Opioidergic and Dopaminergic Modulation of Cost/Benefit Decision-Making in Long Evans Rats

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Highlights

- We assess opioid and dopamine control of food intake with two cost/benefit decision-making tasks
- Naloxone reduces operant responses for palatable food without affecting free chow intake
- Haloperidol shifts behavior away from lever pressing and increases free chow feeding
- Naloxone reduces preference for palatable banana pellets when no effort is involved
- Dopamine antagonism reduces intake of both foods without altering preference

Abstract

Eating disorders are associated with impaired decision-making and dysfunctional reward-related neurochemistry. The present study examined the potential contributions of dopamine and opioid signaling to these processes using two different decision-making tasks. In one task, Long Evans Rats chose between working for a preferred food (high-carbohydrate banana-flavored sucrose pellets) by lever pressing on a progressive-ratio schedule of reinforcement vs. obtaining less preferred laboratory chow that was concurrently available. In a second (effort-free) task, rats chose between the same two reinforcers when they were both available freely. Rats were trained in these tasks before receiving haloperidol (0.00, 0.05, 0.10 mg/kg, intraperitoneally (i.p.)) or naloxone (0.0, 1.5, 3.0 mg/kg, i.p.). In the first task, haloperidol decreased breakpoint, lever presses, number of reinforcers earned, and increased chow intake, whereas naloxone decreased breakpoint and number of reinforcers earned but had no effect on chow consumption. In the effort-free task, haloperidol reduced intakes of both foods without affecting preference, whereas naloxone selectively reduced the consumption of banana-pellets. The present findings support converging evidence suggesting that DA signaling affects processes more closely related to appetitive motivation, leaving other components of motivation unchanged. By contrast, opioid signaling appears to mediate aspects of hedonic feeding by selectively altering intakes of highly palatable foods. For preferred foods, both appetitive and consummatory aspects of food intake were altered by opioid receptor antagonism. Our findings argue against a general suppression of appetite by either compound, as appetite manipulations have been shown to unselectively alter intakes of both types of food regardless of the task employed.

Keywords: Opioids, Dopamine, Motivation, Decision-Making, Food Preference, Eating Disorders

1. Introduction

Binge eating and related food-centered pathologies are marked by a number of behavioral and cognitive symptoms. Among these are intense cravings, recurring bouts of elevated intake of highly palatable foods, loss of control, and the transition from normal consumption patterns to more compulsive-like behaviors [1]. Often, these episodes can reach such intensities that they impair normal decision-making processes and lead to behaviors that mimic those seen during cases of drug addiction [2–6]. Proper treatment of these disorders has become a challenge for clinicians because of their complexity in etiology and presentation. Given the wide range of genetic, biological, and socio-cultural determinants that contribute to disordered eating, it is not likely that a single factor is solely responsible for the development of these disorders. Because of this, it has become increasingly important to develop partial animal models that assess specific behaviors/symptoms and their underlying neurochemical signatures. Of special credence is the contribution of dysregulated reward-related process and decision-making impairments [4].

Those diagnosed with eating disorders often have difficulties with decision-making that requires assessing different behavioral options based on their respective costs and benefits. Because of this, it has been suggested that food based decision-making tests are well suited to model a number of these cognitive, appetitive, and consummatory behaviors in rodents [7,8]. One type of decision-making that merits considerable attention involves situations where organisms must choose between simple actions that yield smaller less desired rewards, or behaviors that result in higher valued options but require significantly more effort. In these tasks, rats choose between a low effort/low reward option (freely available) standard laboratory chow and a higher effort/high reward option (lever pressing to obtain more palatable sucrose pellets).

Eating is controlled by many areas of the brain and neurotransmitter systems involved in motivation, hedonic processing, learning and memory, and homeostatic regulation [for detailed reviews see [9–11]]. For these reasons, it is important to narrow the focus to specific neurotransmitter systems and their dissociated contributions to food motivation. The use of the aforementioned behavioral paradigms in conjunction with pharmacological tools has made it possible to observe the effects of certain manipulations on consummatory and appetitive behaviors independently, and determine precise neurochemical contributions that contribute to these various aspects of food intake and food-related decision-making.

The endogenous opioid system (EOS) consists of a number of opioid peptides and their respective receptors, distributed throughout a number of central and peripheral tissues [12–15]. EOS involvement in a number of biological processes that include analgesia, hormone regulation, motor function, motivation, and hedonic processing can likely be attributed to their vast localization in the brain and periphery [10,16,17]. In addition to these functions, opioid regulation of food intake has also been greatly studied. Opioids have been found to affect the hedonic processing of palatable foods in both rodents and humans [18–23]. Studies using the taste reactivity test, a well validated measure of hedonic responses [16,24,25], have found that opioid stimulation of discrete hedonic spots within the nucleus accumbens (NAc), ventral pallidum (VP) increases facial reactions associated with positive affect that arise from ingestion of sweet palatable solutions [26–28].

It has also been suggested that opioids mediate eating that occurs outside of caloric needs, or hedonic feeding [19,29,30]. Opioid receptor agonists tend to produce increases in food intake in animals while opioid receptor antagonists mostly show inhibitory effects [31–33], but these affects appear to be sensitive to the overall palatability of the reinforcer. Although opioid

agonists and antagonists can affect the intakes of normal foods at higher doses or when those foods are presented alone, their effects are more pronounced on foods that rate higher in palatability, such as those high in fat or rich in carbohydrates [34–38]. It has been then suggested the EOS is likely responsible for regulating intakes of particular macronutrients [39–41]. However, further studies have found that what seems to be important is not a specific macronutrient, but rather the baseline preference of the animal [42–44]. Organisms that prefer foods high in fats will selectively decrease their intake of such foods upon administration of opioid receptor antagonists, and those that prefer high carbohydrate foods will alter their intake of carbohydrates. This baseline preference is also correlated with opioid agonists and antagonists ability to alter taste reactivity [45,46].

Opioid agonists and antagonists have also been shown to affect an animal's willingness to engage in operant behavior [38,47,48], suggesting the EOS goes beyond the processing of the hedonic value of food to also include the motivation to engage in food-reinforced behaviors. These studies have mostly focused on operant actions when a single food option is available. Despite the vast amount of literature devoted to opioid regulation of food intake, it is not well-understood how disruptions to this system affect certain aspects of decision-making and food preference. To this end, the present study was designed to investigate the role of the endogenous opioid system in food preference using two different decision-making tasks. In order to gauge animal's baseline preference of sucrose pellets to standard laboratory chow, we employed a traditional preference test in which both food options were available concurrently. In the other task (progressive ratio/chow feeding or effort condition), animals were given the option of obtaining less palatable laboratory chow that was freely available in the chamber vs. working for a preferred food (high-carbohydrate banana-flavored sucrose pellet) by lever pressing on a progressive ratio (PR) schedule of reinforcement, in which the work requirements increased systematically with each food reinforcer earned. This task was adapted from Salamone and colleagues, originally developed to describe how dopaminergic modulations can affect some aspects of food motivation while leaving others unchanged [49]. Given that dopaminergic involvement in these processes has been greatly characterized [For review see [50,51], we compared the effects of systemic dopamine antagonism to those produced by administration of a nonselective opioid receptor antagonist, naloxone.

2. Materials and Methods

2.1 Animals

Adult male Long Evans rats ($n = 8$) were supplied from Harlan (Indianapolis, IN) and housed in pairs within a temperature controlled (22± 2° C) room on a standardized 12h light/dark cycle (lights on at 7:00 AM). Animals were food restricted throughout the experiment to approximately 85% of their free-feeding weight, and had access to water *ad libitum*. Rats were allowed to consume all food obtained during behavioral tests. They were also given additional access to laboratory chow (Lab Diet 5012, St. Louis, MO) and allowed modest weight gain throughout the duration of the experiment. All procedures were conducted in accordance with the Institutional Animal Care and Use Guidelines of Reed College and the National Institute of Health (NIH) guidelines for the Care and Use of Laboratory Animals.

2.2 Pharmacological Agents

Haloperidol was purchased from Sigma Aldrich (St. Louis, MO) and dissolved in a 0.3% tartaric acid solution. Microliter quantities of 1.0M NaOH were titrated into all solutions to rescue pH (4.0). The 0.3% tartaric solution served as the vehicle control for this condition. Naloxone hydrochloride was obtained from Sigma Aldrich Co. (St. Louis, MO) and prepared in a 0.9% saline solution. Saline served as the vehicle control for all naloxone experiments. Doses of haloperidol and naloxone were selected from previously published data [52–55]. All injections were delivered intraperitoneally (IP).

2.3 Decision-making Task

The basic decision-making task was adapted and modified from those created by Salamone and colleagues [53]. Operant sessions were conducted in two-lever operant chambers (27 cm length x 30 cm width x 29 cm height; Med-Associates, St. Albans City, VT). All tasks were programmed and executed on a 512KE PC using Med-PC software. Behavioral and pharmacological testing occurred Monday through Friday between 11:30 AM and 1:30 PM and sessions were 30 min long. Rats received a single day of magazine training in which 45 mg banana-flavored sucrose pellets (Bio-Serv; Frenchtown, NJ; 3.90 kcal/gm; 0% Protein, 0% Fat, 0% Fiber, 97.5% Carbohydrate) were delivered irrespective of responding every 30 s (i.e., a fixed-time [FT] 30-s schedule of delivery). Animals were then trained to press a lever on a continuous schedule of reinforcement (i.e., fixed-ratio [FR] 1) for 5 sessions before switching to a PR schedule. For PR sessions, the ratio began at 1 FR1 and increased by 1 response every 15 reinforcers (FR 1 x 15, FR 2 x 15, FR 3 x 15, etc). Training sessions were conducted 5 days per week for 5 weeks (25 total sessions) until responding reached a steady state, defined by variability no greater than 15% across sessions and the absence of monotonic trends (confirmed statistically using one-way Analysis of Variance (ANOVA)). At this point, lab chow (Lab Diet 5012; St. Louis, MO; 3.07 kcal/gm; 27.0% Protein, 13.1% Fat, 59.9% Carbohydrates) was introduced concurrently in the chamber with the PR schedule. For this PR/Chow training, 15-20 g of lab chow was weighed and placed in a small, glass, petri dish that was subsequently positioned in the far corner of the operant chamber, underneath the unused left lever. Rats were removed after each 30-min session and chow intake was measured, including any spillage. Training continued for another 4 weeks (24 Total sessions) until the same previously defined stability criteria was established and pharmacological testing began. All pharmacological testing for haloperidol happened first. Animals were then given a week off of pharmacology but continued baseline training and were then tested in naloxone.

2.4 No Effort Intake Test

Food preference in a free feeding situation (30-min sessions; 5 days per week) was assessed in the same group of animals after the conclusion of the PR/Chow phase of the experiment. All sessions were conducted in the same operant chambers previously described. One rat developed respiratory issues at the beginning of the experiment and its data were removed from all further analyses. Prior to a session, 15-20 g of chow and 20-25 g of banana pellets were weighed onto glass petri dishes and placed within the operant chambers. Dish locations coincided with the position of the left and right levers, and location was counterbalanced across individuals. Rats were placed in their respective operant chambers, the house light was turned on, and a timer ran down the 30-min sessions. Animals were then removed and consumption of both food options was measured, including spillage. Preference

training occurred 5 days a week and continued for 2 weeks (10 sessions total) until consumption reached stable conditions. Stability was defined as defined as before, and statistically confirmed using one-way ANOVA (factor: session). Pharmacological testing for naloxone occurred first on the no effort intake test. Animals continued baseline training for a week without any drug treatments and were then tested with haloperidol.

2.5 Experimental Procedures

All experiments used a within-subject design in which each rat received all treatments. Animals received each dose of drug (IP) a single time. For PR/Chow testing, drug was administered on Fridays, with doses counterbalanced across individuals. Rats continued baseline training from Monday through Thursday. Drug testing was conducted on Tuesdays and Fridays for all no effort assessments. The change in drug treatment was justified by the relatively short half-life and action of both compounds [56,57]. Off days were dedicated to additional baseline preference training.

2.5.1 Effects of Opioid Receptor Antagonism on PR/Chow Responding: Comparison to DA Antagonism

Experiment 1 assessed the effects of the dopamine D_2 receptor antagonist haloperidol on PR/Chow performance. Baseline training continued four days a week, and behavioral performance was unaffected by any previous injection. On drug days, rats received IP injections of 0.05 mg/kg haloperidol, 0.10 mg/kg haloperidol, or vehicle 50 min before PR/Chow testing.

Experiment 2 explored the role of the nonselective opioid receptor antagonist naloxone on PR/Chow responding. Animals were given a week off from pharmacology at the conclusion of the first experiment but continued baseline training. Rats received IP injections (saline, 1.5 mg/kg, or 3.0 mg/kg) 30 min before testing began.

2.5.2 Effects of Opioid Receptor Antagonism on Food Preference: Comparison to DA Antagonism

Experiment 3 measured the effects of systemic haloperidol on food preference in a free feeding situation. Animals underwent preference training after the conclusion of Experiment 2 until intakes stabilized. IP injections (0 mg/kg or 0.10 mg/kg haloperidol) were administered to each animals 50 min before each test.

Experiment 4 was carried out to explore the effects of systemic naloxone on food preference. Rats were given a week off from pharmacological testing after the conclusion of Experiment 3 but continued baseline training. They received naloxone (0.0 mg/kg or 3.0 mg/kg) 30 min before behavioral testing.

2.6 Statistical Analysis

The effects of haloperidol and naloxone on breakpoint, number of lever presses, number of reinforcers, banana pellet intake (calories) and chow intake (in grams and calories), and total intakes (in calories) during PR/Chow sessions were recorded using Med-PC software and analyzed using a one-way repeated measures ANOVA. Post hoc Tukey HSD tests were conducted when ANOVA indicated significance. For preference tests, the effects of naloxone and haloperidol on intake, in grams and calories, were analyzed using two-way repeated measures ANOVAs followed by Tukey Tests when necessary. Stability on measures of

breakpoint, lever pressing, reinforcers, and chow intake on the PR/Chow test was determined using one-way repeated measures ANOVA. For the no effort intake test, stability was determined using a 2 x 2 repeated measures ANOVA. All statistical analyses were carried out using STATA SE 13.0 (College Station, TX).

3. Results

3.1 Baseline behavioral training

Animals went through extensive training before any sort of pharmacological testing occurred. Rats were first trained to respond on a PR schedule of reinforcement alone until behavior was stabilized and chow was introduced into the chamber. Stable performance on the PR schedule was statistically confirmed using one-way repeated measures ANOVA on measures of breakpoint, lever presses, and number of reinforcers earned over the last four days of testing. ANOVA revealed no differences in breakpoints [$F(3,18) = 2.11$, n.s] lever presses [$F(3,18) = 2.59$], or number of reinforcers earned [$F(3,18) = 0.59$, n.s] over the last four days of training. Introducing chow into the chamber affected lever pressing measures but overall did not affect the overall intakes of the animals tested. We compared performance on the PR task and PR/Chow Task on a number measures when behavior was stabilized. Dependent measures t-tests showed that the addition of chow decreased lever pressing on the PR schedule [$t(6) = 6.463$, $p < 0.05$, 2-tailed], but had no affect on the animals total intakes in either calories [$t(6) = 0.5457$, n.s] or grams [$t(6) = 0.59$, n.s].

We also assessed stability on the concurrent feeding PR/Chow procedure using separate one-way (factor: session) repeated measures ANOVA on breakpoint, lever pressing, number of reinforcers, and chow intake over the last four days of training. ANOVA revealed no significant effect of day on breakpoint [$F(3,18) = 0.74$, n.s], number of lever presses [$F(3,18) = 0.92$, n.s], number of reinforcers earned [$F(3,18) = 0.62$, n.s], or chow consumption [$F(3,18) = 1.27$, n.s], further suggesting performance had reached stable levels.

3.2 Experiment 1: Effects of systemic haloperidol on PR/Chow Performance

The first experiment analyzed the effects of haloperidol administration on PR/Chow performance. Results from the first experiment can be seen in Figure 1. The repeated measures one-way (factor: haloperidol dose) ANOVA revealed that haloperidol reduced breakpoints [$F(2,12) = 14.04$, $p < 0.05$, see Figure 1A] in drug treated rats. Post hoc Tukey tests indicated both 0.05 mg/kg ($M = 2.86$, $SEM = 0.46$) and 0.10 mg/kg ($M = 1.57$, $SEM = 0.20$) differed ($p < 0.05$) from the vehicle condition ($M = 6.29$, $SEM = 1.19$). Haloperidol reduced the number of lever presses [($F(2,12) = 6.70$, $p < 0.05$, see Figure 1B]. Both doses tested, 0.05 mg/kg ($M = 75.14$, $SEM = 25.17$) and 0.10 mg/kg ($M = 20.43$, $SEM = 5.11$) significantly attenuated lever pressing compared to vehicle ($M = 360.6$, $SEM = 129.9$), ($p < 0.05$). Dopamine D_2 receptor antagonism also decreased the number of reinforcers earned [$F(2,12) = 14.46$, $p < 0.05$, see Figure 1C]. Tukey's test showed that both 0.05 mg/kg ($M = 34.00$, $SEM = 7.05$) and 0.10 mg/kg ($M = 15.14$, $SEM = 3.03$) doses differed significantly ($p < 0.05$) from vehicle ($M = 83.86$, $SEM = 17.53$), but no differences were found in reinforcer attainment between mid and high doses. Finally, systemic administration of haloperidol resulted in marked increases in chow intake [$F(2,12) = 17.51$, $p < 0.001$, see Figure 1D]. Increases in chow consumption ($p < 0.05$) were seen

at both 0.05 mg/kg ($M = 7.85$, $SEM = 0.77$) and 0.10 mg/kg ($M = 8.84$, $SEM = 0.47$) haloperidol doses compared to vehicle ($M = 6.03$, $SEM = 0.70$).

Due to the differences in energy density between the two reinforcer types, we compared the effects of haloperidol treatment on banana pellet, chow, and total intake in calories. There was a significant reduction in calories obtained from banana pellets upon haloperidol treatment [$F(2,12) = 14.46$, $p < 0.05$]. Post hoc comparisons revealed that both 0.05 mg/kg ($M = 6.00$, $SEM = 1.24$) and 0.10 mg/kg ($M = 2.66$, $SEM = 0.53$) differed from vehicle ($M = 14.72$, $SEM = 3.08$). Systemic haloperidol also affected the amount of calories obtained from chow [$F(2,12) = 17.72$, $p < 0.001$]. Chow consumption after 0.05 mg/kg ($M = 24.1$, $SEM = 2.37$) and 0.10 mg/kg ($M = 27.15$, $SEM = 1.45$) was higher than during vehicle conditions ($M = 18.51$, $SEM = 2.15$). Treatment with haloperidol had no effect on the total amount of calories consumed during the PR/Chow task [$F(2,12) = 1.31$, n.s]. No differences in total calories were observed between vehicle ($M = 33.22$, $SEM = 1.82$), mid ($M = 30.07$, $SEM = 2.20$), and high doses ($M = 29.81$, $SEM = 1.63$). When collapsed across all conditions, we found a significant negative correlation ($r = -0.6892$, $df = 19$, $p < 0.0005$) between lever presses and chow intake, highlighting the overall inverse relationship between these two variables.
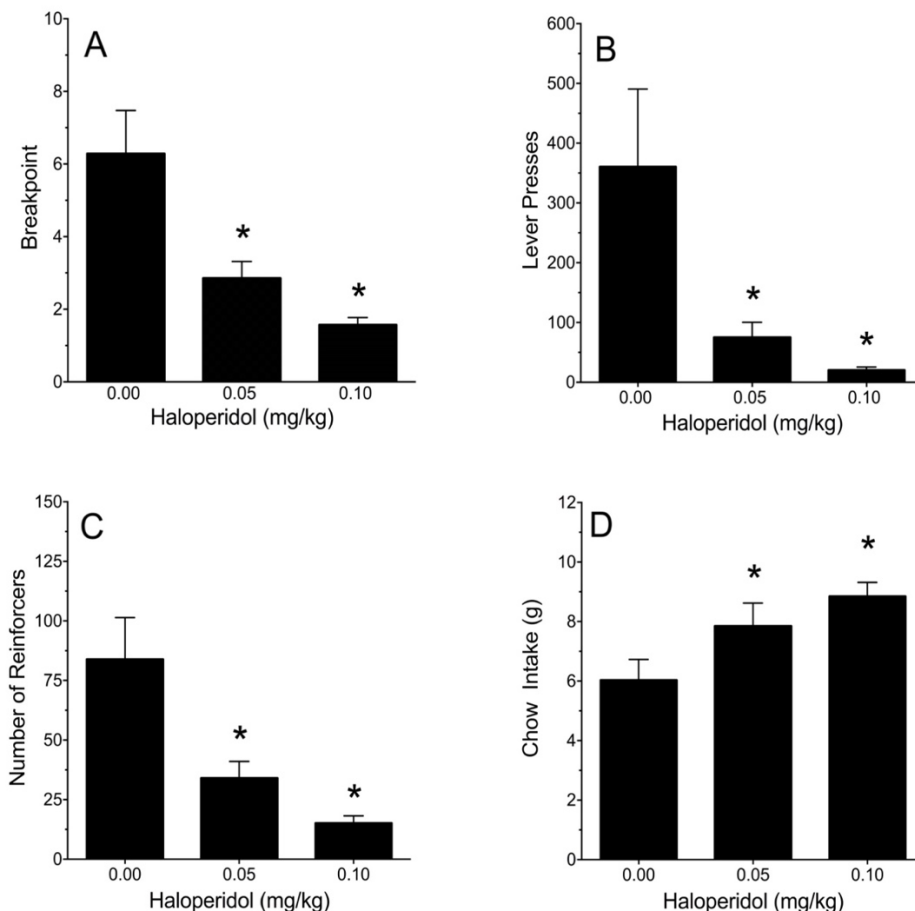

Figure 1. Behavioral Effects of dopamine D$_2$ receptor antagonist haloperidol on the PR/Chow Task. Animals received IP haloperidol (0.00 mg/kg, 0.05 mg/kg, and 0.10 mg/kg) and were tested on the PR/Chow procedure. All data are presented as Means ± SEM. Both doses of

haloperidol decreased A) breakpoint, B) number of lever presses, C) number of reinforcers, and increased D) chow intake (in grams). * $p < 0.05$ compared to 0.00 mg/kg.

3.3 Experiment 2: Effects of Systemic Naloxone on PR/Chow Responding

The continued stability on the PR/Chow task across both pharmacological agents was assessed by comparing breakpoints, lever presses, number of reinforcers earned, and chow intake for haloperidol vehicle and naloxone vehicle treatments. Paired t-tests revealed no significant differences between haloperidol and naloxone treatment on breakpoints [$t(6) = 0.42$, n.s], lever presses [$t(6) = 0.39$, n.s], number of reinforcers earned [$t(6) = 0.12$, n.s], or chow intakes [$t(6) = 0.72$, n.s].

A one-way repeated ANOVA (factor: naloxone dose) showed that naloxone decreased breakpoints [$F(2,12) = 5.03$, $p < 0.05$, see Figure 2A]. Follow up Tukey tests revealed that the 3.0 mg/kg ($M = 3.00$, $SEM = 0.44$) dose differed ($p < 0.05$) from saline ($M = 5.71$, $SEM = 1.30$). Breakpoints in the 1.5 mg/kg condition ($M = 3.42$, $SEM = 0.57$) did not differ from any other treatment. There was a trend towards statistical significance ($p = 0.06$) for naloxone's effect on lever pressing measures [$F(2,12) = 3.40$, n.s., see Figure 2B]; number of lever presses for vehicle, mid, and high doses were ($M=352.14$, $SEM = 150.04$), ($M =110.43$, $SEM = 38.68$), ($M = 87.29$, $SEM = 19.83$), respectively. Opioid receptor antagonism attenuated the number of reinforcers earned [$F(2,12) = 4.46$, $p < 0.05$, see Figure 2C], with Tukey's test revealing that 3.0 mg/kg ($M = 39.00$, $SEM = 6.63$) differed ($p < 0.05$) from vehicle ($M = 78.29$, $SEM = 20.35$), and that banana pellet attainment at the 1.5 mg/kg ($M = 43.71$, $SEM = 8.82$) dose did not differ from any other condition. ANOVA showed no effect of naloxone on chow intake [$F(2,12) = 0.24$, n.s., see Figure 2D] at any of the doses tested ($M = 6.57$, $SEM = 1.10$; $M = 6.76$, $SEM = 0.90$; $M = 6.99$, $SEM = 0.76$ for saline, 1.5 mg/kg, and 3.0 mg/kg, respectively).

Effects of naloxone treatment on number of banana pellets, chow, and total intake, in calories were also analyzed. ANOVA revealed a trend toward significance ($p = 0.06$) regarding naloxone's effects on banana-pellet intake [$F(2,12) = 4.46$, n.s]. Energy obtained from banana-flavored pellets for vehicle, mid, and high doses was ($M = 13.74$, $SEM = 3.37$), ($M= 7.67$, $SEM = 1.55$), ($M = 6.85$, $SEM = 1.16$). Naloxone had no effect on the amount of calories obtained from consuming chow [$F(2,12) = 0.24$, n.s.]. Chow consumption under vehicle ($M = 20.17$, $SEM = 3.37$), 1.5 mg/kg ($M = 20.74$, $SEM = 2.77$), 3.0 mg/kg ($M = 21.45$, $SEM= 2.319$) did not differ. One-way ANOVA showed that naloxone decreased total caloric intake [$F(2,12) = 4.73$, $p < 0.05$].
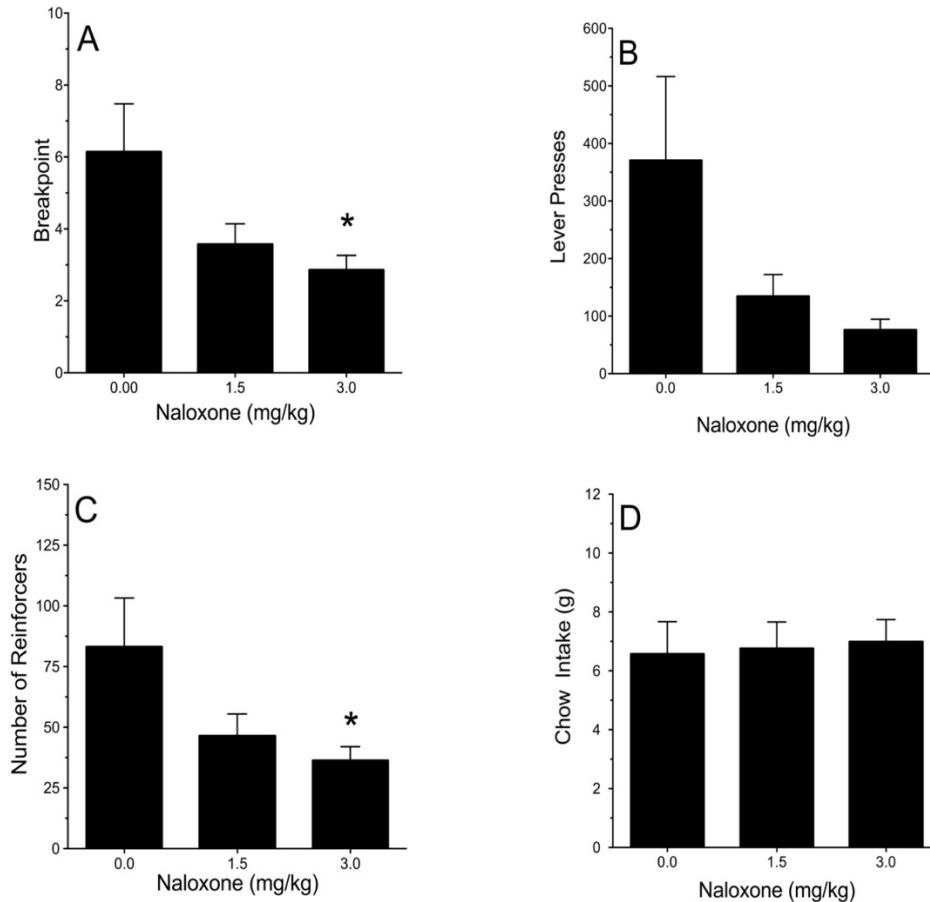
Figure 2. Behavioral Effects of Opioid Receptor Antagonist Naloxone on PR/Chow Task. Rats received IP injections of naloxone (0.00, 1.5 mg/kg, and 3.0 mg/kg) 30 minutes before testing. Data are represented as Means ± SEM. Naloxone (3.0 mg/kg) decreased A) breakpoint and C) number of reinforcers, but had no effect on B) number of lever presses and D) chow intake. * $p < 0.05$ relative to 0.0 mg/kg dose.

3.4 Experiment 3: Effects of Systemic Haloperidol on Food Intake with No Effort

All rats went through extensive preference training in order to ensure stability. In addition to the criterion set by our laboratory, stability was assessed using a 2 (food type) x 4 (session) repeated measures ANOVA over the last 4 days of baseline training. There was no main effect of time [$F(3,18) = 2.272$, n.s], but there was a main effect of food type [$F(1,6) = 27.27$, $p < 0.05$] such that animals preferred to consume banana pellets to regular lab chow. The interaction between the two factors was also not significant [$F(3,18) = 0.1813$, n.s].

A 2 (Dose) x 2 (Food Type) repeated measures ANOVA was used to determine the effects of systemic haloperidol on food intake and preference under free-feeding conditions. ANOVA revealed no significant main effect of dose [$F(1,6) = 4.66$, n.s], a main effect of food type [$F(1,6) = 70.01$, $p < 0.05$], with no significant interaction found [$F(1,6) = 0.7109$, n.s]. Banana pellet consumption was ($M = 8.36$, $SEM = 0.65$) and ($M = 5.56$, $SEM = 1.425$) under

vehicle and 0.10 mg/kg haloperidol, respectively. Chow consumption was ($M = 3.13$, $SEM = 0.44$) and ($M = 1.50$, $SEM = 0.52$) under vehicle and 0.10 mg/kg haloperidol, respectively.

The data was also analyzed by converting banana pellet intake and chow into calories. ANOVA showed no main effect of dose [$F(1,6) = 4.4$, n.s], a significant main effect of food type [$F(1,6) = 76.00$, $p < 0.001$], and no significant interaction [$F(1,6) = 1.16$, n.s]. Banana pellet consumption was ($M = 32.62$, $SEM = 2.54$) and ($M = 21.70$, $SEM = 5.56$) under vehicle and 0.10 mg/kg haloperidol, respectively. Chow consumption was ($M = 9.61$, $SEM = 1.36$) and ($M = 4.58$, $SEM = 1.60$) under vehicle and 0.10 mg/kg haloperidol, respectively.
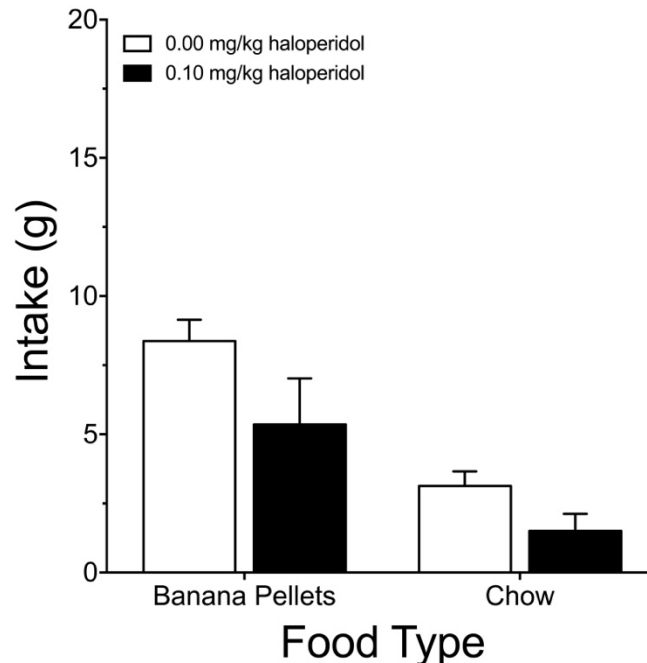

Figure 4. Effects of Systemic Haloperidol Administration on Food Intake and Preference. Rats received IP haloperidol 50 minutes before testing. Intake is presented as Means ± SEM. Haloperidol had an effect on intake without affecting overall preference.

3.5 Experiment 4: Effects of Naloxone on Food Intake with No Effort

We tested the stability of intakes during the no effort task during haloperidol and naloxone treatments. Paired t-tests showed no differences in banana pellet [$t(6) = 1.65$, n.s], chow[$t(6) = 1.18$, n.s], or total intakes [$t(6) = 1.71$, n.s] across the haloperidol vehicle and naloxone vehicle treatments.

The effects of naloxone on free food consumption were assessed with a 2 (Dose) x 2 (Food Type) repeated measures ANOVA, which revealed a main effect of dose [$F(1,6) = 19.12$, $p < 0.05$], a main effect of food type [$F(1,6) = 25.08$, $p < 0.05$], and a significant interaction [$F(1,6) = 6.60$, $p < 0.05$]. Post-hoc Tukey's test revealed that, under vehicle conditions, rats preferred ($p < 0.005$) banana-pellets ($M = 12.44$, $SEM = 2.22$) compared to chow ($M = 2.63$, $SEM = 0.93$). Naloxone (3.0 mg/kg) significantly decreased ($p < 0.05$) banana pellet intake ($M = 4.51$, $SEM = 0.91$) compared to its vehicle counterpart. In contrast, naloxone appeared to have no effect on chow consumption. Intakes of chow after naloxone administration ($M = 2.87$, $SEM = 0.56$) did not differ from chow intakes under the vehicle condition ($M = 2.63$, $SEM = 0.93$). The

magnitude of naloxone's effect was large enough to attenuate banana pellet-preference in drug treated animals.

When analyzed in terms of calories consumed, a 2 (Dose) x 2 (Food Type) repeated measures ANOVA showed a main effect of dose [$F_{(1,6)} = 17.08$, $p < 0.05$], a main effect of food type [$F_{(1,6)} = 35.33$, $p < 0.001$], and a significant dose x food type interaction [$F_{(1,6)} = 7.29$, $p < 0.05$]. Follow up Tukey's test showed that animals consumed more banana pellets ($M = 48.54$, $SEM = 7.31$) than chow ($M = 8.10$, $SEM = 2.40$) under vehicle conditions. Naloxone decreased banana pellet intake ($M = 17.65$, $SEM = 2.99$) selectively, such that there was no difference between that and chow ($M = 8.80$, $SEM = 1.45$).
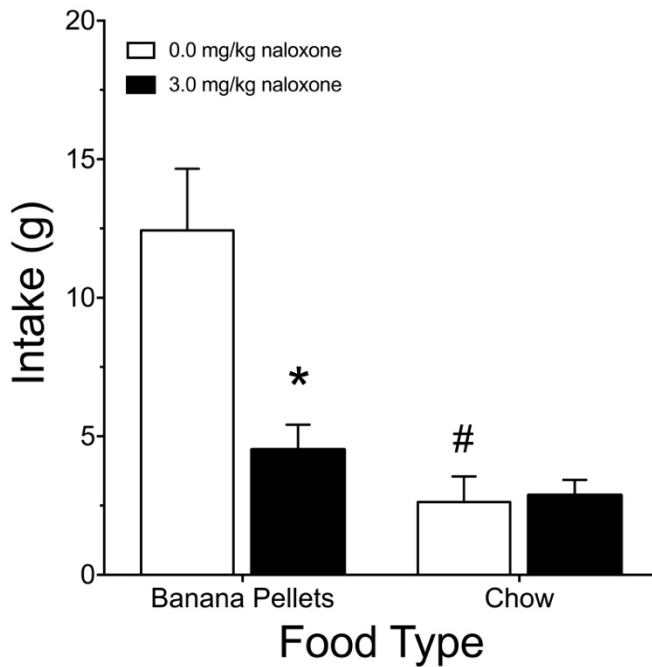

Figure 5. Effects of Systemic Naloxone Administration of Food Preference. Rats received IP injections of Naloxone (0.0 mg/kg and 3.0 mg/kg) 30 minutes before testing. Data are represented as Means ± SEM. Banana pellet intake was greater than chow in the saline solution. # $p < 0.05$ relative to 0.0 mg/kg banana pellet intake. Naloxone also decreased banana pellet intake and eliminated preference. * $p < 0.05$ compared to 0.0 mg/kg banana pellets intake

3.6 Baseline Food Intakes Under Conditions of Effort and No-Effort

We compared the intakes of animals during baseline training in both tasks in order to determine whether there were differences in the amount of food consumed as a function of test type. A 2 (Test Type) x 2 (Food Type) ANOVA revealed no significant effect of food type [$F_{(1,6)} = 2.47$, n.s]. There was a main effect of test type [$F_{(1,6)} = 15.17$, $p < 0.05$] and a significant food and test type interaction [$F_{(1,6)} = 132.3$, $p < 0.0001$]. Planned comparisons showed intakes of chow differed as a function of test type, ($p < 0.05$), as did banana pellet consumption ($p < 0.05$). Similar results were found when consumption was converted into caloric units. ANOVA revealed a main effect of food type [$F_{(1,6)} = 7.27$, $p < 0.05$, see Table 1], a main effect of test type [$F_{(1,6)} = 33.55$, $p < 0.05$], and a significant interaction [$F_{(1,6)} = 119.7$,

p < 0.001]. Follow up planned comparisons disclosed that chow and banana consumption differed as a function effort (*p* < 0.05, *p* < 0.001), respectively. Dependent measures t-tests found total intakes differed between tests of effort and no effort in both grams [*t*(6) = 3.895, *p* < 0.05, 2-tailed, see Table 1] and calories [*t*(6) = 5.792, *p* < 0.05, 2-tailed].

Unit	Test Type	Banana		Chow		Total	
		Mean	SEM	Mean	SEM	Mean	SEM
Grams							
	Effort	3.85*	0.97	7.17 †	1.04	11.02‡	0.68
	No Effort	11.59	0.96	3.15	0.61	14.76	0.60
Calories							
	Effort	15.00*	3.81	20.89 †	2.82	35.89‡	2.42
	No Effort	45.28	3.75	9.66	1.88	54.96	2.54

Table 1. Food intakes by food and test type during baseline training
Food preference differed depending on the type of test that was conducted. Data are presented as Means ± SEM. * *p* < 0.05 in comparison to banana pellet intake in no effort group. † *p* < 0.05 in comparison to chow intake in no effort group. ‡ *p* < 0.05 in comparison to total intake in no effort group.

4. Discussion

The present experiments evaluated the behavioral effects of systemic administration of dopamine and opioid receptor antagonists on cost/benefit decision-making and food preference using tasks with differing levels of effort. Experiment 1 demonstrated that the DA D_2 receptor antagonist haloperidol, at both doses tested, decreased the breakpoint, number of lever presses, number of reinforcers obtained, and increased intake of freely available laboratory chow. Our results are in line with previous work that has carefully described the role of DA mechanisms on this lever pressing and chow feeding task [52,53,58]. DA antagonism and striatal depletions by 6-OHDA or tetrabenazine have been shown to decrease measures of lever pressing while resulting in compensatory increases in chow intake. The focus of the present experiment, however, was not on DA signaling *per se*, but rather, to replicate previous findings in order to better understand the potentially dissociated effects of opioid signaling on food preference and decision-making.

The nonselective opioid receptor antagonist naloxone produced effects that differed slightly from those of haloperidol in Experiment 1. Naloxone, at the highest dose tested (3.0 mg/kg), decreased breakpoint, number of reinforcers obtained, and marginally decreased the number of lever presses. In contrast to the haloperidol treatment, there was no effect on free chow intake. To our knowledge, no work has been done investigating the effects of opioid antagonism on food motivation using this decision-making task, but previous results from our laboratory suggest similar effects can be seen across different schedule requirements [48]. We have found that systemic naloxone also decreases lever pressing for palatable pellets when an FR5/chow schedule of reinforcement is used (without causing any alterations to free chow consumption), suggesting that the motivational effects of opioid receptor antagonism on food

intake are consistent and can be replicated across a number of different schedules of reinforcement. In addition to extending previous results from our laboratory, the data from Experiment 2 are also consistent with research showing that opioid agonists increase, while opioid receptor antagonists decrease, responding on conventional operant tasks across a number of ratio schedules and reinforcer types [33,38,47,54].

Experiments 3 and 4 were carried out in order to determine the effects of haloperidol and naloxone on food intake and preference when no effort is involved. In this task, animals had free access to both banana pellets and laboratory chow within the operant chamber. We found that systemic administration of haloperidol had no significant effect on total food intakes or preference in these experiments. The effects of DA antagonism are in line with previous studies suggesting that this neurotransmitter does not participate in general food preference [59]. By contrast, opioidergic antagonism caused highly selective reductions in food intake by only affecting banana-pellets. The magnitude of these effects was large enough such that, under naloxone, there was no clear preference for either food type. Our experiments converge with lines of preclinical and clinical data suggesting the EOS mediates aspects of hedonic feeding [10,16,19,20,23,29,30].

A question that arises from our data is whether the results of our experiments were due to haloperidol and naloxone acting on central mechanisms that regulate appetite. Our own data, in conjunction with that of others, argues against this being the sole contributor to our results. In Experiment 1, DA receptor antagonism decreased lever pressing for banana pellets, and by extension, consumption of this food type. However, these changes were followed by compensatory increases in free chow intake, suggesting that general appetite was not reduced. This idea is further strengthened by the fact that administration of haloperidol during the PR/Chow task had no effect on the animals' total caloric intake in this task. Haloperidol appears to increase the cost associated with lever pressing, and results in animals reallocating their behavior from palatable pellets to the less costly alternative food source, but overall consuming similar amounts of food. Furthermore, appetite manipulations have been shown to produce different effects from those observed in both the haloperidol and naloxone experiments. Pre-feeding or administration of appetite suppressants do not increase chow intake at the doses that reduce lever pressing. Rather, these manipulations suppress both operant responding and free chow intake [53,59,60].

Previous findings by Randall et al. (2012) using Sprague Dawley rats showed that 0.05 mg/kg of haloperidol had no effect on lever pressing or chow intake, and 0.10 mg/kg decreased lever pressing for banana pellets (without altering chow consumption) [53]. In both our studies and those by Randall et al., freely available chow intake either increased or was unaffected by haloperidol. The present results showed reductions in lever pressing and concurrent increases in chow intake at the 0.05 mg/kg dose, which seems to suggest that Long Evans rats may be more sensitive to the pharmacological effects induced by haloperidol. Generally, DA antagonism has little to no effect on free feeding at the same doses that suppress lever pressing [59,61]. These are findings we replicate in the current study. In Sprague Dawley rats, higher doses than those tested here (0.15 mg/kg) also caused reductions in free food intake, but analyses of feeding microstructures have shown that they are associated with motor impairments that physically prevent animals from consuming food[62]. Because our routes of administration were i.p., we cannot fully exclude motor impairments as a factor that contributed to our results. However, there are several indications suggesting that they were not the key underlying cause of banana pellet intake suppression in Experiment 1. The same dose of haloperidol (0.10 mg/kg) that

reduced lever pressing on the PR/Chow task also produced significant compensatory increases in chow intake and had no significant effect on intakes of either food type in the no effort condition. This indicates that animals' ability to interact and consume food, in general remained intact.

As with the results from the haloperidol treatments, the effects of naloxone cannot be entirely understood as a consequence of appetite modification. While some effects on primary motivation are possible, this does not appear to be the main variable affected. Opioid receptors are localized throughout hypothalamic brain sites that modulate homeostasis [55,63]. It should be noted, however, that Experiment 2 still differs from what has been observed with appetite manipulations, which decrease lever pressing and chow intake on the PR/Chow task and suppress consumption of both food options in no-effort conditions. In both Experiments 2 and 4, naloxone's effects were sensitive to the palatability of the reinforcer, selectively altering consumption of the tastant that animals preferred in effort-free conditions, and not intake in general. In line with this are previous studies suggesting that while high doses of opioid antagonists can decrease intakes of bland foods like water and laboratory chow, their effects are more pronounced on sweet tastants like saccharin and sucrose [64]. In addition to taste, homeostatic state also appears important for opioid modulation of palatable food intake. The anorexigenic effects produced by opioid receptor antagonism are more pronounced in sated than food deprived animals, further evidencing the hypothesis that opioids can regulate reward-related feeding that occurs outside caloric needs [38,47].

A second alternative contributor to our results is that administration of the first pharmacological agent in each task might have somehow contributed to the results of the next task. To elaborate, the possibility exists that haloperidol injections affected the results of naloxone experiments during the PR/Chow task, and vice versa on the no effort task. We argue that this explanation cannot fully account for our results for a number of reasons. First, the order of pharmacology was counterbalanced across our behavioral experiments. Animals were tested first with haloperidol on the PR/Chow task and with naloxone initially during the no effort condition in order to prevent this from being an underlying factor. In addition, during our testing, animals always had at least two days off from pharmacology within each drug and at least a week off between drugs to allow any compound to be fully expelled from their systems. Given the short half-lives of both naloxone and haloperidol [56,57], prolonged drug effects or interactions are unlikely to account for our results. On days in which drug testing did not occur, animals continued their baseline training, during which behavior remained stable and comparable to each vehicle condition [own observations, unpublished]. Our own statistical analyses between naloxone and haloperidol vehicles on each task revealed no differences in performance, suggesting that one drug was not likely affecting any subsequent results.

To our knowledge, this is the first time that opioid regulation of cost/benefit decision-making has been explored in rodents and our results indicate a role for this neurotransmitter system in these processes. It is known that cost/benefit decision-making involves the interaction of a wide network of neuroanatomical and neurochemical systems that includes DA, GABA, and glutamate signaling interactions within the VTA, NAc, VP, BLA, ACC, and mediodorsal thalamus [65–67]. The precise manner in which the EOS fits within this circuitry still remains to be fully determined, but there is evidence that mu-opioid receptor signaling within the NAc, VP, and BLA might be involved. Naloxone is generally regarded as a relatively nonselective opioid receptor antagonist, but research has shown that at low doses, its effects are primarily mediated through mu receptors [68]. These receptors are highly localized within the NAc, VP, and BLA, and their activities have been previously shown to affect both hedonic and motivational

processes related to food consumption [12,19,22,26,28,34,35,69]. Future research focusing on central manipulations will prove fruitful to our understanding of this topic.

5. Conclusions

In summary, DA antagonist haloperidol reduced breakpoint, lever presses, and number of reinforcers earned while increasing chow intake on the PR/Chow task. In contrast, Naloxone decreased breakpoints, and number of reinforcers earned without affecting free chow intake. Haloperidol might be reducing responding on the PR/Chow test by affecting motivational mechanisms related to effort exertion. By contrast, naloxone's effects on this task seem to be multifaceted; the EOS appears to affect motivational mechanisms related to effort, and also those that mediate palatability and hedonic processing. Opioid receptor antagonism might be reducing the palatability of preferred foods such as high sucrose banana-flavored pellets, which in turn reduces an organism's willingness to work to obtain such foods. These experiments are important for understanding how brain circuitry involved in food motivation and decision-making might become dysregulated during pathologies of eating. In line with the clinical literature, our studies indicate a role for DA and EOS mechanisms in regulating the motivation to work for and consume foods. Dysregulation to these systems might produce the compulsive-like patterns of food consumption and impaired decision-making seen during disorders like bulimia nervosa and binge eating disorder [70–74]; they may be responsible for generating increased hedonic responses derived from consuming palatable foods, the development of obsessive desires/cravings to consume foods, or both.

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