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The effects of dose and repeated administration on the longer-term hypophagia produced by amphetamine in rats

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ABSTRACT

Rats are hypophagic approximately 1–3 and 13–27 h after receiving amphetamine (2.0 mg/kg). This study examined how these short- and longer-term phases of hypophagia were affected by repeated administration of different amphetamine doses. Throughout eight five-day tests, the rats could lever press for food pellets for 1-hour periods beginning every three hours. On test day 1, the rats were treated with saline, and on test day 3, they were treated with a dose of amphetamine. Across tests, for one group, treatment on day 3 alternated between 0.0 (saline) and 0.5 mg/kg amphetamine; for a second, group treatment on day 3 alternated between 1.0 and 2.0 mg/kg amphetamine; and for a third group, treatment on day 3 was always 1.0 mg/kg amphetamine. The patterns of food intake following day 1 saline and day 3 treatment were compared. Short-term food intake was abolished by 0.5, 1.0, and 2.0 mg/kg amphetamine, and no tolerance was observed to this effect. Longer-term hypophagia was produced by 1.0 and 2.0 but not by 0.5 mg/kg. Tolerance to longer-term hypophagia was seen when 1.0 mg/kg alone was used as the day 3 treatment, but not when 1.0 and 2.0 mg/kg were alternated across tests as the day 3 treatment. Short- and longer-term hypophagia were dissociated by threshold doses for elicitation and by differential tolerance. Occasional receipt of a higher amphetamine dose may sometimes increase the longer-term hypophagia produced by a lower dose.

1. Introduction

The effects of amphetamine on food intake during the first several hours following administration (short-term intake) have been studied extensively. In rats, amphetamine doses above 0.25 mg/kg are required to reduce short-term intake (Colle and Wise, 1988; Gilbert and Cooper, 1985; Towell et al., 1988; Wolgin et al., 1988). Generally, when amphetamine is repeatedly administered tolerance to reduced short-term intake is observed (Milloy and Glick, 1976; Poulos et al., 1981; Salisbury and Wolgin, 1985). Reduced short-term intake and tolerance have been ascribed to a variety of processes including changes in appetitive or consummatory behavior (Wolgin et al., 1988), elicitation and suppression of stereotyped behaviors that interfere with food intake (Salisbury and Wolgin, 1985; Wolgin et al., 1987), and shifts in hunger or in body weight set point (Caul et al., 1988; Wolgin, 1983). Tolerance has also been ascribed to the development of Pavlovian compensatory responses (Caul et al., 1988; Poulos et al., 1981; Wolgin, 1983).

Fewer studies have looked at how amphetamine affects food intake over longer intervals. Some studies have looked at hourly intake during intervals of four to twelve hours (Blundell et al., 1976; Caul et al., 1988; Jones and Caul, 1989). When the effects of amphetamine across even longer intervals have been considered, investigators have tended to measure total intake at the end of a long interval such as 24 h (Chen et al., 2001; Foltin, 2005). Few studies have periodically sampled food intake during the circadian interval following amphetamine administration. Time–course information is important because it provides a more complete account of a drug's time-dependent effects than does more limited sampling.

One effort to obtain this kind of data was recently described (White et al., 2007). Rats were trained to lever press for food pellets during 1-hour periods that occurred every three hours. When animals were administered 2.0 mg/kg amphetamine, they showed two phases of hypophagia. Short-term hypophagia occurred approximately 1 to 3 h after treatment, and longer-term hypophagia occurred approximately 13–27 h after treatment (White et al., 2007). A moderate dose was given at five-day intervals, mimicking some aspects of human recreational drug use.

The present study used a similar procedure to see how different doses of amphetamine (0.5, 1.0, and 2.0 mg/kg) affected food intake during the circadian interval following administration, and to see how the pattern of food intake changed when doses were administered repeatedly. We were particularly interested in seeing the effects of doses and of repeated administrations on longer-term hypophagia.

Eight five-day tests were conducted. At light onset of test day 1, rats received saline, and lever pressing and food intake were monitored for the next two days. At light onset of test day 3, animals received saline or a dosage of amphetamine, and behaviors were monitored for the next

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three days. For each test, effects following day 1 and day 3 treatments were compared. Each five-day test began with a two-day re-baseline in the eventuality of baseline shifts due to prolonged housing in the apparatus, aging, or shifts in food-intake set point due to repeated drug receipt (Koob and Bloom, 1988). Drug was administered at light onset, the start of the inactive period, so that motivational deficits, which tend to be greatest 18 to 24 h post-treatment, would coincide with the active period and so be easier to detect. The beginning of the inactive period is also the time at which recreational drug use presumably peaks in humans.

The design was intended, in part, to reduce the number of animals used and to provide information that a simple between groups design could not. In one group of animals, the dose given on test day 3 alternated across the eight five-day tests in a counterbalanced fashion between 0.0 mg/kg (saline) and 0.5 mg/kg amphetamine. Tests involving 0.0 mg/kg as the day 3 treatment enabled us to evaluate whether intake was stable over five-day intervals dispersed throughout the entire testing duration. At the same time, the condition allowed us to detect any systematic changes in intake across tests. In a second group, 1.0 mg/kg amphetamine, a dose that produces extreme locomotor activation but little stereotypy, was the day 3 treatment in all eight five-day tests. In a third group, day 3 treatment alternated between 1.0 and 2.0 mg/kg amphetamine across the eight tests. The 2.0 dose was used to replicate our prior results (White et al., 2007). Comparing results for the second and third groups would enable us to evaluate whether responsiveness to 1.0 mg/kg was affected by intermittent administration of a higher dose. The design was also intended to identify a "best dose" that could be used to explore, in subsequent studies, the determinants of amphetamine-induced longer-term deficits. A best dose was considered the lowest dose that produced robust longer-term hypophagia showing minimal tolerance across many tests.

Virtually no work on the determinants of longer-term hypophagia has been done, and so predictions are difficult. In the study by White et al. (2007), little tolerance of longer-term hypophagia was observed when 2.0 mg/kg amphetamine was administered at light onset. On the basis of research using activity as a dependent measure (White and White, 2006), 2.0 mg/kg amphetamine might be expected to produce more robust longer-term hypophagia than lower doses.

In order to show how repeated administration of amphetamine affects responsiveness, studies have typically examined the impact of closely spaced administrations on short-term effects. Generalizations from this research may have limited applicability to this study, which was concerned with the impact of widely spaced administrations on longer-term effects. For example, when administrations are closely spaced, as dose increases, the rate of tolerance can increase (Fernstrom and Choi, 2008; Graham et al., 2008). This dose–tolerance relationship may result, in part, because higher doses produce larger impairments in the mechanisms that underlie normal responsiveness, and with closely spaced administrations these mechanisms may not have sufficient time to normalize before the next administration. On the other hand, widely spaced administrations, which allow more recovery time, may result in a different dose–tolerance relationship. As another example, though tolerance of short-term effects has been ascribed to numerous processes (see above), still other processes could be involved in tolerance in longer-term effects: Though tolerance of longer-term hypophagia could be due to changes in factors that are activated in the short-term and that initiate the cascade of events resulting in longer-term hypophagia, such tolerance could also be due to factors occurring later in the cascade, such as those involved in the proximate expression of longer-term hypophagia.

High doses of amphetamine can augment responsiveness to lower doses (sensitization). This left open the possibility that 1.0 mg/kg amphetamine given in alternation with 2.0 mg/kg would produce more robust long-term hypophagia than 1.0 given alone.

Though this study was primarily concerned with dose and repeated administration effects, other information was sought. Whether short- and longer-term hypophagia are elicited by the same conditions is unknown. By administering a range of doses repeatedly, the present study attempted to dissociate the two phases of hypophagia in terms of their threshold eliciting doses and their rates of tolerance.

Hypophagia is one of several abnormalities present 18 to 24 h post-amphetamine treatment in rats. Others are hypoactivity (White and White, 2006; White et al., 2004) and the presence of internal cues resembling those produced by Haloperidol (Barrett et al., 1992). An analysis of the determinants of longer-term hypophagia might contribute to a better understanding of how these effects are interrelated. If these effects were to show correlated changes in response to different amphetamine doses and repeated administrations, then it would be more likely that, rather than being instigated by independent mechanisms, the effects either were mediated by a unitary mechanism or were hierarchically related.

2. Methods

2.1. Animals

A total of 24 adult male Wistar rats (Harlan, Indianapolis, IN) were used. The study had three successive conditions, and each condition included eight animals. Animals were housed in plastic tubs in a departmental colony having a 12-hour light/12-hour dark cycle and a temperature of 20–22 °C, and they were adapted to this environment for several weeks prior to the start of their condition. Animals had free access to water and food (Purina 5001 Rodent Diet, Lab Diet). Initially, animals were housed in pairs, but a week before the start of their condition they were housed individually. Just prior to the start of their condition, animals were handled and were pre-exposed in their home cages to the pellets that they would consume during the study. Animals weighed between 400 and 450 g at the start of their condition.

2.2. Apparatus

Animals learned to lever press for food pellets in four standard operant conditioning stations (Med Associates). Each station contained a retractable lever, a feeder that dispensed 94-mg pellets, and a bin that could be illuminated and that was equipped with a head-in-bin detector.

The animals were tested in one of eight "24-hour stations" that were designed for long-term housing. Each station consisted of a sound attenuating, wooden compartment (58 cm × 42 cm × 58 cm high) that enclosed a plastic housing cubicle (40 cm x 20 cm x 40 cm high). Each station contained a response lever, a pellet dispenser, and a food bin similar to those in the operant stations. The lever was situated just below the bin in the left half of one end wall of the cubicle. The right half of the end wall contained a drinking tube that was attached to a water bottle. The floor of each cubicle was a black metal pan that contained a thin layer of absorbent micro-waved topsoil. Each compartment had a fan (Sunon, sf11580A) that provided ventilation and that masked noises and a light fixture (Lampi-Pico accent light, 4-W) that produced a 12-hour light/12-hour dark cycle.

Devices in operant conditioning stations and in 24-hour stations were connected to an interface (Med Associates) and a computer. Software (Med Associates) was used to arrange contingencies and monitor behavior. Stations were located in well-isolated temperature- and humidity-controlled rooms (approximately 1.8 m × 2.1 m × 2.6 m high).

2.3. Drug

Powdered D-amphetamine sulfate (Sigma, St Louis) was mixed in saline (0.5, 1.0 and 2.0 mg/ml base). Saline was used as the control treatment (1.0 ml/kg).

2.4. Procedure

Animals in each condition were exposed to the same general procedure.

2.4.1. Lever press training

Each animal was deprived to 85% of its free-feeding body weight and was trained in an operant conditioning station to press the lever for 94-mg pellets (Bio Serv, #F0058). The animals were then placed on free food availability in the colony for 5 days.

2.4.2. Meal training

Animals were then transferred to 24-hour stations for the remainder of the study. Throughout this time animals were on a 12-hour light/12-hour dark cycle and had free access to water. Rats could lever press for food pellets for 1-hour periods ("meal opportunities") that began every three hours. Meal opportunities were scheduled during hours 2, 5, 8 and 11 of the light phase and of the dark phase. The beginning of a meal opportunity was signaled by the delivery of a pellet and the illumination of the feeding bin for 10 s. During a meal opportunity each lever press could result in six 94-mg pellets. The lever press produced the first pellet, and the delivery of each subsequent pellet was contingent on a head-in-bin response. The lever press also turned on the bin light, which remained on until the animal made a head-in-bin response to retrieve the sixth pellet. After the number of pellets consumed each day and the pattern of intake across the light–dark cycle stabilized, testing began.

2.4.3. Testing

Each test was 5 days long. Restricted feeding remained in effect. At light onset of test day 1, each animal was given a control treatment, 1.0 ml/kg saline subcutaneously (s.c.). Two days later, at light onset of test day 3, each animal was given an experimental treatment (0.0, 0.5, 1.0 or 2.0 mg/kg s.c. amphetamine). Each animal received eight tests.

The three conditions differed in the treatments given on test day 3. For one condition (the low doses condition) day 3 treatments were 0.0 and 0.5 mg/kg amphetamine. For half of the eight subjects the treatment during test 1 was 0.0 mg/kg amphetamine, and for the other half it was 0.5 mg/kg. The treatments alternated until a total of eight tests were given (four tests for each dose). Rats in the second condition (the high doses condition) were treated with 1.0 and 2.0 mg/kg amphetamine on a similar schedule. In the third condition (the 1.0 Amph condition) rats again received eight five-day tests, but the treatment on test day 3 was always 1.0 mg/kg amphetamine. This condition was run to see if the effects produced by 1.0 mg/kg amphetamine in the high doses condition might have been affected by its being given in alternation with 2.0 mg/kg amphetamine. Due to a power failure, data for the first day of amphetamine treatment in the third condition were lost, and so the test 1 and 2 averages described below reflect the results of the second amphetamine administration relative to the average of the controls. Test days and tests were successive, except on a couple of occasions when experimenters were unavailable.

Body weights were recorded at the time of saline or amphetamine treatment. Each animal's station was maintained at the same time, and this maintenance involved re-filling the pellet dispenser and water bottle, wiping off the lever, wiping out the feeding bin, and changing the pans and topsoil. Similar station maintenance was done at light onset of non-treatment days, except that pans and bedding were not changed. Otherwise, animals were not disturbed.

Animals in a particular condition were on the same 12–12-hour light–dark cycle from several weeks before the start of their condition until the end of their study. For the low- and high doses conditions the local time of light onset was 2:00 PM, and for the 1.0 Amph condition the local time of light onset was 12:00 noon.

2.5. Compliance statement

The experimental protocol was approved by the Institutional Review Committee for the use of Animal Subjects and was in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.6. Dependent measures

Lever presses, head-in-bin responses, and pellets consumed were monitored throughout Meal Training and Testing.

2.7. Data analysis

Data were analyzed using within, between, or mixed ANOVAs. Significant effects were analyzed with additional ANOVAs, followed up by Fisher's PLSD post hoc comparisons or t-tests (paired or unpaired).

3. Results

3.1. Meal training

Testing did not begin until mean intake did not vary for five consecutive days. This took 15–20 days.

3.2. Testing: low and high doses conditions

All animals continuously gained weight across the eight tests.

Fig. 1 shows the number of pellets consumed on each day of each test for each dose. The low doses group received, on day 3 of tests, counterbalanced treatment with 0.0 and 0.5 mg/kg amphetamine, and the higher doses group received similar treatment with 1.0 and 2.0 mg/kg amphetamine. The data for the different dose conditions were sorted and are displayed in different graphs. For 0.5, 1.0, and 2.0 doses, less intake occurred on the day of amphetamine treatment than on any other test day, $F(4,28) = 14.22$, 53.35 and 27.95, respectively, $ps < .0001$and Fisher's PLSD $ps < .05$. For the 0.0 dose, more intake occurred overall on the day after treatment than on the day of treatment or on the second day after treatment, $F(4,28) = 3.54$, $p = 0.0185$ and Fisher's PLSD $ps < .05$. For 0.0 and 0.5 doses, intake decreased across tests, $F(3,21) = 10.42$ and 5.33, $p = .0002$ and .0069, respectively. For both doses, less intake occurred during tests 3 and 4 than during test 1, Fisher's PLSD $ps < .05$. To summarize, food intake was reduced on the day of treatment with 0.5, 1.0, and 2.0 doses of amphetamine relative to other test days. Also, in the low doses condition, but not in the high doses condition, a reduction in daily intake was seen across tests.

Fig. 2 shows, for each dose condition, the mean number of pellets consumed at each meal opportunity for 2 days following saline control ("Sal") and amphetamine ("Amph"). Each function is an average of the four tests for a dose condition.

Several features of the saline control functions deserve note. For each dose condition, the patterns of intake on each of the two days following saline were similar, and the pattern across the light–dark cycle was typical, with enhanced intake early in the dark period. Following saline control, intake during the first meal opportunity in the light appeared elevated. The saline control patterns for the two low doses conditions were similar, as might be expected given that the conditions involved the same group of rats. The same was true for the control conditions for the two higher dose conditions. On both days following saline control, compared to the low doses group, the high doses group had more intake during the first dark-period meal opportunity, $F(1,14) = 6.84$, $p = .0204$, and $t(14) = 2.59$ and 2.97, $p = .0215$ and .0102, respectively. In summary, the control data

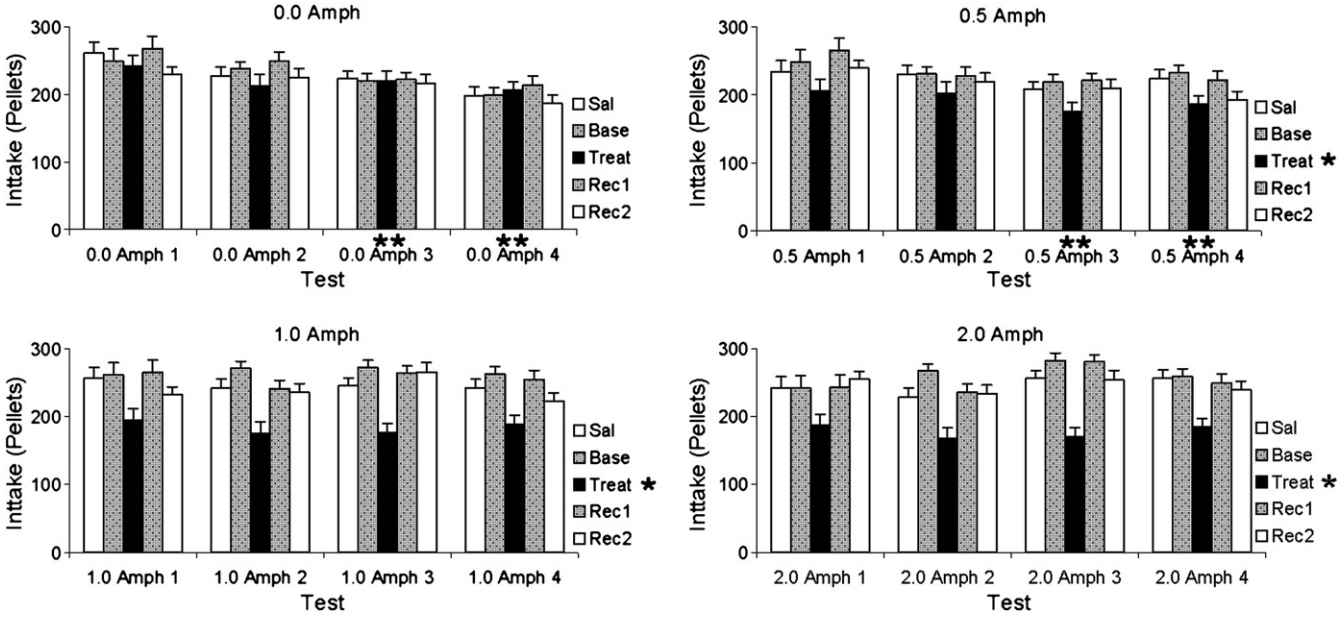

Fig. 1. Mean number of pellets consumed on each day of each test for each dose. "Sal" is the day of saline administration and "Base" is the day after. "Treat" is the day of amphetamine treatment, and "Rec1" and "Rec2" are the first and second recovery days afterwards. "0.0 Amph" is saline treatment. The animals in the low doses condition (upper panels) received alternating treatments of 0.0 and 0.5 mg/kg amphetamine. The animals in the higher doses condition (lower panels) received alternating treatments of 1.0 and 2.0 mg/kg amphetamine. Error bars are standard errors. The * symbol denotes a difference from all other test days. The ** symbol denotes a difference from test Amph 1. All ps<.05.

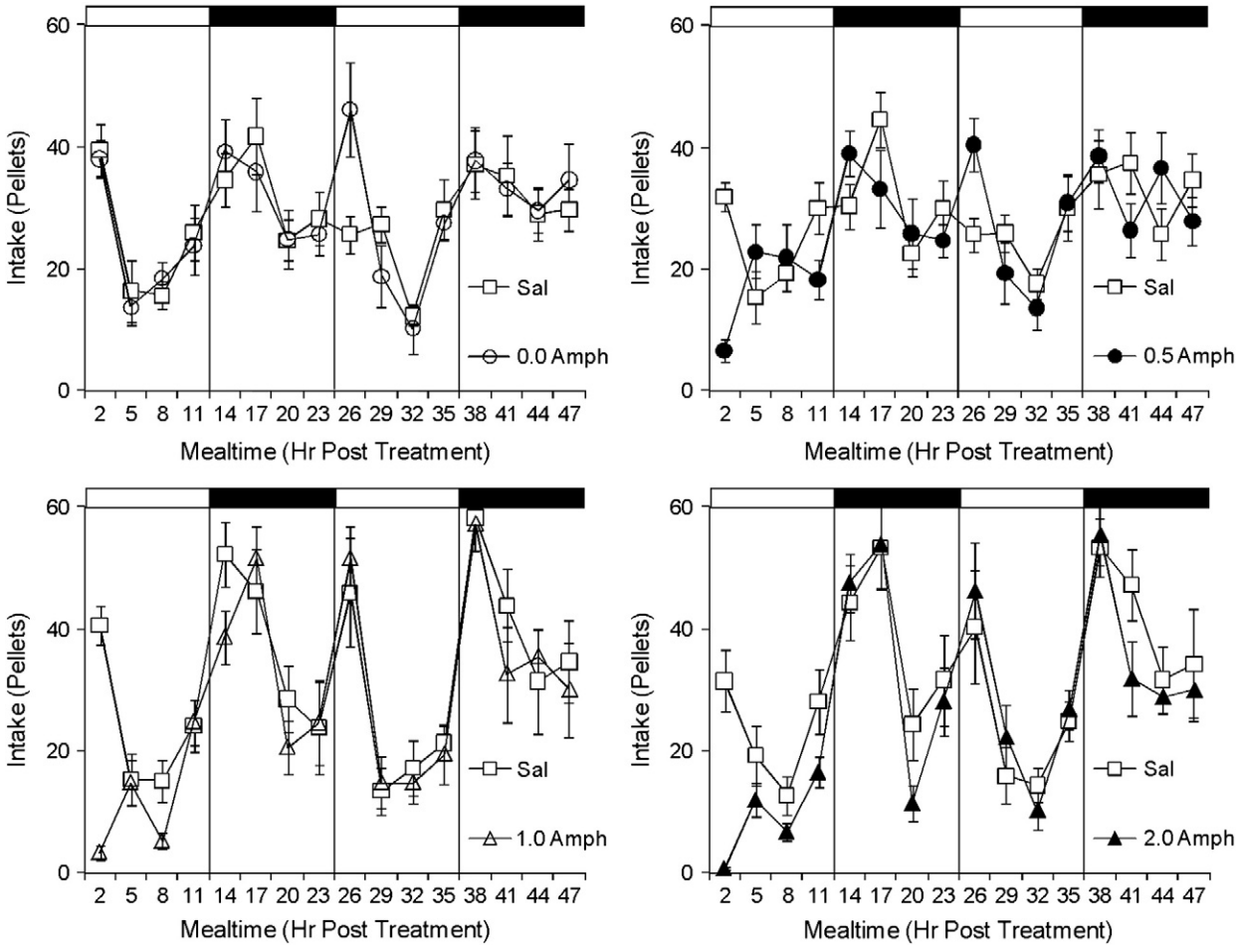

Fig. 2. For each dose condition, the mean number of pellets consumed at each meal opportunity during the two-day period following saline ("Sal") and amphetamine ("Amph"). Each function is an average of the four tests for a dose condition. The bar across the top of a graph indicates when lights were on or off. Error bars are standard errors.

provided a reliable baseline against which to compare the effects of amphetamine administration.

All amphetamine doses (0.5, 1.0, and 2.0 mg/kg) abolished food intake during the first meal opportunity following treatment (hour 2 post-treatment), and doses of 1.0 and 2.0 mg/kg also reduced food intake at other mealtimes, especially during the first post-treatment day (Fig. 2).

In order to make the longer-term effects of amphetamine treatment more evident, for each of the dose conditions shown in Fig. 2, the differences in intake following saline control and amphetamine were cumulated and are shown, in Fig. 3, for the first twelve meal opportunities following treatment. The 0.0 dose did not alter cumulative intake relative to saline. 0.5 mg/kg amphetamine reduced food intake at the first meal opportunity post-treatment, $t(7) = 13.71$, $p < .0001$, but produced no change in cumulative intake subsequently, $F(11,77) = 1.87$, $p = 0.056$. Doses of 1.0 and 2.0 mg/kg amphetamine also reduced intake at meal opportunity 1, $t(7) = 9.59$ and 5.82, $p < .0001$ and $p = .0006$, respectively. In addition, both produced a progressive decrease in cumulative intake, $F(11,77) = 3.60$ and 5.43, $p = .0004$ and $p < .0001$, respectively. Fisher's PLSD post hoc test indicated that for the 1.0 dose, the cumulative decrease in intake continued through mealtime 20 post-treatment and for the 2.0 dose it continued through mealtime 23, $p < .05$. For 0.5, 1.0, and 2.0 doses, all means were less than 0, $t(7) = 3.28$ to 18.98, $p = .0134$ to $< .0001$. At mealtime 23 the cumulative decrease was greater following 0.5, 1.0, and 2.0 doses than following the 0.0 dose, $t(7) = 3.30$, $p = .0132$, $t(14) = 7.84$, $p < .0001$, $t(14) = 5.22$, $p < .0001$, respectively, and the cumulative decrease was greater following the 1.0 and 2.0 doses than following the 0.5 dose, $t(14) = 6.01$ and 3.45, $p < .0001$ and $= .0039$, respectively. Summarizing, the 0.0 dose produced no change in intake, the 0.5 dose produced a short-term change, and 1.0 and 2.0 also produced similar progressive reductions in longer-term food intake.

3.3. Testing: 1.0 amphetamine condition

The results of tests 1 and 2 were averaged, as were the results of tests 3 and 4, 5 and 6, and 7 and 8. Fig. 4 shows the mean number of pellets consumed on each day for each test pair. Intake on the day of treatment with amphetamine was lower than intake on any other day,

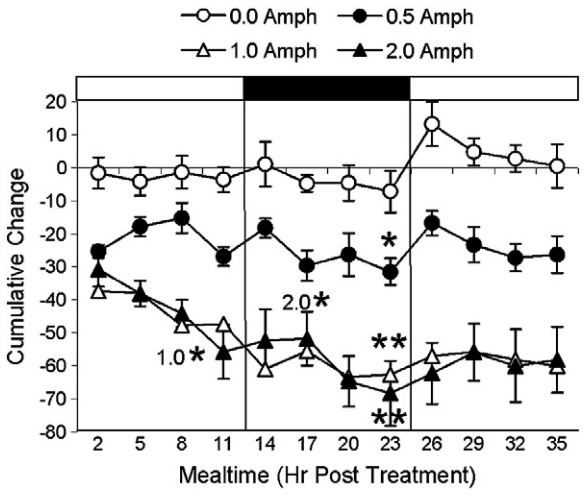

Fig. 3. Difference in cumulative pellet intake across the first twelve meal opportunities following saline and amphetamine. The results for each dose are an average of four tests. The bar across the top indicates when lights were on or off. Error bars are standard errors. At mealtime 23, the * symbol denotes a difference in intake relative to 0.0 Amph, and the ** symbol denotes a difference in intake relative to 0.0 and 0.5 Amph. For 1.0 Amph, only mealtimes 20 and 23 differed from mealtime 8 (denoted with 1.0*), indicating that mealtime 20 corresponded to a nadir in cumulative intake. For 2.0 Amph, only mealtime 23 differed from mealtime 17 (denoted with 2.0*), indicating that mealtime 23 corresponded to a nadir in cumulative intake. All ps < .05.

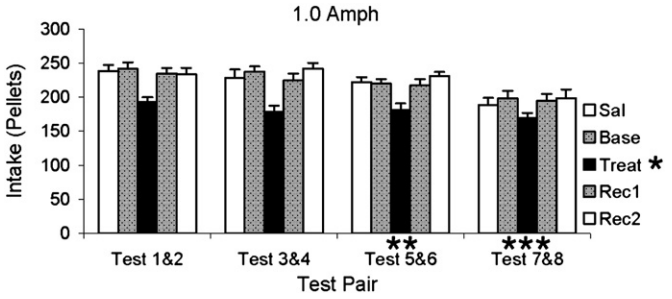

Fig. 4. Mean number of pellets consumed on each day for each successive pair of tests. 1.0 mg/kg amphetamine was the treatment for all eight tests. Error bars are standard errors. The * symbol denotes a difference from all other test days. The ** symbol denotes a difference from tests 1 and 2, and the *** symbol denotes a difference from all other tests. All ps < .05.

$F(4,28) = 29.92$, $p < .0001$ and Fisher's PLSD, ps < .05. Also, intake decreased across test pairs, $F(3,21) = 22.48$, $p < .0001$. Specifically, intake during test pair 7 and 8 was lower than intake during any other test pair, and intake during test pair 5 and 6 was lower than intake during test pairs 1 and 2, Fisher's PLSD, ps < .05. In summary, 1.0 amphetamine reduced food intake on the day of administration, and intake decreased across tests. A similar decrease was seen in the low doses condition, but not in the high doses condition, when 1.0 and 2.0 treatments alternated.

Fig. 5 shows, for each test pair, mean intake at each meal opportunity for 2 days following saline control and amphetamine. Following saline control, the pattern of intake for a particular test pair was similar on day 1 and day 2, and patterns seemed typical for rats, with intake peaking in the early dark period. Though patterns were similar across test pair, some differences were seen. Intake at meal opportunity 1 post-saline was higher for test pairs 3 and 4 and 5 and 6 than for test pairs 1 and 2 and 7 and 8, $t(7) = 3.33$ to 4.90, $p = .0088$ to .0018. Only one difference was seen on both days: Intake was higher at the first meal in the dark during test pairs 3 and 4 than during test pair 7 and 8. To summarize, control data were sufficiently consistent across tests and appeared to be a good baseline against which to compare the effects of amphetamine administration.

Fig. 6 shows, for each test pair, the cumulative difference in intake following amphetamine relative to saline. Amphetamine eliminated intake at meal opportunity 1 post-treatment (hour 2). For each test pair a cumulative reduction in intake was observed through mealtime 23 post-amphetamine, $F(11,77) = 2.16$ to 12.96, $p = .025$ to $< .0001$, and Fisher's PLSD, ps < .05. At mealtime 23 post-treatment, cumulative intake was less attenuated during test pair 7 and 8 than during any other test pairs. The results of tests 7 and 8 were very similar (data not shown). Summarizing, longer-term hypophagia produced by 1.0 amphetamine showed a tolerance-like effect within eight intermittent administrations. Evidence for this kind of tolerance was not seen in the high doses group when treatments with 1.0 and 2.0 were alternated.

4. Discussion

4.1. Summary of results

Doses of 0.5, 1.0, and 2.0 mg/kg amphetamine decreased intake on the day of administration. Overall intake decreased across tests for 0.0 and 0.5 doses and for the 1.0 dose when it was administered by itself. Overall intake did not decrease across tests when 1.0 and 2.0 were alternated in the high doses condition. On control days, the pattern of intake across the light–dark cycle for a condition was consistent from test to test and typical for the rat. 0.0 mg/kg (saline) did not change the pattern of food intake relative to control. 0.5 mg/kg reduced intake at the first meal opportunity, but did not alter intake subsequently,

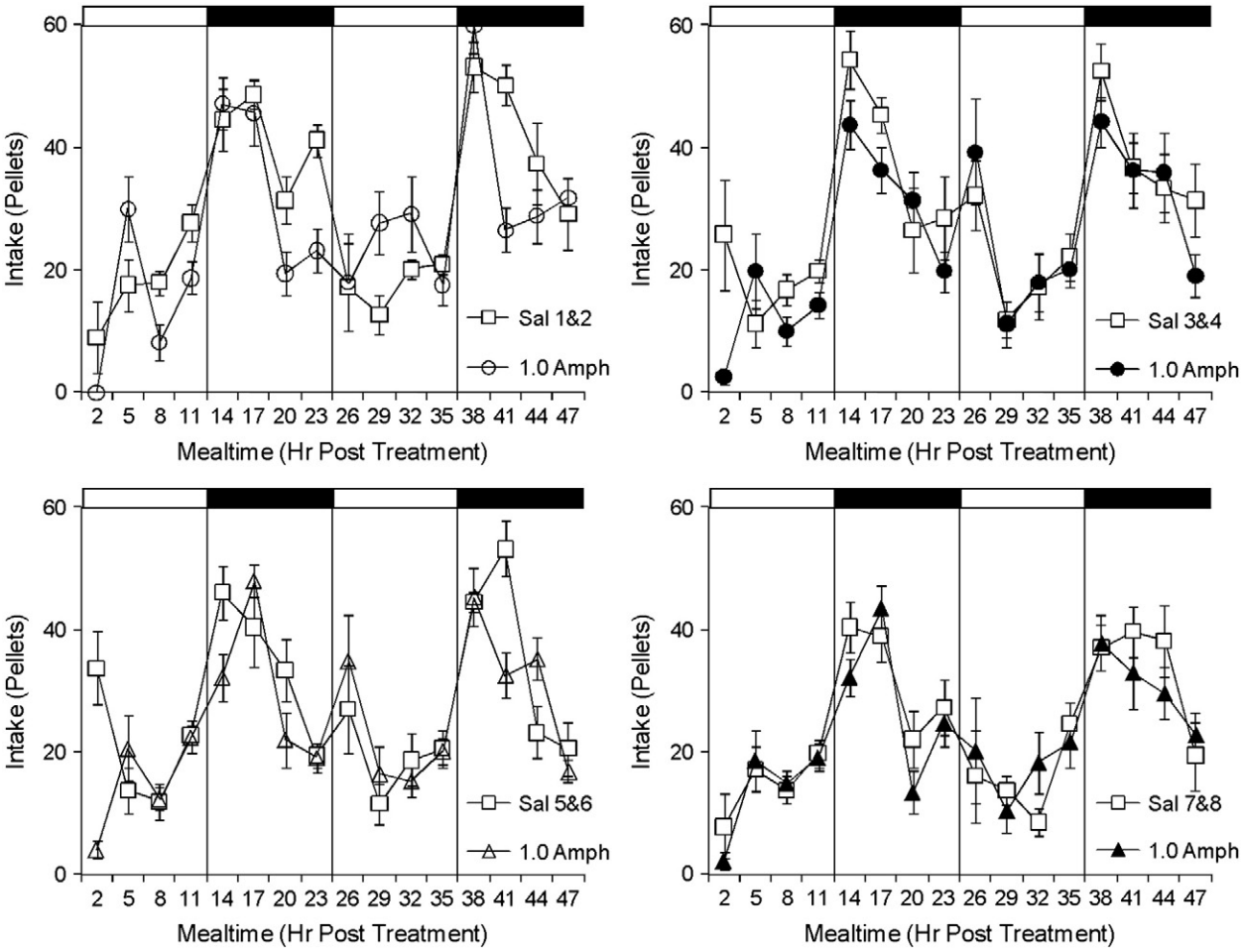

Fig. 5. Mean number of pellets consumed at each meal opportunity during the two-day period following saline control and amphetamine. The results were averaged for successive pairs of tests. The bar across the top of a graph indicates when light were on or off.

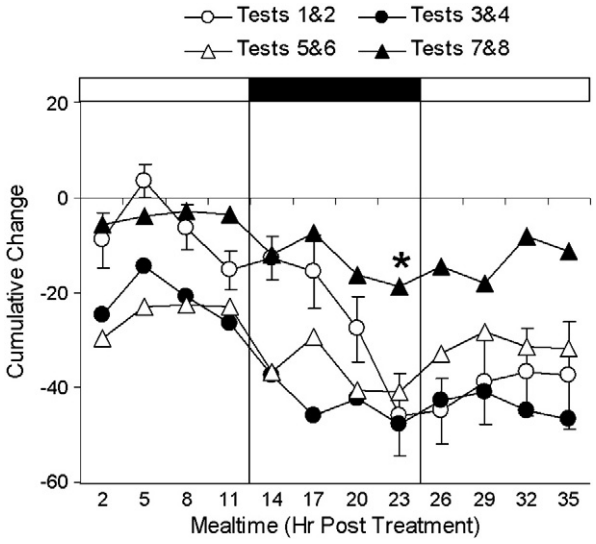

Fig. 6. Difference in cumulative pellet intake across the first twelve meal opportunities following saline and treatment with 1.0 mg/kg amphetamine. The bar across the top indicates when lights were on or off. Standard errors are shown only for the first test pair to make the figure more readable. Standard errors for the other functions were comparable. At mealtime 23, the * symbol denotes a difference in intake during tests 7 and 8 relative to all other tests. All ps < .05.

that is, 0.5 produced short-term hypophagia but not longer-term hypophagia. 1.0 and 2.0 mg/kg produced short-term hypophagia, with both doses abolishing intake at meal opportunity 1 of every test. Both doses also produced longer-term hypophagia. The time of occurrence of this longer-term hypophagia was similar for 1.0 and 2.0, with a minimum in cumulative intake occurring around 20 to 23 h post-amphetamine treatment. When 1.0 and 2.0 were alternated, longer-term hypophagia showed little tolerance, but when 1.0 was administered by itself, longer-term hypophagia showed considerable tolerance.

4.2. Baseline intake

Baseline intake decreased across tests in the low doses group, whereas it was maintained or increased in the high doses group. This pattern may seem counterintuitive at first, because under some circumstances higher doses produce more impairment of intake than lower doses. However, untreated male rats become less active with age and with chronic exposure to a context, and so they normally consume less across days. The low doses regime apparently did not markedly alter this pattern. On the other hand, 2.0 mg/kg amphetamine would be expected to produce more activity and metabolic expenditure than lower doses. It also produced longer-term hypophagia that did not evidence tolerance. Maintained or increased baseline intake across tests in the high doses group may have been a compensatory response to these challenges to body weight maintenance. All groups of subjects showed equivalent levels of continuous weight gain throughout the study, though they probably did so by

playing off intake, activity, and metabolic rate in different ways. The wide spacing of administrations probably contributed to the relationship between doses and baseline intake observed. These interpretations are tentative, and results should be replicated using alternative designs.

4.3. Elicitation of longer-term hypophagia

Longer-term hypophagia was observed following doses of 1.0 mg/kg or higher, doses that would be considered moderate or higher. Longer-term hypophagia may depend on substantial short-term amphetamine "intoxication."

A shift in hunger or in body weight set point could be involved in longer-term hypophagia. Such a shift would account for the fact that following amphetamine administration food intake progressively declined on day 1 following treatment and then tended to normalize without compensation on day 2 (see Figs. 3 and 6 in this study and White et al., 2007).

Certain processes thought to contribute to the reduction in food intake that occurs shortly after treatment with amphetamine were unlikely to have been involved in longer-term hypophagia. Little appetitive behavior was required to obtain food, and animals had ample time at each meal opportunity to procure food. Consummatory behavior, as indicated by the mean time required to consume six-pellet packets is not altered by the longer-term hypophagia produced by 2.0 mg/kg amphetamine (White et al., 2007). Consequently, longer-term hypophagia cannot be ascribed to gross impairments in appetitive and consummatory behavior. Longer-term hypophagia is also not due to the elicitation of stereotyped behaviors that interfere with food intake, because these behaviors are not present at the time of the hypophagia. Generally, explanations that depend upon the presence of high concentrations of amphetamine in the body cannot account for longer-term hypophagia because amphetamine has a half-life of 1–3 h in rats (Fuller et al., 1977; Hutchaleelaha et al., 1994), and negligible amounts of drug would be in the body by the time of the hypophagia's occurrence. Longer-term hypophagia is not complicated by the various activating effects of amphetamine, so in this respect it may be a more tractable phenomenon for the study of amphetamine-induced hypophagia than short-term hypophagia.

4.4. 1.0 Amphetamine and tolerance

When 1.0 mg/kg was given repeatedly by itself, longer-term hypophagia showed tolerance. This tolerance could represent a counter-regulatory response to a shift in hunger or in body weight set point initially produced by amphetamine. Though not necessarily a mutually exclusive explanation, conditioned compensatory responses have also been used to account for tolerance (Caul et al., 1988; Poulos et al., 1981; Wolgin, 1983). In the present study, the best predictors of drug receipt were the stimuli arising from administration, and these would be considered moderate predictors.

Tolerance has both benefits and costs. On the positive side, tolerance indicates that an animal is adjusting to deleterious effects of drug. In the case of the present study, tolerance of longer-term hypophagia enabled animals to maintain food intake. On the negative side, tolerance is achieved through changes in processes that may have wide untoward impact. For example, tolerance may indicate that the capacity to experience reward has been diminished (Kitanaka et al., 2008). 1.0 mg/kg amphetamine is about the optimal dose for producing positive hedonic effects in rats, as suggested by procedures such as conditioned place preference (Bardo et al., 1995). A dose such as 1.0 mg/kg amphetamine may entail a particular risk potential, because it is high in "liking" yet produces regulatory changes after a rather small number of widely spaced administrations.

Tolerance of longer-term hypophagia could be due to changes in as many as three factors: Factors that are activated in the short-term and that initiate the cascade of events resulting in longer-term hypophagia, factors that are involved in the proximate expression of longer-term hypophagia, and intermediary factors that link those involved in initiation and expression.

Kuo and colleagues (Chen et al., 2001; Hsieh et al., 2005; Kuo, 2003) have done a series of studies evaluating the contribution of D1 and D2 receptors and of hypothalamic NPY to amphetamine anorexia and anorexia tolerance. The ability of amphetamine to reduce 24-hour food intake appeared to be blocked by pretreatment with either a D1 or D2 antagonist. The tolerance which developed to amphetamine anorexia also depended on both D1 and D2 receptors (Chen et al., 2001; Kuo, 2003). Amphetamine may produce anorexia and anorexia tolerance via an NPY intermediary (Hsieh et al., 2005; Kuo, 2003). In the present study, dopamine receptor stimulation was probably involved in initiating events that produced longer-term hypophagia. NPY could conceivably have been an intermediary: When rats were given 5.0 mg/kg methamphetamine, serum NPY was reduced for the next 48 h (Kobeissy et al., 2008). Such a reduction could underlie the tendency, described above, for food intake to normalize without compensation following amphetamine treatment.

The diminution in longer-term hypophagia during tests 7 and 8 may have been due, at least in part, to the progressive decline in baseline intake, rather than to a change in the capacity of amphetamine to elicit longer-term hypophagia (tolerance). Additional research is required to assess the contribution of these alternative possibilities to the diminution.

4.5. 2.0 Amphetamine and absence of tolerance

Significant tolerance was not seen to the longer-term hypophagia that was produced by 2.0 mg/kg amphetamine. This replicated a result obtained in a prior study (White et al., 2007). Perhaps this dose posed a regulatory challenge that would have been counteracted only after more administrations. Tolerance was also not seen to the 1.0 dose when it was administered in alternation with the 2.0 dose. The result was reminiscent of sensitization, which can be reflected in an increased response to a lower dose of drug as a consequence of exposure to a higher dose (Robinson and Becker, 1986). Longer-term hypophagia in response to 1.0 mg/kg amphetamine appeared greater when 1.0 was administered in combination with 2.0 (Fig. 3) than when 1.0 was administered alone (Fig. 6), a result that was consistent with sensitization.

In this study, tolerance developed in a group given only 1.0 mg/kg amphetamine, but not in a group that received alternating administrations of 1.0 and 2.0. This outcome may initially seem surprising, because when administrations are closely spaced, a higher dose can produce tolerance more rapidly than a lower dose. Differences in the drug administration interval may account for the different results. Compared to lower doses, higher doses appear to produce larger long-term impairments of behavioral mechanisms, which may produce greater attenuation of the effects of subsequent administrations. Consequently, if administrations were closely spaced, at a 24-hour interval for instance, higher doses would tend to produce more rapid tolerance than lower doses. On the other hand, administration at five-day intervals would allow mechanisms to recover prior to the next administration. Behavioral results obtained would reflect the eliciting capacity of the dose, less contaminated by the carryover effects of prior administrations. Given this, repeated administration of a bigger dose may have larger and more persisting acute impairing effects. Though 2.0 mg/kg amphetamine given at widely spaced intervals may not readily produce chronic dysregulation, it retains the capacity to produce substantial transient impairment. This property may make 2.0 mg/kg well suited for investigating the determinants of amphetamine-induced longer-term deficits.

4.6. Dissociation of short-term and longer-term hypophagia

Short-term hypophagia could be elicited by 0.5 mg/kg amphetamine, whereas longer-term hypophagia required a dose of 1.0 mg/kg. When 1.0 was given alone, short-term hypophagia, as indicated by food consumed at meal opportunity 1, persisted throughout testing, whereas longer-term hypophagia was considerably attenuated. Short- and longer-term hypophagia appear to have different threshold eliciting doses, and they appear to undergo different rates of tolerance, suggesting that their determinants differ.

4.7. Dose dependence of longer-term deficits and implications for symptom organization and treatment

Amphetamine produces several abnormalities 18 to 24 h post administration, including sensations resembling those produced by Haloperidol (Barrett et al., 1992), reduced food intake (White et al., 2007; this study), and reduced activity (White and White, 2006). It is not known how these "symptoms" are interrelated, for example, whether they are due to separable mechanisms or a common mechanism, or whether they are hierarchically related.

Using a standardized set of circumstances to examine how different symptoms are affected by a similar manipulation, such as a variation of eliciting dose, can suggest how the symptoms are interrelated: Symptoms that change in parallel in response to variations of dose are more likely to be mediated by a common mechanism and/or to be related hierarchically than to be mediated by separable mechanisms. A few observations suggest that longer-term Haloperidol-like sensations, hypophagia, and hypoactivity are due to a common mechanism or are hierarchically related: All are elicited by amphetamine doses on the order of 1.0 to 2.0 mg/kg; the times post-treatment at which the symptoms reach their nadirs are similar; and these times seem to be rather independent of dose once a threshold dose is employed (Caul et al., 1997; White and White, 2006; Figs. 3 and 6 of the present study; White et al., 2007). Unfortunately, these or related symptoms have not been investigated under a standardized set of circumstances, so generalizations about dose–response and tolerance/sensitization effects are difficult to make, and symptom organization remains uncertain.

How symptoms are interrelated has important implications for treatment. If symptoms were due to a common mechanism, then the syndrome could be managed by targeting that mechanism. If the symptoms were hierarchically related—if, for example, recuperative processes (Maier and Watkins, 1998), as reflected by Haloperidol-like sensations, inhibited hunger, and this reduced activity—then treatment targeted at the super-ordinate symptom might resolve subordinate symptoms as well.

The methodology used here may serve as the basis for a standardized procedure that can be used to: Investigate the impact of similar manipulations on different symptoms; disclose symptom organization; and guide treatment options.

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