bundle (DBL) used extensively by Mason and colleagues (for review see [14]) in a variety of behavioral situations has demonstrated that rats with large depletions of forebrain NA show an increased resistance to extinction of acquired responses. Although the effects of DB lesions on behavior are typically attributed to the loss of forebrain NA, this assumption may not always be justified. It is known, for example, the DB lesions cause NA levels in the cerebellum and spinal cord to increase and the extent to which these changes may contribute to alterations in behavior has been generally ignored [11]. The present experiments sought to examine this question using a novel neurotoxin for NA neurons, DSP-4 [4, 5, 19, 20].

DSP-4 crosses the blood-brain barrier and a single systemic injection to adult rats causes a marked and prolonged reduction of NA levels in all regions of the central nervous system that are innervated by the locus coeruleus [4, 5, 19]. Thus, the DSP-4 treated animals offer a lesion model similar to that of the bilateral electrolytic lesions of the LC with the advantage of no known non-specific damage. The present experiments were designed to evaluate the performance of DSP-4 treated rats in several behavioral paradigms. Initially rats were tested for exploratory activity that might interfere with performance in learning tasks. Subsequently the acquisition and retention of a running response for food reward and the acquisition and retention of a simultaneous light-dark discrimination in a Y-maze were evaluated. Finally the acquisition and extinction of a bar-press operant response for food reinforcement were studied.

METHOD

Treatment

Male Wistar rats weighing approximately 200 g received two injections (IP) of DSP-4 (50 mg/kg). One week elapsed between the two injections. Control rats were treated with an equal volume of distilled water. Another group was injected with desipramine HCl (DMI, 20 mg/kg, IP) 20 min prior to both DSP-4 injections.

The treatment with DSP-4 alters transiently sympathetic neurons in the periphery and produces a severe and long-

[1]Send reprint requests to Dr. H. C. Fibiger, The University of British Columbia, Faculty of Medicine, Kinsmen Laboratory of Neurological Research, 2255 Wesbrook Mall, Vancouver, B.C., Canada V6T 1W5.

lasting depletion of the central NA [4]. DMI pretreatment antagonizes the neurotoxic effect of DSP-4 presumably by blocking uptake into NA neurons [19]. In this respect the animals pretreated with DMI might represent ideal controls to test for the NA specific effects of DSP-4.

Behavioral Testing

For the behavioral tests three groups were studied (DSP-4; DMI + DSP-4; vehicle), each group containing eight animals. The animals were kept in standard laboratory cages in a temperature-controlled room on a 12 hr light-dark cycle. Water was available ad lib. During the treatment and the first behavioral test (exploration) they were housed five per cage with free access to standard laboratory rat chow (Purina). Subsequently, they were housed singly and given access to food for 2 hr per day. Behavioral experiments were always conducted during the light phase of the light-dark cycle.

Exploratory Behavior

This test was carried out 10–12 days after the second DSP-4 or vehicle injection. The exploratory activity was measured by placing each animal in a Hebb-William maze apparatus [23] with squares, alleyways, start and goal boxes. The animal was placed in the start box of the maze and 3 sec later the door leading to the maze was raised. The number of squares and alleyways entered in successive 3-min periods for a total of 21 min was measured.

Acquisition and Extinction of a Food Reinforced Running Response in an L-shaped Maze

An L-shaped runway was used. It was constructed of wood, with dimensions as follows: height, 15 cm; width, 11 cm; runway, 140 cm. Two photocells, 120 cm apart, located immediately beyond the start box and immediately before the goal box, were utilized to measure running time over the initial long arm of the maze. Training commenced on the fifth day after the food deprivation schedule was initiated (3 weeks after the last DSP-4 injection). On the first experimental day each rat was allowed to explore the runway for thirty min without food present. The day after, each subject was placed in the start box and the guillotine-style door was raised 3 sec later. The running time in the stem was measured using electronic timers triggered by the photocell beam interruptions. On entering the goal box the rat had access to a cup containing five food pellets (45 mg, P. J. Noyes). A manually lowered door constrained the animal to stay in the goal box. The animal was removed after it had consumed the food (2–4 sec). At the end of each trial the subject spent 15 sec in an inter-trial cage. After this time it was returned to the start box for the next trial. Each subject received five trials a day over a period of ten days. If the rat had not reached the goal box within five min the trial was ended.

Extinction was begun on day 11 and the procedure was identical except that no food was present in the goal box. Extinction trials continued until the subject's running time was $\geq$10 times the time on the first extinction trial. After each session the animals were returned to their home cages where food was available for two hours.

Simultaneous Light-Dark Discrimination in a Y-maze

A conventional, grey-painted wooden Y-maze measuring 10 cm wide and 24 cm high, each arm being 65 cm in length, was used. A 40 W light bulb located 30 cm above the midpoint of each arm illuminated the choice area. Training commenced 5 weeks after the last DSP-4 injection. On the first two pretraining days, animals were placed in the apparatus in groups of three for 20 min. The doors of the apparatus remained open and the goal boxes contained food cups with food pellets (Noyes, 45 mg). On the third pretraining day each animal was forced (by barring one arm) to enter a preselected arm and allowed to eat food in the goal box. On this day each rat was then given as many trials as necessary to complete as many trials on the left as on the right side and to reach the goal box in 30 sec for 3 consecutive trials. On the fourth day, the light-dark discrimination training started. The animals were trained on this task with spatial position (left or right arm) irrelevant. The position of the illuminated arm varied on a random basis from trial to trial. Each animal was run ten trials per day with the intertrial interval of 15 sec spent in a waiting box. Half of the animals in each group were trained with food reward in the illuminated arm and the other half were trained with the dark arm rewarded. Each rat was trained to an acquisition criterion of 9/10 correct choices. The percent correct responses per day and the days to reach the criterion were measured. Twelve days were allowed to elapse after the last acquisition day, then the animals were tested for retention in the same paradigm. The number of correct choices, over ten trials, was used as the retention score.

Operant Responding-Lever Pressing (CRF)

Operant testing started 8 weeks after the last DSP-4 injection and took place in standard operant chambers with the behavioral contingencies controlled and the data recorded by an on-line computer. The chambers contained two levers. The left hand side lever was only used to start the session and simultaneously illuminate the house light. The animals were placed on a continuously reinforced (CRF) schedule for 9 consecutive days. Each daily session was conducted with the house light illuminated and lasted 20 min. Lever responses were recorded in 60 sec bins and then cumulated to give the total number of responses over a period of 20 min. At the end of acquisition training the animals were tested in extinction in which a lever press did not deliver a food pellet but the noise of the hopper was still present. Extinction testing continued for two consecutive days. The number of lever presses and the time to reach a criterion consisting of two consecutive minutes without responses being emitted were recorded.

Biochemical Assay

At the completion of behavioral testing half of the animals in each group were sacrificed by cervical fracture. Cortex, hippocampus, cerebellum and spinal cord were dissected on ice. These regions were homogenized in 0.1 N perchloric acid and assayed for endogenous NA content [16].

Histochemistry

Histochemical staining for acetylcholinesterase (AChE) was performed according to Karnovsky and Roots [6] on brain sections at the level of locus coeruleus, in order to examine the effects of DSP-4 treatment on NA perikarya. One week after the second DSP-4 or vehicle injections, the animals were injected IM with di-isopropylphosphorofluoridate (DFP, 2 mg/kg Sigma, dissolved in peanut oil) and IP

TABLE 1

NORADRENALINE VALUES THREE MONTHS FOLLOWING DSP-4 TREATMENT

	Control n=4	DSP-4 n=4	% Control	DMI+DSP-4 n=4	% Control
Hippocampus	410 ± 60	199 ± 60	48.5*	297 ± 30	72.4
Cortex	352 ± 20	126 ± 30	35.8*	173 ± 20	49.0*
Cerebellum	221 ± 70	70 ± 5	21.5‡	111 ± 10	50.2‡
Spinal Cord	208 ± 10	79 ± 10	37.9‡	155 ± 10	74.5†

Values are means (±SE) in ng/g wet weight of tissue.
*Significant at 5% from controls.
†Significant at 1% from controls.
‡significant at 0.1% from controls

with atropine sulphate (5 mg/kg). Five hours following the injection the animals were anesthetized with sodium pentobarbital (50 mg/kg) and perfused intracardially with saline solution followed by 10% Formalin containing 1% $CaCl_2$. The brains were fixed overnight in the same fixative and then kept in 0.88 M sucrose-1% gum arabic. The brainstem was frozen and sectioned at 30 μ and every fourth section through the level of the locus coeruleus was stained for AChE.

RESULTS

Biochemical

The results of post mortem NA assays are shown in Table 1. In accordance with previous results [4,17], DSP-4 produced extensive and widespread reductions in all of the areas that were measured, ranging from approximately 80 percent in the cerebellum to 50 percent in the hippocampus. Pretreatment with the NA uptake inhibitor DMI provided only subtotal protection from the depleting effects of DSP-4 in the different brain regions.

Histochemical

AChE histochemistry demonstrated that NA perikarya in the locus coeruleus remained present one week after the last of two DSP-4 injections (Fig. 1). However, from the limited material that was available it appeared that the NA perikarya were somewhat swollen in the DSP-4 treated animals. To the extent that this may represent early stages of retrograde degeneration after destruction of the NA axons by DSP-4, it will be important to conduct similar histochemical studies at longer intervals after the DSP-4 administration. Such studies are in progress. In any case, the present results render it

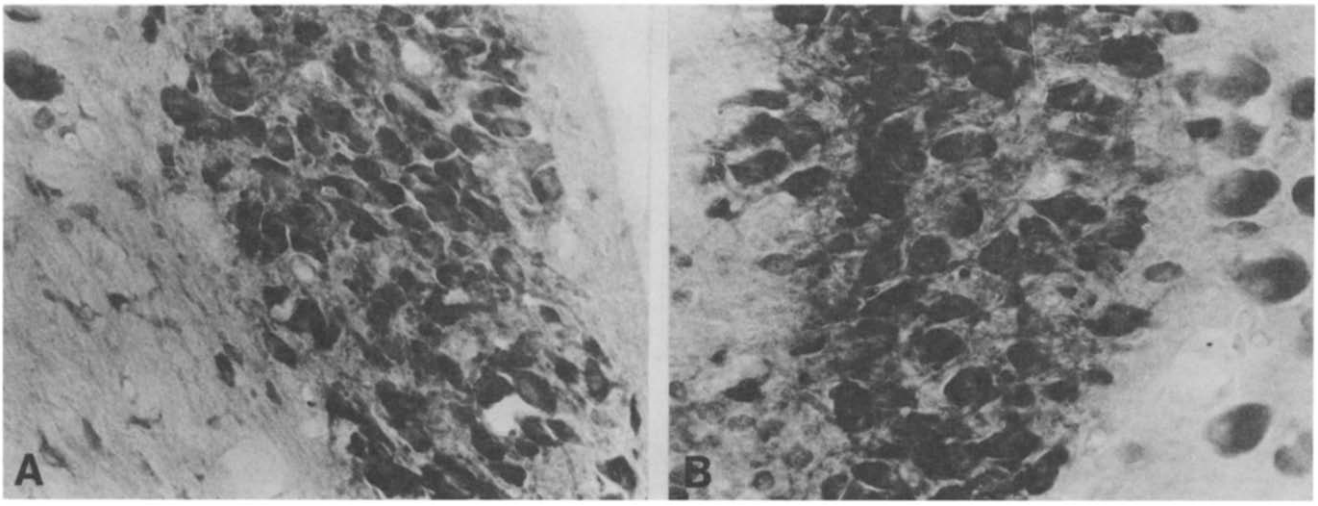

FIG. 1. Sections through the locus coeruleus of a control (A) and a DSP-4 (B) treated animal. DSP-4 (50 mg/kg) was administered twice, one and two weeks prior to sacrifice. Both animals were treated with DFP 5 hours prior to sacrifice and subsequent processing for AChE histochemistry (see text for details). Note the apparent swelling of the perikarya in the locus coeruleus of the DSP-4 treated animal. Bar equals 100 microns.

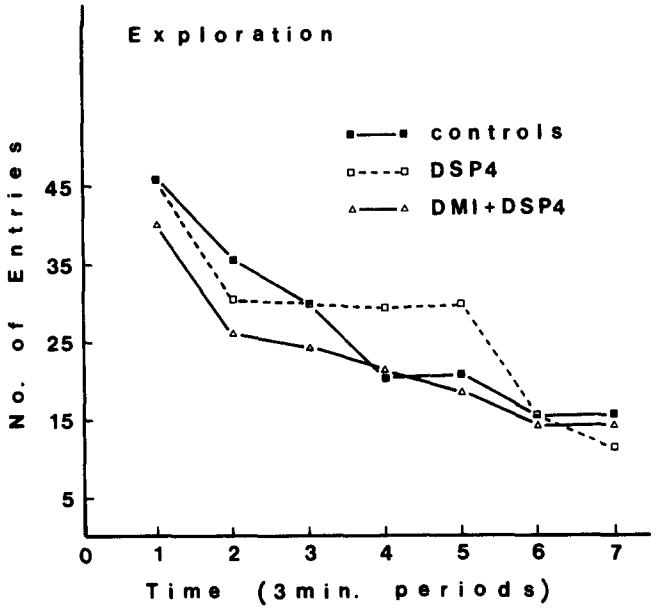

FIG. 2. Mean number of entries (alleyways+squares) per 3-min period for a total of 21 min in a Hebb-William apparatus. (n=7/group).

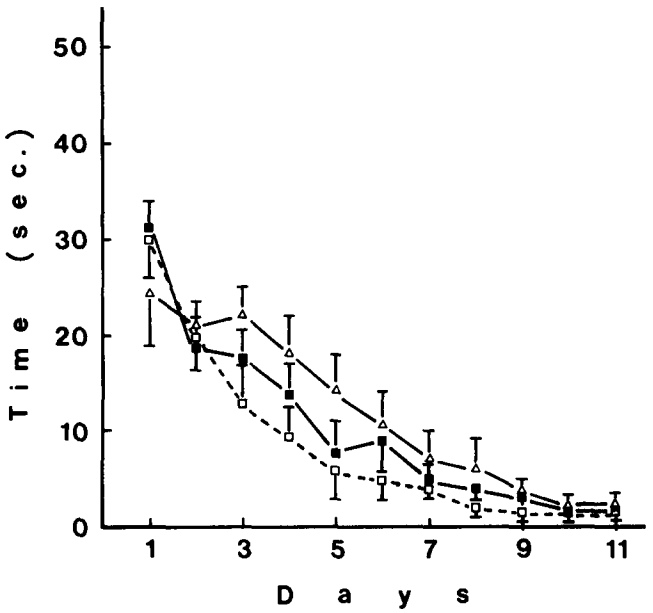

FIG. 3. Acquisition of a running response for food reinforcement in an L-shaped maze. Each rat received 5 trials per day. Data represent running times (means ± S.E.M.) of 8 animals per group. ■——■ Controls, □- - -□ DSP-4, △——△ DSP-4 + DMI.

unlikely that DSP-4 reduces central NA levels by a direct neurotoxic action on the perikarya of the locus coeruleus. Had this been the case, more pronounced effects on the NA perikarya would have been expected.

Behavioral

Exploration. The number of entries per 3-min period is shown in Fig. 2. Exploration decreased across time, $F(2,6)=13.86$, $p<0.01$. Repeated measures analysis of variance revealed no difference between groups and no significant interaction effect ($F<1$). The results indicate that depletion of NA in forebrain, cerebellum and spinal cord does not lead to a deficit in exploration.

Runway-acquisition and extinction. Figure 3 shows the mean running time over days. The data of the three groups were analyzed with a repeated measures analysis of variance design. The statistical analysis revealed a significant days effect, $F(10,20)=48.66$, $p<0.01$, but failed to show a significant difference between groups ($F=1.16$) or a significant interaction effect ($F=1.03$). Single comparisons between the DSP-4 treated group against control groups (vehicle treated and DMI + DSP-4 treated) also failed to reach statistical significance.

On the extinction test the DSP-4 treated rats showed slower running and increased number of trials to reach the extinction criterion (Table 2). One way analysis of variance revealed a significant difference between groups, $F(2,21)=3.67$, $p<0.05$. Post-hoc comparisons indicated that this difference was between DSP-4 animals and controls (vehicle treated: $t=2.15$ $p<0.05$ or DMI pretreated: $t=2.58$, $p<0.05$). No difference was found between the two control ($t=1.32$, n.s.) groups.

Simultaneous light-dark discrimination in Y-maze. The performance of animals in the simultaneous light-dark discrimination task in a Y-maze is shown in Fig. 4. It can be seen that the DSP-4 treated animals did not differ from their controls. This was confirmed statistically by repeated measures analysis of variance. The effect between groups ($F=0.01$) or the group × days interaction ($F=0.56$) was not significant. Learning of the discrimination across days did occur however, $F(10,180)=26.48$, $p<0.001$. Also, no difference was observed between groups with respect to days to criterion ($F=0.08$). The results failed to demonstrate any alteration in the learning of a Y-maze discrimination task for food reward. The test for retention (Table 3) carried out 12 days after the last session of acquisition failed to show a significant difference between groups ($F=0.74$).

TABLE 2

L-MAZE EXTINCTION: TRIALS TO CRITERION

Controls	DSP-4	DMI+DSP-4
13 ± 1.39	18 ± 1.9*	12.5 ± 1.5

Values are means with standard errors of the means for 8 animals per group.

*$p<0.05$ with respect to both controls and DMI+DSP-4.

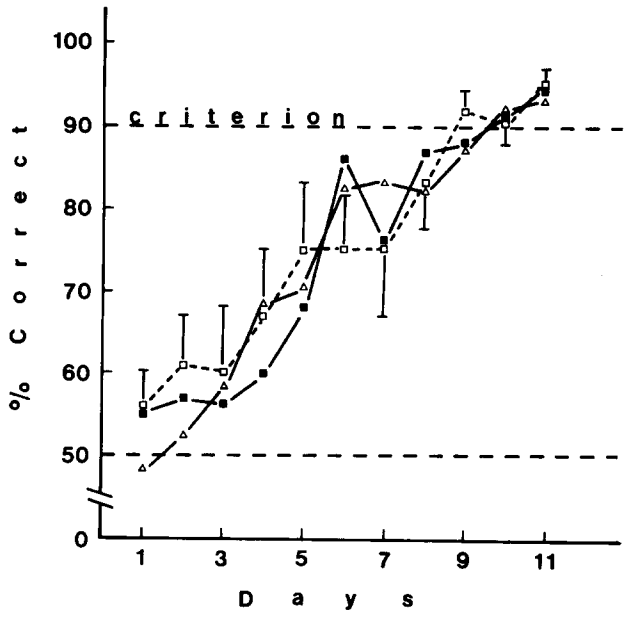

FIG. 4. Acquisition of a simultaneous light-dark discrimination task in a Y-maze with spatial position irrelevant. Mean percent correct responses are plotted as a function of days of training. (n=8/group). ■——■ Controls, □- - -□ DSP-4, △——△ DMI + DSP-4.

TABLE 3

Y-MAZE RETENTION: CORRECT CHOICES OVER TEN TRIALS

Controls	DSP-4	DMI+DSP-4
8.3 ± 0.63	7.25 ± 0.80	8.25 ± 0.68

Values represent means ± SEM for 8 animals per group.
Statistical comparisons between groups with *t*-tests produced no significant differences at the 0.05 level.

Lever pressing-acquisition and extinction. The rate of acquisition of the operant CRF response is shown in Fig. 5. All groups learned this task, Days $F(8,23)=2.87$, $p<0.05$, and in no case did the DSP-4 treated animals differ from the respective controls. Neither the groups nor the groups × days interaction was significant. Similarly, during extinction there was no significant difference between the groups. Analysis of variance with repeated measures on one factor (days) revealed that although significant extinction was established, Days $F(1,23)=24.48$, $p<0.001$, neither the effect of treatment nor the interaction of treatment with sessions reached significance ($F<1$). In addition, no significant difference was observed between DSP-4 treated animals and vehicle controls on the mean number of responses or the mean time to the extinction criterion (2 consecutive minutes without lever pressing).

DISCUSSION

A number of conclusions can be reached on the basis of the present data. First, in agreement with previous reports

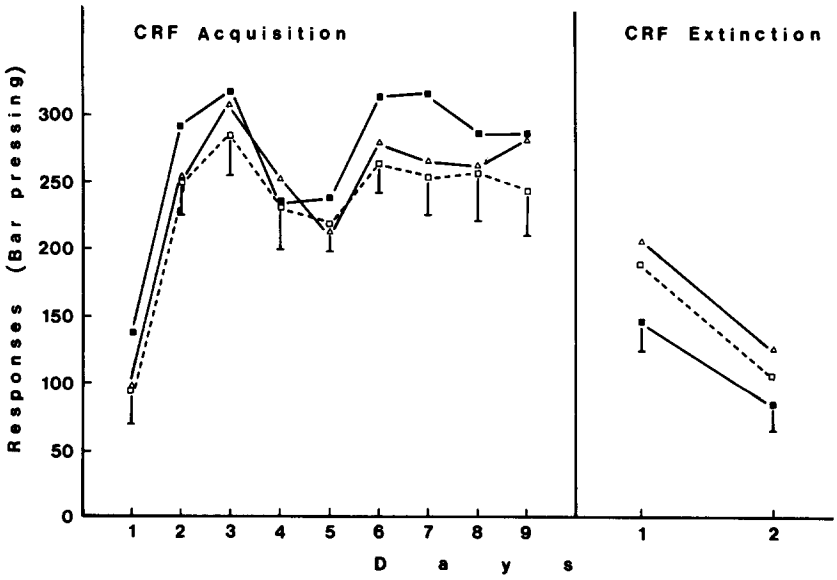

FIG. 5. Acquisition (left panel) and extinction (right panel) of a continuously reinforced (CRF) lever pressing response. Data represent means ± S.E.M. of responses emitted per 20 min daily session. For clarity S.E.M.'s, which were similar for all groups, are shown for one group only. (n=8/group). ■——■ Controls, □----□ DSP-4, △——△ DMI + DSP-4.

[4,19] DSP-4 produces extensive depletion of NA in the forebrain, cerebellum and spinal cord. Second, at the light microscopic level there does not appear to be a reduction in the number of perikarya in the locus coeruleus one week after the second of two DSP-4 injections. Therefore, it appears unlikely that DSP-4 depletes central NA by damaging directly NA perikarya in the locus coeruleus. Rather, this probably occurs through an action of this compound on the NA axons and terminals. Third, DMI pretreatment offers only partial protection from the DSP-4 induced depletion of central NA levels. These findings, in agreement with those reported previously [17,19], indicate that DMI pretreatment does not under all circumstances protect central NA neurons from DSP-4 and therefore inclusion of a DMI-DSP-4 group may not be a particularly useful control. Further studies on DMI-DSP-4 interactions appear to be in order.

Although DSP-4 may impair the acquisition of active avoidance responses [17], this appears not to be due to a general learning deficit but may instead apply only to aversively motivated behaviors. Thus, in the present experiments in no instance was the acquisition of a food-rewarded operant behavior adversely affected by the DSP-4 treatment. These results are therefore in complete agreement with the substantial body of evidence showing that depletion of central NA or destruction of central NA systems do not result in general impairments in learning and memory [8, 13, 18]. The fact that DSP-4 may selectively impair the acquisition of aversively motivated behaviors suggests that central and peripheral NA neurons are involved in the reaction of the organism to stressful events, a proposition for which there is considerable independent evidence [22]. However, the exact roles of these NA systems in stress related phenomena remain to be specified.

Finally, it should be pointed out that the present results provide only a small degree of support for the conclusions that have been reached with animals in which depletions of forebrain NA were produced by 6-OHDA lesions of the dorsal NA bundle. Specifically, although DSP-4 increased resistance to extinction in the L-shaped runway, this was not found in extinction of the bar-press response. Secondly, DBL rats have been reported to show greater exploratory behavior in a complex maze and to be impaired in the acquisition of a simultaneous light-dark discrimination task [10, 12, 15]. Neither of these effects were observed after DSP-4. Several factors may account for these discrepancies. The most obvious is that neither the degree nor pattern of NA depletion is the same in DBL and DSP-4 treated rats. In addition, it is possible that NA levels in DSP-4 treated animals may recover somewhat with time after the drug administration whereas forebrain NA remains permanently low after DB lesions [11]. Equally important, subsequent studies on DB lesioned rats in this laboratory have failed to confirm some of the findings upon which the present experiments were partly based. These include failures to confirm alterations in exploration in a complex maze and the reported deficits in the acquisition of a simultaneous light-dark discrimination task (Pisa, Spyraki and Fibiger, in preparation). In view of these facts, the failure to obtain effects of DSP-4 treatment in these situations is perhaps not surprising.

ACKNOWLEDGEMENTS

Supported by the Medical Research Council. DSP-4 was generously supplied by Astra Laboratories. The skilful technical assistance of Stella Atmadja is gratefully acknowledged. We thank M. Martin-Iverson for helpful discussions.

REFERENCES

1. Amaral, D. G. and J. A. Foss. Locus coeruleus lesions and learning. *Science* **188:** 377–378, 1975.
2. Anlezark, G. M., T. J. Crow and A. P. Greenway. Impaired learning and decreased cortical norepinephrine after bilateral locus coeruleus lesions. *Science* **181:** 682–684, 1973.
3. Crow, T. J. The coerulo-cortical norepinephrine system and learning. In: *Frontiers in Catecholamine Research,* edited by E. Usdin and S. H. Snyder. New York: Pergamon Press, 1973, pp. 723–726.
4. Jaim-Etcheverry, G. and L. M. Zieher. DSP-4: a novel compound with neurotoxic effects on noradrenergic neurons of adult and developing rats. *Brain Res.* **188:** 513–523, 1980.
5. Jonsson, G. Chemical neurotoxins as denervation tools in neurobiology. *A. Rev. Neurosci.* **3:** 169–187, 1980.
6. Karnovsky, M. J. and L. Roots. A "direct-coloring" thiocholine method for cholinesterases. *J. Histochem. Cytochem.* **12:** 219–221, 1964.
7. Kety, S. S. The biogenic amines in the central nervous system: their possible roles in arousal, emotion and learning. In: *The Neurosciences,* edited by F. O. Schmitt. New York: Rockefeller University Press, 1970, pp. 324–336.
8. Koob, G. F., A. E. Kelley and S. T. Mason. Locus coeruleus lesions: learning and extinction. *Physiol. Behav.* **20:** 709–716, 1978.
9. Lindvall, O. and A. Bjorklund. The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. *Acta physiol. scand.* Suppl. **42:** 1–48, 1974.
10. Mason, S. T. and H. C. Fibiger. Altered exploratory behaviour after 6-OHDA lesion to the dorsal noradrenergic bundle. *Nature* **269:** 704–705, 1977.
11. Mason, S. T. and H. C. Fibiger. Neurochemical basis of the dorsal bundle extinction effect. *Pharmac. Biochem. Behav.* **10:** 373–380, 1979.
12. Mason, S. T. and H. C. Fibiger. Noradrenaline and selective attention. *Life Sci.* **25:** 1949–1956, 1979.
13. Mason, S. T. and S. D. Iversen. Learning in the absence of forebrain noradrenaline. *Nature* **258:** 422–424, 1975.
14. Mason, S. T. and S. D. Iversen. Theories of the dorsal bundle extinction effect. *Brain Res. Rev.* **1:** 107–137, 1979.
15. Mason, S. T. and D. Lin. Dorsal noradrenergic bundle and selective attention in the rat. *J. comp. physiol. Psychol.* **94:** 819–832, 1980.
16. McGeer, E. G. and P. L. McGeer. Catecholamine content of the spinal cord. *Can. J. Biochem. Physiol.* **40:** 1141–1151, 1962.
17. Ogren, S. O., T. Archer and S. B. Ross. Evidence for a role of the locus coeruleus noradrenaline system in learning. *Neurosci. Lett.* **20:** 351–356, 1980.
18. Roberts, D. C. S., M. T. C. Price and H. C. Fibiger. The dorsal tegmental noradrenergic projection: an analysis of its role in maze learning. *J. comp. physiol. Psychol.* **90:** 363–372, 1976.
19. Ross, S. B. Long-term effect of N-2-chlorethyl-N-ethyl-2-bromo-benzylamine hydrochloride on noradrenergic neurons in the rat brain and heart. *Br. J. Pharmac.* **58:** 521–527, 1976.
20. Ross, S. B. and A. L. Renyi. On the long-lasting inhibitory effect of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine(DSP-4) on the active uptake of noradrenaline. *J. Pharm. Pharmac.* **28:** 458–459, 1976.
21. Sessions, G. R., G. J. Kant and G. F. Koob. Locus coeruleus lesions and learning in the rat. *Physiol. Behav.* **17:** 853–859, 1976.
22. Usdin, E., R. Kvetnansky and I. Kopin (eds). *Catecholamines and Stress: Recent Advances.* Amsterdam: Elsevier North Holland Inc., 1980.
23. Whishaw, I. Q. and R. M. Cooper. Strychnine and suppression of exploration. *Physiol. Behav.* **5:** 647–649, 1970.