Pharmacology Biochemistry & Behavior, Vol. 9, pp. 395–398. Printed in the U.S.A.

BRIEF COMMUNICATION

Suppression of Fixed-Interval Responding by Flavour-Amphetamine Pairings in Rats

G. D. D'MELLO AND I. P. STOLERMAN

MRC Neuropharmacology Unit, The Medical School, Birmingham B15 2TJ England

(Received 6 April 1978)

D'MELLO, G. D. AND I. P. STOLERMAN. *Suppression of fixed-interval responding by flavour-amphetamine pairings in rats.* PHARMAC. BIOCHEM. BEHAV. 9(3) 395–398, 1978.—Amphetamine is a potent and very effective drug for conditioning taste aversions, but much less is known about the possible effects of flavour-amphetamine pairings on aspects of behaviour other than eating and drinking. Rats were trained to press bars for water reinforcers delivered on a fixed-interval one-min schedule. Flavoured reinforcers were then substituted for the water and post-session injections of amphetamine (1 mg/kg) were given. Even a single flavour-amphetamine pairing produced some disruption of responding for that flavour, whereas 3 pairings almost completely suppressed responding (both bar-pressing and drinking). In the same rats, flavours paired with saline injections did not suppress responding. Amphetamine (1mg/kg) injected before sessions of responding for plain water disrupted the temporal pattern of fixed interval responding without affecting the total numbers of bar-presses or the amounts of liquid consumed. Omitting primary reinforcement (water) throughout a single session also failed to suppress responding. The conditioned effects of the flavour were therefore different from the effects of either the unconditioned stimulus (amphetamine) or of an extinction procedure.

Amphetamine Conditioned taste aversion Conditioned suppression Operant behaviour Fixed-interval schedules

CONDITIONED taste aversions (CTA) have been induced with a wide variety of psychoactive drugs including amphetamine [5,12], but the experimental techniques have generally involved a limited range of standardised measurements. Usually only the gross intakes of food or water have been assessed and much less is known about possible changes in other aspects of behaviour after encounters with drug-paired flavours. We have previously reported that after pairings with amphetamine (1 mg/kg), flavour stimuli can markedly suppress bar-pressing on a fixed-ratio schedule of liquid reinforcement in rats [19]. This dose of amphetamine was selected on the basis of previous, extensive studies of the CTA effect of amphetamine [2, 4, 7, 9]. However, the unconditioned effect of a given dose of amphetamine on operant behaviour can be influenced by factors associated with the schedule of reinforcement [15,18], and it was thought possible that this might also apply to the conditioned response to flavours. We now report an attempt to test this idea by using a fixed interval schedule of liquid reinforcement. For purposes of comparison, the unconditioned effects of amphetamine and the effects of an extinction procedure have also been examined.

METHOD

Four female, hooded rats (150–200 g) were trained in a standard test chamber to press a bar for water reinforcement (5 sec access to distilled water presented in a dipper). A measured amount of tap water was presented in the home cage after each session, such that total daily intake was equal to that previously obtained in daily 1-hr sessions of free access to water. After initial training with continuous reinforcement, a fixed-interval schedule [10] was introduced progressively and the final schedule of fixed-interval 1 min (FI 1) was maintained for several weeks to allow performance to stabilise. In this schedule, reinforcement was given for the first response occurring at least 1 min since the previous reinforcement. Sessions were terminated after 20 reinforcements or 25 min, whichever came first. Fuller details of procedures have been reported [19] and only minimal changes have been made apart from the use of FI 1 instead of fixed-ratio 40.

After responding on the FI 1 schedule had stabilised, chicken or lemon-flavoured water [9,19] was substituted for distilled water as the reinforcer. At the end of each session of responding for flavoured water, either amphetamine (1 mg/kg (+)-amphetamine sulphate dissolved in saline) or isotonic saline was injected intraperitoneally in a volume of 1 ml/kg. For each rat, one of the two flavours was repeatedly paired with amphetamine in this way, whereas the other flavour was similarly paired with saline. A counterbalanced design was used to average out effects of the unconditioned palatabilities of the flavours, which were presented to each rat in an alternating sequence. Distilled water served as the reinforcer on days between flavour presentations, which were separated by at least 72 hr.

After completion of flavour-conditioning, the effects were determined of amphetamine (1 mg/kg IP) or saline injected 5 min before sessions of responding for distilled water. Saline and amphetamine were tested twice in each rat in random order. This work was carried out to test the applicability under our conditions of previous studies of the effects of amphetamine on fixed-interval performance [15,18].

Throughout the experiments, the numbers of bar-presses were recorded separately for five 12-sec periods within each fixed interval of 1 min, and the amounts of liquid reinforcers consumed were estimated by weighing the reservoir. The temporal pattern of bar-pressing was assessed by means of an index of curvature [11]; the index had a value of zero when the numbers of responses were the same in all five periods into which the fixed intervals were divided, and could reach a maximum of 0.8 if all responses were made in the last fifth of each interval.

RESULTS

The mean results for all 4 rats are presented in Fig. 1 and it can be seen that responding for flavours followed by saline injections remained very stable throughout the experiment. However, even a single pairing of a flavour with amphetamine (1 mg/kg) greatly disrupted performance and the effects of repeated pairings were even more marked. Statistical evaluation of the results for each of the three indices shown in Fig. 1 was by two-factor analysis of variance with repeated measures on both factors [21]; the details are not presented since all main effects and interactions were highly significant ($p<0.01$).

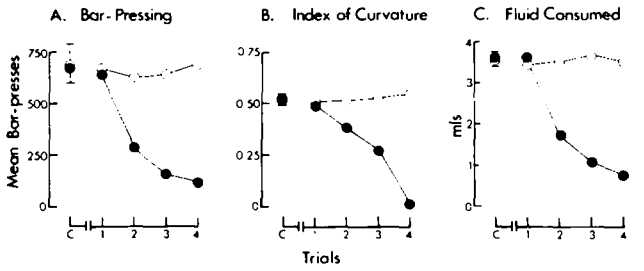

FIG. 1. Flavour-amphetamine pairings disrupted FI 1 bar-pressing for and consumption of flavoured water (●, amphetamine 1 mg/kg; ○, saline). Points represent means from 4 rats over entire sessions except for amphetamine-paired flavours on trial 2, where n=3 due to equipment failure. The points above C on the abscissae show means ± s.d. for distilled water reinforcement on days immediately before flavour presentations. The index of curvature estimates the temporal pattern of responding (see Method).

The relations between the results for the operant and consummatory measures are considered next. For saline-paired flavours, there was no significant relationship between the overall numbers of bar-presses and the amounts of solutions consumed ($r=-0.35$, df 14), a result which is expected since with fixed-interval schedules, the numbers of reinforcers presented are largely independent of the numbers of operant responses. However, bar-pressing for and consumption of amphetamine-paired flavours were highly correlated ($r=0.96$, df 13, $p<0.001$). The reduction in fluid intake was brought about partly by the presentation of fewer reinforcers (e.g. numbers reduced from 20 to an average of 14.5 after

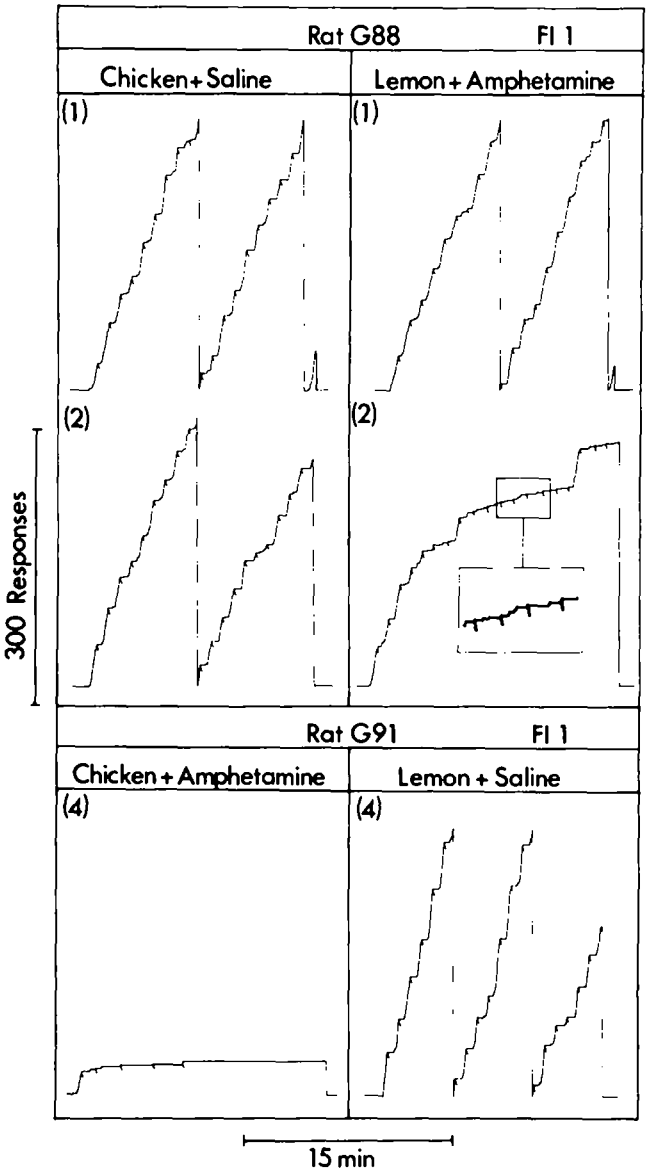

FIG. 2. Cumulative records of complete sessions showing representative performance under the FI 1 schedule. Short diagonal strokes indicate presentations of flavoured water reinforcers. The recorder was reset after 300 responses and when sessions ended. The records for rat G88 show responding for chicken and lemon flavours on their first (1) and second (2) presentations; responding for lemon flavour was disrupted by a single pairing with amphetamine at 1 mg/kg. The more marked effect of repeated flavour-amphetamine pairings is shown in records for rat G91. Taken together, the results for the two rats illustrate that conditioning occurred regardless of which flavour was paired with amphetamine.

two flavour-amphetamine pairings) and partly by reduced mean consumption of the reinforcers which were obtained (from 0.18 ml to 0.07 ml per reinforcer, $p<0.01$). Samples of cumulative records for two rats are shown in Fig. 2 to illustrate the effects of single and repeated flavour-amphetamine pairings on the characteristic pattern of fixed-interval responding. The extremely irregular pattern of responding

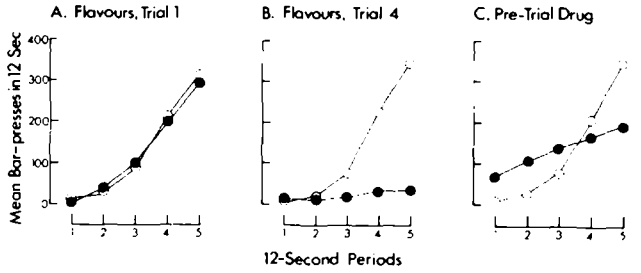

FIG. 3. Mean numbers of bar-presses for flavoured water during 5 successive 12-sec periods within fixed intervals of 1 min, summed across all the 1-min intervals in a session (n=4). (A) Prior to flavour-injection pairings, the usual acceleration of responding within fixed intervals can be seen. (B) The same flavours only suppressed responding after three pairings with amphetamine at 1 mg/kg (●). Flavours paired with saline (○) did not suppress responding. (C) The same dose of amphetamine injected 5 min before sessions of responding for distilled water increased responses early in the fixed interval but decreased responses late in the interval.

after flavour-conditioning can be seen in the enlarged segment of the record for rat G88. A quantitative analysis of the temporal pattern of responding is shown in Fig. 3, where the results within the 1-min fixed intervals have been broken down into five 12-sec periods. Flavours paired with amphetamine (1 mg/kg) did not increase the low rate of responding during the early part of the intervals, but greatly suppressed ($p<0.001$) the normally rapid rate during the second half of the intervals (Fig. 3B).

The results with amphetamine injected before sessions of responding for distilled water are considered next. Figure 3C shows that at the dose used for flavour-conditioning, amphetamine disrupted the temporal pattern of responding ($p<0.001$) by increasing the numbers of responses during the early part of each interval and decreasing responses late in the interval. There was no change in the total number of responses (104.2% as compared with saline injection) or the amount of water consumed (94.9%).

In the final stage of the experiment, the primary reinforcer was omitted for one entire session by presenting only empty dipper cups when a cup of water would otherwise have been presented. This procedure reduced the total number of bar-presses to 84.9% of the number on the preceding day of responding for distilled water ($p<0.05$). There was no significant change in the mean index of curvature, which was 0.46 for responding for empty cups as compared with 0.49 for cups of water.

DISCUSSION

Rats failed to respond for or to consume flavoured solutions when their presentation was followed by injections of amphetamine (1 mg/kg). These observations with a fixed-interval schedule thus confirm and extend previous work with fixed-ratio responding [19]. Explanations of such findings in terms of conditioning assume that the effect of the flavour can be distinguished from any long-term, unconditioned effect of the drug. This assumption is reasonable since responding by the same rats for distilled water and even for saline-paired flavours remained quite constant on days between presentations of drug-paired flavours. It therefore appears that the flavour-amphetamine pairings produced a discriminative, conditioned suppression of bar-pressing which paralleled the discriminative CTA seen when consummatory behaviour was assessed [2, 9, 16].

The question as to how the conditioning is best viewed is considered next. Firstly, explanations [6,7] based on stimulus-substitution views of conditioning can be excluded since the conditioned effect of the flavour was different from the effect of the drug; flavours suppressed responding on both FI and FR schedules of reinforcement, whereas amphetamine (1 mg/kg) injected before sessions produced the well-known, schedule-associated mixture of response-rate increases and decreases. Although unconditioned effects of amphetamine were not studied extensively in the present experiments, the results were fully consistent with previous observations discussed in recent reviews [15,18]. The nature of the disruption of FI responding also differed in the sense that flavours produced irregular responding (Fig. 2) whereas amphetamine typically produces very regular cumulative records. The reductions in the index of curvature after flavour-amphetamine pairings (Fig. 1) arose mainly because the total numbers of responses were reduced (Fig. 3B). Studies with amphetamine, other drugs or x-radiation have all shown that anorexic or hypodipsic potency does not correlate with potency in CTA [2, 13, 14, 20], and thus further support the case that the conditioned and unconditioned responses can differ.

Secondly, the suppression of bar-pressing by flavours did not occur simply because the reduced fluid intake meant that the rats were in effect not being reinforced. After only a single flavour-amphetamine pairing, a brief encounter with the flavour produced marked suppression of responding both in the present study and in that reported previously [19]; in contrast, deliberately failing to provide any liquid reinforcement at all for an entire session had virtually no effect on the number or temporal pattern of responses in the present study. Presumably the characteristic FI performance was maintained by secondary reinforcement associated with presentations of the (empty) dipper cup.

Thirdly, therefore, the possibility has to be considered that bar-pressing was suppressed because it culminated in an encounter with a flavour-stimulus which had acquired aversive characteristics due to pairings with amphetamine. Although this is the conventional explanation for drug-induced CTA [5, 13, 17], it is difficult to support its validity by citing independent evidence from experiments not involving flavour-conditioning. For example, it is known that far from being aversive, sensory stimuli paired with amphetamine can serve as conditioned positive reinforcers in self-administration experiments [8]. Furthermore, in some circumstances stimuli paired with positive reinforcers such as food can suppress responding [1] and, therefore, it is possible to speculate that flavour-conditioning with amphetamine produces a form of such positive conditioned suppression. Several drugs can indeed be used to induce conditioned suppression in the conventional procedures which do not involve flavours [3]. However, the contingencies in flavour conditioning and conventional conditioned suppression procedures differ in several respects and the role of these differences must be analysed if valid analogies are to be made.

ACKNOWLEDGEMENTS

We wish to thank Drs. A. J. Goudie and D. J. Sanger for discussion, Miss J. A. Wall for technical assistance and Professor P. B. Bradley for encouragement of this work. Rats were kindly supplied by the Department of Psychology, University of Birmingham.

REFERENCES

1. Azrin, N. H. and D. F. Hake. Positive conditioned suppression: conditioned suppression using positive reinforcers as the unconditioned stimuli. *J. exp. Analysis Behav.* **12:** 167–173, 1969.
2. Booth, D. A., G. D. D'Mello, C. W. T. Pilcher and I. P. Stolerman. Comparative potencies of amphetamine, fenfluramine and related compounds in taste aversion experiments in rats. *Br. J. Pharmac.* **61:** 669–677, 1977.
3. Cameron, O. G. and J. B. Appel. Drug-induced conditioned suppression: specificity due to drug employed as UCS. *Pharmac. Biochem. Behav.* **4:** 221–224, 1976.
4. Cappell, H. and A. E. Le Blanc. Punishment of saccharin drinking by amphetamine in rats and its reversal by chlordiazepoxide. *J. comp. physiol. Psychol.* **85:** 97–104, 1973.
5. Cappell, H. and A. E. Le Blanc. Conditioned aversion by psychoactive drugs: does it have significance for an understanding of drug dependence? *Addictive Behav.* **1:** 55–64, 1975.
6. Carey, R. J. A comparison of the food intake suppression produced by giving amphetamine as an aversion treatment versus as an anorexic treatment. *Psychopharmacology* **56:** 45–48, 1978.
7. Carey, R. J. and E. B. Goodall. Amphetamine-induced taste aversion: a comparison of *d* versus *l*-amphetamine. *Pharmac. Biochem. Behav.* **2:** 325–330, 1974.
8. Davis, W. M. and S. G. Smith. Blocking effect of alpha-methyltyrosine on amphetamine based reinforcement. *J. Pharm. Pharmac.* **25:** 174–177, 1973.
9. D'Mello, G. D., I. P. Stolerman, D. A. Booth and C. W. T. Pilcher. Factors influencing flavour aversions conditioned with amphetamine in rats. *Pharmac. Biochem. Behav.* **7:** 185–190, 1977.
10. Ferster, C. B. and B. F. Skinner. *Schedules of Reinforcement.* New York: Appleton-Century-Crofts, 1957.
11. Fry, W., R. T. Kelleher and L. Cook. A mathematical index of performance on fixed-interval schedules of reinforcement. *J. exp. Analysis Behav.* **3:** 193–199, 1960.
12. Gamzu, E. The multifaceted nature of taste-aversion-inducing agents: is there a common factor? In: *Learning Mechanisms in Food Selection*, edited by L. M. Barker, M. R. Best and M. Domjan. Dallas: Baylor University Press, 1977, pp. 477–509.
13. Garcia, J., B. K. McGowan and K. F. Green. Biological constraints on conditioning. In: *Classical Conditioning II: Current Research and Theory*, edited by A. H. Black and W. F. Prokasy. New York: Appleton-Century-Crofts, 1972, pp. 3–27.
14. Martin, G. M. and L. H. Storlien. Anorexia and conditioned taste aversion in the rat. *Learn. Motivat.* **7:** 274–282, 1976.
15. McMillan, D. E. and J. D. Leander. Effects of drugs on schedule-controlled behavior. In: *Behavioral Pharmacology*, edited by S. D. Glick and J. Goldfarb. Saint Louis: C. V. Mosby, 1976, pp. 85–139.
16. Reicher, M. A. and E. W. Holman. Location preference and flavour aversion reinforced by amphetamine in rats. *Anim. Learn. Behav.* **5:** 343–346, 1977.
17. Revusky, S. and J. Garcia. Learned associations over long delays. In: *The Psychology of Learning and Motivation: Advances in Research and Theory*, Volume 4, edited by G. H. Bower. New York: Academic Press, 1970, pp. 1–84.
18. Sanger, D. J. and D. E. Blackman. Rate-dependent effects of drugs: a review of the literature. *Pharmac. Biochem. Behav.* **4:** 73–83, 1976.
19. Stolerman, I. P. and G. D. D'Mello. Amphetamine-induced taste aversion demonstrated with operant behaviour. *Pharmac. Biochem. Behav.* **8:** 107–111, 1978.
20. Stolerman, I. P. and G. D. D'Mello. Amphetamine-induced hypodipsia and its implications for conditioned taste aversion in rats. *Pharmac. Biochem. Behav.* **8:** 333–338, 1978.
21. Winer, B. J. Statistical Principles in Experimental Design. First Edition, London: McGraw-Hill, 1962.