Tolerance to Amphetamine Hypophagia: A Microstructural Analysis of Licking Behavior in the Rat

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The development of tolerance to amphetamine-induced hypophagia was assessed by recording changes in lick parameters in rats given chronic administration of the drug (2 mg/kg) and access to sweetened milk. Although licking and milk intake gradually recovered, the volume of milk ingested per lick remained suppressed. Amphetamine had no effect on the interlick interval or the force per lick. In contrast, the drug caused a sustained increase in the number of lick bursts (defined by pause criteria of 0.5–2.0 s) and a decrease in the number of licks per burst (but only at pause criteria of 0.5 and 1.0 s). These results suggest that tolerant rats frequently interrupt licking, resulting in less efficient capture of milk.

Amphetamine is well known for its ability to suppress feeding. The most common explanation for this effect is that the drug produces a decreased appetite for food (anorexia) by acting at feeding-related sites in the brain (Hoebel, 1977; Leibowitz, 1975; Paul, Hulihan-Giblin, & Skolnick, 1982). In addition, however, amphetamine induces locomotion and stereotyped movements, which are incompatible with feeding (Carlton, 1963; Cole, 1978; Joyce & Iversen, 1984; Lyon & Robbins, 1975). The relative contributions of these two mechanisms have been assessed by comparing the effects of amphetamine on the intakes of rats given milk via intraoral cannulas or in standard drinking tubes. Relatively little hypophagia was found in the cannula condition, presumably because intraoral feeding is not incompatible with stereotyped movements (Salisbury & Wolgin, 1985; Wolgin, Thompson & Oslan, 1987). A similar effect was found with cocaine (Wolgin & Hertz, 1995). Indeed, doses of amphetamine and cocaine that produced a 40–50% reduction in intake in bottle-fed rats had virtually no effect on the intake of cannula-fed rats (Wolgin, 2000; Wolgin & Hertz, 1995). The importance of behavioral competition in the suppression of feeding is also underscored by the finding that under both drugs, rats have longer latencies to initiate feeding and frequently interrupt their meals with periods of activity (Blundell & Latham, 1980; Cooper & van der Hoek, 1993; Rosofsky & Geary, 1989), a pattern consistent with a nonspecific disruption of ingestive behavior.

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When rats are given chronic injections of amphetamine or other psychostimulants, they develop tolerance to the initial suppression of feeding provided that they have access to food while in the drugged state (Carlton & Wolgin, 1971; Emmett-Oglesby & Taylor, 1981; Foltin & Schuster, 1982; Woolverton, Kandel, & Schuster, 1978). According to the instrumental learning model (Wolgin, 1989, 2000), such "contingent tolerance" is mediated by the learned suppression of stereotyped movements, which is reinforced by the ingestion of food. Rats given amphetamine in the absence of food lack this incentive to suppress stereotyped movements and, hence, do not develop tolerance despite receiving repeated injections of the drug. This model is supported by the finding that rats injected with amphetamine can learn to maintain a stationary head position when reinforced for doing so with intraoral infusions of milk (Wolgin & Wade, 1995). The rate at which they learn to suppress stereotypy is similar to the rate at which bottle-fed rats develop tolerance. In contrast, rats injected with amphetamine and given noncontingent infusions of milk do not show a decrease in stereotyped head movements over time (Salisbury & Wolgin, 1985; Wolgin et al., 1987).

In general, tolerance to amphetamine-induced hypophagia has been assessed indirectly, by measuring the total amount of food (usually sweetened milk) ingested in daily test sessions. This approach provides little insight into how the organization of feeding behavior is affected by chronic drug treatment. In the present experiment, we addressed this issue by examining the microstructure of licking in rats given daily injections of amphetamine prior to 30 min access to sweetened milk. Previous studies involving the ingestion of sapid solutions by undrugged rats have shown that licking is organized into "bursts" (Davis, 1973, 1996; Davis & Smith, 1992). Interlick intervals (ILI) within a burst are narrowly distributed around a mean of about 160 ms, yielding a lick rate of about 6–7/s. Pauses between bursts range from several hundred milliseconds to seconds or even minutes. Pauses in the millisecond range are thought to reflect a missed contact with the drinking tube, whereas pauses lasting seconds or minutes are thought to reflect the intrusion of noningestive behaviors, such as grooming or locomotion (Davis, 1996). These findings suggest that a microstructural analysis of licking would provide a sensitive assay for

analyzing how amphetamine-induced stereotyped movements interfere with feeding behavior and how feeding recovers during the development of tolerance.

Recently, Badiani and Stewart (1999) reported that rats given chronic injections of amphetamine show sensitization of water licking, characterized by a progressive increase in rate and a shift to the left in the ILI distribution. In this study, the rats were maintained on ad-lib food and water, and their ingestion of water was measured for 6 hr after injection of the drug. In contrast, studies of tolerance to the hypophagic effect of amphetamine typically use food-deprived rats and measure the ingestion of sweetened milk for 30 min after injection of the drug (e.g., Wolgin et al., 1987; Wolgin & Hertz, 1995; Wolgin & Hughes, 1997). Because the microstructure of licking is strongly influenced by deprivation conditions (Davis & Perez, 1993; Spector, Klumpp, & Kaplan, 1998), solute concentration (Davis & Smith 1992; Spector et al., 1998), drug dose (Fowler & Mortell, 1992; Fowler & Wang, 1998; Higgs & Cooper, 1998), and situational variables (Weijnen, 1998), it is uncertain whether the results obtained by Badiani and Stewart (1999) can be generalized to other paradigms. Accordingly, in the present study we attempted to reproduce as closely as possible the procedures used in previous tolerance studies conducted in our laboratory.

Method

Subjects

Seventeen naive male, albino Sprague-Dawley rats (Charles River Laboratories, Portage, MI), weighing 243–321 g at the beginning of the experiment, served as subjects. The rats were housed individually in stainless steel cages with wire mesh floors in a room maintained on a 12-hr light–dark cycle (lights on 0800). The rats were fed three Purina Rat Chow pellets (about 15 g) and given ad-lib water daily in their home cages.

Apparatus

Test sessions were conducted in six experimental chambers (MED-Associates, Georgia, VT), each measuring 30.5 cm long 24 cm wide 29 cm high. The side and top panels of each chamber were clear Plexiglas, the front and rear panels were stainless steel, and the floors were 0.5-cm stainless steel rods spaced 1.5 cm apart. General illumination was provided by a 1.5-W incandescent lamp mounted in the center of the rear wall, 2 cm from the top. The chambers were housed in sound-attenuating enclosures (ENV-018M, MED Associates) equipped with Plexiglas windows and ventilation fans.

Access to a straight stainless steel drinking tube with a 0.2 cm lumen was provided through a 5-cm^2 opening centered in the front panel of each chamber. The opening was covered by a removable aluminum shield until the test sessions began. The drinking tube was mounted behind the panel at a 45° angle so that its tip was positioned 6 cm above the floor and flush with the plane of the front wall. The tube was mechanically connected to a load cell (ENV 251FX, MED Associates) attached to the back of the front wall by a 10-cm-long piece of Delrin plastic with an angled hole drilled at one end. The drinking tube was inserted through the hole and secured in place with a set screw. The other end of the Delrin was attached to the load cell by two screws. Thus, forces applied to the drinking tube were transferred to the load cell by the Delrin "bridge." The output of the load cell was sampled every 10 ms by a Dell OptiPlex 300 computer running commercially available software (Force Lickometer, Version 1.20; MED Associates). Forces of individual tongue contacts with the drinking tube in the range of 0.4–40.0 g were stored in an array for subsequent analysis.

Eagle Brand sweetened condensed milk (Borden, Columbus, OH) diluted with water (1:3) was supplied to the drinking tube through vinyl tubing from a 100-ml calibrated bottle fitted with a curved stainless steel spout. The bottle was held in a clamp attached to a ring stand and positioned above and behind the drinking tube.

Procedure

After habituating the rats to the apparatus, test sessions were conducted 6 days per week. On test days, the rats were transported to the test room in plastic cages, weighed, injected with either physiological saline or amphetamine, and then placed in an experimental chamber. Twenty minutes later, the volume of milk in the bottles was recorded, the houselights were illuminated, the protective aluminum shields were removed, and the rats were given access to the drinking tube for 30 min. At the end of the session, the shields were replaced, the volume of milk remaining in the bottles was recorded, and the rats were returned to their home cages and fed.

The first 44 sessions were used to establish stable levels of milk intake. Saline injections were administered on the last 9 of these sessions, and the means of the final 7 sessions were used as the baseline. Following this phase, an initial dose–response determination (DR 1) was conducted to assess initial sensitivity to the hypophagic effect of amphetamine. Test doses of *d*-amphetamine sulfate (0.5, 1.0, 2.0, and 4.0 mg/kg) and saline were administered in counterbalanced order, with at least 3 days between doses. On the intervening days, saline injections were given. After DR 1, the rats were randomly assigned to one of two groups matched on the basis of their sensitivity to the drug. During the ensuing tolerance phase, one group (n 9) received a daily injection of amphetamine (2 mg/kg) for 30 trials, and the other group (n 8) received a saline injection. At the end of the tolerance phase, a second dose–response determination (DR 2) was conducted, in which test doses of amphetamine and saline were substituted for the usual chronic treatment, with at least 3 days between each dose. The continuation of the chronic treatment on the intervening days was designed to maintain the level of tolerance previously established.

Drugs

Amphetamine (*d*-amphetamine sulfate; Sigma, St. Louis, MO) was dissolved in physiological saline. Doses of the drug are expressed as the weight of the salt. Injections of both amphetamine and saline were given in a volume of 1 cc/kg.

Data Analysis

Milk intakes were calculated by subtracting the volume of milk in the calibrated bottles at the end of each session from the volume that was present at the start of the session. Volumes less than 2 cc were treated as zero intake to allow for the possibility of spillage at the beginning of the session, when the rats were placed in the boxes and the protective guards were removed. In addition to milk intakes, the following dependent measures were calculated from the stored force–time arrays for each subject on each session by means of custom-written software: (a) number of licks, (b) number of bursts (defined by interlick pause criteria of $\geq$ 0.5 s, $\geq$ 1.0 s, $\geq$ 1.5 s, $\geq$ 2.0 s, $\geq$ 2.5 s and $\geq$ 3.0 s), (c) frequency distribution of ILIs $\leq$ 250 ms, (d) mean intraburst interval (based on ILIs $\leq$ 1.0s), (e) frequency distribution of forces per lick, and (f) mean force per lick. ILIs were defined as the time from one tongue contact with the drinking tube (force 0.4 g) to the next such contact. Force was measured as gram-equivalent weights. Finally, two additional measures were derived from the above data: (g) the mean volume per lick (calculated by dividing the amount of milk ingested in each session by the total number of licks in that session) and (h) burst size (licks/burst; calculated by dividing the number of licks in each session by the total number of bursts in that session, for each of the pause criteria). Inspection of the volume per lick data occa-

sionally revealed values that exceeded the normal mean of about 5.5 μl by 50% or more. This occurred six times in 333 trials in the amphetamine group and 12 times in 296 trials in the saline group. Invariably, such cases were found to be associated with abnormally low levels of licks relative to the volume of milk consumed and were, therefore, attributed to equipment failure. These trials were excluded from the statistical analysis of any of the lick parameters.

Statistical analyses were conducted by means of analyses of variance (ANOVA), with adjustments to the degrees of freedom when violations of the circularity assumption were detected (Kirk, 1982). When significant interactions were obtained, tests of simple main effects were performed, followed by individual comparisons using the test of Dunn and Sidak (Kirk, 1982).

Because rats developed tolerance at different rates, the number of amphetamine-treated rats drinking milk on each of the first 15 tolerance trials was variable. Although zero values are legitimate data points for statistical analyses of milk intake and number of licks, null scores in other measures (e.g., ILIs, number of bursts, volume per lick) are not meaningful and distort the group means. Accordingly, for intakes and licks, both the graphic presentations and the statistical analyses included all subjects and all trials during the tolerance phase. For the other dependent variables, the graphs include data (means and standard errors) only from subjects that actually ingested milk on a particular trial, and the statistical analyses were limited to data from the baseline (mean of last 7 trials) and Trials 16–30, when drinking was more consistent. To assess the initial effect of amphetamine on lick parameters, we also calculated the ILI and force distributions on the first trial in which a rat's intake was ≥ 5 cc and compared the pooled data to the baseline distributions for the group.

Results

Milk Intake and Licks

Mean milk intakes during DR 1, conducted prior to the tolerance phase, are shown in Figure 1 (bottom left). Amphetamine produced a dose-dependent decrease in intake in both groups, and at the 2 mg/kg dose, almost completely suppressed drinking. With chronic administration of this dose, intakes gradually recovered but remained below those of the saline group (see Figure 1, top left). Statistical analysis revealed a significant Group Trial interaction, $F(30, 450)$ 8.99, p .01. Post hoc comparisons indicated that intakes in the amphetamine group remained below those of the saline group on all but 6 trials (Trials 16, 19, 24, 25, 27, 28). The development of tolerance in the amphetamine group was confirmed by an upward shift of DR 2, conducted after the tolerance phase (see Figure 1, bottom left). Statistical analysis followed by post hoc comparisons indicated a significant DR Dose interaction, $F(3, 24)$ 10.41, p .02, with significant differences between dose–response determinations at all four doses. In contrast, there were no significant differences between dose–response determinations for the saline group (p .05).

As would be expected, licking was also initially suppressed after injection of amphetamine but recovered with chronic administration of the drug (see Figure 1, top right). Unlike milk intakes, however, licking recovered to the level of the saline group from Trial 13–30, as confirmed by a significant Group Trial interaction, $F(30, 450)$ 9.53, p .01, and post hoc comparisons. The residual deficit in milk intake coupled with the full recovery of licking in the amphetamine group resulted in a decreased volume per lick relative to saline controls (see Figure 1, bottom right). This difference was confirmed by a significant Group Trial interaction, $F(15, 225)$ 3.18, p .01. Post hoc comparisons revealed that the groups differed on Trials 16–30, but not during the baseline. The mean volume of milk per lick for the amphetamine group during these trials was 4.1 μl versus 5.7 μl for the saline group.

ILI

The relative frequency of ILIs of various durations during the baseline period is shown in Figure 2 (top left). The data are grouped in 10-ms bins up to 250 ms, with all remaining intervals combined into a single final bin. For both groups, the ILI distribution was symmetrical, with most of the data falling into a narrow range with a mean of about 150–160 ms. The relative frequency of intervals 250 ms was similar, and relatively small, in both groups.

Amphetamine had almost no effect on the ILI, even early in the tolerance phase, when the rats first began to recover milk intake (see Figure 2, bottom left). For example, on the first trial in which intakes exceeded 5 cc, the amphetamine group showed the same ILI distribution as it had during the baseline for ILIs up to 250 ms. Similarly, there were no differences on the last trial of the tolerance phase (Day 30) compared with the baseline. Although the frequency of ILIs 250 ms was somewhat higher under amphetamine, there were no significant differences between treatment conditions on any trial during the tolerance phase (ps .05). There were also no significant differences in the saline group in the ILI distributions during the tolerance phase and the baseline period (data not shown).

Finally, on the basis of a relatively liberal pause criterion of 1 s to define a burst of licking, the mean intraburst interval did not differ between the groups on either the baseline or on Trials 16–30 of the tolerance phase (p .05; see Figure 2, top right).

Number of Bursts

In order to explore as broadly as possible the effect of amphetamine on the temporal distribution of licking, we used pause criteria ranging from 0.5–3.0 s to calculate the number of lick bursts per session. As shown in Figure 3 and Table 1, amphetamine produced a marked increase in the number of bursts per session up to pause criteria of 2.5 s. Even at pause criteria of 2.5 and 3.0 s, differences between the groups were marginally significant. Visual inspection of Figure 3 also suggests that, during the later trials, differences between the groups in the number of lick bursts tended to increase at the shorter pause criteria, whereas at the longer pause criteria, the number of bursts in the amphetamine and saline groups tended to converge. However, except at the 0.5 s criterion, there were no significant Group Trial interactions (ps .05).

Burst Size

The number of licks per burst at each of the burst pause criteria is shown in Figure 4. Amphetamine produced significant reductions in the number of licks per burst at pause criteria of 0.5 and 1.0 s, but not at pause criteria of 1.5–3.0 s. These impressions were confirmed by statistical tests, which revealed significant main effects of group at the 0.5-s, $F(1, 15)$ 7.46, p .02, and 1-s, $F(1,$

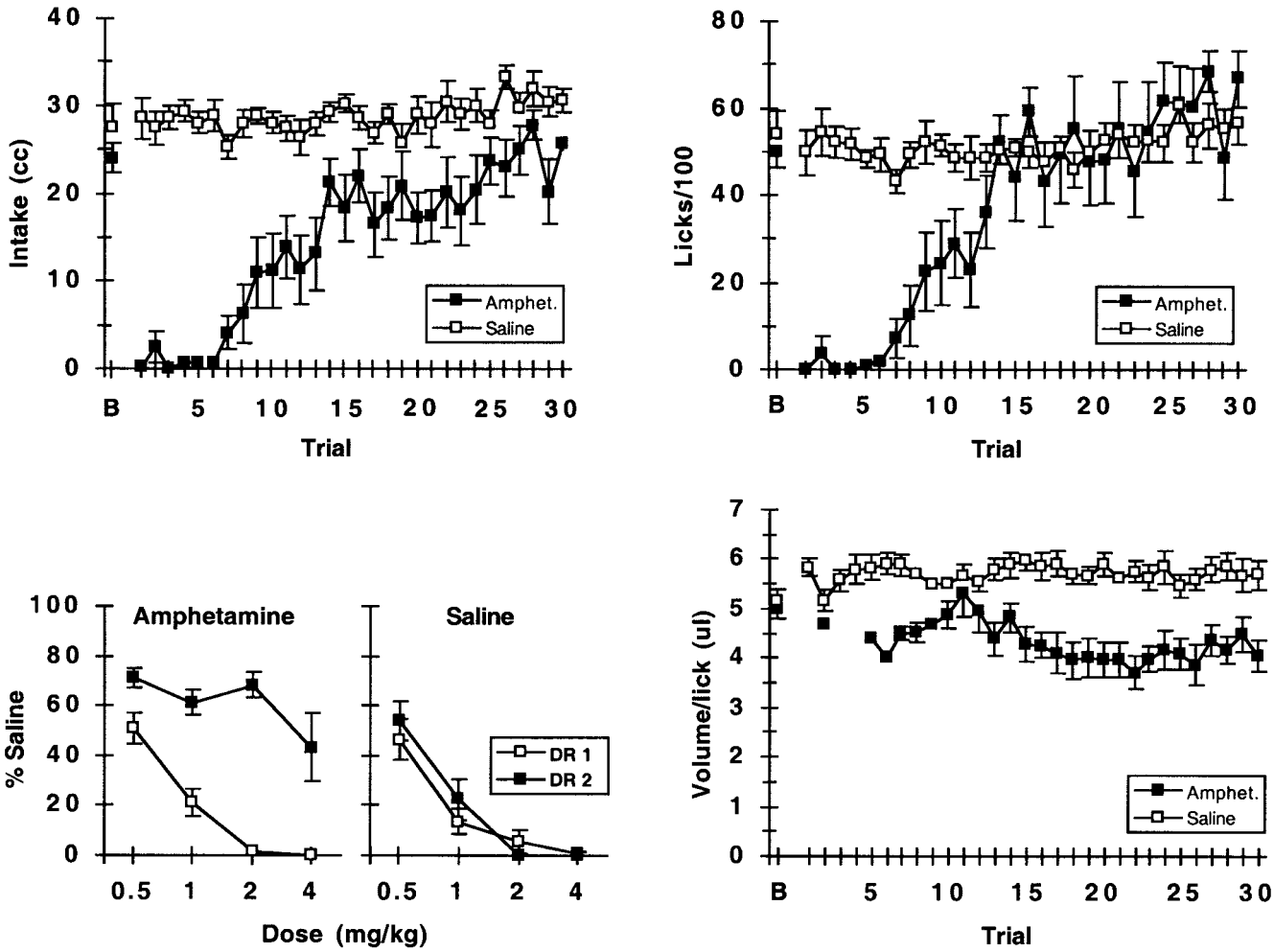

Figure 1. Top left: Mean (*SEM*) milk intakes of amphetamine (Amphet.) and saline groups during the tolerance phase. Bottom left: Effect of various doses of amphetamine on mean (*SEM*) milk intakes in the amphetamine and saline groups prior to (dose–response determination [DR] 1) and after (DR 2) the tolerance phase. The data are expressed as a percentage of intakes under the saline doses for each DR determination. Mean intakes under saline for DR 1 and 2, respectively, for the amphetamine group were 25 and 39 cc; for the saline group, 25 and 31 cc. Top right: Mean (*SEM*) number of licks (100) by rats in the amphetamine and saline groups during the tolerance phase. Bottom right: Volume of milk per lick for rats in the amphetamine and saline groups during the tolerance phase. B mean of the last seven baseline trials.

15) 6.73, *p* .02, criteria. There were no significant Group Trial interactions (*p*s .05 for all pause criteria).

Force per Lick

The effect of amphetamine on peak force per lick is presented in Figure 5. During the baseline period, both the amphetamine group and the saline group displayed similar frequency distributions of force per lick, with modal values of 1.5–2.0 g (see Figure 5, top left). On the first trial in which intakes exceeded 5 cc, as well as on subsequent trials (e.g., Day 20, 25 and 30), amphetamine caused a decrease in the frequency of forces in the 1.5–2.5 g range and on some trials a slight increase in the frequency of forces in the 3.5–6.0 g range (Figure 5, middle and bottom). Similarly, the mean peak force of the amphetamine group during the tolerance phase was slightly higher than that of the saline group (see Figure 5, top right). However, none of these differences was significant (*p*s .05).

Discussion

Historically, the development of tolerance to amphetamine hypophagia has been assessed in terms of the total amount of food consumed during the session (e.g., Carlton and Wolgin, 1971; Poulos, Wilkinson, & Cappell, 1981; Wolgin & Hughes, 1997; Woolverton et al., 1978). Although total food intake is a convenient means of tracking the recovery of feeding, it does not provide information on how the organization of feeding behavior is affected by the drug. In the present study, we have shown that

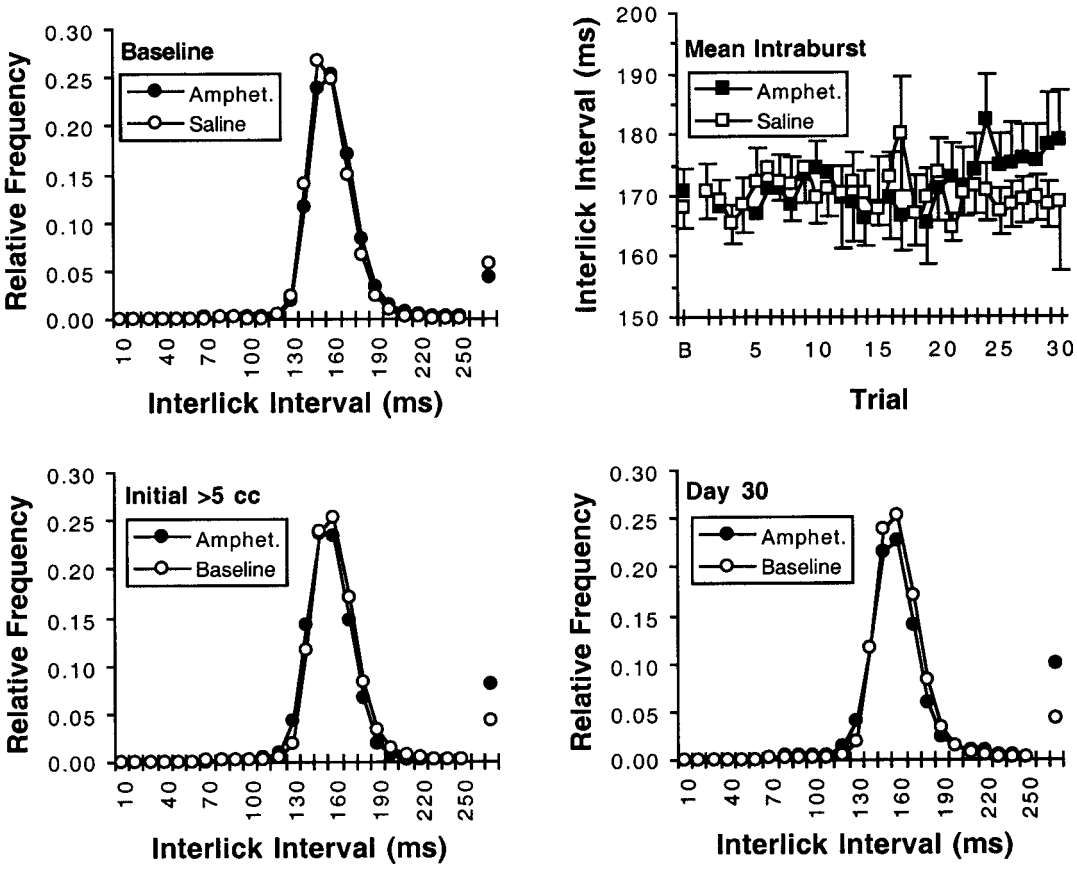

Figure 2. Top left: Frequency distribution of interlick intervals (ILIs) during baseline trials for the amphetamine (Amphet.) and saline groups. ILIs ≤ 250 ms are divided into 10-ms bins; ILIs 250 ms are combined into a single bin. Bottom left: Frequency distribution of ILIs for the amphetamine group on the first trial on which intakes exceeded 5 cc during the tolerance phase and on the baseline trials. Bottom right: Frequency distribution of ILIs for the amphetamine group on the final tolerance trial and on the baseline trials. Top right: Mean (*SEM*) intraburst ILIs for the amphetamine and saline groups during baseline and tolerance trials. A pause criterion of 1 s was used to define a burst of licking. B mean of the last seven baseline trials.

amphetamine has relatively little effect on certain parameters of feeding but has a profound effect on others.

Amphetamine had no significant effect on the ILI distribution. Modal values of this parameter on the first trial in which intakes were at least 5 cc were identical to those during the baseline and remained similar throughout the tolerance phase. Similarly, there were no significant differences between the amphetamine and saline groups during the tolerance phase in the mean ILI calculated for all intervals up to 1 s, a value generally considered the upper limit for intraburst pauses in licking under nondrug conditions (Spector et al., 1998). There results confirm and extend those of Asin, Davis, and Bednarz (1992), who found that acutely administered amphetamine (0.25–1.00 mg/kg) had no effect on the ILI of nondeprived rats drinking a 0.4-M sucrose solution. On the other hand, Badiani and Stewart (1999) reported that rats given chronic injections of amphetamine and access to water showed a progressive increase in the rate of licking and a shift to the left in the ILI distribution relative to baseline levels. There were, however, many procedural differences between the Badiani and Stewart (1999) study and ours, including deprivation conditions (ad libitum vs. deprived), dose of the drug (3 mg/kg vs. 2 mg/kg), test fluid (water vs. milk), and session duration (6 hr vs. .5 hr). Because the microstructure of licking is strongly influenced by each of these variables (see references in the introduction), it is perhaps not surprising that different outcomes were obtained.

Amphetamine also had little effect on the distribution of lick forces. In this case, there was a tendency for the distributions to be flatter and somewhat broader, reflecting a relative shift to higher forces. However, these changes were not statistically significant. Similarly, there were no significant differences between the amphetamine and saline groups in the mean peak force per lick throughout the tolerance phase. The fact that both lick force and the ILI distribution were normal suggests that, at doses typically used in feeding experiments (0.25–2.00 mg/kg), amphetamine does not disrupt the neural system that controls rhythmic licking movements.

In contrast, there were marked effects of amphetamine on the volume of milk ingested per lick. On Trials 16–30, the mean intake per lick for the amphetamine group was 4.1 μl, whereas for the saline group, it was 5.7 μl. Thus rats given amphetamine were

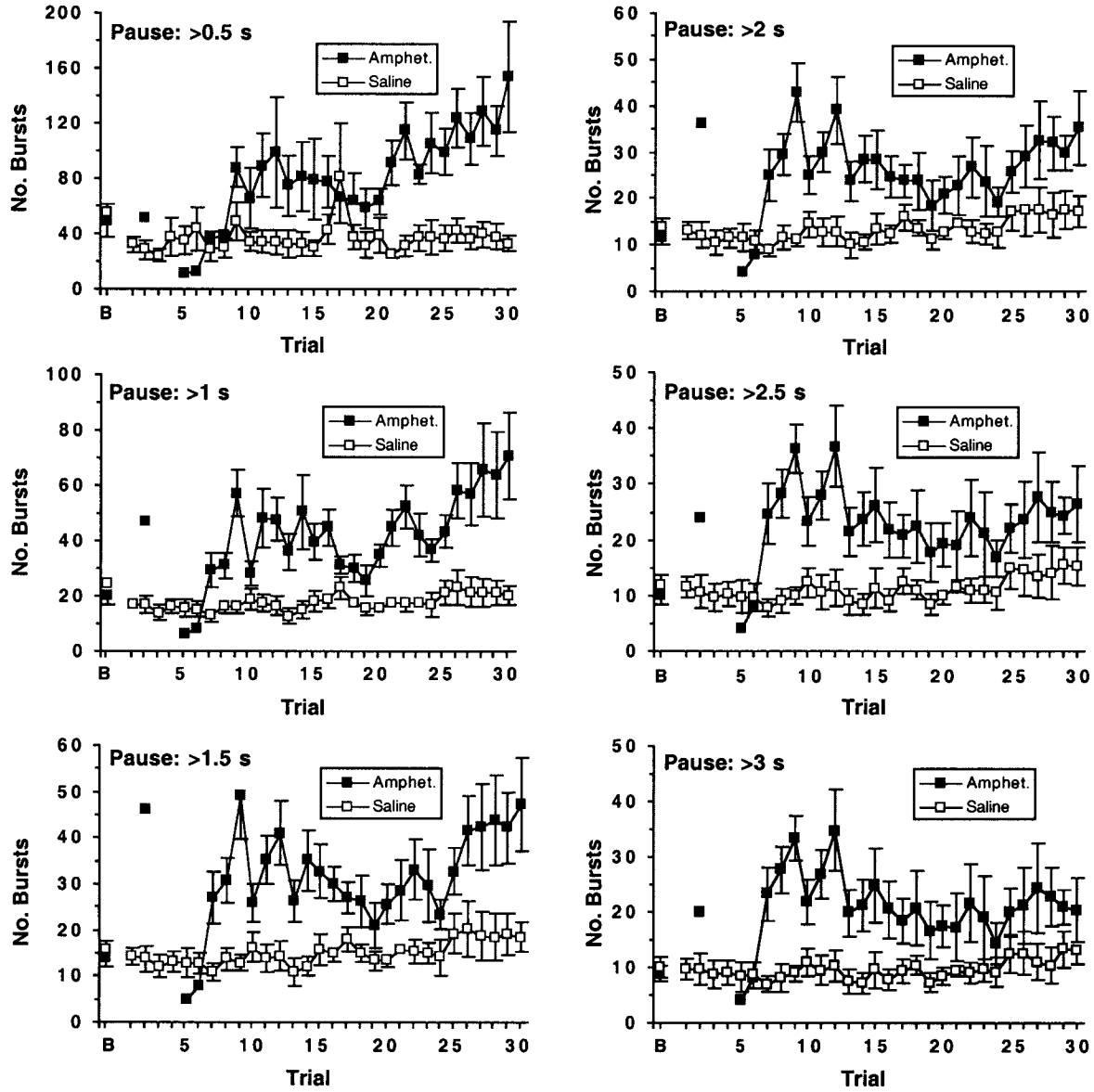

Figure 3. Mean (*SEM*) number of lick bursts during baseline and tolerance trials for the amphetamine (Amphet.) and saline groups. In each panel, a different interlick pause criterion, ranging from 0.5 to 3.0 s, was used to define a burst. B mean of the last seven baseline trials.

less efficient in capturing milk from the drinking tube than were rats given saline. Since the ILIs and peak forces per lick were not significantly different between the groups, this effect cannot be attributed to a deficit in the ability to lick per se. An alternative explanation may be found in the temporal organization of licking, as reflected in the number of lick bursts.

Amphetamine had a profound effect on the number of lick bursts, particularly at pause criteria up to 2.5 s. For example, during the final week of the tolerance phase, the number of bursts in the amphetamine group was roughly three times the number in the saline group. This means that rats given amphetamine interrupted their licking behavior much more frequently than rats given saline. It is apparent from comparison of the data from different

Table 1
Statistical Significance of the Effects of Amphetamine on Burst Number at Various Pause Criteria

Pause criterion (s)	*F*	*df*s	*p*	Effect
0.5	3.28	15, 225	.01	Trials 21–30
1.0	33.69	1, 15	.01	Group
1.5	15.02	1, 15	.01	Group
2.0	6.08	1, 15	.03	Group
2.5	4.33	1, 15	.06	Group
3.0	4.23	1, 15	.06	Group

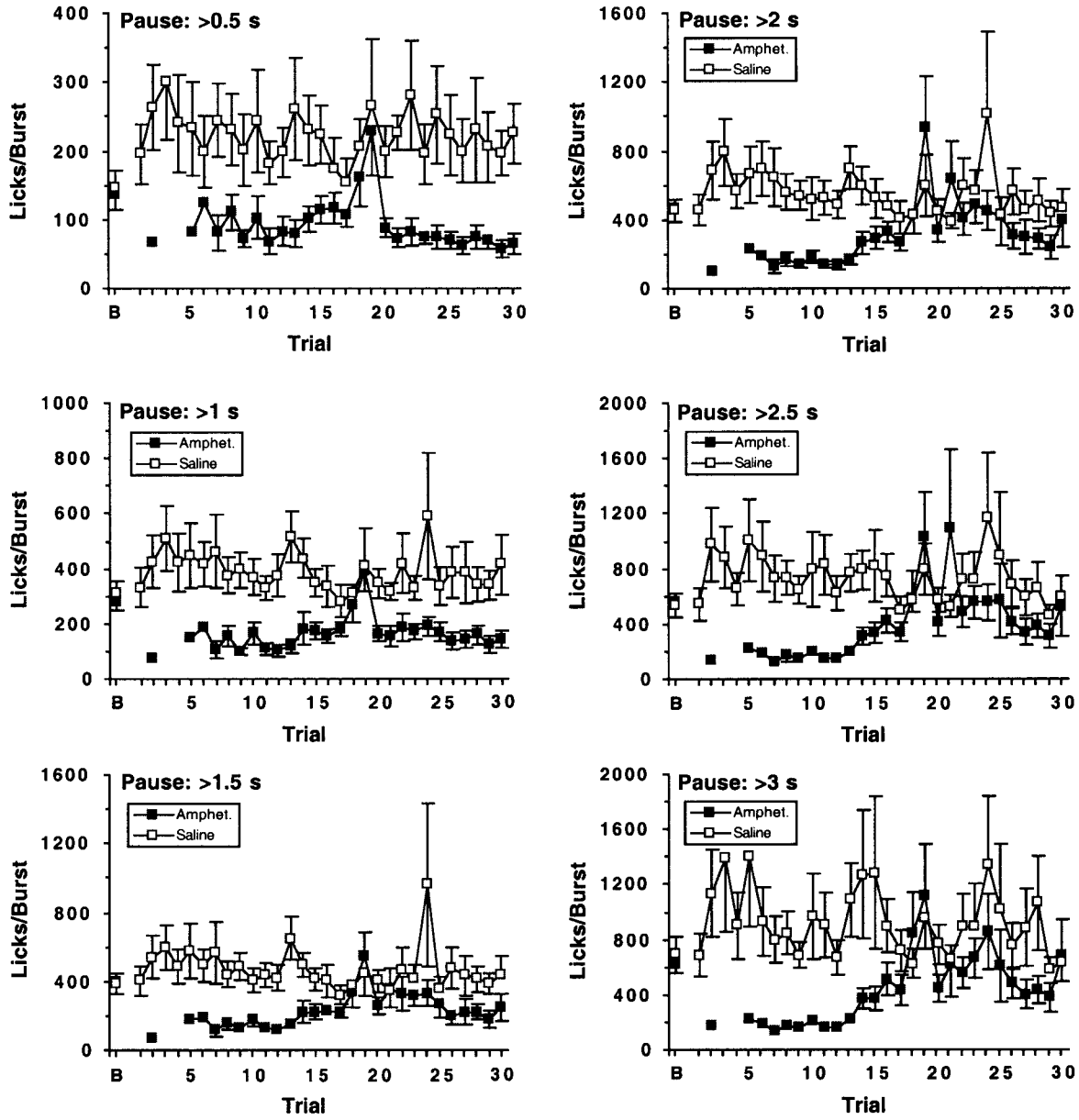

Figure 4. Mean (*SEM*) burst size (number of licks per burst) on baseline and tolerance trials for the amphetamine (Amphet.) and saline groups. In each panel, a different interlick pause criterion, ranging from 0.5 to 3.0 s, was used to define a burst. B mean of the last seven baseline trials.

pause criteria that the majority of these interruptions were fairly short, lasting somewhere between 0.5 and 1.5 s. Beyond that point, the absolute number of bursts in the amphetamine group did not decrease as much with successively longer pause criteria. The frequent interruptions of licking in the amphetamine group may have limited the efficiency with which milk was captured from the drinking tube.

This conclusion is supported by the finding that the number of licks per burst was reduced at the shorter pause criteria in the amphetamine group relative to that of the saline group. It seems reasonable that the efficiency of licking would be enhanced by long bursts of licking because once the head is properly oriented to the drinking tube and the tongue is accurately guided to the lumen via feedback from the initial licks, licking and, therefore, milk intake are optimized. In contrast, frequent interruptions of licking require repeated repositioning of the head and tongue and, consequently, less efficient capture of milk. It is also possible that licking was less accurate under amphetamine.

The present findings offer several new insights into the mechanism of tolerance to amphetamine hypophagia. According to the instrumental learning model, tolerance involves learning to suppress stereotyped movements that interfere with the appetitive phase of feeding (Wolgin, 1989, 2000). Initial evidence supporting this model came from studies showing an inverse relation between

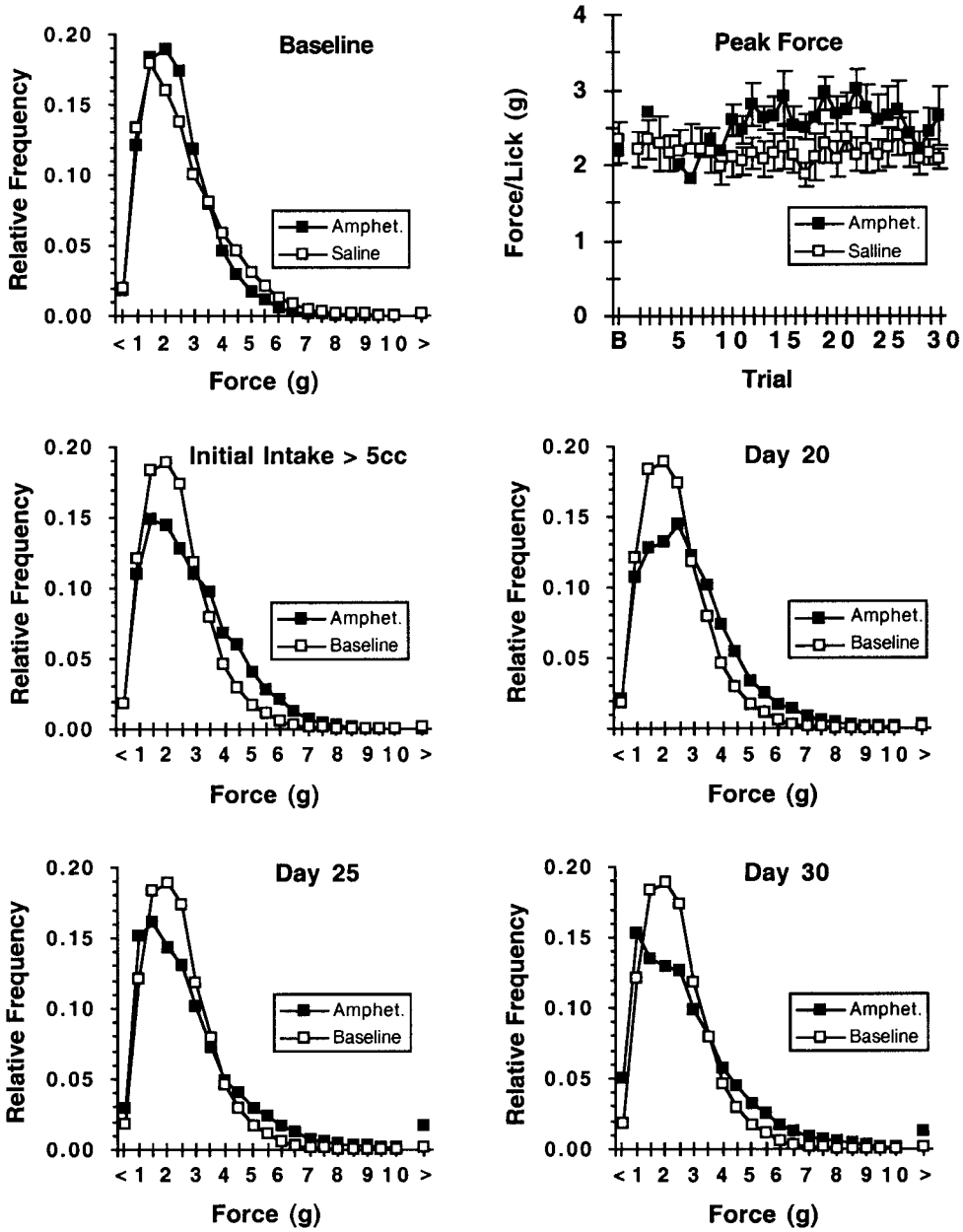

Figure 5. Top left: Frequency distribution of peak forces during baseline trials for the amphetamine (Amphet.) and saline groups. Middle and bottom: Frequency distribution of peak forces for the amphetamine group on baseline trials and on selected trials during the tolerance phase, including the first trial on which intakes exceeded 5 cc and on Days 20, 25, and 30. On the *x*-axes, forces less than 1 g, forces greater than 10 g. Top right: Mean (*SEM*) peak forces during baseline and tolerance trials for the amphetamine and saline groups. B mean of the last seven baseline trials.

total intake per session and the frequency of stereotyped movements (Salisbury & Wolgin, 1985; Wolgin et al., 1987; Wolgin & Hughes, 1997). More persuasive evidence was obtained from an experiment in which rats were reinforced with intraoral infusions of milk for holding their heads stationary within a narrow area of space defined by intersecting photobeams (Wolgin & Wade, 1995). Amphetamine-treated rats learned this task at the same rate as rats that were given milk in bottles. Analysis of the temporal distribution of intraoral infusions within a session, however, revealed marked differences between amphetamine- and saline-treated rats. In particular, tolerance was characterized by a more fragmented pattern of ingestion, with frequent interruptions of milk infusions.

The results of the present study confirm and extend these findings by providing a more quantitative assessment of the effects of amphetamine on feeding behavior. The increased number of

bursts and decreased number of licks per burst at the shorter pause criteria in amphetamine-treated rats are consistent with a pattern of ingestion characterized by frequent interruptions, as has been reported after both acute (Blundell & Latham, 1980; Cooper & van der Hoek, 1993; Rosofsky & Geary, 1989) and chronic (Wolgin & Wade, 1995) injections of stimulant drugs. Although behavioral ratings were not systematically conducted in this experiment, the increase in burst frequency in the amphetamine group, particularly at pause criteria 1 s, was very likely due to the intrusion of stereotyped movements, as documented in previous research (Wolgin et al., 1987; Wolgin & Wade, 1995). It is noteworthy, however, that although the number of bursts was consistently higher in the amphetamine group across all pause criteria, the size of the bursts (i.e., the number of licks per burst) gradually normalized over trials at the longer pause criteria. This means that tolerant rats made more normal-sized bursts of licking than saline controls. Indeed, inspection of Figures 3 and 4 suggests that the primary adjustment rats made during the development of tolerance was to increase the number of licks per burst at the longer pause criteria. However, this conclusion must be considered tentative because milk intakes during the early trials were low and variable, which precluded making statistical comparisons across the entire tolerance phase.

To further explore the implications of these findings, we have begun to examine the temporal distribution of licking responses within sessions using cumulative records generated from the stored force–time arrays. Preliminary analyses revealed that licking responses in amphetamine-tolerant rats were distributed over the entire session, whereas in saline controls licking was typically confined to the first 10–20 min of the session (Wolgin, 2002; Wolgin & Jakubow, 2001). This pattern is consistent with the increased number of lick bursts in tolerant rats. Because the volume per lick was lower in the amphetamine group, the increase in the number of bursts still resulted in mean daily intakes that were slightly lower than those of the saline group.

It should be noted that not all of the interruptions in licking can be attributed to the intrusion of stereotyped movements. This can be deduced from the fact that the number of bursts declined by nearly half when the pause criterion was increased from ≥ 0.5 s to ≥ 1.0 s. Because episodes of stereotyped movements generally last much longer than 1 s, the increased number of bursts in amphetamine-treated rats must be due to the intrusion of brief, nonstereotyped responses as well. Although we do not know the nature of those responses, they apparently occur in nondrugged rats too, as increasing the pause criterion from ≥ 0.5 s to ≥ 1.0 s caused a 50% decline in the number of bursts in the saline group as well.

Davis and Smith (1992), studying the microstructure of licking in nondrugged rats drinking sugar solutions, defined "clusters" as bursts of licking separated by pauses ≥ 0.5 s. The pauses that produce clusters are due to the intrusion of nonlicking responses. Further analysis (Davis, 1996) suggested that these pauses were determined probabilistically and could be subdivided into two classes: those that lasted about 1.5 s and did not vary with the concentration of the solution and those that lasted 50 s or more and did vary with the concentration of the solution. It may be that bursts defined by pause criteria of 0.5–1.0 s in the present study were caused by the intrusion of the short-duration responses described by Davis (1996). The topography of the nonlicking responses responsible for these pauses is unknown. However, it would appear from the present results that their frequency is markedly increased by amphetamine treatment.

In conclusion, the results of this experiment demonstrate that tolerant rats tested with amphetamine (2 mg/kg) have residual deficits in the microstructure of licking. The nature of these deficits, an increase in the number of bursts, a decrease in the number of licks per burst at short pause criteria, and a decrease in the volume of milk per lick, suggest that amphetamine continues to exert effects on feeding even though the amount of milk consumed recovers. To the extent that tolerance represents a learned compensation for the initial effects of the drug, as proposed by the instrumental learning model, these results suggest that the learned compensation is not complete. At the same time, it is also clear that tolerant rats can adjust their pattern of licking to achieve near-normal intakes, despite the ongoing constraints imposed by the behavioral effects of the drug. From this perspective, the development of tolerance represents yet another example of the behavioral flexibility that rats exhibit under a variety of conditions to maintain a normal meal size (cf. Kaplan, Baird, & Grill, 2001).

References

Asin, K. E., Davis, J. D., & Bednarz, L. (1992). Differential effects of serotonergic and catecholaminergic drugs on ingestive behavior. *Psychopharmacology (Berl), 109,* 415–421.

Badiani, A., & Stewart, J. (1999). Long-lasting sensitization to the accelerating effects of amphetamine on the speed of an internal clock. *Behavioural Brain Research, 100,* 217–223.

Blundell, J. E., & Latham, C. J. (1980). Characterisation of adjustments to the structure of feeding behaviour following pharmacological treatment: Effects of amphetamine and fenfluramine and the antagonism produced by pimozide and methergoline. *Pharmacology Biochemistry and Behavior, 12,* 717–722.

Carlton, P. L. (1963). Cholinergic mechanisms in the control of behavior by the brain. *Psychological Review, 70,* 19–39.

Carlton, P. L., & Wolgin, D. L. (1971). Contingent tolerance to the anorexigenic effects of amphetamine. *Physiology & Behavior, 7,* 221–223.

Cole, S. O. (1978). Brain mechanisms of amphetamine-induced anorexia, locomotion, and stereotypy: A review. *Neuroscience and Biobehavioral Reviews, 2,* 89–100.

Cooper, S. J., & van der Hoek, G. A. (1993). Cocaine: A microstructural analysis of its effects on feeding and associated behaviour in the rat. *Brain Research, 608,* 45–51.

Davis, J. D. (1973). The effectiveness of some sugars in stimulating licking behavior in the rat. *Physiology & Behavior, 11,* 39–45.

Davis, J. D. (1996). Deterministic and probabilistic control of the behavior of rats ingesting liquid diets. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 270,* R793–R800.

Davis, J. D., & Perez, M. C. (1993). Food deprivation- and palatability-induced microstructural changes in ingestive behavior. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 264,* R97–R103.

Davis, J. D., & Smith, G. P. (1992). Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. *Behavioral Neuroscience, 106,* 217–228.

Emmett-Oglesby, M. W., & Taylor, K. E. (1981). Role of dose interval in the acquisition of tolerance to methylphenidate. *Neuropharmacology, 20,* 995–1002.

Foltin, R. W., & Schuster, C. R. (1982). Behavioral tolerance and cross-tolerance to *dl*-cathinone and *d*-amphetamine in rats. *Journal of Pharmacology and Experimental Therapeutics, 222,* 126–131.

Fowler, S. C., & Mortell, C. (1992). Low doses of haloperidol interfere

with rat tongue extensions during licking: A quantitative analysis. *Behavioral Neuroscience, 106,* 386–395.

Fowler, S. C., & Wang, G. (1998). Chronic haloperidol produces a time- and dose-related slowing of lick rhythm in rats: Implications for rodent models of tardive dyskinesia and neuroleptic-induced parkinsonism. *Psychopharmacology (Berl), 137,* 50–60.

Higgs, S., & Cooper, S. J. (1998). Effects of benzodiazepine receptor ligands on the ingestion of sucrose, intralipid and maltodextrin: An investigation using a microstructural analysis of licking behavior in a brief contact test. *Behavioral Neuroscience, 112,* 447–457.

Hoebel, B. G. (1977). Pharmacological control of feeding. *Annual Review of Pharmacology and Toxicology, 17,* 605–621.

Joyce, E. M., & Iversen, S. D. (1984). Dissociable effects of 6-OHDA-induced lesions of neostriatum on anorexia, locomotor activity and stereotypy: The role of behavioral competition. *Psychopharmacology (Berl), 83,* 363–366.

Kaplan, J. M., Baird, J.-B., & Grill, H. J. (2001). Dissociation of licking and volume intake controls in rats ingesting glucose and maltodextrin. *Behavioral Neuroscience, 115,* 188–195.

Kirk, R. E. (1982). *Experimental design: Procedures for the behavioral sciences* (2nd ed.). Belmont, CA: Brooks/Cole.

Leibowitz, S. F. (1975). Catecholaminergic mechanisms of the lateral hypothalamus: Their role in the mediation of amphetamine anorexia. *Brain Research, 98,* 529–545.

Lyon, M., & Robbins, T. (1975). The action of central nervous system stimulant drugs: A general theory concerning amphetamine effects. In W. V. Essman & L. Valzelli (Eds.), *Current developments in psychopharmacology* (pp. 78–163). New York: Spectrum.

Paul, S. M., Hulihan-Giblin, B., & Skolnick, P. (1982, October 29). ( )-Amphetamine binding to rat hypothalamus: Relation to anorexic potency of phenylethylamines. *Science, 218,* 487–490.

Poulos, C. X., Wilkinson, D. A., & Cappell, H. (1981). Homeostatic regulation and Pavlovian conditioning in tolerance to amphetamine-induced anorexia. *Journal of Comparative and Physiological Psychology, 95,* 735–746.

Rosofsky, M., & Geary, N. (1989). Phenylpropanolamine and amphetamine disrupt postprandial satiety in rats. *Pharmacology Biochemistry and Behavior, 34,* 797–803.

Salisbury, J. J., & Wolgin, D. L. (1985). Role of anorexia and behavioral activation in amphetamine-induced suppression of feeding: Implications for understanding tolerance. *Behavioral Neuroscience, 99,* 1153–1161.

Spector, A. C., Klumpp, P. A., & Kaplan, J. M. (1998). Analytic issues in the evaluation of food deprivation and sucrose concentration effects on the microstructure of licking behavior in the rat. *Behavioral Neuroscience, 112,* 678–694.

Weijnen, J. W. W. M. (1998). Licking behavior in the rat: Measurement and situational control of licking frequency. *Neuroscience and Biobehavioral Reviews, 22,* 751–760.

Wolgin, D. L. (1989). The role of instrumental learning in behavioral tolerance to drugs. In A. J. Goudie & M. W. Emmett-Oglesby (Eds.), *Psychoactive drugs: Tolerance and sensitization* (pp. 17–114). Clifton, NJ: Humana Press.

Wolgin, D. L. (2000). Contingent tolerance to amphetamine hypophagia: New insights into the role of environmental context in the expression of stereotypy. *Neuroscience and Biobehavioral Reviews, 24,* 279–294.

Wolgin, D. L. (2002). Effects of chronic amphetamine on the appetitive and consummatory phases of feeding. *Appetite, 38,* 221–223.

Wolgin, D. L., & Hertz, J. M. (1995). Effects of acute and chronic cocaine on milk intake, body weight, and activity in bottle- and cannula-fed rats. *Behavioural Pharmacology, 6,* 746–753.

Wolgin, D. L., & Hughes, K. M. (1997). Role of behavioral and pharmacological variables in the loss of tolerance to amphetamine hypophagia. *Psychopharmacology (Berl), 132,* 342–349.

Wolgin, D. L., & Jakubow, J. J. (2001, November). *Tolerance to amphetamine hypophagia: A real time analysis of learning to suppress stereotyped movements*. Paper presented at the annual meeting of the Society for Neuroscience, San Diego, CA.

Wolgin, D. L., Thompson, G. B., & Oslan, I. A. (1987). Tolerance to amphetamine: Contingent suppression of stereotypy mediates recovery of feeding. *Behavioral Neuroscience, 101,* 264–271.

Wolgin, D. L., & Wade, J. V. (1995). Learned suppression of stereotypy in amphetamine-treated rats: Implications for understanding tolerance to amphetamine "anorexia." *Behavioural Pharmacology, 6,* 254–262.

Woolverton, W. L., Kandel, D., & Schuster, C. R. (1978). Tolerance and cross-tolerance to cocaine and d-amphetamine. *Journal of Pharmacology and Experimental Therapeutics, 205,* 525–535.