Br. J. Pharmac. (1978), **64**, 601–605

THE EFFECTS OF DORSAL BUNDLE INJECTIONS OF 6-HYDROXYDOPAMINE ON AVOIDANCE RESPONDING IN RATS

HANS C. FIBIGER & STEPHEN T. MASON

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

1 The effects of injection of 6-hydroxydopamine (6-OHDA) into the fibres of the dorsal noradrenergic bundle on acquisition, retention and extinction of active avoidance in rats were examined.

2 6-OHDA injections severely depleted noradrenaline in all forebrain areas assayed, with the interesting exception of the septum. No significant effect on dopamine concentrations in various forebrain regions was found.

3 Acquisition and retention of active avoidance was not altered by the lesion. Marked resistance to extinction was seen when the unconditioned stimulus (shock) was removed.

4 A comparison with work by other authors in which both forebrain noradrenaline and dopamine were depleted suggest that the alteration in extinction seen in both studies is a noradrenergic effect, whereas the deficits in acquisition and retention found previously are dopaminergic in origin.

Introduction

In 1975 Mason & Iversen found that injection of the selective neurotoxin, 6-hydroxydopamine (6-OHDA), into the fibres of the dorsal noradrenergic bundle in such a way as to deplete forebrain noradrenaline (NA), led to marked resistance to extinction of a food-rewarded runway behaviour. In 1976 Ashford & Jones showed that injection of 6-OHDA into the amygdala in such a way as to deplete forebrain NA and also forebrain dopamine, led to marked deficits in acquisition and retention of active avoidance behaviour and also to resistance to extinction of this behaviour. Specific lesion of brain dopamine has previously been reported to cause impairment in acquisition and performance of active avoidance (Fibiger, Phillips & Zis, 1974; Cooper, Howard, Grant, Smith & Breese, 1974). This would suggest that the acquisition and retention deficits observed by Ashford & Jones may be dopaminergic while the extinction effect may be noradrenergic. The present study examines the effect of a pure NA depletion on acquisition, retention and extinction of active avoidance behaviour in the rat.

Methods

Surgical

Ten male albino Woodlyn rats weighing 300 g at the time of operation were anaesthetized with Nembutal (50 mg/kg intraperitoneally), positioned in a stereotaxic apparatus (Kopf Instruments) and two holes drilled in the skull. A 34 gauge cannula was lowered bilaterally to the following coordinates with the animal's head in the plan of Konig & Klippel (1963), AP + 2.6 mm from interaural line, ML ± 1.1 mm from midline at bregma and DV + 3.7 mm from the interaural line. 6-OHDA 4 μg (weight expressed as free base of 6-OHDA hydrobromide, Regis Chemicals) dissolved in 2 μl of 0.9% w/v NaCl solution (saline) with 0.2 mg/ml ascorbic acid antioxidant were infused at the rate of 1 μl/min over 2 min and the cannula left in place for a further minute to permit diffusion of the drug. Ten control rats received infusion of saline-ascorbate of an equivalent volume. The skin was then sutured and two weeks allowed for anterograde degeneration of the forebrain terminals to run to completion (Ross & Reis, 1974).

Biochemical

At the completion of avoidance testing all animals were killed by cervical fracture and their brains rapidly removed and dissected on ice into the following regions; cortex, hippocampus, hypothalamus, amygdala, septum, striatum, cerebellum and spinal cord. The brain was placed dorsal surface uppermost and the corpus callosum carefully split. The cortical hemispheres were rolled forward and outwards revealing the hippocampus which was blunt dissected from the cortical mass. The amygdala and pyriform cortex were removed by blunt dissection from the

base of the cortical mass. Amygdalae from two rats were combined for amine measurements. The cortex was repositioned, the brain rotated ventral surface uppermost and a coronal cut made at the level of the mammillary bodies. The cerebellum was removed from the posterior portion by section of the peduncles. The anterior portion was split along the midline and the septum removed by a scissor cut from the anterior commissure to the base of the ventricle. The septi from two rats were combined. Two scissor cuts, one in the vertical plane and one in the horizontal plane intersecting at the anterior commissure defined the extent of the hypothalamus, the thalamus was discarded and the striatum separated from the overlying cortex along the white matter of the corpus callosum. The cortex dorsal to the rhinal fissure and posterior to the frontal pole constituted the cortical sample. The spinal cord was removed by retraction of the musculature above the vertebrae which were then opened using rongeurs and a 5 cm sample of the rostral cord removed. These areas were then weighed and homogenized in 0.1 N perchloric acid and the endogenous concentrations of NA and dopamine determined by a spectrophotofluorimetric method (McGeer & McGeer, 1962). This served to confirm the pattern and extent of amine depletion induced by 6-OHDA injections.

Active avoidance

A wooden box measuring 29 cm by 70 cm by 30 cm with a grid floor was used. The box was divided into two across the long axis by a wooden door which could be raised and lowered. The left side of the apparatus could be electrified by a Lafayette a.c. constant current shock generator and scrambler with the intensity set at a nominal 3 mA.

(a) *Acquisition*
The animal was placed in the left side of the box with the centre door closed and after 5 s a tone (Sonalert, 500 Hz) was sounded and the door raised. If after 10 s of tone presentation the animal had not shuttled to the other side, it received electric foot-shock, the current remaining on until the animal escaped to the non-electrified side. The door was then closed and after an intertrial interval of 15 s the animal was returned by hand to the left side of the apparatus where the next trial started. If the animal avoided by shuttling to the safe side within 10 s of the warning tone, the door was immediately lowered and the tone terminated. The next trial followed in 15 s as usual. Training occurred within one day with as many trials being given as required to reach the acquisition criterion of nine consecutive avoidance responses. Testing ceased for that day. Control and lesioned rats were tested alternately.

(b) *Retention*
Twenty-four hours later the animal was replaced in the apparatus and the number of trials required to reattain the criterion of nine consecutive avoidance responses determined. Shock was again presented during this phase. Testing ceased for the day upon reaching this retention criterion.

(c) *Extinction*
On the next day after retention testing the animal was placed in the apparatus as before, except now no shock was presented. Testing continued until three consecutive avoidance failures occurred and this was taken as the extinction criterion.

Shock threshold

The sensory detection threshold to electric footshock was determined as described by Price & Fibiger (1975) which is derived from Evans (1961). Briefly, this comprised presenting the animal with an inescapable series of shocks of ascending intensities and rating the response as a flinch (any bodily movement), jump (hindpaws leave the grid) or vocalisation and determining the intensity at which the behaviour occurred on three or more of the five presentations of that intensity.

Results

Biochemical

The pattern of amine depletion induced as a result of 6-OHDA injection into the fibres of the dorsal noradrenergic bundle is shown in Table 1. Severe and permanent depletion of forebrain NA occurred with no great effect on brain dopamine or descending NA systems. Cortical, hippocampal and amygdaloid NA were all severely depleted, which is consistent with the known projections of ascending noradrenergic fibres (Ungerstedt, 1971; Lindvall & Bjorklund, 1974). Of particular interest in the light of the severe loss of NA in other forebrain regions is the considerable amount of NA remaining in the septum (46%). This would suggest that only part of the septal NA innervation originates from the locus coeruleus via the dorsal bundle and that as much as half may come instead from ventral systems. This is consistent with a recent anatomical investigation of the origin of septal NA (Moore, 1978). Severe damage was also inflicted on the hypothalamic NA innervation, which again is of mixed dorsal and ventral bundle origin. Striatal and hypothalamic dopamine were entirely spared by the 6-OHDA infusion. Some 20% loss of septal and amygdaloid DA occurred, although caution must be expressed about the amygdaloid values which were

barely twice the assay blank. Some of this dopamine loss may reflect the 10% or so which is present as a precursor of NA in noradrenergic neurones. Descending systems such as cerebellum and spinal cord were entirely intact and even showed a slight increase in NA.

Active avoidance

No effect of the severe NA depletions of forebrain regions was seen on either acquisition or retention of the active-avoidance task. Both control and lesioned groups took about 19 trials to reach acquisition criterion and showed significant 24 h retention, requiring only 11 trials to reattain this criterion (Table 2). However, on extinction, when the unconditioned stimulus was no longer present, the lesioned animals continued responding for longer than controls. Extinction criterion was reached in 15 trials for control rats, but the lesioned animals required more than 35 trials to cease responding. Two lesioned animals were still responding at 60 trials, at which point

Table 1 Effects of dorsal bundle 6-hydroxydopamine injections on monoamine concentrations in various brain areas of the rat

Region	*Controls (n = 10)*	*Lesioned (n = 10)*	%
	Noradrenaline		
	(μg/g wet wt.)	*(μg/g wet wt.)*	
Cortex	0.289 ± 0.014	0.009 ± 0.006	3
Hippocampus	0.305 ± 0.018	0.015 ± 0.003	5
Hypothalamus	2.38 ± 0.11	0.86 ± 0.9	36
Amygdala	0.408 ± 0.005	0.061 ± 0.004	15
Septum	0.954 ± 0.029	0.435 ± 0.034	46
Cerebellum	0.219 ± 0.012	0.271 ± 0.008	124
Spinal cord	0.255 ± 0.006	0.307 ± 0.012	120
	Dopamine		
	(μg/g wet wt.)	*(μg/g wet wt.)*	
Striatum	13.57 ± 0.66	12.84 ± 1.23	95
Amygdala	0.088 ± 0.009*	0.066 ± 0.027*	75
Septum	0.65 ± 0.07	0.50 ± 0.02	77
Hypothalamus	0.452 ± 0.019	0.421 ± 0.026	93

Post-mortem amine assays. Values are means ± s.e. means. % column is the percentage of control concentration remaining in lesioned tissues. * indicates that caution is required with this measure since it was barely twice blank. Values of amygdala and septum are the result of pooling two animals per determination.

Table 2 Acquisition, retention and extinction of active avoidance for control and lesioned rats

	Controls (n = 10)	*Lesioned (n = 10)*	P
Acquisition			
Trials to criterion	18.9	18.9	NS
Shocks taken	5.7	6.4	NS
Retention			
Trials to criterion	11.3	12.6	NS
Shocks taken	1.8	2.1	NS
Extinction			
Trials to criterion	15.1	37.9	0.01

P column indicates the significance level (two-tailed) of any group difference. NS = not significant.

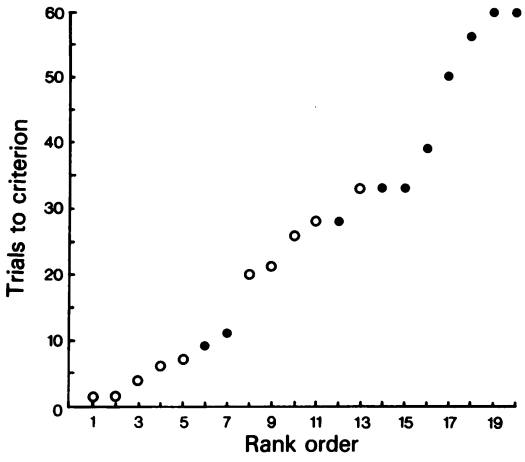

Figure 1 Scatter diagram of trials to extinction criterion for ten control (○) and ten lesioned (●) rats on active avoidance. Display was obtained by ranking all the animals as one group, in order of reaching extinction criterion and then plotting the number of trials to criterion against the rank order, preserving the identity of each point as control or lesioned. The control animals extinguish more quickly and so tend to cluster to the bottom left compared to the lesioned animals on the top right of the figure

testing was abandoned. This difference was highly significant as confirmed by a Mann-Whitney U test (Siegel, 1956) between the two groups, $U = 14$, $P < 0.01$. Figure 1 is a scatter diagram of the number of trials required by animals in the two groups to reach the extinction criterion and it can be seen that the two groups separate with little overlap.

No change in the sensitivity to electric footshock was seen as a result of the lesion (Table 3) with the flinch-jump thresholds being indistinguishable between control and lesioned rats.

Discussion

Despite depletion of forebrain NA in many areas to less than 5% of control concentrations no acquisition or retention deficit was seen in active avoidance learning. This is further evidence that NA is not critical for learning, contrary to suggestions of one recent theory of NA function (Crow, 1973). Further evidence in this vein is available elsewhere (Amaral & Foss, 1975; Mason & Iversen, 1975; 1977a, b & c; Roberts, Price & Fibiger, 1976; Sessions, Kant & Koob, 1976; Koob, Kelley & Mason, 1978).

Comparison of the pattern of amine depletion produced in the present study with that reported by Ashford & Jones (1976) enable us to assign the behavioural deficits observed by these workers to different neurotransmitters. With a pure NA depletion we found no acquisition or retention deficit, thus suggesting that those seen in the former study were due to loss of brain dopamine. This is consistent with reports of acquisition and retention deficits after procedures which selectively deplete dopamine while sparing brain NA (Fibiger, *et al.*, 1974; Cooper *et al.*, 1974). One common element found in both studies was a resistance to extinction. This suggests that it is the NA depletion common to the two studies which underlies the extinction effect. This is consistent with previous data in which resistance to extinction has been obtained in a number of food-rewarded situations after a pure NA depletion which did not affect brain dopamine (Thornton, Goudie & Bithell, 1975; Mason & Iversen, 1975; 1977a & b; Mason & Fibiger, 1977; Tremmel, Morris & Gehart, 1977). Although our present lesions depleted NA from both dorsal and ventral projection systems, previous work has located the resistance to extinction to the dorsal bundle (Mason & Iversen, 1975; 1977b; Tremmel *et al.*, 1976). It is still unknown which particular terminal area innervated by the dorsal bundle is of critical importance although our present data would suggest that it is unlikely to be the septum, which suffered only a 52% depletion. In other systems such a depletion would not be effective in causing any behavioural alteration (Creese & Iversen, 1975).

The mechanism involved in the dorsal bundle extinction effect has been investigated elsewhere (Mason & Iversen, 1977a, & c; 1978a) and the present data add to these results by showing that the mechan-

Table 3 Flinch-jump thresholds for control and lesioned rats in response to inescapable electric footshock

	Controls (n = 10)	*Lesioned (n = 10)*	P
Flinch (microamps)	312	258	NS
Jump (hindpaws)	735	642	NS
Vocalise	660	642	NS

P column indicates that the two groups failed to differ on all measures.

ism must be able to account for the resistance to extinction observed in behaviour maintained by negative reinforcers (footshock) as well as by positive reinforcers. One possibility couched in attentional terms (Segal & Bloom, 1976; Mason & Iversen, 1977a) appears to hold promise (Mason & Iversen, 1978b).

References

AMARAL, D.G. & FOSS, J.A. (1975). Locus coeruleus and learning. *Science*, **188**, 377–378.

ASHFORD, J. & JONES, B.J. (1976). The effects of intra-amygdaloid injections of 6-hydroxydopamine on avoidance responding in rats. *Br. J. Pharmac.*, **56**, 255–261.

COOPER, B.R., HOWARD, J.L., GRANT, L.D., SMITH, R.D. & BREESE, G.R. (1974). Alteration of avoidance and ingestive behavior after destruction of central catecholamine pathways with 6-hydroxydopamine. *Pharmac. biochem. Behav.*, **2**, 639–649.

CREESE, I. & IVERSEN, S.D. (1975). The pharmacological and anatomical substrates of the amphetamine response in the rat. *Brain Research*, **83**, 419–436.

CROW, T.J. (1973). Catecholamine-containing neurones and electrical self-stimulation: II. A theoretical interpretation and some psychiatric implications. *Psychol. Med.*, **3**, 66–73.

EVANS, W.O. (1961). A new technique for the investigation of some analgesic drugs on reflexive behavior in the rat. *Psychopharmac.*, **2**, 318–325.

FIBIGER, H.C., PHILLIPS, A.G. & ZIS, A.P. (1974). Deficits in instrumental responding after 6-hydroxydopamine lesions of the nigro-neostriatal dopaminergic projection. *Pharmac. biochem. Behav.*, **2**, 87–96.

KONIG, J.F. & KLIPPEL, R.A. (1963). *The Rat Brain, a Stereotaxic Atlas*. Baltimore: Williams and Wilkins Co.

KOOB, G.F., KELLEY, A.F. & MASON, S.T. (1978). Locus coeruleus lesions; learning and extinction. *Physiol. Behav.*, **20**, 709–716.

LINDVALL, O. & BJORKLUND, A. (1974). The organization of the ascending catecholamine neuron system in the rat brain as revealed by the glyoxilic acid fluorescence method. *Acta physiol. scand.*, suppl., **412**, 1–48.

MASON, S.T. & FIBIGER, H.C. (1977). Altered exploratory behavior after 6-OHDA lesion to the dorsal noradrenergic bundle. *Nature*, **269**, 704–705.

MASON, S.T. & IVERSEN, S.D. (1975). Learning in the absence of forebrain noradrenaline. *Nature*, **258**, 422–424.

MASON, S.T. & IVERSEN, S.D. (1977a). Effects of selective forebrain noradrenaline loss on behavioral inhibition in the rat. *J. comp. Physiol. Psychol.*, **91**, 165–173.

MASON, S.T. & IVERSEN, S.D. (1977b). An investigation of the role of cortical and cerebellar noradrenaline in associative motor learning in the rat. *Brain Research*, **134**, 513–527.

MASON, S.T. & IVERSEN, S.D. (1977c). Behavioral basis of the dorsal bundle extinction effect. *Pharmac. biochem. Behav.*, **7**, 373–379.

MASON, S.T. & IVERSEN, S.D. (1978a). The dorsal noradrenergic bundle, extinction and non-reward. *Physiol. Behav.*, (in press).

MASON, S.T. & IVERSEN, S.D. (1978b). Reward, attention and the dorsal noradrenergic bundle. *Brain Research*, **150**, 135–148.

MCGEER, E.G. & MCGEER, P.L. (1962). Catecholamine content of spinal cord. *Can. J. Biochem*, **40**, 1141–1151.

MOORE, R.Y. (1978). Catecholamine innervation of the basal forebrain. I. The septal area. *J. comp. Neurol.*, **177**, 665–684.

PRICE, M.T.C. & FIBIGER, H.C. (1975). Discriminated escape learning and response to electric shock after 6-hydroxydopamine lesions of the nigro-neostriatal dopaminergic projection. *Pharmac. biochem. Behav.*, **3**, 285–290.

ROBERTS, D.C.S., PRICE, M.T.C. & FIBIGER, H.C. (1976). The dorsal tegmental noradrenergic projection: an analysis of its role in maze learning. *J. comp. Physiol. Psychol.*, **90**, 363–372.

ROSS, R.A. & REIS, D.J. (1974). Effects of lesions of locus coeruleus on regional distribution of dopamine-beta-hydroxylase activity in rat brain. *Brain Research*, **73**, 161–166.

SEGAL, M. & BLOOM, F.E. (1976). The action of norepinephrine in rat hippocampus. IV. effects of locus coeruleus stimulation on evoked hippocampal unit activity. *Brain Research*, **107**, 513–525.

SESSIONS, G.R., KANT, G.J. & KOOB, G.F. (1976). Locus coeruleus lesions and learning in the rat. *Physiol. Behav.*, **17**, 853–859.

SIEGEL, S. (1956). *Nonparametric Statistics*. New York: McGraw-Hill.

THORNTON, E.W., GOUDIE, A.J. & BITHELL, V. (1975). The effects of neonatal 6-hydroxydopamine induced sympathectomy on response inhibition in extinction. *Life Science*, **17**, 363–368.

TREMMEL, F., MORRIS, M.D. & GEBHART, G.F. (1977). The effect of forebrain norepinephrine depletion on two measures of response suppression. *Brain Research*, **126**, 185–188.

UNGERSTEDT, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand.*, suppl. **367**, 1–49.

(*Received May 2, 1978.*)