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# Enhancement of the Prophagic but Not of the Antidipsogenic Effect of U-50,488H After Chronic Amphetamine

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BADIANI, A. AND J. STEWART. *Enhancement of the prophagic but not of the antidipsogenic effect of U-50,488H after chronic amphetamine.* PHARMACOL BIOCHEM BEHAV 44(1) 77–86, 1993.—Two groups of rats were treated with seven daily injections of either saline or *d*-amphetamine (3 mg/kg IP). On the 2 days following the last injection, rats were tested according to a counterbalanced experimental design, each animal receiving, immediately prior to the beginning of the dark phase, saline on one day and the highly selective κ-opioid agonist *trans*-±3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methanesulfonate hydrate [U-50,488H (U50)] on the other. A microcomputer-controlled data acquisition system was used for the structural analysis of the feeding and drinking responses to amphetamine and U50. U50 enhanced feeding and depressed drinking in the first hour. The increased food intake was probably the result of the effect of U50 on the development of satiation and duration of satiety. Chronic amphetamine potentiated the prophagic effect but not the antidipsogenic effect of U50. The structural analysis demonstrated that the characteristics of the prophagic effect of U50 were amplified but not changed.

U-50,488H κ-Opioids Amphetamine Sensitization Tolerance Anorexia Hyperphagia Drinking Feeding Satiety Satiation Rat

MODERATE doses of amphetamine (2.0–5.0 mg/kg, IP) typically suppress feeding and drinking in the rat. In nondeprived animals, this aphagic phase lasts about 1–3 h, depending upon the dose administered, after which feeding resumes at a level comparable to that of control rats. The feeding and drinking responses to amphetamine undergo profound changes with the repetition of treatments and, within days or weeks, the total amount of food measured in the 5–7 h after amphetamine injection tends toward normal levels, suggesting the development of tolerance to the aphagic effect. In fact, however, this normalization is only apparent; in nondeprived animals, amphetamine continues to induce an initial aphagic response, followed by a progressive increase in the amount of food consumed, leading eventually to hyperphagia (5,29, 39,41). This hyperphagic phase cannot be accounted for entirely by a compensatory response to the drug-induced aphagia because it also develops in rats given access to food several hours after drug treatment, when eating would normally resume (28). Quite independently of the increase in feeding, but around the same time, exaggerated drinking appears (39,46). These effects are somewhat suggestive of other phenomena described in humans. Ford et al. (20) reported that in a number of obese patients receiving long-term treatment with an anorectic agent weight loss was followed by a period of weight plateau and, subsequently, weight regain while the drug was still being administered. Although food intake was not measured, it may be that this increase in weight was related to the development of hyperphagia. Somewhat more speculative is the possible relation of these effects to symptoms of compulsive water drinking seen in some psychotic patients (25). The possibility of such a relation arises from the fact that chronic abuse of amphetamine can lead to psychotic symptoms in humans (44,45).

The mechanisms underlying "tolerance" to the anorectic effects of amphetamine are not understood. Changes in receptor and effector mechanisms have not been consistently found, and though much effort has been placed on trying to understand the behavioral basis of the phenomenon no explanation to date has been able to account for the complexity of the various changes reported [for recent reviews, see (6,15,16,22,30,43,53)]. Recently, however, it has been shown that chronic intermittent treatments with drugs that increase the release of catecholamines at various levels of the CNS, such as amphetamine or cathinone, sensitize the rat to the prophagic effect of the

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highly selective (50) κ-opioid agonist *trans*-±3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methanesulfonate hydrate [U-50,488H (U50)] and of the non-selective opioid agonist morphine, but not to that of the benzodiazepine diazepam (39–41). Stimulation of κ-opioid receptors affects ingestive behavior of the normal rat in a complex manner [for reviews, see (11,12)]. Enhancement of feeding (26,36) and depression of drinking (2,47,48) have been reported. In a previous study (2), we used a microcomputer-controlled data acquisition system to offer a detailed structural analysis of these effects. We found that U50 increases the frequency of feeding bouts, resulting in prolonged meals, and delays considerably the onset of drinking. In the present work, using the same methodology, we investigated which structural aspects of feeding behavior undergo changes after repeated injection of amphetamine and which are responsible for the sensitized prophagic response to U50 following chronic amphetamine treatment. In addition, we studied the interaction between chronic amphetamine and the effects of U50 on drinking. In previous experiments, water intake was either not measured (41) or was considered only as cumulative intake over the 2 and 5 h following treatment with U50 (39,40). Because the antidipsogenic effect of U50 is limited to the first hour posttreatment and can be followed by a phase of polydipsia, possibly related to U50-induced diuresis (1,48) a more detailed recording of the time course of the changes in drinking was appropriate. We compared the structure and time course of the feeding and drinking response to U50 in groups of rats previously given seven daily injections of either saline or amphetamine.

## METHOD

### *Animals*

Male Wistar rats (Charles River Canada, St. Constant, Québec), ranging in weight from 255–285 g at the beginning of the experiment, were used in this study. After their arrival, rats were maintained for 1 week in the general animal facilities to accustom them to the light/dark cycle and the form of food used in this experiment.

### *Apparatus*

A microcomputer-controlled data acquisition system consisting of 10 rat cages, a food delivery controller, drinkometer circuits, and an IBM XT-compatible "turbo" computer running at 8 MHz (21) was used for continuous monitoring of drinking and feeding behavior. In this system, each cage (30 × 30 × 27 cm), built of Plexiglas and aluminum and with a metal grid floor, is equipped with a pellet dispenser, a Plexiglas food cup, and a drinking tube. A photoconductive cell and a light beam placed at the entrance of the food cup activates the pellet dispenser when the beam is broken. The spout of the drinking tube, recessed behind a Plexiglas cover to reduce inadvertent contacts during normal exploratory activity, is connected to noninverting input of an operational amplifier; when the animal, grounded through the metal floor, licks the spout it generates a negative-going pulse at the amplifier outlet. The current passing through the rat body is limited by a 10-MΩ resistor to approximately 1–2 μA. The cages were isolated within a dedicated temperature-controlled room maintained at 22 (±1°C); a ventilation system provided a continuous exchange of air. A 12 L : 12 D cycle (lights on from 1000–2200 h) was in effect at all times. Computer and locally constructed solid interface were located in a separate room. The software program COLLECT (21) monitored the cages during the experiment and recorded the time and identity of every food pellet or lick. In this program, the polling routine is invoked at the standard timer rate of 18.2 times/s, faster than the observed licking rate of rats (51). Each incoming signal from the drinkometer and food delivery circuitry lasts a minimum of one timer cycle, allowing detection by the polling routine.

### *Drugs*

U50, a generous gift from P. F. Von Voightlander, Upjohn Co. (Kalamazoo, MI), and (+)-amphetamine HCl (Smith, Kline, and French, I.A.C., Montréal, Québec, Canada) were dissolved in a 0.9% saline solution. Both drugs were injected IP at the dose of 3.0 mg/kg in a volume of 1.0 ml/kg. The dose of U50 was selected on the basis of a previous study that showed that 3 mg/kg simultaneously enhances feeding and depresses drinking (2). Control treatments consisted of 1 ml/kg saline solution.

### *General Procedures*

The experiment was run using three squads for a total of 28 rats. Notice, however, that occasionally the data from certain cages were eliminated because of failure of either the drinkometer or pellet dispenser. Animals were housed in the cages 1 week before the start of the experiment. Replenishing of the feeding dispenser (with 45-mg Results™ dustless precision pellets, Bio-Serv, Frenchtown, NJ) and water bottles (with tapwater) was performed during the last 30 min of the light phase (0930–1000 h). Animals were handled daily (0930 h) to record the body weight except on test days. The chronic intermittent treatments consisted of seven consecutive daily (1700 h) injections of either saline (group Chron Sal) or amphetamine (group Chron Amph). On the 2 days following the last injection, rats were tested according to a counterbalanced experimental design, each animal receiving, immediately prior to the beginning of the dark phase (1000 h), saline solution (Sal) on one day and U50 on the other day and left undisturbed for the following 24 h.

### *Data Collection and Definitions*

A dedicated software computed the feeding episodes (bouts) according to the following convention: a *bout* ends when the respective input device remains silent for a period of 5 min. This criterion, chosen on the basis of the log survivorship curve of the interpellet intervals, is in agreement with the results of others (8,10). The feeding analysis was also carried out using a second measure of feeding event, to be called a *meal*, according to the criterion proposed by Le Magnen and Devos (32), who argued that the best criterion to separate basic prandial events is a period of 30–40 min of noneating. For the purposes of this study, therefore, a meal was considered terminated after 30 min of noneating. This distinction between bouts and meals must be considered conventional and does not imply any hierarchical relation between the two. We found, however, that, for most individual animals there was a significant positive correlation between size of the meal and postmeal interval, in agreement with the finding of others (33), but not between size of bout and postbout intervals. Thus, even though the analysis at the level of bouts offers a better resolution of the structure of feeding we consider the meal to be a better index of the obtained satiety. To minimize the impact of nonpurposive contacts with the food container

(a problem in particular evident after amphetamine), only bouts consisting of more than five pellets (21) were considered for structural analysis of the ingestive behavior. The dependent variables analyzed were: latency to start drinking and feeding, size, duration (min) and rate of ingestion (pellets/min) of bouts and meals, duration of interbout and intermeal intervals, and *satiety ratio* [duration of the intermeal interval divided by the size of the preceding meal, i.e., min/pellets; see (4)]. Finally, every recorded lick and pellet were considered in the analysis of temporal pattern of drinking and feeding.

*Statistical Analysis*

The time course of the effect of amphetamine on feeding and drinking was assessed using two-way repeated-measures

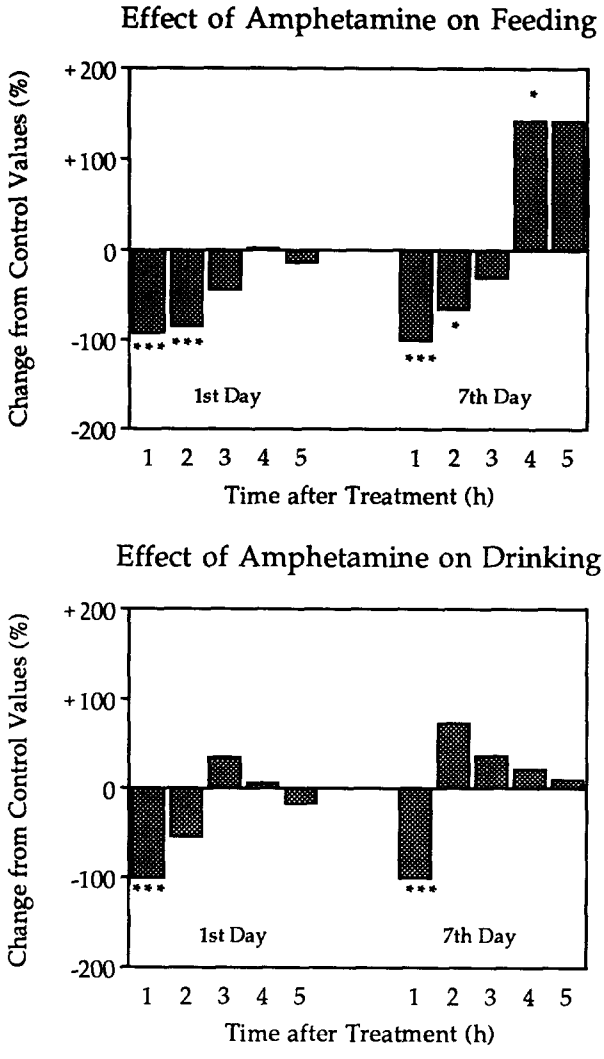


FIG. 1. The time course of the effect of amphetamine on feeding is represented as % change from the values of rats injected with saline. The statistical analyses were carried out on the raw data. On the first day, analysis of variance (ANOVA) showed a significant main effect for treatment, $F(1, 23) = 10.746$, $p = 0.0033$, and time but not for the interaction. On the contrary, on the seventh day only the interaction between treatment and time was significant, $F(4, 25) = 6.894$, $p < 0.0001$. $^{*}p < 0.05$, $^{***}p < 0.001$ vs. saline. For statistical procedures, see the text.

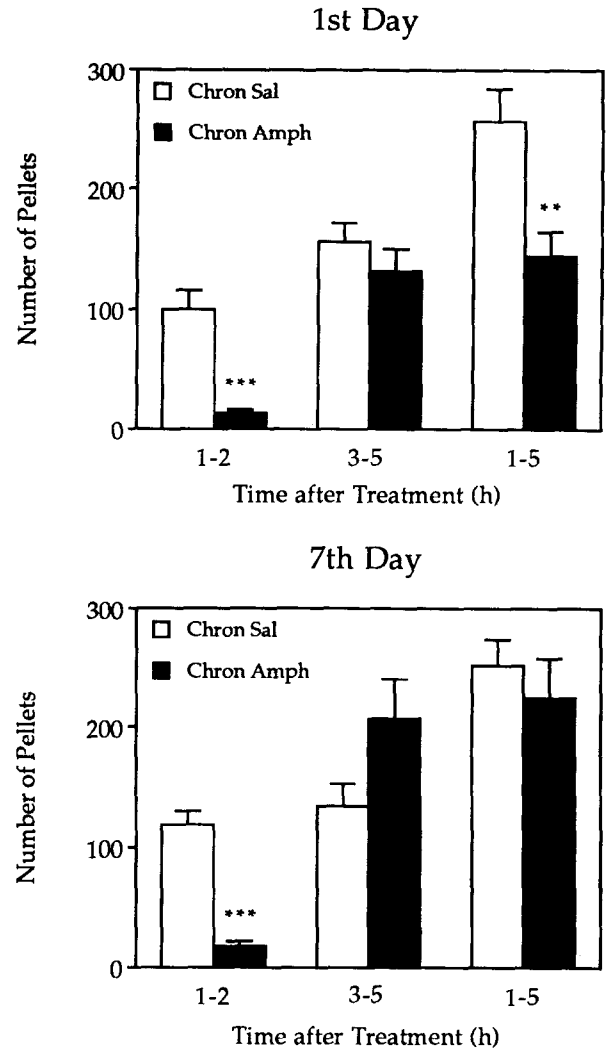


FIG. 2. Cumulative food intake during the 1- to 2-, 3- to 5-, and 1- to 5-h periods following the first (top) and seventh (bottom) treatments. Means ± SE. $^{**}p < 0.05$, $^{***}p < 0.001$ vs. saline group. For statistical procedures, see the text.

analyses of variance (ANOVAs) for treatment (two levels: saline and amphetamine) and time (five levels: first, second, third, fourth, and fifth hour). Because the usual posthoc tests are not appropriate after repeated-measures ANOVA, Student's *t*-tests were used to compare the effects of saline and amphetamine at each hour. To facilitate the comparison of our results with other studies, additional *t*-tests were carried out on the cumulative feeding data of the first 2 h (1–2 h), the following 3 h (3–5 h), and the overall 5-h periods after treatment. The differences in bout and meal size were assessed with *t*-tests. Mann–Whitney *U*-tests were used for analysis of the time, frequency, and rate data.

In the U50 test, each rat served as its own control, receiving both saline and U50 in a counterbalanced order. However, the Latin square repeated-measures design was unsuitable because of the small number of treatments (<3). Therefore, two-way repeated measures ANOVAs for chronic treatment (two levels: Chron Sal and Chron Amph) and acute treatment (two levels: Sal and U50 conditions) were used to analyze the

cumulative number of pellets and licks measured after 15, 30, 45, and 60 min and at the end of each of the following hourly periods. Paired $t$-tests were used for pair-wise comparisons between Sal and U50 conditions. Bout and meal size were evaluated with the same procedure. The differences between Sal and U50 conditions for the measures of time, rate, frequency, and satiety ratio were analyzed by Wilcoxon's signed rank tests. Finally, to study the interaction between chronic treatment and U50 on these measures two-way ANOVAs (in the absence of appropriate nonparametric tests) were carried out on the $1n(1 + x)$ of their raw data to normalize the frequency distributions.

## RESULTS

### *Chronic Amphetamine*

As shown in the top panel of Fig. 1, and again in Fig. 2, amphetamine had a strong aphagic effect leading to a significant reduction in the cumulative food intake over the 5 h, $t(23) = 3.278$, $p < 0.01$. This effect was due to the almost complete suppression of feeding in the first 2 h with only a small (−16%) and nonsignificant decrease in the number of pellets taken in h 3–5. Interestingly, this apparent normalization of intake on the first day was accompanied by an increase in the number of bouts of reduced size (see Table 1 and the top panel of Fig. 3) and a decrease of within-bout rate of feeding. With repetition of the treatment, the feeding response to amphetamine changed such that by the seventh day the phase of aphagia was followed by a significant increase in the number of pellets taken (Fig. 1 top panel and Fig. 2) both in the fourth hour and in the fourth and fifth hours combined [160.5 ± 31.36 vs. 66.23 ± 16.51; $t(25) = -2.6$, $p = 0.015$; data not shown]. As a consequence, by the end of the seventh day no effect on the cumulative intake over the 5 h was evident. Table 1 shows the structural analysis of feeding during the 3- to 5-h posttreatment period when eating resumed. The change that accounts for the "hyperphagia" is the further increase in the time spent feeding accompanied by a normalization of the bout size. By contrast, the effects of amphetamine on the bout number (increase) and within-bout rate (decrease) were unchanged. The meals were larger without changes in their frequency. Finally, an interesting effect of amphetamine is shown in the lower panel of Fig. 3, where the time course and frequency of small bouts are plotted (these are the bouts of ≤5 pellets that were excluded from the structural analysis as described in the Method section). These bouts, consisting primarily of one (60%) or two (20%) pellets, were negligible in saline-injected rats but increased dramatically after amphetamine in the second hour, returning slowly toward control values in the following hours. Even though no attempt was made to record other behaviors, informal observation indicated that most of these pellets were dropped on the floor of the cage or were not removed from the food cup, an effect reported also by others (24). As can be seen from Fig. 3, no tolerance developed to this effect of amphetamine.

The time course of the effect of amphetamine on drinking is shown in the bottom panel of Fig. 1. The pronounced period of adipsia seen on the first day was shortened on the seventh day and was followed by an increase, although nonsignificant, in water intake. As a consequence, total drinking in the 5 h following the first treatment with amphetamine was depressed relative to saline-treated animals [2,037 ± 230 vs. 2,940 ± 317; $t(22) = 3.696$, $p < 0.05$], whereas by the seventh there was no overall difference between groups (2,661 ± 299 vs. 2,607 ± 304).

### *Feeding Response to U50*

As expected, U50 enhanced feeding in all animals but to a greater degree in group Chron Amph (Fig. 4). The increase in the number of pellets taken was evident within 15 min of treatment, $F(1, 25) = 9.88$, $p = 0.0043$, due primarily to increased feeding in group Chron Amph, and was maintained

TABLE 1

EFFECT OF AMPHETAMINE ON THE STRUCTURE OF FEEDING (3–5 h POSTTREATMENT) AT THE BEGINNING (DAY 1) AND AT THE END (DAY 7) OF CHRONIC INTERMITTENT TREATMENT

| | Saline | Amphetamine |
|---|---|---|
| Day 1 | | |
| Number of meals | 1.75 ± 0.25 | 1.54 ± 0.18 |
| Meal size (pellets) | 78.52 ± 10.00 | 76.05 ± 10.68 |
| Meal duration (min) ($Z = -1.747, p < 0.05$) | 12.93 ± 2.58 | 33.07 ± 7.60 |
| Bouts/meal ($Z = -2.038, p < 0.05$) | 1.33 ± 0.10 | 2.45 ± 0.39 |
| Number of bouts ($U = 37.5, p < 0.05$) | 2.75 ± 0.28 | 4.31 ± 0.51 |
| Bout size (pellets) [$t(86) = 4.266, p < 0.0001$] | 56.24 ± 6.49 | 28.51 ± 3.20 |
| Within-bout rate (pellets/min) ($Z = -4.185, p < 0.0001$) | 11.34 ± 0.84 | 7.14 ± 0.66 |
| Total time spent engaging in feeding bouts (min) | 15.16 ± 1.64 | 20.73 ± 3.18 |
| Day 7 | | |
| Number of meals | 1.92 ± 0.24 | 1.79 ± 0.21 |
| Meal size (pellets) [$t(48) = -1.989, p = 0.052$] | 65.88 ± 9.53 | 115.12 ± 22.86 |
| Meal duration (min) ($Z = -2.746, p < 0.01$) | 11.80 ± 3.70 | 36.70 ± 7.63 |
| Bouts/meal ($Z = -2.615, p < 0.01$) | 1.32 ± 0.14 | 2.60 ± 0.42 |
| Number of bouts ($U = 37.5, p < 0.05$) | 2.69 ± 0.38 | 4.71 ± 0.55 |
| Bout size (pellets) | 49.26 ± 6.85 | 42.79 ± 5.76 |
| Within-bout rate (pellets/min) ($Z = -2.912, p < 0.01$) | 13.08 ± 0.96 | 10.2 ± 0.78 |
| Total time spent engaging in feeding bouts (min) ($U = 33, p < 0.01$) | 12.53 ± 2.30 | 26.46 ± 4.36 |

Mean ± SE. For statistical procedures, see the text.

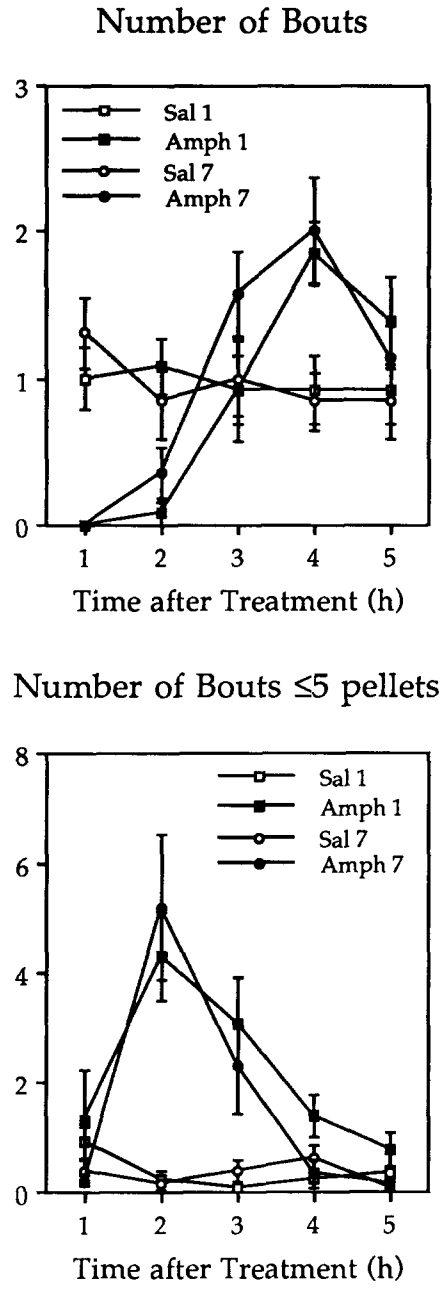


FIG. 3. Time course of the changes in the number of feeding bouts (top) and number of small bouts of ≤5 pellets (bottom). See the text for explanation. Means ± SE saline. (Sal) 1 and Amphetamine (Amph) 1 refer to the first treatments and Sal 7 and Amph 7 to the seventh.

throughout the first hour, $F(1, 25) = 41.503$, $p < 0.0001$. During the second hour, there was a modest but nonsignificant increment (14.9 ± 9.8 pellets in group Chron Sal and 38.3 ± 13.6 pellets in group Chron Amph). However, ANOVAs carried out on the cumulative data for the first 2, $F(1, 25) = 54.459$, $p < 0.0001$, 3, $F(1, 25) = 21.888$, $p < 0.0001$, and 4, $F(1, 25) = 7.739$, $p = 0.01$, yielded significant overall effects. The interaction between the effects of chronic

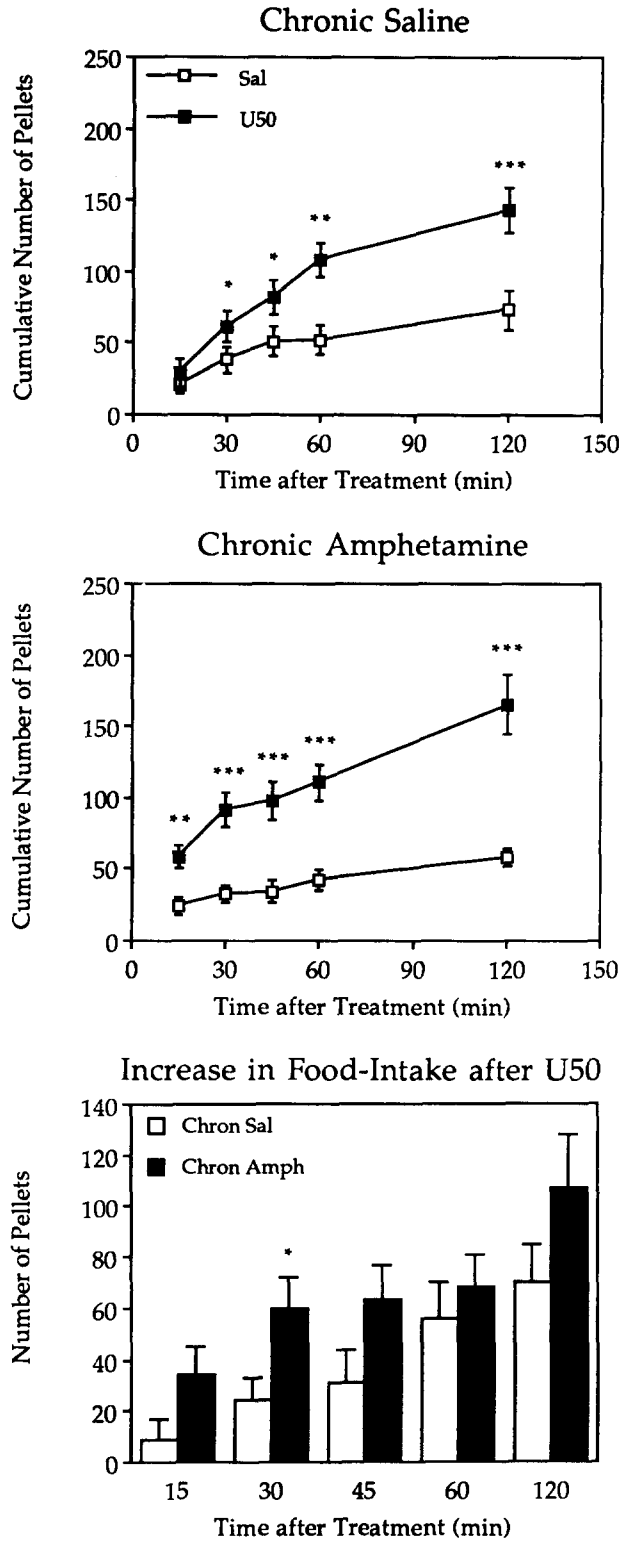


FIG. 4. Time course of the effect of amphetamine (Amph) on feeding in chronic saline (Chron Sal) (top) and Chron Amph (middle). All animals received both Sal and U-50,488H (U50) treatments. The differences between the number of pellets taken after U50 and after saline for animals in groups Chron Sal and Chron Amph are plotted in the lower panel. Means ± SE. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. the Sal condition. For statistical procedures, see the text.

treatment and the acute effects of U50 approached significance at 15 min, $F(1, 25) = 3.657, p = 0.067$, and was significant at 30 min, $F(1, 25) = 5.465, p = 0.028$. This is shown graphically in the lower panel of Fig. 4, where the difference between the number of pellets taken after U50 and after saline is plotted for groups Chron Sal and Chron Amph.

U50 did not reduce the time to take the first pellet nor did it significantly reduce the latency to start the first bout in either group. Figures 5 and 6 summarize the results of the effects of U50 on bouts and meals. U50 increased the size of the *first meal*, $F(1, 25) = 22.437, p < 0.0001$, and significantly more so in group Chron Amph; this is reflected in the significant interaction between U50 and chronic treatment, $F(1, 25) = 5.781, p = 0.024$. U50 increased the length of the first meal in both groups Chron Sal (Wilcoxon's test, $t = 2$, $n = 14$, $p < 0.0001$) and Chron Amph ($t = 6$, $n = 13$, $p < 0.001$). This effect and its potentiation by chronic amphetamine were limited to the first meal, the size of the second meal being almost equal in both groups (Chron Sal-Sal, 67.64 ± 8.75 pellets; Chron Sal-U50, 51.93 ± 12.1 pellets; Chron Amph-Sal, 60.77 ± 9.01 pellets; Chron Amph-U50, 62.08 ± 14.42 pellets). Interestingly, the respective intermeal interval was not altered by U50 (Chron Sal-Sal, 101.52 ± 12.66 min; Chron Sal-U50, 80.92 ± 11.63 min; Chron Amph-Sal, 96.4 ± 11.59 min; Chron Amph-U50, 96.72 ± 19.09 min) despite the fact that meal size and duration increased. As mentioned in the Method section, when a long period of non-eating (30–45 min) is chosen as a criterion to define prandial episodes (as was the case for *meals* in the present experiment) a positive correlation between prandial size and postprandial interval is observed (33). If animals were satiating normally after U50, a longer interval between the first and second meal might have been expected after the larger meal induced by U50. This was in fact confirmed by the analysis of the respective satiety ratios ($t = 11$, $n = 14$, $p < 0.01$ in group Chron Sal; $t = 0$, $n = 13$, $p \approx 0$ in group Chron Amph), as shown in Fig. 5.

The augmentation of the first meal after U50 was the result

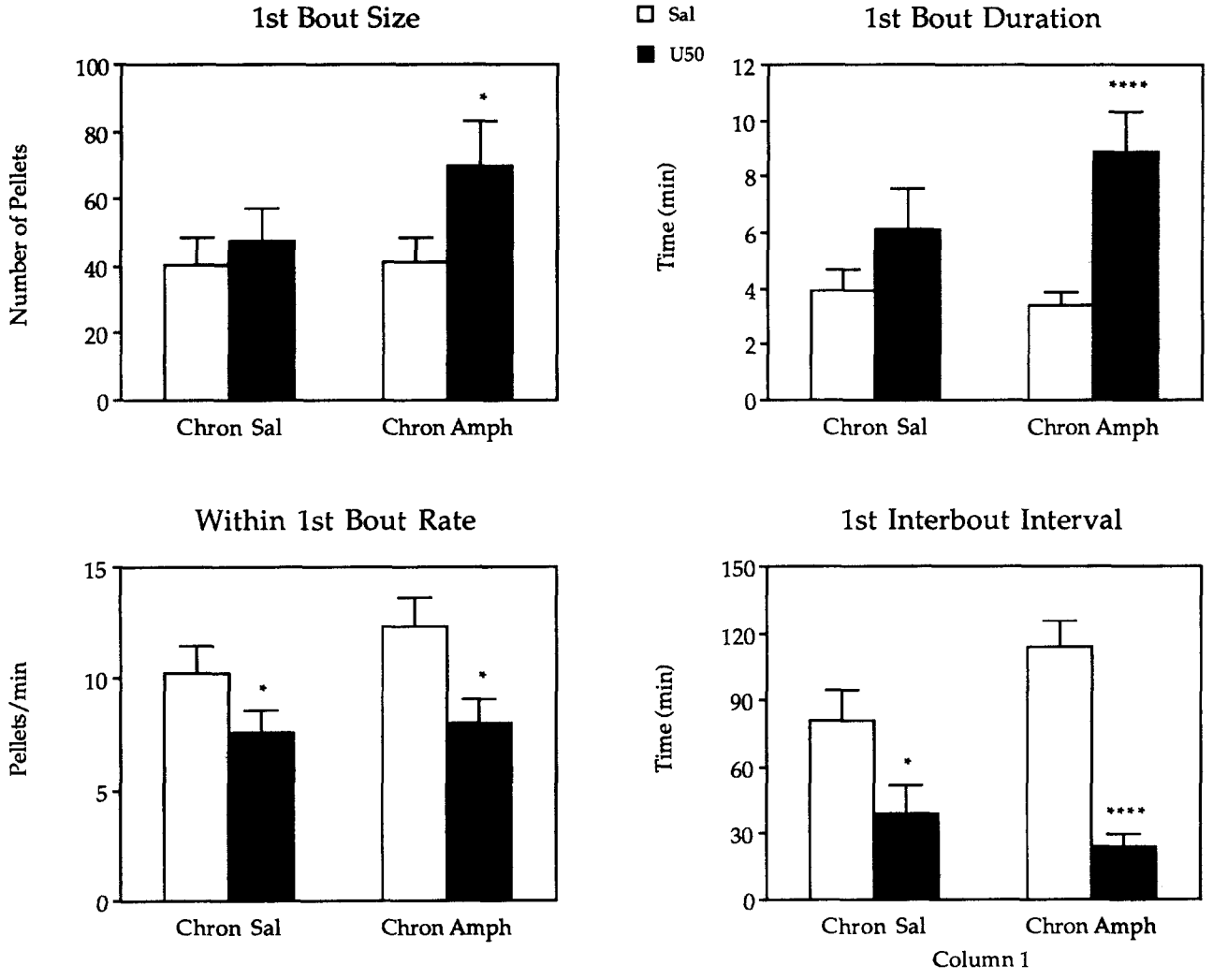


FIG. 5. Comparison of the effect of U-50, 488H (U50) and saline (Sal) on the first bout in groups chronic amphetamine (Chron Amph) and Chron Sal. All animals received both Sal and U50 treatments. Means ± SE. *$p < 0.05$ and ****$p < 0.0001$. For statistical procedures, see the text.

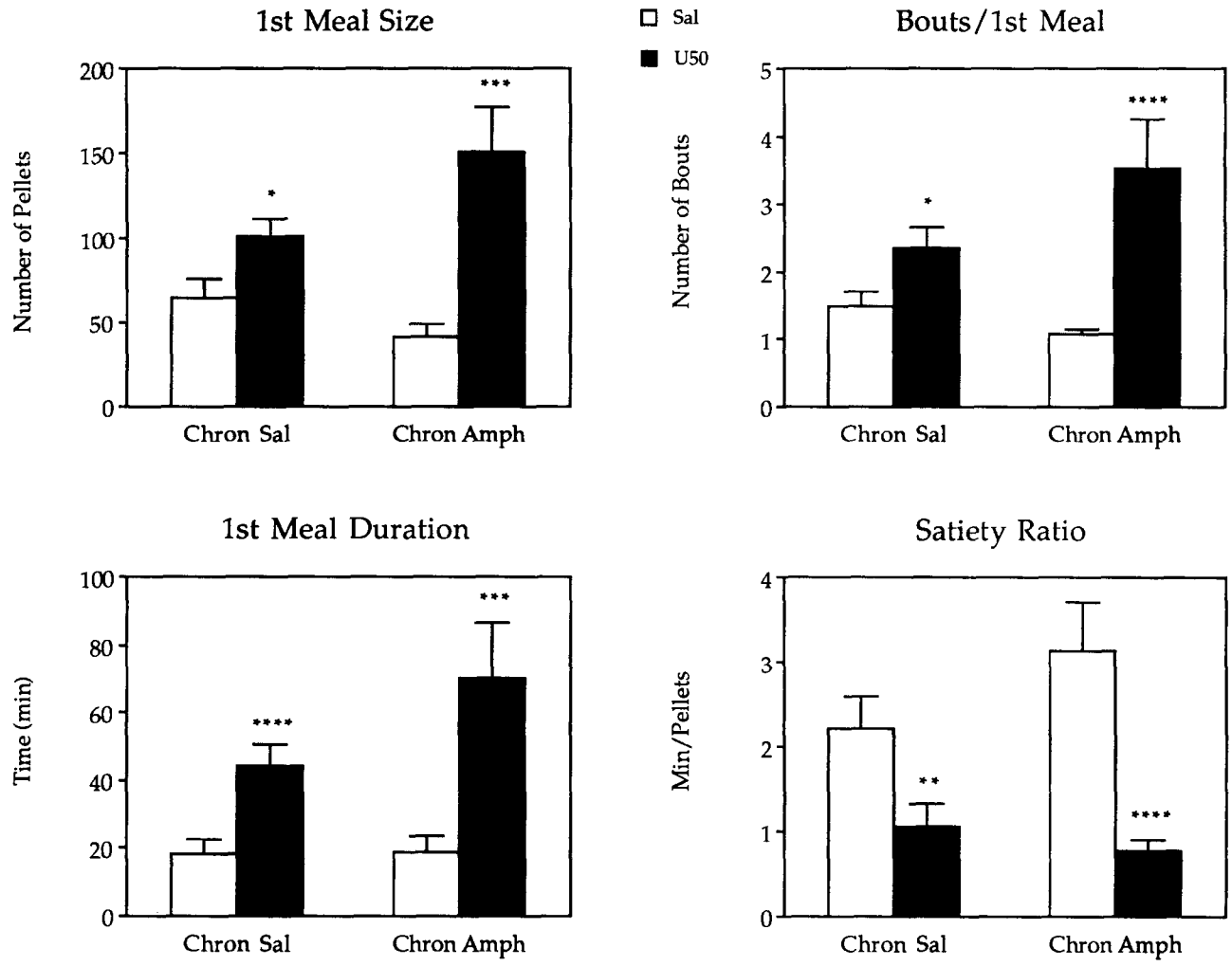


FIG. 6. Comparison of the effect of U-50, 488H (U50) on the first meal in groups chronic amphetamine (Chron Amph) and Chron Saline (Sal). The satiety ratio is obtained dividing the duration of the first intermeal interval (min) by the size of the first meal (pellets). All animals received both Sal and U50 treatments. Means ± SE. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. For statistical procedures, see the text.

of an increased number of bouts. There was a larger number of bouts in the first meal in both groups Chron Sal ($t = 7$, $n = 10$, $p < 0.05$) and Chron Amph ($t = 0$, $n = 10$, $p \approx 0$), and in addition a significant interaction between chronic treatment and U50, $F(1, 25) = 4.479$, $p = 0.044$. Under U50, the first bout tended to be larger and longer, but these effects reached statistical significance only in group Chron Amph. The overall rate of ingestion (pellets per min) during the first bout was depressed by U50 in both groups Chron Sal (7.56 ± 1.02 vs. 10.26 ± 1.2; $t = 18$, $n = 14$, $p < 0.05$) and Chron Amph (7.98 ± 1.08 vs. 12.36 ± 1.26; $t = 14$, $n = 13$, $p < 0.05$). Further, as shown in Fig. 7, if one considers the rate of ingestion during the first minute of the first bout (a parameter considered to reflect the palatability of food) there was no change in group Chron Sal and a small reduction in group Chron Amph.

*Drinking Response to U50*

Water intake was severely depressed by U50 during the first hour in both groups Chron Sal and Chron Amph (Fig. 8). After this period of adipsia, slightly greater levels of drinking occurred in U50 animals so that by the end of the fifth hour there were no differences in either cumulative water intake or water to food ratio between saline and U50 treatments. We have previously reported a detailed analysis of these effects (2). Chronic treatment with amphetamine did not modify the magnitude of the effect of U50 on drinking.

## DISCUSSION

*Chronic Amphetamine*

The first injection of the 3-mg/kg dose of amphetamine used in this experiment resulted in a complete suppression of feeding during the first 2 h (1–2 h) whereas in h 3–5 food intake was equal to that in saline-treated animals. The *normalization* of feeding, however, was only apparent; it was accomplished by an increase in bout number and a reduction in within bout rate so that rats took more time (+37% in comparison to saline-injected rats) to eat the same amount of food. The most likely explanation for this is that the activating

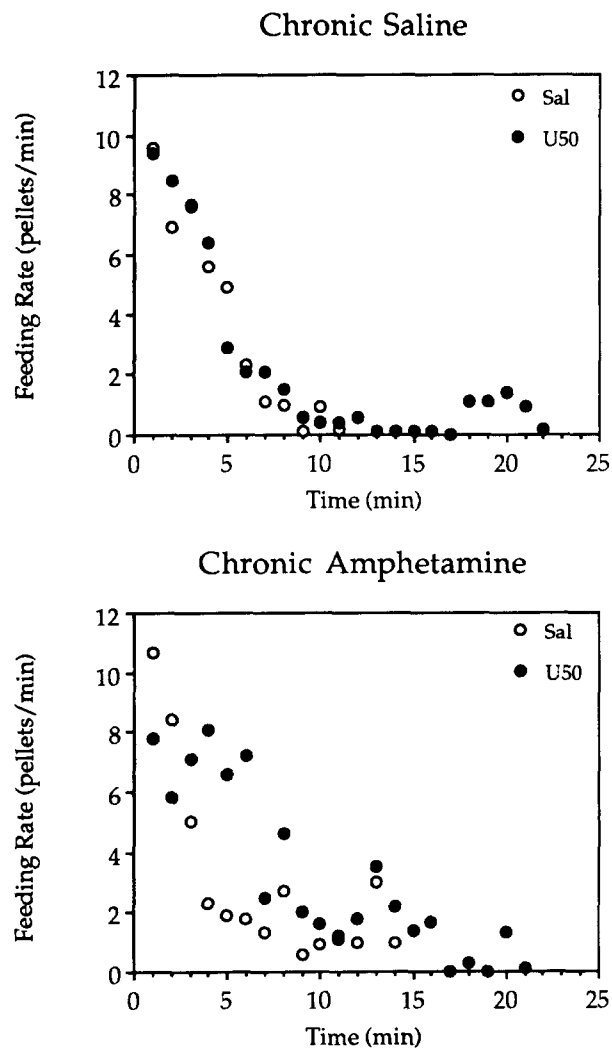


FIG. 7. Time course of the rate of ingestion (pellets/min) during the first feeding bout in groups chronic saline (Chron Sal) and Chron amphetamine (Amph) (bottom).

properties of amphetamine cause rats to continually break off from one activity (such as feeding, locomotion, and grooming) to engage in another, as found by Blundell and McArthur (7) in an experiment in which they used continuous videorecording of behavior. Thus, it seems clear that even after the end of the anorectic action amphetamine has a disruptive effect on the structure of feeding. Demellweek and Goudie (15,16), within the framework of a more general hypothesis about the basis of the behavioral tolerance to psychostimulants, proposed that the development of tolerance to the anorectic effect of amphetamine is a form of behavioral adaptation in which compensatory mechanisms gradually overcome the behavioral disruptions or reinforcement loss induced by amphetamine. Our results do not support this hypothesis, at least in free-feeding animals. In agreement with the work of others (5,9,29,39,41), we find, in fact, that little if any *true* tolerance develops to the amphetamine-induced suppression of feeding in the first 2 h. In the present experiment, the hyperphagia seen after repeated amphetamine resulted from the greater amount of time spent feeding as compared to both saline-treated animals and amphetamine-treated animals on the first day. On the other hand, the effects of amphetamine on number of bouts and within-bout rate persisted unchanged from the first day. If these latter effects are, as suggested by Blundell and McArthur (7), signs of behavioral disruption, it is evident that after 1 week no tolerance developed to them.

*Interaction Between Chronic Amphetamine and U50*

These results confirm those reported previously (39,41) that a chronic intermittent amphetamine pretreatment potentiates the effect of U50 on feeding and show, in addition, that the antidipsogenic effect of U50 was unchanged. The structural analysis demonstrated that the characteristics of the prophagic effects of U50 were amplified but not changed, ruling out the possibility that chronic treatment with amphetamine altered other, previously latent, effects of U50. The delay in

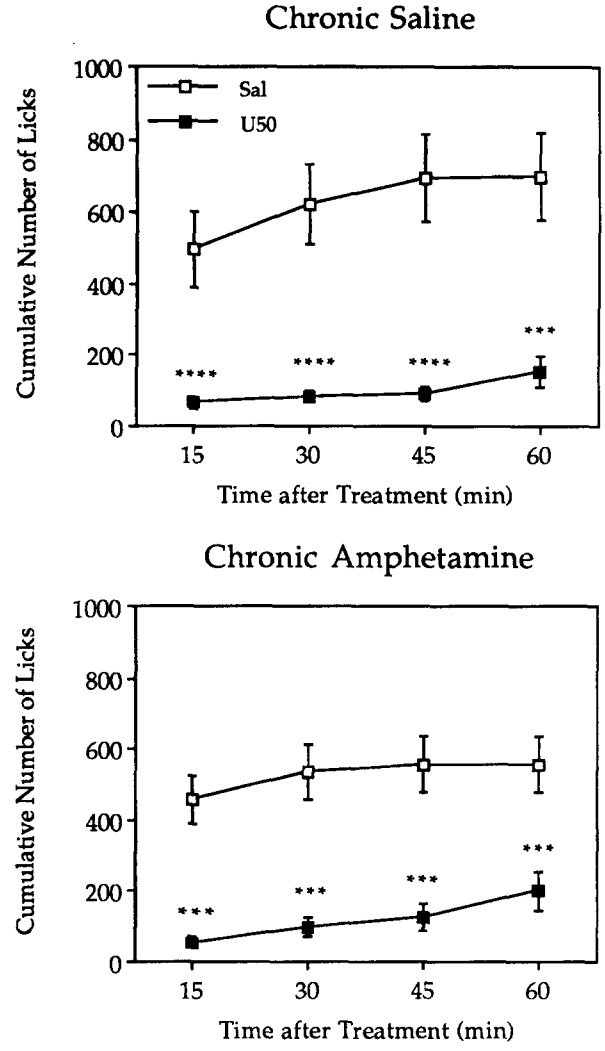


FIG. 8. Comparison of the time course of the effect of U-50, 488H (U50) on drinking in groups chronic saline (Chron Sal) (top) and Chron amphetamine (Amph) (bottom). All animals received both Sal and U50 treatments. Means ± SE. ***$p < 0.001$ and ****$p < 0.0001$. For statistical procedures, see the text.

the termination of the first bout after U50, reduced interbout intervals, and increased number of bouts per meal all suggest that κ-opioid mechanisms are involved in the development of satiation; in addition, the comparatively shorter postmeal interval, as expressed by the satiety ratio, suggests that U50 can reduce the duration of satiety. The fact that chronic amphetamine exaggerated these parameters suggests that catecholamines and κ-opioid mechanisms interact in systems involved in the maintenance of feeding and/or in the transmission of the satiety signals. It is unlikely that the increased food intake following U50, and its enhancement by amphetamine pretreatment, is due to increased palatability of the food inasmuch as U50 does not increase the initial rate of ingestion, a measure considered to reflect palatability (13,14); in addition, U50 has been shown to produce place and taste aversion (3,37). It is interesting to note that there are similarities in the changes in the structure of feeding immediately following U50 alone and in the 3–5 h following amphetamine alone. In both cases, there is an increased number of bouts, bouts per meal, and meal duration and a decreased within-bout rate.

It is still not known where in the CNS U50 acts to modulate feeding or to what extent it acts centrally or peripherally. On the other hand, amphetamine has been shown to modulate feeding at several brain regions. Leibowitz (31) argued that the lateral perifornical region of the hypothalamus (PFH) is the most important brain site for the production of the anorectic effect of amphetamine. By contrast, facilitation of feeding follows injection of amphetamine into the nucleus accumbens (NAC), the terminal region of the mesolimbic dopamine system (18,52). Increased feeding after microinjection of κ-opioids into the ventral tegmental area (VTA), the cell body region of the mesolimbic dopamine system, has been reported (23,27). However, others have failed to produce a robust facilitation of feeding after injection of U50 into either the VTA or NAC (1,34,41,42). In addition, chronic amphetamine did not change the response to U50 injected into the VTA (1,41).

Because the prophagic mechanisms of amphetamine do not seem able to account for the sensitization to U50, it is possible that the changes occur at the level of its "anorectic" mechanisms. The work of Bhakthavatsalam et al. (5) suggests, in fact, that tolerance to the anorectic effect of amphetamine develops after repeated injection into the PFH. High density of cell bodies and terminals containing dynorphin, the putative endogenous ligand for the κ-opioid receptors, has been found in the hypothalamus, including the PFH (19,38). On the contrary, only low to moderate levels of κ-opioid binding sites have been found in the same areas (17,28,35,49). We are currently studying the possible changes in κ-receptors in the hypothalamus and other brain regions occurring after chronic amphetamine. It is hoped that on the basis of these results it will be possible to select the brain regions for further intracranial injection studies.

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