Behavioral/Systems/Cognitive

Peptide YY3-36 Decreases Reinstatement of High-Fat Food Seeking during Dieting in a Rat Relapse Model

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A major problem in treating obesity is high rates of relapse to maladaptive food-taking habits during dieting. This relapse is often provoked by acute re-exposure to palatable food, food-associated cues, or stress. We used a reinstatement model, commonly used to study relapse to abused drugs, to explore the effect of peptide YY3-36 (PYY3-36) on reinstatement of high-fat (35%, 45 mg pellets) food seeking induced by acute exposure to the pellets (pellet priming), a cue previously associated with pellet delivery (pellet cue), or yohimbine (2 mg/kg, a pharmacological stressor). Rats were placed on a restricted diet (16 g of chow per day) and lever-pressed for the pellets for 9–12 sessions (6 h/d, every 48 h); pellet delivery was paired with a tone–light cue. They were then given 10–20 extinction sessions wherein lever presses were not reinforced with the pellets and subsequently tested for reinstatement of food seeking. Systemic PYY3-36 injections (100–200 μg/kg) decreased pellet priming- and pellet cue-induced reinstatement of food seeking but not yohimbine-induced reinstatement. Arcuate nucleus (Arc) injections of PYY3-36 (0.4 μg per side) decreased pellet priming-induced reinstatement. The attenuation of pellet priming-induced reinstatement by systemic PYY3-36 was reversed by systemic (2 mg/kg) but not Arc (0.5 μg per side) injections of the Y2 receptor antagonist BIIE0246. Arc PYY3-36 injections did not decrease pellet cue-induced reinstatement. Finally, systemic PYY3-36 injections had minimal effects on ongoing food self-administration or heroin priming- or heroin cue-induced reinstatement of heroin seeking. These data identify an effect of systemic PYY3-36 on relapse to food seeking that is independent of Y2 receptor activation in Arc and suggest that PYY3-36 should be considered for the treatment of relapse to maladaptive food-taking habits during dieting.

Key words: **arcuate nucleus; heroin self-administration; peptide YY; reinstatement; relapse; stress; Y2 receptors; yohimbine**

Introduction

A main problem in dietary treatment of excessive eating is high rates of relapse to maladaptive eating habits (Peterson and Mitchell, 1999). This relapse is often triggered by re-exposure to palatable foods, food-associated cues, or stress (Herman and Polivy, 1975; Grilo et al., 1989; Drewnowski, 1997). The mechanisms underlying relapse to maladaptive eating habits in humans are unknown, and this topic has rarely been studied in animal models (Horvitz and Ettenberg, 1988; Duarte et al., 2003).

The neuronal mechanisms underlying drug reward overlap with those of food reward (Carr, 2002; DiLeone et al., 2003; Abizaid et al., 2006). Therefore, we recently adapted a reinstatement model, commonly used to study relapse to abused drugs (Shalev et al., 2002) to explore mechanisms underlying relapse to food seeking during dieting (Ghitza et al., 2006). We found that in food-restricted rats, a corticotropin-releasing factor (CRF) receptor antagonist blocks reinstatement of food seeking induced by yohimbine (a pharmacological stressor) but has no effect on reinstatement induced by acute noncontingent exposure to food pellets (pellet priming). These findings suggest that different mechanisms mediate relapse to food seeking induced by stress versus acute food re-exposure. Here, we explored mechanisms underlying relapse to high-fat food by studying the effect of peptide YY3-36 (PYY3-36) on reinstatement of high-fat food seeking.

PYY3-36 is a major circulatory derivative of peptide YY (PYY) (Eberlein et al., 1989), a gastrointestinal-derived hormone released from intestinal L-cells after meals in proportion to caloric intake (Tatemoto and Mutt, 1980; Murphy et al., 2006). PYY3-36 has high affinity for neuropeptide Y (NPY) Y2 receptors and lower affinity for Y1 and Y5 NPY receptors (Grandt et al., 1992; Ballantyne, 2006); NPY is a hypothalamic peptide that increases food intake after hypothalamic or ventricular injections (Leibowitz, 1995). The Y2 receptor is a putative NPY presynaptic inhibitory autoreceptor, whereas Y1 and Y5 are postsynaptic excitatory receptors (Wahlestedt et al., 1986; Larhammar and Salaneck, 2004). Systemic PYY3-36 injections decrease food intake in mice, rats, monkeys, and humans (Batterham et al., 2002, 2003; Moran et al., 2005; Chelikani et al., 2006) [but see Tschop et al. (2004) and Boggiano et al. (2005) for different results]. In rats, PYY3-36 injections into the arcuate nucleus (Arc) inhibit food intake (Bat-

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terham et al., 2002). In mice, the anorexic effects of PYY3-36 are prevented by deletion of Y2 receptors (Batterham et al., 2002; Renshaw and Batterham, 2005).

We initially assessed the effect of systemic PYY3-36 injections on ongoing high-fat pellet self-administration and on pellet priming-induced reinstatement of food seeking. We then assessed the role of Y2 receptors in the inhibition of pellet priming-induced reinstatement of PYY3-36 by using BIIE0246, a selective Y2 receptor antagonist (Doods et al., 1999), and the role of the Arc in this reinstatement by injecting PYY3-36 or BIIE0246 into this brain area. We then assessed the generality of PYY3-36 effects on reinstatement by examining its effect on reinstatement induced by a tone–light cue previously paired with pellet delivery (pellet cue) and the pharmacological stressor yohimbine. Finally, we assessed the specificity of PYY3-36 to reinstatement of food seeking by determining its effect on heroin priming- and heroin cue-induced reinstatement of heroin seeking.

Materials and Methods

Subjects and apparatus

Male Long–Evans rats (total $n = 192$, 300–420 g; Charles River, Raleigh, NC) were housed in self-administration chambers for the duration of the experiment under a reverse 12 h light/dark cycle (lights off at 9:30 A.M.). We excluded 84 rats because of failure to meet an extinction criterion (see below), lever responding that was >3 SDs above the group means, poor health, catheter failure (experiment 7), or Arc cannula misplacement. The rats trained to self-administer food were kept on a restricted diet of 16 g/d [~60–65% of their regular daily Purina (St. Louis, MO) rat chow]. The rats trained to self-administer heroin were mildly restricted to 20–25 g/d of Purina rat chow to maintain stable body weight. The rats' body weights were taken daily, and procedures followed the guidelines outlined in the *Principles of Laboratory Animal Care* (National Institutes of Health publication number 85-23). Experiments were conducted in standard self-administration chambers (Med Associates, Georgia, VT). Each chamber had two levers 9 cm above the floor, but only one lever ("active," retractable lever) activated the pellet dispenser, which delivered 45 mg of chocolate-flavored food pellets containing 35% fat and 45.2% carbohydrate (F05879; Bioserv, San Diego, CA).

Drugs

Drugs were prepared fresh before testing. PYY3-36 (100 and 200 μg/kg, i.p., for systemic injections and 0.4 μg per side for Arc injections; catalog #H-6042; Bachem, Torrence, CA) was dissolved in saline, BIIE0246 (2 mg/kg, i.p., for systemic injections and 0.5 μg per side for Arc injections; catalog #1700; Tocris Bioscience, Ellisville, MO) was dissolved in 30% polyethylene glycol (Sigma-Aldrich, St. Louis, MO), and yohimbine HCl (2 mg/kg, i.p.; Research Biochemicals, St. Louis, MO) was dissolved in distilled water. The injection volumes for systemic injections were 1 ml/kg for PYY3-36 and BIIE0246 and 0.5 ml/kg for yohimbine. The doses of PYY3-36 and BIIE0246 for systemic and Arc injections are based on previous studies (Batterham et al., 2002; Abbott et al., 2005; Boggiano et al., 2005; Scott et al., 2005). The yohimbine dose (2 mg/kg, i.p.) is based on our previous work (Shepard et al., 2004; Le et al., 2005; Ghitza et al., 2006). PYY3-36 was injected systemically either 30 min (pellet self-administration, priming- and cue-induced reinstatement) or 60 min (yohimbine-induced reinstatement) before the test sessions. PYY3-36 was injected into the Arc 5 min before the test sessions. Yohimbine was injected 45 min before the test sessions. BIIE0246 was injected systemically 30 min before PYY3-36 injections. BIIE0246 was injected into the Arc 10 min before PYY3-36, which was injected systemically 5 min before the test sessions.

Intracranial surgery and intracranial injections

The rats were anesthetized with a mixture of sodium pentobarbital and choral hydrate (60 and 25 mg/kg, i.p.). Using a stereotaxic instrument (David Kopf Instruments, Tujunga, CA), they were implanted with guide cannulas (23 gauge; Plastics One, Roanoke, VA) bilaterally 1–2 mm above the Arc: anteroposterior, −2.3 mm; mediolateral, ±2.2 mm; dorsoventral, −7.9 or −8.9 mm (10° angle) (Paxinos and Watson, 2005). Buprenorphine (0.1 mg/kg, s.c.) was given after surgery, and the rats were allowed to recover for 7 d. Arc PYY3-36 injections (0.4 μg per side; injection volume, 0.3 μl) and BIIE0246 injections (0.5 μg per side; injection volume, 0.3 μl) were made with Harvard Apparatus (Holliston, MA) infusion pumps, using 10 μl Hamilton (Reno, NV) syringes that were connected via polyethylene-50 tubing to 30 gauge injectors (Plastics One). The day before the intracranial injections, the rats were habituated to the injection procedure by removing and reinserting the cannula blockers. Injections were performed in the freely moving (nonanesthetized) rats over 1 min, and injectors were left in place for an additional 2 min before being replaced with cannula blockers. After testing, the rats were perfused with paraformaldehyde (Sigma) and decapitated, and the brains were removed. Coronal sections (40 μm) were sliced on a cryostat, stained with cresyl violet (ICN Biomedicals, Aurora, OH), and examined for cannula placement under a light microscope.

Procedures

With the exception of an initial assessment of the effect of PYY3-36 on ongoing food pellet self-administration, we used a reinstatement procedure that included three phases: training for food self-administration (9–12 sessions), extinction of the food-reinforced behavior (10–20 sessions), and tests for reinstatement under extinction conditions (up to 4 sessions). During all phases, the sessions started 30 min after the beginning of the rats' dark cycle (lights off at 9:30 A.M.). Below, we first describe the training and extinction procedures for all experiments and then provide the specific details for the reinstatement phase of each experiment. During the reinstatement phase, we counterbalanced the experimental conditions and the drug administration conditions.

Food self-administration training

All rats were given two to three 6 h daily sessions of "autoshaping" during which pellets were administered noncontingently every 5 min into a receptacle located near the active lever. Pellet delivery was accompanied by a compound 5 s tone (2900 Hz, 20 dB above background)–light (a 7.5 W white light located above the active lever) cue; this cue is termed pellet cue. Subsequently, the rats were trained to self-administer the pellets every other day for 6 h/d (two 3 h sessions separated by 1 h) on a fixed-ratio-1 reinforcement schedule for 9–12 sessions. For the rats that were subsequently tested for food priming- and yohimbine-induced reinstatement, the timeout period after pellet delivery was 20 s. For the rats that were subsequently tested for pellet cue-induced reinstatement, the timeout period was 40 s.

At the start of each 3 h session, a red houselight was turned on, and the active lever was extended. After each pellet delivery, the pellet cue was turned on for 5 s. At the end of each 3 h session, the red houselight was turned off, and the active lever was retracted. During the training days, regular food (16 g of Purina rat chow) was given immediately after the second daily session (~7.5 h into the dark cycle). During the off days, the 16 g of regular food was given at the start of the dark cycle.

We chose this training schedule and these diet conditions because previous nonoperant food-consumption studies have shown that rats placed on a restricted diet and given intermittent access to palatable food develop binge-like eating behavior (Colantuoni et al., 2002; Corwin and Buda-Levin, 2004; Avena et al., 2007) and become hypersensitive to the effect of stress on palatable food intake (Hagan et al., 2002, 2003). We used a 40 s timeout in the pellet cue-induced reinstatement experiment, because we found that cue-induced reinstatement of sucrose seeking is more robust with this timeout duration than with a shorter duration (J. M. Bossert, unpublished observation) (Bossert et al., 2006a).

Extinction of food self-administration

After training, the rats were given 10–20 daily extinction sessions until active lever responding was below 30 presses/3 h for three consecutive sessions (an extinction criterion). For the rats that were subsequently tested for food priming- and yohimbine-induced reinstatement, lever presses led to tone–light cue presentations but not pellet delivery. For the rats that were subsequently tested for pellet cue-induced reinstatement, lever presses had no programmed consequences (i.e., neither the tone–light cue nor pellets were made available). Initially, the rats were given

two 3 h sessions (separated by 1 h) each day for 6 d. Subsequently, they were given one 3 h extinction session per day for an additional 4–14 d until they met the extinction criterion. During the extinction and reinstatement phases, the regular food (16 g) was given approximately at the same time as during training (i.e., ~7.5 h after the onset of the dark cycle).

Experiment 1: effect of systemic PYY3-36 injections on food self-administration

In this initial experiment, we examined the effect of systemic PYY3-36 injections on ongoing food self-administration. After 12 training days, we assessed the effect of PYY3-36 on food pellet self-administration in two 6 h tests (two 3 h sessions separated by 1 h) that were conducted 48 h apart. We used the within-subjects factors of PYY3-36 pretreatment (vehicle, PYY3-36) and session (first 3 h session, second 3 h session) and the between-subject factor of PYY3-36 dose (100 and 200 μg/kg; $n = 8–10$ per dose). Thus, each rat was given an injection of the PYY3-36 vehicle (saline) and a single PYY3-36 dose before the test sessions, which were performed in a counterbalanced order.

Experiment 2: effect of systemic PYY3-36 injections on pellet priming-induced reinstatement

After testing the effect of PYY3-36 on food self-administration (experiment 1), the same rats were given extinction sessions (see above) and were subsequently tested for pellet priming-induced reinstatement in 3 h test sessions in which one food pellet was administered noncontingently just before the start of the test sessions. We tested the effect of PYY3-36 on pellet priming-induced reinstatement in four 3 h test sessions with two sessions run consecutively and 1 extinction day between sets of tests. We used the within-subjects factors of PYY3-36 pretreatment (vehicle, PYY3-36), pellet priming (pellet, no pellet), and session minutes (six 30 min blocks) and the between-subject factor of PYY3-36 dose (100 and 200 μg/kg; $n = 8–10$ per dose). Thus, each rat in the low PYY3-36 dose group was given four counterbalanced test sessions [vehicle-no pellet, vehicle-pellet, PYY3-36 (100 μg/kg)-no pellet, and PYY3-36 (100 μg/kg)-pellet], and each rat in the high-dose group was given four counterbalanced test sessions [vehicle-no pellet, vehicle-pellet, PYY3-36 (200 μg/kg)-no pellet, and PYY3-36 (200 μg/kg)-pellet]. We used a similar experimental design, which is based on previous pharmacological studies using the reinstatement model (Shaham et al., 1997), in the other experiments described below. The rationale for using this mixed experimental design is to decrease the number of rats used while limiting the number of repeated pellet priming-induced reinstatement tests to two (instead of four in the case of a complete within-subjects design) to minimize habituation to the effect of pellet priming (or other stimuli used to induce reinstatement) over repeated testing.

A separate group of rats ($n = 10$) was tested in the same manner as the rats that received 200 μg/kg PYY3-36 (see above), with the exception that this group of rats received an injection of the Y2 receptor antagonist BIIE0246 (2 mg/kg, i.p.) 30 min before injections of PYY3-36 or its vehicle.

Experiment 3: effect of Arc PYY3-36 injections on pellet priming-induced reinstatement

The purpose of experiment 3 was to determine whether the systemic effect of PYY3-36 on pellet priming-induced reinstatement is mimicked by Arc injections of the peptide. We tested the effect of Arc injections of PYY3-36 (0.4 μg per side) on pellet priming-induced reinstatement in four test sessions with two sessions run consecutively and 1 extinction day between each set of tests ($n = 14$). For this purpose, we used the within-subjects factors of PYY3-36 pretreatment (vehicle, 0.4 μg per side), pellet priming (pellet, no pellet), and session minutes.

Experiment 4: effect of Arc BIIE0246 injections on the inhibitory effect of systemic injections of PYY3-36 on pellet priming-induced reinstatement

Based on the results of experiments 2 and 3, in experiment 4 we explored the role of Arc Y2 receptors in the inhibitory effect of systemic injections of PYY3-36 on pellet priming-induced reinstatement. The rats ($n = 10$) received Arc injections of BIIE0246 (0.5 μg per side) or its vehicle 10 min before systemic injections of PYY3-36 (200 μg/kg, i.p.); PYY3-36 was injected 5 min before the test sessions. We tested the rats in four test sessions with two sessions run consecutively and 1 extinction day between each set of tests, using the within-subjects factors of BIIE0246 dose (0 and 0.5 μg per side) and pellet priming (pellet, no pellet). The order of the experimental conditions was counterbalanced.

Experiment 5: effect of systemic and Arc PYY3-36 injections on cue-induced reinstatement

The purpose of experiment 5 was to assess the generality of the effect of PYY3-36 on reinstatement by determining the effect of the drug on reinstatement of food seeking induced by discrete cues previously associated with pellet delivery during training. It is well established that such discrete cues robustly reinstate both food and drug seeking after extinction (De Vries and Schoffelmeer, 2005; See, 2005). As stated previously, during the training phase each pellet delivery was paired with a tone–light cue (pellet cue); this pellet cue was not presented during the extinction phase after lever pressing. During the tests for reinstatement, lever responding led to contingent presentations of the tone–light cue under the fixed-ratio-1 40 s timeout reinforcement schedule. We tested the effect of PYY3-36 on cue-induced reinstatement in four test sessions with two sessions run consecutively and 5 extinction days between sets of tests. We conducted 5 extinction days between sets of tests, because in previous studies we found that this procedure minimizes habituation to the effect of the conditioned cues on reinstatement of sucrose seeking (J. M. Bossert, unpublished observation) (Bossert et al., 2006). We used the within-subjects factors of PYY3-36 pretreatment (vehicle, PYY3-36), pellet cue (cue, no cue), and session minutes and the between-subject factor of PYY3-36 dose (100 and 200 μg/kg; $n = 10$ per dose).

Based on the results of experiment 3, we also assessed the effect of Arc PYY3-36 injections on pellet cue-induced reinstatement of food seeking ($n = 8$). The experimental procedure for self-administration training and extinction of lever responding was the same as described for the rats given systemic PYY3-36 injections. Rats were given injections of PYY3-36 (0.4 μg per side) or its vehicle in four test sessions with two sessions run consecutively and 5 extinction days between sets of tests. We used the within-subjects factors of PYY3-36 pretreatment (vehicle, 0.4 μg per side), pellet cue (cue, no cue), and session minutes.

Experiment 6: effect of systemic PYY3-36 injections on yohimbine-induced reinstatement

The purpose of experiment 6 was to further assess the generality of the effect of PYY3-36 on reinstatement by determining its effect on reinstatement induced by yohimbine, an α-2 adrenoceptor antagonist that induces stress- and anxiety-like responses in both humans and nonhumans (Bremner et al., 1996a,b) and reinstates drug and food seeking in laboratory animals (Lee et al., 2004; Bossert et al., 2005; Le et al., 2005; Nair et al., 2006). We tested the effect of PYY3-36 on yohimbine-induced reinstatement in four test sessions with two sessions run consecutively and 1 extinction day between sets of tests. We used the within-subjects factors of PYY3-36 pretreatment (vehicle, PYY3-36), yohimbine (vehicle, 2 mg/kg), and session minutes and the between-subject factor of PYY3-36 dose (100 and 200 μg/kg; $n = 9–10$ per dose). One outlier rat that lever pressed >350 times in both the vehicle and the PYY3-36 (100 μg/kg, i.p.) condition (>3 SD above the group mean) was excluded.

Experiment 7: effect of systemic PYY3-36 injections on heroin cue- and heroin priming-induced reinstatement of heroin seeking

In this final experiment, we assessed the specificity of PYY3-36 effect to reinstatement of food seeking by determining its effect on heroin priming- and heroin cue-induced reinstatement of heroin seeking. Rats were implanted with intravenous catheters into the jugular vein as described previously (Shaham et al., 1996; Shalev et al., 2001a; Bossert et al., 2006b), and training for heroin self-administration started after 7 recovery days. During this time period and the training phase, catheters were flushed every 24–48 h with gentamicin in sterile saline (0.08 mg/ml). Experiment 7 consisted of three phases: training for heroin self-administration, extinction of the heroin-reinforced behavior, and tests for the effect of PYY3-36 on heroin cue-induced or heroin priming-

induced reinstatement of heroin seeking. All sessions started 30 min after the onset of the dark cycle.

The rats were trained to self-administer heroin for 6 h/d for 10 d under a fixed-ratio-1 schedule. Heroin (diacetylmorphine HCl; National Institute on Drug Abuse) was dissolved in sterile saline and infused in a volume of 65 μl over 2.3 s at a dose of 0.05 mg/kg (first five sessions) and 0.025 mg/kg (last five sessions) per infusion. The heroin training doses are based on previous studies (Shalev et al., 2001b; Bossert et al., 2004), and the unit dose was halved after the fifth day of training to verify that the rats reliably acquired drug self-administration, as indicated by the increase in lever presses for the lower dose (Yokel, 1987). During training, heroin infusions were earned under a fixed-ratio-1 (40 s timeout) schedule and were accompanied by the compound tone–light cue for 5 s. Sessions began with the illumination of the red houselight and the insertion of the active lever. At the end of each session, the houselight was turned off, the active lever was retracted, and the rats were fed 20–25 g to maintain their body weight. During the extinction phase (10 d), procedures were identical to those of training, except that the responses on the active lever did not result in the infusion of heroin or the presentation of the tone–light cue.

Heroin cue-induced reinstatement. We tested the effect of PYY3-36 on heroin cue-induced reinstatement in four test sessions with two sessions run consecutively and 5 extinction days between sets of tests. In the four counterbalanced test sessions, the rats were given injections of vehicle (saline) or PYY3-36 (100 or 200 μg/kg, i.p.) 30 min before the start of the tests, and active lever presses led to the presentation of either the tone–light cue or no cue. We used the within-subjects factors of PYY3-36 pretreatment (vehicle, PYY3-36), heroin cue (cue, no cue), and session minutes and the between-subject factor of PYY3-36 dose (100 and 200 μg/kg; $n = 8$–10 per dose).

Heroin priming-induced reinstatement. At the completion of the heroin cue-induced reinstatement tests, nine rats were given four additional extinction sessions in the presence of the tone–light cue. During two subsequent test sessions, four of these rats were given injections of the PYY3-36 vehicle (saline, intraperitoneally) 30 min before the sessions, and 25 min later they were given injections of heroin (0.25 mg/kg, s.c.) or its vehicle (saline, subcutaneously). The other five rats were given injections of 200 μg/kg PYY3-36 and heroin or saline before the test sessions. Thus, we used the within-subjects factor of heroin priming (0 and 0.25 mg/kg) and the between-subjects factor of PYY3-36 dose (0 and 200 μg/kg). The heroin priming dose is based on previous studies (Shaham et al., 1996; Shalev et al., 2001b).

Statistical analyses

The data on the effect of PYY3-36 on food pellet self-administration (experiment 1) were analyzed separately for the number of pellets earned and timeout nonreinforced active lever responding. The data from the reinstatement experiments were analyzed for nonreinforced lever presses on the previously active lever and on the inactive lever. Because the experimental manipulations had no effect on inactive lever responding, which was very low, these data are not shown.

In reinstatement experiments in which two PYY3-36 doses were used, we tested the effect of PYY3-36 in a factorial design with the within-subjects factors of PYY3-36 pretreatment (vehicle, PYY3-36), stimulus condition [pellet priming (pellet, no pellet), pellet cue/heroin cue (cue, no cue), or yohimbine (vehicle, 2 mg/kg)], and session minutes (six 30 min blocks) and the between-subject factor of PYY3-36 dose (100 and 200 μg/kg). In this analysis, a significant effect of PYY3-36 pretreatment reflects differences between PYY3-36 (100 or 200 μg/kg, or both) and vehicle, whereas a significant effect of PYY3-36 dose reflects differences between the 100 μg/kg dose and the 200 μg/kg dose. The factors used in the statistical analysis of reinstatement of lever responding in which only one PYY3-36 dose was used are described in Results. Because of the complexity of the experimental design used, we primarily report in Results critical significant interactions between the different factors. These significant interactions were followed by *post hoc* PLSD tests (two-tailed), which are indicated in the figures.

Results

During the food training phase, the rats (total $n = 108$) were maintained on 16 g/d of regular chow food and were given 6 h access to the food pellets every other day. They gained weight when pellets were available and lost weight when they were not (Fig. 1*A*). A repeated-measures ANOVA using pellet availability and training day as the within-subjects factors and body weight as the dependent measure revealed a significant interaction between these factors ($p < 0.01$). This weight fluctuation was not observed during the extinction and reinstatement phases when the pellets were not available.

Training and extinction

The rats in experiment 1–6 were trained for 9–12 sessions and demonstrated reliable pellet self-administration and, as in our previous study, a progressive escalation of timeout responding across sessions (Ghitza et al., 2006) (Fig. 1). The rats were then given six 6 h extinction sessions and additional 3 h extinction sessions during which lever presses decreased over time (Fig. 1). The rats that were subsequently tested for pellet priming- and yohimbine-induced reinstatement ($n = 80$) (Fig. 1*A–C*) were trained under a fixed-ratio-1 20 s timeout reinforcement schedule, and their active lever presses were extinguished in the presence of the pellet cue (the 5 s tone–light previously paired with each pellet delivery). The rats that were subsequently tested for pellet cue-induced reinstatement ($n = 28$) (Fig. 1*A*,*D*,*E*) were trained under a fixed-ratio-1 40 s timeout reinforcement schedule, and their active lever presses were extinguished in the absence of the pellet cue.

During the training phase for both experimental conditions, the statistical analyses revealed significant increases over time for both pellets earned and active-lever timeout responding (p values <0.01) but not for inactive lever responding. During the extinction phase for both experimental conditions, the analyses revealed significant decreases over time for active lever responding during the first 6 extinction days when the rats were given two 3 h daily sessions that were separated by 1 h (p values <0.01) but not for inactive lever responding.

Experiment 1: effect of systemic PYY3-36 injections on food self-administration

PYY3-36 modestly decreased the number of pellets earned (Fig. 2) and timeout lever responding (data not shown) during food self-administration; however, this effect did not reach statistical significance and was not dose dependent. Two groups of rats ($n = 8$–10 per group) were given injections of one dose of PYY3-36 (100 or 200 μg/kg) or vehicle 30 min before 2 training days during which the rats lever pressed for the pellets in two 3 h sessions that were separated by 1 h. The statistical analyses for each measure (pellet and timeout responses) included the within-subjects factors of PYY3-36 pretreatment (vehicle, PYY3-36) and session (first 3 h session, second 3 h session) and the between-subject factor of PYY3-36 dose (100 and 200 μg/kg). The analysis for the effect of PYY3-36 on the number of pellets earned revealed an approaching significant effect of PYY3-36 pretreatment ($F_{(1,16)} = 4.1$; $p = 0.061$); the effects of PYY3-36 dose or session or the interactions between the different factors were not significant ($p > 0.05$). The analysis for the effect of PYY3-36 on the number of timeout responses did not reveal any significant effects, with the exception of session ($F_{(1,16)} = 6.3$; $p < 0.05$), because of a greater number of timeout responses in the first session than in the second session, collapsed across PYY3-36 pretreatment conditions (data not shown).

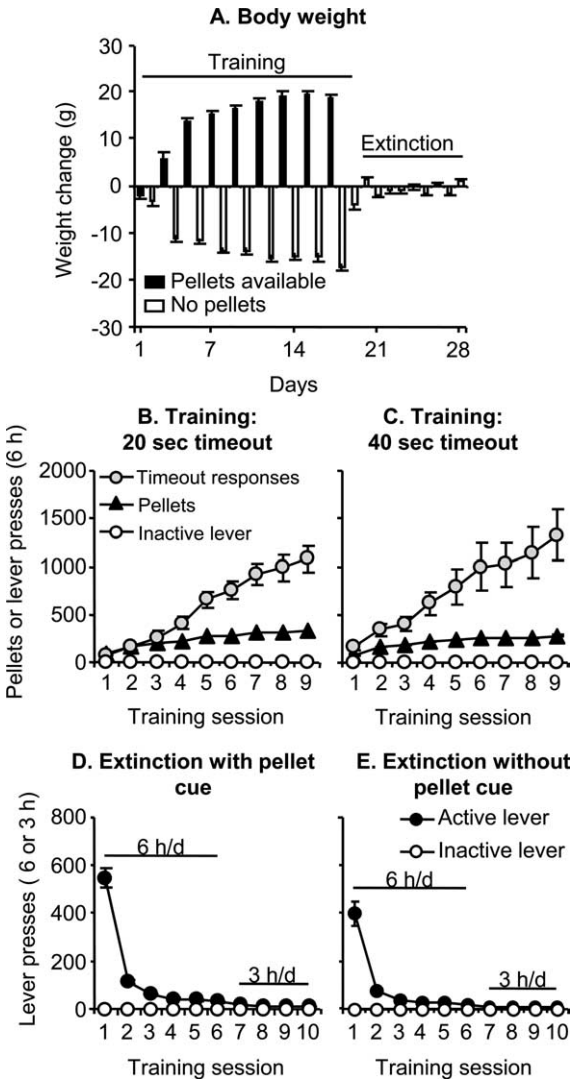

Figure 1. Food pellet self-administration training and extinction of the food-reinforced lever responding. ***A***, Body weight fluctuations during the training and extinction phases. During the training phase, the rats (total $n = 108$) were maintained on 16 g/d of regular chow food and were given 6 h access to the food pellets every other day. During the extinction (and reinstatement) phases, food pellets were not available in the self-administration chambers, and the rats were maintained on 16 g/d of regular food. ***B***, Training. Mean ± SEM number of 35% fat pellets earned, active lever presses, and inactive lever presses during the training sessions over 9 alternating days (two 3 h sessions per day, every other day) for rats that were trained under a fixed-ratio-1 (FR-1) 20 s timeout reinforcement schedule ($n = 80$); these rats were subsequently tested for pellet priming- and yohimbine-induced reinstatement. ***C***, Corresponding training data for rats that were trained under an FR-1 40 s timeout reinforcement schedule ($n = 28$); these rats were subsequently tested for pellet cue-induced reinstatement. ***D***, Extinction with pellet cue. Mean number of presses on the previously active lever or the inactive lever during the extinction phase for rats that had been trained with a timeout period of 20 s after each pellet delivery. Lever presses were extinguished in the presence of the tone–light pellet cue over consecutive days for two 3 h sessions per day on days 1–6 and for 3 h/d for the subsequent days. ***E***, Extinction without pellet cue. Corresponding extinction data for rats that had been trained with a timeout period of 40 s after each pellet delivery and lever presses extinguished in the absence of the pellet cue are shown.

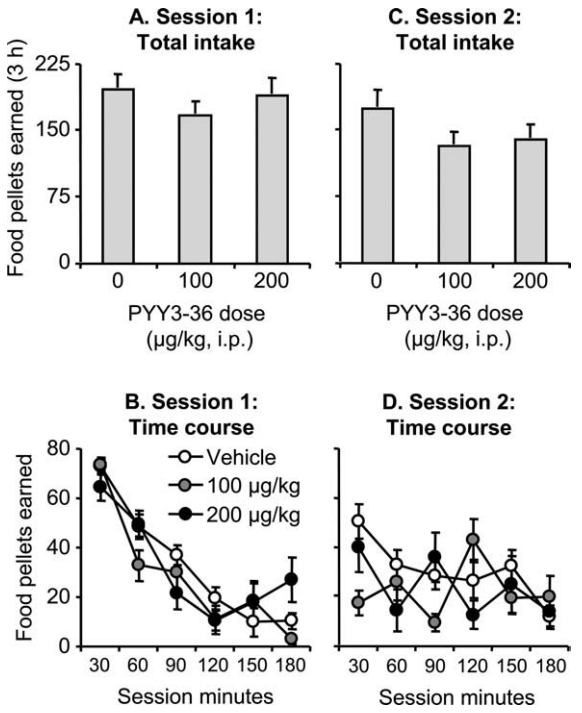

Figure 2. Systemic PYY3-36 injections have a minimal effect on pellet self-administration. ***A***, ***C***, Total pellets. Mean ± SEM number of 35% fat pellets self-administered after vehicle (saline) or PYY3-36 injections during the first (***A***) and second (***C***) 3 h sessions ($n = 8$–10 for each PYY3-36 dose; $n = 18$ for the vehicle condition). ***B***, ***D***, Corresponding time course for the data described in ***A*** and ***C***. PYY3-36 or vehicle was injected 30 min before session 1; session 2 started 1 h after the end of session 1. Each rat was given injections of vehicle and one dose of PYY3-36 (see Materials and Methods and Results for details of the experimental design).

Experiment 2: effect of systemic PYY3-36 injections on pellet priming-induced reinstatement

Systemic injections of both doses of PYY3-36 decreased pellet priming-induced reinstatement of active lever presses, an effect that was most pronounced in the first 30 min of the 3 h test sessions (Fig. 3*A*,*B*). The ANOVA revealed significant interaction effects of PYY3-36 pretreatment × pellet priming ($F_{(1,16)} = 32.5$; $p < 0.01$) and PYY3-36 pretreatment × pellet priming × session minutes ($F_{(5,80)} = 48.3$; $p < 0.01$). Injections of the Y2 receptor antagonist BIIE0246 (2 mg/kg, i.p.) 30 min before PYY3-36 injections reversed the inhibition of PYY3-36 on pellet priming-induced reinstatement (Fig. 3*C*,*D*); injections of BIIE0246 by itself had no effect on pellet priming-induced reinstatement. As described previously, for determining the effect of BIIE0246 on the inhibition of PYY3-36 on pellet priming-induced reinstatement, we pretreated one group of rats during four test sessions with the Y2 antagonist. These rats were given injections of 200 μg/kg PYY3-36 or its vehicle and tested in extinction (no pellet) or after a single exposure to a food pellet (pellet priming). The statistical analysis for this group included the within-subjects factors of PYY3-36 pretreatment (vehicle, 200 μg/kg), pellet priming (pellet, no pellet), and session minutes. This analysis revealed significant effects for pellet priming ($F_{(1,9)} = 59.5$; $p < 0.01$) and session minutes ($F_{(5,45)} = 58.0$; $p < 0.01$) but no significant effects of PYY3-36 pretreatment or interactions between the different factors ($p > 0.05$). These results indicate that in the presence of BIIE0246, systemic injections of PYY3-36 did not attenuate pellet priming-induced reinstatement.

Experiment 3: effect of Arc PYY3-36 injections on pellet priming-induced reinstatement

Arc injections of PYY3-36 decreased pellet priming-induced reinstatement (Fig. 4*A*,*B*), an effect that was most pronounced in the first 30 min of the 3 h test sessions. The statistical analysis included the within-subjects factors of PYY3-36 pretreatment (vehicle, 0.4 μg per side), pellet priming (pellet, no pellet), and

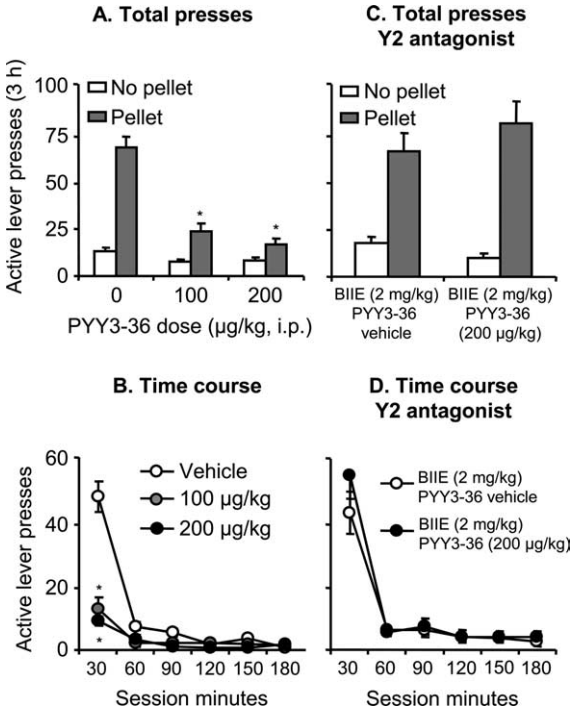

Figure 3. Systemic PYY3-36 injections attenuate pellet priming-induced reinstatement of food seeking, an effect reversed by systemic injections of the Y2 receptor antagonist BIIE0246 (BIIE). ***A***, Mean ± SEM number of nonreinforced active lever presses in the no pellet (extinction) and the pellet-priming conditions after vehicle or PYY3-36 injections ($n = 8$–10 for each PYY3-36 dose; $n = 18$ for the vehicle condition). PYY3-36 or vehicle was injected 30 min before the test sessions. ***B***, Time course of the data described in ***A***. ***C***, Mean number of active lever presses after BIIE0246 pretreatment (30 min) and subsequent injections of PYY3-36 or its vehicle ($n = 10$). ***D***, Time course of the data described in ***C***. *$p < 0.05$, different from vehicle-pellet condition.

session minutes. The ANOVA revealed significant interaction effects of PYY3-36 pretreatment × pellet priming ($F_{(1,13)} = 15.8$; $p < 0.01$) and PYY3-36 pretreatment × pellet priming × session minutes ($F_{(5,65)} = 7.5$; $p < 0.01$). PYY3-36 had no effect on pellet priming-induced reinstatement in 14 rats with cannulas bilaterally in the ventromedial hypothalamus (VMH), or with one cannula in the Arc and the second cannula in the VMH. The mean ± SEM active lever presses per 3 h were 69 ± 7 and 74 ± 16 for the vehicle and PYY3-36 conditions, respectively.

Experiment 4: effect of Arc BIIE0246 injections on the inhibitory effect of systemic injections of PYY3-36 on pellet priming-induced reinstatement

Arc injections of BIIE0246 had no effect on inhibition of pellet priming-induced reinstatement by PYY3-36 (Fig. 4*C*,*D*). In this experiment, all rats were given Arc injections of BIIE0246 or its vehicle and were then given injections systemically with PYY3-36 (200 μg/kg) before the test session. The statistical analysis included the within-subjects factor of BIIE0246 dose (0, 0.5 μg per side) and pellet priming (pellet, no pellet). The ANOVA revealed no significant effects of BIIE0246 dose, pellet priming, or interactions between the two factors (p values >0.1).

Experiment 5: effect of systemic and Arc injections of PYY3-36 injections on cue-induced reinstatement

Systemic injections of both doses of PYY3-36 decreased pellet cue-induced reinstatement of active lever presses, an effect that was most pronounced in the first 30 min of the 3 h test sessions (Fig. 5*A*,*B*). The ANOVA revealed significant interaction effects of PYY3-36 pretreatment × pellet cue ($F_{(1,18)} = 24.9$; $p < 0.01$) and PYY3-36 pretreatment × pellet cue × session minutes ($F_{(5,90)} = 17.4$; $p < 0.01$). In contrast, Arc injections of PYY3-36 did not decrease pellet cue-induced reinstatement of food seeking; surprisingly, these injections appeared to enhance the response to the discrete cue and to induce reinstatement of lever responding in the absence of this cue. The statistical analysis included the within-subjects factors of PYY3-36 pretreatment (vehicle, 0.4 μg per side), pellet cue (cue, no cue), and session minutes. The ANOVA revealed significant effect of pellet cue ($F_{(1,7)} = 17.8$; $p < 0.01$) and PYY3-36 pretreatment ($F_{(1,7)} = 6.7$; $p < 0.05$) but no significant interaction between PYY3-36 pretreatment × pellet cue × session minutes ($p > 0.05$). The significant effect of PYY3-36 pretreatment reflects enhanced nonreinforced lever presses by Arc PYY3-36 injections after extinction, regardless of discrete cue exposure.

Experiment 6: effect of systemic PYY3-36 injections on yohimbine-induced reinstatement

Systemic PYY3-36 injections had no effect on yohimbine-induced reinstatement (Fig. 6). The ANOVA only revealed a significant effect of yohimbine ($F_{(1,17)} = 42.8$; $p < 0.01$) but no interaction effects between the different factors (p values >0.05).

Experiment 7: effect of systemic PYY3-36 injections on cue-induced and heroin priming-induced reinstatement of heroin seeking

The rats in experiment 7 were trained for 10 sessions and demonstrated reliable heroin self-administration and, interestingly, as in the food-trained rats, also demonstrated a progressive escalation of timeout responding across sessions (Fig. 7). The rats were then given six 6 h extinction sessions and additional 3 h extinction sessions during which lever presses decreased over time (Fig. 7). Systemic injections of both doses of PYY3-36 had no effect on heroin cue-induced or heroin priming-induced reinstatement of heroin seeking. For heroin cue-induced reinstatement, the ANOVA revealed a significant effect of heroin cue ($F_{(1,14)} = 21.8$; $p < 0.01$), whereas the effects of PYY3-36 pretreatment or PYY3-36 pretreatment × heroin cue were not significant (p values >0.05) (Fig. 7*C*). For heroin priming-induced reinstatement, the ANOVA revealed a significant effect of heroin priming ($F_{(1,7)} = 6.4$; $p < 0.05$), whereas the effects of PYY3-36 pretreatment or PYY3-36 pretreatment × heroin priming were not significant (p values >0.05) (Fig. 7*D*).

Discussion

We used a reinstatement model to explore PYY3-36 effects on relapse to food seeking in food-restricted rats. We found that systemic PYY3-36 injections attenuated pellet priming- and pellet cue-induced reinstatement of food seeking. In contrast, systemic PYY3-36 injections had minimal effects on ongoing food self-administration, reinstatement of food seeking induced by the pharmacological stressor yohimbine, or heroin priming- or heroin cue-induced reinstatement. We also found that Arc PYY3-36 injections decreased pellet priming- but not cue-induced reinstatement and that the systemic effect of PYY3-36 on pellet priming-induced reinstatement was reversed by systemic but not Arc injections of the Y2 receptor antagonist BIIE0246. These data indicate that PYY3-36 systemic effects on reinstatement of food seeking are likely independent of Y2 receptor activation in Arc.

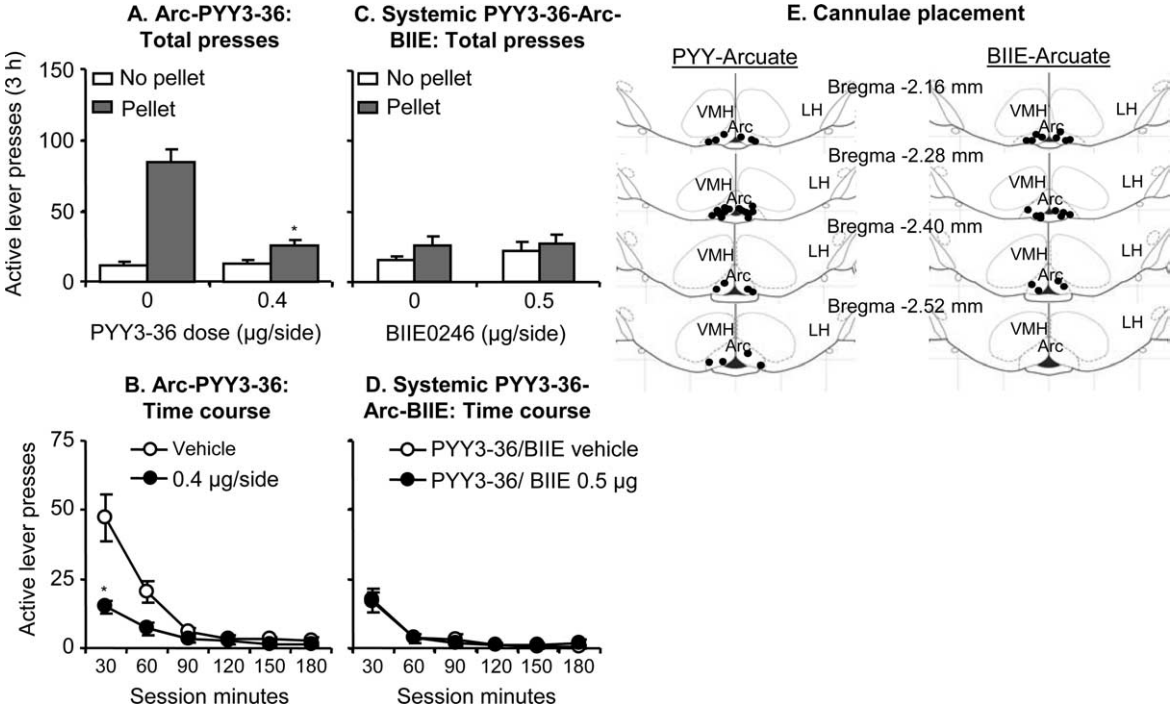

Figure 4. Arc PYY3-36 injections attenuate pellet priming-induced reinstatement, whereas Arc injections of BIIE0246 (BIIE) do not block the inhibition of pellet priming-induced reinstatement by systemic injections of PYY3-36. ***A***, Mean ± SEM number of active lever presses in the pellet-priming and no-pellet conditions. PYY3-36 or vehicle was injected 5 min before the test sessions ($n = 14$). *$p < 0.05$, different from vehicle-pellet condition. ***B***, Time course of the data described in ***A***. ***C***, Mean number of active lever presses in the pellet-priming and no-pellet conditions in rats that received Arc injections of BIIE0246 or its vehicle and, 10 min later, systemic injections of PYY3-36 ($n = 10$). ***D***, Time course of the data described in ***C***. ***E***, Approximate placements of the tip of the injector (Paxinos and Watson, 2005). LH, Lateral hypothalamus.

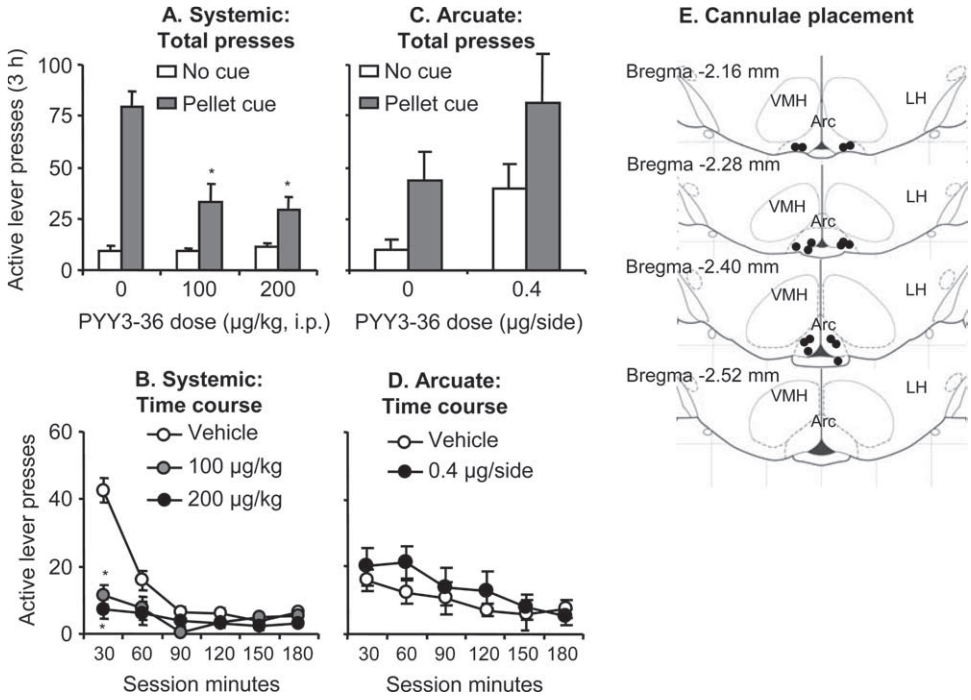

Figure 5. Systemic but not Arc PYY3-36 injections attenuate pellet cue-induced reinstatement of food seeking. ***A***, Mean ± SEM number of active lever presses in the no-cue (extinction) and cue conditions after systemic injections of vehicle or PYY3-36 injections ($n = 10$ for each PYY3-36 dose; $n = 20$ for the vehicle condition). ***B***, Time course of the data described in ***A***. ***C***, Mean number of active lever presses in the pellet-cue and no-cue conditions in rats that received Arc injections of PYY3-36 or its vehicle ($n = 8$). ***D***, Time course of the data described in ***C***. ***E***, Approximate placements of the tip of the injectors of the rats. *$p < 0.05$, different from vehicle-cue condition. LH, Lateral hypothalamus.

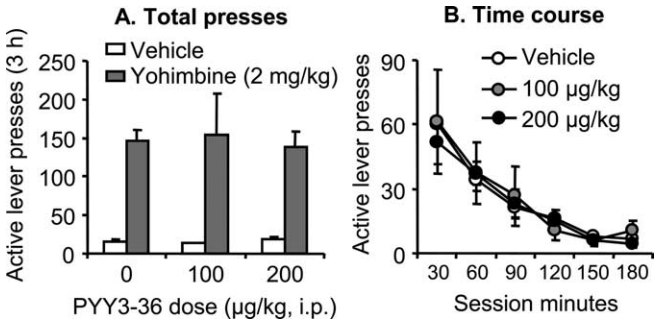

Figure 6. Systemic PYY3-36 injections have no effect on yohimbine-induced reinstatement of food seeking. ***A***, Mean ± SEM number of active lever presses after injections of yohimbine or its vehicle (distilled water) in rats pretreated with PYY3-36 or its vehicle ($n = 9$–10 for each PYY3-36 dose; $n = 19$ for the vehicle condition). PYY3-36 or its vehicle was injected 15 min before yohimbine or its vehicle. Yohimbine was injected 45 min before the session. ***B***, Time course of the data described in ***A***.

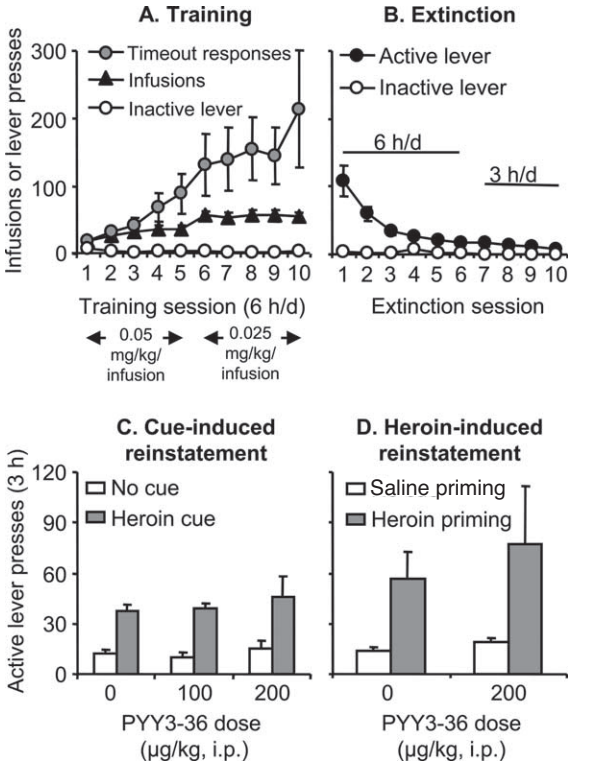

Figure 7. PYY3-36 systemic injections have no effect on cue-induced and heroin priming-induced reinstatement of heroin seeking. ***A***, Training. Mean ± SEM number of infusions, time-out responses, and inactive lever presses over the 10 training days (fixed-ratio-1 40 s timeout reinforcement schedule; $n = 19$). ***B***, Extinction. Mean number of presses on the previously active or inactive lever during the extinction sessions. Lever pressing in the absence of the 5 s tone–light cue was extinguished over consecutive days for 6 h per session on days 1–6 and for 3 h for the subsequent sessions. ***C***, Heroin cue. Mean number of active lever presses in the no-cue (extinction) and the heroin-cue conditions in rats given injections of vehicle or PYY3-36 30 min before the test sessions ($n = 9$–10 for each PYY3-36 dose; $n = 19$ for the vehicle condition). During testing, lever presses led to contingent presentation of the 5 s tone light cue. ***D***, Heroin priming. Mean number of active lever presses in the vehicle (saline) and heroin-priming (0.25 mg/kg) conditions in rats given injections of the PYY3-36 vehicle ($n = 4$) or PYY3-36 ($n = 5$) 30 min before the test sessions. Saline (subcutaneously) or heroin was injected 5 min before the start of the session.

Neuronal mechanisms underlying PYY3-36 effects on reinstatement of food seeking

In initial anatomical characterization of the inhibitory effect of systemic PYY3-36 on reinstatement of food seeking, we examined the role of Arc because Arc PYY3-36 injections inhibit feeding (Batterham et al., 2002) and Arc BIIE0246 injections reverse the inhibitory effect of systemic PYY3-36 on home-cage feeding (Abbott et al., 2005). Our data clearly indicate that Arc does not mediate PYY3-36 systemic effects on pellet cue-induced reinstatement. We also interpret our data to suggest that the PYY3-36 Y2 receptor-dependent systemic effect on pellet priming-induced reinstatement does not involve the Arc. Although Arc PYY3-36 injections decreased pellet priming-induced reinstatement, the systemic effect of PYY3-36 on this reinstatement was reversed by systemic but not Arc injections of BIIE0246. It is unlikely that the lack of effect of Arc BIIE0246 injections on inhibition of pellet priming-induced reinstatement by systemic PYY3-36 is attributable to the intracranial dose of BII0246 used here (0.5 μg per side); at this dose, Arc BIIE0246 injections increased (>250%) feeding in food-sated rats and reversed PYY3-36 systemic effects on home-cage feeding (Abbott et al., 2005).

The site of action of PYY3-36 Y2 receptor-dependent systemic effects on reinstatement of food seeking is unknown. It is unlikely that PYY3-36 peripheral effects mediate conditioned resumption of lever presses induced by acute exposure to food taste (pellet priming) or the tone–light pellet cue, because under our conditions, PYY3-36 had minimal effects on food self-administration. PYY3-36 can reach multiple brain sites after systemic injections (Nonaka et al., 2003) and activate Y2 receptors expressed in hypothalamic nuclei other than the Arc, including lateral hypothalamus and anterior hypothalamic nucleus (Fetissov et al., 2004). Thus, systemic PYY3-36 effects on these hypothalamic sites may mediate its effect on reinstatement of food seeking. Y2 mRNA is also detected in many extrahypothalamic sites (Parker and Herzog, 1999). Stanic et al. (2006) reported that Y2 receptors are expressed at low to moderate levels in extrahypothalamic sites involved in food reward (Kelley and Berridge, 2002; Wise, 2006) and reinstatement of drug seeking (Bossert et al., 2005; See, 2005). These areas include ventral tegmental area (VTA), nucleus accumbens core, cingulate cortex, central amygdala, and ventral pallidum (Stanic et al., 2006). Thus, PYY3-36 systemic effects on reinstatement may be mediated by these extrahypothalamic sites. In a preliminary study, we found that VTA PYY3-36 injections had no effect on pellet priming-induced reinstatement [mean ± SEM for the vehicle and PYY3-36 (0.4 μg per side) were 34 ± 12 and 51 ± 14, respectively]. The role of other mesocorticolimbic sites that express Y2 receptors in the systemic effect of PYY3-36 on reinstatement of food seeking is currently unknown.

An intriguing finding in our report was that PYY3-36 Arc injections decreased pellet priming- but not pellet cue-induced reinstatement of food seeking. A potential mechanism for the local effect of PYY3-36 on pellet priming-induced reinstatement is inhibition of β-endorphin projections from Arc to nucleus accumbens (Finley et al., 1981; Akil et al., 1984). Local PYY3-36 application inhibits Arc pro-opiomelanocortin (the precursor for β-endorphin) neuronal activity (Acuna-Goycolea and van den Pol, 2005). In the context of food-taking behavior, the Arc–accumbens β-endorphin projection is of interest, because stimulation of accumbens μ-opioid receptors (the main receptor target of Arc–accumbens β-endorphin neurons) increases food-taking behavior and food palatability (Zhang et al., 1998; Pecina and Berridge, 2000; Kelley and Berridge, 2002). Published data on the effect of food-taking behavior on β-endorphin release in accumbens are not available. However, there is evidence that β-endorphin accumbens levels are increased during reward

(brain stimulation, heroin, cocaine) seeking, as measured in extinction tests (Roth-Deri et al., 2003; Zangen and Shalev, 2003).

Different effects of PYY3-36 on food- and drug-taking behaviors

Our results revealed several dissociations in PYY3-36 behavioral effects. The first is that PYY3-36, at doses that had minimal effects on ongoing food self-administration, inhibited pellet priming- and pellet cue-induced reinstatement. The self-administration procedure measures the operant reinforcing effects of unconditioned stimuli such as food and drugs (Schuster and Thompson, 1969; Wise, 1989). Thus, our data suggest that mechanisms underlying food reinforcement differ from those underlying food relapse. This notion is in agreement with findings that pharmacological agents that attenuate cue-induced reinstatement of food seeking have no effect on ongoing food self-administration (De Vries et al., 2001, 2005; Baptista et al., 2004; Bossert et al., 2006a). Evidence for dissociation between the effects of pharmacological agents on reinstatement versus self-administration is congruent with findings from studies in which drug self-administration and reinstatement models were used to assess mechanisms underlying drug reinforcement and relapse (De Vries and Shippenberg, 2002; Shalev et al., 2002; Kalivas and Volkow, 2005).

The second dissociation in PYY3-36 effects concerns its selective attenuation of pellet priming- and pellet cue-induced reinstatement but not yohimbine-induced reinstatement. It is unlikely that these results are attributable to different pretreatment times (30 min for cue and priming vs 60 min for yohimbine): PYY3-36 effects on home-cage feeding can last for at least 8 h (Batterham et al., 2002; Challis et al., 2003). Our results on the effects of PYY3-36 on pellet priming versus yohimbine are opposite from those we previously reported with the CRF_1 receptor antagonist antalarmin, which blocks yohimbine- but not pellet priming-induced reinstatement of food seeking (Ghitza et al., 2006). The different effects of PYY3-36 and antalarmin on reinstatement induced by pellet priming versus yohimbine suggest that different mechanisms underlie relapse induced by food reexposure versus stress. This conclusion is not surprising in light of previous results on dissociations in the effects of pharmacological agents on stress- versus drug priming- or drug cue-induced reinstatement (Shaham et al., 2000; Le and Shaham, 2002; Lu et al., 2003; Weiss, 2005).

The third dissociation concerns the effect of PYY3-36 on reinstatement of food seeking versus heroin seeking. Doses of PYY3-36 that inhibited pellet cue- and pellet priming-induced reinstatement had no effect on heroin cue- and heroin priming-induced reinstatement. Although there are several reports that pharmacological and neuroanatomical manipulations that inhibit reinstatement of drug seeking have no effect on reinstatement of food seeking (Kalivas and McFarland, 2003; Schmidt et al., 2005; See, 2005), there are no published reports that a pharmacological manipulation can selectively inhibit reinstatement of food but not drug seeking. One issue to consider here is that although the cue-induced reinstatement manipulation is similar for both food and heroin, this is not the case for the priming manipulation. For food, noncontingent delivery of one 45 mg pellet likely induces reinstatement, because of the taste cue properties of the pellet, whereas for heroin priming, reinstatement is attributable to drug effects on brain opiate receptors (Stewart, 1984).

Methodological considerations

We interpret our data to suggest that systemic PYY3-36 injections decreased the rat's motivation to seek high-fat food after exposure to pellet priming or pellet cues. However, alternative interpretations should be considered. PYY3-36 injections had minimal effects on high rates of responding for food pellets (Fig. 2), making it unlikely that PYY3-36 inhibited reinstatement of food seeking because it disrupted motor performance or induced conditioned taste aversion (Halatchev and Cone, 2005). Blockade of Y2 receptors induces anxiolytic-like effects in rodents (Heilig, 2004). Thus, PYY3-36 (Y2 receptor agonist) effects on reinstatement may be attributable to induction of anxiety-like states that can inhibit operant responding (Geller et al., 1962). This interpretation is unlikely, because in our model yohimbine (a classical anxiogenic drug) potently reinstates food seeking (Fig. 6).

An issue to consider is the anatomical specificity of the effect of Arc PYY3-36 injections on pellet priming-induced reinstatement. Drugs injected into specific brain sites can change behavior by diffusing away from the injection site into either nearby or distal sites (if diffuse into ventricles) (Johnson and Epstein, 1975; Wise and Hoffman, 1992). Our results, however, suggest that the effect of Arc PYY3-36 injections on reinstatement is not attributable to injection site diffusion: missed injections, primarily found in VMH were ineffective (see Results).

Finally, it is unlikely that the different effects of PYY3-36 on reinstatement of food seeking versus heroin seeking are attributable to differences in response rates during training (Fig. 1*B* vs Fig. 7*A*). There is little evidence from drug reinstatement studies that different response rates during training qualitatively influence the effect of pharmacological agents on reinstatement after extinction (Shalev et al., 2002).

Concluding remarks

We identified a novel and potent effect of PYY3-36 on relapse to high-fat food seeking, as measured in the reinstatement procedure. To the degree that this procedure is a valid relapse model (Self and Nestler, 1998; Shaham et al., 2003; Epstein et al., 2006), our findings may have implications for relapse prevention during dieting. There are conflicting reports on PYY3-36 effect on home-cage feeding (Boggiano et al., 2005), and we found that PYY3-36 had weak effects on high-fat food self-administration. Based on these results, our reinstatement data suggest that PYY3-36 may be more effective for the treatment of relapse to maladaptive high-fat food-seeking habits during dieting than for reducing ongoing high-fat food consumption.

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