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Infusions of neuropeptide Y into the lateral septum reduce anxiety-related behaviors in the rat

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ABSTRACT

Neuropeptide Y (NPY) is one of the most abundant peptides in mammalian brain and NPY-like-immunoreactivity is highly expressed in the lateral septum, an area extensively involved in anxiety regulation. NPY counteracts the neurochemical and behavioral responses to acute threat in animal models, and intracerebroventricular (i.c.v.) administration of NPY at low doses is anxiolytic. Less is known about the specific contributions of the lateral septum to NPY-mediated anxiety regulation. In Experiment 1, the effects of infusions of NPY (1.5 μg) into the lateral septum were investigated in three animal models of anxiety: the elevated plus-maze, novelty-induced suppression of feeding, and shock-probe burying tests. Experiment 2 examined the role of the NPY Y1 receptor in these models by co-infusing the Y1 antagonist BIBO 3304 (0.15 μg, 0.30 μg) with NPY into the lateral septum. In the elevated plus-maze, there were no changes in rats' open arm exploration, the index of anxiety reduction in this test. In the novelty-induced suppression of feeding test, rats infused with NPY showed decreases in the latency to consume a palatable snack in a novel (but not familiar) environment, suggesting a reduction in anxiety independent of increases in appetite. This anxiolysis was attenuated by co-infusion with BIBO 3304 (0.30 μg) in Experiment 2. Lastly, rats infused with NPY showed decreases in the duration of burying behavior in the shock-probe burying test, also indicative of anxiety reduction. However, unlike in the feeding test, BIBO 3304 did not attenuate the NPY-induced anxiolysis in the shock-probe test. It is concluded that NPY produces anxiolytic-like actions in the lateral septum in two animal models of anxiety: the novelty-induced suppression of feeding, and shock-probe burying tests, and that this anxiolysis is dependent on Y1 receptor activation in the feeding test.

1. Introduction

Neuropeptide Y (NPY) is a 36-amino acid peptide that is distributed in mammalian brain in higher concentrations than any other peptide studied to date (Gray and Morley, 1986). NPY has many physiological and behavioral functions, including the regulation of stress and anxiety responses and may act endogenously through opposing the behavioral responses of anxiety under acutely stressful conditions (for review: Heilig, 2004). Interestingly, NPY-transgenic rats behave like wild type animals in the elevated plus-maze under normal conditions, but do not display the expected anxiogenic response observed in normal rats following acute restraint stress (Thorsell et al., 2000). On the other hand, intracerebroventricular (i.c.v.) administration of NPY decreases anxiety-like behaviors in many animal models, including the plus-maze, open field, and conflict tests (Britton et al., 2000; Heilig and Murison, 1987; Heilig et al., 1989). Expectedly, NPY-like-immunoreactivity (NPY-IR) is highly expressed in many structures that also regulate these behaviors, including the amygdala, hippocampus, hypothalamus, and lateral septum (Allen et al., 1983; Chronwall et al., 1985; Heilig, 2004; Kask et al., 2002).

The lateral septum regulates anxiety as part of a circuit that includes multiple interconnections with the structures mentioned above (Risold and Swanson, 1997; Sheehan et al., 2004). Both lesions and pharmacological perturbations of the lateral septum reduce anxiety-like behaviors; i.e., by increasing rats' open arm exploration in the elevated plus-maze and reducing defensive burying in the shock-probe burying test (e.g., Bondi et al., 2007; Menard and Treit, 1996; Pesold and Treit, 1992, 1996; Trent and Menard, 2010). Given that the lateral septum has a high density of NPY binding sites (Allen et al., 1983; Larsen et al., 1993; Martel et al., 1986) it seems likely that NPY actions at that site may mediate rats' behavioral responses in anxiety-related paradigms. Thus, infusions of NPY into the lateral septum decreased anxiety in the social interaction and plus-maze tests and antagonized the anxiogenic effects of corticotrophin releasing hormone (Kask et al., 2001; Molina-Hernandez et al., 2010). In the current study, we were interested in

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investigating lateral septal NPY in a wider range of paradigms because not all defensive behaviors share the same neural circuitry (e.g., Pesold and Treit, 1994; Treit and Menard, 1997; Treit et al., 1993). In addition, although the lateral septum has been implicated in both open-arm avoidance and shock-probe burying these responses are nonetheless differentially regulated by distinct receptor types within that structure. For example, lateral septal infusions of either the 5-HT1A receptor agonist, 8-OH-DPAT or the NMDA receptor antagonist, AP-5 suppressed burying while leaving rats' normal levels of open-arm avoidance intact (Menard and Treit, 1998, 2000). By contrast, chronic infusions of the vasopression V1/V2 receptor antagonist, d(CH2)5-D-Tyr(Et)VAMP into the lateral septum increased open-arm avoidance while leaving the burying response intact (Everts and Koolhaas, 1999).

The purpose of the current study was two-fold: to investigate the effects of infusions of NPY into the lateral septum across a range of anxiety-related behaviors (Experiment 1) and to examine the role of the Y1 receptor by pre-treating rats with the Y1 antagonist BIBO 3304 prior to the NPY infusions (Experiment 2). The development of specific NPY receptor antagonists has allowed for a detailed investigation into the role of each receptor subtype. The actions of NPY are mediated through at least four G-protein linked receptors: NPY Y1, Y2, Y4, and Y5 (Dumont et al., 1998; Eaton et al., 2007; Harro, 2006). The Y1 receptor has been the most associated with mediating the anxiolytic actions of NPY (for review: Kask et al., 2002) and is located mostly postsynaptically (King et al., 1999). The lateral septum has a high expression of Y1 receptors (Martel et al., 1986; Dumont et al., 1996, 1998), which may play a putative role in anxiety regulation at this site. In a prior study, co-infusion of the Y1 antagonist BIBO 3304 with NPY in the lateral septum attenuated lateral septal NPY-induced anxiety reduction in the social interaction test (Kask et al., 2001). Infusion of BIBO 3304 into the lateral septum did not alter anxiety on its own, implying that NPY-mediated anxiety regulation may be phasic at this site (Kask et al., 2001). Infusions of Y1 antagonists alone usually have no effect on anxiety, except when infused into the periaqueductal gray or dentate gyrus, where infusions have been found to either increase or decrease anxiety, respectively (Kask et al., 1998a,c; Smialowska et al., 2007).

We tested rats in three paradigms: the elevated plus-maze test, novelty-induced suppression of feeding test, and the shock-probe burying test. In the plus-maze test, a reduction in anxiety suppresses the normal tendency of rodents to avoid open areas, measured as an increase in the proportion of entries or time spent on the open arms of the maze (Carobrez and Bertoglio, 2005; Pellow et al., 1985). In the novelty-induced suppression of feeding test, anxiety reduction is indexed by a decrease in the latency to initiate consumption of a palatable snack in a novel environment without changing latency to snack consumption in the home-cage (Merali et al., 2003). One advantage of this test is that it allows us to examine potential NPY-related changes in appetite. This is important, because NPY is known to increase appetite (e.g., Hanson and Dallman, 1995; Polidori et al., 2000) and exploration-based animal models of anxiety are sensitive to changes in appetitive motivation (Genn et al., 2003a,b; Inoue et al., 2004). For example, chronic food restriction has been shown to selectively increase rats' open-arm exploration in the plus-maze without altering their behavioral responses in the social interaction test (Genn et al., 2003b). Lastly, we also tested rats in the shock-probe burying test, in which anxiety reduction is indexed by a decrease in burying behavior, that is, the natural tendency of rats to bury an electrified probe by using their forepaws to push bedding material towards and over the probe (Treit et al., 1981). Inclusion of this test allowed us to examine the effects of lateral septal NPY on rats' defensive responses to a noxious, localizable threat source. To the best of our knowledge, there have been no prior investigations into the potential anxiolytic effects of lateral septal NPY on either novelty-induced suppression of feeding or shock-probe burying. Given the established role of the lateral septum in anxiety regulation, we hypothesized that infusions of NPY into the lateral septum would reduce rats' anxiety-like behavior in all three animal models: the elevated plus-maze, novelty-induced suppression of feeding and shock-probe burying tests (Experiment 1) and further that this NPY-induced anxiolysis could be attenuated by the Y1 receptor antagonist BIBO 3304 (Experiment 2).

2. Materials and methods

2.1. Subjects

Subjects were naïve, male Long Evans rats from Charles River, Quebec weighing 300–400 g at the time of surgery. Rats were given at least 1 week to acclimatize to the colony conditions before undergoing surgery. Prior to surgery, rats were double housed in polycarbonate cages, given ad libitum food and water, and maintained on a 12:12 light/dark cycle (lights on at 0700 h). The temperature of the colony room was maintained at approximately 21 °C. Following surgery, rats were individually housed under the same conditions as before surgery. The treatment of all animals was in compliance with the guidelines of the Canadian Council on Animal Care, and was approved by Queen's University Animal Care Committee.

2.2. Drugs

Human, rat NPY_{1-36} was obtained from Polypeptide Laboratories in San Diego, CA and BIBO 3304 trifluoroacetate was obtained from Tochris Bioscience in Strasbourg, France. Owing to poor solubility, NPY and BIBO 3304 were dissolved in sterile water rather than physiological saline.

2.3. Surgery

Rats were anesthetized with isoflurane (1.5%–4.5%) in oxygen at a rate of 1.5–2 L/min. Buprenorphine (0.04 mg/kg s.c.) was administered preoperatively to reduce pain. The rats' heads were shaved and they were injected subdermally with the analgesic Marcaine (2 mg/kg). Rats were then placed in a Kopf stereotaxic apparatus. The scalp was thoroughly sterilized and an incision was made to expose the skull. Stereotaxic procedures were used to drill burr holes through the skull, bilaterally, over the right and left lateral septum and two 23-gage stainless-steel guide cannula were implanted, according to flat skull coordinates from Paxinos and Watson (1998) (0.5 mm AP, ±1.2 mm ML, and 3.2 mm DV to bregma at 7° angled medially). Guide cannulae secured by cementing 4 small jeweler's screws to the skull using dental acrylic. At the end of surgery a pin was inserted into each cannula to keep the tract clear of debris. Immediate post-operative care included: analgesic treatment using ketoprofen (5 mg/kg s.c.), rehydration with injection of lactated ringer solution (5 ml s.c.), and maintenance of body temperature by placing the rat under a heat lamp. After animals recovered from anesthesia they were transferred from the surgery room to a recovery room (separate from the home colony) where they remained for a minimum of 3 recovery days. On each recovery day rats were given a morning injection of buprenorphine (0.04 mg/kg s.c.) and afternoon injections of both buprenorphine (0.04 mg/kg s.c.) and ketoprofen (5 mg/kg s.c.). The recovery room temperature was set to approximately 25 °C, which was slightly higher than the regular colony conditions. Once recovery was complete, the animals were returned to the regular colony where they were left undisturbed (except for regular maintenance) for at least 4–6 days prior to behavioral testing.

2.4. Infusions

Following post surgical recovery, rats were randomly assigned to one of the following infusion conditions: Experiment 1: (a) physiological

saline (0.9%) (b) NPY (1.5 μg/side, equivalent to 0.35 nmol); Experiment 2: (a) saline + saline, (b) saline + BIBO 3304 (0.15 μg/side or 0.30 μg/side, equivalent to 0.20 and 0.39 nmol), (c) saline + NPY (1.5 μg/side), (d) BIBO 3304 (0.15 μg/side or 0.30 μg/side) + NPY (1.5 μg/side). All rats received a volume of 0.5 μl/side at an infusion rate of 1 μl/min. Doses used were based on previous studies and modified when necessary (Kask et al., 2001; Olivera-Lopez et al., 2008). Rats were habituated to the infusion procedures for 3 consecutive days prior to testing. This was done by holding them in a gentle, manual towel-wrap restraint and removing and replacing the cannula pins in the infusion room. On testing day, rats were gently hand-held, the pins were removed, and two 30-gage stainless-steel internal cannulae were lowered to 2.0 mm below the tip of the guide cannulae. The internal cannulae were connected to a 10 ml constant rate Hamilton microsyringe with polyethylene tubing and the infusions were delivered using an infusion pump (KD Scientific, MA). The displacement of an air bubble inside the polyethylene tubing was monitored to confirm drug flow. In addition, prior to replacing the stylet, the top of the cannula guide was inspected for fluid efflux. At the end of the 30 s infusion, the microinjector was left in the brain for an additional 1 min to allow for diffusion away from the tips. In Experiment 1, behavioral testing began 15 min after the infusion. In Experiment 2, the second infusion was delivered 15 min after the first infusion, and behavioral testing began 15 min following the second infusion.

2.5. Behavioral testing

Rats were allowed at least 1 week to recover from surgery before behavioral testing commenced. All infusions and testing occurred between 0900 and 1700 h and all behaviors were recorded on videotape for subsequent analysis. The behaviors for the elevated plus-maze and the shock-probe burying test were evaluated using Observer 7 software (Noldus Information Technology, MA).

2.5.1. Elevated plus-maze test

Elevated plus-maze testing occurred 1 week after surgery. The wooden plus-maze consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 50 cm) that form a plus shape, all with open roofs. The maze was elevated 50 cm from the floor and situated in the center of a quiet and dimly lit test room. Rats were placed individually in the center of the maze facing one of the closed arms and allowed 5 min of exploration. During the test, the rats' arm entries were observed and recorded by the experimenter sitting quietly in the corner of the room. After the test was completed the maze was cleaned with distilled water and wiped dry to prevent rats from following each other's scent. Behaviors measured for this test were: (a) number of open arm entries, (b) number of closed arm entries, (c) total number of arm entries (open + closed), (d) time spent in the open arms, (e) time spent in the closed arms, and (f) number of rears. An arm entry was indexed as the rat having all four of its paws on the arm. Open arm activity was quantified as the % open arm time (time on the open arms/time on open + closed arms) and % open arm entries (open entries/open + closed entries). We also measured the frequency of stretched attend postures (SAP; i.e., stretching forward and retracting to its original position). SAPs were differentiated according to where they occurred on the maze. If they occurred in the closed arms or the central area they were classified as "protected" while if they occurred in the open arms they were classified as "unprotected". SAPs were quantified as total SAP (protected + unprotected) and % protected SAP (protected/total) and were used as indices of risk assessment (Rodgers and Johnson, 1995). The index for anxiety reduction in this test is an increase in the proportion of entries into the open arms and/or an increase in the proportion of time spent in the open arms (Pellow et al., 1985). The number of closed arm entries and the total number of entries were used as indices of general exploration/locomotor activity (Pellow et al., 1985; Rodgers and Johnson, 1995). We also measured the number of rears as an additional measure of general exploration (Lever et al., 2006).

2.5.2. Novelty-induced suppression of feeding test

The novelty-induced suppression of feeding test occurred 1 week after elevated plus-maze testing. Habituation for this test consisted of rats receiving half a piece of a graham cracker (Honey Maid Graham Crumbs, Nabisco) in a small dish in the corner of their home cages for 4 consecutive days (days 1–4). The latency to initiate consumption of the palatable snack was recorded each day by an observer standing quietly in the corner of the colony room. Following day 4, the rats were infused with their respective treatment and tested in the home cage test (day 5), whereby rats were given the snack in their cage in the home colony room as usual. The following day at the same time rats were again infused with their respective treatment and tested in the novel cage test (day 6), whereby rats were given the snack in an unfamiliar environment: an opaque cage with an inverted lid in a novel room. In the novelty-induced suppression of feeding test, a decrease in anxiety is indexed by a decrease in the latency to begin consumption of the snack in the novel cage environment, in the absence of changes in the latency to begin consumption in the home cage environment (Merali et al., 2003).

2.5.3. Shock-probe burying test

Shock-probe burying test occurred 1 week after the novelty-induced suppression of feeding test. The apparatus consisted of an electrified copper-wired stationary probe inserted 6 cm through a hole into a transparent (40 × 30 × 40 cm) Plexiglas chamber that contained 5 cm of bedding material (wood chips) spread evenly on its floor. An electrified, wire-wrapped Plexiglas probe (6 × 0.5 × 0.5 cm) could be inserted through a small hole centered 5 cm above the bedding material. An electrical current was distributed through two copper wires wrapped around the probe. Using a 2000 V shock source, the intensity of the shock was set at 2.5 mA. Rats were individually habituated to the test chamber without the shock-probe present for 15 min on each of 4 consecutive days prior to the testing day. On the test day, the shock probe was inserted 6 cm into the chamber and secured in place. At the start of the test, rats were individually placed in the chamber facing away from the electrified probe. The 15 min test began immediately after rats received their first contact-induced shock from the probe. At the end of testing, fecal boluses were removed and the bedding was replaced and smoothed to equal thickness. After being shocked, rats' innate defensive response normally consists of rapidly pushing bedding with their forepaws and shoveling bedding with their head toward the shock-probe (i.e. burying behavior) (Treit et al., 1981). Duration of burying behavior is used as an index of anxiety: a reduction in anxiety is measured as a reduction in the time spent burying. The following behaviors were measured for this test: (a) duration of burying; (b) duration of immobility (e.g. rest, sleep) and (c) number of rears and (d) number of probe-contact induced shocks received. Rats' physical reactivity to the shocks was measured on a four-point scale, according to Treit et al. (1981): small head or forepaw flinch (1), whole body flinch with or without movement away from the probe (2), whole body flinch/jump with movement away from probe (3), whole body flinch and jump with rapid movement away from probe (4). The mean shock reactivity score was calculated for each rat by dividing the total shock reactivity scores by the total number of shocks received. The duration of burying behavior is used as the primary index of anxiety in this test. Reductions in burying that are not associated with changes in associative learning and memory (indexed by probe avoidance, i.e., the number of shocks received), pain sensitivity (indexed by mean shock-reactivity) and locomotor activity (indexed by rears and immobility) are indicative of anxiety reduction in this test (Treit et al., 1981).

2.6. Histology

Following behavioral testing, rats were anesthetized with isoflurane in oxygen and sacrificed with an overdose of chlorohydrate (300 mg/kg, i.p.). Rats were perfused intracardially with 120 ml of 0.9% saline followed by 120 ml of 10% phosphate buffered formalin. The brains were then extracted and placed in small plastic containers filled with formalin. At least 48 h later the brains were frozen with a cryostat and coronal slices (40 μm thick) were made and mounted onto subbed glass slides. The location of the cannulae was examined and transcribed onto atlas sheets (Paxinos and Watson, 1998) while blind to corresponding behavioral data.

2.7. Statistical analysis

Data are presented as mean ± standard error of means (SEM). The results for the elevated plus-maze and shock-probe burying tests were analyzed using a one-way analysis of variance (ANOVA). In order to correct for heterogeneity of variance, the duration of burying scores were transformed (natural log) prior to the ANOVA. The results for the novelty-induced suppression of feeding test were analyzed using an ANOVA with repeated measures, with treatment as a between factor and day as a within factor. All data that reached significance ($p<0.05$) were further analyzed post hoc using LSD tests.

3. Results

3.1. Experiment 1: infusions of NPY into the lateral septum

The cannulae placement sites for infusion into the lateral septum are shown in Fig. 1. Rats with cannulae situated in the lateral septum yielded the following group numbers: saline (n = 10), NPY (n = 12), totaling 22 rats with correct cannula placements.

3.1.1. Elevated plus-maze

As can be seen in Fig. 2, infusions of NPY into the lateral septum had no effect on rats' open arm exploration. An overall one-way ANOVA was performed for % open arm entries, $F(1,20) = 0.19$, $p>0.6$, and for % open arm time, $F(1,20) = 0.02$, $p>0.8$.

Locomotor activity and risk assessment (SAP) behavior are provided in Table 1. There was no NPY-induced change in locomotor activity, measured as the number of closed arm entries, $F(1,20) = 0.64$, $p>0.4$, the number of total arm entries, $F(1,20) = 0.21$, $p>0.8$, and number of rears $F(1,20) = 1.67$, $p>0.20$. There was also no effect of NPY on risk-assessment behavior, measured as total number of SAP, $F(1,20) = 1.78$, $p>0.1$, or % protected SAP, $F(1,20) = 0.96$, $p>0.9$.

3.1.2. Novelty-induced suppression of feeding test

One animal from the NPY group lost its skull cap before the initiation of the novelty-induced suppression of feeding test, yielding the following group numbers: saline (n = 10) and NPY (n = 11).

Fig. 3A shows the latency to begin to consume the snack over the 4 habituation days (Hab Days 1–4), the home cage test (Day 5), and the novel cage test (Day 6). The figure shows the natural decline in latency across the first 2 habituation trials, after which the response latencies in the home-cage stabilized (across Days 3 and 4). A repeated measures ANOVA revealed a main effect of Day, $F(5,95) = 18.37$; $p<0.001$ and Day × Treatment interaction, $F(5,95) = 6.60$, $p = 0.001$. Follow up analysis, using a one-way ANOVA, revealed that compared to saline-treated controls, rats infused with NPY (1.5 μg) into the lateral septum took significantly less time to initiate snack consumption in the novel cage test, $F(1,19) = 13.76$, $p = 0.001$ but not in the home cage test, $F(1,19) = 1.65$, $p>0.2$. Similarly, there were no between group differences on any of the 4 habituation trials (Days 1–4, all ps > 0.30). Further analysis, using difference scores (novel cage latency − home cage latency), similarly underscored that exposure to the novel cage increased latency to snack consumption in saline-treated but not NPY-treated rats, $F(1,19) = 9.15$, $p<0.01$ (see Fig. 3B).

Although the findings above suggest that lateral septal NPY did not affect appetitive motivation, examination of Fig. 3A raised the possibility that the latency to feeding scores of NPY-treated rats (but not saline-treated controls) decreased from baseline measures, taken on habituation Day 4, following their first drug treatment in this test on Day 5 (i.e., the home cage test). Thus, we further analyzed data from Day 4 and the Home-Cage test alone. A repeated measures ANOVA revealed no main effect of Day, $F = 1.64$; $p>0.20$ or Day × Treatment interaction, $F(1,19) = 2.74$, $p = 0.12$. Further a posterior analysis revealed that the experimental rats were significantly faster to initiate snack consumption following their first infusion of NPY on Day 5 (home-cage test) than they were on the last habituation trial on Day 4, $F(1,10) = 9.85$, $p = 0.01$. Similar results were not observed in saline treated controls, $F(1,9) = 0.04$, $p>0.80$.

3.1.3. Shock-probe burying test

One animal from the NPY group lost its skull cap prior to the initiation of the shock-probe testing and a second animal from that group did not receive a satisfactory infusion (one of its cannulae guides was plugged). This yielded the following group numbers: saline (n = 10) and NPY (n = 9).

As shown in Fig. 4, rats that received NPY infusions into the lateral septum spent less time burying the electrified probe than did saline-

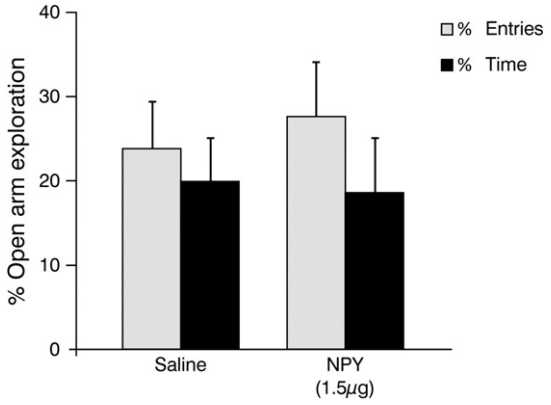

Fig. 2. Mean (± SEM) % of open-arm entries (open squares) and % of open-arm time (closed squares) displayed by rats following bilateral infusions of either saline (n = 10) or NPY (1.5 μg, n = 12) into the lateral septum. NPY = neuropeptide Y.

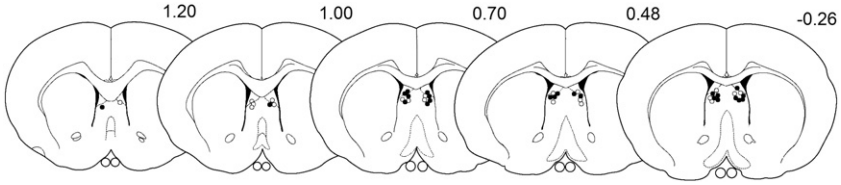

Fig. 1. Histological results for Experiment 1. Circles indicate the location of the cannula tips for bilateral infusions of saline (open circles) or NPY (1.5 μg) (filled circles) into the lateral septum. Atlas plates are adapted from Paxinos and Watson (1998). NPY = neuropeptide Y.

Table 1
Mean (±SEM) plus-maze activity and vigilance scores after infusions of saline or NPY into the lateral septum (Experiment 1).

Behavior	Saline (n = 10)	NPY (1.5 μg) (n = 12)
Closed arm entries	10.10 ± 0.66	9.33 ± 0.68
Total arm entries	13.60 ± 1.25	13.83 ± 1.25
Number of rears	23.60 ± 1.46	20.08 ± 2.15
Total SAP	14.70 ± 1.33	12.08 ± 1.40
% protected SAP	77.04 ± 5.23	76.49 ± 8.88

NPY, neuropeptide Y; SAP, stretch-attend postures.

treated controls. This pattern was confirmed significant by ANOVA: duration of burying (log seconds) $F(1,17) = 34.77$, $p < 0.001$. Table 2 shows the duration of burying in seconds (prior to log transformation), as well as the activity and reactivity scores for this test. Importantly, NPY-induced reductions in burying were not associated with treatment-induced changes in general activity level, indexed as duration of immobility, $F(1,17) = 2.27$, $p > 0.1$ or number of rears, $F(1,17) = 0.17$, $p > 0.6$; mean shock reactivity, $F(1,17) = 3.49$, $p > 0.07$; or the number of shocks received, $F(1,17) = 0.25$, $p > 0.6$.

3.2. Experiment 2: infusions of NPY and the Y1 antagonist BIBO 3304 into the lateral septum

The cannulae placement sites for infusion into the lateral septum for Experiment 2 are shown in Fig. 5. Rats with cannulae situated in the lateral septum yielded the following group numbers: (a) saline + saline (n = 10), (b) saline + NPY (1.5 μg, n = 8), (c) saline + BIBO 3304 (0.15 μg, n = 9), (d) BIBO 3304 + NPY (0.15 μg + 1.5 μg, n = 10) (e) saline + BIBO 3304 (0.30 μg, n = 11) (f) BIBO 3304 + NPY (0.30 μg + 1.5 μg, n = 11), totaling 59 rats that had correct cannula placements.

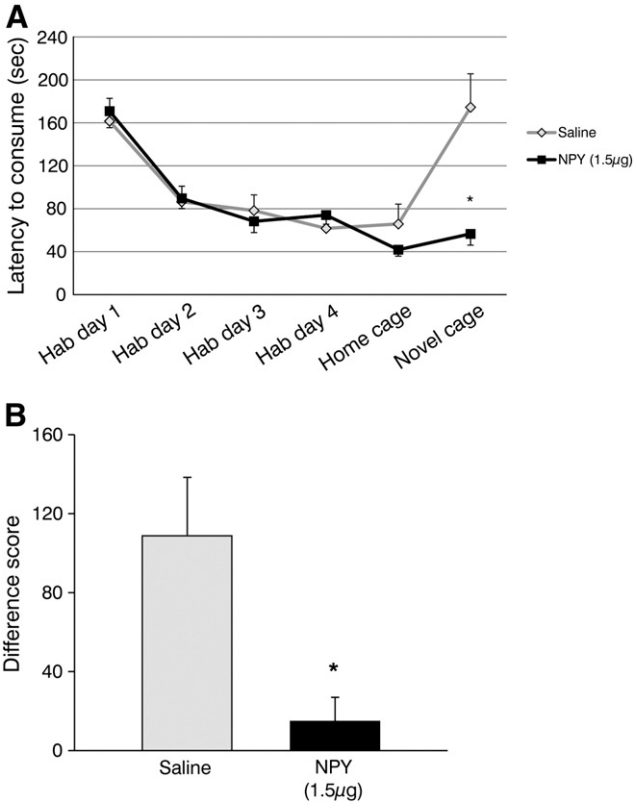

Fig. 3. Mean (±SEM) latency to begin to consume the snack across the 4 habituation trials (Hab days 1–4) and the home and novel cage tests (A) by rats following bilateral infusions of either saline (n = 10) or NPY (1.5 μg, n = 11) into the lateral septum. The Mean (±SEM) difference scores (novel cage latency − home cage latency) are also displayed (B). (*p < 0.05, relative to saline infusions).

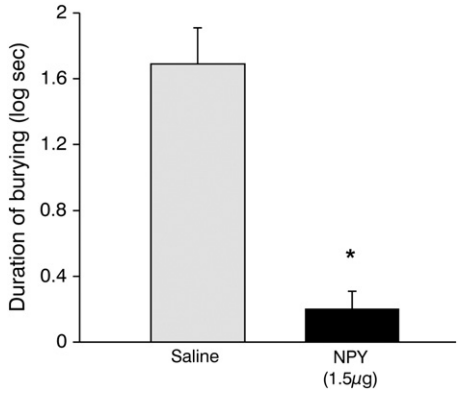

Fig. 4. Mean (±SEM) duration of burying (log seconds) in the shock-probe burying test after bilateral infusions of either saline (n = 10) or NPY (1.5 μg, n = 9) into the lateral septum. (* p < 0.001, relative to saline infusions).

3.2.1. Elevated plus-maze

As can be seen in Fig. 6, there were no group differences on rats' open arm activity, similar to Experiment 1. A one-way ANOVA confirmed that NPY infusions into the lateral septum did not affect the percentage of open arm entries, $F(5,53) = 1.60$, $p > 0.1$, or the percentage of open arm time, $F(5,53) = 0.80$, $p > 0.5$.

Locomotor activity and risk assessment (SAP) behavior are provided in Table 3. There were no group differences in locomotor activity, measured by either the number of closed arm entries, $F(5,53) = 0.17$, $p > 0.9$, the number of total arm entries, $F(5,53) = 1.61$, $p > 0.1$, or the number of rears, $F(5,53) = 1.13$, $p > 0.35$. There was also no effect of NPY on risk-assessment behavior, indicated by total number of SAP, $F(5,53) = 1.07$, $p > 0.3$, or % protected SAP, $F(5,53) = 0.47$, $p > 0.7$.

3.2.2. Novelty-induced suppression of feeding test

As can be seen in Fig. 7A, the pattern of the novelty-induced suppression of feeding test is similar to the pattern observed in Experiment 1 (Fig. 3A) in that there was a general decline in latency over the habituation period in all groups. A repeated measures ANOVA found a main effect of Day, $F(5,265) = 38.13$; $p < 0.0001$ and Day × Treatment interaction, $F(25,265) = 1.88$ $p < 0.01$. Follow up analysis, using a one-way ANOVA, revealed that between group differences in the latency to initiate snack consumption were present

Table 2
Mean (±SEM) activity and reactivity scores in the shock-probe burying test after infusions of saline or NPY into the lateral septum (Experiment 1).

Behavior	Saline (n = 10)	NPY (1.5 μg) (n = 9)
Burying (sec)	134.05 ± 56.71	1.12 ± 7.11
Immobility (sec)	0.77 ± 0.77	6.17 ± 3.70
Number of rears	46.60 ± 5.44	43.44 ± 5.25
Number of shocks	3.40 ± 0.31	3.11 ± 0.51
Shock reactivity	2.12 ± 0.12	1.71 ± 0.18

Note: The duration of burying scores are provided for comparative purposes, only. Data analysis was done on transformed (log 10) burying scores; see Results section for details. NPY, neuropeptide Y.

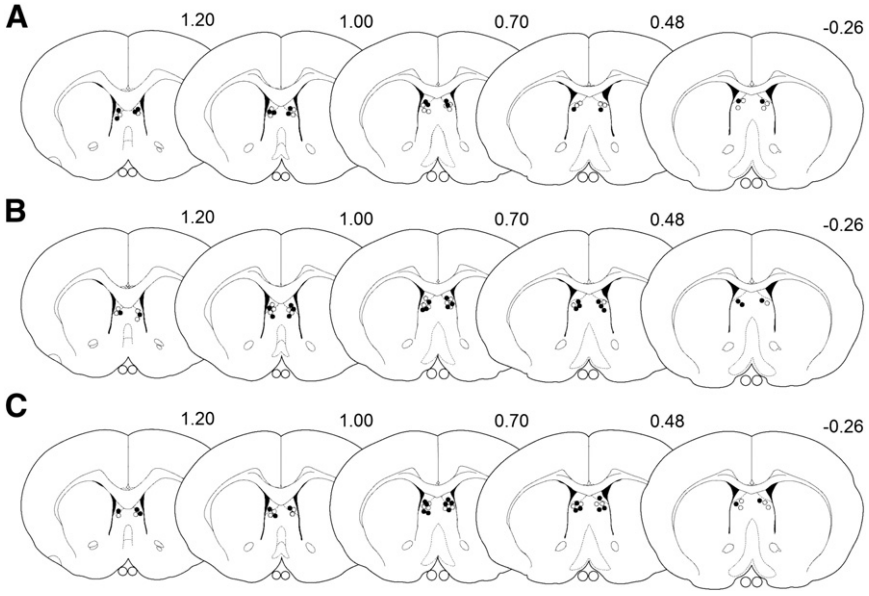

Fig. 5. Histological results for Experiment 2. Circles indicate the location of the cannula tips for rats bilaterally infused with either saline (open circles), NPY (1.5 μg) (filled circles) (A), Y_1 (0.15 μg) (open circles) or Y_1 (0.30 μg) (filled circles) (B), and Y_1/NPY (0.15 μg/1.5 μg) (open circles) or Y_1/NPY (0.30 μg/1.5 μg) (filled circles) (C) into the lateral septum. NPY = neuropeptide Y, Y_1 = Y_1 receptor antagonist BIBO 3304. Atlas plates are adapted from Paxinos and Watson (1998).

for the novel cage test (Day 6), $F(5,53) = 3.48$, $p = 0.009$, but not for the home cage test (Day 5), $F(5,53) = 0.49$, $p > 0.79$. Similarly, there were no between group differences in latency to snack consumption for any of the habituation trials (Days 1–4, all ps > 0.40). Pair-wise comparisons (LSD test) on the novel cage test data confirmed that compared to saline treated controls, rats infused with NPY (1.5 μg) displayed significant reductions in the latency to begin consumption of the snack in the novel cage, $p < 0.001$. The higher dose of BIBO 3304 (0.30 μg) significantly attenuated the effect of NPY on feeding latency in the novel environment, $p > 0.07$ compared with saline and $p < 0.04$ compared with NPY alone, whereas the lower dose of BIBO 3304 (0.15 μg) did not, $p < 0.02$ compared with saline and $p > 0.2$ compared with NPY alone. Unexpectedly, when BIBO 3304 was infused alone at the higher dose (0.30 μg) rats displayed a reduced latency to initiate snack consumption in the novel cage test, $p < 0.02$ compared with saline.

The difference scores for this test (novel cage latency − home cage latency) are displayed in Fig. 7B. An overall one-way ANOVA revealed that the difference scores varied between groups, $F(5,53) = 4.01$, $p = 0.003$. Pair-wise comparisons (LSD test) revealed that, similar to Experiment 1, rats infused with NPY had reduced difference scores compared to saline, $p < 0.001$. BIBO 3304 (0.30 μg) at the higher dose significantly attenuated the effect of NPY-induced reduction in difference score, $p > 0.1$ compared to saline and $p = 0.007$ compared with NPY, whereas the lower dose of BIBO 3304 (0.15 μg) did not, $p = 0.008$ compared with saline and $p > 0.7$ compared with NPY. As above, rats infused with BIBO 3304 alone at the higher dose (0.30 μg) had significantly lower difference scores compared to saline, $p < 0.02$.

To further examine for potential treatment effects on appetitive motivation we analyzed data from Day 4 and the Home-Cage test using a repeated measures ANOVA. With this truncated analysis, there was a main effect of Day, $F(1,53) = 26.64$; $p = 0.00$ and no Day × Treatment interaction, $F(5,53) = 1.10$, $p = 0.37$. The absence of an interaction effect suggests that the decline in latency scores displayed by rats across Day 4 to Day 5 reflected ongoing habituation rather that treatment effects on appetitive motivation.

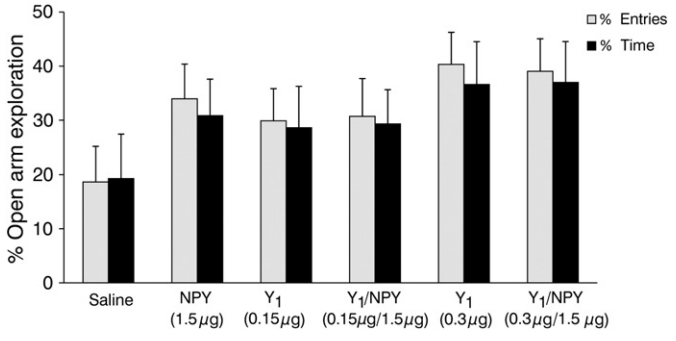

Fig. 6. Mean (±SEM) % of open-arm entries (open squares) and % of open-arm time (closed squares) displayed by rats following bilateral infusions of either saline (n = 10), NPY (1.5 μg, n = 8), Y_1 (0.15 μg, n = 9), Y_1/NPY (0.15 μg/1.5 μg, n = 10), Y_1 (0.30 μg; n = 11), or Y_1/NPY (0.30 μg/1.5 μg, n = 11) into the lateral septum. NPY = neuropeptide Y, Y_1 = Y_1 receptor antagonist BIBO 3304.

3.2.3. Shock-probe burying test

The shock-probe data from Experiment 2 is depicted in Fig. 8. Similar to Experiment 1, a one-way ANOVA indicated that the duration of burying (log seconds) $F(5,52) = 4.94$, $p = 0.001$ varied between groups. Follow-up pair-wise comparisons (LSD test) confirmed that, similar to Experiment 1, rats infused with NPY (1.5 μg) into the lateral septum spent less time burying the shock-probe than did saline treated controls, $p < 0.001$. Neither dose of BIBO 3304 attenuated the effect of NPY infusions on burying behavior; i.e., burying scores of rats that received combined infusions of either BIBO 3304 (0.15 μg) + NPY (1.5 μg) or BIBO 3304 (0.3 μg) + NPY (1.5 μg) did not differ from those of rats treated with NPY alone, both ps > 0.4, but did differ from saline-treated controls, both ps < 0.003. Although the low dose of BIBO 3304 when given alone did not affect burying behavior, $p = 0.10$, the high dose of BIBO 3304 produced significant reductions in burying relative to controls, $p = 0.001$.

Table 4 shows the duration of burying in seconds (prior to log transformation), as well as the activity and reactivity scores for this test. Importantly, there were no group differences in mean shock-reactivity $F(5,52) = 0.20$, $p > 0.9$, or the number of shocks received $F(5,52) = 0.84$, $p > 0.5$. There was however, a significant overall difference in activity level

Table 3
Mean (±SEM) plus-maze activity and vigilance scores after infusions into the lateral septum (Experiment 2).

Behavior	Saline (n=10)	NPY (1.5 μg) (n=8)	Y_1 (0.15 μg) (n=9)	Y_1/NPY (0.15 μg/1.5 μg) (n=10)	Y_1 (0.30 μg) (n=11)	Y_1/NPY (0.30 μg/1.5 μg) (n=11)
Closed arm entries	10.50±1.15	11.50±1.34	10.44±0.85	10.00±0.75	10.30±1.02	9.91±1.01
Total arm entries	12.90±1.09	14.62±1.33	15.22±1.19	18.45±2.03	15.80±1.69	16.64±1.29
Number of rears	22.70±2.88	19.03±2.04	21.78±2.20	18.00±1.15	23.82±1.85	21.46±0.83
Total SAP	12.70±1.12	11.25±1.69	12.78±1.54	10.00±1.49	11.00±1.47	9.36±0.91
% protected SAP	77.23±10.14	64.41±10.74	73.70±6.31	61.28±8.48	69.40±7.94	67.54±7.75

NPY, neuropeptide Y; Y_1, Y_1 antagonist BIBO 3304; SAP, stretch-attend postures.

as measured by duration of immobility, $F(5,52)=3.46$, $p=0.009$ and number of rears, $F(5,52)=2.38$, $p=0.05$. Pair wise comparisons (LSD) revealed that, as in Experiment 1, NPY-treated rats did not differ from saline-treated rats on either the duration of time spent immobile ($p>0.65$) or the number of rears ($p>0.25$). However, BIBO 3304 (0.15 μg)+NPY (1.5 μg) infused rats spent more time immobile compared to all other groups, $p<0.02$. In addition, BIBO (0.15 μg) treated rats made fewer rears relative to saline-treated controls, $p=0.05$, and both BIBO (0.15 μg) and BIBO (0.15 μg)+NPY (1.5 μg) treated rats made fewer rears than NPY-treated rats, both ps<0.05.

4. Discussion

Infusions of NPY into the lateral septum produced anxiolytic-like effects in the novelty-induced suppression of feeding and shock-probe burying tests, but failed to alter rats' open arm avoidance in the elevated plus-maze test. Thus, lateral septal NPY decreased rats' latency to initiate consumption of a palatable snack in a novel environment, without changing their latency to snack consumption in the home cage, and selectively reduced the duration of time spent burying an electrified shock-probe, without affecting general locomotor activity. Pre-infusions of the Y1 antagonist BIBO 3304 (0.30 μg) attenuated the anxiolytic-like effects of NPY in the novelty-induced suppression of feeding test but did not alter the effects of NPY on burying. Together, our findings support the view that activation of NPY receptors in the lateral septum reduced anxiety-related responses, but this effect seems to be test specific.

Although NPY (i.c.v.) is known to enhance appetite (Flood and Morley, 1991; Hanson and Dallman, 1995; Polidori et al., 2000), we saw no consistent evidence of this following NPY infusions into the lateral septum. We note that in Experiment 1, NPY-treated rats (but not saline-treated controls) approached the palatable snack significantly faster during the home-cage test on Day 5, relative to their last habituation trial on Day 4, thus raising the possibility that lateral septal NPY increased appetitive motivation. However, in Experiment 2, both NPY- and saline-treated rats displayed significant reductions in latency to snack consumption from Day 4 to Day 5, suggesting that habituation to snack delivery was ongoing at the time of the home-cage test. Even more importantly, in both experiments, there were no between group differences in latency to snack consumption in the home cage test itself. These null findings are unlikely to reflect a floor effect because the latency scores during the home-cage test were sufficient for examining drug-related changes in either direction (i.e., control baseline latencies were over 1 min in both experiments). Thus, it seems likely that the lateral septum does not mediate the appetite-inducing effects of NPY. In a similar vein, others have found that NPY failed to increase feeding when it was infused into the

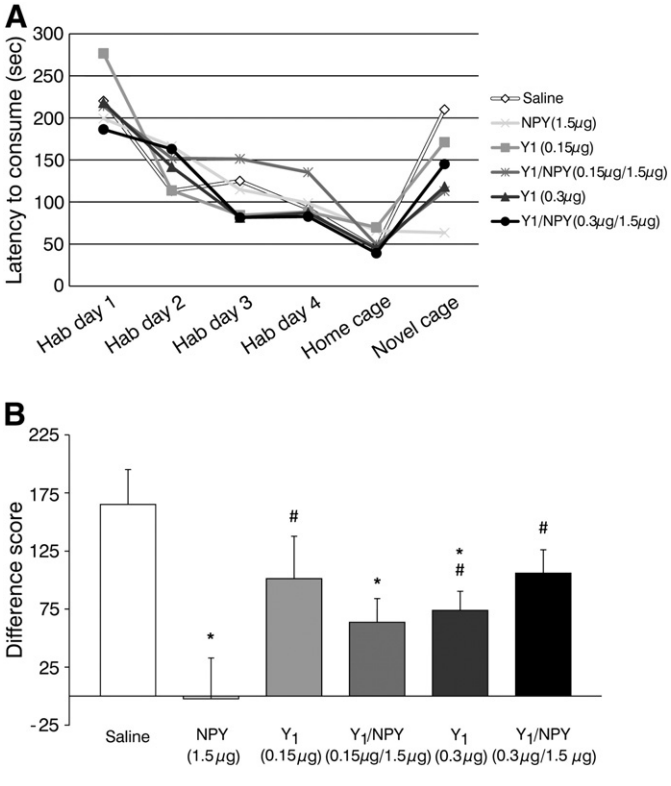

Fig. 7. Mean (±SEM) latency to begin to consume the snack across the 4 habituation trials (Hab days 1–4) and the home and novel cage tests (A) for rats bilaterally infused with either saline (n=10), NPY (1.5 μg, n=8), Y_1 (0.15 μg, n=9), Y_1/NPY (0.15 μg/1.5 μg, n=10), Y_1 (0.15 μg, n=11), or Y_1/NPY (0.30 μg/1.5 μg, n=11) into the lateral septum. The mean (±SEM) difference scores (novel cage latency − home cage latency) are also displayed (B). NPY = neuropeptide Y, Y_1 = Y_1 receptor antagonist BIBO 3304. (*p<0.05 relative to saline infusions, #p<0.05 relative to NPY infusions when given alone).

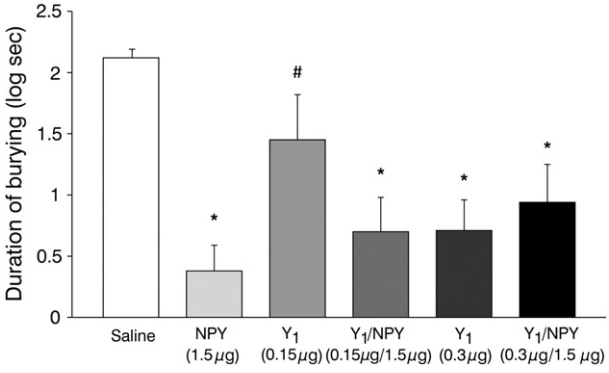

Fig. 8. Mean (±SEM) duration of burying (log seconds) in the shock-probe burying test for rats bilaterally infused with either saline (n=9), NPY (1.5 μg, n=8), Y_1 (0.15 μg, n=9), Y_1/NPY (0.15 μg/1.5 μg, n=10), Y_1 (0.30 μg; n=11), or Y_1/NPY (0.30 μg/1.5 μg, n=11) into the lateral septum. NPY = neuropeptide Y, Y_1 = Y_1 receptor antagonist BIBO 3304. (*p<0.005 relative to saline infusions, #p<0.05 relative to NPY infusions when given alone).

Table 4
Mean (±SEM) activity and reactivity scores for the shock-probe burying test after infusions into the lateral septum (Experiment 2).

Behavior	Saline (n=9)	NPY (1.5 μg) (n=8)	Y_1 (0.15 μg) (n=9)	Y_1/NPY (0.15 μg/1.5 μg) (n=10)	Y_1 (0.30 μg) (n=11)	Y_1/NPY (0.30 μg/1.5 μg) (n=11)
Burying (s)	146.88±25.81	7.01±6.02	122.71±40.51	27.22±15.07	18.00±6.94	62.73±29.81
Immobility (s)	1.24±0.54	9.97±5.84	11.50±4.64	73.15±29.88[a]	8.04±5.92	21.63±11.85
Number of rears	51.33±3.76	58.63±5.67	38.56±3.65[b,c]	41.70±4.58[c]	48.55±5.26	47.09±2.83
Number of shocks	2.56±0.38	2.75±0.49	3.11±0.51	1.90±0.28	2.73±0.36	2.55±0.53
Shock reactivity	2.39±0.27	2.15±0.13	2.18±0.11	2.25±0.25	2.24±0.14	2.32±0.19

Note: The duration of burying scores are provided for comparative purposes, only. Data analysis was done on transformed (log 10) burying scores; see results section for details. NPY, neuropeptide Y; Y_1, Y_1 antagonist BIBO 3304.
[a] $p<0.05$ compared to all other groups.
[b] $p<0.05$ compared to saline vehicle.
[c] $p<0.005$ compared to NPY.

amygdala (Heilig et al., 1993), but led to robust, reliable increases in feeding when it was infused into the hypothalamus (Kask et al., 1998c; Van Dijk and Strubbe, 2003).

A further consideration is whether treatment-induced differences in motivation to explore underlie the group differences we observed in feeding latency in the novel cage. This concern is based on prior work showing that infusions of NPY (i.c.v.) reduced rats' exploration of a novel open-field, as indexed by treatment-induced decreases in both horizontal (total crosses) and vertical (number of rears) activities (Heilig and Murison, 1987). However, NPY did not impair indices of exploration/locomotor activity in the current study, as indexed by either the number of closed arm and total arm entries or the number of rears in the plus maze test and the number of rears and time spent immobile in the burying test. Thus, our overall findings suggest that the observed NPY-induced decreases in latency to feeding in the novel environment were not secondary to changes in general exploration, but rather reflected a specific reduction in the neophagia typically observed when rats are offered food in a novel environment.

It is important to note that in the current study, the home-cage (Day 5) and novel environment (Day 6) trials of the novelty-induced suppression of feeding test were separated by only 24 h. Thus, it might be that our findings in the novel environment were influenced by carry over effects of NPY. Although our data do not directly address this issue, prior reports suggest that the effects of NPY are not long lasting (Hanson and Dallman, 1995; Heilig and Murison, 1987). For example, Heilig and Murison (1987) treated rats with NPY (1 nmol i.c.v.) every 24 h for 3 days and, after each treatment, measured their locomotor activity in an open field test. Relative to saline-treated controls, NPY-treated rats displayed a parallel downward shift in their locomotor activity curve across the 3 test days, suggesting that NPY did not impair normal habituation processes and further that the magnitude of NPY effects on locomotor activity did not change across the repeated daily injections. Thus, although we cannot conclusively rule out whether drug carry-over effects contributed to the NPY-induced reductions in rats' latency to feeding in the novel environment this possibility seems unlikely.

Although the effect of NPY on defensive burying behavior is a novel finding, the observed reduction in burying produced by lateral septal NPY is consistent with prior evidence that pharmacological perturbations of the lateral septum suppress the burying response (e.g., Degroot et al., 2001; Menard and Treit, 1996). NPY has anti-nociceptive properties (Kuphal et al., 2008), which could affect both burying behavior and probe avoidance. However, we saw no evidence that NPY-induced reductions in burying were secondary to changes in pain sensitivity in that there were no between group differences in mean shock reactivity. Furthermore, all rats avoided the probe to a similar degree, as indexed by the number of probe contact-induced shocks. This latter finding further suggests that both groups were able to learn the association between the probe and the shock, ruling out learning and memory deficits as a possible confound. Finally, because NPY has sedative actions at high doses (Heilig and Murison, 1987; Sorensen et al., 2004) it is important to account for potential treatment-induced changes in general exploration/locomotor activity. In the current study, lateral septal NPY did not alter general activity levels suggesting that the NPY-induced reductions in burying were not secondary to treatment-related sedation. Taken together, it appears that NPY produced a selective reduction in defensive behavior in the shock-probe test.

We did not specifically address the issue of neuroanatomical specificity for NPY effects in the current study. One concern is whether the reductions in anxiety-related behaviors that we observed following lateral septal NPY were in fact mediated by diffusion of the drug to the medial septum. We think this is unlikely. Most (if not all) of our injector tips were situated at least 1 mm away from the medial septum, and because we used a relatively small infusion volume (0.5 μl) we think that drug diffusion to that site was likely minimal (Martin, 1991). More importantly, others have described a relative absence/paucity of NPY receptors in the medial septum (Kask et al., 2001; Martel et al., 1986). Consistent with those findings, infusions of NPY into the medial septum failed to alter rats' anxiety-related responses in the plus-maze, social interaction and conflict tests, whereas anxiolytic-like effects were observed in those tests when equivalent doses of NPY were infused into the lateral septum (Kask et al., 2001; Olivera-Lopez et al., 2008). Similarly, we think it unlikely that our results reflect diffusion of NPY from the lateral septum to the lateral ventricles. The effective dose of lateral septal NPY (1.5 μg, 0.35 nmol) in the current study is comparable to doses of NPY (0.2–0.5 nmol, i.c.v.) that failed to alter rats' behavioral responses in the elevated plus-maze, open field and Geller–Seifter conflict tests when administered into the lateral ventricles (Britton et al., 1997; Heilig and Murison, 1987; Heilig et al., 1989). By contrast, higher doses of NPY (0.9–1.0 nmol, i.c.v.) in the lateral ventricles led to anxiolytic-like effects in the plus-maze and conflict tests, as well as effects on exploration in the open-field test (Britton et al., 1997; Heilig and Murison, 1987, Heilig et al., 1989).

Initially, we were surprised that infusions of NPY into the lateral septum did not alter open arm exploration in the elevated plus-maze test, given the involvement of both the lateral septum and NPY in modulating open arm exploration (Heilig et al., 1989; Menard and Treit, 1996; Trent and Menard, 2010). However, when we repeated this work, in Experiment 2, we once again obtained null effects. In contrast, other investigators, using the same dose as in the current study, observed selective increases in open arm exploration following intra-lateral septal NPY (1.5 μg/side for a total dose of 3.0 μg; Molina-Hernandez et al., 2010). These conflicting results might reflect methodological differences between the two studies. Others have shown a U-shaped dose response curve in rats' plus-maze behavior following NPY (i.c.v.), with low doses (7 pmol) decreasing open arm exploration; moderate doses (70 pmol) having no effects, and high doses (0.7 nmol) increasing open arm exploration (Nakajima et al., 1998). Interestingly, the anxiogenic-like effects of the low dose of NPY (7 pmol) were observed with an

infusion-test-interval (ITI) of 10 min but not 30 min, raising the possibility that some effects of NPY are fast acting and show a steep degradation line. It might be that a similar account could explain why Molina-Hernandez et al. (2010) observed effects in the plus-maze (using a 3 min ITI) whereas we did not (using a 15 min ITI). It also might be that a higher dose NPY than the one used here is needed for effects in the plus-maze. However, this seems unlikely given the reliable reductions in anxiety-related behavior we observed in the novelty-induced suppression of feeding test and shock-probe burying test.

An additional possibility is that the elevated plus-maze might be relatively less sensitive to the anxiolytic actions of NPY than other models. Consistent with this possibility, evidence implicating NPY in open arm avoidance is mixed. Although NPY, administered i.c.v., affected open arm exploration (Britton et al., 2000; Heilig et al., 1989), the direction of this change appears to be both dose and time dependent (Nakajima et al., 1998). Infusions of NPY (10 pmol) into the basolateral amygdala failed to increase rats' open arm exploration, but were effective at increasing social interactions between unfamiliar conspecifics (Sajdyk et al., 2008). Lateral septal NPY increased open arm exploration in both female (Molina-Hernandez et al., 2006; Olivera-Lopez et al., 2008) and male (Molina-Hernandez et al., 2010) rats. For females, the effective dose varied according to whether the rats were in metestrus–diestrus (2.0 μg/side) or late proestrus (1.0 μg/side; Molina-Hernandez et al., 2006). The latter finding is consistent with evidence that NPY and estradiol have synergistic effects on anxiety (Olivera-Lopez et al., 2008). Other studies, using NPY-transgenic rats that over express NPY, suggest that prior stress may be necessary for NPY-mediated effects in the plus-maze test (Thorsell et al., 2000). In that study, NPY-transgenic rats displayed normal levels of open arm avoidance but failed to display the expected increases in open arm avoidance that typically follow acute restraint stress (Thorsell et al., 2000). Further studies should examine whether prior exposure to stress similarly influences the effects of lateral septal NPY on open arm activity.

Our findings indicate that the Y1 receptor subtype mediates the anxiolytic-effects of lateral septal NPY in the novelty-induced suppression of feeding test, but not in the shock-probe burying test. In particular, infusions of BIBO 3304 completely attenuated NPY-induced decreases in rats' latency to initiate snack consumption in a novel environment but did not alter NPY-induced reductions in shock-probe burying. The Y1 receptor in the lateral septum also regulates anxiety-like behaviors in the social interaction test, in that infusions of BIBO 3304 at this site attenuated NPY-induced increases in social interaction (Kask et al., 2001). Similarly, infusions of NPY into the basolateral amygdala increased rats' social interactions, and this effect was reversed by co-infusions of BIBO 3304 (Sajdyk et al., 1999). In another study, the increases in open arm exploration produced by infusions of a metabotropic glutamatergic receptor (mGluR) group II agonist into the dorsal hippocampus were attenuated by co-infusions BIBO 3304 (Smialowska et al., 2007). Thus, activation of mGluR present on NPY-expressing interneurons may increase the local release of NPY leading to subsequent anxiolysis (Smialowska et al., 2007). The PAG also seems to be involved in Y1-mediated anxiety regulation, as infusions of the Y1 antagonist BIBP3226 into this region were anxiogenic in both the plus-maze and social interaction tests (Kask et al., 1998a,b). Additionally, mice lacking the NPY Y1 receptor gene failed to display NPY-induced (i.c.v.) increases in open arm exploration in the plus-maze test (Karlsson et al., 2008). Taken together, although it appears that the NPY Y1 receptor contributes to anxiety regulation, its involvement in specific tests might vary according to brain region. Further studies are needed to either refute or confirm this possibility.

The finding that BIBO 3304 failed to attenuate NPY-induced decreases in defensive burying suggests that some of the anxiolytic-like effects of NPY could be mediated by other NPY receptors, such as the Y5 receptor. Consistent with this, the Y1 and Y5 receptors have been implicated in the anxiolytic-like effects of NPY (i.c.v.) in the plus-maze and open field tests (Sorensen et al., 2004). Although moderate densities of the Y5 binding site are present in the lateral septum (Dumont et al., 2004; Morin and Gehlert, 2006), their involvement in the effects of lateral septal NPY has yet to be investigated. Future work should similarly investigate the potential involvement of the Y2 receptor, which is highly expressed in the lateral septum (Dumont et al., 2000), in NPY-induced anxiolysis at that site. Unlike Y1, Y4 and Y5 receptors, which are predominately expressed post-synaptically, the Y2 receptor is a pre-synaptic autoreceptor that inhibits the release of NPY (reviewed in Harro, 2006; Kask et al., 2002). In keeping with the hypothesis that endogenous NPY is anxiolytic, i.c.v. infusions of the Y2 agonist, $NPY_{13–36}$ were anxiogenic in the elevated plus-maze (Nakajima et al., 1998), whereas i.c.v. infusions of the Y2 antagonist, BIIE 0246 were anxiolytic in the elevated plus-maze test (Bacchi et al., 2006). This said, when BIIE 0246 was infused into the lateral septum it failed to alter rats' social behavior in a social interaction test across a range of doses (Kask et al., 2001). Other evidence suggests a possible role of the Y4 receptor in NPY-mediated anxiolysis, as knock-out mice displayed anxiolysis in open field and plus-maze tests (Painsipp et al., 2008), although Y4 has, to date, not been detected in the lateral septum (Dumont et al., 2000).

We found that infusions of BIBO 3304 alone, at the higher dose (0.30 μg), paradoxically decreased neophagia in the novelty-induced suppression of feeding test and decreased burying in the shock-probe test. It is not clear why high doses of the Y1 antagonist alone would mimic the effects of NPY. Furthermore, in the novelty-induced suppression of feeding test, although lateral septal infusions of either NPY or BIBO 3304 alone both reduced neophagia, when they were coinfused together the antagonist completely attenuated the anxiolytic-like actions of NPY. Given the high selectivity and affinity that BIBO 3304 shows for the Y1 receptor (Dumont et al., 2000; Wieland et al., 1998), it seems unlikely that these paradoxical results reflect the activity of BIBO 3304 at receptors other than Y1. Interestingly, others have shown that infusions of either NPY or BIBO 3304 alone into the dentate gyrus increased open-arm activity in the elevated plus maze (Smialowska et al., 2007). In the same study, pre-infusions of the Y2 receptor antagonist, BIIE 0246 into the dentate gyrus attenuated the anxiolytic-like effects of NPY at that site on open-arm activity. Given this pattern of results, those authors suggested that reductions in the ratio of Y1 to Y2 activation in the dentate gyrus might be anxiolytic. More studies are needed to determine whether a similar mechanism could account for the anxiolytic-like effects of lateral septal BIBO 3304.

It was initially suggested that the anxiolytic-like actions of septal NPY are mediated through interactions with cholinergic projections from the medial septum to the hippocampus (Zaborszky and Duque, 2000). However, this no longer seems likely in light of more recent evidence that the medial septum is devoid of NPY receptors (Dumont et al., 2000; Kask et al., 2001). Thus, infusions of NPY into the medial septum failed to reduce anxiety-like behaviors in either the social interaction test or the elevated plus-maze (Kask et al., 2001; Olivera-Lopez et al., 2008). Alternatively, the lateral septum sends GABAergic input to the hippocampus (Risold and Swanson, 1997), possibly to hippocampal interneurons that co-express GABA and NPY (Hendry et al., 1984; Pascual et al., 1999). In return, the lateral septum receives massive glutamatergic input from hippocampal pyramidal cells (Risold and Swanson, 1997). NPY-induced inhibition of lateral septal GABAergic input to the hippocampus could disinhibit hippocampal interneurons, ultimately reducing the release of glutamate in the lateral septum. Although speculative, this could account for the anxiolytic-like effects of NPY we observed in the current study. In partial support, infusions of the NMDA receptor antagonist AP5 into the lateral septum have been shown to suppress defensive burying in the shock-probe test (Menard and Treit, 2000).

To summarize, our findings confirm and extend previous reports that lateral septal NPY regulates anxiety-related behaviors across a range of animal models of anxiety (Kask et al., 2001; Molina-Hernandez

et al., 2010; Olivera-Lopez et al., 2008). Further, our finding that NPY Y1 receptor antagonism attenuated the anxiolytic-like effects of NPY in the novelty-induced suppression of feeding test adds support to the contention that the Y1 receptor contributes to anxiety regulation (Kask et al., 2001). However, we saw no evidence that the Y1 receptor contributes to the anxiolytic actions of lateral septal NPY on defensive burying. This raises the possibility that other NPY receptors, such as the Y2 and/or Y5 receptors might contribute to the anxiolytic effects of NPY. Future studies are needed to confirm or refute these possibilities.

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