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Time-dependent effects of amphetamine on feeding in rats

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Abstract

Following administration of a moderate dose of amphetamine, rats appear to pass through a sequence of physiological/ psychological states, including stimulant and depressant states. The present research evaluated whether these states could be inferred from time-dependent changes in feeding-related measures. Male rats were housed in individual stations (light-dark 12-12 hr, free access to water) where, at three hour intervals, they could respond for food for one hour. The work requirement was fixed ratio 1, and each lever press produced 6 94-mg food pellets. When the pattern of responding for food stabilized across the light-dark cycle, a series of 6 or 7 tests was run. During each test, rats received a saline treatment (1.0 ml/ kg, subcutaneously) followed by a 48-hour monitoring period, and then they received an amphetamine treatment (2.0 mg/ kg, subcutaneously) followed by a 72-hour monitoring period. Different groups were treated at either light onset or light offset. Lever presses and head-in-feeding-bin responses were monitored throughout these tests. Administration of amphetamine at light onset and at light offset produced cumulative food intake functions having four regions: post-treatment hours 1-6 (hypophagia), 7-12 (normal intake), 13-27 (hypophagia), and 28 and beyond (normal intake). The sequence, duration, and quality of the amphetamine-induced changes in food intake resembled those formerly seen in cue state and activity, and provided further evidence of a transient withdrawal state 20-24 hr post-amphetamine treatment.

Section: Regulatory Systems

Keywords

psychostimulant; food intake; circadian; withdrawal; anorexia

1. Introduction

Amphetamine, a psychostimulant, produces activating effects in the short term (the first several or so hours post administration). These short term effects and the mechanisms that mediate them have been extensively studied. Amphetamine and related compounds are used recreationally in part because of such effects (reviewed in Berridge, 2006; Robinson and Berridge, 1993; Segal and Kuczenski, 1994).

Amphetamine produces additional time-dependent effects during the first day or so following administration. Investigations of the effects of amphetamine on cue state and on activity have provided good evidence for this. Barrett, Caul and colleagues have examined the impact of amphetamine on cue state in a series of drug discrimination studies involving amphetamine

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and haloperidol (Barrett et al, 1992; Barrett et al, 2005; Caul et al, 1996; Caul et al, 1997; Stadler et al, 1999). By “cue state,” Barrett, Caul and colleagues meant the distinguishable internal sensations present at a particular time following drug treatment. In one study, rats treated with 10 mg/kg amphetamine responded on an amphetamine-paired lever 4 and 6 hr after treatment, on amphetamine- and haloperidol-paired levers equally 8, 12, and 16 hr after treatment, on a haloperidol-paired lever 20 and 24 hr after treatment, and again on each lever equally 32 hr after treatment (Barrett et al, 1992). White and colleagues have examined the impact of amphetamine on activity. Rats treated with 2.0 or 4.0 mg/kg amphetamine were hyperactive 1 to 6 hr after treatment, normally active 7 to 18 hr after treatment, hypo-active 19 to 24 hr after treatment, and again normally active 25 hr after treatment (White and White, 2006).

Changes in cue state and activity appear correlated across time, and the changes may signify the presence of different amphetamine-induced states. An amphetamine-like cue state and hyperactivity from hours 1 to 6 post-treatment indicate the presence of a stimulant state, whereas a haloperidol-like cue state and hypo-activity from hours 19 to 24 post-treatment may indicate the presence of a withdrawal state. The withdrawal state may be preceded by a latent state, when measures only appear to normalize, and it may be followed by a recovery state, when measures actually do normalize. Withdrawal is comprised of a constellation of symptoms (Barr and Markou, 2005), and one way to bolster the claim that withdrawal is present at a particular time is to show that other symptoms indicative of withdrawal are also present at that time. One characteristic symptom of withdrawal from amphetamine is diminished food intake (hypophagia).

The purpose of this study was to see whether amphetamine altered food intake in a time-dependent manner comparable to that observed for cue state and activity. We were particularly interested in determining whether amphetamine produced hypophagia during the same interval that it reportedly produced a haloperidol-like cue state and hypo-activity.

Certain amphetamine administration regimes, such as regimes involving chronic escalating doses, have been used to produce a relatively prolonged condition that has been likened to depression (Barr and Markou, 2005). In contrast, in this research, a moderate dose of amphetamine, 2.0 mg/kg, was repeatedly administered at intervals of at least 5 days, a regime that is better suited to produce a transient withdrawal. Short-term effects of amphetamine on food intake have been extensively studied, whereas longer-term effects have not been. When longer-term effects have been studied, investigators have tended to measure total intake at the end of a long interval such as 24 hr (Chen et al, 2001). In order to enhance the opportunity to identify time-dependent changes, we assessed the effects of amphetamine on intake frequently and over a long interval. In particular we allowed rats to lever press for food pellets at meal opportunities that began every three hours and that were 1-hr in duration, and we monitored responding and food intake for three days following amphetamine administration. The effects of a drug depend in part on when it is administered in the light-dark cycle (Davis and Wellman, 1991; Reinberg, 1999). To evaluate whether changes could be observed that were independent of administration time, we examined the effects of treating different groups at light onset and at light offset.

2. Results

2.1. Acquisition

Groups of eight rats were treated at either light onset or light offset of the 12-12 hr light-dark cycle during testing. The groups were not tested until they showed stable responding on the feeding schedule. The feeding schedule allowed animals to respond for food for one hour every three hours. During a feeding hour, a lever press could result in a “package” of six 94-mg pellets

(Fixed ratio 1, FR1, or “ratio”). The lever press produced the first pellet, and an animal had to place its head in the feeding bin to produce subsequent pellets in the package. Both groups of rats adjusted to the feeding schedule in a similar manner, and so acquisition data will be shown only for the group eventually treated at light offset during tests.

Figure 1 shows the mean number of ratios the animals completed across days of exposure to the feeding schedule (Training days). The number of ratios completed increased from days 1 to 3, decreased from days 3 to 16, but did not differ from days 16 to 20, $F(7,19) = 29.012$, $p < .0001$ and Fisher’s PLSD post hoc tests. In summary, performance was stable after 15 days of training.

2.2. Testing

Animals received a series of five-day tests. On day 1, different groups were treated, at either light onset or light offset, with saline (Sal). On day 3, they were treated, at the same times, with 2.0 mg/kg amphetamine (Amp). Feeding opportunities were scheduled as before. Figure 2 shows the mean number of ratios each group completed on each day of each test. The upper panel shows results for the group treated at light onset. An ANOVA produced a significant effect of Test, $F(6, 42) = 5.035$, $p < .001$, a significant effect of Test day, $F(4, 28) = 51.448$, $p < .0001$, and a significant interaction, $F(24, 168) = 1.762$, $p < .05$. Fisher’s PLSD for the main effect of Test day indicated that fewer ratios were completed on the day of amphetamine administration than on any other day, $ps < .05$, but that no other days differed. Fisher’s PLSD for the main effect of Test indicated that more ratios were completed during Test 1 than during other tests, $ps < .05$. An ANOVA based on the data of the group treated at light offset (Figure 2, lower panel) produced a significant effect of Test, $F(5, 35) = 7.814$, $p < .0001$, and of Test day, $F(4, 28) = 36.146$, $p < .0001$. Fewer ratios were completed on the day of amphetamine administration than on other days, and more ratios were completed during Tests 1 and 2 than during the other tests (Fisher’s PLSD, $ps < .05$). Overall, for treatment at both light onset and light offset, fewer ratios were completed on the day of amphetamine administration, and more ratios were completed during the earliest tests.

Figure 3 shows the number of ratios each group completed at each meal opportunity during the two day period following saline administration of tests. The time course with which amphetamine affected performance (see below) was evaluated by making comparisons to these baselines. The upper panel shows the data for the group treated at light onset. Three test sets were created by averaging the results of Tests 1 and 2 (Sal1&2), 3 and 4 (Sal3&4), and 5, 6 and 7 (Sal5&6). The circadian pattern of ratios completed appeared to be similar across the two days post saline and across the three test sets. An ANOVA yielded a significant effect of Test set, $F(2, 14) = 10.740$, $p < .005$, a significant effect of Meal opportunity, $F(15, 105) = 12.193$, $p < .0001$, and a significant interaction, $F(30, 210) = 1.725$, $p < .05$. The stability of the circadian pattern was assessed via post hoc comparisons based on the main effect of Meal opportunity. The number of ratios completed was highest at Meal opportunities 1, 5, and 6 following light onset, intermediate at Meal opportunities 4 and 8, and lowest at Meal opportunities 2 and 3 (Fisher’s PLSD, $ps < .05$). This pattern tended to occur on the two days post saline and across the three test sets.

For the group treated at lights offset (Figure 3, lower panel), an ANOVA produced a significant effect of Test set, $F(2, 14) = 26.713$, $p < .0001$, and of Meal opportunity, $F(15, 105) = 17.255$, $p < .0001$. The number of ratios completed was highest at meal opportunities 1 and 2 after light offset, intermediate at meal opportunity 8, and lowest at meal opportunities 4, 5, 6, and 7 (Fisher’s PLSD based on the main effect of Meal opportunity, $ps < .05$). The pattern was observed on the two days post saline and across the three test sets. On the whole, both groups had highly reproducible circadian patterns of responding, but the group treated at light onset had an additional peak of responding at the first meal opportunity in the light period.

On day 3 of tests, groups were treated, at light onset or offset, with 2.0 mg/kg amphetamine, after which they could respond for food on the same schedule of meal opportunities as before: beginning one hour after treatment, one-hour meal opportunities were scheduled every three hours, and during these meal opportunities each lever press produced six 94-mg food pellets. The graphs in Figure 4 show the change in cumulative food intake at the first sixteen meal opportunities following amphetamine administration, relative to saline administration. The upper and middle panels show the results for the groups treated at light onset and offset, respectively. To produce individual functions in these panels, the difference in the number of food pellets consumed at each meal opportunity following saline and amphetamine treatment was found (amphetamine - saline). These differences were then cumulated across the sixteen meal opportunities. The results for sets of tests were then averaged as in Figure 3. Amphetamine produced a progressive reduction in the number of pellets consumed ("Cumulative Pellet Deficit").

An ANOVA for the group treated at light onset (upper panel) produced a significant effect of Test set, $F(2, 14) = 6.047$, $p < .05$, and of Meal opportunity, $F(15, 105) = 12.426$, $p < .0001$. A significant interaction was not obtained. Given that the functions had the same general form, in order to identify the major trends produced by amphetamine, the main effect of Meal opportunity was analyzed with Fisher's PLSD (significant effects were $p < .05$). A mean deficit of approximately 40 pellets was obtained at the end of the first meal opportunity. A significant change did not occur from meal opportunity 1 to 2. However, the deficit increased from meal opportunity 2 to 3, and the deficit was greater at meal opportunity 9 than at meal opportunities 3, 4, 5, and 7, indicating that the deficit progressed from meal opportunity 3 to 9. The deficits at meal opportunities 10-16 were less than or equivalent to the deficit at meal opportunity 9, indicating that this meal opportunity corresponded to a nadir in cumulative intake. The analysis suggested that the overall function relating cumulative pellet deficit to meal opportunity could be divided into four regions corresponding to meal opportunity 1, meal opportunity 2, meal opportunities 3-9, and meal opportunities 10-16.

A parallel ANOVA for the group treated at light offset (middle panel) also produced a significant effect of Test set, $F(2, 14) = 8.345$, $p < .005$, a significant effect of Meal opportunity, $F(15, 105) = 3.073$, $p < .0004$, and no significant interaction. Fisher's PLSD, done on the main effect of Meal opportunity, indicated that the deficit, already present at the end of meal opportunity 1, progressed through meal opportunity 2, diminished at meal opportunities 3 and 4, and progressed again from meal opportunities 5 to 8. The analysis divided the overall function into four regions corresponding to meal opportunities 1-2, 3-4, 5-8, and 9-16.

The lower panel shows the cumulative change in pellet deficit at each meal opportunity averaged across groups and tests. An ANOVA and post hoc comparisons divided the function into regions that included meal opportunities 1 and 2, 3 and 4, 5-9, and 10-16, $F(15, 225) = 4.501$, $p < .0001$, Fisher's PLSD, $ps < .05$. Overall, Figure 4 suggests that amphetamine produced a short-term phase of hypophagia and a longer-term one (approximately 13-24 hr post-treatment) that was preceded and followed by phases of seemingly normal intake.

Though the functions in Figure 4 had some similarities, they also suggested effects on cumulative intake that appeared to depend upon both test set and treatment time. To assess these effects, an ANOVA was done with Treatment time (light onset or light offset) and Test set (Test1&2 or Test5&6/ Test 5&6) as factors. Separate analyses were done for meal opportunities 8 and 16. For meal opportunity 8, only the effect of Test set was significant, $F(1, 14) = 7.582$, $p < .05$. Treatment groups had similar cumulative deficits at meal opportunity 8 during Test1&2, and during the last Test set this deficit was similarly attenuated in both groups. For meal opportunity 16, the effect of Treatment time approached significance, $F(1, 14) = 3.616$, $p = .0780$, and the effect of Test set was significant, $F(1, 14) = 9.866$, $p < .01$. Treatment

time groups did not differ during Test1&2, but did differ during the last test set, $t(14) = 2.689$, $p < .05$. For the group treated at light onset, the change from Test1&2 to the last test set was not significant, whereas for the group treated at light offset, the change was significant, $t(7) = -2.755$, $p < .05$. In summary, repeatedly treating with amphetamine at light offset resulted in a lower deficit in intake two days following treatment than repeatedly treating at light onset.

During a feeding hour, an animal could retrieve and consume six pellets after a lever press. The time from the lever press to the head-in-bin response that retrieved the sixth pellet is a measure of consummatory behavior and will be called consummatory time. Consummatory times over 65 sec were not included in analyses. The remaining durations constituted over 97% of the observations. Figure 5 shows mean consummatory times for groups treated with saline and amphetamine at light onset (upper panels) or light offset (lower panels).

For the group treated at light onset, the upper left panel shows mean consummatory times during light and dark phases of the two days following treatments. No effects of treatment (saline or amphetamine), phase (light or dark), or day (day 1 or 2) were observed. The upper right figure shows mean consummatory times during different regions revealed by the cumulative-pellet-deficit analysis (See Figure 4 lower panel). ANOVA indicated an effect of region, $F(3, 21) = 6.336$, $p < .005$, but no effect of treatment and no interaction. During meals 1-2, consummatory times trended shorter following amphetamine than saline, $t(7) = 2.286$, $p = .0562$.

For the group treated at light offset, phase in the light-dark cycle did have an effect on consummatory times (lower left panel), $F(1,7) = 61.121$, $p < .0001$, and consummatory times were shorter during the dark phase than during the light phase, Fisher's PLSD, $p < .0001$. Saline and amphetamine did not produce different consummatory times during any region following treatment (lower right panel), $t(7)$, $p > .05$, though a main effect of region, $F(3,21) = 27.872$, $p < .0001$, and a treatment by region interaction, $F(3,21) = 4.015$, $p < .05$, were obtained. The latter effects were due to modulation by phases of the light-dark cycle. In sum, consummatory behavior was not differentially affected by saline and amphetamine at any time post treatment, and it was modulated by phase in the light-dark cycle when treatment was given at light offset, but not when treatment was given at light onset.

The mean weight of both groups was around 500 g on the day of the first saline administration, and both groups gained roughly 20 g over the course of the first six tests. During each test, body weight increased from the day of saline treatment to the day of amphetamine treatment, but an increase in body weight was attenuated from the day of amphetamine treatment to the day of saline treatment.

3. Discussion

The group treated at light onset had a peak of ratios completed at the first meal opportunity in the light phase, whereas the group treated at light offset did not. Presumably, this peak was produced by the treatment and the station maintenance that took place near light onset. This peak was observed not only on the day of saline administration, but also on the day after it and on the two recovery days following amphetamine administration, days when treatment and maintenance were not performed. Although the pattern was instigated in part by exogenous events, it provided a stable baseline against which the effects of amphetamine administration could be assessed.

Administration of amphetamine at light onset and at light offset produced cumulative food intake functions having four regions. Looking across both administration times, these regions corresponded very roughly to post-treatment hours 1-6 (hypophagia), 7-12 (normal intake), 13-27 (hypophagia), and 28 and beyond (normal intake). The sequence, duration, and quality

of the amphetamine-induced changes in food intake resembled those formerly seen in cue state (Barrett et al, 1992) and activity (White and White, 2006). The three measures undergo changes consistent with stimulant, latent, withdrawal, and recovery states. An amphetamine cue state, hyperactivity, and hypophagia overlap from hours 1 to 5 post-amphetamine treatment, indicating a stimulant state. A haloperidol-like cue state, hypo-activity, and hypophagia appear to overlap from approximately hours 20 to 24 post-treatment, suggesting that a withdrawal state may be present during this time.

The two phases of hypophagia were expressed in the context of different states and were separated by a latent period when intake appeared to have recovered. Our observations suggest the possibility that the phases of hypophagia are not a unitary response and may be mediated via different processes. Although much research has investigated the determinants of the hypophagia occurring shortly after amphetamine treatment, very little research has evaluated whether these determinants and those of the longer term hypophagia can be dissociated. If the two phases of hypophagia are due to different determinants, then amphetamine-induced hypophagia may be better understood by looking at both short- and long-term hypophagia.

A basic question raised by the results is why intake declined from hours 13-27 post-amphetamine treatment.

The decline cannot be ascribed to a direct effect of amphetamine, because amphetamine has a half-life of 1-3 hr in rats (Fuller et al, 1977; Hutchaleelaha et al, 1994), and negligible amounts of drug would be in the body during this interval.

Withdrawal from amphetamine is thought to reduce food intake by interfering with appetitive behavior (Barr and Phillips, 1999; Orsini et al, 2001), at least under conditions of high response cost (Salamone and Correa, 2002). Whether reduced intake in the present study was due to a change in processes related to appetitive behavior is debatable. In the present study, the impact of appetitive behavior on food intake was limited in several ways: Travel distance was minimal, the ratio requirement was one, each response produced six pellets, and ample time to earn food (one hour) was afforded at each meal opportunity. Nevertheless, an impact of appetitive processes remains possible, because appetitive behavior is expressed via a complex control structure entailing sensory, motor, and integrative processes (Timberlake and Lucas, 1989).

Consummatory times were the duration required to consume five pellets and to retrieve the sixth. Mean consummatory times during the withdrawal region were the same (approximately 30 sec) following both saline and amphetamine. This result suggests that the decline in intake during the withdrawal region was not due to a gross impairment of consummatory behavior. A lack of effect of withdrawal from amphetamine on consummatory behavior has been reported previously (Barr and Phillips, 1999; Salamone and Correa, 2002). In the present study, amphetamine affected consummatory behavior at a higher level of meal organization, such as the number of packages taken.

To our knowledge, no systematic research has been done regarding mechanisms that might mediate longer-term hypophagia produced by widely-spaced administrations of moderate amphetamine doses. Whether this hypophagia reflects malaise (Cole et al, 1995; Turchan et al, 1998), a specific deficit in feeding-related motivation (Stanek, 2006; Vicentic and Jones, 2007), or some other condition is uncertain.

Although intake and weight gain were attenuated by amphetamine administration, animals did not compensate by increasing intake on the following days. A symptom of withdrawal from amphetamine sometimes reported is a rebound hyperphagia (Srisurapanont et al, 1999). Such a rebound was probably not observed in the present study because the drug regimen was mild enough and the feeding schedule was rich enough to preclude significant weight loss.

Administering at different time in the light-dark cycle produced some differences. Boundaries between stimulant, latent, withdrawal, and recovery regions differed somewhat. This was probably the case for a couple of major reasons. First, for each group the cumulative change in food intake due to amphetamine was relative to a different control pattern. Second, for each group amphetamine-induced effects interacted with a different sequence of states entrained by the light-dark cycle. The treatment-time groups also showed some differences in the capacity to recover intake following amphetamine. Groups treated at both times had a smaller deficit in cumulative intake at meal opportunity 8 during later tests. Such tolerance to the anorectic effects of amphetamine has been reported frequently (Caul et al, 1988; Kuo and Cheng, 2002). For the group treated at light offset, a greater attenuation in the cumulative intake deficit was observed from earlier to later tests at meal opportunity 16. The group treated at light offset showed a slightly better capacity to recover, even though moderate doses, widely spaced administrations, and only six tests were employed. For the group treated at light offset, consummatory times were shorter during the active period and longer during the dark period, whereas for the group treated at light onset, this circadian pattern was lost, and consummatory times tended to be uniformly short. Treatment at light onset may more readily disrupt normal circadian patterns.

Other measures show a biphasic response following amphetamine administration and are potential indicators of withdrawal, including the frequency of ultrasonic vocalizations (Covington and Miczek, 2003; Thompson et al, 2006), the threshold of intracranial self-stimulation (Cryan et al, 2003), and the proportion of EEG activity indicative of REM sleep (Edgar and Seidel, 1997). Evaluating whether common conditions produce similar time-dependent effects in different measures would suggest whether amphetamine-induced states were mediated by a unitary mechanism or by distributed mechanisms.

Each of the successive five-day tests began with a two-day re-baseline to which the effects of drug were compared. This design seemed necessary, because amphetamine administration sometimes appears to shift reward-related set points (Koob and Bloom, 1988). The design did not allow effects due to repeated drug administration to be fully revealed.

Several procedural features were selected to model human recreational drug use. A dose of amphetamine was used that a rat might be expected to self-administer over a reasonable period of time (Deminier et al, 1989). Drug was administered repeatedly, but at intervals widely spaced enough (five days) to prevent drug accumulation and to allow intake to recover. In one condition, drug was administered at the start of the inactive period (light onset for the rat), a time at which recreational drug use presumably peaks in humans. Widely-spaced administrations of moderate doses of amphetamine may not produce dramatic withdrawal symptoms, but this and similar research suggests that such regimes significantly impact motivation.

Summary

In the present study, a moderate dose of amphetamine was administered every five or more days. Two phases of hypophagia were observed during the first 24 hours following amphetamine administration, a short-term hypophagia (hours 1-6 post-treatment) and a longer-term hypophagia (hours 13-27 post-treatment). The phases of hypophagia could be mediated by different processes and mechanisms, and a complete understanding of amphetamine-induced hypophagia may not be achievable by investigating only the short-term phase. Minimal appetitive behavior was required to procure food, and the efficiency of consummatory behavior was not impaired during either phase of hypophagia. Consequently, short- and longer-term hypophagia could not be readily ascribed to a gross impairment in either appetitive or consummatory processes. Following repeated amphetamine treatment, longer-term hypophagia was diminished, and the group treated at light offset was able to partially

compensate during the recovery phase. The changes across time in food intake appeared to be indicators of a series of amphetamine-induced states, because they corresponded to changes seen in cue state and activity. Together, the measures indicate that a psycho-stimulant state is present approximately 1-5 hours following amphetamine treatment, and that a transient withdrawal is present 20-24 hours post-treatment. The changes across time in the measures appear to be correlated, suggesting that they may be mediated by a common mechanism. The results may be relevant to the effects of human recreational drug use.

4. Experimental Procedure

4.1. Animals

The study consisted of two conditions that were run successively. Each condition included eight male Wistar rats (Harlan, Indianapolis, IN). Prior to the start of a condition, animals were pair-housed in an animal colony on a 12-hr light/ 12-hr dark cycle and in a temperature of 20 - 22°C. They had free access to food (Purina 5001 Rodent Diet, Lab Diet) and water. Just prior to the start of a 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 300 and 400 g at the start of a condition. Animal care and experimental procedures were in accordance with NIH guidelines.

4.2. Apparatus

Animals were trained in four standard operant conditioning stations (Med Associates) that 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 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. 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-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) arranged contingencies and monitored behavior. Stations were located in well-isolated temperature- and humidity-controlled rooms (approximately 1.8 m × 2.1 m × 2.6 m high).

4.3. Drug

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

4.4. Procedure

Training—Each animal was deprived to 85% of its free-feeding body weight and, in an operant station, was trained in a series of conditions (habituation, bin training, auto-shaping, fixed ratio 1) to press the lever for 94-mg pellets (Bio Serv, #F0058). The animals were then placed on free food availability in the colony for five days.

Acquisition—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 schedule and had free access to water. During the first 24 hours in the stations, the animals could earn food pellets on an FR1 schedule. Each lever press produced six 94-mg pellets. The lever press produced the first pellet, and the delivery of subsequent pellets was contingent on a head-in-bin response. The lever press turned on the bin light, and the light remained on until the animal made a head-in-bin response to retrieve the sixth pellet.

On subsequent days, food access was restricted to one-hour periods spaced three hours apart. One-hour meal opportunities began 1, 4, 7, and 10 hours after light onset and light offset. The beginning of a meal opportunity was signaled by the delivery of a pellet and the illumination of the feeding bin for 10 sec. Animals remained on this schedule until the number of ratios completed per day and the distribution of ratios completed across the light-dark cycle stabilized.

Testing—After intake stabilized, five-day tests were conducted. The prior feeding conditions remained in effect. For one group of eight subjects, at light onset of test day 1, each animal was injected with saline (1.0 ml/kg subcutaneously under the loose skin on the back of the neck). Two days later, at light onset of test day 3, each animal was administered 2.0 mg/kg amphetamine in the same manner. The other group of eight subjects received a similar series of tests, but they were treated at light offset. The group treated at light onset was tested seven times, and the group treated at light offset was tested six times.

Tests and test days were successive, except on a couple of occasions when experimenters were unavailable. Animals treated at light onset received an additional baseline day between saline and amphetamine phases of Test 3. Also, between Tests 3 and 4, they were placed in individual plastic tubs on free access to food and water for five days, after which they received two additional days of baseline.

Body weights were taken at the time of saline or amphetamine treatment. Apparatus maintenance was done at the same time and involved re-filling the pellet dispensers and water bottles and changing the pans and top-soil. Otherwise, animals were not disturbed.

4.5. Dependent measures

Throughout acquisition and testing, the number of ratios completed at each meal opportunity was recorded. The number of non-reinforced lever presses was also recorded every hour, as well as the number of occasions upon which an animal put its head in the feeding bin (“checks”).

4.6. Data analysis

Data were analyzed using within subjects ANOVA. Significant effects were analyzed with additional within subjects ANOVAs, followed up by PLSD pos hoc comparisons or paired t-tests.

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Figure 1.

Mean ratios completed across acquisition days for the group treated at light offset. 1-hr meal opportunities, during which each lever press produced 6 pellets (fixed ratio 1), were scheduled at 3-hr intervals. Arrows indicate training days from which performance on the measure was stable.

Figure 2.

Mean ratios completed during each day of each five-day test for the group treated at light onset (upper panel) and for the group treated at light offset (lower panel). “SalRec” is the recovery or baseline day following the day of saline (“Sal”), and “AmpRec1” and “AmpRec2” are recovery or baseline days following the day of amphetamine (“Amp”) treatment. * indicates tests during which more ratios were completed, and ** indicates days during which fewer ratios were completed.

Figure 3. Mean ratios completed at each meal opportunity during the first two days following saline administration of tests. The upper panel is for the group treated at light onset, and the lower panel is for the group treated at light offset. Results from tests 1 and 2, 3 and 4, and 5-7 or 5 and 6 have been averaged (Sal1&2, Sal3&4, Sal5&6 or Sal5&6, respectively). The bar across the top indicates when lights were on or off. The variability bar ("SEM") shows the mean standard error of the mean.

Figure 4.

Cumulative pellet deficit due to amphetamine at the end of each meal opportunity. The deficit is relative to saline treatment. The upper panel is for the group treated at light onset, and the middle panel is for the group treated at light offset. Tests were averaged as in Figure 3. The bar across the top indicates when lights were on or off. The variability bar ("SEM") shows the mean standard error of the mean. The lower panel is the average for both groups and all tests. The error bars represent standard errors of the mean. Vertical lines show the four regions into which an analysis of pair-wise comparisons divided the function. Each pellet was 94 mg. In the lower panel, * denotes a difference from Meal Opportunity 9 but not from Meal Opportunity 9.

8. In the upper and middle panels, * denotes a difference from Test1&2 to the last test set, and ** denotes a greater difference for the group treated at light offset.

Figure 5.

Consummatory times for groups treated at light onset (upper panels) and offset (lower panels). Left panels show mean consummatory times during phases of the light-dark cycle following saline or amphetamine treatment. The bar across the top indicates when lights were on or off, and variability is standard error of the mean. Right panels show mean consummatory times during different regions revealed by the cumulative-pellet-deficit analysis (See Figure 4 lower panel). Variability is standard error of the mean. Labels above bars indicate meal-opportunity regions having longer consummatory times.