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Learned Taste Aversions Induced by Hypnotic Drugs

JOHN R. VOGEL AND BETH A. NATHAN

Central Nervous System Section, Department of Pharmacology/Toxicology
William H. Rorer, Inc., Fort Washington, Pennsylvania 19034

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VOGEL, J. R. AND B. A. NATHAN. *Learned taste aversions induced by hypnotic drugs.* PHARMAC. BIOCHEM. BEHAV. 3(2) 189–194, 1975. – Taste aversions reflect the association of drug-produced noxious interoceptive stimuli with distinctive tastes. In the present experiments, taste aversions to sweetened condensed milk were induced in rats by a wide range of doses of the hypnotic drugs, hexobarbital, amobarbital, phenobarbital, chloral hydrate, flurazepam and methaqualone and by anesthetization with ethyl ether. Observation of changes in motor behavior following drug administration could not be used to predict the development of taste aversions. Despite the fact that hypnotic drugs have punishing properties, which these experiments reveal, they are widely abused by man and are self-administered by laboratory animals. This apparent paradox is discussed in terms of procedural differences between taste aversion and self-administration experiments.

Learned taste aversions Hypnotic drugs Barbiturates Hexobarbital Amobarbital Phenobarbital Chloral hydrate Flurazepam Methaqualone Ether

ANIMALS learn to avoid distinctive flavors that are associated with administration of certain drugs. These learned taste aversions demonstrate punishing properties of drugs. In rodents these properties are easily conditioned to taste stimuli and taste aversions are often conditioned in a single trial despite delays of minutes or hours between ingestion and drug administration [11].

Learned taste aversions have been induced with a variety of drugs that produce obvious signs of sickness. These include lithium chloride [10], apomorphine [7] and cyclophosphamide [16]. Recent experiments suggest that other less toxic drugs may also induce taste aversions. In fact, many drugs which are self-administered by animals can also induce learned taste aversions [15]. This finding is particularly interesting because studies of psychoactive drugs have largely emphasized rewarding rather than aversive properties of these drugs.

Self-administration of sleep-inducing (hypnotic) drugs has been reported in laboratory animals and in man. Barbiturate hypnotics are self-administered by rats [5] as well as Rhesus monkeys [6]. The present experiments were designed to assess aversive properties of hypnotic drugs. While there has been no previous systematic study of learned taste aversions induced by hypnotic drugs, Roll and Smith [12] and Rozin and Ree [13] did report slight reductions in the consumption of flavors associated with anesthetization by barbiturates. We report here that a single dose of any one of a variety of hypnotic drugs can induce a learned taste aversion.

EXPERIMENT 1

In Experiment 1 administration of a barbiturate drug, hexobarbital, amobarbital or phenobarbital, was paired with ingestion of sweetened condensed milk.

METHOD

Animals

One hundred and twenty-seven male Sprague-Dawley rats, weighing approximately 200–250 g at the start of the experiment, were used. Throughout the experiments all rats were maintained in individual cages with a 12 hr light (6:00 a.m. – 6:00 p.m.) and 12 hr dark (6:00 p.m. – 6:00 a.m.) illumination cycle. Water and food were removed from the cages for the period 24 hr prior to training and test sessions. At all other times water and food (Wayne Lab Chow in metal feeders attached to the cages) were available ad lib.

[1]Parts of this paper were presented at the Eastern Psychological Association Meeting, Philadelphia, 1974, and the Behavioral Pharmacology Society Meeting, Columbia, 1974.

Apparatus

Training and test sessions were carried out in a small cubicle (Scientific Prototype Model A-100) which measured 23.5 X 20.5 X 19.3 cm high. A bottle containing a solution of sweetened condensed milk (1 part Borden's Eagle Brand sweetened condensed milk to 2 parts distilled water at room temperature) was attached to the outside of the cubicle. A metal drinking tube extended up to and was flush with a 19 mm opening (65 mm above the grid floor) in the end wall of the cubicle. Each rat could easily lick the tube while standing on the grid floor. Individual licks were monitored by a drinkometer connected to programming equipment which was located in an adjacent room. The behavior of each rat during training and test sessions was monitored with closed-circuit television.

Procedure

On the training day each rat was removed from its cage, weighed and carried to the room which contained the apparatus. The drinking tube was previously adjusted so that a drop of milk solution hung from the end of the tube and protruded slightly into the cubicle. The rat was placed in the cubicle facing away from the tube. The time to completion of 100 licks was recorded to the nearest 0.1 sec. After completion of 100 licks, the rat was removed from the apparatus, injected according to its group designation and returned to its home cage. All rats were observed in their home cages 30 min after administration of drug; decreased motor activity, ataxia or complete loss of the righting reflex was recorded if present. Food and water were returned to the cages approximately 2 hr after training.

The test session, carried out 7 days after training, was identical in all respects to the training session, except that no drug was administered. If a rat failed to complete 100 licks in 600 sec, it was removed from the apparatus and assigned a score of 600.0.

Drugs

In each of the experiments, drugs were prepared as solutions in room temperature distilled water or as suspensions in room temperature 0.5% methylcellulose. The suspensions were homogenized to a uniform consistency and the drug was kept in suspension by a magnetic stirrer during the time that portions were removed for injection. All rats were injected intraperitoneally at a constant volume of 1.0 ml/kg. Control rats received vehicle alone. The doses are expressed in terms of the free base. Hexobarbital sodium was prepared as a solution and was administered at doses of 4.6, 9.2, 18.3, 36.7, 73.4 and 146.8 mg/kg. Amobarbital was prepared as a suspension and administered at doses of 18.3, 36.7 and 73.4 mg/kg. Phenobarbital sodium was prepared as a solution and administered at doses of 9.2, 18.3, 36.7, 73.4 and 110.1 mg/kg.

RESULTS

Hexobarbital

The results for hexobarbital are shown in Fig. 1. As can be seen from the figure, hexobarbital produced the expected dose-related impairment of motor function ranging from no obvious effect at doses up to 9.2 mg/kg to apparent anesthetization of all animals at 146.8 mg/kg on the training session.

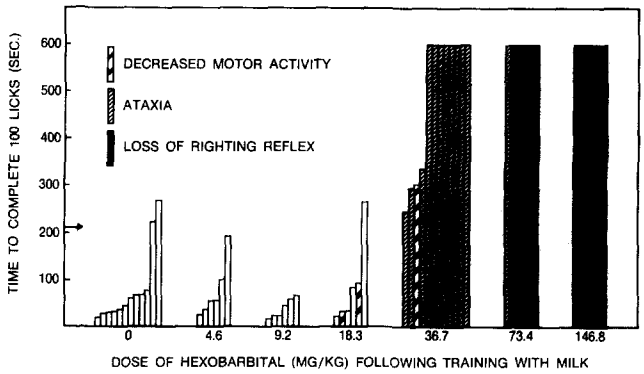

FIG. 1. Effects of doses of hexobarbital on home cage behavior observed 30 min after injection on the training session and individual times to complete 100 licks 1 week after sweetened condensed milk was paired with hexobarbital. Open bars represent normal behavior. The arrow on the ordinate represents the mean time to complete 100 licks on the training session (prior to injection) for all animals shown.

One week after injection, the animals were tested with sweetened condensed milk. On this test, drinking times for the 4.6, 9.2 and 18.3 groups did not differ from those of the control group. A marked taste aversion was indicated in Groups 36.7, 73.4 and 146.8. These animals drank slower than control animals ($p<0.001$ in all cases; Mann-Whitney U Test, 2 tailed). These results indicated that hexobarbital at doses from 36.7 to 146.8 mg/kg induced taste aversions to sweetened condensed milk.

Amobarbital

The results for amobarbital are shown in Fig. 2. Control animals appeared normal 30 min after injection. As can be seen from the figure, amobarbital produced a dose-related decrement of normal function ranging from decreased motor activity and ataxia at 18.3 mg/kg to apparent anesthetization of all animals at 73.4 mg/kg.

When the animals were tested with milk one week later, the drinking behavior of control animals and animals in the 18.3 group did not differ. The 36.7 and 73.4 groups exhibited taste aversions and drank slower than the control group ($p<0.02$; $p<0.002$, respectively). These results indicated that amobarbital induced a taste aversion at doses of 36.7 and 73.4 mg/kg.

Phenobarbital

The results for phenobarbital are shown in Fig. 3. Control animals and animals in Groups 9.2 and 18.3 appeared normal 30 min after injection. Eight out of 12 animals in the 36.7 group also appeared normal, while the remaining 4 animals were ataxic. All of the animals in the 73.4 group were ataxic. All of the animals in the 110.1 group completely lost the righting reflex and appeared to be anesthetized.

One week after injection, the animals were tested with sweetened condensed milk. Groups 9.2 and 18.3 did not differ from the control group. The drinking times for the

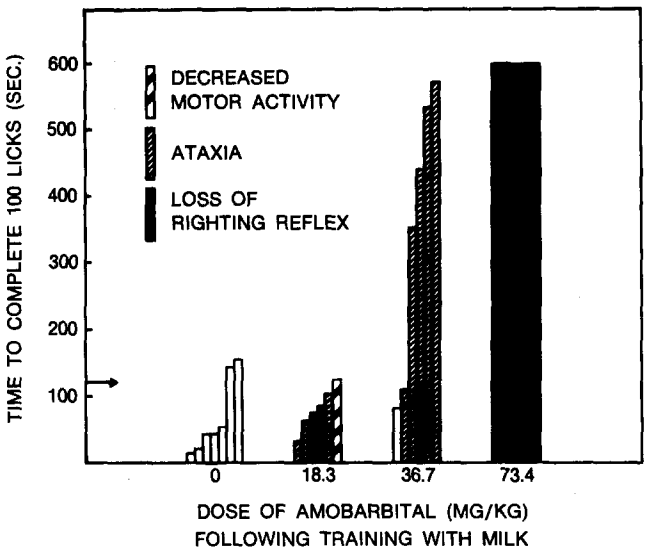

FIG. 2. Effects of doses of amobarbital on home cage behavior observed 30 min after injection on the training session and individual times to complete 100 licks 1 week after sweetened condensed milk was paired with amobarbital. Open bars represent normal behavior. The arrow on the ordinate represents the mean time to complete 100 licks on the training session (prior to injection) for all animals shown.

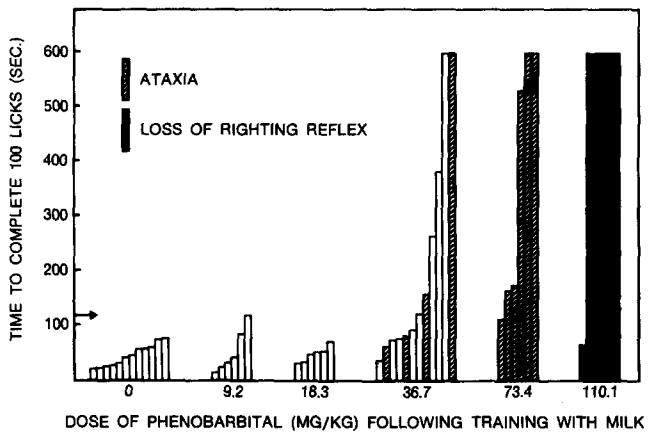

FIG. 3. The effects of doses of phenobarbital on home cage behavior observed 30 min after injection on the training session and individual times to complete 100 licks 1 week after sweetened condensed milk was paired with phenobarbital. Open bars represent normal behavior. The arrow on the ordinate represents the mean time to complete 100 licks on the training session (prior to injection) for all animals shown.

36.7, 73.4 and 110.1 groups were significantly longer than those of the control group ($p<0.002$; $p<0.001$; $p<0.001$, respectively), indicating that these animals developed taste aversions to sweetened condensed milk.

Administration of the barbiturate drugs hexobarbital, amobarbital or phenobarbital to laboratory rats following consumption of sweetened condensed milk led to marked reductions in milk consumption on a subsequent test session (learned taste aversions). The development of taste aversions after injection of amobarbital and phenobarbital was not related to motor depression produced by these drugs; a dose of 18.3 mg/kg amobarbital produced decreased motor activity and ataxia but failed to induce a taste aversion (Fig. 2), and several animals who did not show any motor impairment after a dose of 36.7 mg/kg phenobarbital did, nevertheless, develop taste aversions (Fig. 3). On the test session, all of these animals took a few licks from the tube when they were first placed in the box. Some animals continued to lick; others engaged in approach-avoidance behavior or avoided the tube for the remainder of the session.

EXPERIMENT 2

Learned taste aversions were obtained with equivalent doses of the barbiturate hypnotics hexobarbital, amobarbital and phenobarbital in Experiment 1. The drugs chloral hydrate, flurazepam and methaqualone are widely used non-barbiturate hypnotics. In Experiment 2, various doses of these drugs were tested in the learned taste aversion paradigm.

METHOD

One hundred and ninety male Sprague-Dawley rats weighing approximately 200–250 g at the start of the experiment were used. The rats were maintained as described in the Experiment 1. The apparatus and procedures were similar to those described in Experiment 1 except that records of the behavior of individual rats 30 min after administration of methaqualone were not made. Chloral hydrate was prepared as a solution and administered at doses of 20.0, 40.0, 80.0, 160.0, 320.0 and 480.0 mg/kg. Flurazepam dihydrochloride was prepared as a solution and administered at doses of 5.0, 10.0, 20.0, 40.0, 80.0 and 160.0 mg/kg. Methaqualone was prepared as a suspension and administered at doses of 2.5, 5.0, 10.0, 20.0 and 40.0 mg/kg.

RESULTS

Chloral Hydrate

The results for chloral hydrate are shown in Fig. 4. As can be seen from the figure, chloral hydrate produced a dose-related impairment of motor function similar to the barbiturates, ranging from no obvious signs of impairment at doses up to 40.0 mg/kg, to decreased motor activity and ataxia at 80.0 and 160.0 mg/kg, to loss of the righting reflex and apparent anesthetization at 320.0 and 480.0 mg/kg.

One week after injection, the groups were tested with milk. On this test, control animals and animals in Group 20.0 drank rapidly. Group 40.0 was slightly slower than the control group ($p<0.05$). Doses of 80.0, 160.0, 320.0 and 480.0 mg/kg chloral hydrate induced marked taste aversions; these groups drank significantly slower than the control group ($p<0.001$ in all cases).

Flurazepam

The results for flurazepam are shown in Fig. 5. Thirty min after injection, control animals and animals in Group 5.0 appeared normal. Two animals in Group 10.0 also appeared normal while 3 animals exhibited decreased motor activity and an additional animal was ataxic. All of the

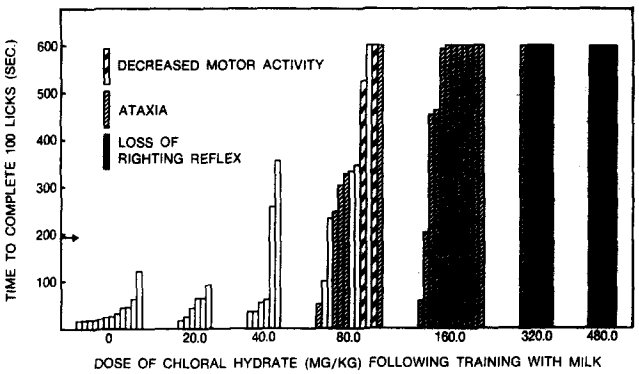

FIG. 4. Effects of doses of chloral hydrate on home cage behavior observed 30 min after injection on the training session and individual times to complete 100 licks 1 week after sweetened condensed milk was paired with chloral hydrate. Open bars represent normal behavior. The arrow on the ordinate represents the mean time to complete 100 licks on the training session (prior to injection) for all animals shown.

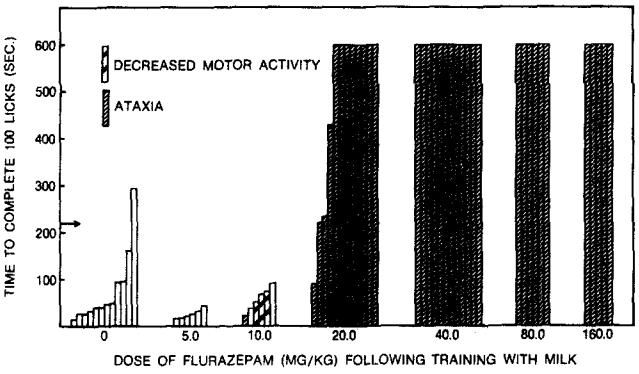

FIG. 5. Effects of doses of flurazepam on home cage behavior observed 30 min after injection on the training session and individual times to complete 100 licks 1 week after sweetened condensed milk was paired with flurazepam. Open bars represent normal behavior. The arrow on the ordinate represents the mean time to complete 100 licks on the training session (prior to injection) for all animals shown.

animals in groups 20.0, 40.0, 80.0 and 160.0 were ataxic. None of the animals given flurazepam exhibited loss of righting reflex.

The groups were tested with milk one week after injection. On this session, Group 5.0 drank slightly faster than the control group ($p<0.05$). Group 10.0 did not differ from the control group. Groups that received 20.0, 40.0, 80.0 and 160.0 mg/kg flurazepam exhibited marked taste aversions ($p<0.001$ in all cases).

Methaqualone

The results for methaqualone are shown in Fig. 6. Individual records of behavior 30 min after injection were not made for these groups; however, casual observation 30 min after injection revealed that Group 10.0 was generally

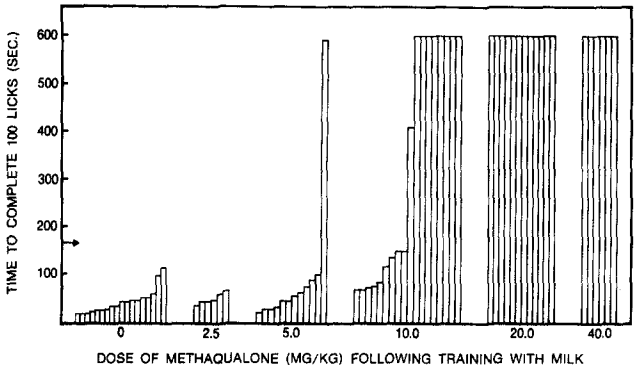

FIG. 6. Individual times to complete 100 licks 1 week after sweetened condensed milk was paired with injections of methaqualone. The arrow on the ordinate represents the mean time to complete 100 licks on the training session (prior to injection) for all animals shown.

hypoactive and Group 20.0 was ataxic. At this time, animals in Group 40.0 appeared anesthetized.

On the test session, which was conduced one week after injection, drinking times for Groups 2.5 and 5.0 did not differ from those of the control group. Taste aversions were exhibited by Groups 10.0, 20.0 and 40.0 which drank slower than the control group ($p<0.002$; $p<0.001$; $p<0.001$, respectively).

Administration of the nonbarbiturate hypnotic drugs chloral hydrate, flurazepam and methaqualone induced aversions to the taste of sweetened condensed milk. As was the case with the barbiturates amobarbital and phenobarbital, the development of taste aversions after injections of chloral hydrate was not related to motor depression produced by the drug; several animals who did not show any motor impairment after a dose of 80.0 mg/kg chloral hydrate did, nevertheless, develop taste aversions (Fig. 4). On the test session all of the animals sampled the sweetened condensed milk, but many did not continue to drink it.

EXPERIMENT 3

Taste aversions were exhibited by animals which appeared to be anesthetized by various hyponotic drugs. This suggested that anesthetization with ether would induce taste aversions. In Experiment 3 we anesthetized rats with ether for various durations after they drank sweetened condensed milk.

METHOD

Twenty-nine male Sprague-Dawley rats weighing approximately 200–250 g at the start of the experiment were used. The rats were maintained as described in Experiment 1. The apparatus and procedures were similar to those described in Experiment 1 except that after completion of 100 licks on the training day, the animals were removed from the apparatus and either: (1) returned to their home cages, (2) placed in a jar containing ethyl ether and removed immediately after loss of the righting reflex, or (3) exposed to ethyl ether for a continuous period of 10 min after loss of righting reflex occurred.

RESULTS

When returned to their home cages, animals which had been removed from the ether jar immediately upon loss of righting reflex regained the ability to right themselves within a minute. Animals which were continuously exposed to ether for a period of 10 min after loss of righting reflex righted themselves after approximately 10 min in their home cages.

The individual times to complete 100 licks of sweetened condensed milk on the test session are shown in Fig. 7. Animals which were simply returned to their home cages following training with milk readily drank the solution on the test session. Animals which were exposed to ether until loss of righting reflex took significantly longer to complete 100 licks than did control animals ($p<0.01$). A more marked taste aversion was present in animals which were continuously exposed to ether for 10 min after loss of righting reflex occurred ($p<0.001$). In this group scores were significantly ($p<0.005$) longer than those in the loss of righting group.

All of the animals took a few licks of milk from the tube on the test session. Several animals engaged in approach-avoidance behavior and eventually completed 100 licks. Two animals in the loss of righting group and 8 out of 9 animals in the loss of righting plus 10 min group avoided the drinking tube after initially sampling the milk solution.

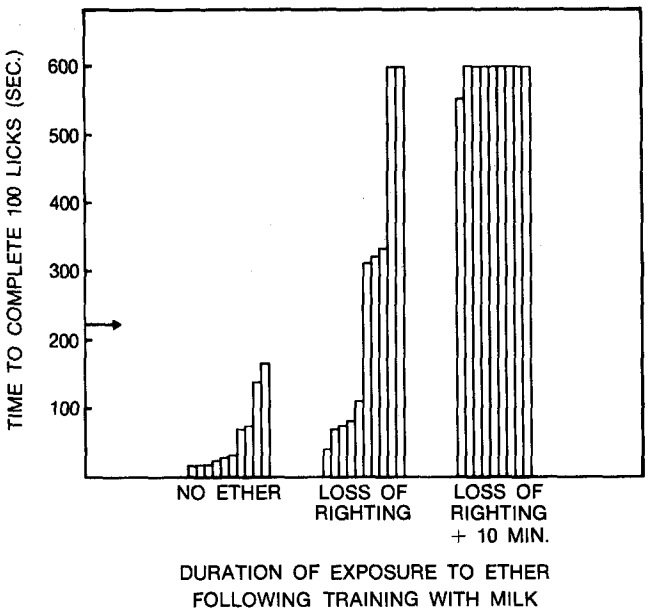

FIG. 7. Individual times to complete 100 licks 1 week after sweetened condensed milk was paired with brief (loss of righting) or sustained (loss of righting + 10 min) exposure to ethyl ether. The arrow on the ordinate represents the mean time to complete 100 licks on the training session (prior to exposure to ether) for all animals shown.

DISCUSSION

These experiments demonstrate that a variety of sleep-inducing (hypnotic) drugs can induce aversions to the taste of sweetened condensed milk. The phenomenon was dose-related: higher doses of drug led to stronger aversions reflected by longer drinking times. The test session was conducted a full week after administration of drug, so it is not reasonable to attribute the inhibition of drinking to a reduction in thirst or any other direct pharmacologic action of the drug. Rather, taste aversions induced by hypnotic drugs appear to reflect some aversive event resulting from administration of these drugs.

The drugs used in these experiments are designed to induce sleep. Their central depressant action leads to decreased motor activity and ataxia at moderate doses; while at higher doses, loss of the righting reflex occurs. Taste aversions induced by these drugs were also dose-related; however, motor impairment following administration of a drug could not be used to accurately predict the development of a taste aversion. This result suggests that, at least in the rat, there may be no relationship between interoceptive cues related to central depressant effects of these drugs and their ability to induce taste aversions (c.f. [11], p. 21–31). On the other hand, it is possible that many of our subjects also experienced noxious visceral cues which have been shown to be easily associated with taste [8].

Doses that appeared to produce anesthesia (loss of righting reflex) nearly always induced learned taste aversions (32 out of 33 animals), suggesting that anesthetization is aversive. However, anesthetization alone cannot fully account for this result, because brief anesthetization with ether induced a smaller taste aversion than did non-anesthetic doses of hypnotic drugs.

These experiments demonstrate that hypnotic drugs have aversive properties. Perhaps because hypnotic drugs are widely abused by humans and are self-administered by laboratory animals as well [4,6], little attention has been given to their aversive properties. These opposing behavioral effects are not simply a function of dose: self-administration of hypnotic drugs is not confined to low doses nor are aversive properties confined to high doses. In fact, Deneau, *et al.* [6] and Yanagita and Takahashi [17] reported that monkeys will self-administer anesthetic doses (i.v.) of the barbiturate hypnotic pentobarbital, while we demonstrated that subanesthetic dose levels of barbiturate drugs are aversive to the rat. Cappell and LeBlanc [1] also suggested that opposing behavioral effects are not simply a function of dose and pointed out that d-amphetamine sulfate can induce a taste aversion at a dose which is smaller than that self-administered by rats in a single session. These results, taken together, indicate that some drugs may have both rewarding and punishing properties at similar doses. This suggests that some behavioral effects of a drug may be determined not by its pharmacologic action alone but by other variables as well.

One such variable is stimulus relevance. Revusky and Garcia [11] suggested that malaise following drug administration is more easily associated with taste than with an instrumental response (p. 30). A similar argument has been made by Jacquet [9] who also suggested that the instrumental conditioning paradigm may favor association of response cues with CNS effects of drugs.

Another variable may also result from procedural differences between self-administration and taste aversion experiments. In self-administration studies animals are allowed to choose the rate at which they receive drugs, while in taste aversion studies drug experience is not controlled by the subject. A recent study suggests that rewarding stimuli may

become aversive when animals are no longer in control of them. Steiner *et al.* [14] hypothesized that opportunity to initiate a train of brain stimulation is an important factor in determining the reinforcing effects of that stimulation. To test this hypothesis, they allowed rats to electrically stimulate their brains and recorded the individual patterns of responses on tape. On a later session, individual patterns were played back to each rat. All animals worked to escape from the same pattern of brain stimulation that they administered to themselves on the previous session, indicating that the stimulation became aversive when animals were no longer allowed to control its initiation.

In view of Steiner, Beer and Shaffer's finding, the opposite drug effects in self-administration and taste aversion experiments may result from the fact that self-administration procedures allow the animal to control drug administration and thereby enhance rewarding effects; whereas, taste aversion procedures deny the subject control over drug administration and thereby enhance punishing effects.

ACKNOWLEDGEMENT

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