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On the Role of the Dorsal Noradrenergic Bundle in Learning and Habituation to Novelty

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PISA, M., M. T. MARTIN-IVERSON AND H. C. FIBIGER. *On the role of the dorsal noradrenergic bundle in learning and habituation to novelty*. PHARMACOL BIOCHEM BEHAV **30**(4) 835–845, 1988.—In Experiment 1, the performance of vehicle control rats and rats with 6-hydroxydopamine-induced lesions of the dorsal noradrenergic bundle (DB) was examined in acquisition and extinction of bar pressing and in spontaneous and food-reinforced alternation in a T-shape maze. Plasma corticosterone levels in basal conditions, after chronic food restriction, after transportation to a novel environment, and after sessions of either rewarded or nonrewarded bar pressing were assayed. DB lesions produced a significant decrease of spontaneous alternation and a significant but small resistance to extinction, without reliably altering either corticosterone responses or instrumental spatial alternation. In Experiment 2, bar-press extinction and instrumental alternation were reexamined in new groups of control rats and rats with DB lesions without any blood collection procedures. The DB lesions did not reliably alter either behaviors on any measures. Taken together, these data indicate no consistent effects of forebrain noradrenaline depletion on either extinction or spatial memory or pituitary-adrenocortical function. However, the impairment of spontaneous alternation found in a previous study was confirmed. These findings are discussed in terms of the proposed roles of the dorsal noradrenergic bundle in learning and habituation to novelty.

Corticosterone Dorsal noradrenergic bundle Extinction Habituation Locus coeruleus Memory Noradrenaline Spatial alternation

PREVIOUS studies have indicated that 6-hydroxydopamine (6-OHDA)-induced lesions of the dorsal bundle (DB), which comprises the ascending fibers of the locus-coeruleus noradrenergic neurons, can retard behavioral habituation to novel stimuli [20,31], aversive stimuli [39], or frustrative stimuli [29] in the rat. A plausible interpretation of these findings is that the dorsal noradrenergic bundle plays a modulatory role in behavioral responses to alarming stimuli [39,40], a proposition for which there is independent biochemical [15,42], pharmacological [52] and electrophysiological evidence [2, 16, 43, 44].

It is also possible that the dorsal bundle influences endocrine responses to alarming stimuli because pharmacological activation of brain adrenergic mechanims has been shown to inhibit the pituitary-adrenocortical response to such stimuli [7, 9, 11, 12, 45, 48]. In a previous study we found, however, that DB lesions, while prolonging behavioral neophobic reactions, did not alter the plasma corticosterone response to a novel environment [20]. Other investigators have also reported that central noradrenaline depletion induced by intraventricular injections of 6-OHDA [19] or intraperitoneal injections of DSP-4 [3,6] do not alter corticosterone responses to aversive stimuli such as immobilization and ether inhalation. Despite this negative evidence, it is still possible that the DB influences the corticosterone response to other kinds of alarming stimuli, such as omission of reward.

It has repeatedly been reported [21, 22 25–28, 32] that DB lesions retard extinction of continuously reinforced bar pressing after omission of food reward. This phenomenon, the dorsal bundle extinction effect (DBEE), might reflect an exaggerated behavioral response to frustrative nonreward. A role of the pituitary-adrenal axis in the DBEE was invoked [32] based on the finding of abolished DBEE in adrenalectomized animals. We sought to determine, therefore, whether an abnormal increase in the level of plasma corticosterone attended the DBEE.

The second objective was to reexamine the role of the DB in spatial memory. Mason and co-workers originally reported [24], and subsequently confirmed [33], that depletions of forebrain noradrenaline impair learning of spatial alternation in a T-shape maze. This effect was interpreted to be consistent with a postulated role of forebrain noradrenergic

activity in selective attention [23]. However, Pisa and Fibiger [37] could not confirm this finding. Furthermore, they could not replicate [36] the detrimental effects of DB lesions on discrimination learning originally reported by Mason and Lin [30]. Thus, no support could be found for the hypothesis [23,24] that DB lesions impair either spatial memory or selective attention. A relative ineffectiviness of the 6-OHDA treatment did not appear to account for the negative behavioral results of Pisa and Fibiger because the depletions of forebrain NE in their studies were similar to those reported by Mason and co-workers. It is possible, however, that Pisa and Fibiger inadvertently introduced some procedural changes that generally prevented DB lesions from affecting behavior in the manner described by Mason and co-workers. To examine this hypothesis, we decided to reinvestigate the performance of rats with DB lesions in both spatial alternation learning and extinction of continuously reinforced bar pressing.

The DBEE might be considered to be a robust phenomenon since Mason unfailingly replicated it many times, in different laboratories, with different co-workers, and with subjects from different litters and sources [21, 22, 24, 25, 27, 28, 32]. Thus, if we succeeded in replicating the DBEE, but not the impairment of alternation learning, we could at least reject the possibility that changes in some uncontrolled variables, including the experimenter's identity, generally invalidated our replication procedures. On the other hand, even more serious doubts could be raised about the reliability of the findings reported by Mason and co-workers if the DBEE was not replicated.

EXPERIMENT 1

In this experiment we examined the effects of 6-OHDA-induced lesions of the dorsal noradrenergic bundle on bar press extinction, spatial alternation learning, and plasma corticosterone response to omission of reward.

METHOD

Subjects

Twenty-five male Wistar rats (175–200 g) obtained from Woodlyn Farms (Guelph, Ontario) were used as subjects. They were housed in individual stainless steel cages in a colony room with temperature of 22–25°C, humidity of 45%, and a 12:12 hr light-dark cycle, and were maintained with ad lib Purina Rat Chow and tap water.

Surgery

After 7 days of acclimatization in the colony room, 12 rats, selected at random, were assigned to a control group, and the other 13 rats (DB rats) to a group for DB lesions. The rats were anaesthetized with sodium pentobarbital (50 mg/kg IP), and positioned in a Kopf stereotaxic instrument with bregma and lambda on the horizontal level. An incision was made in the sagittal midline of the scalp, and two holes were drilled in the skull to allow a 32-g infusion cannula to be lowered 1 mm lateral to either side of the midline, 6 mm posterior to bregma and 5 mm below the dura. The brains of the DB rats were infused with 4 μg of 6-OHDA hydrobromide (Regis Chemicals, weight expressed as the base) dissolved in 2 μl of 0.9% saline solution, with 0.3 mg/ml ascorbic acid added as antioxidant. The brains of the control rats were infused with 2 μl of the saline-ascorbate solution. At the end of the injection, which was made at the rate of 1 μl/min, the cannula was left in place for 1 min to promote local diffusion of the solution. The cannula was then withdrawn, and the scalp sutured.

Apparatus

Four standard operant test boxes (BRS/LVE) enclosed in sound-proof chambers and fitted each with a lever and a dispenser of food pellets were used to examine bar-press performance. The operant boxes were interfaced to a NOVA 4/X minicomputer (Data General) equipped with MANX software and interface (GC Controls), and programmed to control the behavioral contingencies and to record bar presses and interresponse pauses.

A wooden, grey-painted, T-shape maze with a lid of mesh hardware cloth was used to examine alternation learning. The walls of the maze were 15 cm high, and the arms 50 cm long and 13 cm wide. The choice area was a square of 13 cm per side. The start box consisted of the first 20 cm of the start arm. Guillotine doors separated the choice area from the goal arms and the start box from the remainder of the start arm. Illumination came from a 25-W light bulb 1 m above the choice area.

Procedure

Behavioral. Four weeks after surgery, the rats were removed from the home cages, intermittently handled over a period of 5 min, weighed, replaced in the home cages, and twice given five food pellets (45 mg, Noyes) in a plastic dish, with an interval of 5 min between servings. One hour later, they were given 12 g of Purina Rat Chow. This procedure was repeated daily until the rats reduced their weights approximately to 85% of their free-feeding weights, which took about a week. Both handling and the servings of food pellets were discontinued thereafter, and the daily amount of Purina Rat Chow as adjusted to allow an average weekly increase of 7.5 g in body weight.

Daily training in the operant boxes started 5 weeks after surgery and took place between 09:00 a.m. and 12:30 p.m. All training sessions lasted 15 min. In Sessions 1 and 2 the rats had free access to 20 food pellets in the food magazine. In sessions 2 and 3 the hoppers were programmed to deliver food pellets both on a schedule of continuous reinforcement (CRF) for bar pressing and on a fixed time-30 sec schedule. In Sessions 4 to 14, delivery of food was exclusively contingent on bar pressing. In Sessions 15 to 17, an extinction schedule was operative so that no programmed events occurred as a result of bar pressing. To examine spontaneous recovery of bar pressing, a fourth 10-min session of extinction was given after 10 days of rest. The data were bar presses in acquisition, bar presses in extinction, bar presses and latencies to the extinction criterion of no responses for 2 minutes, and interresponse pauses longer or shorter than 60 sec in each 3-min block of the first extinction session.

After rest and ad lib food in their home cages for 10 days, the rats were put on a food-restricted diet until their weights were about 85% of their free-feeding weights.

Training in the T-shaped maze started 12 weeks after surgery. In Session 1 the rats were given five trials of spontaneous (nonfood-reinforced) alternation. In each trial, the rat was inserted in the start box, and the door of the start box was opened after 5 sec. After the rat entered a goal arm with all four feet, the door behind it was closed, the rat was confined in the goal arm for 10 sec, carried to a holding box behind the maze, confined there for a 15-sec intertrial inter-

val (ITI) and then inserted in the start box for the next trial. Alternated choices were taken as data.

In Session 2, twenty 45-mg food pellets were placed in two food cups each at the far ends of the goal arms, four evenly spaced pellets were placed on the floor of each alley, and one pellet in the center of the choice area. The rats were individually placed in the maze for 20 min, and given the opportunity to ambulate in all sections of the maze and to eat food. No data were taken.

In Sessions 3 to 27 the rats were trained on food-reinforced, left-right alternation. The procedure was similar to that of Session 1, with these exceptions: in the first daily trial, the rat was rewarded with food for responding to either the left or the right goal arm. In the subsequent trials, food reward was found only in the goal arm opposite that entered in the previous trial. Reward consisted of 5 food pellets in a cup at the end of the goal arm. In each trial, the rats were confined in the selected goal arm for 10 sec or until they ate the food. Then, they spent a 15-sec ITI in a holding box. To reduce odor cues from the baited cup, a hole 1 cm in diameter was drilled near the bottom of the rear walls of both goal arms, and 50 g of food pellets were placed immediately behind the rear walls on a platform at the same level of the floor of the maze. In each session, training of control rats was alternated to training of rats with DB lesions, and the floor of the maze was thoroughly wiped with paper soaked with a 0.7% acetic acid solution before each rat was trained. The same procedure of food-reinforced alternation was used in sessions 28 to 37 except that the rats were left in the holding box for as short a time as possible (nominally 0 ITI). The data were alternated choices in each session, and errors and days to reach the learning criterion of 85% correct choices over 2 consecutive sessions.

Corticosterone assays. To collect blood samples, the rat was inserted into a restraint jar, its tail was nicked and 200 μl of blood were collected in a heparinized capillary tube within 2 min from the insertion of the rat into the bottle. The procedure described by Glick and co-workers [13] was used for the fluorometric determination of plasma corticosterone levels. The assays were done blind to experimental conditions in which they were taken. Five blood samples were collected from each rat, to examine plasma corticosterone levels in the following conditions: (1) transportation from the animal quarters to the behavioral laboratory and food rewarded bar pressing; (2) transportation from the animal quarters to the behavioral laboratory and bar pressing in the absence of reward; (3) transportation to the behavioral laboratory while on a food-restricted diet; (4) food-restricted diet; (5) ad lib food. Samples 1 and 2 were collected immediately after the eighth session of reward bar pressing and the first session of extinction, respectively. Sample 3 was collected 15 min after transportation of the rats from the animal quarters to the behavioral laboratory, 3 days after the third session of extinction training. Sample 4 was collected in the animal quarters, as soon as the rats were taken out of their home cages, 8 days after the third session of extinction training. Sample 5 was collected in the animal quarters after the rats terminated extinction testing and were kept on ad lib food diet for 10 days. All samples were taken before 1 p.m.

Catecholamine assays. Two rats with DB lesions died before the end of the behavioral tests and their brains could not be recovered for assays. At the end of training, the remaining 11 rats with 6-OHDA injections and a random sample of 5 rats from the control group were sacrificed by cervical fracture. The brains were rapidly removed and dissected on ice into neocortex-hippocampus, hypothalamus and striatum. To determine the extent of catecholamine depletions produced by the 6-OHDA injections, noradrenaline concentrations of the neocortex-hippocampus and hypothalamus, and dopamine concentration in the striatum were assayed by a modification of the spectrofluorometric method of McGeer and McGeer [34]. Cortico-hippocampal noradrenaline was assayed in all tissue samples, hypothalamic noradrenaline and striatal dopamine in tissue samples from the five control rats and 5 rats with 6-OHDA injections, selected at random.

TABLE 1

POST-MORTEM CATECHOLAMINE CONCENTRATIONS IN FOREBRAIN REGIONS OF VEHICLE CONTROL RATS AND RATS WITH 6-HYDROXYDOPAMINE LESIONS OF THE DORSAL NORADRENERGIC BUNDLE

	Vehicle Control	n	Dorsal Bundle Lesion	n	%
Noradrenaline					
Cortex-hippocampus	370 ± 60	5	10 ± 1*	11	2.7
Hypothalamus	1810 ± 240	5	800 ± 390†	5	44.4
Dopamine					
Striatum	8710 ± 630	5	8310 ± 980‡	5	95.4

*$t(14)=6.00$, $p<0.001$; †$t(8)=2.21$, $p<0.05$; ‡$t(8)=0.34$, $p>0.01$.

Values are mean ± S.E. ng/g of fresh tissue. Percentages are relative to control values.

Statistics. Unweighted means solution two-way analyses of variance were made of the behavioral data and the plasma corticosterone levels. Lesion was the between-factor of all analyses. Training Condition was the within-factor in the analysis of the corticosterone data. Training Session was the within-factor in the analyses of the responses in bar press acquisition, the responses in bar press extinction, the responses and the latencies to the criterion of bar press extinction, and the alternated choices in the tasks of delayed and nondelayed alternation. Three-Min Block was the within-factor in the analyses of the interresponse pauses. The Geisser and Greenhouse conservative correction of degrees of freedom was used to test interaction effects in all analyses with more than two repeated measures, as a protection against violation of the assumption of homogeneity of covariance which would result from sequence effects ([54], pp. 305–306). Post hoc multiple comparisons ([53], pp. 474–478) with significance level set at 5% were made in the presence of significant main effects. Student's t-statistic was also used to analyze the data of individual extinction sessions, because this method of multiple comparisons was usually used in the original studies of the DBEE effect. Student's t-tests were made of the regional levels of noradrenaline and dopamine, the alternated choices in the test of spontaneous alternation, and the errors and the days to reach the criterion of instrumental alternation learning. Prior to statistical analysis, the scores of spontaneous alternation were subjected to a variance-stabilizing logit transformation, $0=\log_n [x+0.5/(1-x)+0.5]$, where x is the proportion of alternation responses made in the 4 alternation opportunities.

TABLE 2

PLASMA CORTICOSTERONE LEVELS OF CONTROL RATS AND RATS WITH DORSAL BUNDLE LESIONS

	Vehicle Control (N=12)	Dorsal Bundle* Lesion (N=9)
Basal	20.6 ± 3.46	20.4 ± 2.3
Food deprivation	27.6 ± 1.6	26.7 ± 3.4
Transportation	26.3 ± 1.2	31.8 ± 3.7
Rewarded bar pressing	41.5 ± 2.2	35.1 ± 2.6
Nonrewarded bar pressing	41.0 ± 2.64	39.7 ± 1.4

*Not significantly different from control group in any conditions, $F<1$.

Data are mean ± S.E. μg/100 ml.

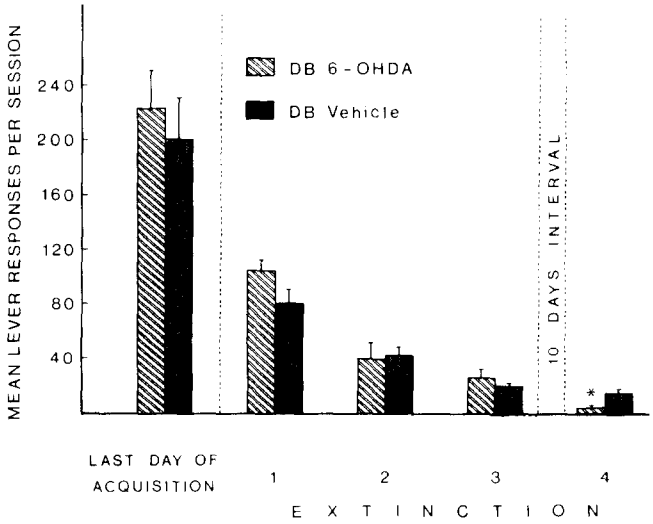

FIG. 2. Mean±S.E. responses of control rats and rats with dorsal bundle lesions in the last session of bar press acquisition (left panel), in Sessions 1, 2, and 3 of extinction (central panel), and in Session 4 of extinction following 10 days of no training (right panel). *Significantly different from control, $p<0.05$, by Student's t-test.

RESULTS

Catecholamine Assays

The effects of 6-OHDA injections on forebrain regional levels of catecholamines are shown in Table 1. The 6-OHDA injections reduced the average noradrenaline levels in the neocortex-hippocampus and hypothalamus to 2.7% and to 44.4% of control values, respectively. These reductions were statistically significant. On the other hand, the 6-OHDA injections did not significantly alter the concentrations of striatal dopamine.

Corticosterone Assays

The results of the corticosterone assays for the rats that learned bar pressing are shown in Table 2. The effect of training condition was highly significant, $F(1,19)=25.93$, $p<0.01$. Multiple comparisons showed that each of the environmental changes caused a significant increase of corticosterone levels relative to basal conditions. The experience of transportation to the behavioral laboratory did not significantly increase corticosterone levels above those found after food deprivation only. The conditions of rewarded bar pressing and nonrewarded bar pressing both increased corticosterone levels significantly above those found after transportation and food deprivation. However, corticosterone levels after nonrewarded bar pressing were not significantly higher than those after rewarded bar pressing.

Neither the main effect of lesion nor the interaction were significant, $F(1,19)=0.09$ and 2.21, respectively, $p>0.05$, indicating that the noradrenaline depletion resulting from dorsal bundle lesions did not reliably affect the corticosterone response to any of the conditions examined.

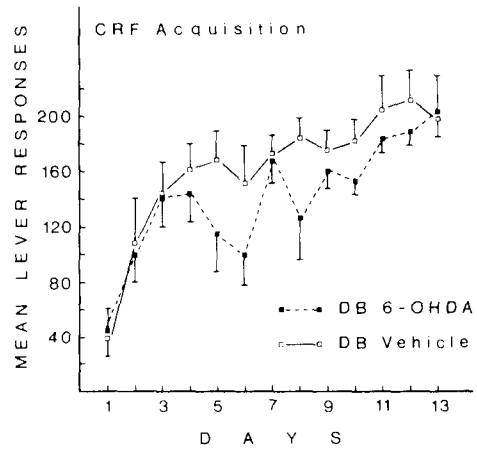

FIG. 1. Mean±S.E. responses of rats with vehicle control injections or 6-OHDA injections into the dorsal noradrenergic bundle (DB) in acquisition of continuously reinforced bar pressing.

Bar Press Acquisition and Extinction

Three DB rats never initiated bar pressing and another DB rat stopped bar pressing after the third session of acquisition. The average responses in acquisition are shown in Fig. 1. Neither the main effect of lesion, $F(1,19)=1.4$, $p<0.05$, nor the interaction of lesion with sessions, $F(1,19)=1.0$, were significant. On the other hand, the main effect of sessions was significant, $F(1,19)=15.2$, $p<0.01$, reflecting the increase of responses with acquisition practice. Figure 2 shows the average responses in the sessions of extinction (center panel) and spontaneous recovery (right panel). Analysis of the responses in the extinction sessions showed a significant main effect of sessions, $F(1,19)=44.1$, $p<0.01$, reflecting the decrease of responses with extinction practice. However, neither the main effect of lesion nor the interaction of lesion with sessions reach statistical significance, $F(1,19)=0.05$ and 1.5, respectively, $p<0.05$. Both groups of rats showed little spontaneous recovery of responses after the 10 days of rest. However, the DB rats responded slightly less than the controls in the recovery session, $t(19)=2.3$, $p<0.05$ (two-tailed test).

Figure 3 shows the average responses and the average times to reach the arbitrary extinction criterion of no responses for 2 consecutive minutes in the extinction sessions. Analysis of the responses to criterion showed that neither

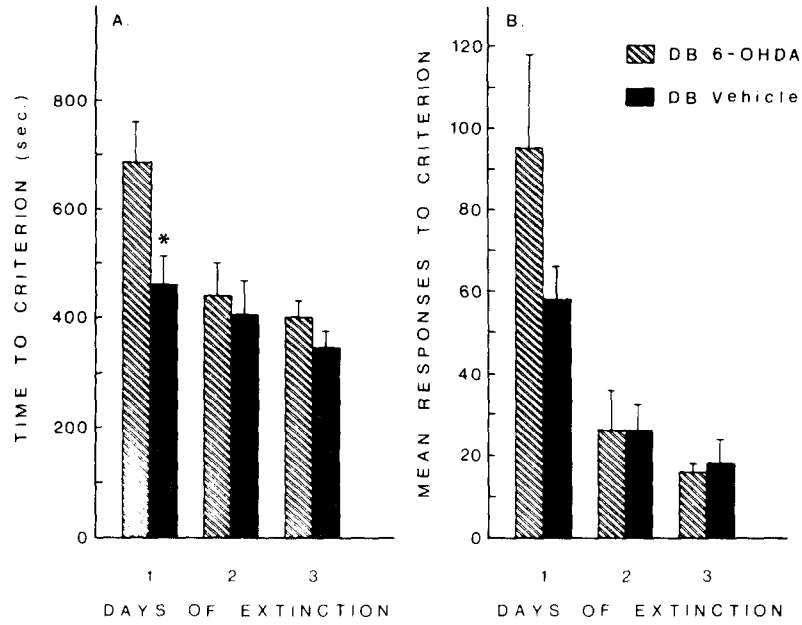

FIG. 3. Mean±S.E. latencies (left panel) and responses (right panel) of control rats and rats with dorsal bundle lesions to reach the extinction criterion of no bar presses for 2 consecutive minutes in Sessions 1, 2 and 3 of bar press extinction. *Significantly different from control, $p<0.05$, by Student's t-test.

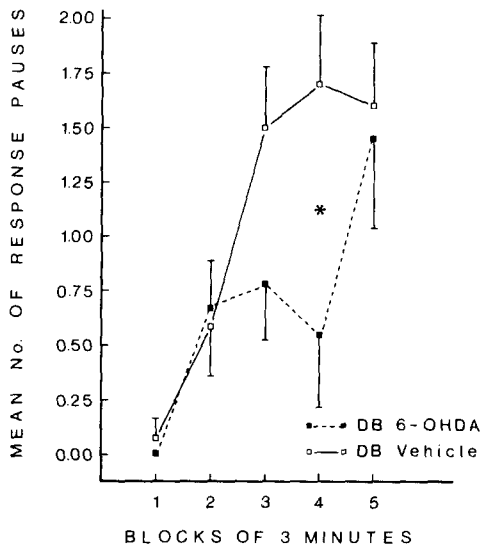

FIG. 4. Mean±S.E. number of interresponse pauses of 1 minute or longer during successive blocks of 3 min in Session 1 of bar press extinction for control rats and rats with dorsal bundle lesions. *Significantly different from control, $p<0.05$, by ANOVA.

the main effect of lesion nor the interaction of lesion with sessions were statistically significant, $F(1,19)=1.0$ and 3.36, respectively, $p>0.05$. Student's t-tests also showed no significant differences between control and DB rats in any sessions, $t(19)=1.5$, 0.1 and 0.3, $p>0.05$.

There was a significant main effect of lesion on the time to reach the extinction criterion, $F(1,19)=4.47$, $p<0.05$. Student's t-tests revealed a significant difference between groups in Session 1, $t(19)=2.44$, $p<0.05$ (two-tailed test), although not in Sessions 2 and 3, $t(19)=0.3$ and 1.3, respectively, $p>0.05$.

Mean interresponse pauses longer than 60 sec (long pauses) in the first session of extinction are depicted in Fig. 4. The main effect of 3-min blocks was significant, $F(1,19)=9.5$, $p<0.01$, reflecting an increase in the number of long pauses with time. The main effect of lesion also was significant, $F(1,19)=4.6$, $p<0.05$. Post hoc comparisons between group means in each 3-min block indicated that the DB rats had a significantly smaller number of long pauses than the control rats only in the fourth 3-min lock, $F(1,19)=8.8$, $p<0.01$. A similar analysis was made of the interresponse pauses shorter than 60 sec in the first session of extinction. Neither the main effect of lesion, $F(1,19)=1.1$, $p>0.1$, nor the interaction of lesion with 3-min blocks $F<1$, were significant.

Spontaneous and Food-Reward Alternation

Two rats with DB lesions were found dead in their home cages after the end of the bar pressing experiment and before the onset of T-maze training. The cause of their death was not established. Twelve control rats and 11 DB rats were tested for spontaneous alternation. Mean±S.E. percentages of spontaneous alternation were 61.3±6.2 and 67.0±8.3 for the rats with DB lesions and the control rats, respectively. One sample t-tests indicated that the control rats alternated significantly above chance levels, $t(11)=2.0$, $p<0.05$ (one-tailed test), whereas the rats with DB lesions did not, $t(10)=1.7$, $p>0.05$.

Two control rats became sick in the course of instrumental alternation training due to tail infection, and were therefore dropped from the experiment. Learning performance of

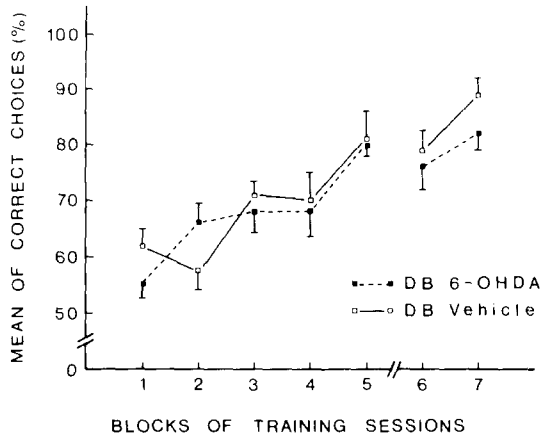

FIG. 5. Mean±S.E. percentages of reinforced choices of control rats and rats with dorsal bundle lesions in consecutive blocks of 5 sessions each of food-reinforced left-right alternation in a T-shape maze. The intertrial interval was 15 sec in blocks 1 to 5, and 0 sec in blocks 6 and 7.

TABLE 4

MEAN ± S.E. RESPONSES AND MEAN ± S.E. TIMES TO THE EXTINCTION CRITERION IN THE FIRST SESSION OF BAR PRESS EXTINCTION OF VEHICLE CONTROL RATS AND RATS WITH 6-OHDA LESIONS OF THE DORSAL BUNDLE TRAINED IN BOTH BAR-PRESS EXTINCTION AND INSTRUMENTAL ALTERNATION

Group	n	Responses	Time
Control	10	58 ± 9.6	493 ± 57.2
DB lesion	8	75 ± 14.8*	659 ± 80.4†

*Not significantly different from controls, $t(1,16)=0.96$, $p>0.05$; †not significantly different from controls, $t(1,16)=1.68$, $p>0.05$.
Time is in sec.

the remaining 10 control rats and 11 DB rats is shown in Fig. 5. Two-way ANOVA of the alternated responses in the sessions with 15-sec ITI, with lesion as between-factor and blocks of sessions as within-factor, showed neither a significant effect of lesion, $F<1$, nor a significant interaction, $F(1,18)=1.2$, $p>0.1$. On the other hand, the main effect of blocks of sessions was significant, $F(1,18)=11.7$, $p<0.001$, reflecting the improvement of performance with practice. A similar lack of statistically significant lesion effect or interaction was obtained in the analysis of the alternated responses in the session with zero delay. As shown in Table 3, the two groups did not significantly differ from each other in either number of days or number of errors to the arbitrary learning criterion of at least 85% correct responses over two consecutive sessions.

Further analyses were made of the bar press extinction data and the alternation learning data of the rats that complete training in both tasks (Table 4). Analyses of the responses and the latencies to the criterion of bar press extinction in the first extinction session showed no significant main effect of lesion on either measure, $F(1,16)=1.5$ and 2.2, respectively $p>0.05$. The interaction of lesion with extinction sessions also failed to reach statistical significance for both measures, $F(1,16)=1.5$ and 1.0, respectively, $p>1$.

TABLE 3

T-MAZE ALTERNATION LEARNING PERFORMANCE OF CONTROL RATS AND RATS WITH DORSAL BUNDLE LESIONS

Group	n	Errors to Criterion	Days to Criterion
Control	10	17.0 ± 3.6	11.7 ± 1.9
Dorsal bundle lesion	11	19.0 ± 2.9*	12.2 ± 1.6†

*Not significantly different from controls, $t(19)=0.332$, $p>0.01$; †not significantly different from controls, $t(19)=0.153$, $p>0.1$.
Values are mean ± S.E. errors and mean ± S.E. days to reach the learning criterion of 85% correct performance (7 alternated responses in 8 consecutive alternation opportunities).

Analyses of the correct responses in the alternation task showed that neither the main effect of lesion nor the interaction of lesion with blocks of sessions reached statistical significance, $Fs<1$.

DISCUSSION

The major findings were as follows: (1) 6-hydroxydopamine injections into the dorsal bundle produced subtotal depletion of noradrenaline in the cortex-hippocampus and partial depletion in the hypothalamus; (2) lesions of the dorsal bundle produced a DBEE on a time measure, but did not alter the plasma corticosterone response to omission of reward; (3) lesions of the dorsal bundle impaired spontaneous alternation but not instrumental alternation in a T-maze.

Catecholamine Depletions

The regional depletions of forebrain norepinephrine were comparable to those reported by Mason and co-workers in the studies in which a DBEE and an impairment of alternation learning were found. Thus, any discrepancies in the behavioral findings between those studies and the present could not be attributed to differences in the effectiveness of the lesion procedure.

Corticosterone Responses

In a previous study, we found that DB lesions reliably prolonged neophobic behavior without significantly altering the corticosterone response to novel stimuli [20]. In the present study, dorsal bundle lesions produced a statistically significant, albeit small, DBEE without reliably altering the corticosterone response to extinction. Taken together, these data appear to indicate that the dorsal noradrenergic bundle may influence behavioral responses, but not pituitary-adrenocortical responses to novel stimuli or omission of reward.

It has recently been reported that 6-OHDA-induced partial depletion of norepinephrine in the paraventricular nucleus of the hypothalamus significantly depressed corticosterone responses to photic, acoustic or sciatic-nerve stimulation [8]. In contrast, 6-OHDA injections into the medial forebrain bundle only suppressed responses to photic stimuli [9]. Thus, the noradrenergic fibers terminating into or around the paraventricular nucleus appear to be especially important in modulating pituitary-adrenocortical responses. Most noradrenergic fibers innervating the paraventricular nucleus

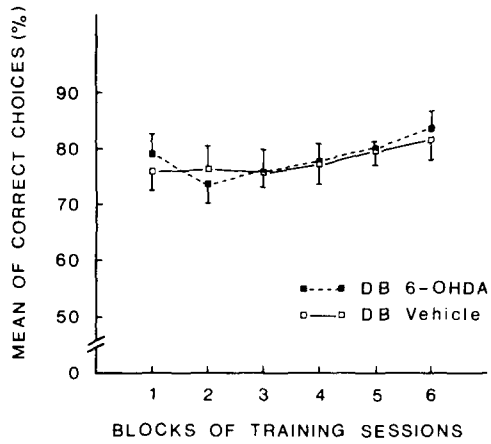

FIG. 6. Mean±S.E. percentages of correct choices of control rats and rats with DB lesions in consecutive blocks of 2 sessions each of food-reinforced spatial alternation in a T-shape maze. The intertrial interval was nominally 0 sec.

originate from medullary nuclei whose projection fibers ascend to the hypothalamus via the ventral noradrenergic bundle rather than the dorsal noradrenergic bundle [46]. It may be speculated, therefore, that dorsal and ventral noradrenergic bundles are predominantly involved in behavioral responses and pituitary-adrenocortical responses to alarming stimuli, respectively.

Starting with the basal condition of ad lib food in the home cage, the rats tended to respond to each additional stimulus with an increase of corticosterone levels. Thus, food deprivation and transportation increased corticosterone levels above baseline, and bar pressing produced a significant increase over these conditions. However, the levels found after the session of nonreward bar pressing were not significantly higher than those after rewarded bar pressing, which does not agree with the findings of Levine and his colleagues [5,14]. The source of the discrepancy is in the corticosterone response to rewarded bar pressing, which, relative to the condition of food deprivation, was significantly elevated in the present study and significantly *decreased* in theirs. Our results are more similar to those of Osborne and co-workers [36] who also found an increase of corticosterone levels after transportation and rewarded bar pressing relative to the condition of food deprivation only. These investigators suggested that food-rewarded bar pressing increases corticosterone levels if the operant sessions are too short to afford satiation. It is worth noting, in this respect, that in the studies of Levine and co-workers the reinforcer was water and the operant sessions lasted 20 min. It is possible, therefore, that satiation readily occurred in those conditions but not in those used here and by Osborne and his colleagues.

Dorsal Bundle, Extinction and Spatial Alternation

Dorsal bundle lesions did not reliably alter the number of responses in any sessions of extinction. This is at odds with the report that a DBEE could be demonstrated by this measure [21]. Mason and co-workers usually reported a significant DBEE in terms of both the time and the number of responses to reach the arbitrary extinction criterion of no responding for 2 min [22, 25–29]. In several such studies they also reported a reliable DBEE in both Sessions 1 and 2, in terms of the responses to criterion [22, 25, 26]. In the present experiment, there was no significant DBEE in terms of the response measure in any sessions. However, a statistically significant DBEE was indicated by the time measure of extinction in Session 1. This effect was related to an increased probability of low-frequency responses late in the session. It appears, therefore, that dorsal bundle lesions induced a reluctance to discontinue responding altogether once reward was omitted. However, the DBEE, as observed in the present experiment, was considerably weaker than that reported by Mason and co-workers. First, it reliably occurred only in the first session. Second, it was reflected in only one of three measures of extinction. Further evidence of the weakness of the DBEE was found in the analysis of extinction that excluded the data of the two control rats and the rat with DB lesions which failed to complete alternation training. This analysis showed no reliable DBEE by any measure. Thus, any evidence of a reliable DBEE as it was found in the original analysis was obliterated by slightly reducing the size of the two groups.

Consistent with the findings of Pisa and Fibiger [40], dorsal bundle lesions did not impair learning of spatial alternation. However, the original objective of examining alternation learning in animals showing a robust DBEE could not fully be met. This is because the DBEE itself turned out to be a weak phenomenon in our hands, being demonstrable to some extent in the original group of DB rats that completed bar press extinction, but not in the subgroup of rats that also completed alternation testing.

In agreement with the findings of Pisa and Fibiger [40] and other investigators (Owen, unpublished Ph.D. thesis, 1979, cited in [17]; see [33], however) rats with dorsal bundle lesions failed to show reliable spontaneous alternation. As discussed in detail elsewhere [39,40], this effect is consistent with the proposed role of the dorsal noradrenergic bundle in habituation to novel stimuli [17, 20, 40]. The finding that four rats with dorsal bundle lesions either did not start or did not persist in operant bar pressing (see [50] for a similar finding) also suggests a detrimental effect of DB lesions on habituation to novel environments.

EXPERIMENT 2

In Experiment 1, lesions of the dorsal bundle in rats produced a small DBEE effect and did not reliably affect spatial alternation learning. These results contrasted with those reported by Mason and co-workers. It is possible, however, that the collection of blood samples interfered with the manifestation of the full effects of DB lesions on performance of these tasks. To examine this hypothesis, both extinction of bar pressing and T maze alternation learning were reexamined in control rats and rats with DB lesions in the absence of blood collection procedures.

METHOD

Male Wistar rats weighing 175–200 g at the time of surgery were assigned at random to groups for bilateral injections of either vehicle solution (N=10) or 6-OHDA (N=9) into the DB. The procedures of housing, surgery, gentling and food restriction, bar press training and food-reinforced, alternation training were similar to those of Experiment 1, with these exceptions: (1) food restriction, bar press training, and T-maze alternation training started 5 weeks, 6 weeks, and 11 weeks after surgery, respectively; (2) the rats were given 11 daily sessions of acquisition and 3 sessions of extinction in the bar pressing task, and 12 daily training ses-

TABLE 5

TOTAL RESPONSES AND RESPONSES AND LATENCIES TO MEET THE CRITERION OF BAR PRESS EXTINCTION IN CONTROL RATS AND RATS WITH DORSAL BUNDLE LESIONS

Group	n	Total Responses	Responses to Criterion	Latency to Criterion
Control	10			
Day 1		74.5 ± 9.2	59.1 ± 12.0	416.9 ± 91.1
Day 2		52.3 ± 10.4	24.9 ± 5.0	310.2 ± 50.2
Day 3		40.6 ± 8.9	25.6 ± 7.3	351.8 ± 84.1
Dorsal Bundle Lesion*	9			
Day 1		94.9 ± 19.1	83.9 ± 19.2	528.5 ± 88.5
Day 2		55.5 ± 8.1	36.7 ± 10.4	390.0 ± 83.4
Day 3		38.4 ± 9.8	17.2 ± 9.6	191.0 ± 56.7

*Not significantly different from controls in any measures on any days, $ps > 0.1$ by ANOVA.

Data are means ± S.E. Latencies are in sec.

sions in the T-maze alternation task, with 11 trials in each session and no interval between trials; (3) adaptation to the conditions of T-maze training consisted of a single 20-min session of free exploration of the maze, with food pellets available in both food cups, the day prior to the first training session. Spontaneous alternation in the T-maze was not examined. At the end of training the rats were sacrificed by cervical fracture. The hippocampi were dissected from the brains of 5 control rats and 5 DB rats selected at random. Hippocampal noradrenaline levels were assayed by the same procedure as in Experiment 1.

TABLE 6

ERRORS AND DAYS TO REACH THE LEARNING CRITERION OF INSTRUMENTAL SPATIAL ALTERNATION IN A T-SHAPE MAZE

Group	n	Errors	Days
Control	10	18.3 ± 4.2	7.2 ± 1.4
Dorsal Bundle*	9	12.3 ± 4.5	5.1 ± 1.2

*Not significantly different from control group in any measures, $ps > 0.1$ by Students t-tests.

Data are means ± S.E.

RESULTS

Catecholamine Assays

Mean±S.E. levels of noradrenaline in hippocampal tissue, expressed as ng/g of fresh tissue were 304.9±18.0 for controls and 38.3±9.0 for DB lesions, $t(8) = 10.18$, $p < 0.001$. Thus, the 6-OHDA injections into the DB produced about 88% noradrenaline depletion in the hippocampus, on the average.

Behavior

During acquisition of bar pressing the rats increased their daily responding from an average of 13 responses in Session 1 to an average of 260 responses in Session 11. Neither the lesion effect nor the interaction of lesion with sessions were significant, $F = 0.08$ and 0.3, respectively. The main effect of sessions was highly significant, $F(1,17) = 55.1$, $p < 0.001$, reflecting the increase of responding with practice. Total responses in each extinction session, and responses and latencies to reach the extinction criterion are shown in Table 5. In Session 1, the DB rats made more bar presses than the controls on the average. However, neither the main effect of lesion nor the interaction of lesion with sessions reached statistical significance, $F = 0.3$ and 0.8, respectively. On the average, the DB rats also made more responses and showed longer latencies than the control rats to reach the extinction criterion in Session 1. However, the main effect of lesion did not reach statistical significance for either measures, $F = 0.5$ and 0.1, respectively. The interaction of lesion with sessions also failed to reach significance for both measures, $F(1,17) = 2.1$ and 3.1, $p > 0.1$. Student's t-tests of the responses and the latencies to the extinction criterion in the first session of extinction confirmed the lack of significant differences between groups on both measures, $t(17) = 1.1$ and 0.8, respectively, $p > 0.1$.

In the task of T-maze alternation the rats of both groups alternated at levels well above chance from the first session (Fig. 5), with only a slight improvement of performance by the end of training, and with no apparent differences between groups developing during training. This pattern of performance was reflected by the lack of significant main effects of both lesion and sessions, $Fs < 1$, as well as by the nonsignificant interaction term, $F(1,17) = 1.6$, $p > 0.1$.

DISCUSSION

The 6-OHDA-induced lesions of the DB resulted in a severe depletion of hippocampal noradrenaline. Yet the DB lesions did not produce either a reliable DBEE or a reliable impairment of spatial alternation.

The lack of effect of DB lesions on instrumental alternation agrees with the findings of Experiment 1 and those of Pisa and Fibiger [40]. These investigators obtained negative results irrespective of manipulations of either the ITI or the

distinctiveness of the goal arms. In the present study, negative results were obtained in both Experiments 1 and 2, despite variable effects of the DB lesions on extinction, different pretraining procedures, and differences in the rates of learning which probably resulted from the use of different intertrial intervals, 15 sec in Experiment 1 and nominally 0 sec in Experiment 2. We feel confident, therefore, that the dorsal bundle does not significantly influence processes of selective attention, learning and memory that are relevant to instrumental spatial alternation, at least in the experimental conditions that we have used. It is appropriate to point out that Mason and co-workers [33] misquoted the preliminary communications of Pisa and co-workers [38,41]. These authors never stated that alternation learning is a *critical* test of the attentional hypothesis [23] of dorsal bundle function. Rather, they indicated ([38, 40, 41], and present study) that, insofar as the finding of impaired alternation learning [25] had been interpreted in terms of the attentional hypothesis [23], their failure to replicate this finding could reasonably be interpreted as negative evidence for that hypothesis.

Mason and co-workers [33] used two distinctive alternation contingencies, respectively labelled as independent alternation and dependent alternation. They found that forebrain depletion of noradrenaline produced a learning impairment only in the task of dependent alternation. By misinterpreting preliminary communications [38,41], they suggested that Pisa and co-workers obtained negative results because they used the task of independent alternation. This is not so: the alternation task that Pisa and co-workers used in their studies, including the present study, is the same as the dependent-alternation task. Thus, other factors must account for the discrepant results of these groups of investigators. In this respect, it should be noted that Pisa and co-workers extensively handled the rats prior to alternation training ([40]; present study), whereas Mason and coworkers apparently did not [24,33]. The possible importance of this factor is highlighted by the finding of Owen and coworkers [37] that DB lesions impaired runway learning only if handling was omitted from the pretraining procedures. It would be worthwhile to examine whether a similar interaction also occurs in the case of alternation learning.

Lesions of the dorsal bundle tended to increase resistance to extinction in both Experiments 1 and 2. This tendency reached statistical significance in one out of three extinction measures in Experiment 1, and in none of the measures in Experiment 2. Since our preliminary communication [41], several groups of investigators have reported weak or no effects of central norepinephrine depletion on extinction of bar pressing in rats [35, 46, 49, 50]. In the light of this body of evidence, it is reasonable to conclude that lesions of the dorsal bundle may increase resistance to extinction, but that this effect is weak and unreliable.

The behavioral studies began at least four weeks after the lesions. Although the levels of forebrain noradrenaline remain stably low after 6-OHDA lesions of the locus coeruleus noradrenergic system [18], other parameters of adrenergic function, including firing rate of locus coeruleus cells [4], receptor sensitivity [18,51], oxidative metabolism of cortex [18], and activity of the catecholamine synthesizing enzyme tyrosine hydroxylase [1] show changes interpretable as functionally compensatory. Thus, it might be argued that neurochemical compensation of the lesion accounted for the lack of the unreliability of behavioral effects of dorsal bundle lesions. This is unlikely, however. First compensatory changes were reported to reverse to prelesion values 4 weeks after locus coeruleus lesions [18], namely before the onset of behavioral training in the present study. Second, Tombaugh and co-workers failed to find a significant DBEE at several lesion-training intervals, ranging from 5 to 110 days [50]. It is of special interest that these investigators found no significant DBEE in animals trained within two weeks of surgery, that is, within the peak period of neurochemical compensation.

It has been proposed that the dorsal bundle has a critical role in inhibition of attention to motivationally irrelevant stimuli [23]. The reliability of the findings [27] thought directly to support this hypothesis has seriously been disputed, however [39,46]. The present results further call into question this hypothesis of dorsal bundle function, by showing that other reported effects of dorsal bundle lesions, such as the DBEE and the impairment of spatial alternation learning, also interpreted in terms of this hypothesis [23], are in fact unreliable phenomena. On the other hand, there appears to be more consensus among several groups of investigators [20, 31, 37, 39, 40, 50] about the reliability of behavior effects of dorsal bundle lesions that suggest a role of forebrain noradrenaline in habituation of aversive reactions to novel stimuli. We propose that effects of dorsal bundle lesions that have been interpreted as an inability to suppress attention to motivationally irrelevant stimuli may reflect instead a failure of habituation to novel and potentially alarming stimuli.

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