THE EFFECT OF D-AMPHETAMINE ON HABITUATION OF SCHEDULE CONTROLLED OPERANT BEHAVIOR

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of Robert Packer find it satisfactory and recommend that it be accepted.

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THE EFFECT OF D-AMPHETAMINE ON HABITUATION

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Abstract

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Amphetamine can increase motor behavior soon after administration and also after brain and plasma levels of the drug have declined to a negligible level. Although the behavioral changes that occur immediately after drug administration can be attributed to the direct effect of the drug, the cause of the behavioral changes that occur after the drug has been nearly eliminated is debated. One theory that attempts to explain the increase in motor activity that occurs when the drug is no longer in the system is that amphetamine disrupts the process of habituation. This "habituation disruption" theory is supported by studies in which the behavior being measured was unlearned behavior such as startle response or pre-pulse inhibition. Habituation can be studied in operant conditioning by analyzing the changes in responding that occur over the course of the session, and spontaneous recovery of responding after an inter-session interval. Experiment 1 tested amphetamine effects on habituation of operant responding both within session and after an inter-session interval of 2 hr. Experiment 2 tested amphetamine effects on spontaneous locomotor activity and stereotypy. In Experiment 1, a decreasing pattern of operant responding was observed after administration of two of four doses of amphetamine, but habituation appeared to be disrupted after 0.5 and 1.0 mg/kg amphetamine. Spontaneous recovery of habituated operant responding also was changed by high doses of amphetamine,

suggesting that amphetamine disrupts some properties of habituation. In Experiment 2, locomotor activity increased for all doses of amphetamine in the drug session, compared to saline, but not in the post-drug session. The high doses of amphetamine resulted in nearly continuous engagement in stereotyped behavior. Because locomotor activity did not increase in the post-drug session this suggests that the increase in responding observed in Experiment 1 post-drug session for the higher doses of amphetamine was not due to a general increase in locomotor activity. Additionally, the stereotypy data from Experiment 2 suggest that the low rate of responding observed in the Experiment 1 drug session after administration of the higher doses of amphetamine is likely due to the onset of stereotyped behavior.

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INTRODUCTION

The psychostimulant amphetamine has been extensively studied for its influence on many types of behavior. Some examples in rats include performance on operant schedules of reinforcement (Bradshaw, Ruddle, & Szabadi, 1981; Morley, Bradshaw, & Szabadi, 1985), latent inhibition (Bethus, Goodall, & Muscat, 2006), startle response (Dieu, Seillier, Majchrzak, Marchand, & Di Scala, 2005; Klamer, Palsson, Revesz, Engel, & Svensson, 2004), peak procedure (Saulsgiver, McClure, & Wynne, 2006), attention (Bizarro, Patel, Murtagh, & Stolerman, 2004), temporal discrimination (Chiang et al., 2000; Odum, Lieving, & Schaal, 2002), open field (Furlan & Brandao, 2001), and wheel running (Belke, Oldford, Forgie, & Beye, 2005).

Amphetamine can increase motor behavior soon after administration (with peak effect at 30 – 60 min), and also after brain and plasma levels of the drug have declined to a negligible level (120 – 180 min post administration) (Irwin & Armstrong, 1961; Maickel, Cox, Miller, & Segal, 1969; Tilson & Rech, 1973). Although the behavioral changes that occur immediately after drug administration can be attributed to the direct effect of the drug, the cause of the behavioral changes that occur after the drug has been nearly eliminated is debated.

One theory that attempts to explain the increase in motor activity that occurs when the drug is no longer in the system is that amphetamine disrupts the process of habituation (a decrease in responding to a stimulus after repeated presentations of the stimulus). According to this theory, when a subject in a drug-free state is presented with a stimulus that was originally presented while the subject was in a drug state, the subject responds as if the stimulus had not previously been presented because amphetamine prevented habituation to the stimulus.

This “habituation disruption” theory is supported by studies in which the behavior being measured was startle response (Dieu et al., 2005) or pre-pulse inhibition (Klamer et al., 2004). Dieu et al. did not intend to investigate habituation. Rather, their first experiment was designed to test a possible mechanism by which amphetamine disrupted latent inhibition in a conditioned fear paradigm. Previous research cited by Dieu et al. suggested that amphetamine disrupts latent inhibition by enhancing the impact or salience of the unconditioned stimulus, thereby enhancing conditioning. Experiment 1 was designed to test how amphetamine would affect fear conditioning to a tone stimulus, depending on the intensity of the shock paired with tone. The relevant finding of this experiment was that, in the test sessions, amphetamine-treated groups showed tone-induced suppression of licking *even when the tone had not been paired with shock*. That is, administration of amphetamine may have prevented habituation to the tone during the conditioning session. Experiment 2 was designed to test this hypothesis.

Experiment 2(a) consisted of eight sessions. The initial five sessions were shaping. Session six was conditioning: amphetamine was given 15 min prior to the session. During the session, a 30 sec tone was presented five times after responding reached a specified level. The rates of licking for the 30 sec before each tone and the 30 sec during the tone were recorded. These values were used to calculate a suppression ratio for each presentation of the tone by dividing the number of licks during the tone by the number of licks during the tone + the number of licks in the 30 sec before the tone. Therefore, a suppression ratio of 0.5 indicated complete habituation, whereas a ratio of zero indicated complete suppression of responding. Session seven was the test: the tone was presented in the absence of amphetamine, and the rate of licking was recorded. All groups showed nearly complete suppression of responding after the first tone, but differed in their reaction to subsequent tones. The vehicle-treated group showed nearly

complete habituation to the tone by the fourth and fifth presentations, with suppression ratios of approximately 0.45. In contrast, the amphetamine-treated groups showed delayed habituation: although suppression ratios increased with each subsequent tone presentation, the increases were significantly smaller than those observed in the vehicle-treated group. The eighth session was a repeat of the seventh. In this session all groups of animals showed complete habituation. Thus, these results suggest that amphetamine delays habituation to a stimulus.

While habituation is often studied using unlearned behaviors such as startle response, habituation can also be studied by examining changes in operant responding. The work of McSweeney and colleagues (see McSweeney, 2004 for review) explains the systematic changes in rate of operant responding throughout a session in terms of sensitization and habituation to the reinforcer. It is typically found that subjects maintained on schedules of reinforcement that provide high rates of reinforcement (such as a variable-interval (VI) 15-sec schedule) show an initial high rate of responding followed by a gradual decrease over the course of the session. Schedules of reinforcement that offer low rates of reinforcement (such as a VI 240-sec schedule) typically result in an initial low rate of responding followed by an increasing or constant pattern over the course of the session. Schedules that offer intermediate levels of reinforcement (such as a VI 60-sec schedule) typically result in a bitonic pattern of responding: an initial increase in responding (reflecting sensitization to the reinforcer) followed by a decrease (reflecting habituation to the reinforcer) over the course of the session. The current study used a VI 60-sec schedule of reinforcement in order to produce this bitonic pattern of responding. Thus, changes in sensitization and habituation caused by amphetamine could be examined in part by determining how amphetamine changed the within session pattern of responding in this operant task.

Systematic changes in responding that occur over the course of an experimental session have been observed for a variety of behaviors and reinforcers, including drugs such as cocaine (Roll, McSweeney, Meil, Hinson, & See, 1996) and ethanol (Murphy, McSweeney, Kowal, McDonald, & Wiediger, 2006). In both of these drug studies, the pattern of operant responding within the session differed significantly between drug-reinforced and non-drug-reinforced rats, suggesting that drugs may alter the processes of sensitization and/or habituation. An operant approach for understanding amphetamine's effects on habituation based on within session changes in responding has not been attempted previously.

The present study also tested the "habituation disruption" theory of amphetamine effect using a two-session design to examine amphetamine's effect on spontaneous recovery. Previous research (McSweeney, 2004; Murphy et al., 2006) has shown that responding within a session, for a given reinforcer, decreases over the course of the session (responding habituates); but after a period of time with no reinforcers, responding increases (spontaneous recovery of responding). That is, spontaneous recovery is a property of habituation (McSweeney, 2004). Thus, habituation disruption can be tested not only by looking for an altered pattern of responding over the course of the first session, but also by analyzing the difference in spontaneous recovery from the first to second sessions between saline- vs. amphetamine-treated rats. It was predicted that saline-treated rats would respond at lower rates during the post-drug (second) session than during the drug (first) session. In contrast, it was predicted that amphetamine-treated rats would respond in the post-drug (second) session at the same rate they respond in the drug (first) session after being administered saline. That is, according to the habituation disruption theory, if amphetamine is disrupting habituation, in the post-drug session the subject will respond as if it had not experienced the drug session.

A 2-hr inter-session interval was chosen based on the duration of amphetamine's behavioral effects. The effect of amphetamine on motor activity has been shown to peak approximately 30 – 60 min after injection, and wear off by 135 min after injection for doses as high as 5.0 mg/kg (Gaytan, Swann, & Dafny, 1998; Mollenbauer, Jackson, & Pollack, 1983). It has also been shown that doses of amphetamine ranging from 0.8-2.4 mg/kg lose their ability to act as a discriminative stimulus at 120 min post-injection in a drug discrimination task in rats (C. N. Jones, Grant, & Vospalek, 1976). Brain and plasma levels of amphetamine after doses of 0.25-2.0 mg/kg decrease to very low levels by 2 hr after administration in rats (Maickel et al., 1969). Murphy et al. (2006) found that operant responding did not fully recover after a 2-hr inter-session interval. Thus, it was concluded that a 2-hr inter-session interval (post-drug session beginning 3 hr after amphetamine administration) would allow sufficient time for the direct effects of amphetamine to wane, while retaining a short enough interval so that complete spontaneous recovery of responding would be unlikely to occur in saline-treated rats.

To compare the effects of amphetamine on operant responding to its effects on an unlearned behavior, spontaneous locomotion, Experiment 2 was conducted. Locomotor activity was measured using a photobeam chamber. Sessions of equal length to those in Experiment 1 were conducted, again separated by a 2-hr inter-session interval, for each dose of amphetamine used in Experiment 1. Stereotyped behavior was also assessed during these sessions to determine at what doses and time points this behavior – which competes with lever pressing in operant sessions (J. Pinkston, personal communication, May 18, 2008) – occurs.

Experiment 1, Operant Testing

Method

Subjects

Twelve experimentally naive male Sprague-Dawley rats, bred in house from Taconic Farms (Germantown, NY) stock, were used as subjects. They were approximately 90 days old at the start of the study. They were housed individually and maintained on a 12:12-hr light/dark cycle, lights on at 6:00 a.m. In the home cage subjects had free access to water at all times and supplemental food as needed to maintain stable body weight throughout the study (Hurwitz & Davis, 1983).

Apparatus

Six identical Med Associates Inc. (MED Associates, St. Albans, VT) modular operant chambers were used. Each chamber was approximately 29 cm long, 24 cm wide, and 29 cm high, and was housed in a sound-attenuating enclosure. The chambers were equipped with two response levers centered 13 cm apart on the front wall and 7 cm above the grid floor, and required ~30 g force to register a response. Only the right lever was active in the current experiment. A 28-V DC lamp was centered 5 cm above this lever. Each chamber also contained a 28-V DC house-light, to provide general illumination. It was located at the top center of the back panel. The light above the lever and house light turned on to signal the beginning of the session. The house light remained on throughout the session. Every time a reinforcer was delivered the light above the lever turned off for 0.5 sec and then turned back on. Extinguishing the light above the lever and house-light signaled the end of the session. Reinforcers were collected from a rectangular opening (6.5 cm wide by 4.2 cm high), centered on the front wall between the levers with its bottom edge 2 cm above the grid floor. A ventilation fan in each

chamber provided white noise and masked extraneous sounds. Control of experimental events and data recording were conducted with MED Associates interfacing and programming.

Procedure

Testing was completed during the light part of the light/dark cycle between the hours of 10:00 a.m.- 3:30 p.m. The rats were trained to lever press for food (Noyes pellets, 45 mg) by being placed on a progressive ratio schedule for 10 hr. Reinforcers for all subjects were scheduled according to a VI 30-sec schedule on a Fleshler and Hoffman (1962) 25-interval series. To be sure that responding was stable, subjects were trained for at least 20 days on this schedule. Responding was considered to be stable when the session response rates from the last 5 sessions fell within the range of the first 15 sessions. Sessions were 60 min long.

Sessions were conducted five days per week. After stability was reached, two sessions per day were conducted on Tuesdays and Fridays, "treatment days." On all days other than treatment days, a single session was conducted. On treatment days saline or one of four doses of amphetamine (0.25, 0.5, 1.0, or 2.0 mg/kg) was administered 10 min prior to the start of the drug (first) session in the home room. The post-drug (second) session began 2 hr after completion of the drug session. Between sessions subjects were returned to the home cage. Each subject was tested with each dose two times. The order that the doses were administered was randomized for each subject, using the RAND() function in Microsoft Excel, with the constraint that subjects did not receive the same dose consecutively.

Drug

d-Amphetamine (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline. All injections were administered s.c. in volumes of 1.0 ml/kg.

Data Analysis

Responding was analyzed at the bin and session levels. For each rat, response rate (responses/min) at the bin level was calculated as the total number of responses emitted in a 5-min bin divided by five. Mean responses in a session was calculated for each rat by averaging responses across the 12 bins for each session at a given dose. At both bin and session levels, responses/min was then averaged across the two sessions conducted at each dose. Graphs depict the mean of all six rats.

To determine whether amphetamine affected rate of operant responding (in responses/min) differentially during the drug vs. post-drug sessions and differentially over time within each session, ANOVA was conducted with three repeating factors: time (bin, 12 levels), dose (5 levels), and session (2 levels), with significance level set at 0.05. For data collapsed over time, a 2-way ANOVA was conducted with two factors: dose (5 levels) and session (2 levels), with Dunnett's post hoc tests to determine which amphetamine doses differed from saline.

Additionally, to test the *a priori* prediction that responding in the post-drug (second) session for each dose of amphetamine would be different than responding during the drug (first) session for saline, paired samples t-tests were conducted on session response rates to compare responding during the drug (first) session after saline administration vs. responding during the post-drug (second) session after each of the amphetamine doses.

Results

Figure 1 shows rate of operant responding for drug and post-drug sessions at each 5-min time bin, at each dose of amphetamine. The repeated measures ANOVA indicated that the 3-way interaction between time, dose of amphetamine, and session was significant [$F(44, 220) = 2.33$, $p<0.001$]. That is, amphetamine's effect on operant responding depended on dose, time

within the 60-min session, and session (drug or post-drug). For example, in the drug (first) session, the lowest dose of amphetamine, 0.25 mg/kg, increased responding compared to saline, whereas the highest amphetamine dose, 2.0 mg/kg, decreased responding; in contrast, in the post-drug (second) session, the highest dose of amphetamine increased responding compared to the saline condition, in the first half of the session. In regard to amphetamine's effect on the within session pattern of responding in the drug session, responding after 0.5 mg/kg amphetamine did not decrease in the latter half of the session as it did with saline. In other words, there was a dose by time interaction when comparing the saline to the 0.5 mg/kg conditions within the drug session [$F(11,55) = 2.37$, $p = 0.018$].

Figure 2 shows operant responding collapsed over time, to better illustrate the amphetamine by session interaction [$F(4, 20) = 4.23$, $p = 0.012$]. In the drug (first) session, the lowest dose of amphetamine, 0.25 mg/kg, tended to increase responding compared to the saline condition, whereas the highest dose, 2.0 mg/kg, tended to decrease responding. In the post-drug session, amphetamine increased responding in a roughly dose dependent manner. The *a priori* comparison showed that responding after 1.0 mg/kg amphetamine in the post-drug session was increased compared to saline in the drug session [$t (5) = -5.43$, $p = 0.003$]. Thus, spontaneous recovery of operant responding in the post-drug session was not the same after amphetamine compared to saline treatment.

The pattern of increased responding in the post-drug session for progressively higher doses of amphetamine could have been due to a lingering motor stimulating effect of amphetamine. Experiment 2 was designed to assess the degree to which motor effects of amphetamine could still be detected at 3-4 hr post-injection, as well as the degree to which stereotypy may have interfered with operant responding in the drug session.

Experiment 2, Locomotor and Stereotypy Testing

Method

Subjects

Forty two experimentally naive male Sprague-Dawley rats were procured and housed in the same manner as in Experiment 1, but with free access to food and water at all times except during testing.

Apparatus

Locomotor activity was measured using a clear Plexiglas cage (20 × 40 × 23 cm) placed into a photobeam apparatus (Opto-Varimex, Columbus, OH); the 15 photobeams that crossed the width of the cage were 2.5 cm apart and 8 cm above the cage floor.

Procedure

The drug (first) session was conducted between 11:00 a.m.-12:30 p.m. The post-drug session commenced 2 hr later, between 2:00 - 2:30 p.m. Subjects were randomly divided into five groups of six rats (saline, 0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, and 2.0 mg/kg groups); thus each group was tested with only one dose.

Each rat was weighed, injected with saline or a single dose of amphetamine (0.25, 0.5, 1.0, or 2.0 mg/kg), and returned to the home cage. After 10 min, rats were taken to the room where the locomotor chambers were located and placed into the locomotor chambers. The number of photobeams broken was recorded every 5 min for 60 min. Duration and type of stereotyped behavior was identified by visual observation, and recorded at each 5-min interval. The observer was blind to treatment group assignment. The following behaviors were defined as stereotypy (Pechnick, Janowsky, & Judd, 1979; Roffman & Raskin, 1997):

- continuous sniffing

- continuous biting
- gnawing or licking
- repetitious head movement
- repetitious body or extremity movements
- repeated or extended rearing.

Upon completion of the drug session subjects were returned to their home cages. Two hr later, subjects were again taken to the room where the locomotor chambers were located and placed into the chambers for the post-drug session. The number of beam breaks was recorded as in the drug session.

Drug

Same as experiment 1.

Data Analysis

Locomotor activity was analyzed similarly to Experiment 1, by calculating the mean number of beam-breaks for each bin within a session, and mean responding per minute for the session as a whole, for each rat. ANOVA was conducted on the number of beam-breaks with two repeating factors (time and session), and one non-repeating (between-subjects) factor (dose: each rat received only a single administration of amphetamine). No stereotypy was noted in the first few rats examined during the post-drug session, so stereotypy was not scored in the post-drug session for the rest of the rats. Thus, a two-way ANOVA was conducted on the stereotypy data with time as a repeated factor and dose as a between-subjects factor. The *a priori* predictions that responding in the post-drug (second) session for each dose of amphetamine would be different from responding during the drug (first) session for saline was tested with a paired samples t-test, using beam breaks per min, averaged across all bins (collapsed across time).

Results

Figure 3 shows the effect of amphetamine on locomotor activity in each 5-min bin of the 60-min session, for drug and post-drug sessions. The ANOVA indicated that the 3-way interaction between time, dose of amphetamine, and session was significant [$F(44, 220) = 1.88$, $p < 0.001$]. This indicates that amphetamine's effect on locomotion depended on dose, time within the 60-min session, and session (drug or post-drug). For example, in the drug (first) session, amphetamine increased locomotion as compared to saline at some time points, whereas in the post-drug (second) session, locomotion was more comparable between previously saline- vs. amphetamine-treated rats, across all doses and time points.

Figure 4 shows locomotor activity collapsed over time to better illustrate the amphetamine by session interaction [$F(4, 20) = 13.28$, $p < 0.001$]. In the drug session, amphetamine increased locomotion in a roughly dose-dependent manner. In contrast, in the post-drug session, no dose of amphetamine significantly altered locomotion compared to saline.

Figure 5 shows the time engaged in stereotyped behavior during the drug session for each dose of amphetamine, in each 5-min bin of the 60-min session (no stereotyped behavior was noted in the post-drug session). Results of the ANOVA indicated that stereotypy increased over the course of the 60-min session [$F(11, 275) = 30.16$, $p<0.001$]. The amount of time engaged in stereotyped behavior also increased with the dose of amphetamine [$F(4, 25) = 24.55$, $p < 0.001$], and the time engaged in stereotyped behavior over the course of the 60-min session changed according to the dose that was given [dose x time interaction, $F(44, 275 = 3.29$, $p<0.001$]. For example, 2.0 mg/kg resulted in very rapid maximal engagement in stereotyped behavior, whereas 0.25 mg/kg took longer to produce peak durations of stereotyped behavior.

DISCUSSION

The purpose of the present study was to test the "habituation disruption" theory of amphetamine's effects on behavior. Disruption of habituation was tested in two ways: by determining whether the pattern of decreasing responding over the course of the drug (first) session was altered by amphetamine, and by comparing response rate for each dose of amphetamine in the post-drug (second) session to responding after administration of saline in the drug (first) session. There were mixed findings in support of the "habituation disruption" theory.

In regard to within session patterns of responding in the drug (first) session, the theory was not strongly supported in that at 2 of the 4 amphetamine doses, responding decreased over the course of the session similar to what was observed in the saline condition; thus habituation arguably occurred at some amphetamine doses. Other operant conditioning research with amphetamine has also shown a decrease in responding over the course of the session (Chiang et al., 2000). However, in the current experiment, the doses 0.5 mg/kg and 1.0 mg/kg resulted in steady responding across the session, suggesting that habituation of operant responding was disrupted at those doses.

In contrast to the within session effects of amphetamine, the analysis of spontaneous recovery did support habituation disruption. Whereas responding slightly declined from the drug session to the post-drug session in saline-treated rats (indicating incomplete spontaneous recovery of habituated responding), responding *increased* from the drug session to the post-drug session in rats treated with 1.0 or 2.0 mg/kg amphetamine. Responding in the post-drug session after administration of 1.0 mg/kg amphetamine was greater than responding in the drug session

for saline, which suggests that spontaneous recovery of habituated responding was disrupted at these doses.

There are alternative explanations for why responding increased in the post-drug session at the high doses of amphetamine, such as drug lingering in the brain at levels sufficient to induce higher rates of activity but not stereotypy. While it is true that there is residual amphetamine in the brain 3 – 4 hr after administration (Maickel et al., 1969), Kuczenski & Segal (1999) found that stereotyped behavior occurred after levels of dopamine and amphetamine in the brain had been reduced to levels that are not sufficient to initiate stereotyped behavior. This suggests that in the current study, lingering amphetamine in the brain in the post-drug session is not a sufficient explanation for the increase in behavior observed in that session after administration of 1.0 and 2.0 mg/kg amphetamine. On the other hand, the results with 0.25 and 0.5 mg/kg amphetamine counter the *a priori* prediction that responding in the post-drug session would be different from responding in the saline drug session: rats treated with these low amphetamine doses responded during the post-drug session as if they had not already experienced a session that day (not significantly different from responding after administration of saline in the drug session).

Another possible explanation for the increased responding in the post-drug session, compared to saline, is that responding did habituate, but had spontaneously recovered (completely) during the inter-session interval. However, previous studies on spontaneous recovery of habituated responding have shown that 2 hr is not enough time for habituated behavior to completely recover. Murphy (2003, experiment 3) showed that rats' responding for ethanol did not completely recover after a 2-hr inter-session interval. The saline condition of the current study likewise resulted in responding in the post-drug session that had not spontaneously

recovered to the level of responding in the drug session. Therefore, the increase in responding in the post-drug sessions after amphetamine was administered is probably not due to more rapid spontaneous recovery.

An alternative theory for explaining the increase in behavior after amphetamine's direct effects have subsided is drug-place conditioning. After repeated exposure to a place soon after a drug has been administered, the place can become a conditioned stimulus that elicits drug-like effects (Irwin & Armstrong, 1961; Tilson & Rech, 1973; van der Kooy, 1987). Thus, when the subject is then re-exposed to the place in a drug-free state, drug-like effects on behavior are elicited. This theory is supported by studies in which the behavior being measured was an unlearned behavior, such as exploring an environment (Ahmed, Oberling, Di Scala, & Sandner, 1996; Furlan & Brandao, 2001). However, in operant responding, using rats as subjects, drug-place conditioning has previously been shown only when the drug state was paired with a distinct stimulus (a red light) in the experimental chamber (Watanabe, 1990). Thus, it is unlikely that this sort of conditioning led to the observed results in the present study. The likelihood of developing drug-place conditioning was also decreased in the present study by having subjects respond during at least 20 training sessions in the experimental chamber while establishing stable responding, prior to administration of amphetamine, and administering amphetamine only two out of the five days a week that the rats were run in the chamber. Because the majority of the rat's exposure to the experimental chamber was in a drug-free state, latent inhibition should decrease drug-place conditioning. Bethus et al. (2006) found that amphetamine modulated latent inhibition only if administered before each pre-exposure session, or before the last pre-exposure session.

The results of Experiment 2 (motor activity) suggest that the increased operant responding observed in the post-drug session of Experiment 1 was not simply due to a general increase in motor activity. In the locomotor activity test, activity in the post-drug session was *not* increased in previously amphetamine-treated rats relative to the saline condition. In contrast to the elevated operant responding observed in amphetamine-treated rats during the post-drug session, locomotor activity *decreased* from the drug to post-drug sessions.

Observations made during the drug session of Experiment 2 confirm that at higher amphetamine doses, focused stereotypy was the predominant behavior from 10-70 min post-injection. Thus, it is likely that stereotypy interfered with operant responding in rats treated with 1.0 and 2.0 mg/kg amphetamine in Experiment 1, and that is why these rats showed decreased operant responding relative to saline-treated rats during the drug session. It should also be noted that observation confirmed that the large increases in photobeam breaks produced by 1.0 and 2.0 mg/kg amphetamine during the drug session were due in large part to repeated breaks of the same beam produced by stereotyped behaviors, rather than simply increased horizontal activity.

One theory on the effects of amphetamine on behavior (Lyon & Robbins, 1975) can account for the differences between the operant and locomotor data in the drug session. As applied to the current experiment, Lyon & Robbins' theory suggests that because lever pressing on a VI 30-sec schedule is a repetitive, focused behavior, it is expected to increase at low doses of amphetamine and decrease at higher doses. Lyon and Robbins also stipulate that unconditioned behaviors that require longer pauses, such as exploration of a novel environment, are reduced by low doses of amphetamine and further reduced as the dose increases. These behaviors are replaced by behaviors that include shorter and shorter response sequences, locomotion and rearing and then sniffing, licking, and biting. However, the preference for these

simpler, less perseverative behaviors as dose increases does not explain the increased operant responding seen in the post-drug session.

An alternative explanation for the high rate of responding in the post-drug session for 1.0 and 2.0 mg/kg may be related to amphetamine-induced hyperphagia. Studies have found that 2.0 mg/kg amphetamine nearly completely suppressed food consumption in the first hour after administration, but increased food consumption in the fourth hour (Caul, Jones, & Barrett, 1988; J. R. Jones & Caul, 1989), the time frame of the post-drug session in the present study. Thus, perhaps amphetamine-induced hyperphagia caused the increase in responding in the post-drug session. J. Pinkston (personal communication, May 18, 2008) found similar effects when water instead of food was used as the reinforcer for operant responding, which suggests that amphetamine may increase consummatory behavior in general.

Several limitations to this study should be noted. Because the rats in Experiment 1 were given more than one dose of amphetamine, sensitization to the drug may have changed responding. Behavioral sensitization to amphetamine has been demonstrated in many previous studies examining many different behaviors (Kuczenski & Segal, 1999; Robinson & Becker, 1986). The effects of sensitization were controlled for by administering the doses in a random order to each rat, but a better method may have been to give each dose only one time (as was done in Experiment 2). Or, each dose could have been administered six times, as sensitization has been shown to peak after four drug administrations (J. Pinkston, personal communication, May 18, 2008). Administering each dose multiple times also would increase the face validity of the study in terms of implications for effects of amphetamine on human operant responding. Alternatively, the locomotor experiment could have been done testing each amphetamine dose multiple times. Whichever method is chosen it would be important in the future to use the same

dosing method for operant and locomotor experiments so that differences in amphetamine effect on each type of behavior could be more directly compared.

The length of the inter-session interval could also be varied in future research to better interpret the effect of amphetamine on habituation of operant responding. Shorter inter-session intervals could reveal any changes in responding as the amount of amphetamine in the brain decreased. Longer inter-session intervals could be used to ensure that changes in responding were not due to residual brain amphetamine. However, the work of J. Pinkston (personal communication, May 18, 2008) supports the use of a 2-hr inter-session interval. Pinkston ran a 4-hr continuous session using 5 mg/kg amphetamine, and found similar results to the current study for the higher doses: an increase in stereotypy in the first hr after administration of the drug followed by an increase in operant responding in the fourth hr after administration of the drug. This suggests that at least at higher doses, a 2-hr inter-session interval was appropriate for encapsulating the range of amphetamine's behavioral effects.

The results of the current experiment showed a decreasing pattern of responding after administration of two of four doses of amphetamine in the operant experiment, and all doses in the locomotor experiment, thus demonstrating that habituation does occur after administration of amphetamine, in both operant responding and locomotion. However, because responding was stable rather than decreasing over the course of the session for 0.5 and 1.0 mg/kg, it could be argued that amphetamine disrupted habituation at these doses. In addition, the higher than expected rate of responding in the post-drug session, compared to responding in the saline condition, suggests spontaneous recovery of habituated operant responding was changed by high doses of amphetamine. Therefore, the present study provides evidence that, at some doses, amphetamine disrupts some properties of habituation.

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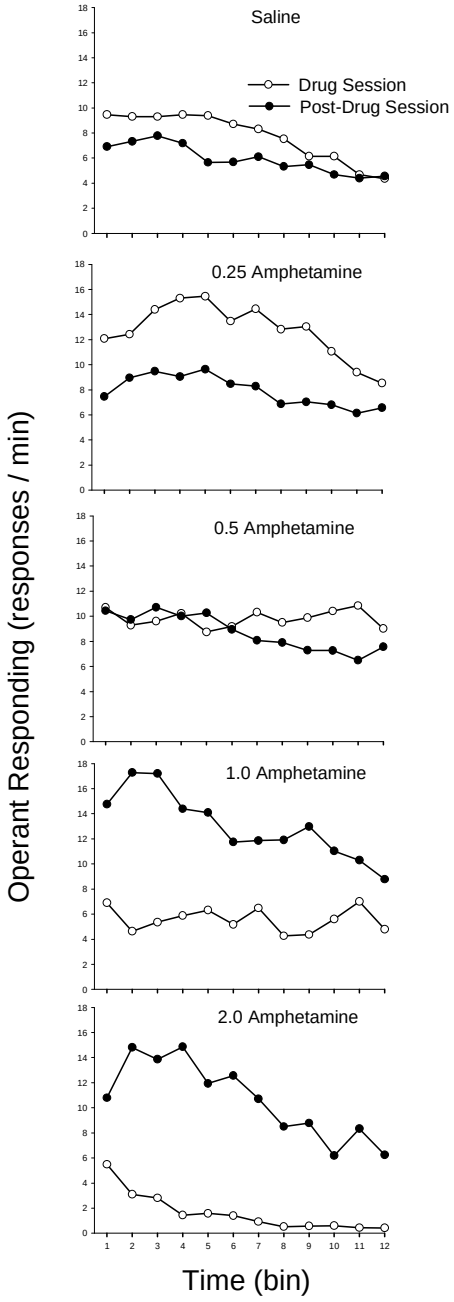

Figure 1. Rate of operant responding (responses/min) during successive 5-min intervals (bins) in rats responding in the drug (first) session (open circles) and post-drug (second) session (closed circles). Each session was 60 min long, and the inter-session interval was 2 hr. Each panel presents the results for a different amphetamine dose. Each point is the mean response rate of 6 rats.

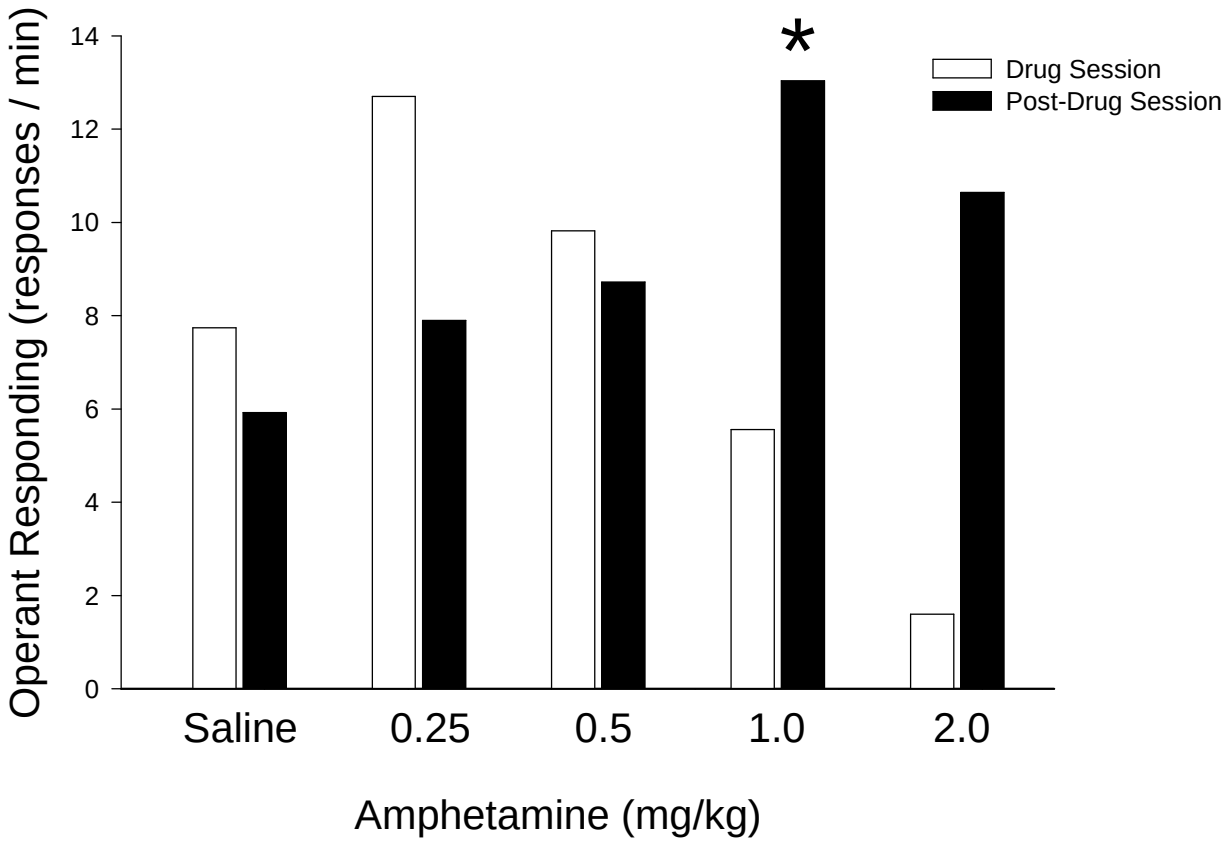

Figure 2. Average rate of operant responding collapsed across time for drug (open bars) and post-drug sessions (closed bars), for each dose of amphetamine, based on data from Figure 1. Each bar is the mean response rate of 6 rats. *responding significantly different from saline condition within same session, $p<0.05$, Dunnett's test.

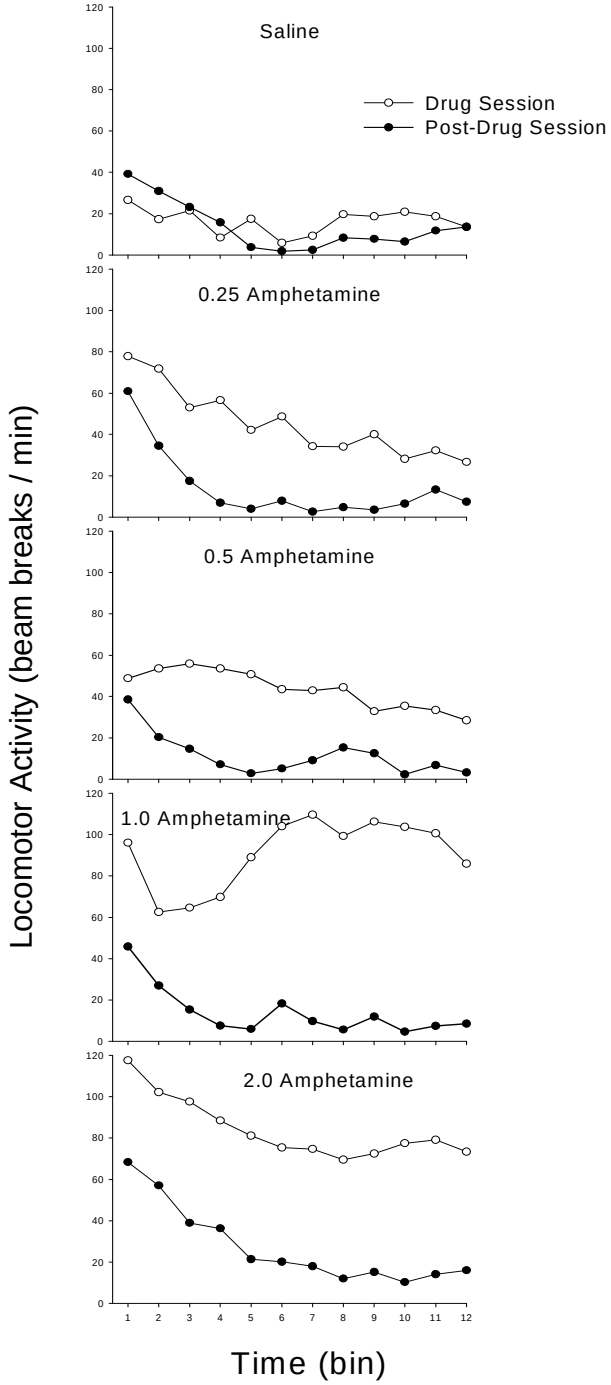

Figure 3. Locomotor activity (photobeam breaks/min) during successive 5-min intervals (bins) in rats responding in the drug session (open circles) and post-drug session (closed circles). Each session was 60 min long, and the inter-session interval was 2 hr. Each panel presents the results for a different amphetamine dose. Each point is the mean photobeam breaks of 6 rats.

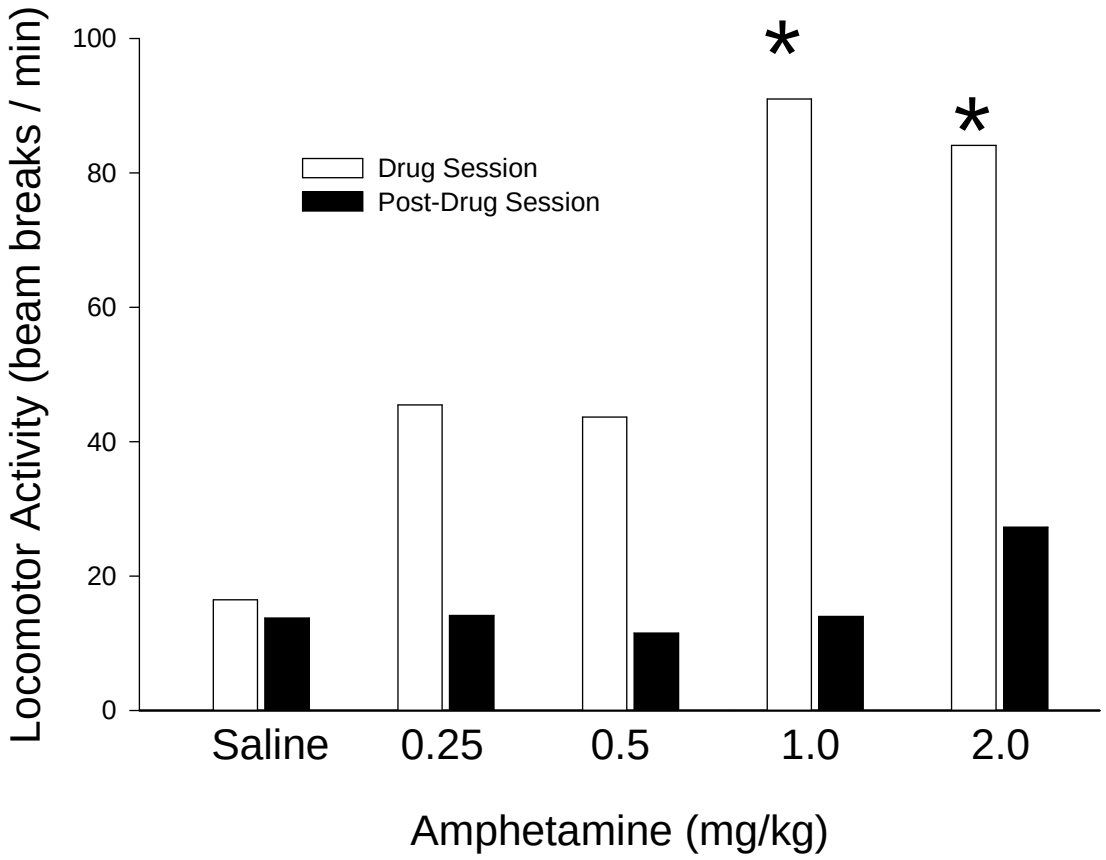

Figure 4. Average locomotor activity collapsed across time for drug (open bars) and post-drug sessions (closed bars), for each dose of amphetamine, based on data from Figure 3. Each bar is the mean locomotor activity of 6 rats. *locomotor activity significantly greater than saline condition within same session, $p<0.05$, Dunnett's test.

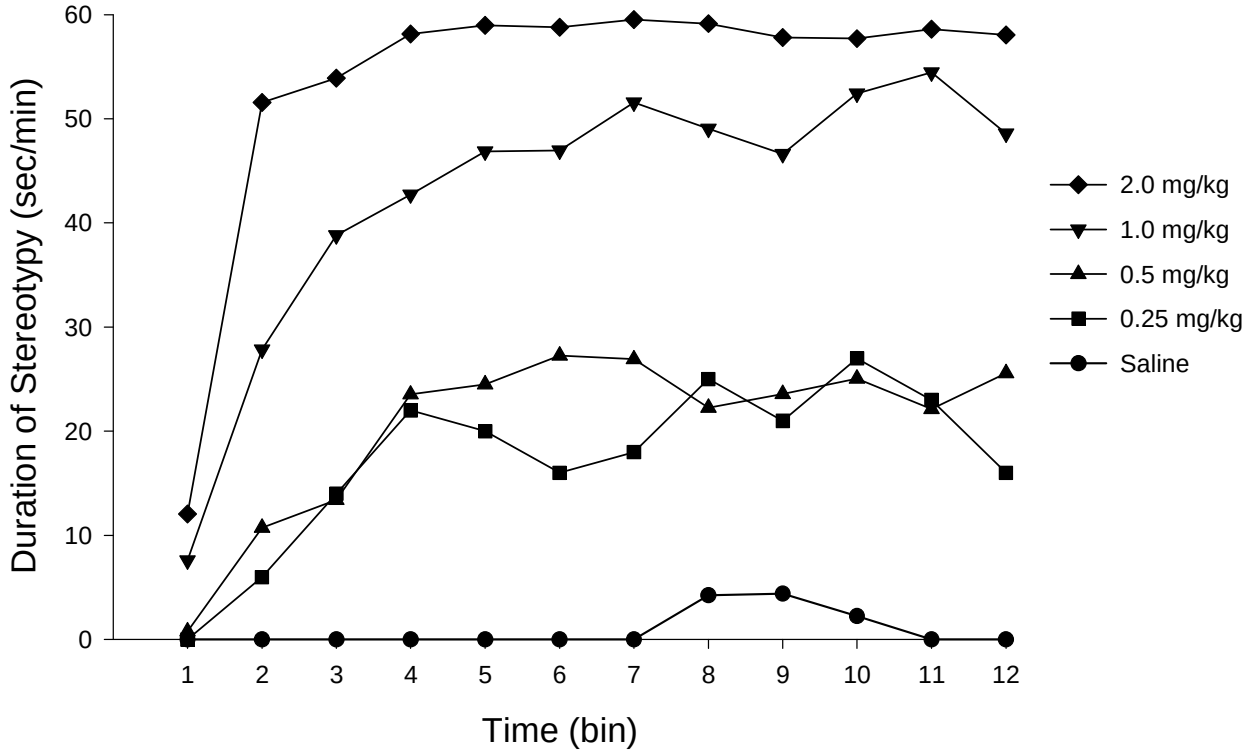

Figure 5. Time engaged in stereotyped behavior for each dose of amphetamine during the drug (first) session of the locomotor activity test. Each point is the mean duration of stereotyped behavior/min of 6 rats.