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Nicotine and Amphetamine Decrease Sucrose Self-Administration

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Nicotine and Amphetamine Decrease Sucrose Self-Administration

by

Cameron Fulco

Under the Direction of Aaron Roseberry, PhD

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

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ABSTRACT

Limited research exists on how amphetamine and nicotine, drugs with potent influences on feeding behaviors, impact the rewarding and pleasurable aspects of feeding. As the rewarding aspects of feeding are thought to be significant drivers of the ongoing obesity epidemic, we sought to test 1-whether amphetamine or nicotine affect the rewarding aspects of food, and 2-whether there were sex differences in these effects. In these studies, we tested whether amphetamine or nicotine altered sucrose self-administration using an FR3 schedule. Amphetamine elicited a dose dependent decrease in responding for sucrose, whereas nicotine also decreased responding, but only with the highest dose tested. Furthermore, males and females showed similar responses to both drugs. These results suggest that the mechanisms regulating the addictive qualities and appetite-suppressing effects of these drugs may be distinct, indicating potential targets for future obesity therapeutics.

INDEX WORDS: Amphetamine, Nicotine, Food self-administration, Hedonic feeding, Sex differences, Dose dependency, Dopamine

Nicotine and Amphetamine Decrease Sucrose Self-Administration

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1   INTRODUCTION

Obesity is a progressively expanding health concern that affects over 500 million people worldwide (23). Epidemiological studies have demonstrated that weight increase to overweight and obese levels are key factors leading to a shortened lifespan. Furthermore, obesity causes and exacerbates other health problems such as diabetes mellitus, heart disease and sleep disorders (54). The rise in obesity over the years has been attributed primarily to altered feeding behaviors. Feeding behaviors can be broadly bifurcated into two primary components: feeding for need to maintain stable energy balance, which is also called homeostatic feeding, and feeding for pleasure or reward, which is also called hedonic feeding. A comprehensive understanding of how these different systems operate under normal and obese conditions, along with the impact of potential therapeutic strategies targeting feeding alterations in obesity, is critical in the development of effective interventions.

Homeostatic feeding is largely driven by the need to maintain energy balance in the body. Historically this aspect of feeding was primarily thought to be regulated by the hindbrain and ventromedial hypothalamus. In classic models, gastrointestinal and other peripheral signals would alert these brain areas about energy levels throughout the body, initiating food seeking behaviors in instances of low energy stores or increasing energy expenditure in instances of high energy states (48). This model contrasts with hedonic feeding, which pertains to the motivational and rewarding aspects of feeding—i.e. consuming highly palatable foods even in times of high energy stores. Historically this feeding aspect was believed to be regulated primarily by limbic and paralimbic brain systems linked with motivation, reward, and cognitive behavioral control such as the orbitofrontal cortex, medial prefrontal cortex, amygdala, and striatum (48). While these feeding aspects have often been considered separate, recent research suggests that the

brain's homeostatic and hedonic systems are not entirely distinct (24). Therefore, studying each

of these systems in isolation as well as how they interact is important for understanding how to

combat the altered feeding systems associated with obesity.

1.1   The Mesocorticolimbic Dopamine System

The mesolimbic dopamine system (MLDA) and mesocortical dopamine system (MCDA)

have been implicated in both the hedonic and homeostatic control of feeding. The MLDA is

primarily associated with reward-related processes such as incentive salience, pleasure response

and reinforcement which can influence the neuronal regulation of feeding behaviors. In the

MLDA, dopamine is synthesized in the ventral tegmental area (VTA) and projects to the nucleus

accumbens of the striatum, amygdala, hippocampus, and olfactory tubercle. The MCDA,

associated with cognitive control and motivation, also influences feeding behaviors regulation.

Here, dopamine is synthesized within the VTA and projects to the prefrontal cortex (72).

Although it is understood that dopamine plays a primary role in reward-related cognition in these

areas, the specifics become more complex depending on the distinct neural circuit and dopamine

receptors involved. Despite this fact, research supports the idea that reward salience is linked to

the amount of dopamine within the synaptic cleft and postsynaptic dopamine receptor D2

densities within these regions (67).

Strong evidence confirms that the MLDA and MCDA systems are heavily involved in the

regulation of hedonic and homeostatic feeding. For instance, dopamine levels in the nucleus

accumbens (69), amygdala (70), and prefrontal cortex (71) rise in response to feeding.

Experiments reveal that dopamine within these areas is necessary for the modulation of feeding

behaviors. When rats are administered with the neurotoxic compound, 6-hydroxydopamine,

which induces apoptosis in catecholaminergic neurons, free feeding is dramatically impaired and

bodyweight decreases (39, 40). In transgenic animals of which tyrosine hydroxylase, the enzyme which synthesizes dopamine, is removed, eating and drinking behaviors are substantially reduced. However, these behaviors are restored when these animals are given the dopamine precursor, L-DOPA (41, 42). Although dopamine is important for feeding, an excess of dopamine in the MLDA and MCDA pathways inhibits feeding. Experiments which flood synapses with dopamine by amphetamine, prevent dopamine reuptake via cocaine, amphetamine, or other DAT inhibitors, or broadly activate dopamine receptors with exogenous all inhibit feeding behaviors (41).

The modulation of feeding by dopamine is also affected by metabolic hormones. For example, circulating signal hormones such as leptin and ghrelin predominantly regulate homeostatic feeding behaviors through the hypothalamus. However, these hormones have also been shown to regulate feeding though the VTA (35, 36, 37). Activation of dopamine receptor D2 (D2R) via a D2R agonist, bromocriptine, results in reduced levels of leptin in obese individuals (38).

There is also evidence from human studies that the MLDA system can affect feeding and could potentially contribute to conditions with altered body weight, such as obesity. Individuals with obesity exhibit a disruption of the MLDA system, potentially leading to the perpetuation of the disease (19). For example, PET imaging studies show that individuals with obesity display low baseline DA and decreased D2R availability in the striatum which are negatively correlated with BMI (20, 79). Interestingly, similarly reduced levels of D2Rs in the striatum have been observed in in individuals addicted to drugs of abuse such as cocaine and opiates (81, 82). Individuals with obesity are more likely than the general population to have a family history of drug abuse (80) and low levels of striatal D2Rs have been demonstrated to lead to excessive food

intake (21). Given that eating increases dopamine release, pathological eating may be a means to compensate for decreased reward circuit activation (79).

1.2 Amphetamine

While there are some effective treatments for obesity such as bariatric surgery and GLP1 analog drugs, the pursuit of additional and potentially more accessible treatment options remains essential. Given the complexity of obesity and its impact on other health problems it is important to diversify the therapeutic options available for treatment of the disease. Given the significance of the MLDA system in the control of both homeostatic and hedonic feeding and its changes associated with obesity, it may be a good target for new approaches to combat obesity. Several drugs of abuse, which act primarily through the mesolimbic dopamine system, have also been shown to affect feeding (1-2).

Amphetamine (AMPH), for example, was one of the first drugs used to treat obesity. However, due to its potential for addiction and harmful side effects, it was removed as a treatment option. Thus, understanding how AMPH reduces feeding may help us develop new effective treatments to reduce food intake without its addictive and other harmful effects. AMPH acts on central neurons to increase extracellular norepinephrine, dopamine, and serotonin. It increases extracellular dopamine via two avenues. First, AMPH promotes vesicle binding to release dopamine. Second, AMPH reverses the action of dopamine transporters (DAT) on the presynaptic terminal so that instead of retaking dopamine into the neuron terminal, dopamine is released in the cleft via the transporter (73). AMPH's anorexic effect has been attributed to its increase of DA release in the striatum and subsequent inhibitory action on the activity of orexigenic neuropeptide Y (NPY). AMPH has been shown to reduce the expression of NPY at translational and post translational levels (43,44). Furthermore, AMPH administration to the

lateral hypothalamus, a region implicated in the control of feeding behaviors, also decreases food intake (45). Finally, lesioning of the nigrostriatal dopamine system also leads to an attenuated AMPH-induced anorexic response (46). Thus, the wide effects of amphetamine have made it difficult to identify the exact mechanisms by which AMPH decreases feeding.

These and other prior studies focused primarily on amphetamine's effect on homeostatic feeding and did not test whether amphetamine also affects feeding under hedonic conditions (8-12). Our lab has previously shown, in a study conducted by West et al., supporting evidence that AMPH has a dose-dependent bidirectional effect on two models of hedonic feeding. In this study, AMPH was shown to increase hedonic feeding with low dose AMPH administration and decrease hedonic feeding with high dose AMPH administration. However, it is unclear if these results were due to changes in consummatory behavior or the rewarding nature of food, however (7, 13). AMPH also has been shown to reduce DA reuptake in high fat diet (HFD) conditions which may contribute to changes seen in binge feeding models (14). Furthermore, dopamine release in the dorsal striatum (DS) has been shown to encode the nutritional value of food reward whereas release in the nucleus accumbens (NAc) may encode the hedonic value (47). Studies have already shown AMPH contributions to obesity treatment; manipulations of AMPH's side chain and ring structure have shown decreased chances of abuse while maintaining its appetite suppression qualities (11). However, this drug was linked to valvular heart disease and pulmonary hypertension and thus removed from public availability.

1.3   Nicotine

Nicotine is another widely abused drug that both acts on the MLDA system for its rewarding and reinforcing properties and has been shown to decrease feeding (29, 49, 50). Stimulation of proopiomelanocortin (POMC) neurons by nicotine leads to increased circulating

levels of α-MSH and adrenocorticotropic hormone, both peptides which lead to decreased feeding behaviors (51, 52, 89). Nicotine may also regulate levels of circulating leptin and NPY to modulate feeding behaviors (49, 53, 55). Similar to amphetamine, little research has been done into the effects of nicotine on reward-based feeding however, as most studies have focused on its effect on homeostatic regulation of feeding. Nicotine produces widespread activation of monoaminergic areas throughout the central nervous system including the lateral hypothalamus which may largely play into nicotine's anorexic effect on homeostatic feeding (56). However, nicotine also activates DA neurons in the VTA which may lead to hedonic control of feeding as well, but this remains to be determined (57).

1.4   Sex Differences

It has been widely reported that there are robust sex differences in both the control of feeding and body weight and the mesolimbic dopamine system and the responses to abused drugs. Preclinical trials have shown that when females are presented with palatable foods, they exhibit a heightened activation of neural reward circuitry (74). Moreover, female rats are more likely to develop binge eating disorder when presented with intermittent highly palatable food (75). In humans, females are a far greater risk to develop eating disorders with the female-to-male ratio varying from 2:1 to 10:1 depending on the specific disorder (76).

Animal models examining drugs of abuse indicate that females tend to acquire self-administration more rapidly and take larger amounts of drugs after acquisition (27). Both AMPH and nicotine have also shown sex-based differences in modulation of the DA system. AMPH induces increased locomotion of both ambulation and rearing with a more significant effect in females versus males. Males contain higher levels of D1Rs in the stratum, and females show blunted striatal DA release versus males when given AMPH (3, 5, 34). Similarly, nicotine seems

to lead to an increase of energy expenditure in females that is not seen in males (29). Females also show greater conditioned place preference to nicotine administration, while males show greater response to the pharmacological aspects of nicotine at high concentrations (4). Females also show changes to AMPH responses based on the estrus cycle. Female mice in estrus display increased DA release in the striatum during estrus compared to non-estrus cycles and display more intense AMPH-induced behaviors during estrus (77). Alongside the sex differences observed in multiple aspects of the control of feeding and energy homeostasis, these differences in drug responses suggest the possibility that amphetamine and nicotine could show sex-dependent effects on hedonic feeding as well, but this is unknown.

AMPH and nicotine clearly influence DA pathways and impact feeding partly through their actions on these pathways. However, more research is needed to investigate their effects on reward-based feeding. In this study, we examined whether AMPH and nicotine dose-dependently and/or sex-dependently affected hedonic feeding using a food self-administration model. We hypothesized that high doses of AMPH and nicotine would reduce sucrose SA while a low dose of AMPH would enhance SA feeding. We also hypothesized that female mice would show greater sensitivity to the effects of these drugs compared to male mice.

2   METHODS

2.1   Animals

Young adult male and female C57Bl/6J mice (N=29, M=15, F=14) (Jackson

Laboratories, Bar Harbor, Maine) were used for these experiments. Mice were ten weeks of age

at the start of all experiments and were housed on a reverse light-dark cycle (11:00am lights off

and 11:00pm lights on) with *ad libitum* access to standard chow and water throughout the study.

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.*

2.2   Sucrose Self Administration

Mice underwent operant conditioning (food self-administration) for 20 mg sucrose pellets

in these studies. Food self-administration trials (SA) were administered in operant test boxes

enclosed in sound-attenuating chambers (Med Associates, Fairfax, Virginia). The chambers

contained two nose poke ports with a food receptacle located between them. Trials consisted of

one-hour sessions. During the trials, both nose poke port lights, the house light, and a 2900 Hz

tone were on except during timeout. Entry into the active port led to the delivery of one 20mg

sucrose pellet to the receptable port and was accompanied by activation of the receptacle port

light, whereas activation of the inactive port had no consequence. Pellet delivery resulted in a 20

second timeout, where the house light, tone, and nose poke port lights turned off and a light in

the food receptacle turned on. The food port light was only illuminated until a head entry was

detected into the port. The light tone and nose poke port lights remained off for the full 20

seconds of the time out period. Nose poke entries into the active port during the time out period

were recorded but did not have any consequence. Mice underwent self-administration sessions 5 days/week (M-F).

At the onset of self-administration training mice first received a single 1-hour magazine training session. During magazine training mice were placed into the operant chambers with cues and timeout mimicking that of the SA trials. However, mice did not need to poke for delivery of a sucrose pellet; instead one pellet was delivered automatically every two minutes for the duration of the trial. The mice were then shifted to a fixed ratio (FR) 1 schedule, where one poke of the active port resulted in the delivery of one pellet, until a 3:1 active port to inactive port interaction was reached with less than 20% variance in the number of pellets eaten between sessions. Upon reaching these criteria, mice were moved to a FR3 schedule, where three pokes of the active port resulted in the delivery of one pellet until stable responding was obtained at this ratio (< 20% variance of eaten pellets over two days). Once this criterion was reached for the FR3 schedule, mice underwent experimental testing. Test days were separated by 3-4 days (i.e. Tuesday and Friday) and mice continued FR3 sessions on non-test days (M-F). On test days, mice received intraperitoneal injections (IP, 10 microL/g body weight) of saline or drug 10 minutes prior to the onset of SA testing. The order of injections was counterbalanced so that half of the mice received injection of a single dose of drug on each test day, and all mice received all drug doses in different order. The experimenter was blind to the doses during treatment but became unblinded for analysis of results. Mice were tested in multiple individual cohorts, with both males and females included in each cohort.

2.3   Lavage

Vaginal cells were collected once a day for three days during the experimental phase for each group of female mice. The method used in this study matched that proposed by McLean et

al. (2012). Cells were flushed by administering a small amount of distilled water through a pipette and placing a few drops of cell suspension on a glass slide for observation. The droplets were allowed to dry before observation via microscope. Leucocytes, cornified epithelia cells and nucleated epithelial cells were all identified and compared to estrus stage identified in McLean et al. (2012) for confirmation of estrus stage.

2.4   Data Analysis

All data are presented as mean ± SEM. Experiments were conducted using a within-subjects design so that each mouse received all treatments. Data was compiled using Microsoft Excel, analyzed using Python, and statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, New York). For analysis of both consumed pellets and correct nose pokes, a linear mixed model was used, with drug dose and sex as independent variables, followed by a Sidak post hoc tests for multiple comparisons.

3   RESULTS

In this study, we tested whether amphetamine or nicotine affected sucrose self-administration. Upon successful acquisition to the food self-administration paradigm (Figure 1), mice were injected i.p. with either amphetamine (0, 0.5, 1, 2, 5 mg/kg) or nicotine (0, 0.1, 0.5, 1, 2, 5 mg/kg), and the amount of active and inactive nose pokes, and pellets obtained and consumed were measured.
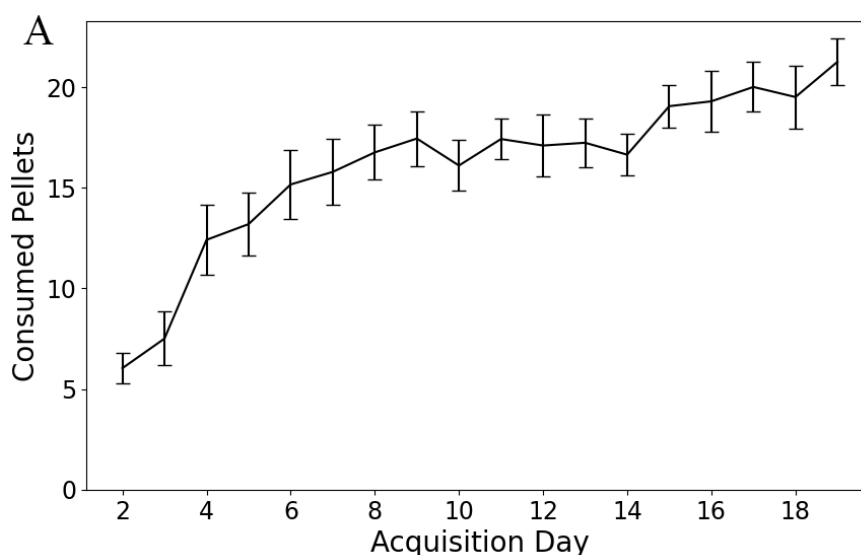

Figure 1. Intake during the acquisition phase.
(A) Mean total sucrose pellet consumption during the acquisition phase. Day 1-14: n=29; Day 15: n=22; Day 16: n=16; Day 17-19: n=8. Mice were run through acquisition trials until all responding mice reached stable levels of responding.

In the amphetamine trials, an analysis of active nose pokes across all mice (independent of sex) revealed a main effect of dose ($F_{4,37}=109.176$; $p=<.001$). Post hoc analysis revealed that 1 mg/kg, 2 mg/kg, and 5 mg/kg effectively reduced operant nose poking for sucrose pellets (Figure 3A). A similar effect was found in the analysis of sucrose pellet consumption, with a main effect of dose as well ($F_{4,35}=119.989$; $p=<.001$). Once again, post hoc analysis revealed that 1 mg/kg, 2 mg/kg, and 5 mg/kg all reduced consumption of sucrose pellets. (Figure 2A) However, there was

no statistical effect of 0.5 mg/kg dose on either consumption or operant nose poking (Figure 2A

& 3A). Last, analysis of food port entries, a measure of food seeking motivation, revealed a main

effect of dose ($F_{4,37}=13.844$; $p=<.001$). Post hoc analysis revealed that 1 mg/kg, 2 mg/kg, and 5
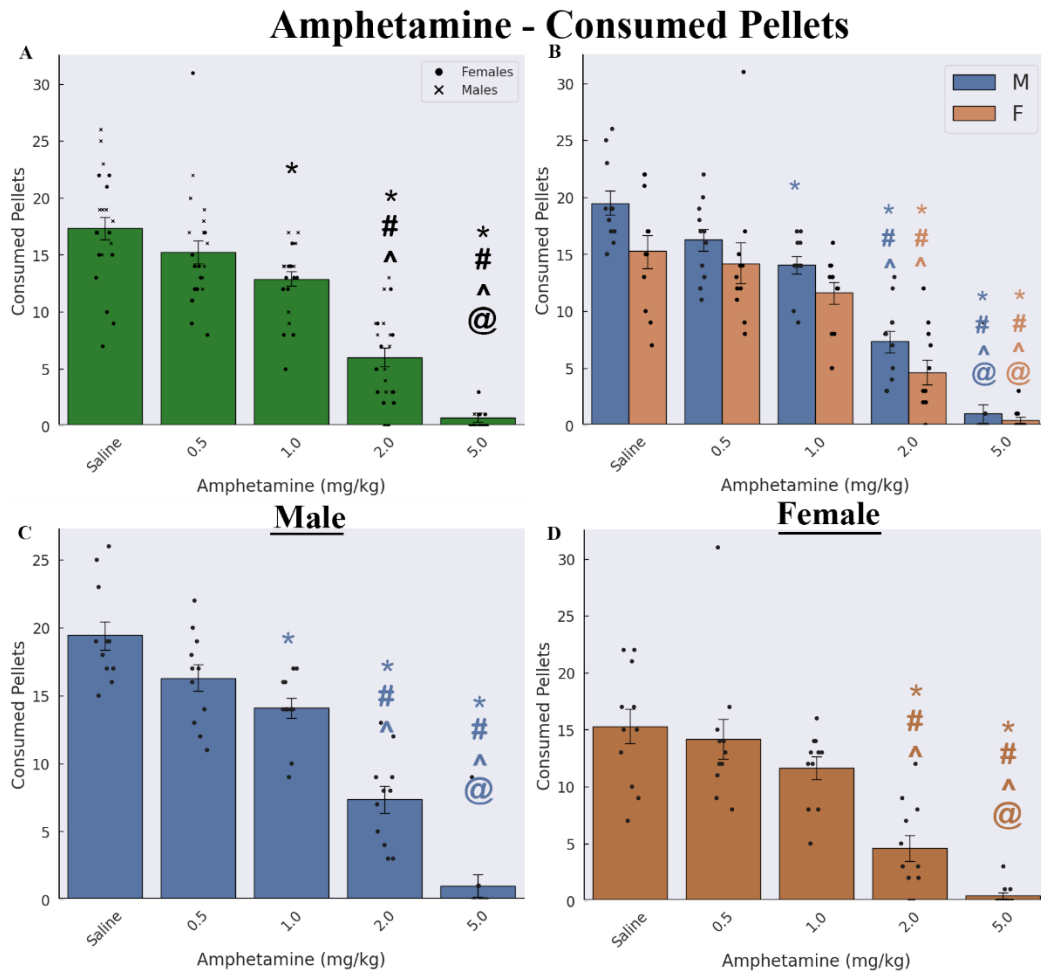
mg/kg all reduced food port entries (Figure 4A).

Figure 2. Amphetamine significantly reduced sucrose pellet consumption.
(A) Mean consumption of sucrose pellets across all mice after amphetamine treatment. All:
N=22; males: n=11; females: n=11. (B) Mean consumption of sucrose pellets in each sex after
amphetamine treatment. males: n=11 (blue bars); females: n=11 (orange bars). (C) Mean
consumption of sucrose pellets of males after amphetamine treatment. n=11. (D) Mean
consumption of sucrose pellets of females after amphetamine treatment. n=11. *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.
Blue symbols in panel B reflect significant differences between doses for males, and orange
symbols in panel B reflect significant differences between doses for females.
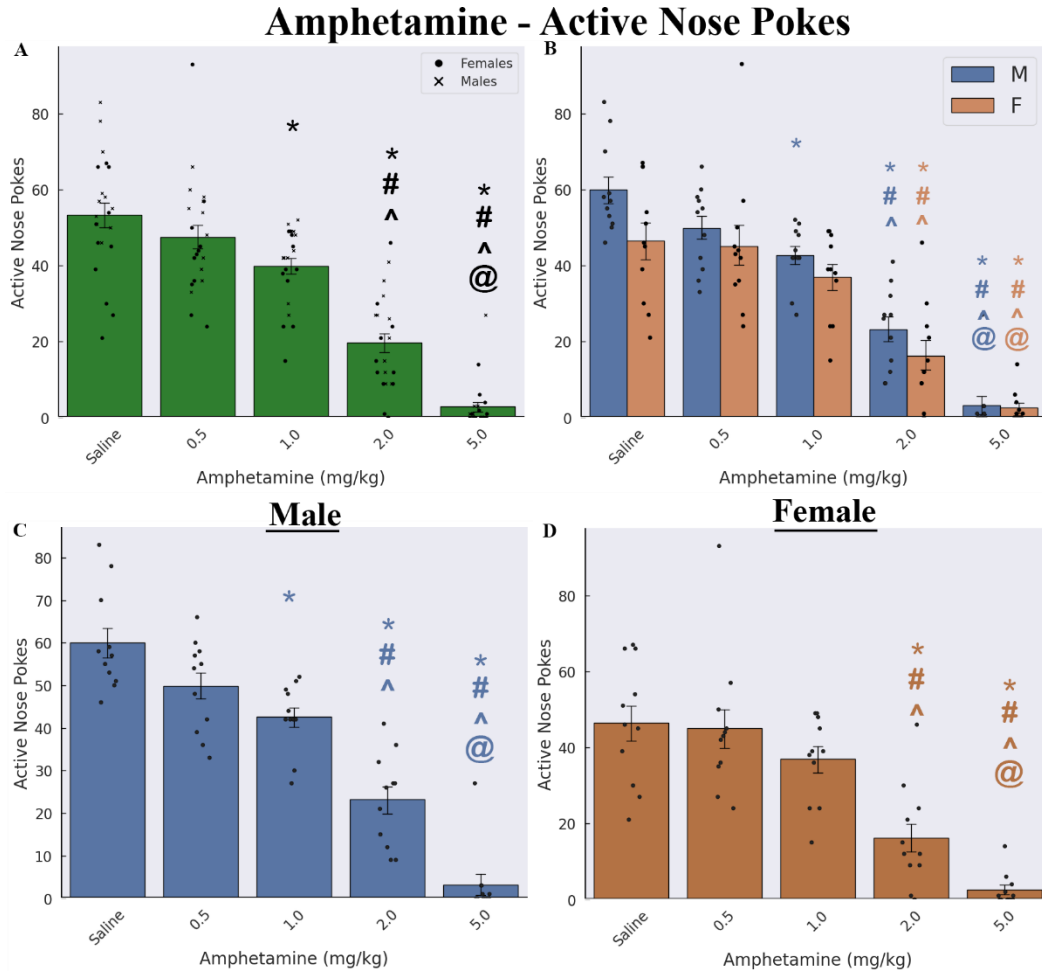
Figure 3. Amphetamine significantly reduced active nose pokes.
(A) Mean active nose pokes across all mice after amphetamine treatment. All: N=22. (B) Mean active nose pokes in each sex after amphetamine treatment. males: n=11 (blue bars); females: n=11 (orange bars). (C) Mean active nose pokes of males after amphetamine treatment. n=11. (D) Mean active nose pokes of females after amphetamine treatment. n=11. *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. Blue symbols in panel B reflect significant differences between doses for males, and orange symbols in panel B reflect significant differences between doses for females.
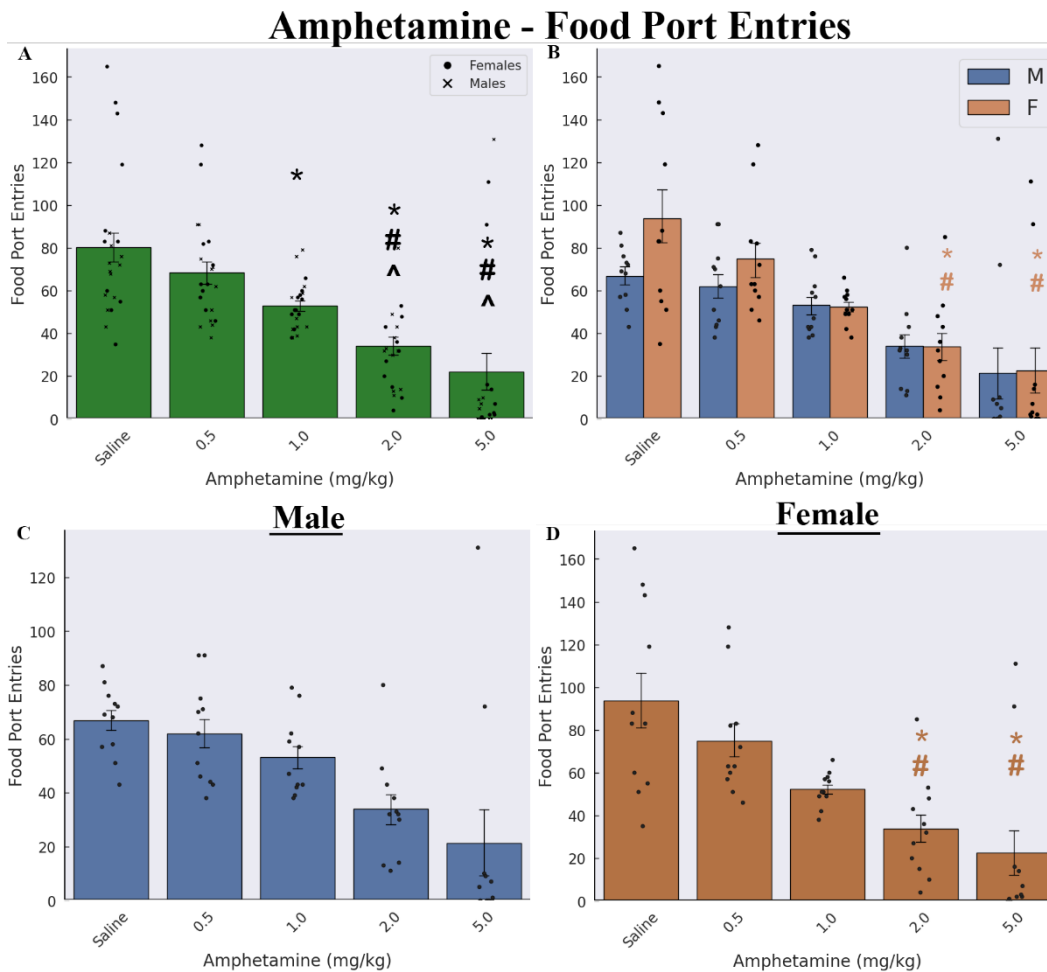
Figure 4. Amphetamine significantly reduced food port head entries.
(A) Mean food port entries across all mice after amphetamine treatment. All: N=22. (B) Mean food port entries in each sex after amphetamine treatment. males: n=11 (blue bars); females: n=11 (orange bars). (C) Mean food port entries of males after amphetamine treatment. n=11. (D) Mean food port entries of females after amphetamine treatment. n=11. *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. Orange symbols in panel B reflect significant differences between doses for females.

In the nicotine trials, an analysis of active nose pokes across all mice (independent of sex) revealed a main dose effect ($F_{5,37}=37.704$; $p=.002$). Post hoc analysis revealed that 5 mg/kg reduced operant nose poking for sucrose pellets (Figure 6A). An analysis of sucrose intake also revealed a main dose effect ($F_{5,37}=5.166$; $p=.001$). Again, post hoc analysis revealed that 5 mg/kg reduced consumption sucrose pellets. (Figure 5A). Last, analysis of food port entries revealed a main effect of dose ($F_{5,36}=4.57$; $p=.002$). Again, post hoc analysis revealed that 5 mg/kg reduced food port entries. (Figure 7A) Doses of 0.1, 0.5, 1, or 2 mg/kg showed no significant effect on sucrose consumption, operant nose poking, or food port entries
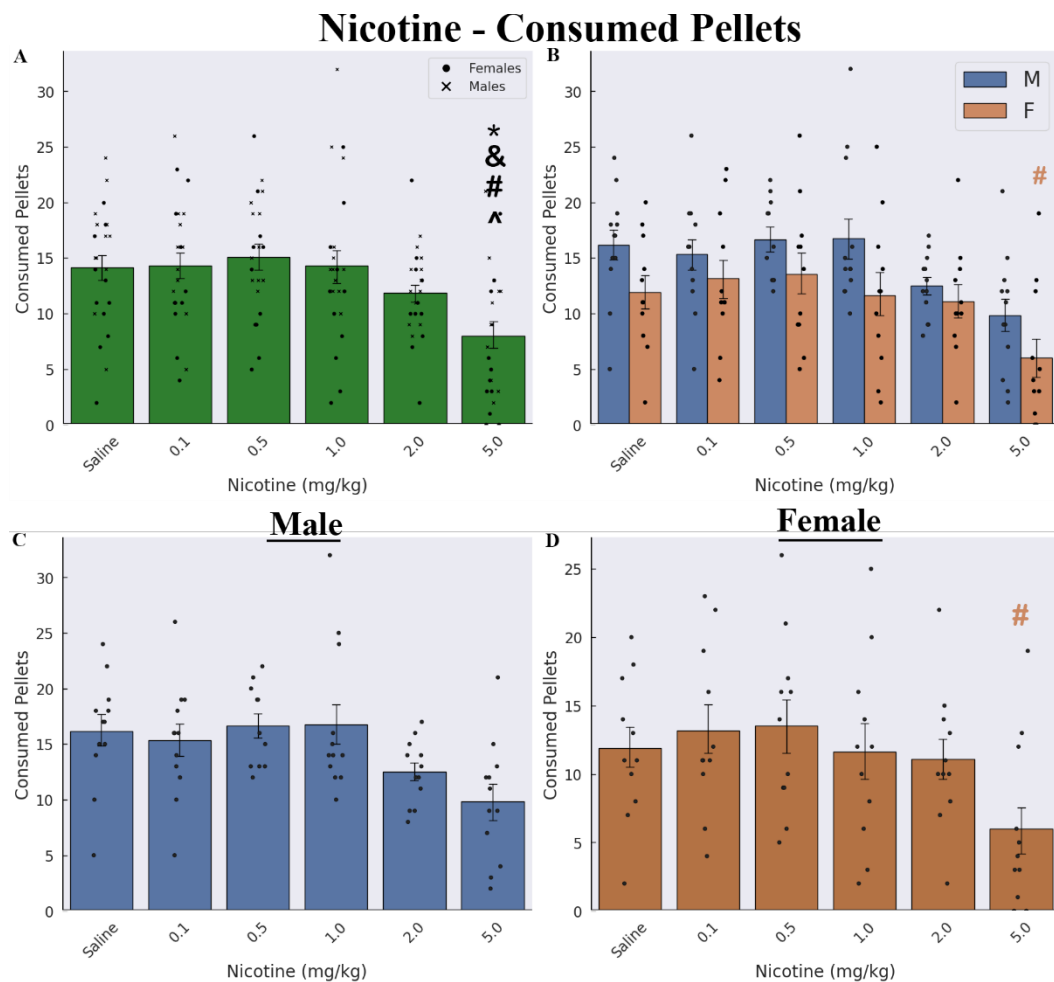

Figure 5. Nicotine significantly reduced sucrose pellet consumption in high dosage. (A) Mean consumption of sucrose pellets across all mice after nicotine treatment. All: N=23; males: n=12; females: n=11. (B) Mean consumption of sucrose pellets in each sex after nicotine treatment. All: N=23; males: n=12 (blue bars); females: n=11 (orange bars). (C) Mean

*consumption of sucrose pellets of males after nicotine treatment. males: n=11. (D) Mean consumption of sucrose pellets of females after nicotine treatment. females: n=12. *P < 0.05 vs. saline; &P < 0.05 vs. 0.1-mg/kg dose; #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. Orange symbols in panel B reflect significant differences between doses for females.*

Figure 6. Nicotine significantly reduced active nose pokes in high dosage.
*(A) Mean active nose pokes across all mice after nicotine treatment. All: N=23; males: n=12; females: n=11. (B) Mean active nose pokes in each sex after nicotine treatment. All: N=23; males: n=12 (blue bars); females: n=11 (orange bars). (C) Mean active nose pokes of males after nicotine treatment. males: n=11. (D) Mean active nose pokes of females after nicotine treatment. females: n=12. *P < 0.05 vs. saline; &P < 0.05 vs. 0.1-mg/kg dose; #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. Orange symbols in panel B reflect significant differences between doses for females.*
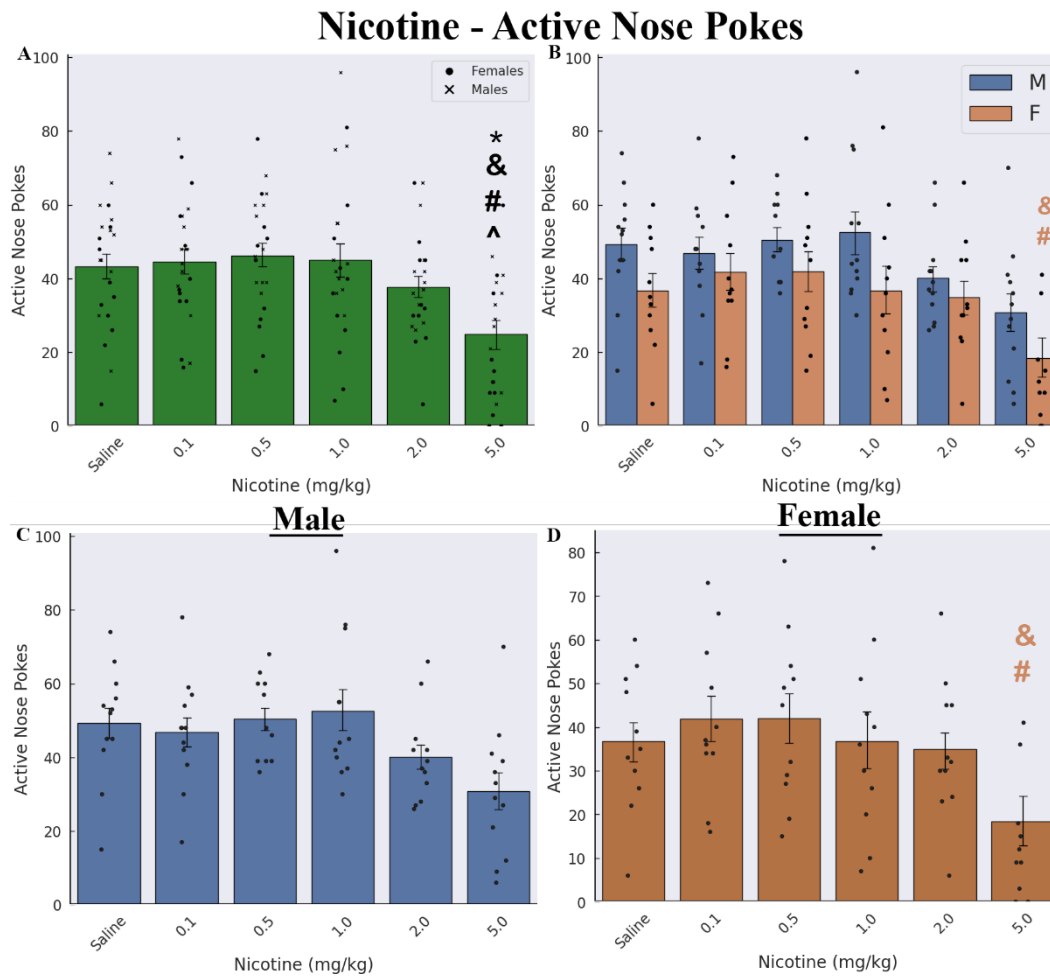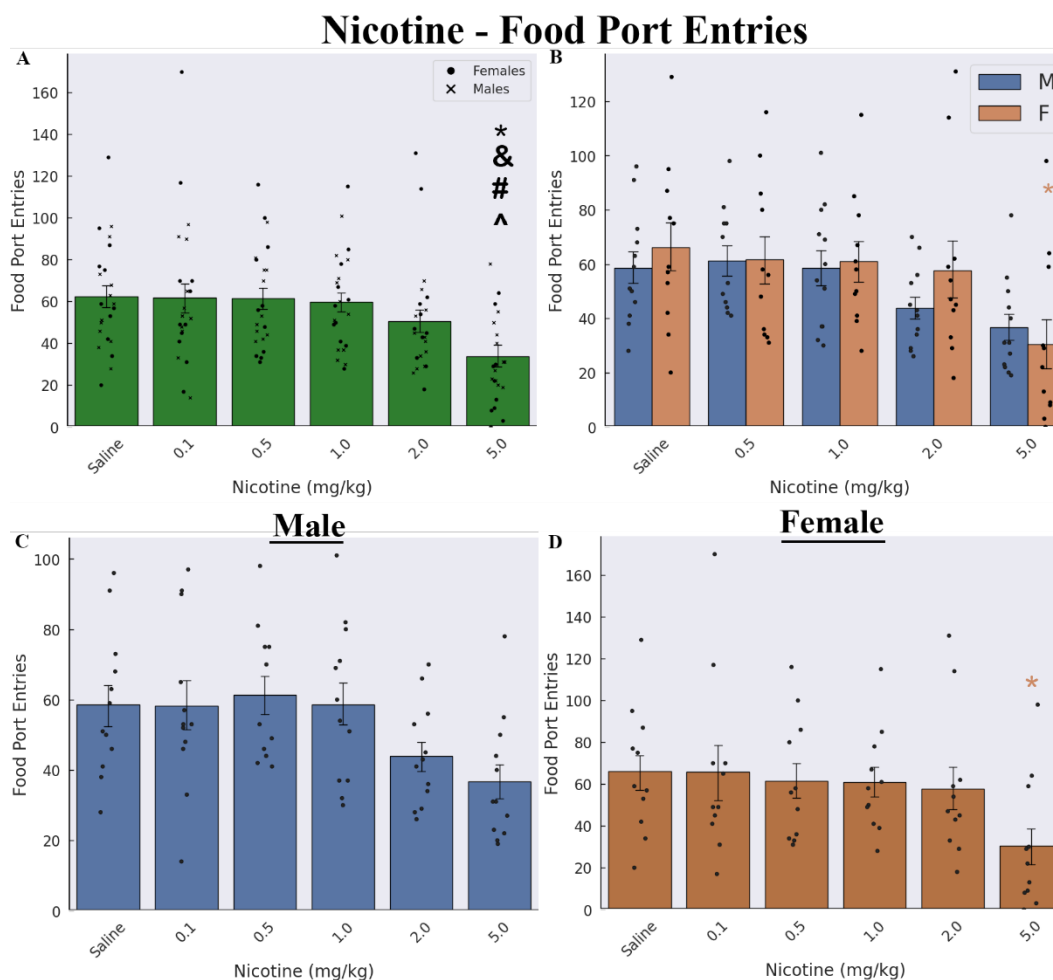
*Figure 7. Nicotine significantly reduced food port head entries in high dosage.
(A) Mean food port entries across all mice after nicotine treatment. All: N=23; males: n=12;
females: n=11. (B) Mean food port entries in each sex after nicotine treatment. All: N=23;
males: n=12 (blue bars); females: n=11 (orange bars). (C) Mean food port entries of males after
nicotine treatment. males: n=11. (D) Mean food port entries of females after nicotine treatment.
females: n=12. *P < 0.05 vs. saline; &P < 0.05 vs. 0.1-mg/kg dose; #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. Orange symbols in panel B
reflect significant differences between doses for females.*

(Figure 5A, 6A, & 7A).

We also tested whether the effects of each drug differed between male and female mice.

When we included sex as a variable in a linear, mixed models analysis, there was no significant

interaction between dose and sex for either amphetamine ($F_{4,35}=1.081$; $p=.380$) or nicotine

($F_{5,37}$=.403; p=.844). For AMPH, analysis of each sex independently with linear mixed models analyses did show significant effects of dose for each sex (Males: $F_{4,35}$=50.534; p=<.001; Females: $F_{4,35}$=70.537; p=<.001). Post hoc analyses revealed a significant reduction in active lever presses and pellets eaten (vs saline) for the 2 mg/kg, and 5 mg/kg doses of amphetamine in females, whereas the 1 mg/kg, 2 mg/kg, and 5 mg/kg doses of amphetamine significantly reduced active nose pokes and pellets eaten (vs saline) in males (Figure 2B & 3B). Furthermore, analysis of each sex independently with linear mixed models analyses for nicotine trials indicated significant effects of dose for each sex (Males: $F_{5,36}$=3.045; p=.021; Females: $F_{5,36}$=2.543; p=.045). However, post hoc analyses revealed no significance reduction in pellet consumption or active nose pokes in any doses when independently analyzing for sex. Although analysis of food port entries revealed that 5 mg/kg doses of nicotine significantly reduced food port entries for females. We also monitored estrus cycle in female animals on non-test days during the test periods (not shown). All females were in different stages of estrus cycle, and therefore, it is unlikely that there were any estrus cycle-dependent effects in the data.

We also measured inactive nose pokes, active nose pokes during timeout, and inactive nose pokes during timeout as a control against non-specific responding, and these data are shown in Table 1.1. No significant main dose effect was observed for either amphetamine or nicotine in inactive nose pokes during the timeout. Similarly, inactive nose pokes did not demonstrate a significant main dose effect for nicotine. However, a significant main effect was observed for amphetamine ($F_{4,27}$=8.052; p=<.001). Although, because the rate of inactive nose poking was quite low across all trials, averaging approximately 1.6 responses per trial, it is unlikely that this effect carries any substantial weight. Active nose pokes during timeout revealed a significant main dose effect for both amphetamine and nicotine (Amphetamine: $F_{1,1606}$=27.207; p=<.001;

Nicotine: $F_{5,40}=2.988$; $p=.022$). While the significant effect observed in active nose pokes during

timeout could suggest a decrease in compulsive behaviors (93), given the also low response rates

in this category, this effect is unlikely to hold significant implications. We also tested whether

the drug order (i.e. amphetamine before nicotine) affected the responses to the 2nd drug tested.

Half of the mice were tested with AMPH first followed by nicotine and the order of drug

treatment was reversed (i.e. nicotine then amphetamine) for the other half of the mice. Overall,

drug order did not affect the responses to the 2nd drug tested (not shown).

Table 1.1 Table of Other Parameters Measured During Food Self-Administration Assays

		Inactive Nose Pokes			Active Nose Pokes During Timeout			Inactive Nose Pokes During Timeout		
		All	**Male**	**Female**	**All**	**Male**	**Female**	**All**	**Male**	**Female**
Amph. (mg/kg)	**0.5**	2.41	2.91	1.91	4.24	2.55	6.10	0.68	0.45	0.91
	1	1.09	0.90	1.27	3.95	4.00	3.91	0.14	0.18	0.09
	2	0.64	0.36	0.90	3.45	4.36	2.55	0.18	0.18	0.18
	5	0.27	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	3.55	2.73	4.36	16.32	17.18	15.45	0.23	0.36	0.09
Nicotine (mg/kg)	**0.1**	2.88	1.67	4.09	14.96	18.92	10.64	0.13	0.00	0.27
	0.5	1.91	1.09	2.73	10.36	8.45	12.27	0.23	0.36	0.09
	1	2.59	1.08	4.09	5.78	9.17	2.09	0.13	0.00	0.27
	2	1.19	0.75	1.64	4.78	4.83	4.73	0.09	0.08	0.09
	5	1.40	1.83	1.00	5.00	7.83	1.91	0.00	0.00	0.00
	0	2.69	1.92	3.46	10.39	15.33	5.00	0.17	0.17	0.18

4   DISCUSSION

The primary objective of this study was to investigate whether nicotine or amphetamine inhibits hedonic feeding in a sex-dependent manner using a sucrose self-administration model. The results of this study revealed three major findings. First, amphetamine and nicotine both reduced sucrose intake in a self-administration model, but this was only dose-dependent for amphetamine. Second, no significant sex differences were identified in the response to either drug. Finally, no significant increase of hedonic feeding resulted from low doses of amphetamine.

Amphetamine was observed in this experiment to decrease hedonic feeding in a dose-dependent manner. Prior studies have also looked at amphetamine's ability to reduce feeding behaviors, however they have mainly focused on the reduction of homeostatic feeding (8-12). Given that amphetamine's impact on homeostatic feeding behaviors is due in part to an increase in extracellular dopamine (42) and dopamine plays a significant role in the reinforcing qualities of drugs of abuse, this physiological change implies a plausible effect of amphetamine on reward-based feeding behaviors (18). Moreover, AMPH can decrease orexigenic NPY in the stratum and NAc (84). As these regions are known to regulate the motivation for sucrose consumption via NPY action which further suggests AMPH's potential role in modulating reward-based feeding through this system (83). The findings of this study align with these reasonings and are consistent with a previous study in our lab demonstrating that high doses of amphetamine can decrease hedonic feeding in a dose dependent manner in a two-bottle choice test and binge feeding model (13). The results of sucrose self-administration experiment however demonstrate this effect on a more hedonic paradigm than used in the West et al. study. The dose-dependent effects of amphetamine observed in this study also align with the results of previous

research demonstrating similar effects. (8-11). Notably, though these studies focused on the change to homeostatic feeding, the finding that systemic administration of AMPH causes a decrease in intake and responding for food as dose increases from 1 mg/kg to 5 mg/kg is also seen in this study of hedonic feeding. This suggests that AMPH might impact the mechanisms regulating both homeostatic and hedonic feeding in a similar way. Since AMPH has been determined to dose-dependently effect homeostatic feeding, future studies should look to determine by which mechanisms AMPH regulates hedonic feeding and if this system is the same or distinct from that by which AMPH effects homeostatic feeding. This is important to determine because if there is a difference between these mechanisms, this could reveal a unique target for the regulation of hedonic feeding without affecting homeostatic feeding. If this system is targeted primarily, it could potentially aid in the control of overeating driven by hedonic motivation, as observed in obesity models (17).

The results of this study propose that AMPH can decrease motivational behavior for palatable food rewards. However, other studies examining the effect of AMPH on other types of motivational behavior demonstrate that AMPH can increase motivational behaviors associated with central DA systems. For instance, AMPH can increase rewarding electrical self-stimulation in the lateral hypothalamus and substantia nigra with similar doses used in this study (85). Also, rats pre-exposed to AMPH show a higher willingness to work for cocaine in a self-administration model (86). Consequently, it is possible that the mechanistic systems in which AMPH can regulate reward-based feeding may differ from those regulating other types of reward motivation.

Nicotine was found to reduce sucrose self-administration only at the highest dose. As with amphetamine, previous studies primarily focused on nicotine's ability to reduce homeostatic

feeding. The suppression of feeding behaviors by nicotine is attributable to the widespread activation of monoaminergic neurons, including those in brain areas regulating feeding. This extensive activation includes dopaminergic neurons in the mesocortical and mesolimbic systems associated with reward suggesting a potential role for nicotine to regulating reward-based feeding behaviors, although this has not been previously studied. Our results suggest that reward-based feeding is affected by nicotine. However, it is unclear by which pathway nicotine can regulate this type of feeding given its involvement in several related pathways. For example, nicotine can regulate NPY expression in a dose-dependent manner. Additionally, nicotine downregulates plasma leptin, which can act on DA neurons in the VTA (53). Furthermore, systemic administration of nicotine causes decreased striatal β-endorphin which acts on opioid receptors that regulate taste palatability (88).

It is also possible that the nicotine effects observed here were due to changes in locomotor activity. Following the administration of the highest nicotine doses, a notable decrease in motor activity was observed in the mice, a condition that gradually improved during the 1-hour trials. Past studies have shown that high doses of nicotine administered systemically may lead to seizure-induced inactivity potentially explaining the decreased nose poking observed in high doses of nicotine as the doses used in this project approached those doses which have the possibility to cause nicotine-induced seizures (92). However, we did not quantify motor activity in these studies, so we cannot conclusively determine whether the decreased responding with the highest dose of nicotine was due to direct effects on food self-administration or whether these were secondary to gross effects on motor activity.

The effects of nicotine in this study are consistent with previous studies demonstrating the dose dependent effects of acute nicotine administration (29, 30, 90, 91). Although these

studies focus on the homeostatic regulation of feeding, the acute effects of low dose nicotine (< 3 mg/kg) do not affect feeding, while high doses (> 3 mg/kg) lead to a reduction in feeding. This observation suggests that the mechanisms regulating homeostatic feeding and the mechanisms regulating hedonic feeding may be affected by nicotine administration in a similar way. Like with AMPH, future studies should look to specify how nicotine affects hedonic feeding and if this system is the same or distinct from that by which nicotine effects homeostatic feeding.

Interestingly, neither drug showed sex differences in their effect on sucrose self-administration. Although the 1 mg/kg amphetamine dose significantly decreased nose pokes, pellets eaten, and receptacle entries in males but not females, the lack of an overall dose x sex interaction demonstrates that there were no overall sex differences in these results. The lack of a dose-sex interaction was unexpected because there are clear sex difference in both the neural control of feeding and metabolism and within the mesocortical and mesolimbic dopamine systems (58,59). For instance, women become addicted to nicotine faster than men and have greater difficulty quitting (60). Rodent studies similarly indicate that females show a stronger response to the conditioned rewarding effects of nicotine (4). With amphetamine, females show greater behavioral responses and locomotion in response to the drug. They also show a quicker acquisition and greater drug taking in a drug self-administration model suggesting a sex difference in the rewarding qualities of amphetamine (61). However, the results of this study show no such sex differences in the ability of nicotine and amphetamine to reduce hedonic feeding in a self-administration paradigm. This result is consistent with the results found in the study by West et al. which also showed no sex by dose interaction with amphetamine on hedonic feeding. Furthermore, it is unlikely this result was due to any effects of the estrus cycle as estrus monitoring performed during the experiment showed varied estrus cycle stages across female

mice during each treatment trial. This may mean that the mechanisms behind the rewarding qualities of these drugs and the mechanisms affecting hedonic feeding may be distinct. This finding poses a potential target for therapeutic treatment which can alter hedonic feeding behaviors without the additive qualities seen in nicotine and amphetamine.

Another noteworthy result within the study was the lack of a bidirectional effect of amphetamine to decrease hedonic feeding at high doses and increase this feeding at low doses. While there is consistent evidence that high doses of amphetamine reduce feeding behaviors, there are conflicting results regarding the effects of low doses. Some studies have shown that in low doses, amphetamine reduces feeding (63,64) while other studies have shown that low doses of amphetamine increase feeding (62,13). A prior study in our lab did not show the increase of feeding caused by low dose amphetamine until 2-hours post systemic injection (13). Therefore, it is possible that this effect was not detected because self-administration trials were started 10 minutes post amphetamine injection and lasted only one hour. Additional experiments testing the effects of amphetamine at later time points after injection will be required to test this, and while preliminary testing of this hypothesis is ongoing, it does not currently contain a large enough sample size to make a conclusion (not shown). Furthermore, a study conducted by Sills and Vaccarino (1995) suggested that low dose amphetamine can either increase feeding or have no impact on feeding, depending on individual variances of baseline sucrose consumption in rats. It is therefore possible that no significant increase of feeding was detected due to individual differences in the effects of low-dose amphetamine across the groups.

This study is slated for continuation, employing the use of a progressive ratio schedule within a sucrose self-administration model, as well as using chow and sucrose self-administration in a home-cage setting. The use of a progressive ratio schedule will facilitate the

observation in changes to maximum effort expended to attain a food reward (i.e. motivation). Also, by using devices which allow for the administration of self-administration trials in the home cages, we can evaluate how these drugs effect hedonic feeding over several hours and affirm that the doses used in this study inhibit normal chow intake on a free feeding paradigm. The purpose of the chow intake study is to create a positive control test to confirm these drugs effect homeostatic feeding in the same way as hedonic feeding.

In conclusion, this study adds to the body of evidence that amphetamine and nicotine are both able to reduce hedonic feeding. This effect was only dose-dependent for amphetamine, and there was also no evidence of a sex difference in the regulation of this feeding behavior. When considering the sex differences already evident within the addictive actions of these drugs, it is likely that the mechanisms regulating its additive qualities and anorexic effects are distinct. Although further research is needed to discover the precise mechanisms by which these drugs decrease hedonic feeding, this study points to a potential treatment avenue for obesity that could avoid the negative addictive side effects of both drugs.

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