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# Lateral septal infusions of the neuropeptide Y Y2 receptor agonist, $NPY_{13–36}$ differentially affect different defensive behaviors in male, Long Evans rats

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## HIGHLIGHTS

- Lateral septal $NPY_{13–36}$ had anxiolytic-like effects in the plus-maze and burying test.
- This same treatment did not affect novelty-induced suppression of feeding.
- Infusions of BIIE 0246 reversed the effects of $NPY_{13–36}$, but only in the plus-maze.
- NPY-Y2 receptor mediation of the effects of lateral septal $NPY_{13–36}$ is test-specific.

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## ABSTRACT

The lateral septum has been extensively implicated in regulating anxiety-related defensive behaviors in the rat. Neuropeptide Y (NPY) contributes to anxiety, likely through activity at the NPY Y1 and/or Y2 receptor binding sites. Although the lateral septum contains the highest density of Y2 receptors in brain, the involvement of this receptor in anxiety-related defensive behaviors is not clear. Thus, the purpose of the current study was to characterize lateral septal Y2 receptor contributions to rats' defensive responses to threat and/or potentially threatening environments. We investigated this by infusing the NPY Y2 agonist $NPY_{13–36}$ into the lateral septum and testing rats across a battery of animal models of anxiety (Experiment 1). To verify the role of Y2 in mediating the observed effects, rats were pre-infused with the potent and highly selective Y2 antagonist BIIE 0246 prior to infusion with $NPY_{13–36}$ (Experiment 2). Infusions of $NPY_{13–36}$ into the lateral septum increased rats' open-arm exploration in the elevated plus-maze test ($p<0.01$) and decreased the proportion of rats' that buried ($p<0.05$) as well as their latency to initiate burying in the shock-probe burying test ($p<0.01$). By contrast, $NPY_{13–36}$ did not affect either anxiety- or appetite-related responses in the novelty-induced suppression of feeding test (all $ps>0.3$; Experiment 1). Pre-treatment with the Y2 antagonist BIIE 0246 prevented the anxiolytic-like actions of $NPY_{13–36}$ in the plus-maze but not in the shock-probe test (Experiment 2). Thus, it appears that the anxiolytic-like actions of lateral septal $NPY_{13–36}$ are mediated by the Y2 receptor in a test-specific manner.



## 1. Introduction

Neuropeptide Y (NPY), one of the most abundant and widely distributed peptides in mammalian brain, is implicated in anxiety [1–3]. Consistent with clinical evidence linking low levels of NPY to pathological anxiety disorders [4–6], NPY knockout mice display increased anxiety-related behavior in the elevated plus-maze, open field, and light–dark test [7,8]. Conversely, NPY-transgenic animals with an over-expression of NPY are resistant to the effects of acute restraint stress in the elevated plus-maze and punished drinking test [9,10], and intra-hippocampal infusions of NPY conferred greater behavioral resilience to the lasting impact of stress (10 min exposure to soiled cat litter), in a rat model of post-traumatic stress disorder [11]. As well, extensive evidence implicates NPY in rodents' immediate, anxiety-related responses to threats; i.e., NPY i.c.v. decreases anxiety-like behaviors in the open field and Vogel's conflict test in rats [12,13], and the plus-maze and light–dark exploration test in mice [12–14]. Subsequent work found that site-specific injections of NPY into the locus coeruleus, periaqueductal gray, amygdala, hippocampus, and lateral septum also decrease anxiety-related defensive behaviors [15–18].

NPY acts through at least four G-protein linked receptors in the rat: NPY Y1, Y2, Y4, and Y5 [1,2]. Of these, the Y1 and Y2 receptors have received the most attention with regard to anxiety regulation [2]. Y1 receptors are primarily expressed post-synaptically [19]. By

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contrast, Y2 receptors are primarily expressed pre-synaptically, either as autoreceptors that inhibit the release of NPY or as heteroreceptors that can inhibit the release of glutamate [20–23] and/or increase the release of GABA [24,25, but see 26]. Particularly high concentrations of Y1 and Y2 binding sites are found in structures that regulate the expression of anxiety-related defensive behaviors, including the periaqueductal gray, locus coeruleus, hippocampus, amygdala, hypothalamus, and lateral septum [27–29]. Administration of NPY directly into these structures decreases rats' anxiety-related defensive behaviors via activation of the Y1 receptor [15–18,30,31]. However, in the lateral septum, Y1-mediated behavioral defense regulation appears to be test-specific, with infusions of the Y1 antagonist BIBO 3304 blocking the anxiolytic-like effects of NPY in the novelty-induced suppression of feeding and social interaction tests, but not the shock-probe burying test [17,31]. This raises the possibility that although the Y1 receptor mediates rats' defensive responses in some tests, other NPY receptors might make either a complimentary or unique contribution to lateral septal regulation of behavioral defense.

One possible contender is the Y2 receptor binding site. The role of the Y2 receptor in behavioral defense regulation is not well-defined and appears to differ across species, regions, and tests. Whereas Y2 knockout mice displayed reduced defensive responding in many tests [32,33] and Y2 agonist $NPY_{13–36}$-treated mice displayed higher levels of open-arm avoidance in the plus-maze test [34], i.c.v. infusions of the Y2 agonists $NPY_{13–36}$ and C2-NPY, across a range of doses, have failed to alter rats' defensive behaviors in various rodent models of anxiety [13,35–37]. On the other hand, site-specific infusions of Y2 agonists into the amygdala, locus coeruleus, and dentate gyrus altered rats' anxiety-related responses, with effects differing depending on the region investigated and/or the test employed. Specifically, infusions of $NPY_{13–36}$ into the amygdala increased rats' anxiety-related behaviors at low doses but decreased these same behaviors at high doses in the social interaction test [38]. Infusions of $NPY_{13–36}$ into the locus coeruleus decreased rats' open-arm avoidance in the plus-maze test [30]. Further, infusions of the Y2 antagonist BIIE 0246 into the rat dentate gyrus blocked the anxiolytic-like effects of NPY at that site in the plus-maze test [18].

The lateral septum contains one of the highest densities of Y2 receptors in rat brain, and the density of Y2 receptors at that site are markedly greater than that of the Y1 and Y5 receptor subtypes [39]. Notably, the lateral septum has been extensively implicated in behavioral defense regulation. Lesions and/or pharmacological inhibition of the lateral septum decrease rats' defensive behaviors in various animal models of anxiety, including the elevated plus-maze, shock-probe burying, social interaction and conflict tests [40–45]. Notably, as indicated above, the lateral septum is sensitive to the anxiolytic actions of NPY [17,31,46]; however there is a surprising lack of information regarding the specific involvement of lateral septal Y2 receptors in rats' defensive responses to threat and/or potentially threatening environments.

Thus, the purpose of the current study was to examine the potential contributions of the Y2 receptor to lateral septal regulation of behavioral defense. We investigated this by infusing the Y2 agonist $NPY_{13–36}$ into the lateral septum and testing rats across a battery of ethologically-based animal models of anxiety (Experiment 1). We then verified the role of Y2 in mediating the observed effects by pre-infusing rats with the potent and highly selective Y2 antagonist BIIE 0246 [47] prior to infusion with $NPY_{13–36}$ (Experiment 2). Rats were tested in three paradigms: the elevated plus-maze, novelty-induced suppression of feeding, and shock-probe burying tests. To the best of our knowledge, there are no prior investigations into the role of the lateral septal Y2 receptor in the regulation of anxiety-related behaviors in any of these tests. Given the high density of Y2 receptors in the lateral septum [48], and on the basis of prior evidence that NPY-induced reductions in open-arm avoidance and burying behavior [31] do not appear mediated at the Y1 receptor, we hypothesized that $NPY_{13–36}$ at that site would reduce rats' anxiety-related defensive behaviors in the elevated plus-maze and shock-probe burying tests and further that these effects would be blocked by pre-infusions of BIIE 0246. By contrast, based on our prior evidence that the anxiolytic-like effect of intra-lateral septal infusions of NPY in that test was blocked by pre-infusions of the Y1 antagonist BIBO 3304 [31], we did not anticipate that the Y2 agonist would affect rats' neophagia in the novelty-induced suppression of feeding task.

## 2. Method

### 2.1. Subjects

Subjects were 74 naïve, male Long Evans rats from Charles River, Quebec that weighed 300–400g and were between 60 and 65 days old 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 food (LabDiet 5001; Aberfoyle, ON) and water, ad libitum, 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 postsurgical recovery, rats were individually housed under the same conditions as before surgery. The treatment of all animals was in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals, and the Canadian Council on Animal Care, and was approved by the Queen's University Animal Care Committee.

### 2.2. Drugs

The selective NPY Y2 agonist $NPY_{13–36}$ (porcine) and the potent, selective and competitive non-peptide Y2 antagonist BIIE 0246 were obtained from Tochris Bioscience in Ellisville, Missouri. All drugs were dissolved in artificial cerebral spinal fluid (aCSF), which also served as vehicle.

### 2.3. Surgery

Rats were anesthetized with isoflurane in oxygen (0.5–4.5%) and administered the analgesic Metacam (2 mg/kg, s.c.) to reduce pain during surgery. The rats' heads were shaved and injected subdermally with the analgesic Marcaine (2 mg/kg). Rats were then placed in a Kopf stereotaxic apparatus. Body temperature was maintained by placing a heating pad under the surgical bed. The scalp was disinfected 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-gauge stainless-steel guide cannulae were implanted, according to flat skull coordinates from Paxinos and Watson [49] (0.5 mm AP, ± 1.2 mm ML, and 3.2 mm DV to bregma at 7° angled medially). Guide cannulae were 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 Tramadol (20 mg/kg, s.c.), rehydration with lactated ringer solution (5 ml, s.c.), and maintenance of body temperature by placing the rat into its home cage under a heat lamp. After animals recovered from anesthesia they were transferred from the surgery room to the recovery room (separate from the home colony) where they remained for a minimum of 3 recovery days. On each recovery day rats were given daily injections of both Tramadol (20 mg/kg, s.c.) and Metacam (1 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 until infusions and behavioral testing commenced.

### 2.4. Infusions

Rats were randomly assigned to one of the following infusion conditions: Experiment 1: (a) aCSF (b) $NPY_{13–36}$ (0.75 μg); Experiment 2: (a) aCSF + aCSF, (b) aCSF + $NPY_{13–36}$ (0.75 μg), (c) BIIE 0246