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Schedule Dependent Changes in Operant Responding after Lesions of the Dorsal Tegmental Noradrenergic Projection

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PRICE, M. T. C., G. N. MURRAY AND H. C. FIBIGER. *Schedule dependent changes in operant responding after lesions of the dorsal tegmental noradrenergic projection.* PHARMAC. BIOCHEM. BEHAV. 6(1) 11–15, 1977. – The present experiments examined the role of the dorsal tegmental noradrenergic bundle (DTNB) in operant responding for food under various schedules of reinforcement. This catecholaminergic neuronal system originates in the nucleus locus coeruleus and has diffuse projections to hippocampus and cerebral cortex. Bilateral stereotaxic injections of 6-hydroxydopamine into the DTNB of rats reduced hippocampal-cortical noradrenaline to less than 5% of control levels. Animals with these lesions acquired a continuously reinforced (CRF) bar-press response at the same rate as controls. Compared to controls rats with DTNB lesions responded at significantly lower rates on a variable interval 30 (VI 30) schedule. Extinction after VI 30 responding did not differ significantly between control and lesioned animals. In another experiment no significant difference was observed between DTNB lesioned and control groups on the rate of responding on a fixed ratio 30 (FR 30) schedule. The results are discussed with reference to previous reports indicating changes in operant responding after intraventricular injections of 6-hydroxydopamine. The data failed to support the hypothesis that the DTNB is critically involved in learning and memory.

Central catecholaminergic neurons Learning Operant responding 6-hydroxydopamine
Schedules of reinforcement

CENTRAL catecholaminergic (CA) systems appear to be involved in the performance of operant responding. Data suggesting such a role for these systems have been obtained by a number of different experimental approaches. First, peripheral administration of drugs that reduce brain levels of dopamine (DA) and noradrenaline (NA) has been shown to result in decreases in operant responding [17,22]. Secondly, performance of an operant response appears to be accompanied by an increased utilization of catecholamines [3, 9, 12, 22, 26]. A third approach used in this area of investigation has been to examine schedule controlled behaviour following the destruction of CA neurons by intraventricular injections of the neurotoxin 6-hydroxydopamine (6-OHDA). Using this method, Cooper, Grant and Breese [4] reported that responding on a schedule of continuous reinforcement (CRF) was not altered by substantial central depletions of NA and DA produced by 6-OHDA. The 6-OHDA treated animals were however, more sensitive to the rate-decreasing effects of α-methyl-para-tyrosine. In contrast to this report of no direct effects on responding on a CRF schedule, other investigators have reported increases in responding on schedules of partial reinforcement. Shoenfeld and Uretsky [23] reported that intraventricular 6-OHDA produced a 4-fold increase in responding maintained by a variable interval (VI) schedule of reinforcement. This effect occurred whether 6-OHDA was administered prior to training or after 4 weeks of training. Similarly, Peterson and Sparber [18] reported that intraventricular 6-OHDA injections caused rats which were trained to respond on a fixed ratio (FR) schedule of reinforcement to perform at significantly higher rates than vehicle injected controls. Inasmuch as this facilitation of responding was in evidence for the 5.5 month duration of the experiment, it appeared to be permanent.

The present experiments sought to extend these studies by investigating the role of a specific NA projection, the dorsal tegmental bundle, in schedule controlled behaviour. This system which originates in the nucleus locus coeruleus, projects diffusely to the telencephalon [13,27]. By stereotaxic injection of microliter quantities of 6-OHDA, it is possible to lesion completely and selectively this NA projection [19]. Compared to intraventricular 6-OHDA injections, this approach offers the obvious advantage that it is possible to attribute behavioural changes to damage to a specific neuronal system rather than to catecholaminergic neurons in general.

METHOD

Variable Interval 30

Thirty-two male Wistar rats weighing 300–320 g at the beginning of the experiment were maintained individually

in stainless steel cages with food and water available ad lib. Throughout the experiment, colony lighting was on from 8:00 a.m. to 8:00 p.m. daily.

Sixteen animals were anaesthetized with Nembutal (50 mg/kg), prepared for surgery in a Köpf stereotaxic instrument, and then given bilateral injections of 6-hydroxydopamine hydrobromide into the dorsal tegmental NA bundle. The injection coordinates according to Konig and Klippel [11] were A + 2.6 mm; L ± 1.1 mm; and DV + 3.7 mm. Each injection was made with 4 μg of 6-OHDA (dosage expressed as the free base) dissolved in 2 μl of a solution containing 0.15 M NaCl and 0.2 mg/ml ascorbic acid. Injections were made at the rate of 0.2 μl/min. Control animals (N = 16) had burr holes drilled bilaterally in the skull at the same coordinates, but did not receive intracerebral injections.

Two standard operant chambers (Lehigh Valley Electronics, Inc.) enclosed in sound-attenuating chambers were used in these investigations. Food reinforcement was provided by the delivery of a 45 mg food pellet (P. J. Noyes Co.) to a cup between 2 levers. Responses on the lever to the right of the cup resulted in food reinforcement. The left lever was inoperative.

One week after surgery, animals were put on a 22 hr food deprivation schedule which remained in effect for the duration of the experiment. After 5 days on this feeding schedule, acquisition training on a CRF schedule was initiated. On each of the 8 days of CRF, 2 food pellets were placed on top of the lever at the beginning of each daily 30 min session and in addition, the experimenter delivered a pellet to the rat on the first two occasions when the rat raised itself up onto the lever. After each daily session the rats were returned to their home cages and given free access to food for 2 hours. After acquisition of responding on the CRF schedule, animals with dorsal NA bundle lesions and controls were placed on variable interval schedules of reinforcement and gradually trained to respond on a VI 30 schedule. Each daily session lasted for 30 min. After the VI schedule had been in effect for 4 weeks, 4 consecutive days of extinction sessions were given. During these sessions, food was removed from the feeder so that bar-pressing no longer resulted in food reinforcement. Following behavioural testing, the animals were killed and the hippocampus and cerebral cortex were assayed for NA content [14].

Fixed Ratio 30

Materials and methods were as described above with the following changes. Sixteen male Wistar rats received bilateral 6-OHDA lesions of the dorsal NA bundle and eleven rats served as sham operated controls. Training on a CRF schedule was initiated two weeks after the lesions. Following the CRF sessions, the rats were gradually trained on increasing fixed ratio schedules until they learned to respond on a FR 30 schedule. This schedule continued to be in effect for 30 min per day on Mondays through Fridays for approximately 4 weeks. Upon completion of behavioural testing the animals were killed by cervical fracture and the hippocampus and cerebral cortex were dissected out on ice. The combined tissues were assayed for NA [14].

Data were analyzed statistically using a repeated measures two-way analysis of variance and Student's *t*-test.

RESULTS

Variable Interval 30

The mean number of bar presses for the 2 groups over the 8 days of acquisition training on the CRF schedule are presented in Fig. 1. No statistically significant differences between the groups were observed.

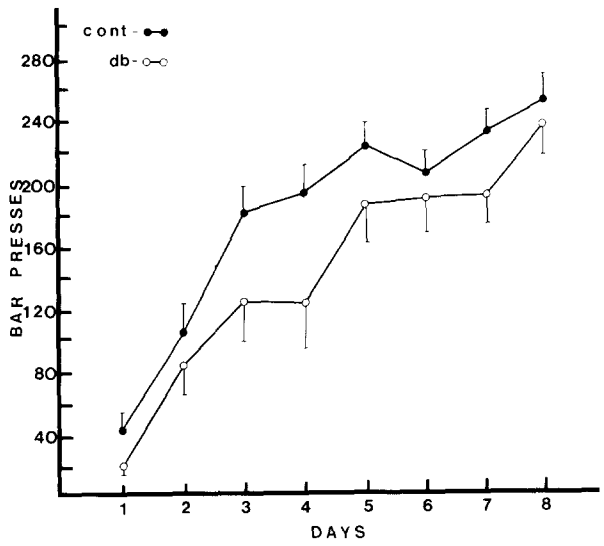

FIG. 1. Effect of lesions of the dorsal tegmental NA bundle on the acquisition of a bar-pressing response for food on a continuous reinforcement schedule. Each point represents the average daily rate of responding (± SEM) for control (n = 16) and lesioned (n = 16) groups.

The mean number of par presses on the VI 30 schedule are presented in Fig. 2. The plotted points in this figure each represent the mean of three 30 min sessions obtained over consecutive 3-day periods. The control group showed a significant increase in the rate of responding over days, whereas dorsal NA bundle lesioned animals did not. This difference in responding between controls and dorsal NA bundle lesioned animals was significant ($p<0.005$).

As shown in Fig. 3, the withdrawal of food reward during the 4 days of extinction resulted in significant decreases in operant responding ($p<0.001$). The groups did not differ significantly in the number of responses emitted on any of the extinction days.

The dordal NA bundle lesions reduced hippocampal-cortical NA to 4.7% of the control values (controls = 0.43 ± 0.03 μg/g; dorsal NA bundle lesions = 0.02 ± 0.002 μg/g). Although not measured in the present experiments in previous work we found that identical 6-OHDA lesions of the dorsal NA bundle did not significantly affect striatal DA levels [19].

Fixed Ratio 30

The mean number of bar presses emitted daily by each group on the FR 30 schedule are presented in Fig. 4. Each point represents the mean of 3 daily 30 min sessions obtained over consecutive days. Although there was an overall tendency for the lesioned animals to respond at higher rates than the controls, this trend failed to reach statistical significance ($p = 0.087$). Despite the absence of

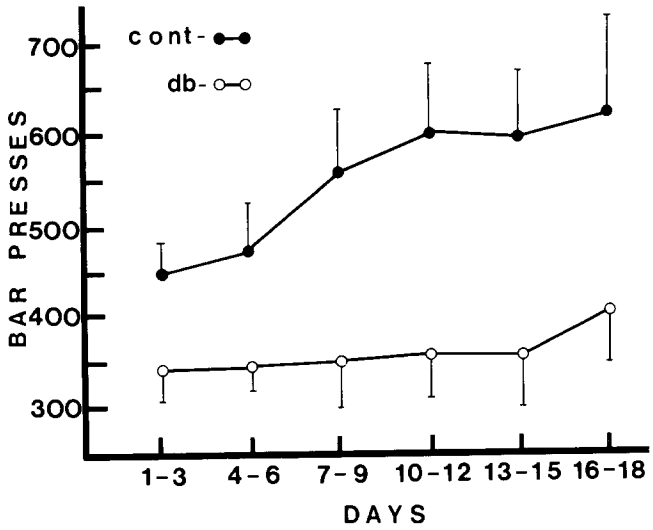

FIG. 2. Effect of lesions of the dorsal tegmental NA bundle on the rate of responding for food on a VI 30 schedule. Each point represents the average daily rate of responding (± SEM) over 3 days for control (n = 16) and lesioned (n = 16) groups.

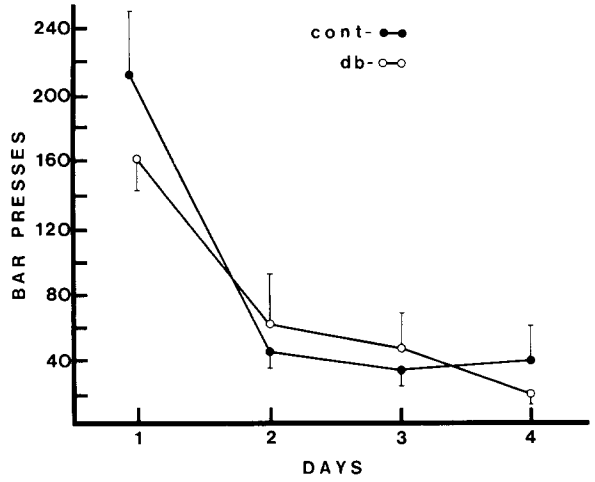

FIG. 3. Effect of lesions of the dorsal tegmental NA bundle on extinction of a bar-pressing response. Before extinction the groups had been responding on a VI 30 schedule of reinforcement (Fig. 2). Each point represents the average daily rate of responding (± SEM) of control (n = 16) and lesioned (n = 16) groups.

statistical significance when all data points were included in the analysis, the lesioned rats did respond significantly more than controls on each of the last 3 days of the experiment, Days 16–18 ($p<0.05$).

For controls, the mean NA content of hippocampus plus cortex was 0.42 ± 0.02 μg/g. The mean value for the lesioned group was 0.01 ± 0.001 μg/g, which represents a significant reduction to approximately 2% of control values ($p<0.001$).

DISCUSSION

In the present experiments, rats with DTNB lesions were found to respond at significantly lower rates than controls on a VI 30 schedule. This effect was to some extent due to

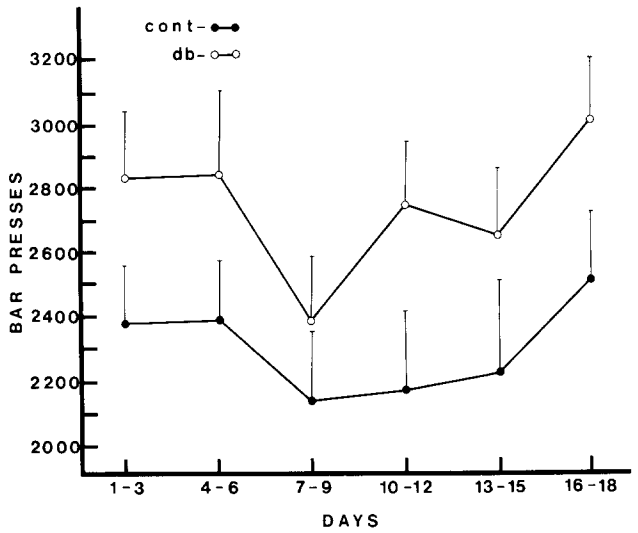

FIG. 4. Effect of lesions of the dorsal tegmental NA bundle on the rate of responding for food on a FR 30 schedule. Each point represents the average daily rate of responding (± SEM) over 3 days for control (n = 11) and lesioned (n = 16) groups.

the fact that the lesioned group did not significantly increase its rate of responding during the last 18 days of the experiment (Fig. 2), while the control animals increased their rates of responding by 33% during this period. By the end of the experiment the lesioned animals were responding at an average of 12 responses/min while the controls performed at approximately 20 responses/min. Schoenfeld and Uretsky [23] reported that intraventricular injections of 6-OHDA, which reduced whole brain levels of NA and DA to 12 and 21 percent of control levels respectively, significantly increased the rate of responding for water on a VI 90 schedule. Although the many differences between the present study and that reported by Schoenfeld and Uretsky [23] make direct comparisons difficult, our observations suggest that the increased responding observed in the latter study was due to damage to catecholamine containing neurons other than the dorsal tegmental NA projection. This suggestion is supported by a subsequent communication by Schoenfeld and Zigmond [24] in which it was reported that selective 6-OHDA induced depletions of brain DA produced similar rate-increasing effects on a VI 90 schedule as did depletion of both NA and DA.

These considerations argue therefore, for a dopaminergically mediated increase in responding on a VI 90 schedule after intraventricular 6-OHDA. In this regard, there is considerable evidence indicating that ascending dopaminergic projections are critically involved in the initiation and maintenance of operant responding [4, 6, 7, 20]. The fact that near complete lesions of these dopaminergic systems disrupt operant behaviour [7] while Schoenfeld and coworkers [23,24] observed increases in rates of responding after partial 6-OHDA lesions is consistent with their suggestion that the remaining catecholamine stores together with the development of postjunctional supersensitivity may have been responsible for their behavioural observations. It is possible that the concomitant damage to the dopaminergic systems masked any rate-decreasing effects on VI responding which may

have resulted from extensive damage to the dorsal tegmental NA projection alone.

Kety [10] and Crow and Arbuthnott [5] have independently suggested that the dorsal tegmental NA bundle is an important neural substrate of learning and long-term memory. In support of this hypothesis, Anlezark, Crow and Greenway [2] reported that animals with electrolytic lesions of the locus coeruleus, which depleted cortical NA by 71% failed to acquire a food-reinforced running response in an L-shaped maze. These observations were not confirmed by others however [1, 15, 21] and it appears that rats will learn a variety of tasks in the virtual absence of telencephalic NA [8]. The present results showing an unimpaired acquisition of the bar-pressing response on the CRF schedule in the lesioned animals again point to the nonessential role of this system in learning and memory. In fact, it could be argued from the results of the FR 30 and VI 30 experiments that lesioned animals were more sensitive to the conditions of reinforcement. Thus, in the FR 30 condition, where the amount of reinforcement was related directly to the number of bar presses, the lesioned animals tended to respond at higher rates than controls. On the other hand, in the VI 30 situation where the number of reinforcements was not directly related to the number of bar presses, the lesioned group worked more efficiently by responding significantly less than controls and yet receiving the same number of reinforcements (58 out of a possible 60 for both groups on the last 12 days of the experiment).

The extinction curves for the control and dorsal NA bundle lesioned groups were virtually identical (Fig. 3), again indicating that these lesions did not impair learning. These results do not lend support to a recent report by Mason and Iversen [16] in which it was observed that while rats with dorsal NA bundle lesions acquired a lever pressing response on a CRF schedule at a rate which was not different from controls, the lesioned animals were significantly slower to extinguish than controls. In the present experiments, extinction was not studied after CRF responding. The failure to observe changes in extinction after VI 30 responding suggests however, that the impaired suppression of responding during extinction observed by Mason and Iversen [16] may not be a general phenomenon which can be demonstrated under a variety of experimental conditions.

Peterson and Sparber [18] reported that intraventricular injections of 6-OHDA, which depleted NA in the cerebral hemispheres by about 60%, produced a long-lasting (5 month) increase in responding for food on an FR 30 schedule. One month after the intraventricular injections, the 6-OHDA treated animals responded 16% faster than controls while 3 months after the injections their rate was 33% greater than controls. In the present experiments, stereotaxic injections of 6-OHDA into the dorsal NA bundle produced near complete reductions (98%) in hippocampal-cortical NA. These lesions resulted in a rate of responding on an FR 30 schedule which was approximately 20% greater than that of the controls. However, this apparent increase in the rate of responding failed to reach statistical significance until the last 3 days of the experiment. Clearly, additional experiments will be required to determine the reliability and validity of this observation. It is noteworthy however that the increase in responding observed by Peterson and Sparber [18] did not reach statistical significance until 3 months after the intraventricular injections. It is possible therefore, that greater differences between the groups in the present experiment may have emerged if testing had been continued to 3 months.

At present, the reasons for the differing effects of dorsal NA bundle lesions on VI 30 and FR 30 responding remain obscure. It is noteworthy that these two schedules generate very different response rates (compare Figs. 2 and 4) and this may have had a bearing on the results. It appears most unlikely that the significantly reduced rates of responding found in the experimental group on the VI 30 schedule resulted from lesion-induced motor deficits. The motor output was considerably greater on the FR 30 schedule and if the DTNB lesions did result in some form of motor impairment then this should have been particularly evident in the FR 30 experiment.

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