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THE EFFECTS OF INTRA-AMYGDALOID INJECTIONS OF 6-HYDROXY-DOPAMINE ON AVOIDANCE RESPONDING IN RATS

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1 The effects of bilateral intra-amygdaloid injections of 6-hydroxydopamine (6-OHDA) on shuttle box avoidance acquisition, retention, and extinction, and passive avoidance acquisition were examined in rats.

2 Intra-amygdaloid 6-OHDA injections produced catecholamine depletion in and around the amygdalae but failed to reduce striatal dopamine concentrations.

3 Conditioned avoidance acquisition was markedly inhibited in 6-OHDA-treated rats whereas retention and extinction were only slightly impaired.

4 Passive avoidance acquisition was slightly but significantly improved in rats with amygdaloid 6-OHDA lesions.

5 Treated rats showed no motor abnormalities, they were not hypoactive in a photocell activity cage and they performed as well as controls on a rotating rod.

6 It is suggested that the conditioned avoidance acquisition deficit in rats with amygdaloid 6-OHDA lesions may be related to an impairment of associative learning rather than to perceptual or motor disturbances.

Introduction

Although the distribution of the main catecholamine-containing pathways in the brain is now well established (Fuxe, Hökfelt & Ungerstedt, 1970; Ungerstedt, 1971), a formidable amount of work remains to be undertaken to discover the functions of these pathways. We are particularly interested in the functions of the catecholaminergic projections to limbic areas in relation to behaviour and our present studies have been confined to the amygdala, an area rich in noradrenaline and dopamine-containing terminals (Ungerstedt, 1971).

Among the numerous aspects of behaviour modified by stimulation or ablation of the amygdala, avoidance responding has been studied extensively (see Richardson, 1973). Furthermore, it has been demonstrated recently that this particular parameter can be modified by specific lesions of catecholaminergic pathways in the brain produced by stereotactic injections of 6-hydroxydopamine (6-OHDA) (Fibiger, Phillips & Zis, 1974; Cooper, Howard, Grant, Smith & Breese, 1974). It was pertinent therefore, to study avoidance responding in rats with amygdaloid 6-OHDA lesions.

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Accordingly, attempts were made to produce specific bilateral lesions of the catecholaminergic projections to the amygdala in rats by injection of 6-OHDA directly into the terminal areas. Subsequently, the rats were tested for acquisition, retention and extinction of a two-way shuttle box avoidance response and acquisition of a passive avoidance response.

Methods

Male hooded rats, weighing between 200 and 220 g at operation were used throughout these studies.

Intra-amygdaloid 6-hydroxydopamine injections

6-OHDA was injected bilaterally into the amygdalae using stereotactic surgery under ether anaesthesia. The coordinates used were determined histologically but were based on the atlas of König & Klippel (1963), viz: 4.6 mm anterior, 3.4 mm lateral and 2.3 mm below zero.

6-OHDA was dissolved in 0.9% w/v NaCl solution (saline) containing 0.2 mg/ml ascorbic acid and kept cool on ice; 4 µl of solution was injected from an 'Agla' micrometer syringe at the rate of 0.2 µl/10 seconds. Control animals received identical injections of the vehicle alone.

6-OHDA hydrobromide was obtained from Sigma Chemical Co. and doses quoted (4, 8 or 16 μg) refer to the base.

Monoamine determinations

Following completion of the behavioural experiments, rats were stunned, decapitated and their brains were removed and dissected rapidly at 0°C. The dissection procedure was modified from the method of Glowinski & Iversen (1966). From the area designated 'cortex' by these authors, the portions immediately adjacent to the hypothalamus and bounded laterally by the external capsule were designated 'amygdalae' and the portions immediately lateral to the 'amygdalae' were referred to as 'periamygdaloid cortex'. In addition, the rostral and caudal portions of the remaining 'cortex' were not pooled, but referred to as 'frontal cortex' and 'cortex' respectively. The 'hippocampus' was included in the 'midbrain' portion.

Immediately after dissection, tissues were frozen and stored in liquid air. After homogenization in 0.4M perchloric acid, samples were centrifuged. Separation of the monoamines in the supernatant was achieved by ion-exchange chromatography (Atack & Magnusson, 1970). Noradrenaline was assayed by the method of Euler & Lishajko (1961), dopamine according to Atack (1973) and 5-hydroxytryptamine by the method of Maickel, Cox, Saillant & Miller (1968).

Conditioned avoidance experiments

A standard Ugo Basile rat shuttle box was used throughout the experiments.

(a) *Acquisition* Fourteen days after surgery, rats were given 5 sessions of 50 trials in the shuttle box, one per day for 5 consecutive days. Each trial consisted of a 5 s conditioned stimulus (CS) (light and buzzer), followed by a 10 s unconditioned stimulus (UCS) (0.5 mA footshock) during which the CS remained on. The sequence was terminated at any time by the rat crossing into the other compartment. Trial frequency was once every 30 seconds.

(b) *Retention* Before surgery, the rats were conditioned for 5 daily sessions in the shuttle box as above. Operations were performed one day after the last session and a further 14 days later, the animals were tested again for 5 daily sessions.

(c) *Extinction* Extinction of response was examined in the rats tested for acquisition and retention by subjecting them to a final 5 sessions in which the UCS was not applied.

Passive avoidance acquisition

The shuttle box was modified so that the whole of the grid floor was continuously electrified. The central partition was replaced by a wooden platform, 6 cm wide and 2 cm high, which spanned the width of the cage.

Fourteen days after operation, each rat was placed on the central platform and left in the apparatus for 3 minutes. The number of times the rats stepped on to the grid and the total time spent on the grid were recorded. A total of 5 consecutive daily sessions were given.

Determination of pain threshold

Immediately before the first conditioned avoidance acquisition trial, each rat was placed in the shuttle box and footshock was applied in increasing intensity, stepwise at 0.05 mA every 30 s, until the animals showed visible signs of discomfort. The current intensity at this point was considered to provide an estimate of pain threshold.

Food and water intake

Food and water were provided *ad lib* but the amounts removed by the rats were determined daily. The estimates of consumption were approximate, because spillage was not taken into account.

Spontaneous locomotor activity

Fourteen days after operation, each rat was placed in a photocell activity cage (Lehigh Valley Electronics). After a 10 min acclimatization period, the number of beam interruptions occurring in 20 min was recorded. This procedure was repeated daily for a further 4 days.

Performance on a rotating rod

The apparatus consisted of a motor-driven 6 cm diameter acrylic rod with a finely corrugated surface. The speed of rotation was controlled manually through a 'Variac' variable voltage transformer.

Fourteen days after operation, rats were placed individually on a partitioned 10 cm section of the rod. According to the principle of Jones & Roberts (1968), the speed of rotation was increased approximately linearly from rest to 28 rev/min in 40 s by pre-determined manual adjustments of the speed control. The times at which the rats fell from the rod were recorded.

Results

Effects on brain monoamine concentrations

Intra-amygdaloid injections of 6-OHDA produced depletion of noradrenaline and dopamine in the amygdala but also in some of the surrounding areas

(Table 1). Confined but slight depletion was obtained with 4 μg 6-OHDA, less confined but greater depletion with 8 μg and widespread depletion was observed with 16 μg.

5-Hydroxytryptamine concentrations were not significantly altered by 6-OHDA.

Conditioned avoidance

6-OHDA produced a dose-related inhibition of conditioned avoidance acquisition (Figure 1), but retention of an acquired response was impaired only by the high dose (8 μg) (Figure 2). Regardless of whether the response had been fully acquired or not, 6-OHDA produced a slight but significant antagonism of response extinction (Figures 1 and 2). However, the effect did not appear to be dose-related.

None of the rats showed any overt signs of motor abnormality, nor was there any indication that their perception of the CS was impaired. However, the 6-OHDA-treated rats exhibited less fearfulness than controls on presentation of the CS during the early stages of acquisition.

Some experiments were performed on rats treated with 16 μg 6-OHDA and there was an almost complete absence of acquisition. However, since the biochemical data indicated that this treatment produced widespread catecholamine depletion, it was subsequently abandoned.

Passive avoidance acquisition

All rats fully acquired the response by the third session. Although there was no difference between the

Table 1 Effects of intra-amygdaloid 6-hydroxydopamine (6-OHDA) injections on monoamine concentrations in various parts of rat brain

	Controls	*4 μg 6-OHDA*	*8 μg 6-OHDA*	*16 μg 6-OHDA*
(a) Noradrenaline (n = 15)				
Medulla	0.58 ± 0.03	0.50 ± 0.02	0.55 ± 0.03	0.55 ± 0.04
Cerebellum	0.28 ± 0.04	0.27 ± 0.03	0.29 ± 0.03	0.30 ± 0.04
Hypothalamus	3.14 ± 0.12	2.65 ± 0.14*	1.49 ± 0.13*	1.06 ± 0.16*
Amygdala	1.74 ± 0.12	1.00 ± 0.14*	0.75 ± 0.03*	0.68 ± 0.08*
Periamygdaloid cortex	0.91 ± 0.04	0.49 ± 0.09*	0.35 ± 0.03*	0.30 ± 0.05*
Striatum	0.37 ± 0.03	0.35 ± 0.03	0.36 ± 0.03	0.23 ± 0.06*
Midbrain	0.57 ± 0.08	0.55 ± 0.07	0.42 ± 0.05	0.30 ± 0.07
Frontal cortex	0.50 ± 0.08	0.28 ± 0.04*	0.25 ± 0.02*	0.28 ± 0.03*
Cortex	0.25 ± 0.03	0.23 ± 0.04	0.18 ± 0.03*	0.16 ± 0.05*
(b) Dopamine (n = 9)				
Medulla	0.30 ± 0.02	0.40 ± 0.05	0.42 ± 0.02	0.42 ± 0.06
Cerebellum	0.31 ± 0.03	0.29 ± 0.03	0.29 ± 0.02	0.27 ± 0.03
Hypothalamus	1.65 ± 0.15	1.43 ± 0.11	1.25 ± 0.15*	1.09 ± 0.04*
Amygdala	1.60 ± 0.21	1.10 ± 0.09*	0.92 ± 0.03*	0.80 ± 0.02*
Periamygdaloid cortex	1.27 ± 0.20	0.82 ± 0.04*	0.78 ± 0.08*	0.61 ± 0.07*
Striatum	5.52 ± 0.63	5.49 ± 0.38	5.40 ± 0.02	4.65 ± 0.02*
Midbrain	0.50 ± 0.03	0.52 ± 0.10	0.56 ± 0.08	0.35 ± 0.07*
Frontal cortex	2.18 ± 0.19	1.95 ± 0.40	1.00 ± 0.06*	0.80 ± 0.05*
Cortex	0.84 ± 0.05	0.81 ± 0.16	0.82 ± 0.03	0.59 ± 0.02*
(c) 5-hydroxytryptamine (n = 15)				
Medulla	0.66 ± 0.05	0.69 ± 0.02	0.60 ± 0.04	0.63 ± 0.04
Cerebellum	0.22 ± 0.07	0.19 ± 0.05	0.20 ± 0.03	0.19 ± 0.04
Hypothalamus	3.10 ± 0.58	3.88 ± 0.80	3.68 ± 0.61	2.83 ± 0.71
Amygdala	1.30 ± 0.16	1.23 ± 0.17	1.40 ± 0.13	1.33 ± 0.17
Periamygdaloid cortex	0.82 ± 0.15	0.83 ± 0.10	0.70 ± 0.09	0.75 ± 0.02
Striatum	1.65 ± 0.12	1.32 ± 0.12	1.53 ± 0.11	1.50 ± 0.12
Midbrain	1.05 ± 0.21	1.26 ± 0.18	1.18 ± 0.09	1.00 ± 0.26
Frontal cortex	0.74 ± 0.08	0.73 ± 0.04	0.78 ± 0.04	0.70 ± 0.05
Cortex	0.37 ± 0.05	0.33 ± 0.04	0.34 ± 0.02	0.36 ± 0.05

Figures are means (μg/g) ± s.e. mean.
* Significantly different from control ($P < 0.05$) as determined by t tests.

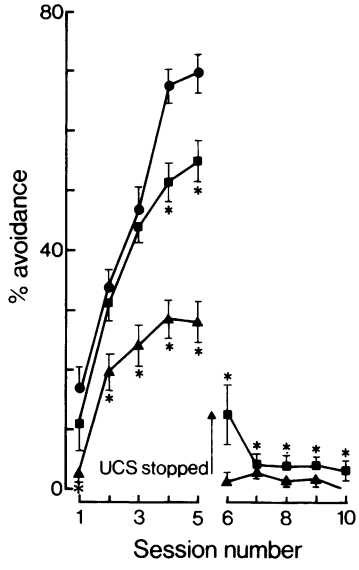

Figure 1 The acquisition and extinction of shuttle-box avoidance responses in rats after intra-amygdaloid injections of 6-hydroxydopamine (6-OHDA). Fourteen days after surgery, rats were given daily sessions (1–5) of 50 trials on 5 consecutive days. Subsequently, 5 sessions (6–10) were performed without the unconditioned stimulus (UCS). Extinction of response in controls was complete in session 6. (●) Control; (■) 4 μg 6-OHDA; (▲) 8 μg 6-OHDA. Points represent mean responses of 10 rats and the vertical bars show s.e. mean. *Significantly different from controls ($P < 0.05$) as determined by U tests.

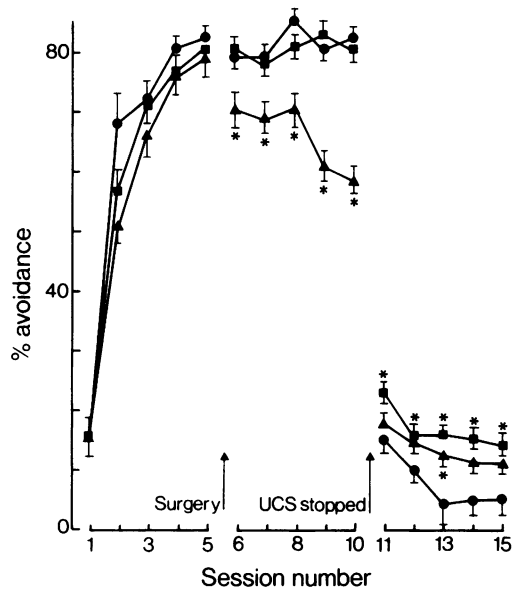

Figure 2 The retention and extinction of shuttle-box avoidance responses in rats after intra-amygdaloid injections of 6-hydroxydopamine (6-OHDA). Rats were given daily sessions (1–5) of 50 trials on 5 consecutive days. 6-OHDA was injected one day after session 5 and a further 14 days later, the rats were subjected to 5 more daily sessions (6–10). A final series of 5 sessions was performed without the unconditioned stimulus (UCS). (●) Control; (■) 4 μg 6-OHDA; (▲) 8 μg 6-OHDA. Points represent mean responses of 10 rats and the vertical bars show s.e. mean *Significantly different from controls ($P < 0.05$) as determined by U tests.

performances of the controls and the 4 μg 6-OHDA-treated group, the rats treated with 8 μg 6-OHDA exhibited a significantly greater tendency to avoid the shock on the second session (Table 2).

Pain threshold

6-OHDA-treated animals were indistinguishable from controls with respect to the intensity of footshock required to elicit a pain response (Table 3).

Food and water intake

Temporary inhibition of both food and water intake was observed in 6-OHDA-treated animals, with a complete return to normal intake six days after operation (Table 4). Although no mortalities occurred in the reported experiments, it has been observed in subsequent experiments that a mortality rate of up to 20% may be encountered with 8 μg 6-OHDA unless forced feeding is instituted.

Spontaneous locomotor activity

The data in Table 5 are presented as logarithms because this transformation was found to be most suitable for reducing heterogeneity of variance (Jones, Tolman & Roberts, 1970). The 6-OHDA-treated rats tended to be slightly more active than controls but on no occasion was there a statistically significant difference.

Performance on a rotating rod

There were no significant differences in the mean times at which controls and 6-OHDA-treated rats fell from the rotating rod (Table 6).

Table 2 The effects of intra-amygdaloid 6-hydroxydopamine (6-OHDA) injections on passive avoidance acquisition

(a) Mean number of shocks received (± s.e. mean) during 3 min

Session number	*Controls (n=15)*	*4 μg 6-OHDA (n=15)*	*8 μg 6-OHDA (n=14)*
1	11.1 ± 1.7	11.6 ± 1.2	8.6 ± 0.9
2	3.6 ± 1.1	4.4 ± 1.3	1.7 ± 0.5*
3	0	0	0
4	0	0	0
5	0	0	0

(b) Mean duration of shock received during 3 min (s ± s.e. mean)

Session number	*Controls*	*4 μg 6-OHDA*	*8 μg 6-OHDA*
1	126 ± 29	147 ± 21	102 ± 19
2	13.9 ± 5.8	16.0 ± 7.2	3.0 ± 2.4*
3	0	0	0
4	0	0	0
5	0	0	0

* Significantly different from controls ($P < 0.05$) as determined by *t* tests.

Table 3 The effects of intra-amygdaloid 6-hydroxydopamine (6-OHDA) injections on pain threshold

Control	*4 μg 6-OHDA*	*8 μg 6-OHDA*
0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01

The figures are mean threshold footshock intensities in mA ± s.e. mean. $n = 10$ in each case.

Table 4 The effects of intra-amygdaloid injections of 6-hydroxydopamine (6-OHDA) on food and water intake

(a) Mean daily food consumption (g/kg bodyweight ± s.e. mean)

	A	B	C	D
Controls	105 ± 5	91 ± 4	107 ± 2	109 ± 3
4 μg 6-OHDA	104 ± 4	73 ± 5*	102 ± 31	103 ± 4
8 μg 6-OHDA	109 ± 4	71 ± 5*	92 ± 3	108 ± 2

(b) Mean daily water consumption (cm^3/kg bodyweight ± s.e. mean)

	A	B	C	D
Controls	145 ± 8	164 ± 7	162 ± 6	143 ± 3
4 μg 6-OHDA	136 ± 7	126 ± 3*	149 ± 5	127 ± 5
8 μg 6-OHDA	151 ± 5	110 ± 9*	156 ± 3	137 ± 3

A = consumption on days 1–5 before surgery; B = consumption on days 1–5 after surgery; C = consumption on days 6–10 after surgery; D = consumption on days 11–15 after surgery.
$n = 10$ in each case. *Significantly different from controls ($P < 0.05$) as determined by *t* tests.

Table 5 The effects of intra-amygdaloid 6-hydroxydopamine (6-OHDA) injections on spontaneous locomotor activity

Session number	*Controls*	*4 μg 6-OHDA*	*8 μg 6-OHDA*
1	2.269 ± 0.063	2.345 ± 0.078	2.348 ± 0.038
2	2.184 ± 0.119	2.215 ± 0.132	2.359 ± 0.054
3	1.822 ± 0.159	1.863 ± 0.204	1.988 ± 0.154
4	1.861 ± 0.113	1.884 ± 0.195	2.140 ± 0.109
5	1.705 ± 0.188	1.935 ± 0.216	1.770 ± 0.145

Figures are the mean numbers of beam interruptions (as logarithms) ± s.e. occurring in 20 minutes. $n = 20$ in each case.

Table 6 The effects of intra-amygdaloid 6-hydroxydopamine (6-OHDA) injections on performance on a rotating rod

	Mean time of falling from rod (s) ± s.e. mean
Controls	28.6 ± 4.7
4 μg 6-OHDA	25.6 ± 3.1
8 μg 6-OHDA	28.3 ± 5.3

$n = 10$ in each case.

Discussion

The catecholamine depletion following intra-amygdaloid injections of 6-OHDA extended to extra-amygdaloid areas, so it was not possible to draw any conclusions about catecholamine function within the amygdala from our present results. Nevertheless, the extent of depletion was considerably less than would be obtained by intraventricular or intranigral injections of 6-OHDA.

The latter procedure is normally adopted for producing destruction of the nigro-striatal dopaminergic pathways, but amygdaloid projections are closely associated with these pathways (Ungerstedt, 1971) and would be expected to be destroyed also.

Recently, it was shown that bilateral intranigral injections of 6-OHDA impaired the acquisition of a one-way conditioned avoidance response (CAR) in rats (Fibiger *et al.*, 1974). This effect could be almost completely overcome by the administration of L-DOPA (Zis, Fibiger & Phillips, 1974), suggesting that depletion of striatal dopamine was possibly the most significant contributory factor to the CAR impairment. Support for this conclusion came from the work of Cooper, *et al.* (1974), who demonstrated pronounced CAR acquisition impairment produced by either destruction of the nigro-striatal pathways or intracaudate 6-OHDA injections.

We have demonstrated that impairment of CAR acquisition can be produced by bilateral intra-amygdaloid injections of 6-OHDA, which fail to produce striatal dopamine depletion. It is therefore possible that the deficit obtained by the above authors may be partly related to catecholamine depletion in extra-striatal areas.

Impairment of CAR acquisition could be due to a number of causes. Since our results were obtained in a two-way avoidance situation, we considered the possibility that the 6-OHDA-treated rats had an enhanced passive avoidance component of the shuttle response, i.e. they had an increased reluctance to return to the compartment in which they were shocked. We attempted to test this theory directly by studying a passive avoidance response. However, such a simple response is rapidly acquired by normal rats and improvements are therefore not readily discernible. Nevertheless, the rats treated with 8 μg 6-OHDA exhibited a slight but significant improvement in passive avoidance acquisition. This result cannot be considered definitive, but there is little doubt that 6-OHDA-treated rats did not have a passive avoidance deficit, indicating that there was no general learning impairment. Similar results were obtained by Cooper, Breese, Grant & Howard (1973) following intra-cisternal injection of 6-OHDA in rats. Their subjects failed to acquire either a one-way or a two-way active avoidance response yet were as capable as controls in learning a passive avoidance response. Such a profile of behavioural responses could simply reflect an impaired motor performance and this criticism is particularly relevant to studies in which the nigro-striatal dopamine pathways have been destroyed, since hypokinesia and rigidity may ensue (Jurna, Ruždić, Nell & Grossmann, 1972; Ungerstedt, Avemo, Avemo, Ljungberg & Ranje, 1973).

Our biochemical results indicated that intra-amygdaloid injections of 6-OHDA had spared the nigro-striatal pathways. Furthermore, 6-OHDA-treated rats showed no motor defects and they could

not be differentiated from controls in spontaneous locomotor activity and rotating rod experiments.

The slight but significant inhibition of retention caused by intra-amygdaloid 6-OHDA injections was similar to the effect seen by Fibiger *et al.* (1974) following intranigral administration of 6-OHDA. The importance of this finding cannot be assessed because retention was studied two weeks after the last acquisition trial and a certain amount of re-acquisition may have been required. Thus, an acquisition deficit could readily account for the small changes observed.

An obvious possible cause of failure to acquire a CAR in the presence of an intact escape response could be an inability to perceive the CS. However, observation of rats with amygdaloid 6-OHDA lesions during presentation of the CS indicated that they perceived the CS although they appeared less fearful than controls. Lack of fearfulness is unlikely to be the direct cause of the acquisition failure, however, because lesioned rats exhibited sufficient fear of footshock to acquire rapidly a passive avoidance response. The apparent lack of fearfulness may merely reflect a failure to associate the CS with impending footshock. Indeed, it is possible that a disruption of the associative learning required to perform a CAR is the cause of the acquisition failure in rats with amygdaloid 6-OHDA lesions.

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