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Reinstatement by Caffeine of an Extinguished Conditioned Dopaminergic Drug Response

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CAREY, R. J. *Reinstatement by caffeine of an extinguished conditioned dopaminergic drug response.* PHARMACOL BIOCHEM BEHAV **36**(1) 127–132, 1990. — An experimental study of extinction of conditioned drug-induced effects was carried out to determine: 1) duration of the extinction effect; and 2) stability of extinction as determined by a challenge with a stimulant drug. Twelve animals with unilateral 6-hydroxydopamine (6-OHDA) substantia nigra lesions were assigned to paired and unpaired treatment groups (n = 6) in a Pavlovian conditioning paradigm. The paired animals received apomorphine (0.05 mg/kg SC) immediately prior to placement into a test chamber and the unpaired animals received the apomorphine 30 min following test chamber placement. The two groups were matched for apomorphine-induced contralateral rotation prior to the conditioning treatment. Following Pavlovian conditioning, the paired group, but not the umpaired group, exhibited contralateral rotation in a nondrug test trial. This conditioned response underwent extinction after one nondrug extinction trial and the extinction effect persisted for 2 months. When tested with caffeine (10 mg/kg), the paired animals again exhibited substantial contralateral rotation. In contrast, the unpaired animals showed only an increase in ipsilateral rotation in response to the caffeine treatment. The drastically different responses to caffeine in the paired and unpaired animals was not due to prior apomorphine exposure per se or due to 6-OHDA lesion-induced differences in striatal dopamine depletion. Rather, the effect of caffeine on rotation behavior was determined by the Pavlovian drug conditioning procedures carried out several months earlier prior to caffeine testing.

Drug conditioning Extinction Apomorphine Caffeine 6-OHDA lesions Nigrostriatal tract

IT is well-established that dopamine agonist drug effects can become conditioned to the situation where the drug is being administered and that such conditioned effects have clinical importance for drug-related behavioral processes such as withdrawal and craving (1, 3–8, 12, 15, 21). At the clinical level, results of attempts to eliminate conditioned drug responses with extinction procedures have shown that conditioned cocaine responses can be attenuated. The extinction effects, however, are labile inasmuch as conditioned cocaine responses can be reinstated by stress (21). This latter finding is consistent with early observations by Pavlov in which extinguished conditioned responses could be reinstated by novel activating stimuli (24,25). Despite the obvious importance of these observations for drug conditioning, there has been little systematic experimental work directed at the effects of extinction on conditioned drug responses. The present study was undertaken to examine extinction effects using a conditioned dopaminergic drug response in an animal conditioning model.

An obvious advantage of using an animal model is that exposure to the conditioned and unconditioned stimuli is under experimental control. The specific conditioning preparation used was that of apomorphine-induced contralateral rotation which has been repeatedly shown to be a highly effective drug conditioning procedure (3–7). A particularly useful advantage of this specific model is that the drug-induced response of rotation is unambiguous and can be quantitated objectively in detail. Furthermore, the response is uniform across animals and does not occur spontaneously. Moreover, we have shown in previous studies that the conditioned and unconditioned rotational responses are identical, thereby fulfilling a requisite feature of Pavlovian conditioning (8). Using this animal model, the present study examined both the persistence and stability of extinction effects over a period of several months. Duration of extinction efficacy was evaluated by retesting for conditioning several months after the extinction treatment. Stability of the extinction effects was assessed by subjecting animals to a pharmacological challenge with caffeine at a dose level which induces arousal effects (19, 20, 28).

METHOD

Animals

Adult male 400–500 g Sprague-Dawley rats were used. The

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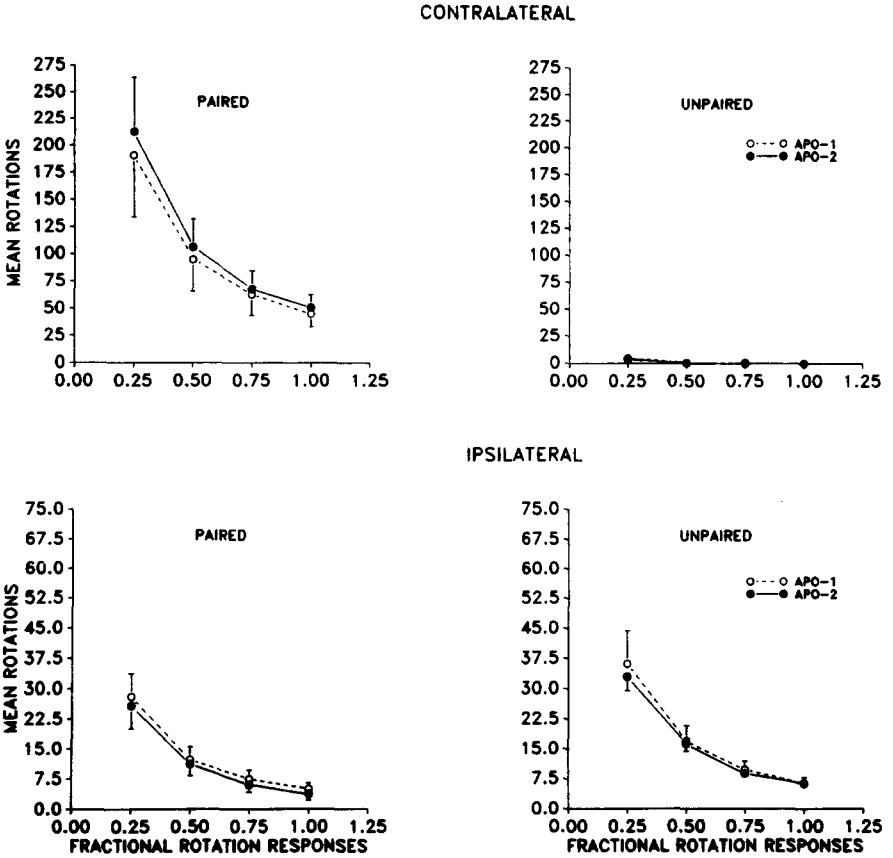

FIG. 1. Means and SEMs of fractional rotation responses of <20 cm diameter. The effects of 0.05 mg/kg apomorphine given immediately prior to placement into the test chamber (paired group treatment) are compared with the same apomorphine treatment given 30 min after removal from the test chamber (unpaired group treatment). APO-1 is the fourth drug conditioning session and APO-2 is the reconditioning session. Contralateral rotation was only elicited in the paired group and the differences in contralateral rotation between the paired vs. unpaired group of animals were statistically significant ($p<0.01$ Mann-Whitney U-test).

animals were housed in individual cages with a continuous access to food and water. Cages were in a climate- (22 ± 1°C) and light-controlled room with 12-hr light and dark cycles. Testing was conducted during the light cycle.

Surgical and Biochemical Procedures

6-Hydroxydopamine (6-OHDA) was injected stereotaxically in the vicinity of A_9 and A_{10} areas of the substantia nigra using the following coordinates: A_9, 4.0 mm posterior to bregma, 7.5 mm below dura and 2.0 mm lateral to the midline suture; A_{10}, 4.0 mm posterior to bregma, 8.0 mm below dura and 1.0 mm lateral to the midline (the incisor bar was placed 3.2 mm above the interaural plane). At each site, 1.5 μl of 3 μg/μl (calculated as a free base) of 6-OHDA-HBR (Sigma) which was dissolved in 0.15 M NaCl containing 0.2 mg/ml of ascorbic acid was injected at a rate of 0.5 μl/min. The injections were started 1 min after the cannula was fixed in position and removed 3 min after completion of injection. Equithesin anesthesia (3 ml/kg) was used in all surgeries. Surgery was aseptic and antibiotics were administered to all animals following surgery and for one week postoperative.

At the conclusion of the experiment, the rats were injected with Equithesin 3 ml/kg and 3 min later were sacrificed by decapitation. According to Schwarting and Huston (26) this procedure induces sedation without altering brain catecholamine content. The brains were removed over ice and brain tissue was transected coronally at the level of the optic chiasma. The striata were dissected from each hemisphere of the anterior section and used for biochemical analysis while the posterior portion was used for histological evaluation of the injection site. The samples were then centrifuged and stored at −70°C. Within two days, the samples were assayed for catecholamines and indoleamines using high performance liquid chromatography with electrochemical detection (HPLC-EC) (23). The amines were separated with a reverse phase column using a mobile phase prepared from acetonitrile and tetrahydrofuran solvents with sodium octyl sulfite added as an ion pair reagent. A glassy carbon working electrode was used in conjunction with a Bioanalytical Systems 4B detector. The detector potential was set at +0.8 V with respect to an Ag/AgCl reference electrode. To evaluate μg/g wet tissue concentrations, the amine peak heights to internal standard (dihydroxybenylamine) peak height ratios were determined.

Drugs

Apomorphine HCl (Sigma) was dissolved in a solution containing 40 mg ascorbic acid/l of distilled water and was administered by SC injection. A dosage of 0.05 mg/kg (0.05 mg/ml

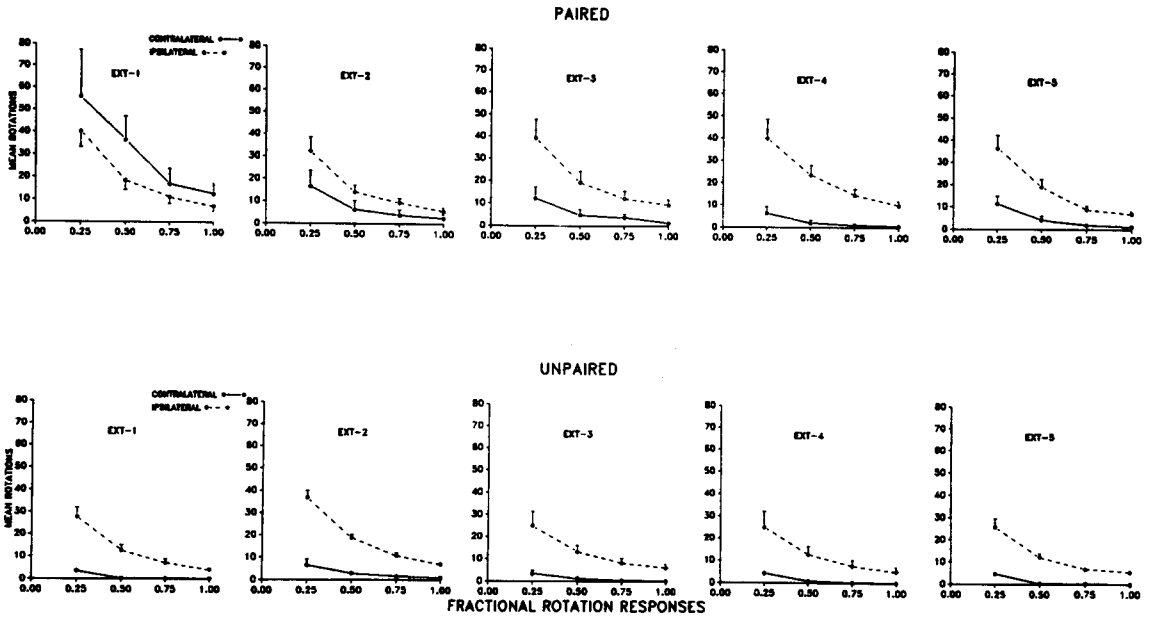

FIG. 2. Means and SEMs of fractional contralateral and ipsilateral rotation responses of <20 cm diameter. Extinction tests 1–5 refer to days 21, 22, 23, 42 and 90, respectively, from the last drug injection. On extinction test 1, the paired group emitted more contralateral rotation responses than the unpaired group ($p<0.01$ Mann-Whitney U-test). On the subsequent 4 extinction tests, both groups emitted more ipsilateral than contralateral rotation responses ($p<0.01$) and there were no statistical differences between paired vs. unpaired groups.

concentration) was used throughout. Caffeine (Sigma), dissolved in distilled, deionized H_2O, was administered IP in 10 mg/kg dosage (10 mg/ml concentration).

Apparatus and Behavioral Measurement

The rotational behavior was measured in a black, dimly lit (red light), 60 cm square enclosure. In this chamber, spontaneous and drug-induced rotations of the animals were measured automatically with the recently developed Video Image Analyzing System (VIAS) (2). This system incorporates an on-line digitizing of analog images of a freely moving animal which are disc-stored. The stored records are subsequently evaluated by a computer software program developed specifically for the system. The computer evaluation of the stored data provides measurement of the linear distance traversed, direction of rotation, number and diameter of ¼, ½, ¾ and full 360° rotations. The rotation diameter scores include circles of <20 cm, 20–<30 cm, 30–<55 cm and 55–<60 cm.

Pavlovian Conditioning Procedures

Three weeks postsurgery and following a week of daily 5-min handling treatment, the animals were given 0.05 mg/kg of apomorphine in their home cage and the number of contralateral rotations were recorded for a 10-min period. A minimal standard for inclusion was 10 contralateral (360°) rotations. The selected animals were then matched on contralateral rotation frequency, and assigned 6 animals each to a paired and to an unpaired treatment group. These matched groups were then administered the paired and unpaired drug-test environment treatment in a Pavlovian conditioning protocol. For the paired group of animals, each animal was injected with apomorphine (0.05 mg/kg SC) immediately prior to placement into the conditioning chamber for a 10-min duration. The animal was then removed and placed back into its home cage. Animals in the unpaired groups received exactly the same treatment except that the apomorphine injection occurred 30 min after removal from the conditioning chamber. This treatment was repeated for four daily sessions. It is important to recognize that the duration of apomorphine-induced rotation is approximately one hour. Thus, for about ⅝ of the apomorphine treatment, the paired and unpaired animals had an equivalent home cage experience with the drug.

Four weeks later, all animals received a 10-min nondrug test trial placement into the test environment in which rotation, rotation direction, and diameter of rotation were automatically measured by the VIAS system. This step was taken to validate that the procedure did in fact induce a conditioned contralateral rotation response. On the next day, the animals were given 1 drug reconditioning trial as in the acquisition phase. Three weeks later, all animals were tested in the test environment without drug on three successive days and once again two weeks, and again two months later. Three days after the final nondrug test, the animals were injected (IP) with 10 mg/kg caffeine 20 min prior to testing. At the end of the experiment, all animals were injected with 0.05 mg/kg apomorphine and placed into the test environment to behaviorally assess 6-OHDA lesion efficacy. Three days later, all animals were sacrificed and the histological and biochemical assays were carried out.

RESULTS

As expected from earlier experiments (5,6), the Pavlovian conditioning protocol generated conditioned contralateral rotation in a nondrug test in the paired group, while the unpaired group exhibited only ipsilateral rotation. Statistical comparison of contralateral and ipsilateral rotation rates during the 10-min test trial revealed significantly higher rates of contralateral rotation in the

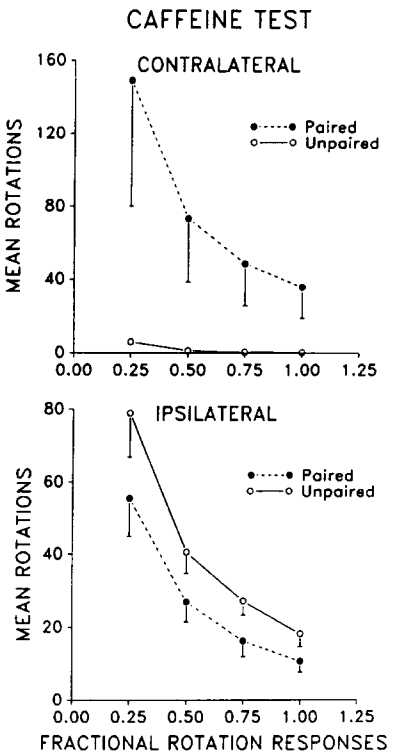

FIG. 3. Means and SEMs of fractional contralateral and ipsilateral rotation responses in the paired and unpaired groups following saline or 10 mg/kg caffeine treatments. In the paired group, caffeine increased contralateral rotation and this increase was greater than both the contralateral rotation and ipsilateral rotation in the unpaired group ($p<0.01$ Mann-Whitney U-tests) and the ipsilateral rotation of the same group ($p<0.01$ *t*-test for differences scores). Caffeine enhanced ipsilateral rotation in both groups ($p<0.01$, *t*-test for difference scores).

paired vs. the unpaired treatment group ($p<0.05$ Mann-Whitney U-test). Ipsilateral rotation rates did not differ statistically. Because extinction can occur with as little as one trial, a reacquisition test trial was administered to reestablish the conditioned contralateral rotation response. Figure 1 shows the effects of the paired and unpaired apomorphine treatment on rotation behavior during the reconditioning test trial. As expected, only animals in the paired group exhibited contralateral rotation as they were given apomorphine before testing while animals in the unpaired group exhibited only ipsilateral rotation as they were given the apomorphine after testing.

Figure 2 shows the rotation responses of the paired and unpaired groups in the 5 nondrug extinction test trials. On the first extinction test trial (EXT-1), the paired groups exhibited more contralateral than ipsilateral rotation responses. In contrast, the unpaired group exhibited substantially more ipsilateral than contralateral rotations. Statistical comparison of paired and unpaired groups in terms of contralateral rotation showed that the differences were statistically significant across all fractional rotation responses ($p<0.01$ Mann-Whitney U-test). These results showed that the conditioned contralateral response had been reestablished in the paired group. On the four subsequent extinction test trials the difference in contralateral rotation between the paired and unpaired groups was not statistically significant ($p>0.20$). On all five extinction trials, however, the paired and unpaired groups exhibited approximately equivalent levels of ipsilateral rotation. The finding that no recovery of contralateral rotation occurred even in the 2-month extinction test shows the relative persistence of the extinction effect. This result contrasts with an earlier report that the nondrug contralateral rotation following apomorphine injections is permanent (27). In this report, however, only one test trial was conducted. As the extinction results show, the effects of paired apomorphine treatment are not to be understood as inducing fixed behavioral modifications but rather as inducing conditioned effects which can be readily extinguished and that the extinction effects are persistent.

Figure 3 shows the effects of caffeine on the rotational behavior of the paired and unpaired groups. While caffeine enhanced ipsilateral rotation in both groups over nondrug levels ($p<0.01$ *t*-test for difference scores), only the paired groups exhibited contralateral rotation to the caffeine. The caffeine-induced contralateral rotation for the paired group was even greater than its rate of ipsilateral rotation under caffeine ($p<0.01$ *t*-test for difference scores). Indeed, the caffeine-induced contralateral rotation rates were approximately half as large as the rotation rate exhibited in response to apomorphine shown in Fig. 1.

At the completion of the experiment, when the paired and unpaired animals were compared directly for their response to 0.05 apomorphine in the test environment, both groups exhibited similar rates and there were no statistical differences between groups. Additionally, the biochemical measurements showed that the DA levels in the striatum of the 6-OHDA-treated hemisphere were reduced to less than 5% of the intact hemisphere in both groups and group differences were not statistically significant ($p>0.25$).

DISCUSSION

In agreement with a number of previous reports, the present study shows that apomorphine-induced contralateral rotation in unilateral 6-OHDA rats can be conditioned to a testing situation, extinguished and reconditioned (3–8, 27). Furthermore, the extinction effects observed persist for several months. Taken together, these observations firmly link the contralateral rotation response associated with apomorphine to conditioning processes. In agreement with clinical observations, the effects of extinction seemed to be labile in that caffeine was able to reinstate and exaggerate the conditioned drug response. Since the stimulant properties of caffeine appear to be mediated by an antagonism of brain adenosine receptors, this effect of caffeine on conditioned behavior suggests a possible role for adenosine in the pharmacology of drug conditioning (10, 11, 19, 28).

Before examining in greater detail the possible connection between caffeine and conditioning processes, a more prosaic consideration needs to be examined; namely, the possibility that caffeine might directly stimulate the striatal supersensitive dopamine receptors and evoke the contralateral response without involvement of the conditioned test stimuli (14,17). This possibility, however, can be discounted on the basis of several observations. First of all, studies have shown that caffeine does not induce contralateral rotation in drug-naive animals with 6-OHDA lesions (14). Secondly, in every case where caffeine has been shown to elicit contralateral rotation, the possibility for conditioning existed since the animals had previously been treated with apomorphine in the same test situation (14,17). Moreover, in a previous report, we found that animals with unilateral 6-OHDA lesions responded differentially to caffeine depending upon the apomorphine link to the test situation (4). In animals that had been previously administered the apomorphine in a temporally contiguous relation to the test environment, caffeine evoked contralateral rotation, whereas the same animals rotated ipsilaterally to caffeine in a test situation that was unpaired to apomorphine administration. Furthermore, in the present study, animals with functionally and biochemically

equivalent 6-OHDA lesions and identical apomorphine treatment differed in their response to caffeine depending upon whether their apomorphine treatment had been paired or unpaired to the test situation. Paired animals rotated contralaterally and unpaired animals rotated ipsilaterally. Altogether, these observations appear to establish that caffeine-induced contralateral rotation involves an interaction with drug conditioning processes.

In general, the present findings appear in agreement with Pavlov's observations which indicate that extinction can suppress but not eliminate a conditioned drug response (24,25). The activation and augmentation of the conditioned rotational response by caffeine in the present report appears analogous to the reinstatement of extinguished conditioned cocaine effects by stress observed in a recent clinical study (21). In addition to acting as a stressor and stimulant, however, caffeine also has interoceptive cue properties (18). In the context of the present experimant, it is possible that the caffeine cue substituted for the apomorphine cue and by virtue of its cue property caffeine reinstated the drug response. While a cue-effect interpretation might account for the contralateral rotation induced by caffeine, it does not explain its selectivity for the paired versus the unpaired drug treatment response. Since both groups had identical exposure to apomorphine, the presentation of the caffeine cue to both groups would be expected to induce the conditioned apomorphine response in both groups albeit at an attenuated level for the unpaired group. Furthermore, studies of comparative cue properties of drugs have shown that the apomorphine cue does not substitute for the caffeine cue (18).

While there is evidence that the mechanisms which mediate conditioned drug responses are pharmacologically distinct from the mechanisms which mediate unconditioned drug responses (1), this important differentiation only highlights the current obscurity regarding central mediation of conditioned drug effects. Thus, the finding that caffeine could activate a conditioned drug response suggests that caffeine mechanisms may offer a potential clue to the pharmacological identity of conditioned drug response processes. This conclusion is strengthened by the fact that caffeine activated the conditioned contralateral rotational response after it had been extinguished. Following extinction, the paired animals reverted to their strong spontaneous response bias for the opposite response of ipsilateral rotation. Had the animals been tested prior to extinction when conditioned effects were optimal, and exaggeration of the contralateral rotation response by caffeine might have simply indicated that caffeine amplified the existing response bias. That is, the conditioning treatment would have made contralateral rotation the dominant response and caffeine, being a stimulant drug, would have been expected to exaggerate this response bias. However, the animals were tested after extinction, when the response bias had already shifted toward ipsilateral rotation, and caffeine actually reversed this response bias selectively in the paired animals. It is this selective reversal of the response bias in favor of the previously conditioned response which makes the caffeine link to drug conditioning promising. In unpaired animals, caffeine enhanced the ipsilateral response bias, whereas in paired animals caffeine actually reversed this response bias. Seemingly, the mechanisms mediating caffeine central effects are implicated in the conditioned drug effects induced by dopaminergic drugs although, paradoxically, they appear to be disassociated from dopaminergic mechanisms. While substantial evidence suggests that caffeine stimulant effects are mediated by adenosine antagonism (9, 11, 19, 28), the central effects of caffeine are complex and diverse and include inhibition of phosphodiesterase (13), calcium blockade (16) as well as noradrenergic effects (18). The possible contribution of these caffeine effects to drug conditioning processes at this point remains to be determined.

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REFERENCES

1. Beninger, R. J. The role of dopamine in locomotor activity and learning. Brain Res. Rev. 6:173–196; 1983.
2. Bonatz, A.; Steiner, H.; Huston, J. P. Video image analysis of behavior by microcomputer: categorization of turning and locomotion after 6-OHDA injection into the substantia nigra. J. Neurosci. Methods 22:13–26; 1987.
3. Carey, R. J. A conditioned anti-parkinsonian drug effect in the hemi-parkinsonian rat. Psychopharmacology (Berlin) 89:269–272; 1986.
4. Carey, R. J. Antiparkinsonian effects of caffeine depend upon Pavlovian drug conditioning processes. Brain Res.; in press.
5. Carey, R. J. Application of the unilateral 6-hydroxydopamine rat model of rotational behavior to the study of conditioned drug effects. J. Neurosci. Methods 22:253–261; 1988.
6. Carey, R. J. Conditioned rotational behavior in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. Brain Res. 365: 382; 1986.
7. Carey, R. J. Neuroanatomical and neuropharmacological dissociation of unconditioned versus conditioned apomorphine induced behavioral effects. Behav. Brain Res.; in press.
8. Carey, R. J. Stimulant drugs as conditioned and unconditioned stimuli in a classical conditioning paradigm. Drug Dev. Res. 16:305–315; 1989.
9. Choi, O. H.; Shamim, M. T.; Padgett, W. L.; Daly, J. W. Caffeine and theophylline analogues: correlation of behavioral effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors. Life Sci. 43:397–398; 1988.
10. Coffin, V. L.; Carney, J. M. Effects of selected analogs of adenosine on schedule-controlled behavior in rats. Neuropharmacology 10: 1141–1147; 1986.
11. Daly, J. W.; Padgett, W. L.; Shamin, M. T. Analogues of caffeine and theophylline: effects of structural alterations on affinity at adenosine receptors. J. Med. Chem. 29:1305–1308; 1986.
12. Drew, K. L.; Glick, S. D. Classical conditioning of amphetamine-induced lateralized and nonlateralized activity in rats. Psychopharmacology (Berlin) 92:52–57; 1987.
13. Freedholm, B. B.; Fluxe, K.; Agnati, L. Effect of some phosphodiesterase inhibitors on central dopamine mechanisms. Eur. J. Pharmacol. 38:31–38; 1976.
14. Fluxe, K.; Ungerstedt, U. Action of caffeine and theophyllamine on supersensitive dopamine receptors: Considerable enhancement of receptor response treatment with DOPA and dopamine receptor agonists. Med. Biol. 52:48–54; 1974.
15. Gold, M. S.; Byron, C. A.; Dackis, C. A.; Sweeny, D. R. Paraphernalia-induced cocaine craving. Soc. Neurosci. Abstr. 12:936; 1986.
16. Hagiwara, S.; Byerly, L. Calcium channel. Annu. Rev. Neurosci. 4:69–125; 1981.
17. Herrera-Marschitz, M; Casas, M. Ungerstedt, U. Caffeine produces contralateral rotation in rats with unilateral denervation: Comparison with apomorphine induced responses. Psychopharmacology (Berlin) 94:38–45; 1988.
18. Holtzman, S. G. Discriminative stimulus properties of caffeine in the rat: Noradrenergic mediation. J. Pharmacol. Exp. Ther. 239:709–714; 1986.
19. Katims, S. J.; Annau, Z.; Snyder, S. Interactions in the behavioral effects of methylxanthines and adenosine derivatives. J. Pharmacol. Exp. Ther. 227:167–173; 1983.
20. Katz, J. L.; Goldberg, S. R. Psychomotor stimulant effects of caffeine alone and in combination with adenosine analog in the squirrel monkey. J. Pharmacol. Exp. Ther. 242:179–187; 1987.
21. McClellen, A.; Childress, A. Ehrman, R.; O'Brien, C. Opiate and

cocaine related stimuli elicit craving and physiological responses in drug-abuse patients. Presented at the international meetings of Society for Stimulus Properties of Drugs, Cape Cod, MA, June 28, 1988. 93:275–280; 1988.
22. Mattingly, B. A.; Gotsick, J. E.; Salamanca, K. Latent sensitization to apomorphine following repeated low doses. Behav. Neurosci. 102:553–558; 1988.
23. Mayer, G. S.; Shoupe, R. E. Simultaneous multiple electrode liquid chromatography-electrochemical assay for catecholamines, indoleamines and metabolites in brain tissue. J. Chromatogr. 255:533–534; 1983.
24. Pavlov, I. P. Conditioned reflex. London: Oxford University Press; 1927.
25. Pavlov, I. P. Lectures on conditioned reflexes. New York: International; 1928.
26. Schwarting, R.; Huston, J. P. Short-term effects of ether, equithesin, and doperidol/fentanyl on catecholamine and indoleamine metabolism in the brain in the rat. Neuropharmacology 26:457–461; 1987.
27. Silverman, R. S.; Ho, B. T. Persistent behavioral effects apomorphine in 6-hydroxidopamine-lesioned rats. Nature 294:457–461; 1981.
28. Snyder, S. H.; Katims, S. J.; Annau, Z.; Bruns, R. F.; Daly, J. W. Adenosine receptors and behavioral actions of methylxanthines. Proc. Natl. Acad. Sci. USA 78:3260–3264; 1981.