Published in final edited form as:

Physiol Behav. 2015 November 1; 151: 345–354. doi:10.1016/j.physbeh.2015.08.013.

Acute withdrawal-related hypophagia elicited by amphetamine is attenuated by pretreatment with selective dopamine D1 or D2 receptor antagonists in rats

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Abstract

After receiving 2.0 mg/kg amphetamine, rats show two phases of reduced food intake, short-term hypophagia, during the first several hours after treatment, and longer-term hypophagia, approximately 19 to 26 hours after treatment. The longer-term hypophagia may be an indicator of an acute withdrawal. This study assessed whether D1 and D2 receptor activation were important early events in the elicitation of longer-term hypophagia. Throughout a series of five-day tests, rats could lever press for food pellets for one-hour periods beginning every three hours. On test day 1, rats were given a saline pretreatment, and fifteen minutes later they were given a saline treatment. On test day 3, they were given a pretreatment of either saline or a selective dopamine receptor antagonist, and fifteen minutes later they were given a treatment of either saline or amphetamine (2.0 mg/kg). In Experiment 1, pretreatments included 3, 12, 31, and 50 μ g/kg of the selective D1 receptor antagonist SCH 23390. In Experiment 2, pretreatments included 25, 50, and 100 μ g/kg of the selective D2 receptor antagonist eticlopride. Distance moved was monitored for the first six hours following pretreatment-treatment combinations to obtain an indirect behavioral measure of receptor blockade (antagonist attenuation of amphetamine hyperactivity). Food intake at each meal opportunity was monitored throughout each five day test. Patterns of food intake following day 1 saline-saline and day 3 pretreatment-treatment were compared. The combination saline-amphetamine produced short-term and longer-term hypophagia. Combinations involving antagonist-saline did not produce longer-term changes in food intake. Pretreatment with 12 to 50 μ g/kg of SCH 23390 produced substantial blockade of amphetamine hyperactivity and prevented amphetamine-induced acute-withdrawal-related longer-term hypophagia. Eticlopride produced a partial blockade of longer-term hypophagia. Both D1 and D2 receptor activation are required for full expression of longer-term hypophagia following amphetamine administration.

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Keywords

Amphetamine; Withdrawal; Food intake; SCH 23390; Eticlopride

1. Introduction

When rats are administered 2.0 mg/kg amphetamine at intervals of several or more days, they show two phases of reduced food intake (hypophagia) during the 24 hours, approximately, after each treatment. The first phase (short-term hypophagia) occurs during the first several hours after drug treatment and is one aspect of the psychomotor stimulant state. The second phase (longer-term hypophagia) tends to be most prominent between, roughly, hours 19 to 26 post-treatment (White et al., 2010; White et al., 2007). Other motivational and affective impairments are observed during the same time period (Barrett et al., 1992; White et al., 2004; White and White, 2006), and so longer-term hypophagia may be an aspect of an acute withdrawal or “hangover” syndrome. Because the regime described above involves intermittently administering a moderately-high but nontoxic dose, it mimics some features of recreational drug use. A similar regime was employed in the experiments described below.

In the present study, time-dependent effects will be described in terms of two post-treatment intervals, short-term and longer-term intervals. The intervals are distinguished on the basis of the effects observed in prior studies following 2.0 mg/kg amphetamine (White et al., 2004; White et al., 2010; White et al., 2007; White and White, 2006). The short-term interval includes hours 1–6 post-treatment. The interval is distinguished by biphasic locomotion that peaks above baseline and then returns to baseline. Effects occurring sometime during this interval will be referred to as “short-term effects.” One such effect is short-term hypophagia, which occurs during the first several hours of the short-term interval. The longer-term interval includes hours 7–26 post-treatment. Effects occurring sometime during this interval will be referred to as “longer-term effects.” Longer-term hypophagia is a longer-term effect that occurs near the end of the longer-term interval.

Amphetamine affects several major neurotransmitters in the brain, including dopamine, norepinephrine, and serotonin. Amphetamine is an indirect agonist of dopamine: Administration of amphetamine results in increased concentrations of dopamine in the synaptic cleft. Dopamine acts at two classes of receptors, the D1-like receptor (D1 and D5) and the D2-like receptor (D2, D3, and D4). The dopaminergic system consists of major pathways that originate in the midbrain and that project to areas including the prefrontal cortex, the dorsal striatum, the nucleus accumbens, and the hypothalamus. Dopaminergic pathways are the primary sites of amphetamine’s actions (Feldman et al., 1997). Many of amphetamine’s major short-term effects, including hyperactivity, hypophagia, and reward-related phenomena, depend upon activation of both D1 and D2 receptors (Waddington, 1993).

Longer-term hypophagia occurs many hours after amphetamine administration, and the phenomenon may have at least two sets of determinants linked by a cascade. The first set of determinants would begin to act immediately after drug receipt and would initiate the

cascade. The second set of determinants would be involved in the proximate expression of longer-term hypophagia. In this research, we assessed the involvement of D1 and D2 receptor activation in initiating the cascade that results in amphetamine-induced longer-term hypophagia. Rats were pretreated with different doses of D1 (Experiment 1) or D2 (Experiment 2) receptor antagonist 15-minutes prior to treatment with 2.0 mg/kg amphetamine. The impacts of these pretreatments on longer-term hypophagia were observed. If D1 antagonist pretreatment were to prevent longer-term hypophagia, then this would suggest that D1 receptor stimulation by amphetamine was involved in initiating the cascade that resulted in longer-term hypophagia. The same was true for D2 antagonist pretreatment. If both D1 and D2 antagonist pretreatment were to prevent longer-term hypophagia, then this would further suggest that a receptor interaction was involved.

Some studies have examined the impact of dopamine antagonists on food intake. Results indicate that D1 and D2 antagonists do not reduce food intake during the longer-term interval, though higher doses can reduce intake during the short-term interval (Clifton et al., 1991; Hobbs et al., 1994; Rusk and Cooper, 1994; Terry and Katz, 1994). Zigmond et al. (1980) focused on intake shortly after treatment with a dopamine antagonist in combination with amphetamine. They suggested that an optimal level of dopaminergic activity mediated feeding, and that increases and decreases from this optimum following amphetamine, dopamine agonists, dopamine antagonists, and some combinations of dopamine antagonists and amphetamine may disrupt feeding. Very few studies have examined the impact of combinations of dopamine antagonists and amphetamine on feeding over longer intervals. Chen et al. (2001) found that pre-treatment with a D1 or D2 antagonist blocked amphetamine-induced reductions in 24-hour food intake. Because intake was measured at only one time point (24 hours post treatment), whether antagonist pretreatment prevented short-term hypophagia, longer-term hypophagia, or both is unclear. To decide the issue measurements must be made at multiple time points during the 24 hour interval after treatment, particularly at those times that could reflect short- and longer-term hypophagia.

The present study employed a procedure that measured short- and longer-term effects (White et al., 2010; White et al., 2007). Throughout a series of five-day tests, rats could lever press for food pellets during one-hour intervals that began every 3 hours. Each five-day test began with a two-day re-baseline in the eventuality of baseline shifts due to prolonged housing in the apparatus, to aging, or to 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 due to amphetamine administration, which tend to be greatest 19 to 24 hours 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.

2. Materials and Methods

2.1. Animals

A total of sixteen adult male Wistar rats (Harlan, Indianapolis, IN) were used. The study consisted of two experiments, and each experiment included eight animals. Animals were housed in plastic tubs in a departmental colony having a 12-hr light/12-hr 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 chow (Purina 5001 Rodent Diet, Lab Diet; composition by calories: 30% protein, 13% fat, 57% carbohydrate). Initially, animals were housed in pairs, but a week before the start of their study they were housed individually. Just prior to the start of their study, animals were handled and were pre-exposed in their home cages to the pellets that they would consume during the study. Animals in Experiment 1 weighed between 430 and 520 g at the start of their study, and animals in Experiment 2 weighed between 490 and 600 g.

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-hr 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 × 20 cm × 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. The floor of the pan was covered with black grip tape and 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-hr light/12-hr dark cycle.

Devices in operant conditioning stations and in 24-hr stations were connected to an interface (Med Associates) and a computer. Software (Med Associates) was used to arrange contingencies and monitor lever presses and head-in bin responses.

In the ceiling of each compartment, and centered 50 cm above the floor of each cubicle, was a monochrome infrared camera (Cat eyes, PC184IR). The cameras were connected to a monochrome multiplexer (Robot-Duplex Digital Video Multiplexer, DMV16Q), which combined images from the cameras in each of the stations into one image for quantification. The multiplexer was connected to a monitor (36 cm Trinitron high resolution video monitor, ECM-1402H) and a computer. The computer (Dell, M782p) contained a piccolo frame grabber. An Etho Vision Pro 3.0 Video Tracking, Motion Analysis, and Behavior Recognition System collected and analyzed the data. Each animal was tracked within an area corresponding to the dimensions of the cubicle floor. The system was programmed to take two samples per second. The gray scaling method was used in object detection.

The eight stations were located in a well isolated temperature- and humidity-regulated room (approximately 1.8 m × 2.1 m × 2.6 m high).

2.3. Drugs

All drugs were obtained in powdered form from Sigma (St Louis) and were mixed in saline. The following drug concentrations were used: 2.0 mg/ml d-amphetamine base; 3, 12, 31, and 50 µg/ml SCH 23390 hydrochloride (D1 antagonist); and 25, 50, and 100 µg/ml eticlopride hydrochloride (D2 antagonist). Saline was used as the control treatment (1.0 ml/kg).

2.4. Procedure

The study consisted of two experiments that were run successively. Animals in each experiment were exposed to the same general procedure. Conditions included lever press training, free feeding in the animal colony, meal training, and testing. Table 1 shows the number of days of each condition.

2.4.1. Lever Press Training—Each animal was deprived to 90% 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; composition by calories: 21% protein, 14% fat, 65% carbohydrate). The animals were then placed on free food availability in the colony for five 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-hr light/12-hr dark cycle and had free access to water. Rats could lever press for food pellets for one-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 sec. 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. When mean ratios completed in a day did not change statistically for three consecutive days testing began.

2.4.3. Testing—The feeding schedule remained in effect. Animals received a series of tests. Generally, tests were five days in duration, though a few were six days (see below). A typical test is shown in Figure 1. At light onset of test day 1, each animal was given a control pretreatment-treatment combination: Each animal was administered saline (1.0 ml/kg), and 15 min later each animal was again administered saline. Two days later, at light onset of test day 3, each animal was given an experimental pretreatment-treatment combination. Pretreatments were either saline or a selective dopamine receptor antagonist, and they were followed 15 minutes later by treatments of saline or 2.0 mg/kg amphetamine. All treatments were given subcutaneously, under the loose skin on the back of the neck. Body weights were recorded at the time of pretreatment.

Each animal’s station was maintained at the time of pretreatment, 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 top-soil. 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.

2.4.3.1. Experiment 1. Dopamine D1 receptor antagonist pretreatment: Experiment 1 included 10 tests. Table 2 shows the experimental pretreatment-treatment combination that was given on the experimental treatment day (generally day 3) of each test. Feeding data for Test 1 Day 3 and for Test 8 Day 1 were lost due to a power failure and an experimenter error, respectively, and so results of those tests will not be shown. During Test 9, an extra baseline day occurred between the control treatment (Saline-Saline) on Day 1 and the experimental treatment (3 µg/kg SCH 23390-Amphetamine).

2.4.3.2. Experiment 2. Dopamine D2 receptor antagonist pretreatment: Experiment 2 included 8 tests. Table 3 shows the experimental pretreatment-treatment combination for each test. During Tests 4 and 7 an extra baseline day occurred between control and experimental treatments.

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. Distance moved was monitored for the first six hours after treatments on control and experimental days.

2.7. Data Analysis

Data were analyzed using repeated measures 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. Experiment 1: Dopamine D1 receptor antagonist pretreatment

Animals gained an average of 50.5 g from test 1 to test 10.

3.1.1. Short-term activity—The upper panel of Figure 2 shows total distance moved during the first six hours following each pretreatment-treatment combination. A one way repeated measures ANOVA compared total distance moved for each of the five combinations involving saline treatment. Activity following 50 SCH-SAL was higher than activity following any other pretreatment – saline combination, $F(7, 4) = 6.278$, $p = .001$, Fisher's PLSD, $ps < .05$. Activity following the other pretreatment – saline combinations did not differ. Another one way repeated measures ANOVA compared total distance moved for each of the five combinations involving AMPH treatment. As SCH dose increased, blockade of amphetamine-induced activity increased, up to a dose of 31 µg/kg, $F(7, 4) = 105.538$, $p =$

$<.0001$, Fisher's PLSD, $ps < .05$. A series of five paired t-tests compared total distance moved when a pretreatment was followed by SAL or by AMPH. Activity following 50 SCH-SAL and 50 SCH-AMPH did not differ, whereas for every other specific pretreatment, activity following saline and amphetamine did differ, $ts(7) = -21.390$ to -3.739 , $ps <.0001$ to $.0073$.

The middle and lower panels of Figure 2 show activity broken down into one-hour bins. The lower panel shows data for combinations involving D1 antagonist pretreatment and amphetamine treatment. SAL-SAL and SAL-AMPH are shown for reference. For each of the six hours, a one way repeated measures ANOVA compared distance moved following each of the six combinations. SAL-AMPH and 3 SCH-AMPH produced similar activity during hours 1 and 2: Otherwise, activity following SAL-AMPH was elevated relative to other conditions through hour 5. Furthermore, 3 SCH-AMPH and 12 SCH-AMPH produced similar activity during hour 1: Otherwise, activity following 3 SCH-AMPH was elevated relative to remaining conditions through hour 4. In addition, 12 SCH-AMPH was elevated relative to remaining conditions through hour 3. Finally, SAL-SAL, 50 SCH-AMPH, and 31 SCH-AMPH produced similar time courses of activity, except during hour 2, when SAL-SAL and 31 SCH-AMPH differed. For hours 1 to 5, $F_s(7, 4) = 6.184$ to 102.343 , $ps < .0003$ to $.0001$, Fisher's PLSDs, $ps < .05$.

Overall, Figure 2 indicates that pretreatment with 50 SCH and 31 SCH produced substantial blockade of amphetamine-induced activity, 12 SCH produced intermediate blockade, and 3 SCH produced negligible blockade. These degrees of blockade presumably reflected ordinal differences in the magnitude of D1 receptor occupancy.

3.1.2. Feeding—All tests were a minimum of five days long. Day 1 was a baseline during which animals were treated with saline. In order to evaluate stability of food intake, for each pair of successive tests Day 1 intakes were compared. On average, Day 1 intake during a subsequent test was 99% of Day 1 intake during a prior test. In other words, at the start of successive tests, intake was comparable: Recovery periods appeared to be long enough for intake to normalize from one test to the next (for discussions of within-test stability see White et al., 2010; White et al., 2007).

Feeding results will be shown in average cumulative change functions. Figure 3 shows how these functions were produced. The figure shows the results for one rat during Test 4, during which SAL-SAL was given on day 1 (control), and SAL-AMPH was given on day 3. The left panel shows pellet intake at the first nine meal opportunities following each combination. To produce the function in the right panel, the difference in intake was found at each meal opportunity, and then these differences were cumulated across meal opportunities. For each test, functions like these were produced for each animal and were then averaged. Displaying results as an average cumulative change from control has a variety of advantages: it removes much of the variation in behavior entrained by the light-dark cycle, better revealing the magnitude of the effects of drug; it condenses the data, yielding a single function with a relatively simple form that can be readily analyzed statistically; and it facilitates comparisons across conditions. Longer-term effects were identified by comparing the cumulative change in intake at meal opportunity 2, the end of

the short-term interval, to the cumulative change in intake at meal opportunities near the end of the longer-term interval. For example, a longer-term effect would be indicated if the cumulative reduction in intake were significantly greater at meal opportunity 8 than at meal opportunity 2.

Analysis will focus on meal opportunities 1 to 9, because the major effects occurred during this time. The graphs in Figure 4 show how pretreatment with different doses of D1 antagonist affected patterns of intake obtained after treatment with saline or amphetamine. Results of 31 SCH-SAL are not shown in the lower left panel because the data were lost. The effects of SAL-AMPH are shown on each graph for comparison.

The upper left panel shows results for tests involving pretreatment with 3 SCH. To assess whether SAL-AMPH and 3 SCH-AMPH produced different effects on cumulative change in intake, a two way repeated measures ANOVA was done, where one factor was treatment combination (two levels) and the other factor was meal opportunity (eight levels). The overall effects of these combinations did not differ, $F(1, 7) = .023$, $p = .8828$, and intake changed across meal opportunities, $F(8,56) = 7.699$, $p < .001$. For each combination, an additional one way repeated measures ANOVA was done to see how cumulative intake changed across meal opportunity. Each combination was followed by a reduction in intake from meal opportunity 2 through meal opportunity 8, $F_s(8,56) = 2.660$ and 11.761 , $p_s = .0151$ and $<.0001$ and Fisher's PLSD $< .05$, and the minima at meal opportunity 8 did not differ, $t(7) = 1.963$, $p = .0904$. Amphetamine produced longer-term hypophagia when pretreatment involved either saline or a very low dose of SCH 23390.

Higher doses of SCH produced similar effects, and the results for these doses were averaged and are shown in Figure 5. The results for 12 SCH-SAL and 50 SCH-SAL were averaged to produce the "12,50 SCH-SAL" function. The results for 12 SCH-AMPH, 31 SCH-AMPH, and 50 SCH-AMPH were averaged to produce the "12,31,50 SCH-AMPH" function. The SAL-AMPH function shows results of Test 4 and is the same function seen in Figure 4.

The functions were compared using a two way repeated measures ANOVA (three treatment conditions by eight meal opportunities). Overall intake depended on condition, $F(2,14) = 66.997$, $p < .001$, overall intake varied across meal opportunity, $F(8, 56) = 2.276$, $p = .0349$, and the pattern of intake depended on treatment condition, $F(16,112) = 2.838$, $p = .0007$. For each of the two conditions involving SCH pretreatments, an additional one way repeated measures ANOVA was done to see how cumulative intake changed across meal opportunity. The SCH-AMPH function was flat, $F(8,56) = 2.074$, $p = .0538$. Pretreatment with SCH 23390 doses of 12 to 50 $\mu\text{g/kg}$ blocked amphetamine-induced longer-term hypophagia. The SCH-SAL function varied across meal opportunity, $F(8,56) = 2.861$, $p = .0097$, but this was because SCH decreased intake at meal opportunity 1 and increased it at meal opportunity 9, PLSDs $> .05$. SCH 23390 doses from 12 to 50 $\mu\text{g/kg}$ did not produce persistent longer-term hypophagia in their own right. A series of one way repeated measures ANOVAs was done at each meal opportunity to assess whether treatment conditions produced differences in intake. Differences occurred at each meal opportunity, $F_s(2,14) = 13.136$ to 48.709 , $p_s = .0006$ to $<.0001$. At all meal opportunities, SAL-AMPH and SCH-AMPH produced a greater

reduction in cumulative intake than SCH-SAL, Fisher's PLSDs $< .05$. At meal opportunity 8, SAL-AMPH produced a lower level of cumulative intake than SCH-AMPH, PLSD $< .05$.

The trends seen in Figure 5 were also present in the data for the individual SCH doses shown in Figure 4. 12 SCH-AMPH, 31 SCH-AMPH, and 50 SCH-AMPH did not produce a change in intake from meal opportunities 1 to 9, $F_s(8,56) = .836 - 1.277$, $p_s = .5752 - .2743$.

3.2. Experiment 2: Dopamine D2 receptor antagonist pretreatment

Animals gained an average of 2.6 g from test 1 to test 8.

3.2.1. Short-term activity—The upper panel of Figure 6 shows total distance moved during the first six hours following each pretreatment-treatment combination. A one way repeated measures ANOVA compared total distance moved for each of the four combinations involving saline treatment. Pretreatment-SAL combinations did not produce different levels of activity, $F(7, 3) = 2.673$, $p = .0763$. Another one way repeated measures ANOVA compared total distance moved for each of the four combinations involving AMPH treatment. As eticlopride pretreatment dose increased, blockade of amphetamine-induced activity generally increased, $F(7, 3) = 64.051$, $p < .0001$, and Fisher's PLSDs $< .05$, though pretreatment with 25 ETI and 50 ETI produced similar blockade. A series of four paired t -tests compared total distance moved when a pretreatment was followed by SAL or by AMPH. Activity following 100 ETI-SAL and 100 ETI-AMPH did not differ, $t(7) = -1.542$, $p = .1669$, whereas for every other specific pretreatment, activity following amphetamine was greater than activity following saline, $t_s(7) = -8.590$ to -4.272 , $p_s = < .0001$ to $.0037$.

The middle and lower panels of Figure 6 show activity broken down into one-hour bins. The lower panel shows data for combinations involving D2 antagonist pretreatment and amphetamine treatment. SAL-SAL and SAL-AMPH are shown for reference. For each of the six hours, a one way repeated measures ANOVA compared distance moved following each of the five combinations. Activity following SAL-AMPH was elevated relative to other combinations, except during hour 2 when 50 ETI-AMPH and 25 ETI-AMPH were similar, and hour 3 when 50 ETI-AMPH was similar. 25 ETI-AMPH and 50 ETI-AMPH had similar time courses, except during hour 1 when 50 ETI-AMPH was lower, and hour 2 when 50 ETI-AMPH was higher. 25 ETI-AMPH and 50 ETI-AMPH were followed by more activity than both 100 ETI-AMPH at hours 1–4 and SAL-SAL at hours 2–4. SAL-SAL and 100 ETI-AMPH had similar effects except at hour 1, when activity following 100 ETI-AMPH was suppressed. For hours 1 to 6, $F_s(4, 7) = 4.727$ to 88.263 , $p_s < .0048$ to $.0001$, Fisher's PLSDs, $p_s < .05$.

Overall, Figure 6 indicates that pretreatment with 100 ETI produced substantial blockade of amphetamine-induced activity, and 25 ETI and 50 ETI produced intermediate blockade.

3.2.2. Feeding—In order to evaluate stability of food intake, the amount of food consumed on Day 1 of successive tests was compared. On average, Day 1 intake during a subsequent test was 97% of Day 1 intake during a prior test.

The upper left panel in Figure 7 shows the cumulative change in pellet intake, relative to SAL-SAL control, during tests 1 and 6. These tests involved giving SAL-AMPH as the day 3 pretreatment-treatment combination. A two way repeated measures ANOVA (two combinations by nine meal opportunities) indicated that similar effects were obtained during both tests. The combinations did not produce an overall difference in intake, $F(1, 7) = .713$, $p = .4262$, an overall effect of meal opportunity was obtained, $F(8, 56) = 18.576$, $p < .0001$, and a combination by meal opportunity interaction was not obtained, $F(8, 56) = 1.660$, $p = .1289$. Amphetamine produced similar effects early and late in the test sequence. Because effects were similar, the results of the two tests were averaged. The remaining panels of Figure 7 show how pretreatment with different doses of D2 antagonist affected patterns of intake obtained after treatment with saline or amphetamine. The average effects of the SAL-AMPH tests are shown on each graph for comparison.

Additional analyses focused on meal opportunities 1 to 9, because the major effects occurred during this time. Eticlopride doses produced similar effects, and the results for these doses were averaged and are shown in Figure 8. The results for 25 ETI-SAL, 50 ETI-SAL, and 100 ETI-SAL were averaged to produce the “25,50,100 ETI-SAL” function. The results for 25 ETI-AMPH, 50 ETI-AMPH, and 100 ETI-AMPH were averaged to produce the “25,50,100 ETI-AMPH” function. The SAL-AMPH function is the average of tests 1 and 6 and is the same function shown in upper right and lower panels of Figure 7.

The functions in Figure 8 were compared using a two way repeated measures ANOVA (three treatment conditions by nine meal opportunities). Overall intake depended on condition, $F(2, 14) = 33.490$, $p < .0001$, overall intake varied across meal opportunity, $F(8, 56) = 15.074$, $p < .0001$, and the pattern of intake depended on treatment condition, $F(16, 112) = 7.725$, $p < .0001$. For each treatment condition, an additional one way repeated measures ANOVA was done to see how cumulative intake changed across meal opportunity. The SAL-AMPH function progressively declined from meal opportunity 2 (post-injection hour 5), and the cumulative decline in intake reached a minimum at meal opportunity 9 (hour 26), $F(8, 56) = 18.576$, $p < .0001$, and Fisher's PLSDs, $ps < .05$. The ETI-AMPH function progressively declined from meal opportunity 2 through meal opportunity 8, $F(8, 56) = 7.507$, $p < .0001$, and Fisher's PLSDs, $p < .05$. The ETI-SAL function did not vary across meal opportunity, $F(8, 56) = 1.548$, $p = .1618$, indicating that eticlopride doses from 25 to 100 $\mu\text{g}/\text{kg}$ did not produce a progressive decline in longer-term intake in their own right. A series of one way repeated measures ANOVAs was done at each meal opportunity to assess whether treatment conditions produced differences in intake. ETI-AMPH was followed by higher levels of intake than SAL-AMPH at meal opportunities 7 and 9, and ETI-SAL was followed by higher levels of intake than ETI-AMPH and SAL-AMPH at all meal opportunities, $F_s(2, 14) = 4.349$ to 41.970 , $ps = .0340$ to $< .0001$, and Fisher's PLSDs, $ps < .05$. Taken together, results suggested that D2 antagonist partially blocked longer-term hypophagia.

The trends seen in Figure 8 were also present in the data for the individual ETI doses shown in Figure 7. Separate one way ANOVAs indicated a change in cumulative intake across meal opportunities for 50 ETI-AMPH, $F(8,56) = 4.309$, $p = .0004$, and 100 ETI-AMPH, $F(8,56) = 4.549$, $p = .0003$, and a trend for 25 ETI-AMPH, $F(8,56) = 1.968$, $p = .0690$. In all

three cases, a significant decline in cumulative intake occurred from meal opportunity 2 to meal opportunity 8, PLSDs, $p < .05$.

In both experiments, when rats pressed the lever to initiate pellet receipt, they virtually always immediately retrieved and consumed all six pellets in a package.

4. Discussion

4.1. Summary

Saline pretreatment followed 15 minutes later by 2.0 mg/kg amphetamine treatment (SAL-AMPH) produced hyperactivity during the first four to five hours following treatment. Saline-Saline (SAL-SAL) control and dopamine antagonist pretreatment followed by saline treatment generally produced comparable levels of activity during the first six hours following treatment. Generally, as the dose of D1 or D2 antagonist pretreatment increased, blockade of amphetamine-induced hyperactivity increased.

SAL-AMPH abolished food intake at meal opportunity 1 (short-term hypophagia), which occurred during the second hour of the psychomotor stimulant state, and the combination was also followed by longer-term hypophagia, a second phase of reduced intake that occurred, roughly, from hours 17 to 26 post-treatment. D1 and D2 antagonists followed by saline reduced intake only when higher antagonist doses were used, and no antagonist-saline combination produced a further decrease in food intake in the longer-term. Pretreatment with a very low dose of D1 antagonist (3 µg/kg SCH 23390) followed by amphetamine treatment, a combination that had produced negligible blockade of amphetamine hyperactivity, did not block longer-term hypophagia. However, pretreatment with higher D1 antagonist doses, which produced substantial blockade of amphetamine hyperactivity, appeared to block longer-term hypophagia. Though these higher doses blocked short-term amphetamine hyperactivity to different degrees, they appeared to produce similar blockade of longer-term hypophagia. All three of the D2 antagonist doses used as pretreatments produced substantial blockade of amphetamine hyperactivity, though the degree of blockade differed, and they appeared to partially block to a comparable degree longer-term hypophagia.

4.2. Saline - Amphetamine treatment and food intake

In both Experiments 1 and 2, SAL-AMPH reduced short-term intake and produced a further progressive decline in intake to around the end of day 1 post-treatment (longer-term hypophagia). The results were very similar to those previously obtained in studies involving treatment with 2.0 mg/kg amphetamine alone (White et al., 2010; White et al., 2007). The goal of this study was to assess involvement of D1 and D2 receptor stimulation in initiating the sequence of events resulting in amphetamine-induced longer-term hypophagia.

4.3. DA antagonist - Saline treatment and food intake

D1 and D2 antagonists followed by saline reduced intake only when higher antagonist doses were used, and no antagonist-saline combination produced a further decrease in food intake in the longer-term. When followed by saline treatment, 50 µg/kg of the D1 antagonist

SCH23390 and 50 and 100 µg/kg of the D2 antagonist eticlopride reduced intake at meal opportunity 1 (hour 2 post treatment). Intake recovered at the next meal opportunity, except when 100 µg/kg eticlopride was the pretreatment. In the case of this latter dose, animals did not appear to compensate for lost intake over the next two days. A similar absence of compensation has been observed following treatment with 2.0 mg/kg amphetamine (White et al., 2010; White et al., 2007). The similarity suggests that D2 receptor manipulations may play a role in the absence of compensation. This absence may have contributed to the reduced weight gain in the group treated with D2 antagonist relative to the group treated with D1 antagonist. Because the two groups differed in average starting weights, this suggestion is very tentative. Longer-term intake was neither decreased nor increased by any combination of D1 or D2 antagonist pretreatment and saline treatment. Prior research has indicated that D1 and D2 antagonists given alone tend to reduce intake shortly after administration but not later, so the results obtained in this study were consistent with prior research (Clifton et al., 1991; Hobbs et al., 1994; Rusk and Cooper, 1994; Terry and Katz, 1994). The absence of a longer-term decrease in intake suggests that D1 and D2 antagonists were not present in pharmacologically significant amounts during the longer-term interval. The absence of a longer-term increase in intake suggests that the antagonists did not produce a rebound in intake during this interval. Longer-term rebounds have been observed in other measures: Barrett et al. (1992), using a drug discrimination procedure, observed a longer-term amphetamine cue state following treatment with a high dose of the dopamine antagonist haloperidol. For the purposes of this study, antagonist-saline combinations were important controls which showed that D1 and D2 antagonists did not produce significant longer-term effects in their own right. Instead, these controls suggested that longer-term hypophagia was not observed following antagonist-amphetamine combinations, because antagonists blocked some short-term effect of amphetamine administration.

4.4. DA antagonist - Amphetamine treatment and short-term hypophagia

All combinations of D1 or D2 antagonist pretreatment and amphetamine treatment impaired short-term food intake, an expected outcome. Normal food intake depends on an interaction of D1 and D2 receptors (Waddington, 1993) and an optimal level of D1 and D2 receptor stimulation (Zigmond et al., 1980). No combination of D1 or D2 antagonist and 2.0 mg/kg amphetamine would be expected to result in normal short-term intake, because any combination would result in over- or under-stimulation of receptors for purposes of normal feeding.

4.5. DA antagonist - Amphetamine treatment and longer-term hypophagia

Pretreatment with the three highest doses of SCH 23390 (12, 31, and 50 µg/kg) prevented amphetamine treatment from producing a progressive decline in intake from around meal opportunity 5 (hour 14 post treatment). In other words, D1 antagonist pretreatments blocked amphetamine-induced longer-term hypophagia. The three dose of the D2 antagonist eticlopride (25, 50, and 100 µg/kg) appeared to partially block the hypophagia. Amphetamine impacts several major neurotransmitters including dopamine, norepinephrine, and serotonin. The results of this study suggested that stimulation of dopamine receptors in particular was an important early event in the sequence necessary to produce longer-term hypophagia. Pretreatment with either a D1 antagonist or a D2 antagonist affected longer-

term hypophagia, suggesting that an interaction of these receptor subtypes may have initiated the cascade that resulted in longer-term hypophagia.

Chen et al. (2001) was one of the very few studies to examine the impact of pretreatment with D1 and D2 receptor antagonists on amphetamine-induced changes in 24-hour food intake. In their study, rats were pre-treated with SCH23390 (0.8 mg/kg) or pimozone (0.4 mg/kg), followed 30 minutes later by amphetamine (2.0 mg/kg), and 24 hours later the amount of freely available chow consumed since treatment was measured. Each group was studied for only a single day. The amount of chow consumed by these groups was greater than the amount of chow consumed by a group treated only with amphetamine, but it was comparable to the amount consumed by a control group. In our study, in contrast, when animals were treated with D1 or D2 antagonist-amphetamine, by the end of day 1 they consumed more pellets than they had in the amphetamine-only condition, but fewer than they had in the control condition. We believe this outcome occurred because antagonist pretreatment blocked the longer-term reduction in food intake, but did not block the short-term reduction, and did not enable animals to make up lost intake. Our study and the study by Chen et al. had several methodological differences, and identifying the factors that produced the discrepant results is difficult. The different results obtained with D2 antagonist may have been due to the different antagonists used. The different results obtained with the D1 antagonist SCH23390 may have been related to the doses used. The dose used by Chen et al. (0.8 mg/kg) was sixteen times higher than the highest dose we used (0.05 mg/kg). Such a dose would almost certainly reduce short-term intake, whether given by itself or given as a pretreatment prior to 2.0 mg/kg amphetamine. For 24-hour intake to be comparable to controls, the high SCH23390 dose used by Chen et al. may have produced a longer-term positive rebound in feeding. To evaluate these possibilities, intake would have to be measured at multiple times of day. Compared to the Chen et al. study, our study showed that blocking effects can be obtained with a much lower range of doses; that forms of blocking different from those obtained by Chen et al. appear to occur; that blocking effects can be obtained not only acutely, but can be replicated within subjects; and that measuring food intake at multiple times can disclose time-dependent phenomena that are missed when intake is measured only at the end of a 24 hour period (White et al., 2010; White et al., 2007).

Different D1 and D2 antagonist doses produced similar degrees of blockade of longer-term hypophagia, even though the doses varied across a wide range and produced differing (though substantial) degrees of attenuation of amphetamine hyperactivity. Apparently, longer-term hypophagia requires substantial threshold levels of D1 and D2 receptor activation, below which it does not occur.

4.6. Unexpected outcomes and study limitations

50 µg/kg eticlopride produced less blockade of amphetamine-elicited hyperactivity than 25 µg/kg eticlopride during hour 2 post-treatment (see Figure 6, lower panel). Perhaps at the time of the 50 ETI-AMPH test (Test 4, see Table 3) animals were more highly sensitized to the effects of amphetamine than they were at the time of the 25 ETI-AMPH test (Test 2), and the effects of amphetamine were less opposable even by the higher dose. From Test 2 to

Test 4, animals had two additional treatments with eticlopride and one additional treatment with amphetamine. Repeated amphetamine administration and pretreatment with D2 antagonist both sensitize the locomotor response to amphetamine administration (Tanabe et al., 2004).

SCH 23390 has been characterized as a selective D1 antagonist. Consequently, attenuation of amphetamine-induced hypophagia by SCH 23390 was consistent with blockade of D1 receptors. However, SCH 23390 has also been reported to be an agonist at the 5HT_{2c} receptor (Millan et al., 2001; Zarrindast et al., 2011). An agonist effect by SCH 23390 at the 5HT_{2c} receptor could conceivably have contributed to the attenuation of hypophagia. Moreover, amphetamine affects the release not only of dopamine but of serotonin and norepinephrine, and so the possible contribution of these neurotransmitters to longer-term hypophagia should be investigated.

In the short term, amphetamine impacts a range of functions other than food intake, including activity, sensing (vision, audition, and olfaction), grooming, sleep, water intake, and exploration. Changes in any of these could conceivably impact longer-term food intake.

In preliminary research, we examined the degree to which different doses of SCH23390 and eticlopride blocked short-term amphetamine hyperactivity (data not shown). The lowest SCH23390 and eticlopride doses that reliably produced substantial blockade were used as the initial doses in Experiments 1 and 2. Whether an antagonist dose was increased or decreased from one test to the next depended on the magnitude and direction of the short- and longer-term effects obtained in prior tests. The present results need to be replicated using designs that counterbalance doses or use different dose groups.

4.7. Amphetamine hangover

In this study and in two prior studies (White et al., 2010; White et al., 2007) we attempted to mimic human recreational psychostimulant use by intermittently administering a moderately high but nontoxic dose of amphetamine to rats. This kind of drug regime produces several motivational deficits that appear to have maximum expression near the end of the first day following administration. These deficits include hypoactivity, hypophagia, and a negative cue state (Barrett et al., 2005; Barrett et al., 1992; White et al., 2007; White and White, 2006). The deficits occur following the first administration of amphetamine, and they may be aspects of an amphetamine-induced acute withdrawal or “hangover” syndrome. Whether a recreational drug administration regime produces a similar hangover in humans does not seem to have been systematically studied.

4.8 Amphetamine hangover as a model for understanding the development of withdrawal

Studying the “hangover” syndrome produced by amphetamine could have some important uses beyond the search for hangover cures.

First, amphetamine hangover may prove to be useful as a model for understanding the development of psychostimulant withdrawal in dependent individuals. Interestingly, humans who have used psychostimulants chronically, and who are dependent, have been reported to show the most intense withdrawal-related symptoms near the end of the first day following

drug termination (McGregor et al., 2005; Walsh et al., 2009). The similarity in time course and in symptoms of amphetamine hangover in rats and of withdrawal in humans suggests that the phenomena are related. The study of acute amphetamine hangover might suggest factors that, as a result of chronic use, undergo plastic change and produce full blown withdrawal (Swift et al., 1998).

4.9 Amphetamine hangover as a model for studying the organization of symptoms in a syndrome

Second, amphetamine hangover may prove useful as a model for studying how the symptoms of a syndrome are mechanistically related and organized. The amphetamine hangover syndrome has several features that make it useful for this purpose. In addition to consisting of multiple symptoms, some of which may be mechanistically related, the syndrome has a well-defined inducer (amphetamine administration), a predictable time of occurrence and dissipation, and a relatively short time course (around one day) from induction to recovery.

4.10 Strengths of the feeding procedure

In the current study, rats could earn packets of six pellets by making a lever-press response and then a series of head-in-bin responses. They could earn packets during one-hour meal opportunities that began every three hours. This procedure had some advantages. First, a two-hour deprivation intervened between meal opportunities to increase the likelihood that a sample of feeding behavior would be obtained. Second, meals opportunities were scheduled at a range of times post-drug treatment and at specific times that might correspond to maximum short- and longer-term effects. Third, a pelleted diet (94 mg/pellet) was used rather than chow, to increase the precision and ease with which intake was measured. Fourth, pellet receipt was made to depend primarily on head-in-bin responses to minimize the response requirement. Finally, the procedure was also designed to establish the feasibility of scheduling instrumental tasks every three hours.

Acknowledgments

This research was supported by grants DA015351 and RR016481. Cory Ruffing and Nicholas Rudd assisted with Experiment 1. Jeremy Bowen assisted with Experiment 2.

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Highlights

- Longer-term hypophagia occurred 19–26 hours after amphetamine in rats
- Pretreatment with dopamine D1 or D2 receptor antagonists attenuated amphetamine-elicited longer-term hypophagia
- Dopamine receptor activation by amphetamine is an early event in the sequence producing longer-term hypophagia

Figure 1.

Diagram of a typical test. Most tests were five days long. At light onset of Day 1, all subjects received a control treatment that consisted of saline followed 15 min later by saline (C in the diagram). On Day 3 subjects received an experimental treatment (E in the diagram, see Tables 2 and 3 for treatments). The numbers across the top show the hour since light onset. The light-dark bar shows when light were on or off. Grey squares represent meal opportunities. Throughout the five day period, animals could lever press for food pellets for one-hour intervals occurring every three hours.

Figure 2.

Distance moved during the first six hours following each pretreatment-treatment combination. The upper panel shows total distance moved, and lower panels show distance moved every hour. The middle panel includes data for combinations involving different doses of D1 antagonist (SCH 23390, “SCH”) pretreatment followed by saline (“SAL”) treatment, and the lower panel includes data for combinations involving different doses of D1 antagonist pretreatment followed by amphetamine (“AMPH”) treatment. 3, 12, 31, and 50 refer to $\mu\text{g/kg}$ doses of SCH 23390 that were used as pretreatments. “AMPH” refers to 2.0 mg/kg amphetamine treatment, and “SAL” refers to 1.0 ml/kg saline pretreatment or treatment. SAL-SAL and SAL-AMPH are shown in middle and lower panels for comparison. Error bars are standard errors. An * indicates a pretreatment-AMPH combination that produced more activity than the corresponding pretreatment-SAL combination. A text label above a bar for a pretreatment-AMPH combination indicates a lower dose pretreatment-AMPH combination that produced a higher level of activity.

Figure 3.

Food intake following amphetamine treatment compared to saline treatment. The figures show the results for one subject during test 4, when pretreatment-treatment combinations on days 1 and 3 were saline-saline (“SAL-SAL”) and saline-amphetamine (“SAL-AMPH”), respectively. The left panel shows mean pellet intake at the first nine meal opportunities following each of these combinations. The right panel shows the cumulative difference in intake across meal opportunities. The bars across the tops of the figures indicate when lights were on or off.

Figure 4.

Cumulative change in food intake, relative to SAL-SAL control, for each pretreatment-treatment combination. Each panel shows the effects of pretreatment with different D1 antagonist dose ($\mu\text{g/kg}$ SCH 23390, “SCH”) on treatment with saline (“SAL”) and amphetamine (“AMPH”). Data for the combination involving saline pretreatment followed by amphetamine treatment (“SAL-AMPH”) is shown in each panel for comparison. The bars across the tops of the figures indicate when lights were on or off. Error bars are standard errors. The effects of the combination involving 31 $\mu\text{g/kg}$ SCH 23390 pretreatment and saline treatment are not shown in the lower left panel, because the data were lost.

Figure 5.

Cumulative change in food intake, relative to SAL-SAL control, averaged across pretreatment-treatment combinations having similar effects. Data are shown through meal opportunity 9 (hour 26 post treatment), because the major effects had occurred by this time. The bar across the top of the figure indicates when lights were on or off. Error bars are standard errors. The SCH-AMPH function is an average of the data for tests involving 12 SCH-AMPH, 31 SCH-AMPH, and 50 SCH-AMPH. The SCH-SAL function is an average of the data for tests involving 12 SCH-SAL and 50 SCH-SAL. An * indicates a meal opportunity at which antagonist followed by amphetamine produced more intake than SAL-AMPH.

Figure 6.

Distance moved during the first six hours following each pretreatment-treatment combination. The upper panel shows total distance moved, and lower panels show distance moved every hour. The middle panel includes data for combinations involving different doses of D2 antagonist (eticlopride, “ETI”) pretreatment followed by saline (“SAL”) treatment, and the lower panel includes data for combinations involving different doses of D2 antagonist pretreatment followed by amphetamine (“AMPH”) treatment. 25, 50, and 100 refer to µg/kg doses of eticlopride that were used as pretreatments. “AMPH” refers to 2.0 mg/kg amphetamine treatment, and “SAL” refers to 1.0 ml/kg saline pretreatment or treatment. SAL-SAL and SAL-AMPH are shown in middle and lower panels for comparison. Error bars are standard errors. An * indicates a pretreatment-AMPH combination that produced more activity than the corresponding pretreatment-SAL combination. A text label above a bar for a pretreatment-AMPH combination indicates a lower dose pretreatment-AMPH combination that produced a higher level of activity.

Figure 7.

Cumulative change in food intake, relative to SAL-SAL control, for each pretreatment-treatment combination. Upper right and lower panels each show the effects of pretreatment with a different D2 antagonist dose ($\mu\text{g/kg}$ eticlopride, “ETI”) on treatment with saline (“SAL”) and amphetamine (“AMPH”). The upper left panel show the results of test 1 and 6, which involved saline pretreatment and amphetamine treatment. The average of these two tests (“SAL-AMPH”) is shown in the upper right and lower panels for comparison. The bars across the tops of the figures indicate when lights were on or off. Error bars are standard errors.

Figure 8.

Cumulative change in food intake, relative to SAL-SAL control, averaged across pretreatment-treatment combinations having similar effects. Data are shown through meal opportunity 9 (hour 26 post treatment), because the major effects had occurred by this time. The bar across the top of the figure indicates when lights were on or off. Error bars are standard errors. The ETI-AMPH function is an average of the data for tests involving 25 ETI-AMPH, 50 ETI-AMPH, and 100 ETI-AMPH. The ETI-SAL function is an average of the data for tests involving 25 ETI-SAL, 50 ETI-SAL and 100 ETI-SAL. An * indicates a meal opportunity at which antagonist followed by amphetamine produced more intake than SAL-AMPH.

Table 1

Number of Days in Each Condition for Experiment 1 and Experiment 2

Condition	Experiment 1	Experiment 2
Lever Press Training	5	5
Free Feeding in Colony	5	5
Meal Training	13	14
Testing	51	42

Note. Experiment 1 included 10 tests. During Test 9, an extra baseline day occurred between the control treatment (Sal-Sal) on Day 1 and the experimental treatment (3.1 µg/kg Eticlopride-Amph). Experiment 2 included 8 tests. During Tests 4 and 7 an extra baseline day occurred between control and experimental treatments.

Table 2

Experiment 1 Day 3 Pretreatment-Treatment Combination for Each Test

Test	Pretreatment	Treatment
1	1.0 ml/kg Saline	2.0 mg/kg Amphetamine
2	50 µg/kg SCH 23390	2.0 mg/kg Amphetamine
3	50 µg/kg SCH 23390	1.0 ml/kg Saline
4	1.0 ml/kg Saline	2.0 mg/kg Amphetamine
5	12 µg/kg SCH 23390	2.0 mg/kg Amphetamine
6	12 µg/kg SCH 23390	1.0 ml/kg Saline
7	31 µg/kg SCH 23390	2.0 mg/kg Amphetamine
8	31 µg/kg SCH 23390	1.0 ml/kg Saline
9	3 µg/kg SCH 23390	2.0 mg/kg Amphetamine
10	3 µg/kg SCH 23390	1.0 ml/kg Saline

Table 3

Experiment 2 Day 3 Pretreatment-Treatment Combination for Each Test

Test	Pretreatment	Treatment
1	1.0 ml/kg Saline	2.0 mg/kg Amphetamine
2	25 µg/kg Eticlopride	2.0 mg/kg Amphetamine
3	25 µg/kg Eticlopride	1.0 ml/kg Saline
4	50 µg/kg Eticlopride	2.0 mg/kg Amphetamine
5	50 µg/kg Eticlopride	1.0 ml/kg Saline
6	1.0 ml/kg Saline	2.0 mg/kg Amphetamine
7	100 µg/kg Eticlopride	2.0 mg/kg Amphetamine
8	100 µg/kg Eticlopride	1.0 ml/kg Saline