Pharmacology Biochemistry & Behavior, Vol. 35, pp. 89–92. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Conditioned Aversion After Delay Place Conditioning With Amphetamine[1]

PAUL J. FUDALA*[2] AND EDGAR T. IWAMOTO†[3]

**Graduate Center for Toxicology, †Department of Pharmacology and the Tobacco and Health Research Institute, University of Kentucky, Lexington, KY 40536*

Received 19 April 1989

FUDALA, P. J. AND E. T. IWAMOTO. *Conditioned aversion after delay place conditioning with amphetamine*. PHARMACOL BIOCHEM BEHAV **35**(1) 89–92, 1990.—Male, Sprague-Dawley rats received subcutaneous injections of either dextroamphetamine sulfate (AMP; 3.0 mg/kg) or vehicle [VEH (phosphate buffer); 1 ml/kg] immediately before (standard conditioning) or after (delay conditioning) conditioning sessions in a place-conditioning paradigm. AMP was paired for 4 conditioning sessions with one compartment of a three-compartment place-conditioning apparatus; VEH was paired for 4 conditioning sessions with another compartment. Animals were then tested for place preference or aversion by determining the proportion of time spent in each compartment during a 15-minute test session. Standard conditioning with AMP produced a place preference while delay conditioning produced a place aversion. Similar findings had earlier been reported from studies involving conditioned place preferences and aversions with nicotine. These studies demonstrated that the time of drug administration can be as strong a determinant of place-conditioning effects as the drug itself.

Amphetamine Rat Conditioned place preference Reinforcement Conditioned place aversion

WE have previously reported the induction of both a conditioned place preference (7,9) and place aversion (8) by nicotine in the place-conditioning paradigm. Both conditioned responses were dose related and dependent upon the time of nicotine administration relative to the conditioning sessions. Nicotine administered immediately prior to the sessions (standard conditioning) resulted in a conditioned place preference, while nicotine given immediately or shortly following sessions (delay conditioning) resulted in a conditioned place aversion. Additionally, both conditioned responses appeared to be centrally mediated. Different findings have been obtained with morphine and diazepam, which were effective in producing conditioned place preferences in the standard-conditioned paradigm, but were ineffective in producing conditioned responses using the delay-conditioning procedure (1, 25, 29, 36). Amphetamine has been shown to reliably condition place preferences using the standard-conditioning procedure (5, 10, 21, 30, 35). The present study was conducted to determine if delay-conditioned place aversion is a phenomenon peculiar only to nicotine. In this study, we tested amphetamine using both standard and delay place-conditioning paradigms.

METHOD

Animals

Experimentally naive, adult male Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) were used in all experiments. The animals were initially quarantined for 10 days before being housed in groups of two. The rats were then individually housed 24–48 hours prior to the beginning of each experiment. Food and water were freely available in the home cages and the animals were kept on a 12-hour light/dark cycle.

Apparatus

The place-conditioning apparatus was constructed of Plexiglas and has previously been described in detail (9). It consisted of three distinctive interconnected chambers. One chamber was cubical (25.4 cm sides) with black walls and a grid floor. The middle chamber was $10.2 \times 10.2 \times 25.5$ cm high with gray walls and a wood floor. The third chamber had a equilateral triangular mesh floor 25.4 cm on a side with white walls 25.4 cm in height.

[1]This work was presented in part at the Seventy-First Annual Meeting of the Federation of American Societies for Experimental Biology, Washington, DC, March 29–April 2, 1987.

[2]Current address: Department of Health and Human Services, National Institute on Drug Abuse, Addiction Research Center, Francis Scott Key Medical Center, Baltimore, MD 21224.

[3]Requests for reprints should be addressed to Edgar T. Iwamoto, Department of Pharmacology, Albert B. Chandler Medical Center, Room MS-305, University of Kentucky, Lexington, KY 40536.

Sliding removable doors separated the middle chamber from the other two. The doorways measured 10.2 by 12.7 cm. A 0.32 cm thick clear Plexiglas hinged door covered the top of the apparatus.

Circular transparent areas (1.9 cm in diameter and centered 1.6 cm above the floor) were used to accommodate the paths of photobeam detectors and transducers (Coulbourn Instruments, Lehigh Valley, PA) used to monitor the position of the animals in the apparatus. The photobeams lay in a horizontal place: one beam bisected the gray chamber and one beam each traversed the white and black chambers 6.35 cm from the doorways. A cumulative timer (Coulbourn Instruments) was activated when the infrared beam passing through the white chamber was initially interrupted. Subsequent interruptions did not modify the timing. Concurrent interruptions of the white and gray chamber beams, or of the gray chamber beam alone, stopped the white chamber timer. Timing was reinstated when the white chamber beam was again broken. A cumulative timer for the black chamber operated in an analogous manner.

Drug

Dextroamphetamine sulfate was obtained from Sigma Chemical Co. (St. Louis, MO) and the dosage is expressed as the salt. All solutions were prepared fresh daily in phosphate buffer vehicle (0.46% monobasic sodium phosphate + 1.8% dibasic sodium phosphate, approximate pH = 7.1). All injections were made subcutaneously and injection volumes were 1 ml/kg.

Procedure

One group of 7 and three groups of 8 rats were used. One group was administered VEH prior to conditioning in both the white and black chambers (standard conditioning) and another was given VEH after conditioning in both chambers (delay conditioning). Similarly, one group was administered amphetamine sulfate, 3 mg/kg (AMP), or VEH, prior to conditioning in the black and white chambers, respectively. The final group was conditioned with AMP or VEH in a similar manner, except that the solutions were administered following the conditioning sessions.

Standard Conditioning

The body weights of two rats were recorded. One was injected with VEH and placed into the closed (doors in place) white chamber. The other was injected with AMP and placed into the closed black chamber. Conditioning time was 20 min per day. The conditioning chambers and treatments were reversed on the following day. Over the eight conditioning days (Tuesday through Friday, then Monday through Thursday), a given rat in the experimental, drug-treatment group received a total of four AMP-black chamber pairings and four VEH-white chamber pairings. In contrast, a rat in the VEH-control group received four VEH-black chamber pairings and four VEH-white chamber pairings. One-half of the subjects within a given experimental group began their conditioning in the white chamber and the other half started in the black chamber. The daily order of conditioning the 31 rats was randomized over the eight-day period.

On preference testing day (DAY 9, Friday), one rat was placed into the closed, central gray chamber of the apparatus. The sliding doors were removed and the amount of time (in sec) spent in the white and black chambers was automatically recorded over the 900-sec testing period.

Delay Conditioning

The procedure used was similar to the one for standard conditioning. On conditioning days, one rat was placed in the closed white chamber for 20 min. Following removal from the chamber, the rat was injected with VEH and immediately returned to the home cage. Similarly, another rat was placed in the closed black chamber for 20 min and subsequently injected with AMP. All injections were given immediately after removing the rats from the apparatus. The subjects were subsequently tested for place preference as previously described.

TABLE 1

STANDARD AND DELAY PLACE CONDITIONING WITH AMPHETAMINE SULFATE

Conditioning Treatment	Residence Ratio
VEH/PRE	−0.09 ± 0.06
AMP/PRE	0.37 ± 0.07*
VEH/POST	0.02 ± 0.09
AMP/POST	−0.28 ± 0.06†

Mean residence ratios (±SE) for groups of rats (N = 7 or 8) that received 3.0 mg/kg amphetamine sulfate (AMP) or vehicle (VEH) immediately prior to (PRE) or following (POST) place-conditioning sessions.

*AMP/PRE was significantly greater than VEH/PRE, indicative of a place preference.

†AMP/POST was significantly less than VEH/POST, indicative of a place aversion.

Statistical Treatment of the Data

A quantity called the residence ratio (RR; 7–9) was calculated for each rat in the AMP-treatment groups as follows: RR = (B − W)/(B + W), where B = the time in sec spent in the black (drug-paired) chamber and W = the time in sec spent in the white (VEH-paired) chamber. RRs were similarly calculated for VEH-controls. In all cases, positive RR values (relative to controls) indicate a place preference for the previously drug-paired chamber and negative values indicate a place aversion. For example, RR values of +1 and −1 indicate that animals spent all time during the preference testing sessions in either the previously drug-paired or vehicle-paired chambers, respectively. A RR value of zero indicates that animals spent equal time in each of the chambers.

Variability of RRs due to treatment effects was analyzed using a two-factor (treatment and time of treatment administration) ANOVA (39). Pairwise comparisons (following a significant ANOVA) were performed using the least squares means analysis (27,28). Variability in the time animals spent in the gray chamber due to treatment effects was analyzed in an analogous manner.

RESULTS

AMP produced a place preference when administered prior to the conditioning sessions and a place aversion when given after the conditioning sessions (Table 1). An ANOVA indicated a significant pre- versus postconditioning effect, $F(1,27) = 14.3$, $p < 0.009$. There was not a significant effect for treatment (AMP or VEH), $p > 0.05$. However, the factor interaction between treatment and time of treatment administration (pre- or postconditioning) was significant, $F(1,27) = 27.8$, $p < 0.0001$. Pairwise comparisons indicated that subjects administered AMP prior to conditioning showed a place preference when compared to their corresponding VEH controls, $t(27) = 4.5$, $p < 0.002$. Similarly, subjects given AMP following conditioning showed a place aversion when compared to their VEH controls, $t(27) = 3.0$, $p < 0.007$. Animals administered VEH following conditioning sessions were not found to be different from those given VEH prior to the sessions, $p > 0.3$.

There were no differences between groups with respect to time spent in the gray chamber.

DISCUSSION

Amphetamine produced a place preference when administered prior to conditioning sessions (standard conditioning) but a place aversion when given following the sessions (delay conditioning). Based on classical conditioning principles alone (3,22), the delay-conditioning procedure was expected to condition a place preference. In delay conditioning, the interval between the onset of the conditioned and unconditioned stimuli may be discriminated, thereby predicting the occurrence of the unconditioned stimulus. The conditioned responses observed from both the standard- and delay-conditioning procedures would thus be expected to be analogous.

The results of the present investigation are like those previously reported for nicotine (7–9), but unlike those for morphine (1, 25, 29) or diazepam (36), substances which produced no place-conditioning effects when administered following conditioning sessions. Nicotine- and amphetamine-induced delay-conditioned place aversions may represent a response contingent on the administration of the unconditioned stimulus (the drug) after conditioning sessions. This concept is consistent with data that indicate that various drug and nondrug stimuli may have different, and possibly opposite effects, depending on how their administration is scheduled with respect to behavior. For example, nicotine and cocaine, as well as electric shock and intracranial brain stimulation, have been shown to maintain responding which produces their presentation under certain reinforcement schedules, while maintaining responding that postpones their presentation under different schedules of reinforcement (11, 12, 20, 32, 33, 37). Thus, the time of drug administration with respect to conditioning may be an important factor in determining whether place preferences or aversion will occur.

That amphetamine is self-administered by laboratory animals (26,34), but also produces conditioned aversions to flavored solutions (4, 6, 18, 24) may seem paradoxical since amphetamine thus produces both apparent reward and aversion. Wise and colleagues (38), using rats initially trained to lever-press for amphetamine, demonstrated that apomorphine can be both positively reinforcing (as assessed by its self-administration) and aversive (as evidenced by the production of a conditioned taste aversion) in the same animals during the same test session. These investigators provided evidence that procedural differences between the self-administration and taste-aversion paradigms did not totally account for the seemingly opposite effects observed in the different paradigms. Hunt and Amit (17) later argued that the capacity of reinforcing drugs to induce conditioned taste aversions may reflect a functionally protective "taste shyness" rather than a form of conditioned sickness, emphasizing the potential importance of how the complex motivational properties of amphetamine and other psychoactive drugs may affect behavior. Our data extend the above findings by showing that the same doses of amphetamine can have opposite effects (place preference- and place aversion-producing) in the same paradigm depending on the time of drug administration.

The present investigation and previously reported data suggest a possible aversive component of amphetamine and nicotine place conditioning, but the significance of this aversion is unclear. Although amphetamine has sometimes been noted to produce dizziness and nervousness in human clinical trials, most subjective reports of the drug's effects have been positive, with persons reporting increased energy, wakefulness, and elevated mood (2, 13, 19, 23, 31). Previous workers using human subjects found that intravenous nicotine produced dose-related effects which included nausea, respiratory distress, lightheadedness, and fear (14–16).

It cannot be readily determined whether nicotine- and amphetamine-induced conditioned place preferences and aversions are secondary to drug-induced positive and negative feeling-states in rats. The conditioning cues of the place-conditioning apparatus in the present experiment were the same in both the standard- and delay-conditioning procedures. We hypothesize that the cues may have been perceived differently depending on the time of drug administration, or that the cues may have been paired with one or more components of the multitude of behavioral effects that may be induced by nicotine or amphetamine. A further characterization of the mechanisms mediating the production of conditioned responses in the place-conditioning paradigm will aid in assessing the usefullness of this model in the study of drug reinforcement and reward.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. Phyllis Finch for her assistance in the preparation of this manuscript and Mr. Edwin C. Williamson for his technical assistance. This study was supported in part by a grant from the University of Kentucky Tobacco and Health Research Institute.

REFERENCES

1. Bardo, M. A.; Miller, J. S.; Neisewander, J. L. Conditioned place preference with morphine: The effect of extinction training on the reinforcing CR. Pharmacol. Biochem. Behav. 21:545–549; 1984.
2. Bigelow, G. E.; Griffiths, R. R.; Liebson, I.; Kaliszak, J. E. Double-blind evaluation of reinforcing and anorectic actions of weight control medications: Interaction of pharmacological and behavioral treatments. Arch. Gen. Psychiatry 37:1118–1123; 1980.
3. Bugelski, B. R. Principles of learning and memory. New York: Praeger Press; 1979.
4. Carey, R. J.; Goodall, E. B. Amphetamine-induced taste aversion: A comparison of d- versus l-amphetamine. Pharmacol. Biochem. Behav. 2:325–330; 1974.
5. Di Scala, G.; Martin-Iverson, M. T.; Phillips, A. G.; Fibiger, H. C. The effects of progabide (SL 76002) on locomoter activity and conditioned place preference induced by d-amphetamine. Eur. J. Pharmacol. 107:271–274; 1985.
6. D'Mello, G. D.; Stolerman, I. P.; Booth, D. A.; Pilcher, C. W. T. Factors influencing flavour aversions conditioned with amphetamine in rats. Pharmacol. Biochem. Behav. 7:185–190; 1977.
7. Fudala, P. J.; Iwamoto, E. T. Further studies on nicotine-induced conditioned place preference in the rat. Pharmacol. Biochem. Behav. 25:1041–1049; 1986.
8. Fudala, P. J.; Iwamoto, E. T. Conditioned aversion after delay place conditioning with nicotine. Psychopharmacology (Berlin) 92:376–381; 1987.
9. Fudala, P. J.; Teoh, K. W.; Iwamoto, E. T. Pharmacologic characterization of nicotine-induced conditioned place preference. Pharmacol. Biochem. Behav. 22:237–241; 1985.
10. Gilbert, D.; Cooper, S. J. Beta-phenylethylamine-, d-amphetamine- and l-amphetamine-induced place preference conditioning in rats. Eur. J. Pharmacol. 95:311–314; 1983.
11. Goldberg, S. R.; Spealman, R. D. Maintenance\ınd suppression of behavior by intravenous nicotine injections in squirrel monkeys. Fed. Proc. 41:216–220; 1982.
12. Goldberg, S. R.; Spealman, R. D.; Risner, M. E.; Henningfield, J. E. Control of behavior by intravenous nicotine injections in laboratory animals. Pharmacol. Biochem. Behav. 19:1011–1020; 1983.
13. Griffith, J. D.; Nutt, J. G.; Jasinski, D. R. A comparison of fenfluramine and amphetamine in man. Clin. Pharmacol. Ther. 18:563–570; 1975.
14. Henningfield, J. E.; Goldberg, S. R. Control of behavior by intravenous nicotine in human subjects. Pharmacol. Biochem. Behav.

19:1021–1026; 1983.
15. Henningfield, J. E.; Miyasato, K.; Jasinski, D. R. Cigarette smokers self-administer intravenous nicotine. Pharmacol. Biochem. Behav. 19:887–890; 1983.
16. Henningfield, J. E.; Miyasato, K.; Jasinski, D.R. Abuse liability and pharmacodynamic characteristics of intravenous and inhaled nicotine. J. Pharmacol. Exp. Ther. 234:1–11; 1985.
17. Hunt, T.; Amit, Z. Conditioned taste aversion induced by self-administered drugs: Paradox revisited. Neurosci. Biobehav. Rev. 11:107–130; 1987.
18. Infurna, R. N.; Spear, L. P. Developmental changes in amphetamine-induced taste aversion. Pharmacol. Biochem. Behav. 11:31–35; 1979.
19. Johanson, C. C.; Uhlenhuth, E. H. Drug preference and mood in humans: Repeated administration of d-amphetamine. Pharmacol. Biochem. Behav. 14:159–163; 1981.
20. Kelleher, R. T.; Morse, W. H. Schedules using noxious stimuli III. Responding maintained with response-produced electric shock. J. Exp. Anim. Behav. 11:819–838; 1968.
21. Mackey, W. B.; van der Kooy, D. Neuroleptics block the positive reinforcing effects of amphetamine but not of morphine as measured by place conditioning. Pharmacol. Biochem. Behav. 22:101–105; 1985.
22. Mackintosh, N. J. The psychology of animal learning. New York: Academic Press; 1974.
23. Martin, W. R.; Sloan, J. W.; Supira, J. D.; Jasinski, D. R. Physiologic, subjective and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. Clin. Pharmacol. Ther. 12:245–258; 1971.
24. Miller, D. B.; Miller, L. L. Buproprion, d-amphetamine, and amitriptyline-induced conditioned taste aversion in rats: Dose effects. Pharmacol. Biochem. Behav. 18:737–741; 1983.
25. Mucha, R. F.; Iversen, S. D. Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: a procedural examination. Psychopharmacology (Berlin) 82:241–247; 1984.
26. Pickens, R.; Harris, W. C. Self-administration of D-amphetamine by rats. Psychopharmacologia 12:158–163; 1968.
27. SAS user's guide: Statistics, 1982 edition. Cary, NC: SAS Institute, Inc.; 1982.
28. Searle, S. R.; Speed, F. M.; Milliken, G. A. Population marginal means in the linear model: An alternative to least squares means. Am. Stat. 34:216–221; 1980.
29. Sherman, J. D.; Pickman, C.; Rice, A.; Liebeskind, J. D.; Holman, E. W. Rewarding and aversive effects of morphine: Temporal and pharmacological properties. Pharmacol. Biochem. Behav. 13:501–505; 1980.
30. Sherman, J. E.; Roberts, T.; Roskam, S. E.; Holman, E. W. Temporal properties of the rewarding and aversive effects of amphetamine in rats. Pharmacol. Biochem. Behav. 13:597–599; 1980.
31. Silverstone, T.; Wells, B.; Trenchard, E. Differential dose response effects of dextroamphetamine sulphate on hunger, arousal and mood in human volunteers. Psychopharmacology (Berlin) 79:242–245; 1983.
32. Spealman, R. D. Behavior maintained by termination of a schedule of self-administration of cocaine. Science 204:1231–1233; 1979.
33. Spealman, R. D. Maintenance of behavior by postponement of scheduled injections of nicotine in squirrel monkeys. J. Pharmacol. Exp. Ther. 227:154–159; 1983.
34. Spealman, R. D.; Goldberg, S. R. Drug self-administration by laboratory animals: control by schedules of reinforcement. Annu. Rev. Pharmacol. Toxicol. 17:313–339; 1978.
35. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Res. 253:185–193; 1982.
36. Spyraki, C.; Kazadjian, A.; Varonos, D. Diazepam-induced place preference conditioning: Appetitive and antiaversive properties. Psychopharmacology (Berlin) 87:225–232; 1985.
37. Steiner, S.; Beer, B.; Shaffer, M. M. Escape from self-produced rates of brain stimulation. Science 163:90–91; 1969.
38. Wise, R. A.; Yokel, R. A.; DeWitt, H. Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. Science 191:1273–1275; 1976.
39. Zar, J. H. Biostatistical analysis. Englewood Cliffs, NJ: Prentice Hall, Inc.; 1974.