Role of Anorexia and Behavioral Activation in Amphetamine-Induced Suppression of Feeding: Implications for Understanding Tolerance

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In order to gain further insight into the mechanism of contingent tolerance to amphetamine anorexia (Carlton & Wolgin, 1971), an attempt was made to determine the role of anorexia and behavioral activation (increased locomotion and/or stereotypy) in the initial suppression of feeding produced by the drug. Rats administered chronic injections of either saline or amphetamine (2 or 4 mg/kg) were given milk either directly into the mouth through an intraoral cannula or in a standard drinking tube. It was reasoned that although drug-induced anorexia would affect intake with both methods of feeding to the same degree, the disruptive effect of behavioral activation would be greater in bottle-fed rats. The results revealed that bottle-fed rats given amphetamine showed substantially greater suppression of intake than cannula-fed rats. Saline-treated rats showed almost identical milk intake with the two methods. Recovery of intake occurred in all drugged rats except those given 4 mg/kg and fed by bottle. In the tolerant groups, rats fed by bottle and given 2 mg/kg recovered at a faster rate than cannula-fed rats at either dose. These results demonstrate that in the normal drinking condition, the initial suppression of intake is caused by a combination of anorexia and behavioral interference and that tolerance occurs to both of these effects.

Contingent tolerance to amphetamine refers to a phenomenon in which rats given chronic injections of the drug prior to feeding become tolerant to the initial suppression of intake whereas rats given the same number of injections but outside the feeding situation do not (Carlton & Wolgin, 1971). Although such tolerance is well documented (see review by Demellweek & Goudie, 1983b), the mechanism by which animals recover from the initial "anorexic" effect of the drug is still not understood. Attempts to account for the phenomenon in terms of adaptation to drug-induced cues (Wolgin, 1983), classical conditioning (Poulos, Wilkinson, & Cappell, 1981), or a lowered set point for body weight regulation (Stunkard, 1981) have not been entirely successful (see, e.g., Demellweek & Goudie, 1983a; Wolgin, 1983; Wolgin & Salisbury, 1985). A more recent proposal, that tolerance involves adaptation to drug-induced response disruption (Demellweek & Goudie, 1983b), is consistent with much of the literature but does not specify *how* the adaptation is achieved.

In our view, an adequate theory of amphetamine tolerance requires an understanding of the mechanisms by which the drug initially suppresses feeding. Two such mechanisms have been considered. First, there is considerable evidence that amphetamine produces anorexia by acting directly on feeding-related neural circuits in the hypothalamus (see review by Hoebel, 1977). The recent discovery of stereospecific binding sites in the hypothalamus for amphetamine and related phenylethylamine anorectics (Paul, Hulihan-Giblin, & Skolnick, 1982) is consistent with this view. Second, intake may be suppressed by amphetamine because feeding is incompatible with the behavioral activation (reflected in increased locomotion and/or stereotyped movements) produced by the drug (Blun-

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This research was submitted by Juanita J. Salisbury to Florida Atlantic University in partial fulfillment of the requirements for a master's degree.

We thank Tom Resnick for preparing the figures, and two anonymous reviewers for helpful suggestions.

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dell & Latham, 1980; Carlton, 1963; Cole, 1978; Joyce & Iversen, 1984; Lyon & Robbins, 1975). Although these two effects can be dissociated anatomically and pharmacologically (see reviews by Cole, 1978; Hoebel, 1977), both factors would be expected to contribute to the disruption of feeding in neurologically normal rats given amphetamine in doses of 1 mg/kg or greater. Consequently, the development of tolerance may involve changes affecting either or both of these variables. The problem is that the relative contributions of anorexia and behavioral activation to the suppression of feeding by amphetamine have not been determined.

As a first approach to resolving this question, we examined the effect of amphetamine in two ingestional paradigms having different appetitive requirements. Specifically, rats were given sweetened milk either through an intraoral cannula or in a standard drinking tube. If amphetamine acts primarily by reducing the motivation for food (i.e., through drug-induced anorexia), then intake should be suppressed to an equivalent degree with both methods. However, if amphetamine disrupts feeding in part by interfering with appetitive behavior (e.g., through the accompanying behavioral activation), then the suppression of intake should be greater with the drinking tube, the condition in which the rats must actively approach the milk.

Method

Subjects

The subjects were 48 adult male Sprague-Dawley albino rats housed individually in wire mesh cages in constant illumination. The rats were maintained on three Purina Lab Chow pellets (approximately 15 g) and ad-lib water daily.

Implantation of Cannulas

Intraoral cannulas were implanted under sodium pentobarbital anesthesia (50 mg/kg) by the method of Phillips and Norgren (1970). The cannulas were constructed from polyethylene tubing (PE 90) with a heat flare at one end. A plastic washer slipped over the tubing rested against the flared end. The open end of the tubing was then fitted over the blunt end of a curved surgical needle. The needle, attached to the cannula, was inserted into the mouth and through the tissue just lateral and rostral to the first molar, and then guided subcutaneously along the side of the head to emerge at an incision made along the midline of the skull. The flared end of the cannula was pulled against the inside of the cheek, and the open end was cut and fitted over one end of an L-shaped piece of 19-ga. stainless steel tubing. The tubing was then embedded in cranioplastic cement on the skull, with the open end extending about 5 mm above the cement. The cannulas were flushed with tap water each day to keep them clean.

Procedure

The rats were first given five preliminary tests with each method of milk delivery to familiarize them with the procedure and to establish baseline levels of intake. During these tests, each rat was weighed and injected with isotonic saline (1 ml/kg, ip) 20 min before receiving sweetened condensed milk diluted with water (1 part milk to 3 parts water) for 30 min. All tests were conducted in cages identical to those used to house the rats. In the drinking tube condition, milk was provided in calibrated bottles with stainless steel spouts attached to the fronts of the cages. In the cannula condition, milk was infused by gravity from a 50-ml disposable syringe positioned 12 in. (30.5 cm) above the test cages. PE 90 tubing connected the syringe to the stainless steel extension of the cannula atop the rat's head. The height of the syringe allowed milk to flow through the cannula at a rate of 1 ml/min. However, the rats could increase the rate of flow by creating negative pressure within the mouth. Spillage was recovered in plastic weighing dishes placed beneath the test cage. The amount of milk ingested was calculated by subtracting spillage, if any, from the difference in the volume of the syringe before and after the session.

Following these preliminary trials, the rats were divided into eight equal groups (ns = 6), matched on the basis of milk intake and body weight. Three groups were given daily ip injections of either saline or one of two doses of *d*-amphetamine sulfate (2 or 4 mg/kg) 20 min before receiving milk by cannula. Three other groups were given similar injections of saline or amphetamine 20 min before receiving milk in bottles. Two additional groups were included to determine whether cannula-fed rats, particularly those showing amphetamine-induced stereotyped movements, could voluntarily avoid ingesting milk if they chose to do so. Rats in these groups were injected with either saline or 4 mg/kg amphetamine and given milk adulterated with quinine hydrochloride (0.25% w/v) through their cannulas for 5 consecutive days. At the conclusion of the experiment, half of the cannula-fed rats injected with 2 mg/kg amphetamine were also given quinine-adulterated milk for one additional trial. Throughout the experiment, 3 animals per group were observed during each session, and their behavioral responses were noted.

Statistics

The data were analyzed by means of analyses of variance for repeated measures. When warranted by significant F ratios, individual comparisons were

made with the least significant difference (LSD) test (Winer, 1971).

Results

Cannula-Fed Groups

Milk intake. The milk intakes of rats fed by cannula are presented in Figure 1 (top). Statistical analysis confirmed a significant Dose × Trials interaction, $F(34, 255) = 1.62$, $p < .02$. On the first drug day, both amphetamine groups ingested significantly less than the saline group, with more suppression in the group given 4 mg/kg (LSD = 4.72). Both groups subsequently developed tolerance to the drug, but to different degrees. Rats given 2 mg/kg reached saline levels of intake on 4 of the last 7 days, whereas rats given 4 mg/kg showed much less recovery, never exceeding 67% of control levels of intake.

Marked behavioral differences were noted between rats given saline and those given amphetamine. Rats given saline continuously licked their paws while drinking, whereas rats given amphetamine were never observed to lick their paws. Instead, they engaged in repetitive head scans in the lateral, vertical, and longitudinal planes, usually along the walls and floor of the cage. There was no apparent diminution in these responses during the course of the experiment. In addition, amphetamine-treated rats allowed some of the milk to spill from their mouths so that their snouts were typically covered with milk. Saline-treated rats drank all of the milk; none of it spilled from their mouths.

Rats injected with saline and given quinine-adulterated milk ingested an average of 3.6 ml per day. Rats injected with 4 mg/kg amphetamine averaged only 1.0 ml/day, whereas rats injected with 2 mg/kg and tested at the conclusion of the tolerance trials drank 0 ml. Again, there were marked differences in the behavioral responses of rats given saline and those given amphetamine. Saline-treated rats showed species-typical aversive responses to quinine similar to those described by Grill and Norgren (1978). In contrast, amphetamine-treated rats showed continuous stereotyped head scanning movements similar to those of drugged rats in the other groups.

Body weight. The body weights of rats fed by cannula are presented in Figure 1 (bottom). Statistical analysis revealed a significant Dose × Trials interaction, $F(34, 255) = 1.86$, $p < .004$. During the course of

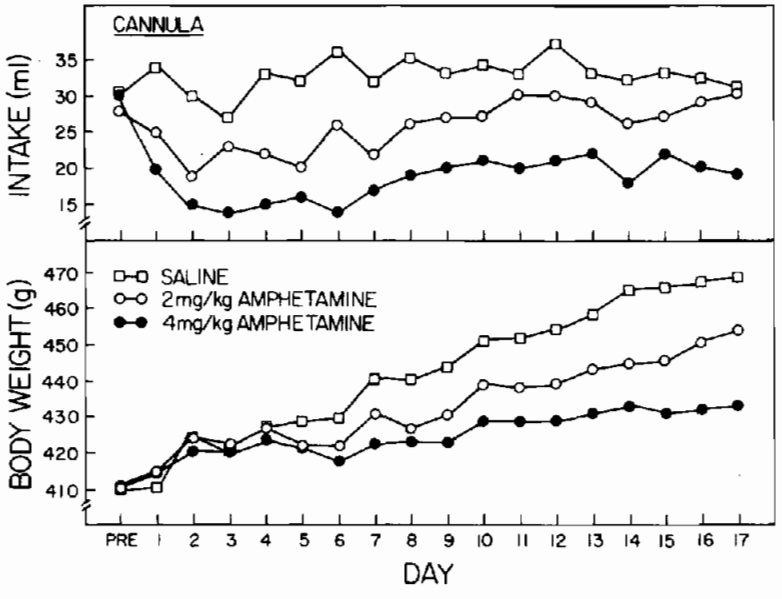

Figure 1. Mean milk intake and body weight of cannula-fed rats given chronic injections of saline or 2 mg/kg or 4 mg/kg *d*-amphetamine sulfate. (PRE = mean of last three preliminary trials.)

the experiment, both amphetamine groups gained weight, but at a slower rate than the saline control group. Differences in weight gain did not appear until Day 7, however. During the last week, rats given 2 mg/kg remained at about 97% of control levels, whereas rats given 4 mg/kg remained at about 93%. The body weights of the amphetamine groups differed significantly from Day 13 to Day 17 (LSD = 11.55). Consistent with previous observations (Wolgin & Salisbury, 1985), there were substantial individual differences in weight gain among rats in each group. Some rats gained weight, others did not, and some lost weight over the course of the experiment.

Bottle-Fed Groups

Milk intake. The milk intakes of rats fed by drinking tube are presented in Figure 2 (top). Statistical analysis confirmed a significant Dose × Trials interaction, $F(34, 255) = 7.44$, $p < .001$. Amphetamine produced a dose-dependent decrease in milk intake on the first drug day (LSD = 4.72). Rats given 2 mg/kg gradually increased their intake on Days 5–8 and then stabilized below control levels, whereas with the exception of Day 11 (when 1 rat drank 19 ml), rats given 4 mg/kg drank nothing during the course of the experiment. Both groups of amphetamine-treated rats showed stereotyped head scanning movements throughout the experiment, although such movements were suppressed in rats given 2 mg/kg while they drank.

Body weight. As shown in Figure 2 (bottom) and confirmed by a significant Dose × Trial interaction, $F(34, 255) = 14.95$, $p < .001$, there were marked differences in body weight between the groups (LSD = 8.90). In contrast to the control group, which gained weight throughout the experiment, rats given 2 mg/kg remained relatively constant through Day 6 and then steadily gained weight at about the same rate as controls. Rats given 4 mg/kg also

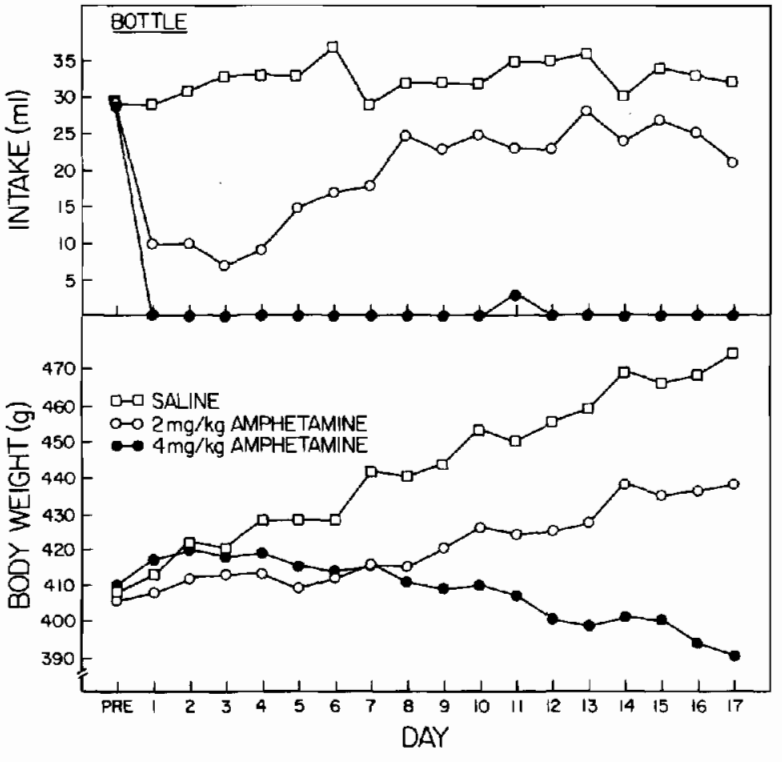

Figure 2. Mean milk intake and body weight of bottle-fed rats given chronic injections of saline or 2 mg/kg or 4 mg/kg *d*-amphetamine sulfate. (PRE = mean of last three preliminary trials.)

remained fairly constant during the first week but then steadily lost weight during the remainder of the experiment. Again, there were substantial differences in the rates of weight gain and loss among individual rats.

Comparisons Between Feeding Methods

Specific comparisons between the cannula and bottle methods of feeding at each dose level are presented in Figures 3, 4, and 5. As shown in Figure 3, the method of feeding had no effect on either the milk intake or the body weight of rats given injections of saline. On the other hand, the feeding method was critically important for rats given amphetamine. As shown in Figure 4, and confirmed by a significant Method × Trials interaction, $F(17, 170) = 2.24$, $p < .005$, rats given 2 mg/kg and fed by cannula initially showed much less suppression of intake than rats fed by bottle (LSD = 5.70). However, bottle-fed rats rapidly developed tolerance over Days 5–8, so that their intakes were comparable to those of cannula-fed rats on 7 of the last 10 days. These differences in intake did not differentially affect the rate of weight gain, however. Statistical analysis revealed a significant effect of trials, $F(17, 170) = 15.47$, $p < .001$, but a nonsignificant effect of feeding method and a nonsignificant Method × Trials interaction (both $ps > .05$).

The differences between cannula- and bottle-fed rats were more extreme at the 4 mg/kg dose (see Figure 5). Rats fed by cannula showed relatively moderate suppression of intake and subsequently achieved some degree of tolerance, whereas rats fed by bottle ingested virtually nothing throughout the experiment. As a result, the body weights of the two groups gradually diverged so that whereas cannula-fed rats ultimately gained weight, bottle-fed rats lost weight. These differences between the groups were confirmed by statistically significant Method × Trials interactions for both milk intake, $F(17, 170) = 3.02$, $p < .001$ (LSD = 4.67), and body weight, $F(17, 170) = 4.15$, $p < .001$ (LSD = 12.04).

Discussion

Although amphetamine-treated rats showed reduced milk intake with both methods of feeding, the degree of suppression was greater at each dose level in the bottle-fed rats. Because saline-treated rats showed almost identical intake and weight gain under the two methods, these results cannot be attributed to differences in the methods per se. Moreover, the greater intake of cannula-fed rats was not due to their inability to avoid swallowing the milk

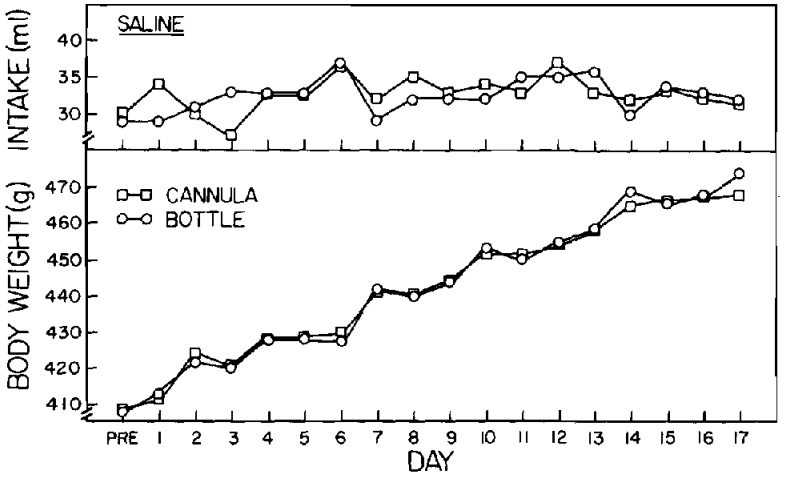

Figure 3. Mean milk intake and body weight of rats injected with saline and given milk by cannula or drinking tube (bottle).

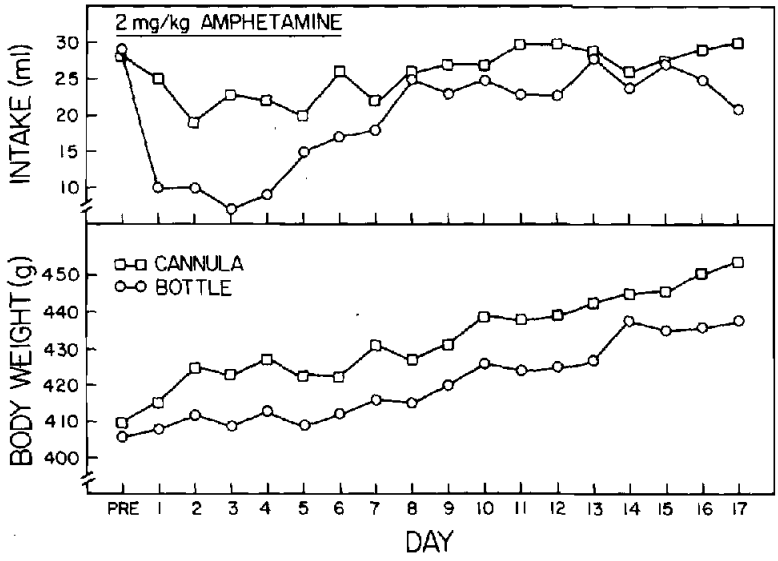

Figure 4. Mean milk intake and body weight of rats injected with 2 mg/kg *d*-amphetamine sulfate and given milk by cannula or drinking tube (bottle).

because rats given quinine-adulterated milk readily rejected the bitter fluid. Thus, the data suggest that amphetamine suppresses feeding both by reducing appetite (i.e., producing anorexia) and by interfering with appetitive behavior. In cannula-fed rats, for which appetitive behavior was largely circumvented, the relatively modest suppression of intake can be attributed to drug-induced anorexia. In bottle-fed rats, however, feeding was contingent on approaching the milk. Hence, the greater degree of suppression in these animals can be attributed to a combination of anorexia and behavioral interference.

Although it is possible that amphetamine directly inhibits the neural mechanisms responsible for the production of appetitive

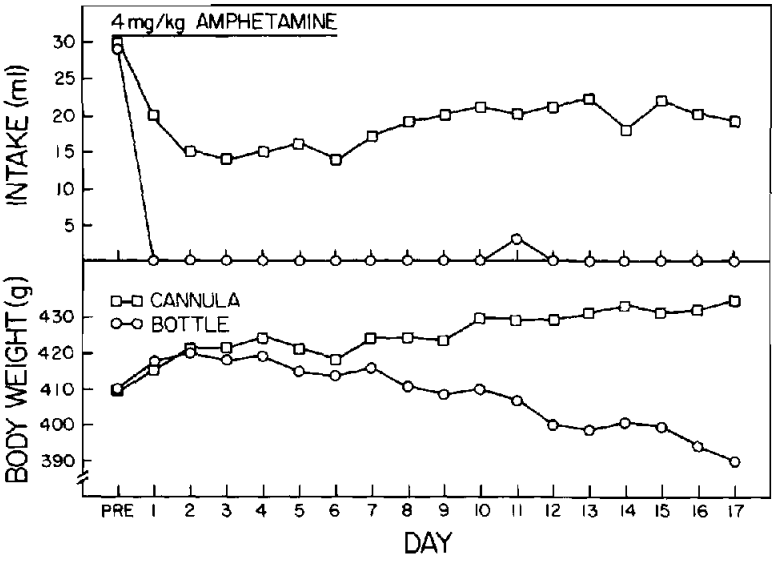

Figure 5. Mean milk intake and body weight of rats injected with 4 mg/kg *d*-amphetamine sulfate and given milk by cannula or drinking tube (bottle).

behavior, a more widely held view is that the drug induces behavioral responses or patterns of responding that are incompatible with feeding (Carlton, 1963; Cole, 1978; Lyon & Robbins, 1975). The results of several studies support this view; for example, significant negative correlations between activity and feeding in amphetamine-treated rats have been reported (Cole, 1977, 1979). Analysis of the microstructure of feeding in such animals has revealed a pattern of ingestion characterized by bouts of rapid eating interrupted frequently by periods of activity (Blundell & Latham, 1980). The results of the present study are consistent with these reports. Although we did not quantify the behavioral activation produced by amphetamine, a reciprocal relation was observed between stereotyped head scans and feeding behavior. This relation was particularly evident at intermediate levels of tolerance when rats showed incessant head scanning except for brief periods during which they lapped milk from the drinking tube. Reciprocal changes in feeding and activity have also been reported in brain-damaged subjects. Following damage to the dopaminergic nigrostriatal system, rats given amphetamine showed reduced stereotypy (but increased locomotion) and increased food intake (Joyce & Iversen, 1984). Conversely, following kainic acid-induced damage to the striatum amphetamine-treated rats showed increased stereotypy (Mason, Sanberg, & Fibiger, 1978) and reduced food intake (Sanberg & Fibiger, 1979).

In addition to interfering with appetitive behavior, amphetamine has a direct effect on feeding-related neural circuits in the brain (cf. Hoebel, 1977). Recently, specific receptor sites for amphetamine and related phenylethylamines were demonstrated in the hypothalamus and brain stem (Paul et al., 1982). The anorectic (but not the stimulant) potencies of these drugs were highly correlated with their affinities for the receptor site in vitro. Taken together, these data suggest that amphetamine-induced anorexia may be mediated by the hypothalamus[1] whereas the activating effects of the drug, which secondarily interfere with feeding, may be mediated by the striatum.

The fact that amphetamine disrupts feeding by more than one mechanism has several implications for understanding the process of tolerance. Because both cannula-fed and bottle-fed groups of rats given 2 mg/kg showed recovery of intake, it appears that tolerance develops to both anorexia and behavioral interference. It is interesting in this regard that bottle-fed rats showed a more rapid rate of recovery than cannula-fed rats. If it can be assumed that this difference is not merely an artifact of the different initial levels of intake,[2] this finding implies that overcoming the behavioral activation produced by the drug played a greater role in the development of tolerance than did recovery from anorexia. A similar conclusion may be drawn from the fact that bottle-fed rats given 2 mg/kg developed tolerance faster than cannula-fed rats given 4 mg/kg despite the fact that the former group initially showed greater suppression of intake (cf. Figures 4 and 5). Thus, again, tolerance developed more rapidly when interference with appetitive behavior was more prominently involved.

The hypothesis that tolerance involves overcoming the behavioral activation produced by the drug is also supported by observations made in this and previous experiments (Wolgin, 1983; Wolgin & Salisbury, 1985), that amphetamine-tolerant rats given milk in drinking tubes show a suppression of drug-induced stereotyped movements while milk is available, although both prior and subsequent to the session such movements are unabated. In contrast, the cannula-fed rats in this experiment showed no diminution in stereotyped head scans. Indeed, in rats given bitter milk, such movements seemed to suppress the expression of many of the

[1] A peripheral mechanism, involving sympathetic stimulation of the liver, may also contribute to anorexia, particularly with low doses (< 0.5 mg/kg) of amphetamine (Tordoff, Hopfenbeck, Butcher, & Novin, 1982; Tordoff, Novin, & Russek, 1982).

[2] The problem alluded to here is the possibility that the initial level of intake may in itself determine the rate of tolerance. Comparing groups of bottle- and cannula-fed rats matched on initial intake would help to resolve this ambiguity. It should be noted, however, that in the present case the rates at which the two groups developed tolerance were inversely related to their initial levels of intake.

stereotyped aversive responses to quinine. Thus, the mechanism by which rats are able to control the behavioral activation produced by amphetamine appears to be central to an understanding of tolerance. Further insight into this process will require detailed monitoring and quantification of the behavioral effects of amphetamine during the period in which feeding recovers.

Finally, it should be noted that the data pose additional problems for the set point theory of amphetamine anorexia (Stunkard, 1981; cf. Wolgin & Salisbury, 1985). According to this view, amphetamine acts primarily to lower a neural set point for the regulation of body weight; drug-induced anorexia is viewed merely as a vehicle for achieving the lower weight level. If this view were correct, then rats given a particular dose of the drug should become anorexic and lose weight regardless of the method of feeding. In the present case, however, cannula-fed rats given 4 mg/kg amphetamine gained weight, whereas bottle-fed rats given the same dose lost weight. Because saline-treated rats showed identical intakes and weight gains with the two methods, these results cannot be attributed to differences in the methods per se.

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