BEHAVIOURAL PHARMACOLOGY OF 6-HYDROXYDOPAMINE

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THE ABILITY of 6-hydroxydopamine (6-HDA) to produce a specific degeneration of central catecholamine-containing neurons offers a unique opportunity to study the consequences of loss of these neurons on the performance of a conditioned response. At the same time we are provided with a useful experimental tool for testing present theories of the mechanism of action of drugs whose behavioural effects are thought to be mediated via an effect on these neurons. The present report will discuss several experiments utilizing 6-HDA treated rats, which were designed with this dual significance in mind.

6-HDA AND OPERANT BEHAVIOUR

Studies of the effects of drugs on operant behaviour have shown that the schedule of reinforcement maintaining behaviour is a prime determinant of drug action (KELLEHER and MORSE, 1968). The principle of schedule-dependency extends to the behavioural effects of amine-depleting drugs. Reserpine (SMITH, 1964), tetrabenazine (MCKEARNY, 1968) and α-methyltyrosine (SCHOENFELD and SEIDEN, 1967, 1969) have all been shown to decrease responding maintained by some schedules at a dose which had less or no effect on responding maintained by other schedules. In light of the accumulating evidence that the turnover of brain dopamine (DA) and norepinephrine (NE) is increased during performance of a conditioned response (FUXE and HANSON, 1967; SCHOENFELD and SEIDEN, 1969; ARBUTHNOTT *et al.*, 1971; LEWY and SEIDEN, 1972), it has been suggested that behaviour maintained by schedules generating higher turnover rates would be most sensitive to these drugs (HARVEY, 1971). Although any proposed mechanism is speculative, it remains essential that we recognise the existence of schedule-dependency. It is for this reason that we have studied the effects of 6-HDA on behaviour maintained by several different schedules of water reinforcement.

(1) *Fixed ratio* (FR)

This schedule generates a high response rate that can be maintained throughout the experimental session. After performance had stabilised on an FR-20 schedule, two doses of 250 μg 6-HDA or vehicle were administered intraventricularly to groups of six rats. Twenty-four hours after the second injection responding was reduced to 51·8± of the pre-injection control rate in the 6-HDA group, while the vehicle group responded at control rate. Responding during the next session (48 hr post-injection) was decreased to 67·1 ± 19% of control in the 6-HDA group, but by the third day (72 hr post-injection) responding had returned to control level and remained at this level for the duration of the experiment (Fig. 1a). Acquisition of performance on this schedule was unimpaired by administration of 6-HDA prior to training.

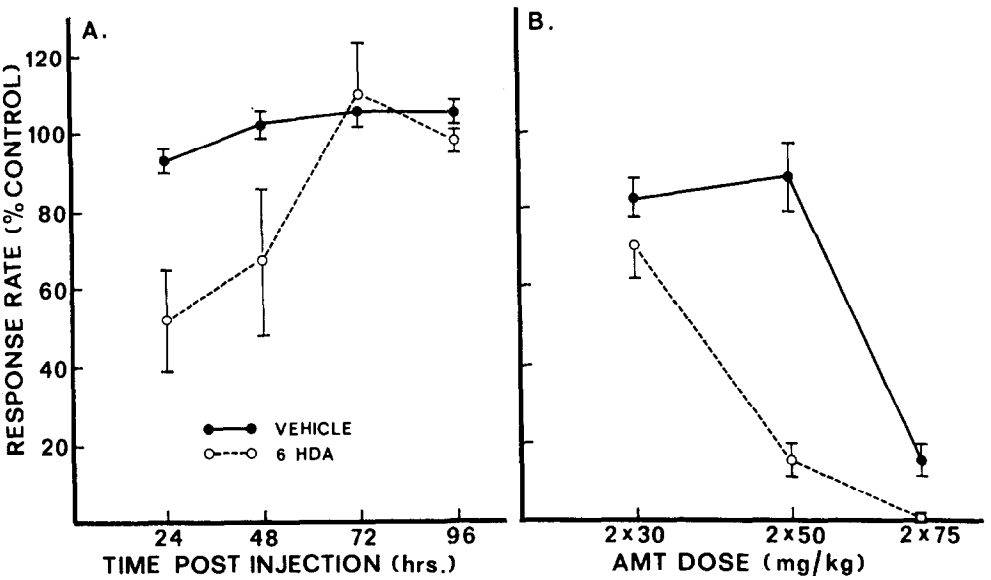

FIG. 1a.—Effect of 6-HDA on FR-20 performance. 250 μg of 6-HDA or vehicle was injected into alternate lateral ventricles (48 hr apart) under ether anesthesia. Twenty-four hr after the second injection testing was continued. Each point represents the mean (±S.E.M.) response output as a percentage of each group's mean response output for three days prior to injection ($N = 6$).

b. Effect of α-methyltyrosine (AMT) on FR-20 performance of 6-HDA- and vehicle-treated rats. D, L- α-methyl-tyrosine or saline was administered 6 and 3 hr prior to the session. Each point represents the mean (±S.E.M). response output as a percentage of the previous day's response output ($N = 6$).

Next we examined the effect of repeated injection (intracisternal) of 200 μg 6-HDA, given at weekly intervals, on FR 20 performance. When tested 1 hr after the first injection, responding was completely abolished in the 6-HDA-treated group. As after the 2 × 250 μg intraventricular treatment, this was followed by recovery to control levels of responding within 3 days. One week after the first injection (when responding was at control levels), a second 200 μg was administered followed by a third injection one week later. Again, after each injection, responding was abolished 1 hr post-injection, followed by recovery to control levels within a few days (SCHOENFELD and ZIGMOND, 1970).

(2) *Fixed interval* (FI)

On this schedule, responding is very low immediately following reinforcement and then rapidly accelerates to a final high rate that is sustained until the next reinforcement. This pattern of low followed by high response rates is repeated throughout the session. Rats treated with 6-HDA prior to training on an FI 3 min schedule developed a pattern of responding identical to control rats.

(3) *Variable interval* (VI)

Rats trained on this schedule have a moderate rate of responding that is sustained throughout the session. When 6-HDA was administered prior to training, the

response rate stabilised after 6 weeks (30 sessions) at a level approximately four times higher than control rats. If 6-HDA was administered to rats already trained on a VI 1·5 min schedule, responding increased over a period of 6 weeks after an initial decrease, until it again stabilised at a level approximately four times higher than control rats. Superimposing a shock contingency on the VI schedule (every 30th response results in administration of an electric shock) was effective in suppressing responding of the 6-HDA-treated rats. Introduction of a time-out (TO) period into the VI schedule (during the middle 9 min of the session responding had no schedule consequences) also diminished responding, although it took several days for the 6-HDA-treated group to reach the same low level of responding observed in the control group (SCHOENFELD and URETSKY, 1972a).

TABLE 1. EFFECT OF DIFFERENT 6-HDA TREATMENTS ON VARIABLE INTERVAL PERFORMANCE AND BRAIN AMINE LEVELS

Treatment	Responses/Session*	Dopamine†	Norepinephrine
Vehicle	557 ± 107	0·46 ± 0·027	0·30 ± 0·009
3 × 50μg 6-HDA	525 ± 93	0·49 ± 0·021 (106·5%)	0·15 ± 0·007 (51·4%)
2(25mg/kg DMI + 250μg 6-HDA)	1459 ± 106	0·09 ± 0·008 (14·2%)	0·27 ± 0·019 (87·4%)

*Values represent mean (±S.E.M.) number of responses during the *fortieth* daily session following treatment ($N = 5$).

†Values represent mean (±S.E.M.) brain amine level (uncorrected for recovery) of groups of rats ($N = 5$ or more) treated identically to those used in behaviour experiment.

Values in parenthesis indicate percentage of control group.

The 6-HDA treatment used in the VI experiment, two injections of 250 μg 6-HDA, produced an 80–90 per cent decrease in brain NE and a 70–80 per cent decrease in brain DA. We have since attempted to reproduce the effect of 6-HDA on VI performance in rats selectively depleted of either DA or NE. Groups of five rats were treated with either three injections of 50 μg 6-HDA, to destroy NE-containing neurons, or two injections of 250 μg 6-HDA 1 hr after 25 mg/kg of desmethylimipramine (DMI), to affect only DA-containing neurons. The group treated with 3 × 50 μg 6-HDA, which lowered whole brain NE by 50 per cent, responded at control rates while the group treated with 2 × DMI + 250 μg 6-HDA, which decreased brain DA to 14 per cent of control, increased their response rate so that after 40 sessions they were responding at a level three times higher than the control group (Table 1).

In summary, 6-HDA produced an initial decrease in responding when administered to rats trained on FR and VI schedules. A few days after administration, responding on the FR schedule returned to normal. Normal response patterns also developed on the FI schedule as well as when as time-out or shock contingency was added to the VI schedule. These results demonstrate that except for the initial deficits observed, the 6-HDA treatment had little effect on the control of behaviour by environmental stimuli. In these animals reinforcing stimuli still maintain responding, discriminative stimuli still control responding (TO schedule) and aversive stimuli still suppress responding (shock schedule). Consequently, it might be concluded that catecholamine-containing neurons are not involved in these behavioural processes, which would be at variance with much experimental evidence to the contrary (see

above). Another possibility is that compensatory mechanisms exist to maintain normal response patterns in spite of the marked destruction of DA- and NE-containing neurons produced by 6-HDA.

Only when rats were responding on a VI schedule, where the relationship between external events and responding provides few cues to the organism, was a prolonged effect observed. Under these conditions 6-HDA-treated rats did not maintain the low response rate of control rats but gradually reached a new level of responding several times higher than controls. This occurred when DA neurons were selectively destroyed but not when only NE neurons were affected, suggesting that dopamine-containing neurons are involved in controlling the level of performance.

6-HDA AND DRUG EFFECTS ON OPERANT BEHAVIOUR

α- *Methyltrosine*

This drug has been shown to produce a specific depletion of DA and NE as a consequence of inhibition of catecholamine (CA) synthesis (SPECTOR *et al.*, 1965). It has been shown to decrease responding maintained by several different schedules of reinforcement (POSCHEL and NINTEMAN, 1966; FUXE and HANSON, 1967; SCHOENFELD and SEIDEN, 1967). That the effect of α-methyltyrosine (AMT) on operant behaviour is a consequence of CA depletion is supported by the finding that L-dopa can restore lever-pressing in AMT-treated rats (SCHOENFELD and SEIDEN, 1969).

We have found that AMT is effective in decreasing responding of 6-HDA-treated rats. In fact, 6-HDA-treated rats ($2 \times 250\ \mu g$) responding on an FR-20 schedule were affected by AMT at a dosage level that had no effect on control rats (Fig. 1b). This increased sensitivity to AMT has also been demonstrated in 6-HDA-treated rats responding on a VI schedule (SCHOENFELD and URETSKY, 1972a), on an FI schedule (SCHOENFELD, unpublished results) and on a CRF schedule (COOPER *et al.*, 1972). Considering the selectivity of the depletion produced by AMT and the fact that dopa can reverse its effects in normal rats, we take the effect of AMT on 6-HDA-treated rats as evidence that CA-containing neurons surviving 6-HDA treatment are involved in maintaining responding in these rats.

d-Amphetamine

d-Amphetamine has both rate-increasing and rate-decreasing effects on lever-pressing. These effects depend on the schedule of reinforcement maintaining responding (see KELLEHER and MORSE, 1968). Pre-treatment with AMT blocks the rate-increasing effect of *d*-amphetamine (RECH, 1970; MAICKEL *et al.*, 1970) but not the rate-decreasing effect (HEFFNER and ZIGMOND, unpublished observations), suggesting that the former effect is mediated via release of catecholamines while the latter effect is due to a direct action on CA receptors or involves some other neuronal population. We have studied both the rate-increasing and rate-decreasing effects of *d*-amphetamine in 6-HDA-treated rats trained on different schedules of reinforcement.

(1) FR: *d*-Amphetamine decreased responding on this schedule. There was no difference in the dose–response curves obtained from vehicle- and 6-HDA-treated rats (Fig. 2a).

(2) VI: A dose of 0·56 μg/kg of *d*-amphetamine increased responding of vehicle-treated rats on this schedule to approximately 160 per cent of the control rate. No

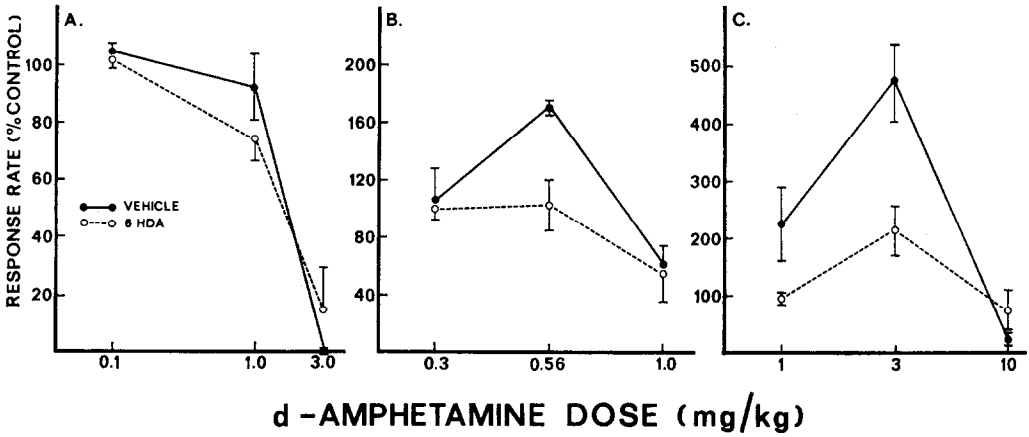

FIG. 2.—Effect of 6-HDA pre-treatment on the rate-increasing and rate-decreasing effect of *d*-amphetamine. *d*-Amphetamine sulphate was administered immediately before the test session to 6-HDA- and vehicle-treated rats ($N = 4$–6) performing on FR-20 (a), VI-1·5 min. (b), or FI-3 min. (c) schedules of reinforcement. Each point represents the mean (±S.E.M.) response output as a percentage of control for the entire session, except FI where the effect of *d*-amphetamine on responding during the initial 1·5 min of each 3 min interval is reported as a percentage of control responding during this time.

increase was observed in 6-HDA-treated rats at this dose or lower doses. Both groups showed a similar decrease in responding after 1 mg/kg *d*-amphetamine (Fig. 2b).

(3) FI: In vehicle-treated rats, *d*-amphetamine produced a large increase in the low rates of responding which occur during the initial part of each interval. Although an increase was observed in 6-HDA-treated rats, it was much less than that obtained in vehicle-treated rats (Fig. 2c).

The reduced effectiveness of *d*-amphetamine to increase responding on the FI schedule, in spite of the low response rate of 6-HDA-treated rats suggests that the loss of CA-containing neurons prevents the expression of its rate-increasing effect. This is supported by the inability of *d*-amphetamine to increase responding on the VI schedule. In this case, however, the higher response rate of 6-HDA-treated rats may make this schedule more like the FR schedule, in which case only rate-decreases would be expected. The unaltered rate-decreasing effect obtained on all schedules tested supports the suggestion that some action other than the release of catecholamines mediates this effect of *d*-amphetamine.

6-HDA AND CENTRAL DENERVATION SUPERSENSITIVITY

Several mechanisms may participate in the recovery of performance following 6-HDA treatment. One possibility is that changes in the synaptic region produce an increased sensitivity to released catecholamines. Based on the effects of apomorphine and L-dopa in 6-HDA-treated rats, it has been suggested that 6-HDA produces a post-synaptic type of supersensitivity in the CNS (UNGERSTEDT, 1971, SCHOENFELD and URETSKY, 1972b). However, determination of the dose–response curve for the effects of dopa on motor activity indicated that 6-HDA shifts the curve by a factor of ten toward lower doses. The time course of the increase in activity and brain

catecholamines produced by 100 mg/kg L-dopa, suggested that the effect was mediated by the conversion of L-dopa to DA, which did accumulate in 6-HDA-treated rats although to a lesser extent than in control rats. The increase in activity following this dose of L-dopa was apparent as soon as 24 hr after the second dose of 6-HDA ($2 \times 250\ \mu g$, 48 hr apart). At this time the uptake of ^{3}HDA was decreased by approximately 50 per cent. Consequently, on the basis of these results, it appears that a pre-synaptic type of supersensitivity to catecholamines is also a consequence of 6-HDA treatment (URETSKY and SCHOENFELD, 1971; SCHOENFELD and URETSKY, 1973).

SUMMARY

The long-term effect of 6-HDA on operant behaviour is schedule-dependent; normal response patterns are maintained on FR and FI schedules, while an increase in responding gradually develops on a VI schedule. This effect appears to be related to altered function of central dopamine-containing neurons. 6-HDA-treated rats are more sensitive to AMT, suggesting that CA-containing neurons surviving 6-HDA treatment participate in maintaining the normal patterns of responding observed on the FR and FI schedules. Maintenance of normal function may also involve the development of central denervation supersensitivity which, as indicated by experiments with L-dopa, occurs soon after 6-HDA treatment. The rate-decreasing effect of *d*-amphetamine is unaltered in 6-HDA-treated rats while the rate-increasing effect is attenuated, consistent with proposals that the rate-increasing effect is mediated by release of catecholamines.

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