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$PYY_{(3–36)}$ Into The Arcuate Nucleus Inhibits Food Deprivation-Induced Increases In Food Hoarding and Intake

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Abstract

Central administration of neuropeptide Y (NPY) increases food intake in laboratory rats and mice, as well as food foraging and hoarding in Siberian hamsters. The NPY-Y1 and Y5 receptors (Rs) within the hypothalamus appear sufficient to account for these increases in ingestive behaviors. Stimulation of NPY-Y2Rs in the Arcuate nucleus (Arc) has an anorexigenic effect as shown by central or peripheral administration of its natural ligand peptide YY (3–36) and pharmacological NPY-Y2R antagonism by BIIE0246 increases food intake. Both effects on food intake by NPY-Y2R agonism and antagonism are relatively short-lived lasting ~4 h. The role of NPY-Y2Rs in appetitive ingestive behaviors (food foraging/hoarding) is untested, however. Therefore, Siberians hamsters, a natural food hoarder, were housed in a semi-natural burrow/foraging system that had a) foraging requirement (10 revolutions/ pellet), no free food (true foraging group), b) no running wheel access, free food (general malaise control) or c) running wheel access, free food (exercise control). We microinjected BIIE0246 (antagonist) and $PYY_{(3–36)}$ (agonist) into the Arc to test the role of NPY-Y2Rs there on ingestive behaviors. Food foraging, hoarding, and intake were not affected by Arc BIIE0246 microinjection in fed hamsters 1, 2, 4, and 24 h post injection. Stimulation of NPY-Y2Rs by $PYY_{(3–36)}$ inhibited food intake at 0–1 and 1–2 h and food hoarding at 1–2 h without causing general malaise or affecting foraging. Collectively, these results implicate a sufficiency, but not necessity, of the Arc NPY-Y2R in the inhibition of food intake and food hoarding by Siberian hamsters.

Keywords

appetitive behavior; Siberian hamster; Peptide YY; BIIE0246; food foraging; food deprivation

INTRODUCTION

In modern industrialized nations, the incidence of obesity has increased markedly over the last few decades and has led to a rise in severe secondary health consequences. Given that most animals forage for food, including humans [for reviews see:[7,31]], we postulated recently that a largely ignored set of related factors leads to sizeable food hoards and has helped propel the obesity crisis: a) size of refrigerators, freezers and pantries, b) processes that extend the shelf lives of food well beyond that of 25–50 years ago, and c) ample and

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inexpensive calorically dense food stuffs[7]. Therefore, a deepened understanding of food foraging and hoarding may lead to behavioral and/or pharmacological treatments for overweight/obese humans, as we have suggested previously[5,7,31].

Using Wallace Craig's[14] division of animal behavior into appetitive (behavior leading to the goal) and consummatory (realization of the goal) phases, ingestive behavior is dichotomized as food foraging/hoarding (appetitive phase) and food intake (consummatory phase). We know considerably more about consummatory ingestive behaviors than appetitive behaviors because the most commonly studied animals in ingestive behavior research are laboratory rats and mice. They are not natural hoarders [for review:[7]] and are typically housed in standard cages that do not permit a significant effort to obtain food. We are able to measure food foraging, hoarding, and intake using our simulated burrow system[17] and Siberian hamsters (*Phodopus sungorus*), as they hoard food in nature[49] and in the laboratory (for review see:[7,31])

Unlike laboratory rats and mice that overeat after a fast [*e.g.*,[27,53]], food deprived Siberian hamsters do not overeat, nor do humans, once access to food is restored but instead 'overhoard', as do humans [for review see:[7]]. Therefore, we reasoned that other stimuli that increase food intake by laboratory rats and mice may trigger increases in food hoarding by these hamsters. Indeed, we launched several studies of the peptidergic control of food hoarding guided by this premise. Some of these studies focused on the Arcuate nucleus (Arc) and the neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons found therein[15,16,19,20,28,29]. As in laboratory rats[41,42,44], and mice[8], NPY and AgRP are nearly exclusively co-localized in neurons within the medial portions of the Arc in Siberian hamsters and Arc NPY and AgRP synthesis is stimulated by food deprivation in Siberian hamsters[22,25,34] making them a possible mediator of food deprivation-induced increases in foraging/hoarding.

NPY is a powerful orexigenic peptide when applied centrally in laboratory rats [*e.g.*,[33,43] and other species [for review see:[6]]. Moreover, NPY is not only a powerful orexigenic peptide in Siberian hamsters[10,15], but also is a powerful short-term (1–4 h, but up to 24 h) stimulator of food hoarding[15,16,20,28,29]. NPY has several receptor (R) sub-types (NPY-Y1-5) that are broadly distributed and their stimulation results in a diverse range of functions [for review see:[48]]. The NPY Y1- and Y5-R have been implicated in the control of food intake in laboratory rats and mice [for review see:[21]]. Microinjections of a Y1-R agonist into the PVH or PFA triggers a dose-dependent increase in food intake in laboratory rats[45] and, conversely, prior or co-injection of a NPY Y1-R antagonist into the PVH blocks the ability of PVH NPY injections to increase food intake[50,51]. NPY Y1-R agonism primarily increases food hoarding, whereas NPY Y5-R agonism primarily increases food intake in our foraging/hoarding model using Siberian hamsters[20,29].

Another NPY receptor subtype that has been strongly implicated in food intake, the NPY Y2-R, is located presynaptically and found in a number of CNS sites, including the Arc and appears to function as an autoreceptor on NPY/AgRP neurons to inhibit their activity and thereby inhibit food intake[11]. A naturally-occurring ligand for the NPY Y2-R is peptide tyrosine-tyrosine (PYY), a gut-derived hormone released from L cells in the intestine after a meal primarily in the form of $PYY_{(3–36)}$ [2]. $PYY_{(3–36)}$ is a selective agonist for the NPY-Y2R resulting in inhibition of food intake, both endogenously and exogenously[1,9]. Consistent with these effects, antagonism of the NPY-Y2R using the Y2-R selective antagonist BIIE0246, increases food intake, adding further support for a role of NPY-Y2R in the cessation of food intake[1]. Therefore, the purpose of the present experiments was to test the role of the NPY Y2-R in food foraging, food hoarding, and food intake in Siberian hamsters. To do so we asked two questions: 1) Does antagonism of NPY Y2-R using

BIIE0246 increase ingestive behaviors in fed animals and 2) Does agonism of NPY Y2-R using the naturally-occurring $PYY_{(3–36)}$ inhibit the food deprivation-induced increases in ingestive behaviors?

MATERIALS AND METHODS

Animals and Housing

Two separate cohorts of 40 male Siberian hamsters 2.5–3 months of age and weighing 35–45 g were selected from our breeding colony. After weaning animals were group housed according to sex and raised in a long day photoperiod (16L:8D, light offset: 1900) with *ad libitum* access to rodent chow (LabDiet® 5001, Purina, St. Louis, MO) and tap water unless otherwise indicated. Room temperature was maintained at 21 ±2 °C. Each cohort was treated identically. All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee and were in accordance with Public Health Service and United States Department of Agriculture guidelines.

Foraging and Hoarding Apparatus

Animals were transferred to the foraging and hoarding room where they were singly housed in shoebox cages 290 × 180 × 130 mm (length × width × height), maintained in a 16L:8D photoperiod (light offset: 1330), and with *ad libitum* access to the pelleted test diet (DPPs, Purified 75 mg pellets; Bio-Serve, Frenchtown, NJ) and water. After two weeks to acclimate to the new light offset, animals were placed into the foraging and hoarding apparatus modified from Perrigio and Bronson[39] and previously described[19]. Briefly, a bottom, “burrow”, cage 290 × 180 × 130 mm (length × width × height) containing Alpha-Dri bedding (Specialty Papers, Kalamazoo, MI) and one cotton nestlet (Anacare, Belmore, NY). The bottom cage was opaque and covered to simulate the darkness of a burrow. The top, “foraging”, cage 456 × 234 × 200 mm (length × width × height) was equipped with a pellet dispenser, running wheel (525 cm circumference), and *ad libitum* access to water. The two cages were connected via convoluted polyvinyl chloride tubing (38.1 mm inner diameter and ~1.52 m long). Wheel revolutions were counted using a magnetic detection system with monitoring by a hardware/software computer interface (Med Associates, Georgia, VT). Hamsters were acclimated/trained to this apparatus for one week prior to and after cannulation (see below).

We used an acclimation/training regimen that minimizes changes in body mass and food intake that can occur when initially housed in the foraging and hoarding apparatus. Specifically, hamsters were given free access to food pellets and were able to earn a food pellet for every 10 wheel revolutions. After the first two days the free access to food was removed and all food had to be earned (1 pellet/10 wheel revolutions) for 5 d, during which body mass, wheel revolutions, pellets earned (food foraging), food intake, and food hoarding were measured daily. After the 7 d acclimation/training period, animals were placed temporarily back into the shoebox cages before cannula implantation (see below).

Foraging Groups and Measurement of Foraging, Food Hoarding, and Food Intake

Three foraging groups were used as in our first of many reports of these groups[17]. When foraging effort is required beyond traversing the tubing, then completion of a programmed number of wheel revolutions triggers food pellet delivery, usually 10, as >10 inhibits hoarding due to decreased payoff – this is the 10 revolution per pellet group (10REVS). Two non-foraging conditions critical to interpreting the 10REVS foraging results were included. In the Free Wheel (FW) condition, food (300 pellets) was presented in the cage non-contingently and independent of wheel running, but wheel running was allowed (controlling for non-specific locomotor stimulation/inhibition thereby providing insight into earned food

by the 10REVS group). In the Blocked Wheel (BW) condition, food (300 pellets) also was presented noncontingently, but the wheel was blocked (controlling for locomotor activity-induced changes – *i.e.*, sedentary controls).

Foraging (pellets earned) was defined as the number of pellets earned (10REV) and food hoarding was defined as the number of pellets found in the bottom cage plus those removed from the cheek pouches. For the BW and FW groups where food was given non-contingently, food intake was defined as the number of pellets supplied (300 pellets/day) minus the total pellets hoarded or left in the top cage (surplus pellets). In the 10REV group, food intake was defined as the number of pellets earned minus the total pellets hoarded or left in the top cage (surplus pellets). The electronic scale used to weigh the food pellets was set to "parts" measurement, resulting in one 75 mg food pellet = 1 with fractions of pellets computed by the scale.

Cannula Implantation, Injections, and Verification

Cannulae were stereotaxically implanted aimed unilaterally at the ventromedial aspect of the Arc (posterior to bregma: −1.4 mm, lateral to midline: 0.3 mm, and ventral to skull: −8.0 mm), because this region shows the densest NPY-Y2R expression in rats[37] and mice[23] under isoflurane (Aerrane, Baxter Healthcare Corporation, Deerfield, IL) inhalation anesthesia as previously described[19]. In brief, each animal had hair removed from the top of their head, skull exposed, and were placed into a stereotaxic surgical apparatus (David Kopf Instruments, Tujunga, CA). A guide cannula (26 gauge stainless steel; Plastics One, Roanoke, VA) was lowered into place and secured to the skull using cyanoacrylate ester gel, 3/16 mm jeweler's screws, and dental acrylic. The opening in the guide cannula was sealed using a removable obturator throughout the experiment except during parenchymal injections. Hamsters received buprenorphine (0.2 mg/kg body mass, s.c.) to minimize discomfort and apple slices to facilitate food and water intake immediately following surgery and for the following 2 d. Animals were housed in shoebox cages for 2 wks following surgery before being returned to the foraging and hoarding apparatus.

Each animal was "mock-injected" daily in the week before a test day, where the obturator was removed and the animal was lightly restrained for 1 min to acclimate the animal to the injection procedure. On test days, an inner cannula (33 gauge stainless steel, Plastics One, Roanoke, VA) was connected to a Hamilton syringe via PE-20 tubing and inserted into the guide cannula, extending 0.5 mm below the guide cannula tip. All injections were given at light offset (1330 EST). Each injection (200 nl) of neurochemical or vehicle was delivered over 30 s and the injection needle remained in place for ~30 s before removal, as done previously [*e.g.*,[15,19]].

Following the final test day, animals were injected with 300 nl bromophenol blue dye to mark the location of the cannula tip and animals were then given an overdose of pentobarbital sodium (100 mg/kg), transcardially perfused with 100 ml of heparinized saline followed by 125 ml of 4% paraformaldehyde in phosphate buffered saline, pH=7.4. The brains were then removed and post fixed in a 4% paraformaldehyde solution for 2 d, followed by a 30% sucrose solution until sectioning, replacing the sucrose solution after 24 h. Brains were sectioned at 80 μm for cannula location verification using light microscopy. Cannulae were considered an Arc hit if the blue dye was visible in the ventromedial aspect of the Arc and only these animals were included in the analyses (n = 75, see Figure 1 for cannula locations).

Baseline Data and Foraging Groups

At the conclusion of the acclimation/training period animals were separated into one of the three foraging groups (10REV, FW, BW) described above. Animals were separated into the groups matched for body mass, food intake, and food hoarding and were allowed 2 wks to acclimate to their foraging treatment group.

Experiment 1: NPY-Y2R Antagonism by BIIE0246 in Fed Animals

Arc injections consisted of one of three doses of BIIE0246 (0.1, 1.0, 5.0 nmol in 200 nl) or vehicle (5% DMSO), with vehicle choice and doses based on effective Arc delivered drug in laboratory rats[1]. Each animal received all injections in a counterbalanced-within subjects design. A washout period of 1 wk separated individual injections to ensure all measures had returned to baseline values similar to our previous work[29]. On injection days, animals were provided with a clean burrow cage and access to food was prevented by blocking access to the top cage 2 h before injections. Animals were injected at light offset and access to food was returned. Wheel revolutions, food foraging, food hoarding, and food intake were measured at 1, 2, 4, 24 h and each day post-injection until the next test day (final group sizes BW: n=21, FW: n=22, and 10REV: n=26).

Experiment 2: NPY-Y2R Agonism by $PYY_{(3–36)}$ in Food-Deprived Animals

After the week-long washout period following the final BIIE0246 test day, animals were maintained in their foraging treatment group for an additional week and then assigned to an injection treatment group (see below) balanced for body weight, food intake, and food hoarding as above. The animals were then food deprived for 56 h (IACUC approved), a time length previously shown to maximize food hoarding[4,18]. Before access to food was returned at light offset, half of the animals received an injection of $PYY_{(3–36)}$ (0.1 nmol in 200 nl), the active form of the peptide for satiation[52], into the Arc and the other half received the saline vehicle. Wheel revolutions, food foraging, food intake, and food hoarding were measured at 1, 2, 4, 24 h and each day post-injection until all animals returned to pre-injection levels. After the animals returned to behavioral baseline, brain tissue was collected to verify cannula location (Fig. 1; 69 hits and 11 misses or removed their cannula; final group sizes: $PYY_{(3–36)}$: BW: n=12, FW: n=11, and 10REV: n=13 and vehicle: BW: n=9, FW: n=11, and 10REV: n=13).

Statistics

Raw data from *Experiment 1* were transformed for each individual into percent change from vehicle before statistical analyses using the formula: [((X-Vehicle)/Vehicle)*100], where “X” equals the value measured in response to the dose of BIIE0246 and “Vehicle” equals the value measured for that individual after vehicle injection. No statistical comparisons were made among the time intervals because the intervals were of unequal duration. No statistical comparisons are reported across test days in this counterbalanced-within subject design, as repeated measures two-way ANOVA (Foraging Treatment × Arc-Injection) showed no effect of injection order. The data were analyzed using a two-way ANOVA (Foraging Treatment × Arc-Injection; 3 × 4). For *Experiment 2*, data were not transformed into percent change from vehicle, because animals only were food deprived once and therefore could not serve as their own control, and the absolute values were analyzed using a two-way ANOVA (Foraging Treatment × Arc-Injection; 3 × 2) within each individual time point for the same reason as above. All statistical analyses were performed using NCSS (version 2007, Kaysville, UT). Exact probabilities and test values were omitted for simplicity and clarity of presentation. Differences were considered statistically significant if $P<0.05$. Tukey-Kramer Multiple Comparison Tests were used for post hoc tests when appropriate. Misplaced cannulae were not included in the final statistical comparisons.

RESULTS

Experiment 1: NPY-Y2R Antagonism with BIIE0246 in Fed Animals

Wheel Running—At each time interval, Arc injection of BIIE0246 did not significantly stimulate wheel running activity compared to vehicle injection at any of the three doses tested (0.1, 1.0, and 5.0 nmol; Fig. 2A). The lack of wheel running increase in the FW group, where food delivery was not contingent upon wheel running, suggests that there was not non-specific stimulation of locomotor activity.

Food Foraging—Arc injection of BIIE0246 did not significantly increase food foraging (wheel running contingent food delivery) at any time point examined (Fig. 2B) above vehicle-treated animals.

Food Intake—Food intake was not stimulated above vehicle at any time interval examined (0–1, 1–2, 2–4, 4–24 h) by Arc injection of BIIE0246 for all three foraging treatments (10REV, FW, and BW; Fig. 3A–C).

Food Hoarding—BIIE0246 injection into the Arc did not stimulate food hoarding compared to vehicle injection at any time point examined for each foraging treatment (10REV, FW, and BW; Fig. 4A–C). During the 2–4 h interval after the 5.0 nmol BIIE0246 injection, hoarding in the 10REV group approached significance (p=0.059) when compared with vehicle injection.

Experiment 2: NPY-Y2R Agonism using $PYY_{(3–36)}$ in Food-Deprived Animals

Wheel Running—$PYY_{(3–36)}$ treatment inhibited the food deprivation-induced increases in wheel running during the 0–1 h interval (Fig. 5A). The inhibition of wheel running is not indicative of malaise caused by $PYY_{(3–36)}$, because the inhibition also was not seen in the 10REV group (see below).

Food Foraging—Arc injection of $PYY_{(3–36)}$ did not result in a significant inhibition of food foraging compared to saline injection at any time point measured (0–1, 1–2, 2–4, 4–24 h as well as during the next 6 d; Fig. 5B).

Food Intake—Arc injection of $PYY_{(3–36)}$ attenuated food intake after food deprivation compared with Arc saline injection at 0–1 and 1–2 h for the 10REV foraging treatment (Fig. 6C), but not in the BW or FW group (Fig. 6A and B); no significant differences were present after the first two h of refeeding for any group.

Food Hoarding—In the 10REV group, agonism of the NPY-Y2R in the Arc using $PYY_{(3–36)}$ inhibited food hoarding upon refeeding compared with saline injection at 1–2 h and approached significance at 0–1 h (p=0.07; Fig. 7C). Significant differences were not seen at any other time point or foraging treatment (Fig. 7A and B).

DISCUSSION

Controlling ingestive behavior is a vital aspect of preventing/treating obesity and accordingly a concerted effort has been made to describe the mechanisms involved in food intake. NPY is the most potent central orexigenic neurochemical in laboratory rats[13,33,43] with marked increases in food hoarding and intake occurring with stimulation of Y1-R and Y5-R, respectively in Siberian hamsters[20]. The NPY-Y2R agonism/antagonism had not been tested for its role in the appetitive ingestive behaviors of food hoarding or foraging[14] in any species before the present study. Here we found for the first time that agonism of the Y2-R

by $PYY_{(3–36)}$ inhibited food intake and hoarding early (first few hours) after refeeding following food deprivation and that antagonism of the Y2-R by BIIE0246 did not affect appetitive or consummatory ingestive behaviors in fed hamsters. These results suggest a possible inhibitory role of $PYY_{(3–36)}$ in food hoarding.

Antagonism of the Y2-R in the Arc using BIIE0246 causes short term increases in food intake by laboratory rats[1], effects similar to those of NPY Y1-R and Y5-R agonism [*e.g.*,[24,45]]. NPY-Rs have a dual role in the control of food intake, where Y2-R and Y4-R agonism is anorexigenic and Y1-R and Y5-R agonism is orexigenic in other rodents[3,21,32]. This dualism only partially extended to Siberian hamsters here as $PYY_{(3–36)}$ microinjections into the Arc inhibited food intake and especially food hoarding, but the NPY-Y2R antagonist BIE0246 did not stimulate food foraging, intake, or hoarding. This lack of effect of BIIE0246 on baseline food intake also has been reported for laboratory rats[40]. It is possible that our BIIE0246 dose was insufficient to block endogenous Y2 signaling, although this seems somewhat unlikely because we used a dose 5-times greater than a dose effective in rats[1]. In two pilot studies, we injected the highest dose of BIIE0246 (5.0 nmol) used here into the Arc followed 2–3 minutes later by $PYY_{(3–36)}$ peripherally (7.5 nmol/kg) or into the Arc (0.1 nmol). In both studies, BIIE0246 co-administered with $PYY_{(3–36)}$ resulted in no significant change in ingestive behaviors when compared to saline-treated Siberian hamsters. These data suggest that our dose BIIE0246 is able to prevent the inhibition of ingestive behaviors caused by Y2 agonism. The present data suggests that there is not a chronic stimulation of Y2 signaling in non-energetically challenged (*i.e.*, ad libitum-fed) Siberian hamsters similar to laboratory rats[40], which is unlike the apparent underlying inhibition of ingestive behaviors by leptin[30] and cholecystokinin[46] in Siberian hamsters.

It is worth noting the large standard error values found in most of the variables measured after Arc administration of the Y2 antagonist. The animals exhibited a dichotomous split into high and low levels of food foraging, intake, and hoarding, but hamsters showing high or low levels of one behavior did not necessarily predict high or low levels of the other behaviors as seems apparent for food hoarding by Syrian hamsters[12]. In addition, the exact location of the cannula within the Arc also was not associated with a particular ingestive behavioral response or the magnitude of the response. Large variations in food hoarding both within and between animals from day-to-day are common, quite unlike that of food intake studies in this species in our experience. The cause of the variations in spontaneous food hoarding by Siberian hamsters remains a mystery presently and is not due to differences in body fat (for example: fat hamsters hoarding less than lean animals because they possess greater internal energy stores).

The second experiment was designed to test the inhibitory role of the Y2-R signaling using the naturally occurring NPY Y2-R agonist $PYY_{(3–36)}$. $PYY_{(3–36)}$ has potent anorexigenic effects whether administered peripherally and centrally in laboratory rats and mice [for review:[35]], with few exceptions[47]. The anorexic effects of $PYY_{(3–36)}$ are diminished when animals are stressed[26], which may account for the studies where $PYY_{(3–36)}$ was unable to inhibit food intake[47]. Because of the possible effects of stress interacting with the $PYY_{(3–36)}$ treatment, our animals were habituated to the injection protocol. As noted above, when food deprived Siberian hamsters are refed, large increases in food hoarding and foraging occur persisting for ~7 d, whereas food intake does not increase beyond the first few h[18]. Arc injected $PYY_{(3–36)}$ inhibited food intake and food hoarding in the true foraging group (10REV) during the first few hours of refeeding, a timeframe of effectiveness similar to that of 24 h food-deprived-refed laboratory rats after $PYY_{(3–36)}$ treatment for food intake[9]. The finding that the effect of $PYY_{(3–36)}$ only was seen in the hamsters ‘earning’ their food via foraging (wheel running; *i.e.*, 10REV) is consistent with our findings with other anorexigenic peptides that exhibit their greatest inhibition in the 10REV group including the

NPY Y1-R antagonist 1229U91[29], leptin[30], melanocortin 4-R agonism [melanotan II[28]] as well as triggering the greatest increases in food hoarding for orexigenic peptides administered centrally [NPY[15,20], AgRP[19]]. The reason that 'earned' food elicits both larger decreases and increases in food hoarding is not clear. It is not that these animals are in any greater increase in negative energy balance due to the wheel running because the FW group runs approximately the same number of wheel revolutions as the 10REV group, although food is not contingent on the wheel running. Thus, some factor(s) associated with foraged ('earned') food rather than freely available food seems in play in the present and our previous studies that certainly warrants further study.

Collectively, the present data indicate that NPY Y2-R agonism inhibits food intake and hoarding, albeit in the short term (0–2 h) with refeeding after food deprivation. In addition, there does not appear to be underlying NPY Y2-R signaling inhibiting ingestive behaviors in this species because the antagonism of NPY Y2-R signaling does not increase appetitive or consummatory ingestive behaviors in ad libitum-fed hamsters. The short term nature of $PYY_{(3–36)}$ is not unique to this study, and as such seems to be limited in its ability to decrease foraging/hoarding in Siberian hamsters. Longer lasting NPY Y2-R agonists are being developed[36], however, some of which may have the potential for therapeutic use to curtail food intake and hoarding in humans.

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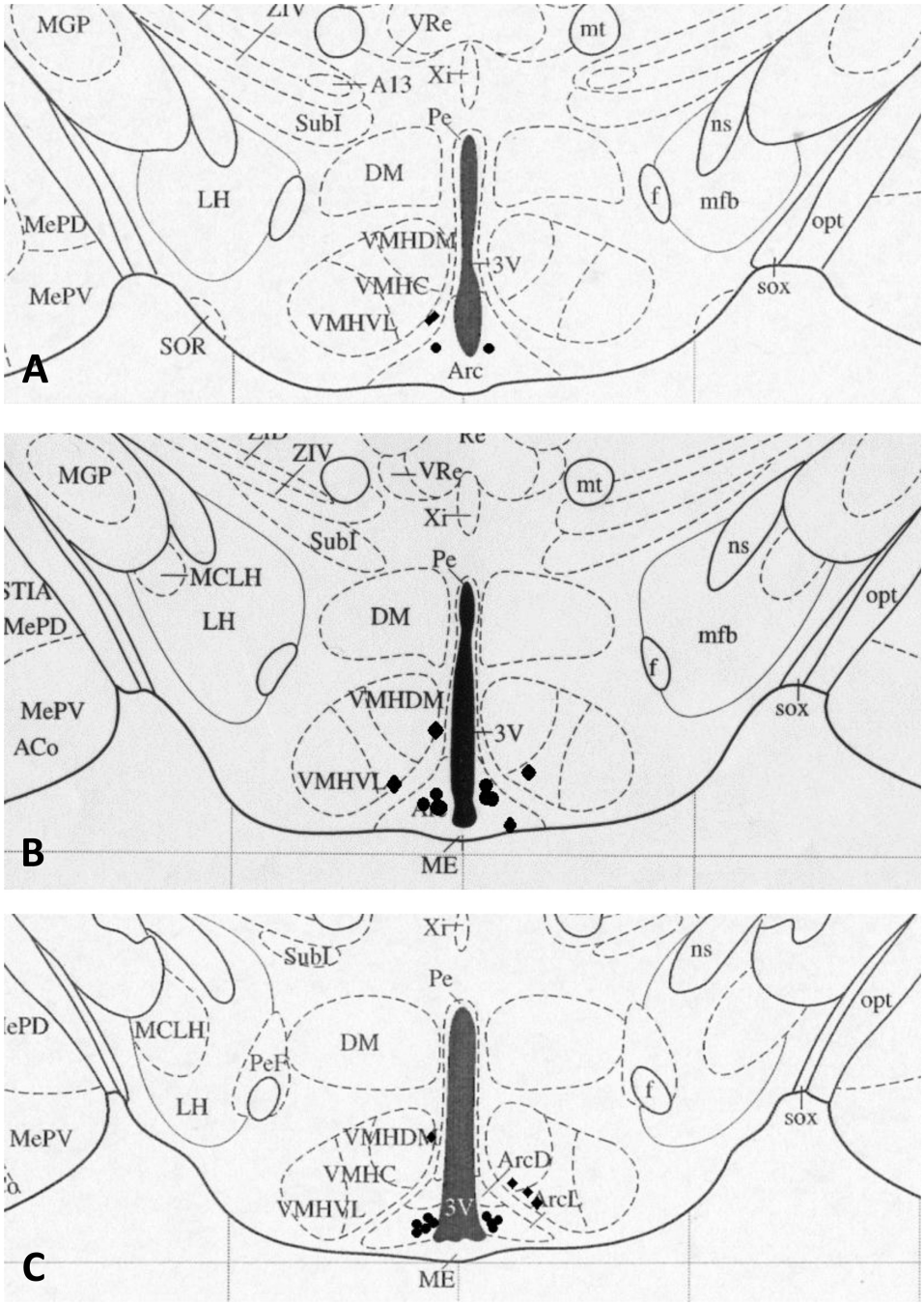
- Arcuate antagonism of NPY-Y2Rs, with BIIE0246, did not stimulate ingestive behavior
- Arcuate agonism of NPY-Y2Rs, with $PYY_{(3–36)}$, inhibited food intake and hoarding
- NPY-Y2R signaling is sufficient, but not necessary, to inhibit ingestive behavior



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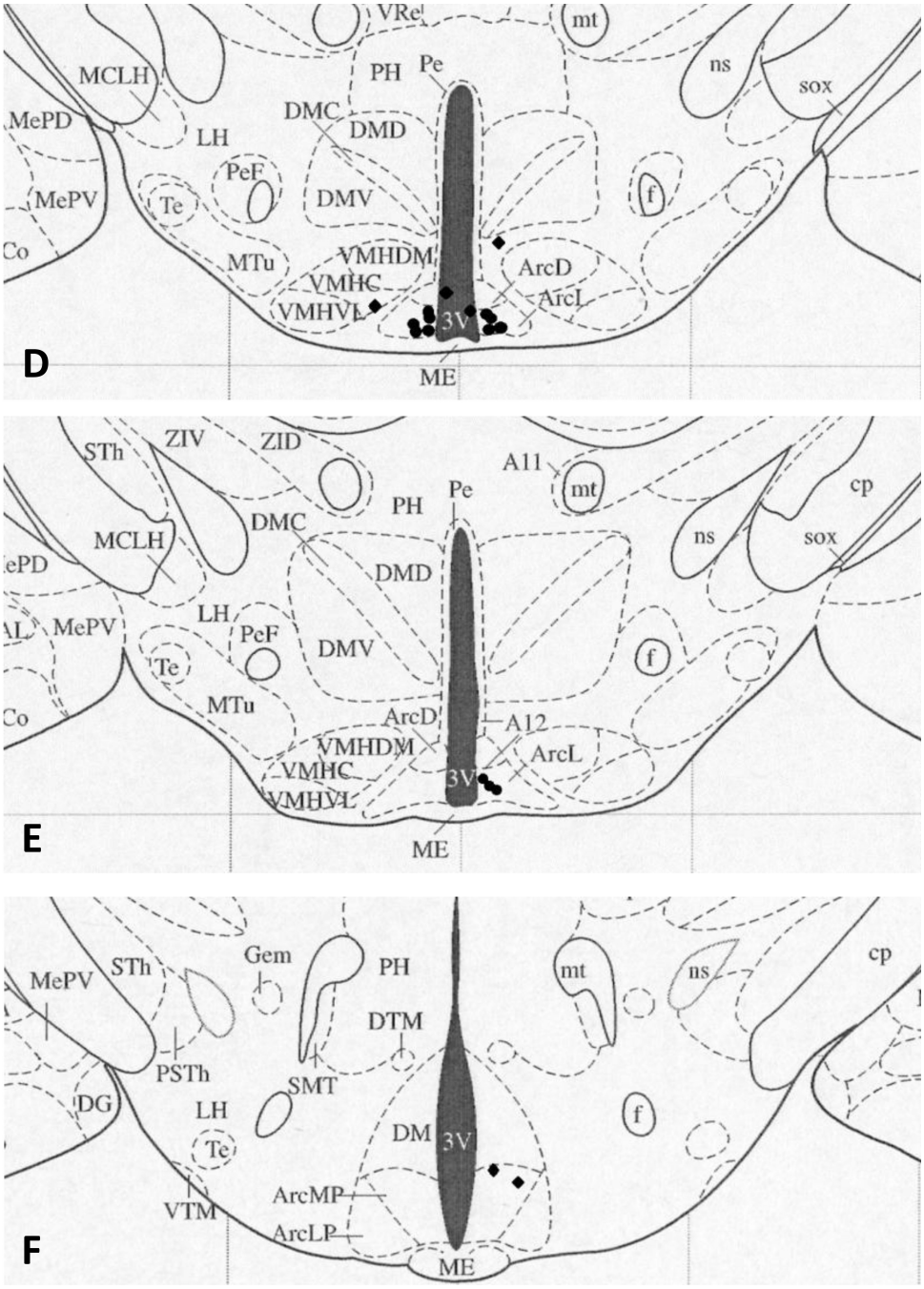

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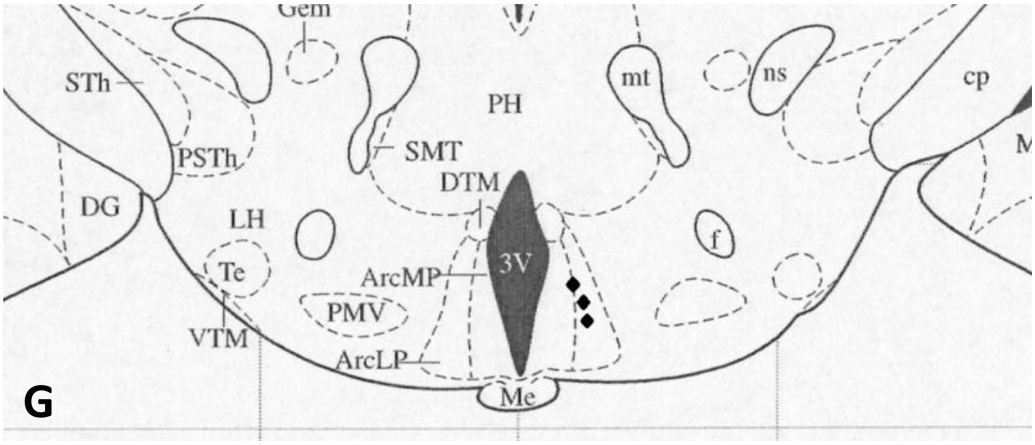

Figure 1.

Cannula location plotted onto schematic brain section diagrams (taken from Paxinos and Watson[38]) with filled circles (•) representing the tip of a correctly placed cannula and filled diamonds (♦) representing misplaced cannula. A. Plate 43, B. Plate 44, C. Plate 45, D. Plate 46, E. Plate 47, F. Plate 49, and G. Plate 50. Some circles represent more than one cannula. 3V, third ventricle; Arc, Arcuate nucleus; ArcD, dorsal Arcuate nucleus; ArcL, lateral Arcuate nucleus; ArcLP, lateroposterior Arcuate nucleus; ArcMP, medioposterior Arcuate nucleus; DM, dorsomedial hypothalamus; ME, median eminence; VMHC, central ventromedial hypothalamus; VMHDM, dorsomedial ventromedial hypothalamus; VMHVL, ventrolateral ventromedial hypothalamus.

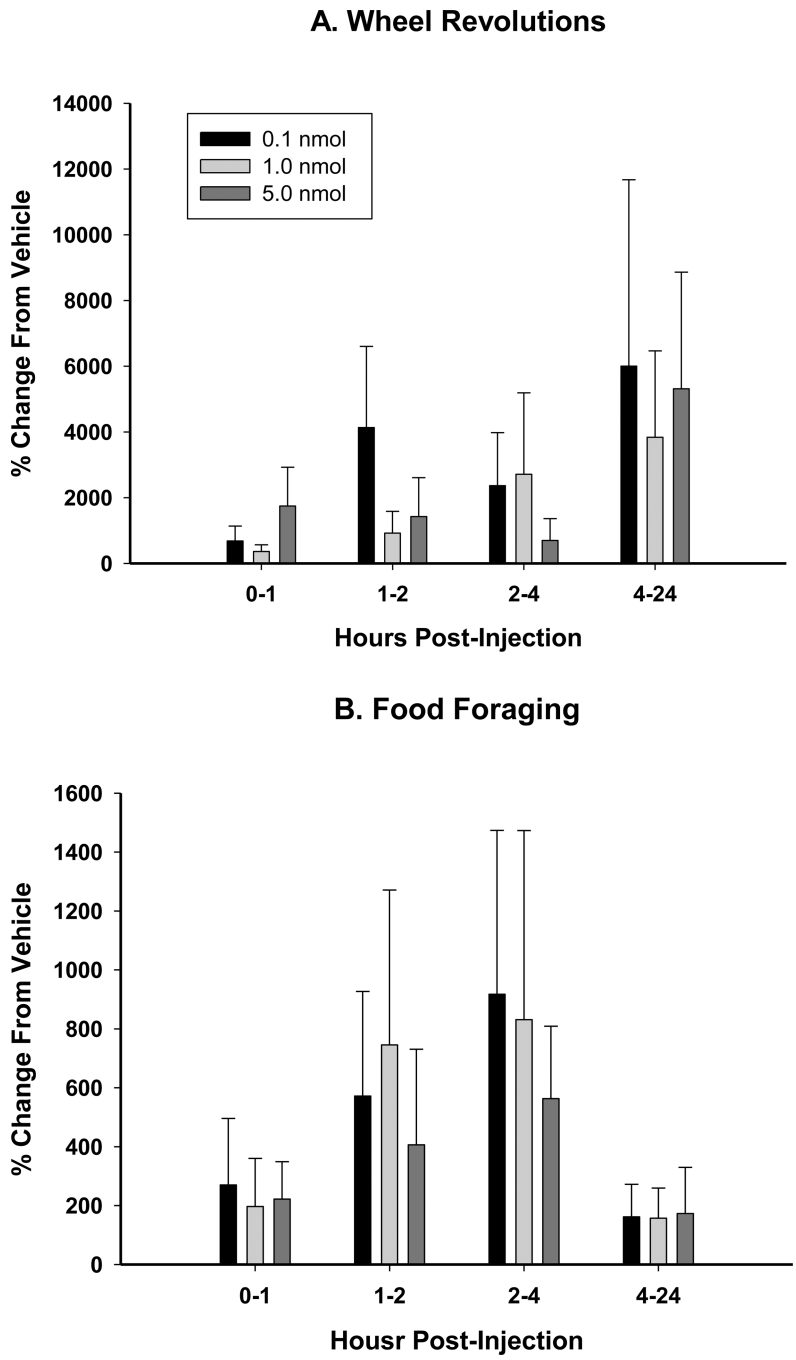

Figure 2.

Mean ± SEM percent change from vehicle (5% DMSO) in response to BIIE0246 (0.1, 1.0, and 5.0 nmol) of A. wheel revolutions in hamsters with no foraging requirement and a functional running wheel [Free Wheel (FW)] and B. Foraging [pellets earned; in hamsters with a 10 wheel revolutions per pellet foraging requirement (10REV)].

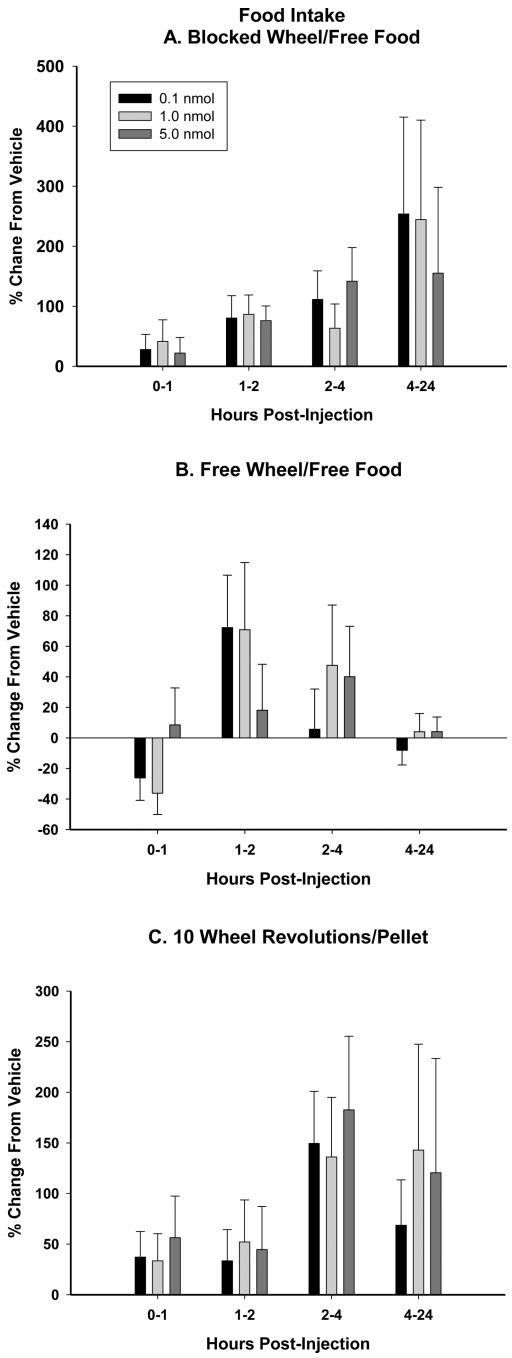

Figure 3.

Mean ± SEM percent change from vehicle (5% DMSO) in response to BIIE0246 (0.1, 1.0, and 5.0 nmol) of food intake with A. no foraging requirement and a stationary running wheel [Blocked Wheel (BW)], B. no foraging requirement and a functional running wheel [Free Wheel (FW)], and C. a 10 wheel revolutions per pellet foraging requirement [10 revolutions/pellet (10REV)].

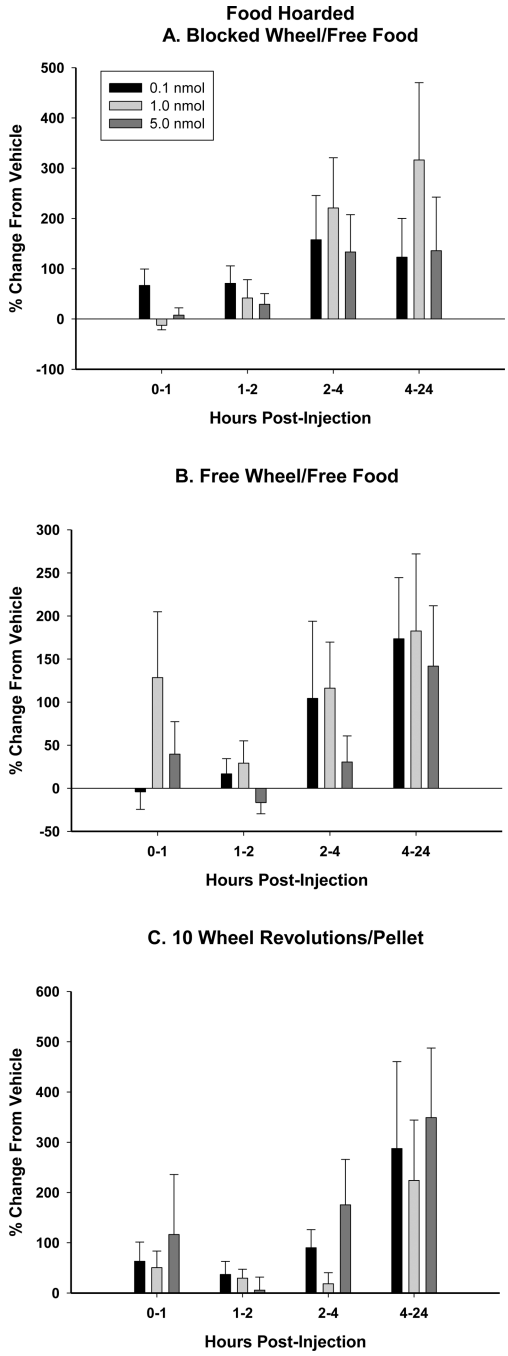

Figure 4.

Mean ± SEM percent change from vehicle (5% DMSO) in response to BIIE0246 (0.1, 1.0, and 5.0 nmol) of food hoarded with A. no foraging requirement and a stationary running wheel [Blocked Wheel (BW)], B. no foraging requirement and a functional running wheel [Free Wheel (FW)], and C. a 10 wheel revolutions per pellet foraging requirement [10 revolutions/pellet (10REV)].

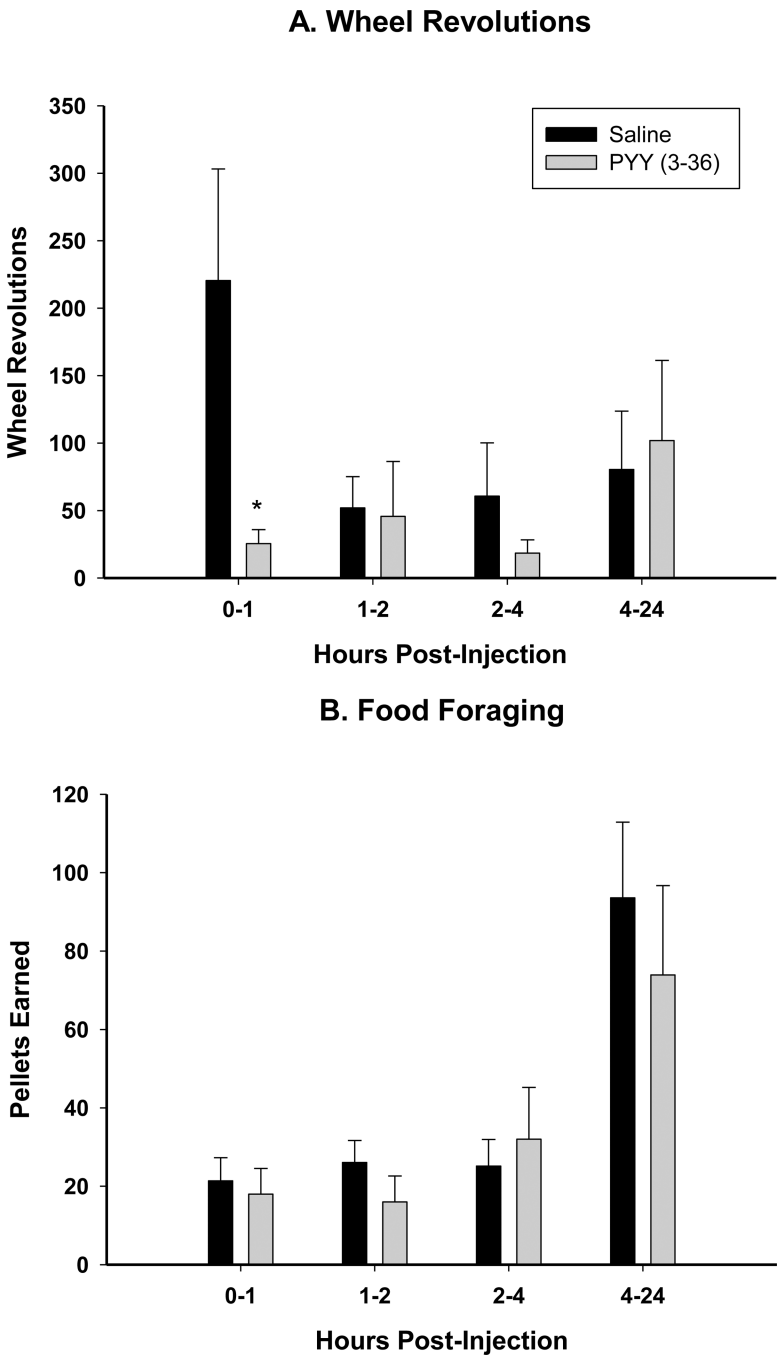

Figure 5.

Mean ± SEM of Arc injection of PYY (3–36) (0.1 nmol) or Saline post-food deprivation of A. wheel revolutions in hamsters with no foraging requirements and a functional running wheel [Free Wheel (FW)] and B. foraging (pellets earned) in hamsters with a 10 wheel revolutions per pellet foraging requirement [10 revolutions/pellet (10REV)]. *= $P<0.05$ vs Saline.

Figure 6.

Mean ± SEM of Arc injection of PYY (3–36) (0.1 nmol) or Saline post-food deprivation of food intake with A. no foraging requirement and a stationary running wheel [Blocked Wheel (BW)], B. no foraging requirement and a functional running wheel [Free Wheel (FW)], and C. a 10 wheel revolutions per pellet foraging requirement [10 revolutions/pellet (10REV)]. *= P<0.05 vs Saline.

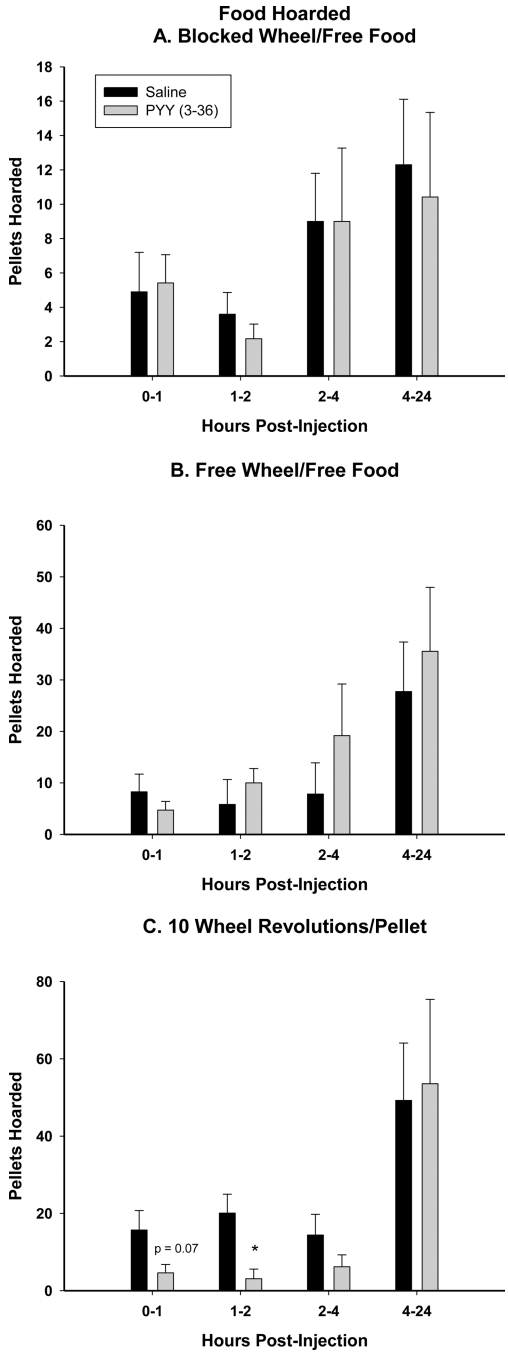

Figure 7.

Mean ± SEM of Arc injection of PYY (3–36) (0.1 nmol) or Saline post-food deprivation of food hoarded with A. no foraging requirement and a stationary running wheel [Blocked Wheel (BW)], B. no foraging requirement and a functional running wheel [Free Wheel (FW)], and C. a 10 wheel revolutions per pellet foraging requirement [10 revolutions/pellet (10REV)]. *= P<0.05 vs Saline.