Both Positive Reinforcement and Conditioned Aversion from Amphetamine and from Apomorphine in Rats

Abstract. *Rats learned to press a lever for intravenous injections of amphetamine or apomorphine. They also learned to avoid the taste of saccharin which was associated with experimenter-administered amphetamine or with self-administered apomorphine. Thus these, and presumably other, self-administered drugs serve as compound pharmacological stimuli, having both positively reinforcing and aversive properties.*

A major focus of current studies on drug abuse assesses the stimulus properties of drugs. It has been established over the last several years that drugs of abuse such as opiates, psychomotor stimulants, and ethyl alcohol can have either positively reinforcing or aversive stimulus properties, depending on the test used. It is known, on the one hand, that intravenous injections of these agents can provide powerful reinforcement. Animals will learn to press a lever for such injections (*1*), and previously neutral auditory or visual stimuli will acquire secondary (learned) reinforcing properties if they are associated with such injections (*2*). On the other hand, animals will learn to avoid taste stimuli which have been associated with similar doses of the same agents (*3*). This presents a conceptual paradox. How can a neutral stimulus become aversive because of an association with another stimulus that is itself positively reinforcing? How can a drug injection serve as a positive unconditioned stimulus for establishing secondary reinforcement in some cases and serve as a negative unconditioned stimulus for establishing learned aversion in others? The possibility that the same abused drug might serve as both a positive reinforcer and an aversive stimulus poses a problem for theories of drug abuse that are couched simply in terms of the laws of positive reinforcement.

One approach to resolving this apparent paradox involves analyzing methodological differences between the paradigms in which the reinforcing and aversive properties of drugs have been demonstrated. For example, in the conditioned taste aversion paradigm animals are usually drug-naive, while in the reinforcement paradigm they are drug-experienced. The drug-experienced animals may be tolerant to, and in some cases dependent on, the drug in question. Thus what is nominally the same dose of the same agent in the two paradigms may not in fact be equivalent; aversive doses for naive animals may have much less impact on experienced animals, and this could conceivably explain their aversive effects in the aversion paradigm (*3*).

We now report that procedural differences between paradigms cannot fully account for the fact that drug injections are aversive in one test and reinforcing in another. While the aversive effects of drugs are stronger in naive animals, we have found that the reinforcing and aversive effects of amphetamine and apomorphine can be demonstrated in the same animals at the same stage of drug experience. In fact, we have found animals to demonstrate both the reinforcing and aversive properties of apomorphine with a single test exposure to the drug; these animals lever-pressed for the very injections that caused learned aversion to associated tastes.

In our first experiment we asked whether a conditioned taste aversion to saccharin could be established by associating saccharin with amphetamine in amphetamine-experienced animals. The classic demonstration of amphetamine reinforcement involves allowing animals to lever-press for intravenous amphetamine injections (*1*); we selected animals that had shown such amphetamine self-administration in eight to 14 prior self-administration sessions (*4*). Thus the animals were all experienced with and perhaps tolerant to intravenous amphetamine, and had already demonstrated the reinforcing property of intravenous amphetamine. For the conditioned taste aversion paradigm, animals were given a 10-minute baseline saccharin drinking test after 16 hours of water deprivation; the saccharin drinking was immediately followed by a 1.0 mg/kg injection of *d*-amphetamine sulfate (*5*). The animals were again deprived of water for post-conditioning saccharin drinking tests on the next day (*6*).

Normally, when initial saccharin exposure is not followed by injection of drugs or other aversive agents, rats double or triple their saccharin intake on their second saccharin exposure. Our control animals were given saline injections rather than amphetamine injections after their saccharin exposure, and these animals showed the normal doubling of saccharin intake (Table 1). In contrast, animals that received intravenous amphetamine injections after their initial saccharin exposure showed only a trivial increase in saccharin intake in their saccharin tests. Thus the association of amphetamine with saccharin caused a moderate suppression of saccharin intake, even in animals with considerable amphetamine experience. Stronger aversions were seen in another group of animals that were given their saccharin-paired amphetamine by the unfamiliar intraperitoneal route of injection, and a total saccharin aversion was seen in a group of amphetamine-naive animals that learned to lever-press for amphetamine in sessions after the conditioned aversion testing (Table 1).

Table 1. Post-amphetamine saccharin intake as percentage of baseline intake for animals in six conditions of treatment (drug versus placebo injection), amphetamine history, and route of injection. Abbreviations: I.V., intravenous; I.P., intraperitoneal; *N*, number (sample size).

Treatment group	Amphetamine history		
	Experienced		Naive
	I.V.	I.P.	I.V.
Experimental*	115% (N = 5)	30% (N = 4)	4% (N = 4)
Control†	225% (N = 4)	491% (N = 2)	338% (N = 2)

*Amphetamine injection. †Placebo injection.

These data suggest that much of the aversive effect of drug injections in the usual conditioned aversion study can be attributed to the fact that the drug and route of administration are novel to the animals (*7*). However, our data clearly indicate that some degree of taste aversion is still caused by saccharin-amphetamine pairings even when animals are quite used to amphetamine injections. Thus factors like drug novelty and drug tolerance which usually differ between the two paradigms are not sufficient to explain the difference in apparent valence of the same drugs tested in the two paradigms; both reinforcing and aversive properties of amphetamine can be demonstrated in amphetamine-experienced animals.

Our amphetamine data do not, however, establish that a particular drug injection can be both reinforcing and aversive. It is clear that the injections we gave were mildly aversive, but the fact that the animals had previously lever-pressed for similar injections (*5*) does not necessarily mean that the experimenter-administered injections in our experiments were reinforcing. There are arguments in psychological theory, and data from intracranial self-stimulation experiments, which suggest that events which are reinforcing when the animal expects them may not be reinforcing when unexpectedly presented by the experimenter (*8*). Thus we next examined saccharin intake before and after animal-administered drug injections.

This experiment was difficult to design because of two conflicting constraints. First, it was necessary to give the conditioned taste aversion tests early in the ani-

mals' history of drug self-administration, since prior drug experience greatly reduces the aversive drug effect. Ideally, one would hope to give the conditioned aversion tests on the first day in which the reinforcing effects of the drug were demonstrated. The second constraint was that the initial saccharin exposure had to be given before the drug injections, in the usual relation of conditioned to unconditioned stimulus. Since it was not possible to predict accurately the first day on which an animal would learn to self-administer amphetamine, it was not possible to anticipate the appropriate time for the initial saccharin exposure. Because of this problem we decided to first train animals to lever-press for one psychomotor stimulant, and then to use another for our reinforcement and taste aversion experiment.

For this second experiment we initially trained naive animals to lever-press for amphetamine. Then for our experiment proper we used apomorphine in place of amphetamine reinforcement (*9*). Apomorphine is self-administered by the rat presumably because it activates the same central mechanism as does amphetamine (*10*), and rats trained to lever-press for amphetamine rapidly learn to lever-press for apomorphine (*11*). Since we expected most rats to show stable apomorphine self-administration on the first day of apomorphine testing, we preceded this first apomorphine session with baseline saccharin intake tests, and gave final saccharin tests on the following day (*12*). Apomorphine self-administration sessions were continued for some animals in order to verify that lever-pressing was truly sustained by apomorphine reinforcement, rather than by the habit established under amphetamine reinforcement.

Not all the animals learned to self-administer apomorphine in the 1 day of testing. Reliable and stable apomorphine self-administration patterns which could be taken as clear evidence that apomorphine was reinforcing were seen in only four animals (Fig. 1, a–d), although several other animals showed patterns which were reasonably stable. After the second saccharin test was completed three animals were tested in conditions that provide a more stringent test of the reinforcing effects of apomorphine. These animals did not continue to respond when saline was substituted for apomorphine; rather, they showed a burst of responding and then stopped responding, as is typical of extinction under conditions of nonreward (*4, 10*). These animals also showed compensating increases in rate of responding when the dose per injection of apomorphine was reduced. This is typical for stimulant self-administration (*5*), and indicates

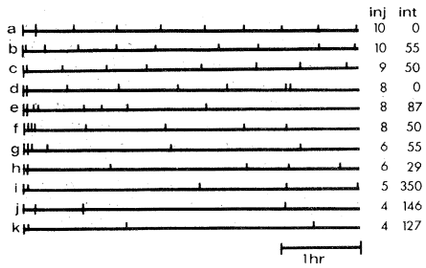

Fig. 1. Apomorphine self-administration records. Upward pen deflections indicate self-administered apomorphine injections (0.5 mg/kg); downward deflections indicate injections that were given by the experimenter. Total number of apomorphine injections (*inj*) and subsequent saccharin intake (*int*) are given in the two right-hand columns; saccharin intake is expressed as a percentage of pre-apomorphine saccharin baseline levels. The pattern seen in animals a, b, c, and d is typical of stable stimulant self-administration in that the injections were well paced; apomorphine is argued to have been reinforcing for these animals in this test. The pattern seen in animals j and k, for example, is not convincing evidence of apomorphine reinforcement.

that apomorphine was not simply activating the habitual level-pressing response by some nonspecific arousal action; if lever-pressing were caused by a simple arousal effect, the rate of response should have varied directly, not inversely, with the dose of apomorphine per injection (*10*). Thus the lever-pressing patterns seen in some of our animals indicated reinforcing effects of apomorphine in our tests. The lever-pressing patterns for all animals are shown in Fig. 1, ranked in order of self-administration reliability.

Eleven animals were tested, and only one showed the doubling or tripling of saccharin intake which was seen in control animals; the other animals showed varying degrees of saccharin aversion. The degree of saccharin aversion was strongest in the animals that self-administered the most apomorphine (rho = .54, $P < .05$, Fig. 1). Only minor evidence of aversion was seen in the two animals (j and k) that took only one and three injections above the experimenter-administered injections (*9*); with one exception (i), strong aversions were seen in the nine animals that took four or more earned injections. This is an important point, since it indicates that only a portion of the taste aversions seen can be accounted for by the unexpected apomorphine injections given at the beginning of the sessions. The apomorphine injections taken by the animals showing the strongest self-administration caused the most significant taste aversions.

These data, then, demonstrate for the first time that the same drug injections can be both positively reinforcing and aversive. The demonstration of both properties in the same animals, in the same test session, rules out arguments that differences in paradigms can account for the fact that in one paradigm a drug seems aversive while in the other paradigm the same drug seems reinforcing. Thus it must be concluded that injections of abused drugs do not represent simple positive pharmacological stimuli; rather, drug injections must be viewed as compound stimuli with both positive and negative elements. With this view in mind it is not difficult to understand why some stimuli associated with a given drug injection acquire positive valence while others acquire negative valence, since it is well established that the elements of a compound stimulus can be differentially conditioned (*13*).

ROY A. WISE
ROBERT A. YOKEL*
HARRIET DEWIT

Center for Research on Drug Dependence, Department of Psychology, Concordia University, Montreal, Quebec, Canada H3G 1M8

References and Notes

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4. R. A. Yokel and R. A. Wise, *Science* **187**, 547 (1975). The animals were implanted with indwelling venous catheters which were connected through a feed-through swivel to a syringe pump. They were housed with free access to food and water in cages equipped with an operant lever, and were trained to lever-press for amphetamine injections (0.25 mg/kg) on a continuous reinforcement schedule. Each injection was given in 0.25 ml of saline over an injection time of 30 seconds. The previous amphetamine self-administration sessions had been 8 to 16 hours in length and had occurred on alternate days in the weeks immediately preceding the present experiment. Each animal was used only once in the present study, 2 days after the earlier series of sessions.
5. The dose of amphetamine was 1 mg/kg, which was four times the dose per injection that was given in the self-administration testing. It was chosen because animals usually take at least four 0.25 mg/kg injections at the beginning of a self-administration session, when there is no amphetamine in the blood. The 1.0 mg/kg dose used was thus intended to approximate the early intake in self-administration sessions, and was, moreover, within the range of doses established as reinforcing in other experiments [R. A. Yokel and R. Pickens, *J. Pharmacol. Exp. Ther.* **187**, 27 (1973)]. The blood levels produced by these injections were below that which is maintained by self-administering animals in the face of varying conditions of dose per injection (*ibid.*).
6. The saccharin concentration was 0.1 percent in water. This is a very palatable solution, and intake levels are usually high when the solution is familiar to the animals. An unfamiliar solution was used in the present study because novel tastes are most susceptible to taste aversion conditioning [M. E. P. Seligman, *Psychol. Rev.* **72**, 406 (1970); S. J. Shettleworth, in *Advances in the Study of Behavior*, D. S. Lehrman and E. Shaw, Eds. (Academic Press, New York, 1972), vol. 4, pp. 1–68; J. Garcia and F. Ervin, *Commun. Behav. Biol.* **1**, 389 (1968)].
7. It is well known that a familiar aversive agent serves less effectively in the conditioned taste aversion paradigm than does a novel one. This is true for both abused drugs and poisons [A. E. LeBland and H. Cappell (*3*); D. S. Cannon, R. F. Berman, T. B. Baker, C. A. Atkinson, *J. Exp. Psychol.: Anim. Behav. Processes* **104**, 270 (1975)].

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9. After stabilization of amphetamine self-administration the animals were tested in the present paradigm with no previous apomorphine experience. They were placed in the self-administration chambers 24 hours after their last amphetamine session and were given two experimenter-administered "priming" injections of apomorphine (0.5 mg/kg per injection); then they were allowed to self-administer apomorphine at this dose on a continuous reinforcement schedule for 4 hours.
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12. Saccharin intake tests followed 16 hours of water deprivation, in the same paradigm as was used in the amphetamine study.
13. In fact it is already known that when compound stimuli are associated with injections of poison, the taste elements of the compound are readily associated with the aversive effects of the poison, but auditory and visual elements are not. Conversely, auditory and visual elements are associated with the aversive effects of footshock, while taste elements are not [Garcia and Ervin (*6*)].
14. Supported by the Non-Medical Use of Drugs Directorate of Canada. We thank Smith Kline & French for the amphetamine, Merck Frosst for the apomorphine, and Jane Stewart and Barry Hoffer for comments on the manuscript.
* Present address: Department of Pharmacology, University of Cincinnati, Cincinnati, Ohio.
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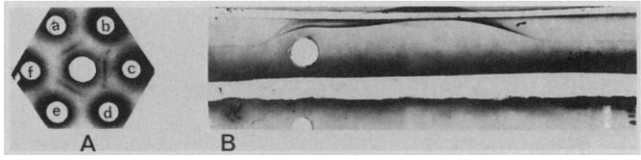
Cobra Venom Factor: Evidence for Its Being Altered Cobra C3 (the Third Component of Complement)

Abstract. *Evidence is presented that cobra venom factor, the anticomplementary protein in* Naja naja *venom, is modified cobra C3 (the third component of complement). Antiserum to the cobra venom factor cross reacts with human C3. A protein in cobra serum reacts strongly with antiserum to the venom factor and the former protein, like human C3, is converted by incubation of cobra serum with endotoxin, hydrazine, or simple storage at 37°C. Incubation of cobra venom factor with cobra serum destroys the C3 cleaving activity of the venom factor in human serum, whereas human C3b inactivator is ineffective. Thus, the cobra venom factor appears to be a form of C3 (perhaps C3b); its potent action in human serum probably derives from its lack of sensitivity to human C3b inactivator.*

Interest in the anticomplementary activity of snake venom spans virtually the entire history of the study of complement itself. A medical officer in the American Army, Captain C. B. Ewing, observed in 1894 that venom from some poisonous snakes destroyed the bactericidal activity of serum (*1*). Around the turn of the century, Flexner and Noguchi (*2*) demonstrated that these venoms acted by destroying complement activity in vitro. Ritz, in 1912 (*3*), showed that snake venom did not destroy either the first or second components of complement, the only components known at that time, and he therefore defined a third component. Some 50 years later, when it had become evident that this "third component" was complex and, in fact, consisted of several different proteins, Klein and Wellensieck (*4*) demonstrated that the attack by venom was directed against what we now call C3. Nelson (*5*) and Müller-Eberhard and his colleagues (*6*) characterized and isolated from snake venom the protein (cobra venom factor or CoF) which induced C3 cleavage and showed that this attack on C3 was not direct but required at least one normal human serum protein. Factor B of the properdin system (*7–9*) was shown to be required for the CoF-mediated attack on C3 (*9, 10*). There was, initially, controversy about whether a complex was formed between factor B and CoF. Götze and Müller-Eberhard obtained evidence that the purified proteins formed an equimolar complex (*9*), but neither we (*11*) nor Hunsicker *et al.* (*10*) could show such a complex. There is now evidence that a complex of CoF and factor B does form in the presence of factor D (*12*). In whole serum only a small fraction of the factor B is involved (*11*). There is thus no CoF-binding protein distinct from factor B as previously postulated by us and by Hunsicker and co-workers. The identification of a positive feedback loop within the properdin or alternative pathway of complement activation, triggered by C3b (*13*) prompted Lachmann and Nicol (*14*) to draw an analogy between the action of purified CoF and human C3b. We now report studies which strongly suggest that CoF is, in fact, an altered form of cobra C3.

A potent antiserum to isolated CoF from the venom of *Naja naja*, the Asiatic hooded cobra, reacted, on Ouchterlony analysis, with normal human serum and highly purified C3 but not with C3-deficient serum (*15*) (Fig. 1). To rule out the possibility that human serum and C3 in particular had somehow contaminated the CoF preparation used as antigen, normal human serum was placed in one trough of an immunoelectrophoresis slide (Fig. 1), and antiserum to CoF was placed in the opposite trough and allowed to diffuse against purified CoF subjected to electrophoresis from the center well. The faint line produced by the human serum reacting with the antiserum to CoF was not continuous, but fused with the very strong arc due to reaction of CoF and its antiserum, indicating that the reaction with human C3 was induced by antibody to CoF and not by a contaminating antibody. Further evidence that this reaction did not result from antibody to a contaminating human antigen is that, after the antiserum to CoF was absorbed with lyophilized whole cobra ven-



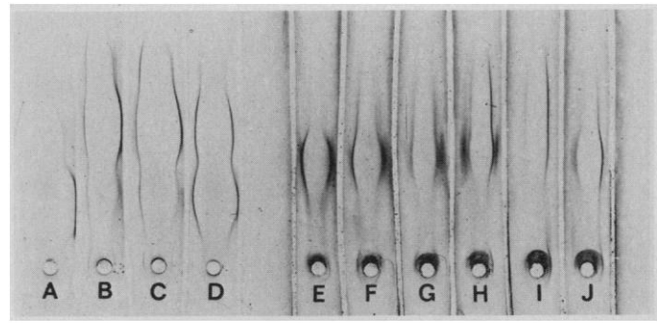


Fig. 1 (left). (A) Ouchterlony analysis in agarose gel in 0.05*M* barbital buffer (*p*H 8.6) containing $10^{-2}M$ disodium ethylenediaminetetraacetate. The central well contained rabbit antiserum to CoF. Peripheral wells a, c, and e contained normal human serum with a C3 concentration of 150 mg/100 ml. Wells b, d, and f were filled with serums from patients with diminished C3 serum concentrations (4, 30, and < 0.25 mg/100 ml, respectively). Reactions were observed only with the normal serum. (B) In immunoelectrophoresis performed in agarose gel, purified CoF was placed in the central well and subjected to electrophoresis. The upper trough was filled with rabbit antiserum to CoF and the lower trough with normal human serum. After diffusion was complete, the gel was washed, dried, and stained. Fig. 2 (right). Immunoelectrophoresis of human serum developed with antiserum to human C3 (left-hand series) and cobra serum developed with antiserum to CoF (right-hand series). The samples placed in the antigen wells were as follows: (A) normal human serum (NHS); (B) NHS incubated with *Escherichia coli* 026 : B6 endotoxin (1 mg/ml) for 30 minutes at 37°C; (C) conditions as in (B) except incubation for 1 hour; (D) NHS incubated alone for 1 hour at 37°C; (E) cobra serum (CS); (F) CS incubated with endotoxin at 0.2 mg/ml for 30 minutes at 37°C; (G) conditions as in (F), except endotoxin concentration was 1 mg/ml; (H) CS incubated with endotoxin at 0.2 mg/ml for 1 hour at 37°C; (I) conditions as in (H) except endotoxin concentration was 1 mg/ml; (J) CS incubated alone for 1 hour at 37°C.