Pharmacology Biochemistry and Behavior, Vol. 44, pp. 595-600, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Fenfluramine-Induced Place Aversion in a Three-Choice Apparatus

ANGELA M. DAVIES AND LINDA A. PARKER[1]

Department of Psychology, Wilfrid Laurier University, Waterloo, Ontario N2L 3C5, Canada

Received 2 June 1992

DAVIES, A. M. AND L. A. PARKER. *Fenfluramine-induced place aversion in a three-choice apparatus.* PHARMACOL BIOCHEM BEHAV **44**(3) 595-600, 1993.—The hedonic properties of the anorectic agent fenfluramine (0.25, 1.0, 2.5, 5.0, and 10.0 mg/kg) were assessed in two experiments in a place conditioning paradigm. After four conditioning trials, rats were tested for their preference for a drug-paired chamber, saline-paired chamber, and a novel chamber. Fenfluramine produced a place aversion at doses of 2.5–10 mg/kg.

Fenfluramine Serotonin Place conditioning Feeding Anorexia

DRUGS that are reinforcing in the drug self-administration paradigm also tend to produce a conditioned place preference [for review, see (35)]. Because drugs that have reinforcing properties are also considered to have a higher probability of promoting addiction and substance abuse, it is important to determine whether clinically effective drugs produce a place preference. Of interest in the present study is the reinforcing/aversive efficacy of fenfluramine, an agent used clinically as an anorectic agent to suppress appetite. The anorectic properties of fenfluramine have been attributed to its ability to act as a serotonergic agonist; fenfluramine nonspecifically facilitates the release of serotonin both peripherally and centrally (4).

The reinforcing efficacy of drugs are typically tested in the behavioral paradigms that include drug self-administration, facilitation of intracranial electrical self-stimulation, and place conditioning. Fenfluramine does not appear to produce or maintain drug self-administration (1,9,13,28,34) or facilitate responding for electrical stimulation to the lateral hypothalamus (17). When assessed in these paradigms, fenfluramine does not serve as a reinforcing agent. The ability of fenfluramine to produce place conditioning has not been tested.

In assessing its aversive properties, it has been demonstrated that fenfluramine effectively establishes a conditioned aversion to a taste with which it is paired [conditioned taste avoidance (CTA)] at doses ranging from 3–9 mg/kg (6,8, 11,12). It is uncertain whether fenfluramine-induced CTA is produced as a result of its anorectic effects or some other effect such as its ability to produce nausea. However, fenfluramine has been demonstrated to produce a CTA at doses well below those that suppress water intake (2 mg/kg) after only one conditioning trial, suggesting that fenfluramine-induced CTA is not mediated by "conditioned anorexia" (32).

There is evidence that a fenfluramine-induced CTA is motivated by an hedonic shift in the palatability of the drug-paired flavor (24). When assessed by the taste reactivity (TR) test, devised by Grill and Norgren (15) to directly measure the palatability of a flavored solution, rats react to fenfluramine- and lithium-paired flavors with the aversive TR pattern elicited unconditionally by bitter quinine. On the other hand, rats do not respond to flavors paired with the reinforcing drugs amphetamine (22,27,36) or morphine (24) with the aversive response pattern, although they avoid consuming the flavors. On the basis of this pattern of results, it was suggested that only drugs that are nonreinforcing are capable of producing aversive taste reactivity responses. If this analysis is correct, then fenfluramine, which produces aversive TR responses when paired with a flavored solution, should not be reinforcing in tests that assess drug reinforcement.

The results of all these experiments consistently suggest that fenfluramine is nonreinforcing and/or aversive. One of the primary paradigms used to examine whether a drug is reinforcing or aversive is the place conditioning paradigm. The hedonic properties of fenfluramine, however, have not been assessed in this paradigm. The advantage of using the place conditioning paradigm is its sensitivity not only to reinforcing properties but also to the aversive properties of drugs (35). It is, therefore, conceivable that fenfluramine may produce a conditioned place aversion.

The present investigation assessed the hedonic properties of various doses of fenfluramine in the place conditioning paradigm. In Experiment 1, three moderate to high doses (2.5, 5, and 10 mg/kg) of fenfluramine were used, and in Experiment 2 two lower doses (0.25 and 1 mg/kg) of fenfluramine were used to expand the range of doses tested in this paradigm.

Place conditioning was assessed in our previously described

[1] To whom requests for reprints should be addressed.

(25) three-choice apparatus with a central choice chamber. This apparatus provides the rat with the opportunity to select among a drug-paired, saline-paired, and novel chamber. Scoles and Siegel (30) suggested that morphine-induced place conditioning may actually reflect a rat's preference for novelty. If the drug interferes with habituation to the chamber with which it is paired, then it may maintain the novelty of the drug-paired chamber. When rats are subsequently tested for their relative preference for a drug- or a saline-paired chamber, they may select the drug-paired chamber not because it has become associated with the positive hedonic properties of the drug but because it is relatively more novel (3). The present experiment allows the rat to select among a drug-paired, a saline-paired, and a novel chamber from the central choice chamber. A place preference is defined as a greater preference for the drug-paired chamber than the novel or the saline-paired chamber. A place aversion is defined as a greater avoidance of the drug-paired chamber than the saline-paired or novel chamber.

In addition, while rats were tested for place preferences their activity level in each chamber was monitored. It has previously been demonstrated that rats tend to be most active while in their least preferred chamber and least active while in their most preferred chamber (3,23).

METHOD

Subjects

In Experiment 1, 36 male Sprague-Dawley rats weighing between 247–282 g served as subjects. Rats were maintained on ad lib access to Purina Lab Chow and water. Twelve rats were conditioned and tested 3 weeks after the first 24 rats were tested. In Experiment 2, 24 male Sprague-Dawley rats weighing between 259–281 g were treated identically to those of Experiment 1 except as indicated.

Apparatus

The apparatus was a three-arm maze with a central choice area (25 × 25 cm). The wooden walls of each chamber (35 × 25 × 30 cm) and the floor of the central choice area were painted flat black. The floors of the chambers differed both tactually and visually. The three different floors were as follows: black plastic, small grid (1/4 cm), 5-cm strips of black sandpaper located 10 cm apart on the black wooden floor. The chambers were covered with clear plastic lids. In conditioning trials, rats were confined to each chamber with a divider painted flat black. The room was illuminated by two fluorescent ceiling lights.

Rats' behavior during testing was recorded by a videocamera located in the ceiling of the testing room. The videocamera sent an image of the largest part of the rat's body to an activity tracking apparatus, Videomex V (Columbus instruments, Columbus, OH), which then sent a signal to an IBM microcomputer for later analysis. The activity tracking apparatus provided not only the amount of time spent in each test chamber (and the central choice chamber) but also the frequency of crossings among four quadrants within each chamber as a measure of activity level during the preference testing while in each chamber.

Procedure

One week after rats arrived, the conditioning trial cycles began. In Experiment 1, there were three fenfluramine dose conditions (2.5, 5.0, and 10.0 mg/kg), and in Experiment 2 there were two fenfluramine dose conditions (0.25 and 1.0 mg/kg), with 12 rats in each condition. All injections were administered IP. The procedures employed for both experiments were identical. Rats received four conditioning trial cycles (fenfluramine trial/saline trial) with 2–4 days intervening between each cycle to permit clearance of fenfluramine. On the first trial of a cycle, rats were injected with fenfluramine or saline solution 5 min prior to being placed in one of the chambers of the three-arm maze for 30 min. On the following day, they were injected with the alternate solution and placed in an alternate chamber in the three-arm maze for 30 min. The third chamber remained novel. The order of drug conditioning within a cycle and the chambers paired with the drug or saline were completely counterbalanced. The test trial occurred 3 days after the final conditioning trial cycle. During the test trial, rats were placed in the central choice area of the three-arm maze with the barriers between chambers removed, and the video-tracking apparatus monitored the movement of the rat for 30 min.

Data Analysis

For Experiment 1, the amount of time (seconds) spent in each of the three test chambers was analyzed as a 3 × 3 mixed-factor analysis of variance (ANOVA) with the between-groups factor of dose of fenfluramine (2.5, 5.0, and 10.0 mg/kg) and the within-groups factor of chamber (drug paired, saline paired, and novel). The activity measure was converted into a score that reflected the amount of time spent in the appropriate chamber. The frequency of quadrant crossings within each chamber was converted to a rate measure determined by dividing the frequency of crossings while in each chamber by the amount of time spent in the chamber, and these scores were analyzed as a 3 × 3 mixed-factor ANOVA in the same manner as the measure of the amount of time spent in each chamber. Subsequent posthoc comparison tests were conducted with a Newman-Keuls analysis. For all statistical tests, the significance level was set at $p < 0.05$.

For Experiment 2, the mean time spent in each test chamber and the activity while in each chamber were analyzed as 2 × 3 mixed-factor ANOVAs with the between-groups factor of dose (0.25 and 1.0 mg/kg) and the within-groups factor of chamber (fenfluramine paired, saline paired, and novel). Subsequent comparison tests were conduced by Newman-Keuls tests.

The amount of time spent in the central choice chamber during testing was also analyzed in both experiments by a single-factor within-groups ANOVA. In neither experiment did groups significantly differ in the amount of time spent in the choice chamber.

RESULTS

Experiment 1

Place conditioning. Figure 1 presents the mean number of seconds spent in each chamber during the place preference test for rats conditioned with each dose of fenfluramine in Experiment 1. The solid bars represent the fenfluramine-paired chamber, the open bars represent the saline-paired chamber, and the hatched bars represent the novel chamber. The 3 × 3 mixed-factor ANOVA revealed a significant chamber main effect, $F(2, 66) = 13.09$, $p < 0.01$. The dose × chamber interaction was not significant, $F(4, 66) = 0.29$. Subsequent Newman-Keuls analyses of the main effect of

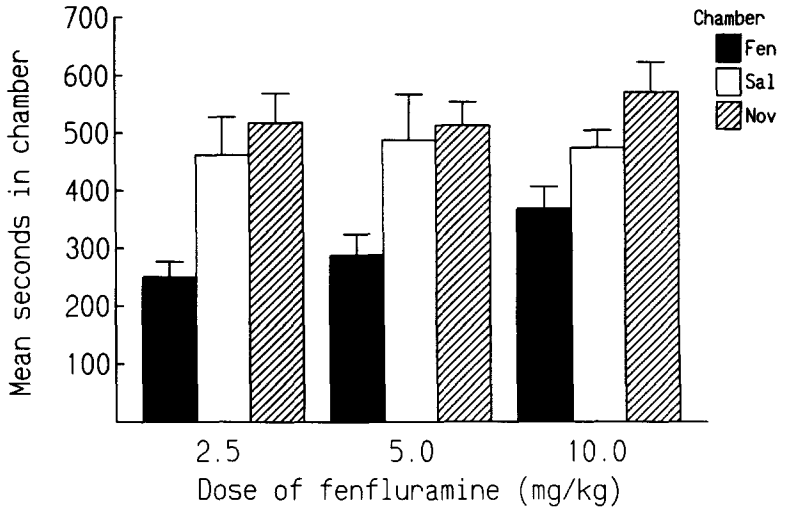

FIG. 1. Mean (±SE) number of seconds rats spent in each chamber of a three-choice apparatus following place conditioning with 2.5, 5.0, and 10.0 mg/kg fenfluramine in Experiment 1.

chamber revealed that rats spent significantly less time in the drug-paired chamber than either the saline-paired or the novel chamber ($p < 0.01$). The rats' preference for the saline-paired chamber did not differ from their preference for the novel chamber.

Activity measure. Figure 2 presents the mean number of quadrant crossings per minute displayed by rats while in each chamber during the place preference test of Experiment 1. The 3 × 3 mixed-factor ANOVA for each of these behaviors revealed a significant chamber main effect only, $F(2, 66) = 18.06$, $p < 0.01$. Subsequent Newman-Keuls analyses for the chamber main effect revealed a significantly higher rate of quadrant crossing in the drug-paired chamber than either the saline-paired or the novel chambers ($p < 0.01$). The rate of quadrant crossing was also significantly greater in the saline-paired chamber than in the novel chamber ($p < 0.01$).

Experiment 2

Figure 3 presents the mean amount of time (seconds) rats conditioned with 0.25 or 1.0 mg/kg fenfluramine spent in

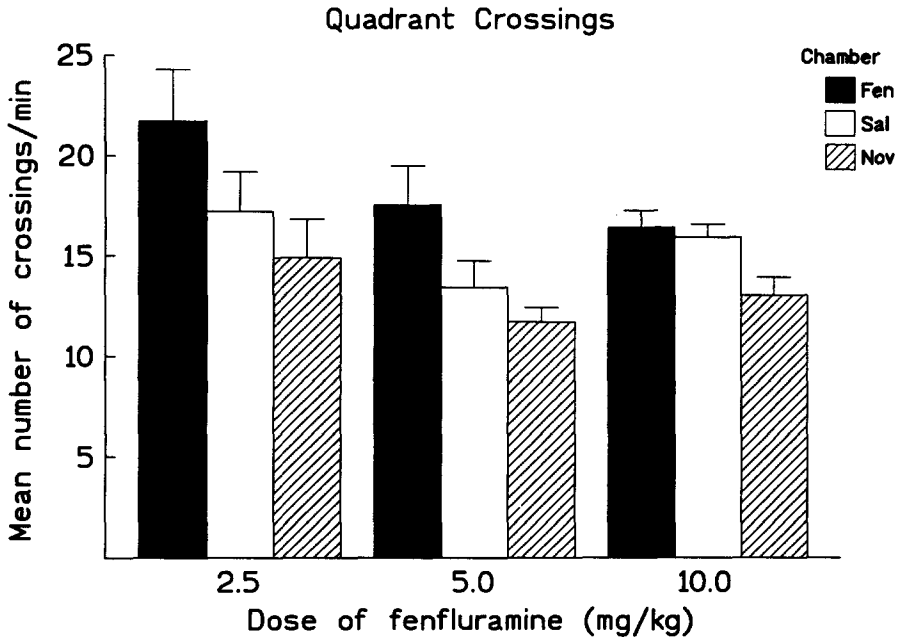

FIG. 2. Mean (±SE) number of quadrant crossings per minute in each chamber of a three-choice apparatus following place conditioning with 2.5, 5.0, and 10.0 mg/kg fenfluramine in Experiment 1.

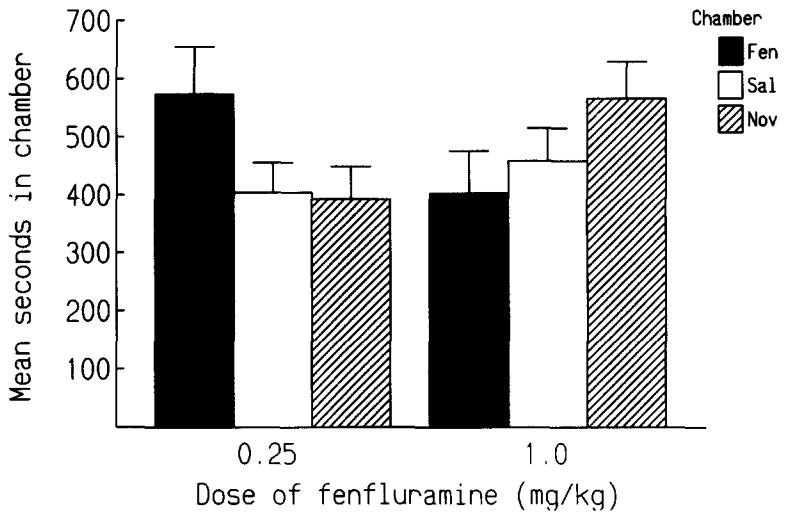

FIG. 3. Mean (±SE) number of seconds rats spent in each chanber during Experiment 2.

each of the chambers during the place preference test of Experiment 2. The 2 × 3 mixed-factor ANOVA revealed no significant effects, although the dose × chamber interaction approached significance, $F(2, 44) = 2.63$, $p < 0.085$. A single-factor within-groups ANOVA for the chamber effect for each dose group revealed no significant effects. The activity measure also revealed no significant effects.

DISCUSSION

Previous research has consistently shown that fenfluramine is not reinforcing. Fenfluramine does not maintain self-administration even at the minimum response requirement (1, 9,13,28,34) and does not facilitate self-stimulation responding (17). It also effectively establishes a CTA (6,8,11,12,32) and produces aversive taste reactivity responses (24), suggesting that a fenfluramine-paired flavor becomes unpalatable to rats. Therefore, it was anticipated that, like lithium (which shows a similar pattern of results in these paradigms), fenfluramine would also produce a place aversion in the place conditioning paradigm. The results of the above experiments revealed that doses of fenfluramine between 2.5–10 mg/kg produce a place aversion.

Because in Experiment 1 the dose of fenfluramine did not effect the strength of the place aversion, it is possible that 2.5 mg/kg is an asymptotic aversive dose; hence, an increase in dosage above 2.5 mg/kg does not produce an increase in aversive hedonic properties. In Experiment 2, doses of 0.25 and 1.0 mg/kg fenfluramine produced neither a place preference nor a place aversion. It does not appear that fenfluramine produces a positive hedonic effect, even at low doses that are unlikely to deplete serotonergic stores (29); however, it is conceivable that doses lower than 0.25 mg/kg would produce a conditioned place preference.

The activity measure employed in the present experiments serves as another measure of the reinforcing/aversive properties of a drug. When conditioned with a reinforcing drug such as morphine or amphetamine, rats spend more time in the drug-paired chamber than in the saline-paired chamber and are also less active while in the drug-paired chamber than while in the saline-paired chamber (3,25), regardless of the unconditioned effects of that agent on activity. On the other hand, when conditioned with an aversive drug (lithium chloride) rats spend less time in the drug-paired chamber and are more active while in that chamber than while in the saline-paired chamber (25). In the above experiment, rats were more active while in the fenfluramine-paired chamber than while in either the saline-paired or novel chambers. Because rats also demonstrated a fenfluramine-induced place aversion, this finding replicates a previous report (25) that rats are most active in the most aversive chamber during a place preference test.

Because fenfluramine has never been tested in a place conditioning paradigm, this experiment provides evidence that not only is fenfluramine not reinforcing to rats [as previously demonstrated by self-administration studies (1,10,13,14,17, 28,34)] but also it is aversive. It is unclear whether the aversive properties of fenfluramine measured in the place conditioning paradigm are related to its anorectic properties (5,14). However, it has been reported (35) that cholecystokinin (CCK), the satiety hormone released by the duodenum after completion of a meal, also produced a place aversion. Because at some doses CCK also produces anorexia when administered by peripheral injection, the anorexia produced by CCK may be the result of nonspecific malaise rather than the result of specific effects on the hunger system (35). It is possible that a similar explanation may account for the suppression of feeding by fenfluramine at doses ranging from 2.5–10.0 mg/kg. However, it has been reported that a dose of fenfluramine as low as 0.5 mg/kg modifies macronutrient intake (36). This dose was ineffective in Experiment 2 in producing a conditioned place aversion. Therefore, at low doses modification of appetite by fenfluramine pretreatment is unlikely to be the result of its nonspecific aversive properties.

The hedonic properties of some serotonergic agents, other than fenfluramine, have been assessed in the place conditioning paradigm. It has been demonstrated that serotonin transmission is important for the establishment of morphine-induced place preferences because 5-hydroxytryptamine$_2$ (5-HT$_2$) and 5-HT$_3$ receptor antagonists disrupt the formation of a place preference conditioned with morphine, nicotine, and amphetamine (7,16,23). However, it has also been reported that zimeldine, a serotonergic agonist, suppressed the

establishment of an amphetamine-induced conditioned place preference but not a morphine-induced conditioned place preference (18). When the direct reinforcing properties of serotonergic agonists and antagonists have been assessed in the place conditioning paradigm, although some investigators have reported that 5-HT agonists such as zimelidine (18) and 5-HT_2 and 5-HT_3 antagonists such as ritanserin (22) and ICS-205,930 and MDL72222 (7) produce no place conditioning, it has recently been reported that the 5-HT_3 antagonist ondansetron produces a place aversion (16). Other investigators, however, have reported that both buspirone, a selective 5-HT_{1A} antagonist (21), and DPAT, a selective 5-HT_{1A} agonist (31), both produce a place preference (16). The relevance of the relationship between the hedonic properties of fenfluramine and selective 5-HT agonists and antagonists requires further investigation.

ACKNOWLEDGEMENTS

The authors thank Morven Rennie for skilled technical assistance in conducting the experiments. The research was supported by funding from the National Institute on Drug Abuse (NIDA Grant 06559).

REFERENCES

1. Aigner, T. G.; Balster, R. L. Rapid substitution procedure for intravenous self-stimulation studies in rhesus monkeys. Pharmacol. Biochem. Behav. 10:105–112; 1979.
2. Barnfield, A.; Clifton, P. Flavour aversion conditioned by *dl*-fenfluramine: A volume independent mechanism. Psychopharmacology 98:108–112; 1989.
3. Bardo, M. T.; Neisewander, J. L.; Pierce, R. C. Novelty-induced place preference behavior in rats: Effects of opiate and dopaminergic drugs. Pharmacol. Biochem. Behav. 32:683–687; 1990.
4. Blundell, J. E. Serotonin and appetite. Neuropharmacology 23: 1537–1551; 1984.
5. Booth, D. A.; Gibson, E. L.; Baker, B. J. Gastromotor mechanism of fenfluramine anorexia. Appetite 7:57–69; 1986.
6. Booth, D. A.; Pilcher, C. W.; D'Mello, G. D.; Stolerman, I. P. Comparative potencies of amphetamine, fenfluramine and related compounds in taste aversion experiments in rats. Br. J. Pharmacol. 61:669–677; 1977.
7. Carboni, E.; Acquas, E.; Leone, P.; di Chiara, G. 5HT_3 receptor antagonists block morphine- and nicotine- but not amphetamine-induced place conditioning. Psychopharmacology (Berl.) 97:175–178; 1989.
8. Fletcher, P. J.; Burton, M. J. Effects of manipulations of peripheral serotonin on feeding and drinking in the rat. Pharmacol. Biochem. Behav. 20:835–840; 1984.
9. Gotestam, K. G.; Andersson, B. E. Self-administration of amphetamine analogues in rats. Pharmacol. Biochem. Behav. 3:229–233; 1975.
10. Gotestam, K. G.; Gunne, L. M. Subjective effects of two anorexigenic agents—fenfluramine and AN 448—in amphetamine dependent subjects. Br. J. Addict. 67:39–44; 1972.
11. Goudie, A. J.; Taylor, M.; Atherton, H. Effects of prior drug experience on the establishment of taste aversions in rats. Pharmacol. Biochem. Behav. 3:947–952; 1975.
12. Goudie, A. J.; Thornton, E. W.; Wheeler, T. J. Drug pretreatment effects in drug induced taste aversions: Effects of dose and duration of pretreatment. Pharmacol. Biochem. Behav. 4:629–633; 1976.
13. Griffiths, R. R.; Brady, J. V.; Snell, J. D. Progressive-ratio performance maintained by drug infusions: Comparison of cocaine, diethylpropion, chlorphentermine, and fenfluramine. Psychopharmacology (Berl.) 56:5–13; 1978.
14. Griffiths, R. R.; Winger, G.; Brady, J. V.; Snell, J. D. Comparison of behavior maintained by infusions of eight phenylethylamines in baboons. Psychopharmacology (Berl.) 50:251–258; 1976.
15. Grill, H. J.; Norgen, R. The taste reactivity test. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res. 143:263–279; 1978.
16. Higgins, G. A.; Joharchi, N.; Nguyen, P.; Sellers, E. M. Effect of the 5-HT_3 receptor antagonists, MDL72222 and ondansetron, on morphine place conditioning. Psychopharmacology (Berl.) 106:315–320; 1992.
17. Kornblith, C. L.; Hoebel, B. G. A dose-response study of anorectic drug effects on food intake, self-stimulation and stimulation-escape. Pharmacol. Biochem. Behav. 5:215–218; 1976.
18. Kruszewska, A.; Romandini, S.; Samanin, R. Different effects of zimelidine on the reinforcing properties of *d*-amphetamine and morphine on conditioned place preference in rats. Eur. J. Pharmacol. 125:283–286; 1986.
19. Montgomery, A. M. J.; Wilner, P. Fenfluramine disrupts the behavioral satiety sequence. Psychopharmacology (Berl.) 94:397–401; 1988.
20. Mucha, R. F.; Van der Kooy, D.; O'Shaughnessy, M.; Bucenieks, P. Drug reinforcement studied by the use of place conditioning in rats. Brain Res. 243:91–105, 1982.
21. Neisewander, J. L.; McDougall, S. A.; Bowling, S. L.; Bardo, M. T. Conditioned taste aversion and place preference with buspirone and gepirone. Psychopharmacology (Berl.) 100:485–490; 1990.
22. Nomikos, G. G.; Spyraki, C. Effects of ritanserin on the rewarding properties of *d*-amphetamine, morphine and diazepam revealed by conditioned place preference in rats. Pharmacol. Biochem. Behav. 30:853–858; 1988.
23. Parker, L. A. Nonconsummatory and consummatory behavioral CRs elicited by lithium and amphetamine paired flavored solutions. Learn. Motiv. 13:281–303; 1982.
24. Parker, L. A. Positively reinforcing drugs may produce a different kind of CTA than drugs which are not positively reinforcing. Learn. Motiv. 19:207–220; 1988.
25. Parker, L. A. Place conditioning in a 3 and 4 choice apparatus: The role of stimulus novelty in drug-induced place conditioning. Behav. Neurosci. 106:1–13; 1992.
26. Parker, L. A.; Carvell, T. Orofacial and somatic responses elicited by lithium, nicotine and amphetamine-paired sucrose solution. Pharmacol. Biochem. Behav. 24:883–887; 1986.
27. Reicher, M. A.; Holman, E. W. Location preference of flavor aversion reinforced by amphetamine in rats. Anim. Learn. Behav. 5:343–346; 1977.
28. Risner, M. E.; Silcox, D. L. Psychostimulant self-administration by beagle dogs in a progressive-ratio paradigm. Psychopharmacology (Berl.) 75:25–30; 1981.
29. Rowland, N. E. Effect of continuous infusion of dexfenfluramine on food intake, body weight and brain amines in rats. Life Sci. 39:2581–2586; 1986.
30. Scoles, M.; Siegel, S. A potential role of saline trials in morphine-induced place preference conditioning. Pharmacol. Biochem. Behav. 35:583–587; 1986.
31. Shippenberg, T. S. Conditioned reinforcing effects of 8-hydroxy-2-(di-*N*-propylamino) tetralin: Involvement of 5-hydroxytryptamine 1A and D_1 dopamine receptors. Neurosci. Lett. 121:136–138; 1991.
32. Stolerman, I. P.; D'Mello, G. D. Amphetamine-induced hypodipsia and its implications for conditioned taste aversion in rats. Pharmacol. Biochem. Behav. 8:333–338; 1978.
33. Stunkard, A.; Rickels, K.; Hesbacher, P. Fenfluramine in the treatment of obesity. Lancet 1:503–505; 1973.
34. Tessel, R. E.; Woods, J. H. Fenfluramine and *N*-ethyl amphetamine: Comparison of the reinforcing and rate-decreasing actions in the rhesus monkey. Psychopharmacologia 43:239–244; 1975.
35. van der Kooy, D. Place conditioning: A simple and effective method for assessing the motivational properties of drugs. In:

Bozarth, M. A., ed. Methods of assessing the reinforcing properties of abused drugs. New York: Springer-Verlag; 1987:229–240.
36. Weiss, G. F.; Rogacki, N.; Fueg, A.; Buchen, D.; Leibowitz, S. F. Impactof hypothalamic *d*-norfenfluramine and peripheral *d*-fenfluramine injection on macronutrient intake in the rat. Brain Res. Bull. 25:849–859; 1990.
37. Zalaquett, C. P.; Parker, L. A. Further evidence that CTAs produced by lithium and amphetamine are qualitatively different. Learn. Motiv. 20:413–427; 1989.
38. Ziance, R. J.; Sipes, I. G.; Kinnard, W. J.; Buckley, J. P. Central nervous system effects of fenfluramine hydrochloride. J. Pharmacol. Exp. Ther. 180:110–117; 1972.