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Amphetamine Dose-Dependently Decreases and Increases Binge Intake of Fat and Sucrose Independent of Sex

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Abstract

Objective—Amphetamine was formerly used as a treatment to combat obesity, but amphetamine's use as an appetite suppressant was discontinued due to its significant abuse potential. Most of the rewarding and reinforcing effects of amphetamine differ by sex, with females showing higher levels of drug intake and amphetamine-induced motivation, relapse and locomotion, but it is unknown if amphetamine's effects on feeding also differ by sex. Furthermore, previous research on the anorexic effects of amphetamine has focused primarily on its effects on baseline, homeostatic feeding, but it is unknown whether amphetamine also affects hedonic, reward-related feeding, which is an important factor driving the rise in obesity levels.

Methods—Here we tested whether amphetamine alters food intake in a sex-dependent manner in two reward-related feeding paradigms, sucrose two-bottle choice tests and a high fat/high sugar binge intake model.

Results—Amphetamine altered food intake equally in males and females in both paradigms, with higher doses significantly inhibiting feeding and low doses of amphetamine increasing feeding at later time points.

Conclusions—Thus, amphetamine's effects on feeding and drug reward may be mediated by distinct mechanisms, which could allow for the development of new approaches to combat obesity with limited abuse and addiction-related side effects.

Keywords

amphetamine; feeding; binge; sucrose; fat; sex

Introduction

Historically, amphetamine was used to treat obesity until its abuse potential and addictive side effects precluded further use as a therapeutic aid to reduce feeding and body weight.

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The specific mechanisms responsible for amphetamine's ability to inhibit food intake have not been elucidated, however. Thus, identifying how amphetamine inhibits feeding and reduces body weight may allow for the development of new treatments to combat obesity without the negative side effects associated with amphetamine.

Much of the research examining the neural control of feeding has considered homeostatic feeding (i.e. feeding required to maintain normal physiological function), independent of the rewarding or hedonic aspects of feeding (1). It has become clear, however, that there are significant interactions and overlap in the neural circuits controlling homeostatic and reward-related feeding (1, 2). For example, although the mesolimbic dopamine system is the primary neural circuit mediating motivated and reward-related behavior, it also interacts with many of the neural circuits controlling homeostatic feeding (1, 2, 3), and it plays an important role in both reward-related (2, 4, 5, 6) and homeostatic feeding (2, 7, 8).

The rewarding and reinforcing effects of amphetamine appear to be due mainly to its ability to increase the extracellular concentration of dopamine through blockade of dopamine transporters and reverse transport of dopamine out of the cell and axon (9, 10, 11, 12, 13, 14, 15). Similarly, evidence indicates that amphetamine's effects on feeding are due primarily to its increase in extracellular dopamine levels (16, 17). Most of the studies examining the effects of amphetamine on food intake have focused on feeding under homeostatic conditions, however (16, 17, 18, 19), and it is unknown if amphetamine alters the rewarding and motivational aspects of feeding as well, which would be expected if amphetamine inhibits feeding through its elevations in extracellular dopamine.

There are significant sex differences in both the regulation of feeding, metabolism, and body weight (20), and in the responses to abused drugs such as amphetamine (21). Males and females differ in the peripheral and central mechanisms controlling feeding, metabolism, and body weight including their responses to various hormones, neurotransmitters and neuropeptides that control feeding, metabolism and body weight (20, 22). Similarly, sex differences in the responses to abused drugs have been observed in both humans and animal models, with females responding more strongly to drugs of abuse (21). Females have higher levels of drug intake, increased locomotor responses to psychostimulants, higher motivation for drugs, increased escalation of drug intake, increased withdrawal symptoms, and higher relapse and reinstatement to drug taking (21, 23, 24, 25). Thus, there are clear sex differences in both the regulation of feeding and in the responses to abused drugs such as amphetamine, but it is unclear whether there are sex differences in the ability of amphetamine to inhibit feeding. In these studies, we tested whether amphetamine inhibits feeding in a sex-dependent manner under reward-related conditions using both two-bottle choice tests for an appetizing sucrose solution and a high fat/high sugar binge intake model.

Methods

Reagents

Sterile bacteriostatic saline was from Patterson Veterinary Supply, Inc. d-Amphetamine was from Sigma. All other reagents were from common commercial sources.

Animals

Young adult male and female C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME, USA) were used for these experiments. Mice were 5–6 weeks old upon arrival and were approximately 10–12 weeks old during testing. Separate cohorts of mice (6 males and 6 females for each) were used for the sucrose two-bottle choice and binge intake tests. Mice were individually housed in ventilated polycarbonate Animal Care System cages in a temperature- and humidity-controlled room on a 12:12 light/dark cycle with *ad libitum* food (standard laboratory chow; Purina rodent diet #5001; 3.36 kcal/g) and water, except for the acclimation and testing periods. All protocols and procedures were approved by the Institutional Animal Care and Use Committee at Georgia State University and conformed to the National Research Council of the National Academies *Guide for the Care and Use of Laboratory Animals.*

Two-Bottle Choice test

For all two-bottle choice tests, mice were taken from their home room to a dedicated testing room and transferred to a conventional polycarbonate shoe box cage with normal bedding, a wire cage top, and no food. Mice were given access to two identical drinking bottles, one filled with normal water and the other filled with a 10% sucrose solution, and the positions of the bottles were alternated in each two-bottle access period to prevent the development of side bias. The bottles were modified 25 ml pipettes with attached sipper tubes that allowed for direct measurement of fluid volume at a resolution of 0.2 ml. For each measurement, the bottles were gently adjusted to a vertical position (without removing the bottle), the volume was recorded, and the bottle gently replaced to its original position. Measurement occasionally led to loss of a small amount of fluid (1–2 drops), which was not measured or accounted for. The caloric content of the 10% sucrose solution was 0.3872 kcal/ml. Mice were acclimated to the two-bottle test for a total of 9 sessions to ensure that their sucrose and water intake reached a stable level before test injections were administered (Figure 1A–B). The volumes of water and sucrose solution ingested were measured at 0.5, 1, 2, and 4 hours. At the end of the test, mice were returned to their home cage and home room where they had normal *ad libitum* access to water and chow until the next two-bottle choice test.

Binge Feeding Model

The binge intake tests were done in the home cage and home room. A pre-weighed amount of an appetizing high fat/high sugar diet was provided in a petri dish magnetically attached to the floor of the cage. The diet was comprised of 30% vegetable shortening, 20% sucrose, and 50% normal chow (ground to a fine powder) (26) and was made in the laboratory. The caloric content of the binge diet was 5.204 kcal/g. Mice had normal *ad libitum* chow and water access during the duration of the binge food access. Mice were acclimated to the binge access for a total of 9 sessions to ensure that their high fat/high sugar binge food intake reached a stable level before test injections were administered (Figure 1A,C). The amount of binge food eaten was measured at 0.5, 1, and 2 hours after food presentation. At the end of the test, the binge diet was removed until the next binge access session, with mice retaining *ad libitum* access to normal chow and water.

Experimental Design

The general timeline for the experiments is presented in Figure 1A. All tests were started early in the light phase. For both the two-bottle choice tests and the binge intake tests, mice were exposed to the test protocol for a total of 9 sessions during the acclimation phase and then 4 days per week (M-Th) during the testing phase. Thus, mice underwent the same two-bottle or binge diet access on the non-testing days (M-Th) throughout the testing period. Sucrose and binge intake during the acclimation phase are shown in Figure 1B–C for reference. For both tests, mice were injected with the different amphetamine doses in a counterbalanced order so that the order of doses tested differed between mice. For example, half of the male and half of the female mice received the doses in the following order: 2 mg/kg, saline, 1 mg/kg, 5 mg/kg, 0.5 mg/kg, whereas the other half of the mice received the injections in the following order: Saline, 2 mg/kg, 1 mg/kg, 0.5 mg/kg, 5 mg/kg. Injections were separated by 2–6 days (Figure 1A) to allow for recovery from the prior test before the next injection. Mice were injected intraperitoneally (IP) with d-amphetamine or saline in a volume of 10 μl/g body weight. A delay of 5–15 minutes occurred between the end of the amphetamine injection and the presentation of the sucrose/water bottles or the binge food. At the end of the test, mice were returned to their home cage and home room with a set amount of normal chow, which was measured daily during the testing phase. The researcher measuring sucrose and binge intake was blind to the amphetamine/saline treatments until the conclusion of the experiment.

Data Analysis

All data are presented as mean +/− SEM. Experiments were conducted using a within-subject design so that each mouse received all treatments. Total caloric intake in kcal for the sucrose solution, the binge diet, and post-test chow intake are presented in Supplementary Table 1 for comparison of total caloric intake between tests. Data were analyzed with Microsoft Excel and statistical analysis was performed using IBM SPSS Statistics 25 and SigmaStat (v11.0, Systat Software, Inc.). For analysis of both sucrose and binge intake, a general linear model with repeated measures analysis was used with drug dose, time, and sex as independent variables, followed by Sidak post-hoc tests corrected for multiple comparisons. For analysis of post-test home-cage chow intake, a two-way repeated measures ANOVA was used. A significance level was set at $p<0.05$ *a priori* for all analyses.

Results

We initially tested whether amphetamine affected the intake of sucrose using a two-bottle choice paradigm. After acclimating the mice to the two-bottle choice test (Figure 1A–B), mice were injected IP with different doses of amphetamine (0, 0.5, 1, 2, 5 mg/kg) and their sucrose and water intake was measured at intervals over the following four hours. Analysis of sucrose intake for all mice revealed significant main effects of dose ($F(4,44)=14.036$, $p=0.000$), time ($F(3,33)=239.793$, $p=0.000$) and a significant dose*time interaction ($F(12,132)=12.728$, $p=0.000$). Post-hoc analyses showed that the 1 mg/kg, 2 mg/kg, and 5 mg/kg doses all acutely inhibited sucrose intake, but the 1 mg/kg dose only inhibited intake at 0.5 hours, whereas the 2 mg/kg dose inhibited intake at both 0.5 and 1 hours, and the 5 mg/kg dose inhibited intake at 0.5, 1, and 2 hours, with the overall intake for each dose

returning to control levels at later time points (Figure 2A). Interestingly, the 0.5 mg/kg dose did not affect sucrose intake at early time points but led to a significant increase in intake at later time points (Figure 2A). The 1 mg/kg dose also appeared to cause an increase in intake at later time points, but this was not statistically significant. There was no effect of amphetamine on water intake for any of the doses tested (Figure 3).

We next analyzed whether males and females differed in the effects of amphetamine on sucrose intake. The effects of amphetamine on sucrose intake by sex are shown in Figure 2B–D, and the intakes for males and females at each specific dose are shown in Figure 4A–E. Although there were significant main effects of dose ($F(4,40)=14.297$, $p=0.000$), time ($F(3,30)=267.573$, $p=0.000$), and a significant dose*time interaction ($F(12,120)=13.305$, $p=0.000$), there were no significant effects of sex. Post-hoc analyses demonstrated that within each sex, there were dose-dependent effects that were similar to the effects seen when all mice were analyzed together (Figure 2B–D). For both males and females, the 2 mg/kg and 5 mg/kg doses significantly inhibited sucrose intake compared to the saline and 0.5 mg/kg injected mice of the same sex at 0.5 and 1 hour (2 & 5 mg/kg) and 2 hours (5 mg/kg). In addition, the 0.5 mg/kg and 1 mg/kg doses both increased sucrose intake at 4 hours compared to saline injected mice but only for males and not females. As can be seen in Figure 4A–E, there were no sex differences in sucrose intake for any of the doses tested. Finally, we also measured home-cage chow intake for the 20-hour period after the conclusion of the sucrose test, and there were no differences in chow intake for any of the doses tested (Figure 4F). Thus, amphetamine dose-dependently alters sucrose intake with higher doses of amphetamine inhibiting sucrose intake at different magnitudes and for differing lengths of time that were related to the dose (i.e. higher dose = greater and longer inhibition), and low doses of amphetamine causing a delayed increase in intake.

We next tested whether amphetamine inhibited the intake of a high fat/high sugar food in a binge intake model using a separate cohort of mice. After acclimating the mice to the binge-intake model (Figure 1A,C), mice were injected IP with different doses of amphetamine (0, 0.5, 1, 2, 5 mg/kg) and their binge intake was measured at intervals over the following two hours. Analysis of binge intake for all mice revealed significant main effects of dose ($F(4,40)=35.924$, $p=0.000$), time ($F(2,20)=394.168$, $p=0.000$), and a significant dose*time interaction ($F(8,80)=9.171$, $p=0.000$). Post-hoc tests demonstrated that both the 2 mg/kg and 5 mg/kg doses significantly decreased binge intake with the 2 mg/kg dose lasting for the first hour and the 5 mg/kg dose significantly decreasing intake for the full test (Figure 5A). There was also a trend for the 0.5 mg/kg dose to increase intake at 2 hours, but this did not reach statistical significance ($p=0.121$).

We next analyzed whether males and females differed in the effects of amphetamine on binge intake. The effects of amphetamine on binge intake by sex are shown in Figure 5B–D, and the intakes for males and females at each specific dose are shown in Figure 6A–E. There were significant main effects of dose ($F(4,36)=34.454$, $p=0.000$), time ($F(2,18)=383.625$, $p=0.000$), and a significant dose*time interaction ($F(8,72)=9.143$, $p=0.000$), but there were no significant effects of sex. Post-hoc analyses demonstrated that there were significant differences within each sex that were similar to when all mice were analyzed together. The 2 mg/kg and 5 mg/kg amphetamine doses significantly reduced binge intake at 0.5 and 1 hour

for both males and females compared to the same sex saline-injected controls (Figure 5B–D). Binge intake also appeared to be slightly increased for the 0.5 mg/kg dose at 2 hours for both males and females compared to their same sex controls, but these increases were not statistically significant. Despite the lack of a main effect of sex, planned post-hoc tests showed a significant difference in the effects of amphetamine between sexes for the 2 mg/kg dose of amphetamine (Figure 6), as females showed a significantly higher reduction in total cumulative binge intake at 2 hours (Figure 6D). This difference should be interpreted cautiously, however, as there were no main effects of sex. Finally, we also measured 24-hour home cage chow intake for the period following the start of the binge tests, and there were no differences in home cage chow intake for any of the doses tested (Figure 6F). Thus, amphetamine dose-dependently inhibited food intake in a binge-intake model, with the low dose of amphetamine showing a non-significant trend toward increased intake.

Discussion

In these studies, we have tested whether amphetamine inhibits feeding in a sex-dependent manner in two models of reward-based feeding, sucrose two-bottle choice tests and a high fat/high sugar binge intake model. The important results of these studies are as follows. First, amphetamine significantly inhibited the intake of both sucrose in the two-bottle choice tests (Figure 2) and the high fat/high sugar food in the binge intake model (Figure 5). Second, there were no sex differences in the response to amphetamine in these tests. Third, there was a bidirectional response to amphetamine, with higher doses decreasing intake and the lowest dose causing a delayed increase in intake.

A key result of these experiments was that amphetamine effectively inhibited the intake of both sucrose (Figure 2 & 4) and the high fat/high sugar binge diet (Figure 5–6). Amphetamine was previously used as a treatment for obesity in humans until its abuse potential was realized, and amphetamine has been widely shown to inhibit feeding in animal models. These previous studies focused on the ability of amphetamine to inhibit normal chow intake under baseline, homeostatic conditions, however (16, 17, 18, 19). Amphetamine appears to inhibit feeding through its elevation of dopamine levels (16, 17), and dopamine is highly involved in motivated and reward-related behavior and the rewarding, reinforcing, and addictive qualities of amphetamine. Therefore, it would be expected that amphetamine would also affect reward-related feeding, but this had not been tested to date. The studies presented here demonstrate that amphetamine does indeed inhibit the intake of appetizing foods under more reward-related conditions. There has been an increasing amount of evidence demonstrating that dopamine circuits are altered with obesity, and it has been proposed that alterations in reward-related feeding (‘hedonic’ feeding) have contributed to the rise in obesity levels (4, 5, 6). Thus, demonstrating that amphetamine can inhibit reward-related feeding in addition to homeostatic feeding is an important advance in our understanding of the neural control of feeding.

Although we attempted to isolate reward-related feeding in these studies, it is possible that the effects observed here were due to changes in basic consummatory behavior rather than reward. Multiple features of these studies were designed to attempt to focus on reward-related intake, however. For example, these studies were performed early in the light phase

when mice normally consume little food, which should have allowed for the rewarding qualities of the diets to be the main driver of their intake. The intake of the 10% sucrose solution also contributed a relatively small amount (~10%) to the mice's daily caloric intake (see Supplementary Table 1), suggesting that mice consumed the sucrose solution due to its palatability (and thus reward) rather than its caloric content. Feeding has both consummatory and rewarding components, however, even under the conditions used here. Thus, we cannot rule out potential effects on consummatory behavior independent of reward. Future studies directly testing effects on food reward, such as in conditioned-place preference or food self-administration assays, will be required to more conclusively demonstrate amphetamine's effects on reward-related feeding.

One of the most interesting results from these experiments was the lack of sex differences in the ability of amphetamine to inhibit sucrose or binge food intake. Although there was a significant difference in total cumulative intake between males and females for the 2 mg/kg dose at the 2-hour time point in the binge intake model, no other sex differences were observed. The specific difference for the 2 mg/kg dose should be interpreted cautiously, however, as there were no significant main effect of sex and no significant sex interactions in the original analyses. This overall lack of sex differences is surprising considering the fact that sex differences have been widely observed in both the neural and physiological control of feeding, metabolism and body weight (20, 22), and in the responses to abused and addictive drugs such as amphetamine (21). For example, female mice show increased amphetamine-induced locomotion (25) and increased cocaine-induced conditioned-place preference (CPP) (23) compared to males. Furthermore, multiple lines of research have demonstrated that females show increased drug intake, increased escalation of drug use, more motivation for drugs, higher withdrawal symptoms and enhanced drug reinstatement in both humans and animal models (21). Thus, there are clear sex differences in the rewarding, reinforcing, and abuse related effects of amphetamine, yet amphetamine inhibited feeding equally in females and males in both of the paradigms tested in these studies. These results indicate that the mechanisms by which amphetamine inhibits feeding are likely distinct from those that mediate its rewarding and reinforcing effects. Thus, it may be possible to target these pathways for new approaches to combat excessive feeding and obesity without the negative side-effects associated with amphetamine.

Some of the increased responses to amphetamine in females and some of the differences in the sex-dependent regulation of feeding appear to be due to sex hormones, as estrogen levels have been widely shown to influence different aspects of feeding (20), and both cocaine conditioning and dopamine circuit activity have been shown to be sensitive to estrus cycle stage (23). Thus, it is possible, that the lack of differences observed in these studies could have been influenced by the estrus cycle. Although we did not monitor the estrus cycle during these studies, it seems unlikely that differences in estrus stage could have resulted in the lack of sex differences observed in these studies, as the counterbalanced injections and timing of the different test days should have led to testing across different stages of the estrus cycle.

The doses of amphetamine that were effective at decreasing intake in these studies have also been widely shown to be rewarding and reinforcing and to induce both conditioned-place

preference and locomotor sensitization ((27) & see (28) and references therein). This corresponds well with the addictive potential of effective anorectic doses of amphetamine previously used in humans, which complicates the potential translational therapeutic potential of amphetamine. These studies indicate that there are likely differences in the mechanisms governing amphetamine reward *vs* anorexia, however. So determining the mechanisms of amphetamine's anorexic effects that are distinct from its rewarding qualities may allow for identification of new strategies to decrease feeding.

Another interesting result of these studies is that there was a bidirectional response to amphetamine, with higher doses inhibiting intake and lower doses actually increasing intake at later time points. For example, 0.5 mg/kg amphetamine increased sucrose intake in the two-bottle choice tests at both 2 and 4 hours post-injection, and the 1 mg/ kg dose showed a bidirectional response by itself, with a strong inhibition of intake at 0.5 hours, followed by a non-significant trend ($p=0.109$) toward increased intake at 4 hours post-injection (Figure 2). Similarly, the 0.5 mg/kg dose also showed a slight increase in intake in the binge model at 2 hours (Figure 5), although this was not statistically significant. These results are consistent with the complex role of dopamine in the control of feeding (2, 3, 4, 5, 6, 7), as increased dopamine release has been shown to both decrease and increase feeding, depending on the magnitude of the increase in dopamine and the stimulus driving its release. For example, low doses of amphetamine injected IP or into the nucleus accumbens increased feeding, yet higher doses injected into the same location inhibited feeding (18, 19). Similarly, a number of feeding-related peptides have been shown to cause reductions in feeding by either increasing or decreasing dopamine neuron activity (3). Thus, the results presented here are in agreement with previous studies showing that low doses of amphetamine, which likely lead to small increases in dopamine levels, increase feeding, whereas higher doses, which lead to robust increases in dopamine levels, inhibit feeding. It is also possible that the increased feeding caused by low dose amphetamine at later time points could have been due to compensation for reduced intake at early time points or increased energy expenditure caused by amphetamine induced locomotion. It appears unlikely that either of these was the cause of this increase, however. 0.5 mg/kg amphetamine did not decrease intake at early time points, which makes it unlikely that there would be a compensatory increase in intake at later time points. Also, although we did not measure locomotor activity in these studies, prior research has shown that the low doses of amphetamine that increase food intake have no effect on locomotor activity (18, 19). Thus, it is unclear how low doses of amphetamine cause a delayed increase in intake, and future studies will be required to examine this in more detail.

In summary, we have demonstrated here that amphetamine bidirectionally regulates feeding in two reward-related feeding paradigms, sucrose intake in two-bottle choice tests, and high fat/high sugar intake in a binge intake model, with low doses of amphetamine increasing intake at later time points and higher doses robustly inhibiting intake. In contrast to the established differences between females and males in the rewarding and reinforcing effects of amphetamine, there were no significant sex differences in the ability of amphetamine to inhibit intake of the appetizing, palatable food in these tests, indicating that the rewarding/ reinforcing and anorexic effects of amphetamine may be mediated by distinct molecular mechanisms. If confirmed, this may allow for the development of new therapeutic

approaches to combat obesity in both males and females with limited abuse potential. Further work will be required to identify the specific mechanisms mediating the anorexic effects of amphetamine independent of its rewarding and reinforcing effects, however.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Importance

- Amphetamine strongly inhibits baseline, homeostatic food intake, but it is unknown if it also affects reward-related feeding. Similarly, amphetamine's rewarding and reinforcing effects are sex dependent, but it is unknown if amphetamine's effects on feeding differ by sex.
- Here, we demonstrate that low doses of amphetamine increased feeding whereas higher doses inhibited feeding in 2 reward-related paradigms with no sex differences in amphetamine's effects on feeding in either paradigm.
- These results demonstrate that amphetamine has a bidirectional effect on feeding depending on the dose used and indicate that amphetamine's effects on feeding and drug reward may be mediated by distinct mechanisms.

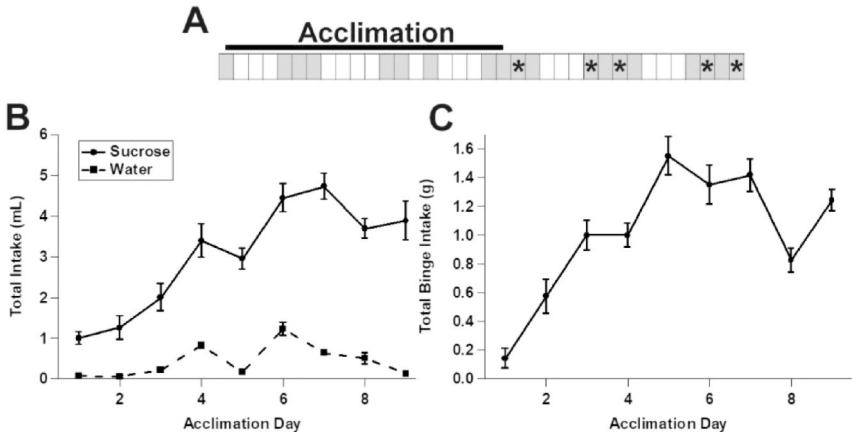

Figure 1:

Experimental timeline and intake during the acclimation phase. A. Timeline for the experiments. Each box represents an individual day. Gray boxes indicate days the mice underwent the sucrose 2-bottle and binge access tests, and days with a * indicate test days measuring the effects of amphetamine or saline injections. B-C. Mean total intake during the acclimation phase for the sucrose 2-bottle test (B) and the binge access test (C).

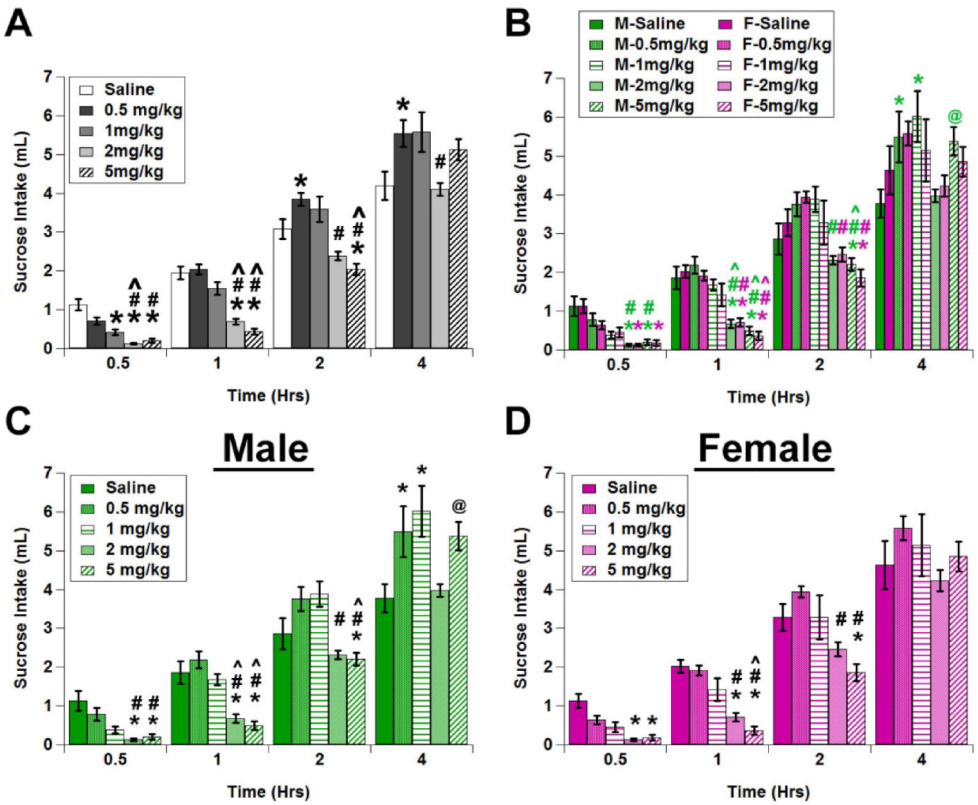

Figure 2:

Amphetamine significantly inhibited sucrose intake in two-bottle choice tests in both males and females. A. Mean cumulative sucrose intake for all mice after amphetamine treatment. B-D. Mean cumulative sucrose intake in males (B-C; green) and females (B, D; magenta) after amphetamine treatment. All: n=12, Male: n=6, Female: n=6. *p<0.05 vs Saline, #p<0.05 vs 0.5 mg/kg dose, ^p<0.05 vs 1 mg/kg dose, @p<0.05 vs 2 mg/kg dose. Green symbols in B reflect significant differences between doses for males, and magenta symbols in B reflect significant differences between doses for females.

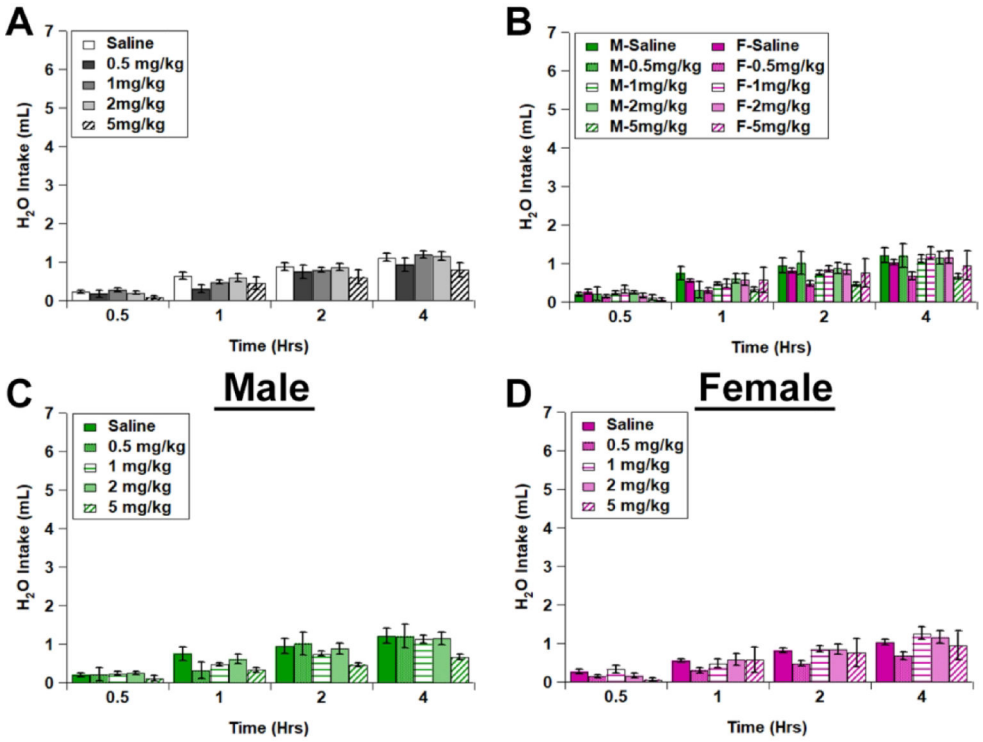

Figure 3:

Amphetamine did not affect water intake in two-bottle choice tests. A. Mean cumulative water intake for all mice after amphetamine treatment. B-D. Mean cumulative water intake in males (B-C; green) and females (B, D; magenta) after amphetamine treatment. All: n=12, Male: n=6, Female: n=6.

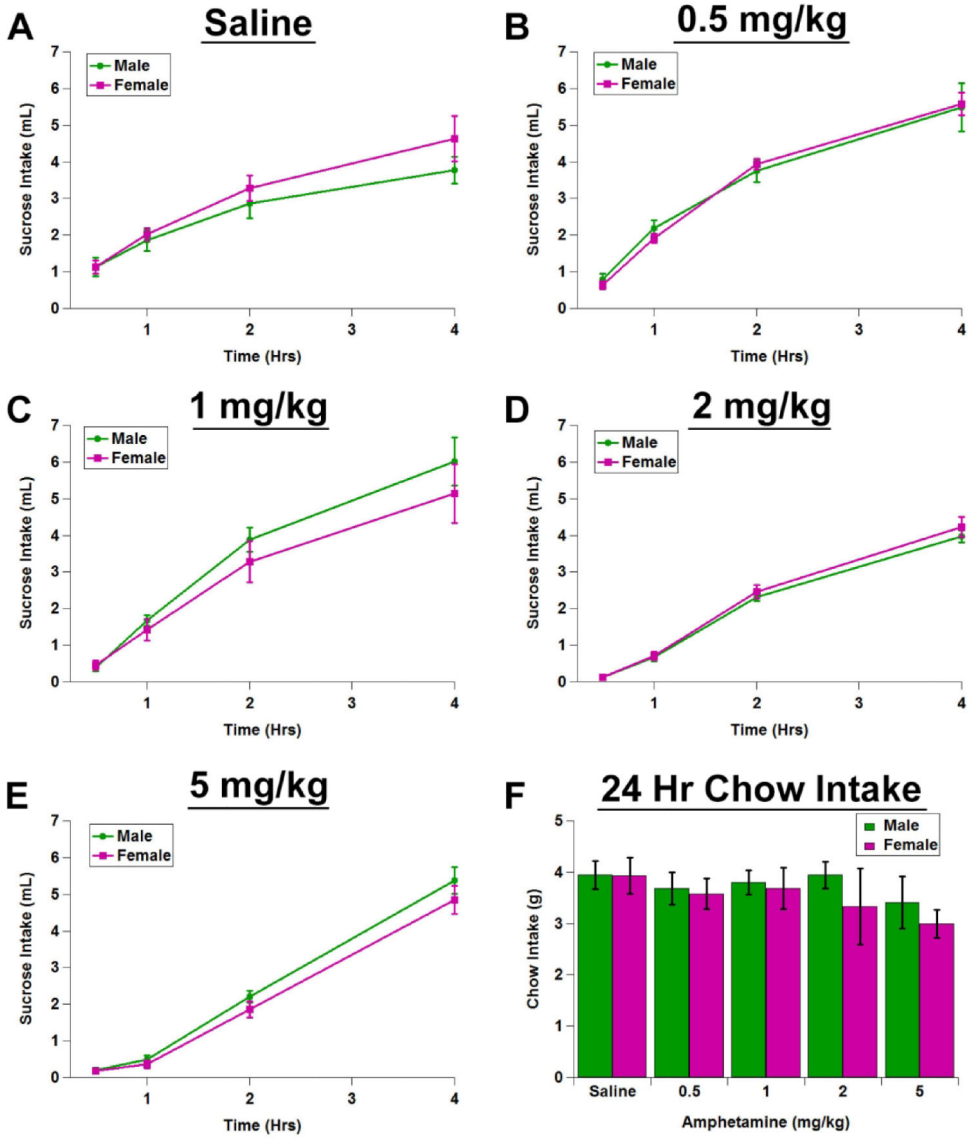

Figure 4:

All amphetamine doses inhibited sucrose intake equally in males and females and did not alter post-test home cage chow intake. A-E. Mean cumulative sucrose intake in male (green) and female (magenta) mice for the saline (A), 0.5 mg/kg (B), 1 mg/kg (C), 2 mg/kg (D), and 5 mg/kg (E) doses. F. Amphetamine did not affect post-test home cage chow intake at any dose in either males or females. Male: n=6, Female: n=6.

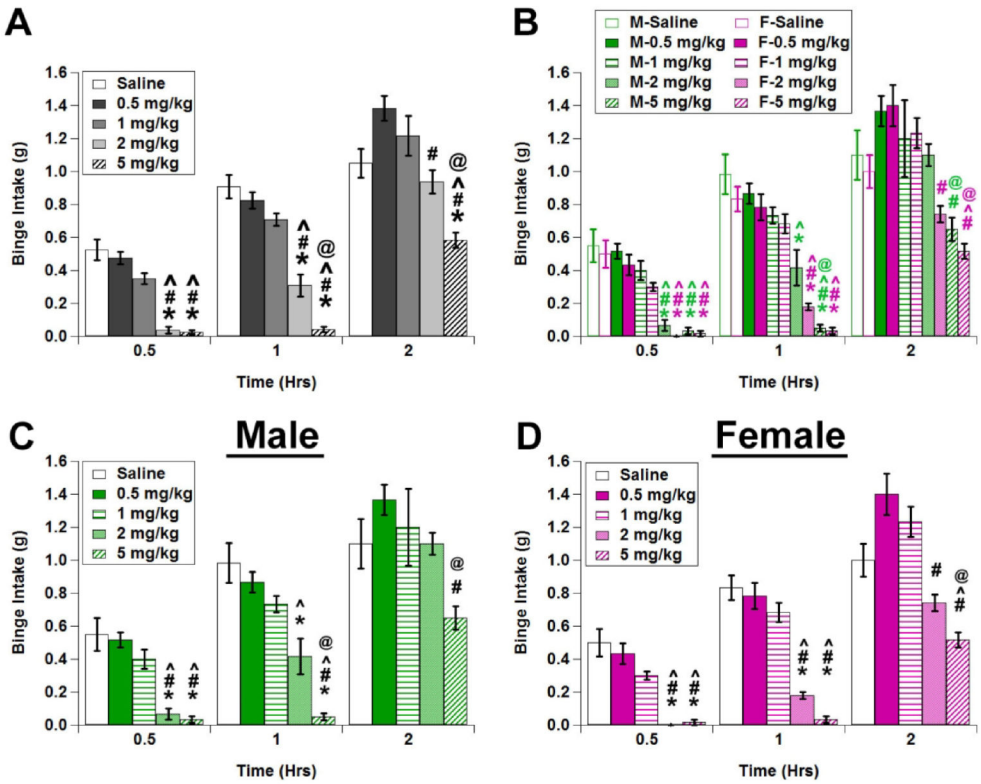

Figure 5:

Amphetamine inhibited binge intake in both males and females. A. Mean cumulative binge intake for all mice after amphetamine treatment. B-D. Mean cumulative binge intake in males (B-C; green) and females (B,D; magenta) after amphetamine treatment. All: n=12, Male: n=6, Female: n=6. *p<0.05 vs Saline, #p<0.05 vs 0.5 mg/kg dose, ^p<0.05 vs 1 mg/kg dose, @p<0.05 vs 2 mg/kg dose. Green symbols in B reflect significant differences between doses for males, and magenta symbols in B reflect significant differences between doses for females.

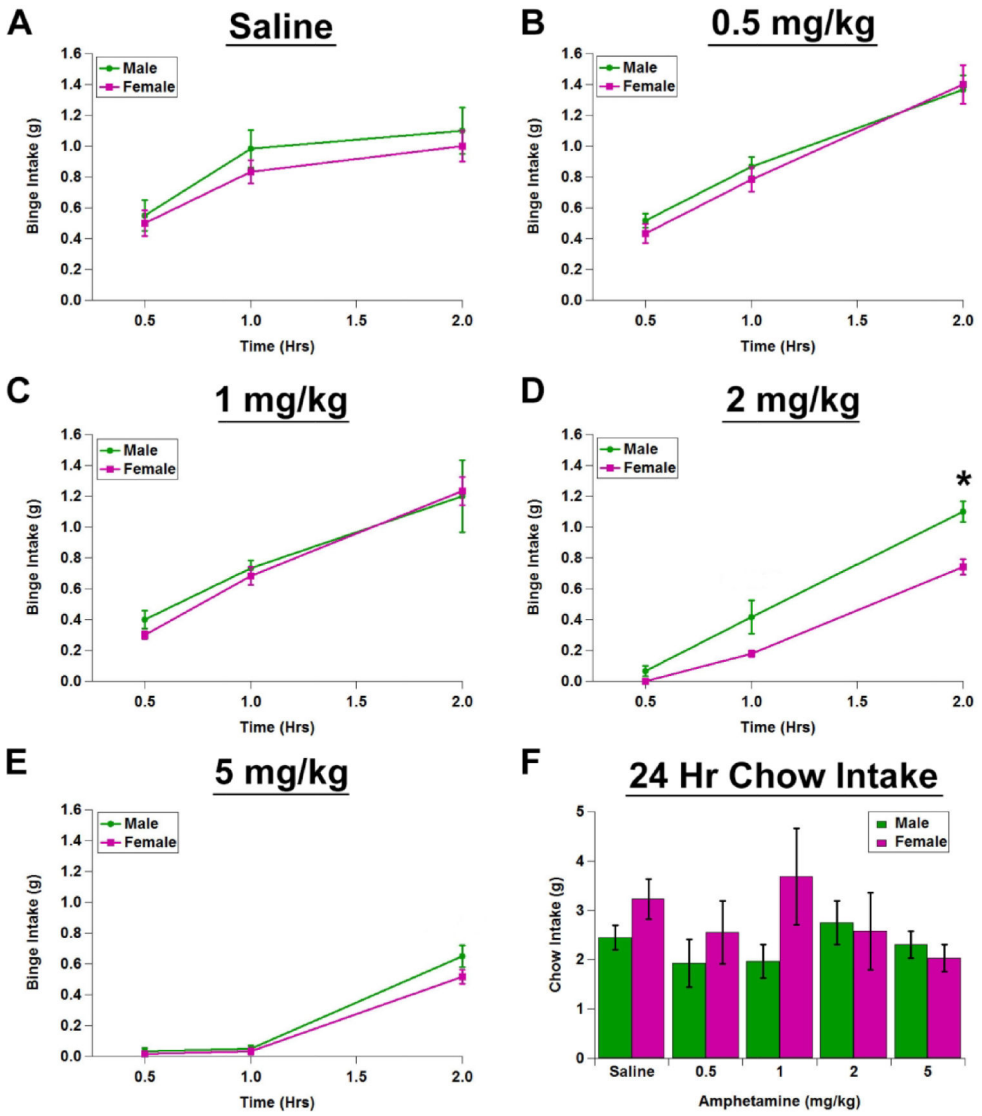

Figure 6:

2 mg/kg amphetamine significantly inhibited binge intake differently in males and females with no significant differences at any other dose. A-E. Mean cumulative binge intake in male (green) and female (magenta) mice for the saline (A), 0.5 mg/kg (B), 1 mg/kg (C), 2 mg/kg (D), and 5 mg/kg (E) doses. F. Amphetamine did not affect post-test home cage chow intake at any dose in either males or females. Male: n=6, Female: n=6. *p<0.05 male vs female.