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Anxiolytic-like actions of centrally-administered neuropeptide Y, but not galanin, in C57BL/6J mice

Rose-Marie Karlsson[a,b,*], Andrew Holmes[b], Markus Heilig[a], Jacqueline N. Crawley[b]

[a]*Division of Psychiatry, NEUROTEC, Karolinska Institute, Stockholm/Laboratory of Clinical Science, NIAAA, NIH, Bethesda, MD, USA*
[b]*Laboratory of Behavioral Neuroscience, National Institute of Mental Health, Building 10 Room 4D11, Bethesda, MD 20892-1375, USA*

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Abstract

Neuropeptide Y (NPY) and galanin (GAL) are densely localized in brain regions subserving stress, fear and anxiety. While previous research supports a role for both neuropeptides in the mediation of rodent emotional behaviors, there is currently a lack of information on the effects of central administration of NPY and GAL on fear- and anxiety-related behaviors in mice. In the present study, the effects of intracerebroventricularly administered NPY and GAL were assessed in C57BL/6J mice on a battery of tests for fear- and anxiety-related behavior. NPY (0.5, 1.0 nmol) produced clear anxiolytic-like effects in the elevated plus-maze and light↔dark exploration test, whereas GAL (0.5, 1.0 nmol) was without effect. NPY (0.5 nmol) also increased locomotor activity in the open field test. In the fear conditioning paradigm, NPY administered prior to training reduced freezing to context (0.5, 1.0 nmol) and auditory cue (1.0 nmol). Pre-training GAL (0.5 nmol) treatment reduced freezing to context. Taken together, results demonstrate robust effects of centrally-administered NPY, but not GAL, on anxiety-like behaviors and fear conditioning in mice. These findings provide a basis for future studies of mice with targeted gene mutations, directed at delineating the anatomical regions and receptor subtypes mediating the effects of NPY and GAL on emotion.

Keywords: Anxiety; Mouse; Neuropeptide Y; Galanin; Fear conditioning; Stereotaxic; Intraventricular; Behavior; Neuropeptides; Stress; Nociception

1. Introduction

Neuropeptide Y (NPY) and galanin (GAL) are highly conserved and abundantly expressed in the central nervous system of mammals, including rats, mice and humans (De Quidt and Emson, 1986a,b; Melander et al., 1986). Both neuropeptides have been implicated in the regulation of behaviors related to cognition, nociception, feeding, and emotionality (for review, NPY: (Hökfelt et al., 1999; Kask et al., 2002), galanin: (Crawley, 1995; Hökfelt et al., 1999; Wrenn and Crawley, 2001)). Targeting NPY and GAL and their receptors may therefore offer opportunities for developing novel psychopharmacological treatments for various psychiatric disorders, with anxiety and depression being two potential applications (Holmes et al., 2003a).

GAL and NPY share similar neuroregulatory features, such as inhibition of cAMP production. In addition, GAL and NPY and their receptors show overlapping patterns of distribution in the rodent brain and both are densely localized in regions known to modulate stress, anxiety, and depression, such as the locus coeruleus, dorsal raphe nucleus, hypothalamus, hippocampus, bed nucleus of the stria terminalis (BNST), and amygdala. Within these regions, NPY exerts numerous effects on neurotransmitter systems, including the facilitation of inhibitory GABAergic interneurons and, acting as a presynaptic heteroreceptor, inhibition of excitatory glutamatergic release (Bleakman et al., 1992; Vezzani et al., 1999). GAL is also a potent modulator of neurotransmission, inhibiting the release of norepinephrine, serotonin (5-HT) glutamate and acetylcholine (Pieribone et al., 1995; Seutin et al., 1989; Kehr et al., 2002; Melander et al., 1985; Zini et al., 1993).

* Corresponding author. Laboratory of Behavioral Neuroscience, National Institute of Mental Health, Building 10 Room 4D11, Bethesda, MD 20892-1375, USA. Tel.: +1 301 4964838; fax: +1 301 4801164.
E-mail address: rosemariekarlsson@mail.nih.gov (R.-M. Karlsson).

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A growing literature supports a major role for NPY in the mediation of stress and anxiety. Abnormally low levels of plasma and cerebrospinal fluid levels of NPY have been found in patients with depression and anxiety disorders (Heilig et al., 2004; Rasmusson et al., 2000). In rats, central administration of NPY has been shown to decrease anxiety-like responses in a variety of tasks, including the Vogel conflict test, elevated plus-maze, fear potentiated startle and fear conditioned responses (Heilig et al., 1989, 1992; Broqua et al., 1995; Tovote et al., 2004). In mutant rats and mice engineered with genetic alterations in NPY or one of its six receptors (Y1–Y6), phenotypic abnormalities have been detected in rodent stress and anxiety-related behaviors. Knockout (KO) mice lacking NPY exhibit increased anxiety-like behavior in the open field and acoustic startle tests (Bannon et al., 2000), while transgenic rats over expressing NPY are resistant to stress-induced increases in anxiety-like behavior (Thorsell et al., 2000; Carvajal et al., 2004). The NPY Y1 and Y5 receptor subtypes have been implicated in the stress- and anxiety-related actions of NPY (Heilig, 1995; Redrobe et al., 2002; Sajdyk et al., 2002a). Recent evidence also supports a major role for the NPY Y2 receptor subtype, which acts as a presynaptic autoreceptor in the brain, in stress and anxiety. NPY Y2 KO mice show anxiolytic-like behaviors in the elevated plus-maze, light ↔dark exploration task, and open field, and a stress-resistant-like profile in the forced swim task (Redrobe et al., 2003; Tschenett et al., 2003). This phenotype is consistent with the anxiogenic-like effects of intra-amygdala treatment with Y2-preferring agonists in the rat social interaction test (Sajdyk et al., 2002a,b).

A role for galanin in the mediation of emotional behaviors has also been suggested by recent research in rodents. Depending on the brain region targeted, administration of GAL can produce increases or decreases in rat anxiety-like behavior. For example, intracerebroventricular (icv) administration of GAL exerts an anxiolytic-like effect in the Vogel conflict test, while the intra-amygdala GAL produced an anxiogenic-like response in the same task (Bing et al., 1993; Möller et al., 1999). GAL also appears to modulate the anxiogenic-like effects of stress in a region-specific manner. In rats, administration of the peptidergic GAL antagonist, M40, blocked stress-induced anxiety-like responses when injected into the BNST, but had opposite effects when microinjected into the amygdala (Khoshbouei et al., 2002a,b). A stress-modulating function for GAL is consistent with brain GAL upregulation in response to a variety of different stressors in rats (for review, see Holmes et al., 2003a) and the anti-stress effects of treatment with galanin or novel nonpeptidergic GAL antagonists in the rat forced swim test (Bartfai et al., 2004; Weiss et al., 1998). While the mechanisms subserving GAL's effects on stress remain to be elucidated, recent in vivo microdialysis studies in both rats and mice suggest that GAL effectively inhibits stress- and antidepressant-induced increases in 5-HT and norepinephrine in forebrain regions mediating emotion, such as the hippocampus (Yoshitake et al., 2004a,b). Lastly, while it remains unclear as to which of the three galanin receptor subtypes (GAL-R1, GAL-R2, or GAL-R3) mediate GAL's effects on stress and anxiety, a recent study in GAL-R1 KO mice suggests a role for this subtype (Holmes et al., 2003b).

To date, pharmacological studies of the stress- and anxiety-related effects of NPY and GAL have largely been conducted in rats. With the continued generation of NPY and GAL mutant mice to study the anxiety-related functions of these neuropeptide systems, it is critical to establish the pharmacological effects of NPY- and GAL-acting compounds on anxiety-related tasks in mice, in order to extend and accurately interpret findings in mutants. Studies assessing the anxiety-related effects of NPY and GAL in mutant mice lacking specific NPY or GAL receptor subtypes could provide insight into the subtypes mediating these effects. Such research would be particularly valuable given the current paucity of brain-penetrant, receptor subtype-selective agonists and antagonists with which to probe NPY and GAL. Confirming anxiety-related effects in mice is also important in the context of recent evidence that there may be differences between rats and mice in the localization of neuropeptides in regions mediating emotion (Larm et al., 2003). The aim of the present study was to assess the fear- and anxiety-related effects of centrally-administered NPY and GAL in C57BL/6J mice. The C57BL/6J strain was chosen because it is the most commonly used background strain in behavioral studies of mutant mice. The effects of icv-administered NPY and GAL were directly compared on a battery of fear- and anxiety-related tasks: elevated plus maze, light↔dark exploration test, open field test, and cued and contextual fear conditioning.

2. Materials and methods

2.1. Subjects

Male C57BL/6J mice, obtained from The Jackson Laboratory (Bar Harbor, ME), were housed in a temperature and humidity controlled vivarium, under a 12-h light/dark cycle (lights on 0600 h) and had free access to food and water in the home cage. Surgical procedures began following a habituation period of one week after arrival, at the age of 7–8 weeks. All experimental procedures were approved by the National Institute of Mental Health Animal Care and Use Committee and followed the NIH guidelines "Using Animals in Intramural Research."

2.2. Surgery and drug administration

Mice were anesthetized with isoflurane (5% for induction, 2.5% for maintenance) (Baxter Healthcare, Deerfield,

IL) and securely placed in a stereotaxic apparatus (Cartesian Research, Sandy, OR). A 22-gauge stainless-steel cannula (Plastics One, Roanoke, VA) was implanted into the lateral ventricle, at coordinates 1.00 mm lateral and −0.05 mm anterior to bregma, and −2.60 mm ventral to the skull surface (Paxinos and Franklin, 1997). After recovering from surgery, mice were individually housed for 7 days prior to behavioral testing. On test days, mice were taken into the experimental room to habituate for at least 1 h. NPY and GAL (American Peptide, Sunnyvale, CA) were dissolved in deionized water and administered in a total volume of 0.5 μl. Delivery was via a stainless-steel injector (Plastics One, Roanoke, VA), projecting 1 mm below the tip of the guide cannula. The mouse was gently held in the hand while the injector was inserted. Injections were given through a 33-gauge internal cannula attached to a Hamilton syringe with polyethylene tubing to allow the animals to move freely during the injection procedure. The solution was slowly injected over 35 s, and the microinjector left in place for a further 25 s to allow diffusion into the ventricle. After the injection the guide cannula was closed with a dummy cannula (Plastics One, Roanoke, VA) and mice were returned to the home cage.

Mice were tested on the elevated plus maze, light↔dark exploration, hot plate test, open field test, and cued and contextual fear conditioning paradigm, in that order, with a 6–10 day washout period between experiments. For each experiment, mice were randomly assigned to treatment with NPY, GAL or deionized water vehicle, and tested in an order pseudorandomly counter-balanced for drug treatment. Doses of 0.5 nmol and 1 nmol were used for NPY and GAL. These doses were chosen on the basis of pilot experiments conducted in our laboratory. The interval of 15 min between microinjections and the start of behavioral testing was chosen from a range previously used in behavioral studies with centrally administered NPY and GAL (Kinney et al., 2002; Heilig et al., 1989).

2.3. Elevated plus-maze

The elevated plus maze test was performed as previously described (Holmes et al., 2002, 2003b). The apparatus (San Diego Instruments, San Diego, CA) comprised two open arms (30×5 cm) and two closed arms (30×5×15 cm) that extended from a common central platform (5×5 cm). A small raised lip (0.5 cm) around the edges of the open arms helped prevent mice from slipping off. The apparatus was constructed from polypropylene and Plexiglas, with a white floor and clear walls, and elevated to a height of 38 cm above floor level. Fifteen minutes after injection, the mouse was placed on the center square facing an open arm and allowed to freely explore the apparatus under a light intensity of 200 lux for 5 min. The apparatus was cleaned with 70% ethanol solution between subjects. Behavior was scored by a trained observer using "Hindsight" (Scientific Programming Service, Wokingham, U.K.). Behaviors scored were open and closed arm entries (an arm entry was defined as all four paws into an arm) and the time spent in the open arms.

2.4. Light↔dark exploration test

The light↔dark exploration test was conducted as previously described (Crawley, 1981; Holmes et al., 2002). The apparatus consisted of a polypropylene cage (44×21×21 cm) separated into two compartments by a partition, with a rectangular opening (12×5 cm) at floor level. The larger compartment (28 cm long) was open-topped, transparent, and brightly lit (900 lux). The smaller compartment (14 cm long) had black painted sides and was covered at the top with black Plexiglas. Fifteen minutes after injection, the mouse was individually placed in the light compartment, facing away from the partition, and allowed to freely explore the apparatus for 10 min. The apparatus was cleaned with 70% ethanol after each subject. The number of light↔dark transitions between the two compartments, and the total time spent in the light compartment, was scored by a trained observer using "Hindsight" (Scientific Programming Service, Wokingham, U.K.). An entry into a compartment was defined as all four paws in the area. Risk assessment (also known as "scanning") was scored when the mouse had its head and two paws in the light compartment, but the rest of its body in the dark compartment.

2.5. Open field test

Open field was performed as previously described (Holmes et al., 2002). Spontaneous exploratory activity was assessed in a Digiscan automated open field (Accuscan, Columbus, OH). The open field was a square arena (40×40×35 cm) with clear Plexiglas walls and floor, evenly illuminated by overhead fluorescent white room lighting (550 lux). Eight sets of photocell beams and detectors were arrayed on each side of the arena, at right angles to one another, forming a grid of 64 equally sized squares. To detect vertical movement, a third set of eight photocell beams was located above the square grid. Fifteen minutes after injection, the mouse was placed in the center of the open field and allowed to freely explore for 15 min. The apparatus was cleaned with 70% ethanol solution after each subject. The number of horizontal and vertical beam breaks was taken as a measure of horizontal and vertical activity, respectively, following standards in the literature (Prut and Belzung, 2003). Time spent in the central square (20×20 cm) of the open field was recorded by the Digiscan system as center time.

2.6. Cued and contextual fear conditioning

Cued and contextual fear conditioning was conducted using methods established in the literature and previously

employed by our laboratory (Kinney et al., 2002). Training was conducted in a (26×23×17 cm) chamber with clear Plexiglas walls and a metal grid floor for foot shock delivery (Freeze Monitor, San Diego Instruments, San Diego, CA). Cued fear conditioning was measured in a novel chamber; a (50×35.5×17 cm) triangular box constructed of solid white plastic, scented with (~0.5 ml) of vanilla extract. A Dell Optiplex computer connected San Diego Instruments software to a shock stimulator and speaker delivered the tone (CS) and shock (US). The tone CS generated by the speaker was 80 dB at a frequency of 810 Hz. The shock was 0.2 mA current delivered for 2 s.

For training, the mouse was placed in the conditioning chamber 15 min after drug treatment. After a 120 s acclimation period, the CS was presented for 30 s and co-terminated with the 2 s US. A further 2 such CS-US pairings were given, with an inter-pairing interval of 90 s. Following the third pairing, mice remained in the chamber for an additional 120 s before being returned to the home cage. During training, freezing was scored during the first 120 s and last 120 s of the session. Twenty-four hours later, mice were returned to the training chamber for 300 s and measured for contextual conditioning. Forty-eight hours after training, mice were placed in the novel context, located in a different room to that used for training and context testing. After 180 s, the tone CS was delivered for 180 s to measure cued conditioning. Throughout training and testing, the presence or absence of freezing behavior was scored every 10 s, defined as the absence of any movement except respiration (Kim and Fanselow, 1992; Wehner et al., 1997). Data were calculated as the proportion of observations in which the subject was scored as freezing. After each subject completed the training session, the chamber was cleaned with 70% ethanol.

2.7. Hot plate test

In order to test whether the doses of NPY and GAL used in this study altered nociception and, therefore, might influence pain-related responses during fear conditioning, the response to an acute thermal stimulus was measured using hot plate test. Fifteen minutes after injection, the mouse was placed on a (254×254×19 mm) metal surface (Columbus Instruments, Columbus, OH) maintained at 53 °C and surrounded by a transparent plastic barrier. The latency to the first paw lick, jump or vocalization was measured using a foot pedal-controlled timer. A cut-off time of 30 s was used to prevent the risk of tissue damage to the paws.

2.8. Histology

At the completion of behavioral testing, mice were euthanized via cervical dislocation and rapid decapitation. Brains were immediately removed and placed in an 8% formaldehyde solution. Tissue was sectioned at a thickness of 50 μm, mounted on gelatin-coated slides and stained with 0.1% thionin. Sections were examined under a stereoscopic light microscope to verify cannula placement. A mouse that did not display a clear cannula track into the lateral ventricle was scored as a "miss" and the behavioral data of those subjects were removed from the data analysis. A total of 58 mice were used in the final statistical analyses, with treatment group sizes of n=8–13.

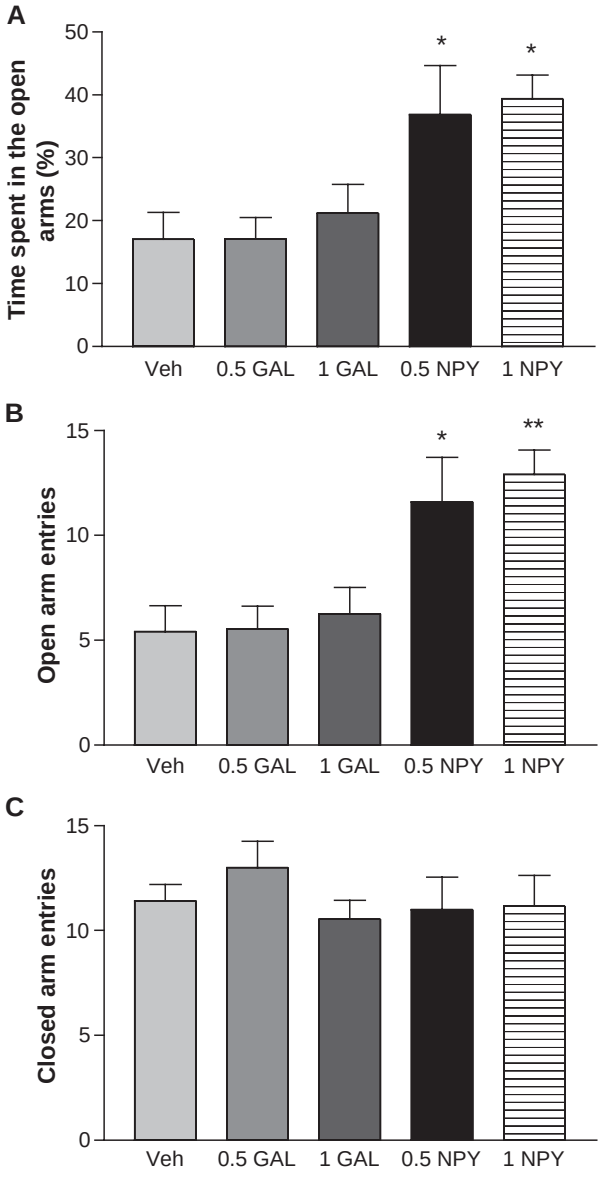

Fig. 1. Elevated plus-maze. The percentage time spent (A) and the number of entries (B) to the open arms were significantly increased in mice treated with 0.5 and 1.0 nmol NPY compared to vehicle treated control mice. GAL treatment did not produce any changes in these parameters. No change was seen in closed arm entries by either treatment (C). (n=10–12/group). Data are expressed as the mean±SEM, *p<0.05 and **p<0.01 for a peptide dose as compared to the deionized water vehicle control group.

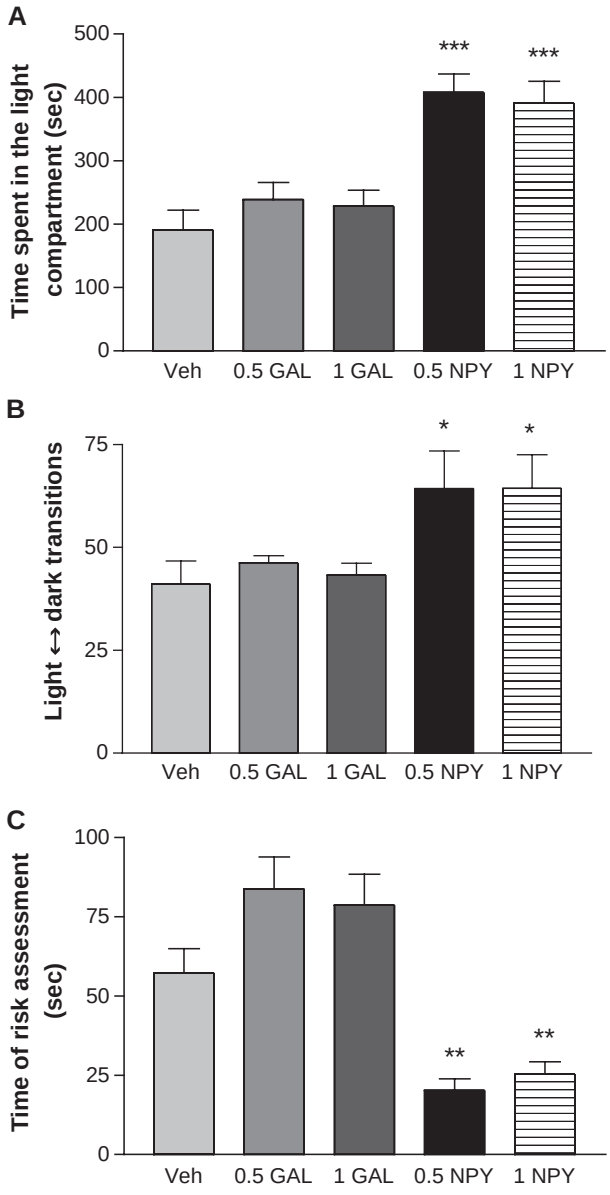

Fig. 2. Light↔dark exploration. Mice treated with NPY at an icv dose of 0.5 and 1 nmol showed a significantly more time spent in the brightly-lit open area (A) and in the number of transitions between the two compartments (B). Risk assessment was measured as an attempt to enter the light compartment, and was significantly lower between NPY dose and vehicle treatments. GAL did not produce any significant effects in this test. (n=8–12/group). Data are the mean±SEM, *p<0.05, **p<0.01 and ***p<0.001 for a peptide dose as compared to the deionized water vehicle control group.

2.9. Statistical analysis

Data were analyzed using One-way Analysis of Variance (ANOVA) and Newman-Keuls post-hoc comparisons in the presence of significant ANOVA effects, using the STATISTICA 6.0 (StatSoft, Tulsa, OK) software package. Statistical significance was set at p<0.05.

3. Results

3.1. Elevated plus-maze

In agreement with previous observations of sedative effects of high does of NPY (Heilig and Murison, 1987), a small number of mice receiving either dose of NPY exhibited clear signs of motor sedation during plus-maze testing. To avoid these data obscuring the anxiety-related effects of NPY, sedated animals were defined as those making <2 open arm entries and excluded from further analysis. The data from three mice were excluded for this reason. The resultant analysis showed that there was a

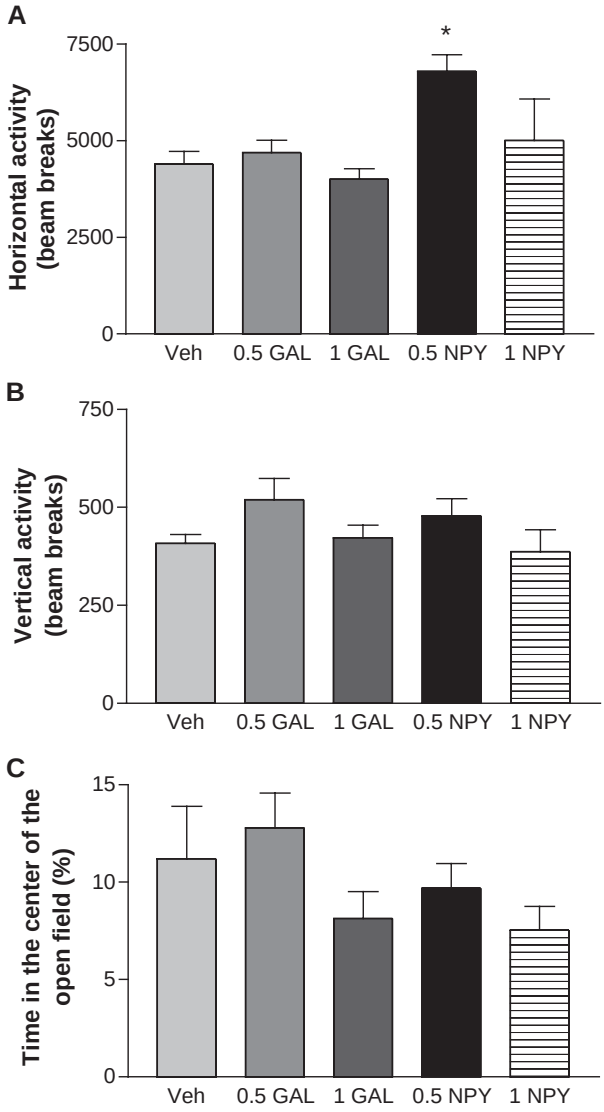

Fig. 3. Open field. NPY at a dose of 0.5 nmol, but not 1.0 nmol, produced higher levels of horizontal beam breaks (A) compared to vehicle controls. GAL did not produce any effects in this test. There were no differences in vertical activity (B) or percentage center time (C) between treatment groups across the 15 min test session. (n=11–13/group). Data are the mean±SEM, and *p<0.05 for a peptide dose as compared to the deionized water vehicle control group.

significant effect of treatment for the % time spent in the open arms (time spent in the open arms/by total time×100) ($F_{4,50}$=5.06, p<0.01) and open arm entries ($F_{4,50}$=6.77, p<0.01), but not closed arm entries. As shown in Fig. 1A–C, mice treated with 0.5 or 1 nmol NPY spent significantly more time and made significantly more entries into the open arms than vehicle controls (p<0.05), while GAL did not affect behavior at the doses tested.

3.2. Light↔dark exploration test

Six mice receiving NPY showed signs of profound sedation (i.e., made 0 transitions) and were excluded from further analysis. There was a significant effect of treatment for time spent in the light ($F_{4,47}$=10.95, p<0.01), light↔dark transitions ($F_{4,47}$)=4.04, p<0.01) and % risk assessment (time with head and two paws in light compartment/total time×100) ($F_{4,47}$=12.01, p<0.01). As shown in Fig. 2A–C, treatment with 0.5 or 1 nmol NPY significantly increased time spent in the light and light↔dark transitions, and significantly decreased % risk assessment. GAL did not significantly alter behavior in this test.

3.3. Open field test

There was a significant effect of treatment for total horizontal activity ($F_{4,53}$=3.85, p<0.01), but not for vertical activity or % time spent in the center. As shown in Fig. 3A–C, 0.5 nmol NPY significantly increased total horizontal activity, in comparison to vehicle-treatment (p<0.01), while vertical activity and % time in the center were unaffected.

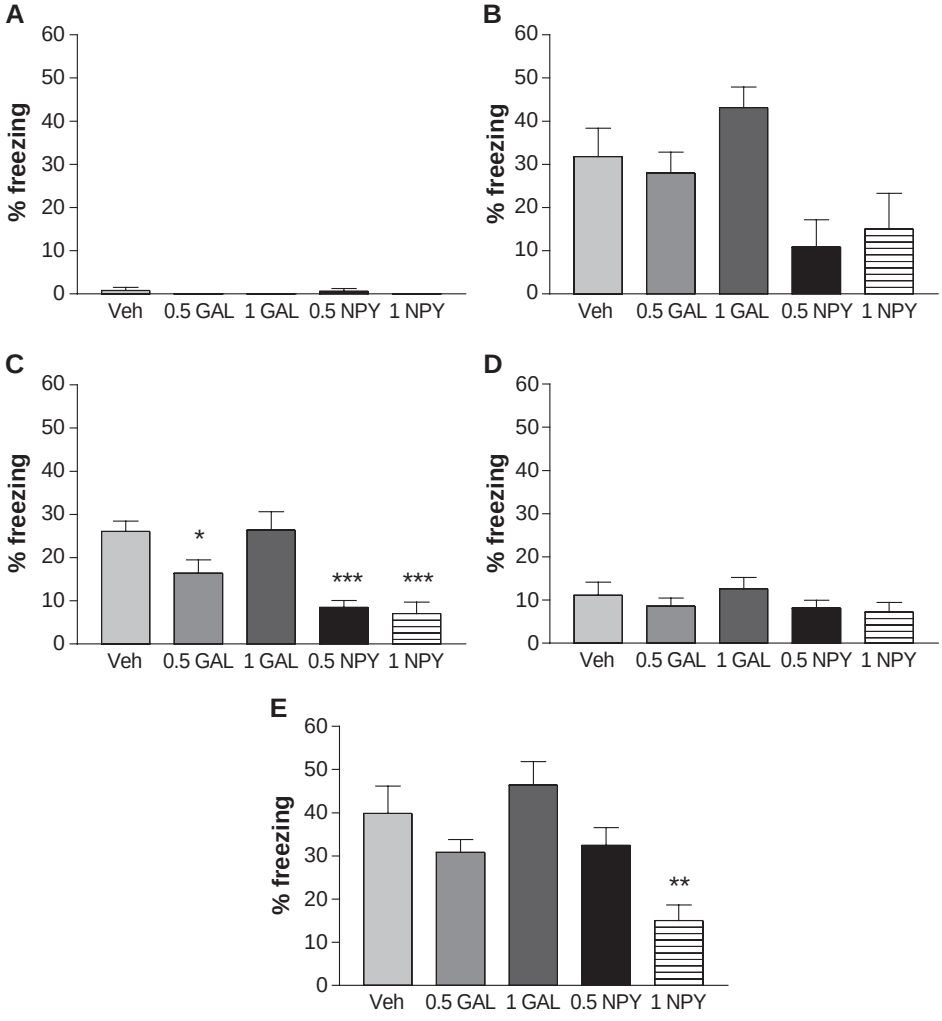

Fig. 4. Cued and contextual fear conditioning. Peptide treatment on day 1 did not alter freezing behavior during the first 2 minutes, before CS-US stimulation (A) nor did the mice respond differently during the last 2 minutes after CS-US training (B), compared to vehicle treated mice. Twenty-four hours later, mice that had previously been treated with NPY, and with GAL at a dose of 0.5 nmol, showed a decrease in freezing behavior when placed in the same context where training had been conducted (C). When placed in a novel context, 48 h after training, there were no significant difference between treated mice and vehicle controls (D). When the auditory cue, CS, was presented in the novel context, NPY-treated mice, at a dose of 1.0 nmol showed less freezing behavior than vehicle controls (E). (n=10–13/group). Data are the mean±SEM, and *p<0.05, **p<0.01 and ***p<0.001 for a peptide dose as compared to the deionized water vehicle control group.

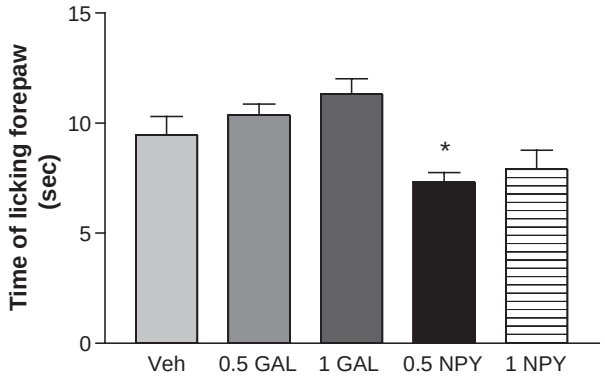

Fig. 5. Hot plate. NPY at the dose of 0.5 nmol produced shorter latencies to forepaw licking compared to vehicle treated animals. GAL did not produce any difference in latency to respond. (n=11–13/group). Data are the mean±SEM, and *p<0.05 for a peptide dose as compared to the deionized water vehicle control group.

3.4. Cued and contextual fear conditioning

During training, two mice receiving NPY showed clear sedation (i.e.>50% immobility during the pre-training acclimation period) and were excluded from further analysis. There was no treatment effect on freezing during the pre-training acclimation period or immediate-post-training period during training (Fig. 4A, B). Treatment significantly affected freezing during context testing ($F_{4,51}$=10.10, p<0.01); mice treated with 0.5 or 1 nmol NPY, or 0.5 nmol GAL, froze significantly less than vehicle controls (p<0.05). During cued testing, treatment did not affect freezing during the pre-CS period, but significantly altered freezing in response to the tone CS ($F_{4,51}$=5.99, p<0.01). Mice treated with the 1 nmol dose of NPY showed significantly less freezing to the tone CS than vehicle controls (p<0.01).

3.5. Hot plate test

There was a significant effect of treatment for response latency ($F_{4,52}$=5.60, p<0.01), due to a significantly reduced latency in mice treated with 0.5 nmol NPY (p<0.05) (see Fig. 5).

4. Discussion

The neuropeptides NPY and GAL exhibit overlapping patterns of distribution in regions of the rodent brain subserving emotion. Consistent with a functional role for NPY and GAL in mediating emotion, previous studies have shown that pharmacological modulation of these systems produces anti-stress and anti-anxiety effects in rats (Heilig et al., 1989, 1992; Bing et al., 1993; Broqua et al., 1995; Möller et al., 1999). Given the current lack of information on the effects of central administration of these neuropeptides on fear-and anxiety-related tasks in mice, the present study evaluates the effects of NPY and GAL in the mouse using a battery of anxiety- and stress-related tasks.

In the elevated plus-maze test for anxiety-like behavior, intracerebroventricular administration of two doses of NPY (0.5 nmol, 1.0 nmol) produced significant increases in open arm exploration, as compared to vehicle-treated controls. Importantly, closed arm entries, a measure of general changes in locomotor activity in this test, were not altered by NPY. This profile of behavioral changes is consistent with a clear and selective anxiolytic-like effect of NPY. Our results in mice confirm earlier work in rats, showing that NPY has robust anxiolytic-like effects in this task (Broqua et al., 1995; Heilig et al., 1989). In the same experiment, GAL (0.5 nmol, 1.0 nmol) did not alter elevated plus-maze behavior. Previous studies in rats have shown GAL fails to alter anxiety-like behavior in the elevated plus-maze under non-stressed conditions (Möller et al., 1999).

A developing literature suggests that GAL is preferentially recruited for its anxiolytic actions under conditions of high stress. This is well illustrated by the finding that administration of the GAL antagonist, M40, into the rat BNST failed to alter anxiety-like behavior in the elevated plus-maze under baseline conditions, but produced significant anxiolytic-like effects in rats stressed by immobilization prior to testing (Khoshbouei et al., 2002b). Similarly, GAL-overexpressing transgenic mice exhibit normal anxiety-like behavior under baseline conditions, but are resistant to the anxiogenic-related effects of noradrenergic activation (Holmes et al., 2002; Zachariou et al., 2003). Rat studies have also demonstrated that galanin gene expression is upregulated by strong stressors like social defeat, but not by less stressful events such as wheel running (reviewed in Holmes et al., 2003a). The stress-dependent effects of GAL on emotion parallel neurophysiological data demonstrating that galanin is preferentially released under conditions of high neuronal activity, such as those likely to occur in response to stress (Consolo et al., 1994; Hökfelt et al., 1987; Lundberg et al., 1983). The novel demonstration that centrally-mediated GAL fails to alter anxiety-like behavior under baseline conditions in mice extends this research and has important implications for the design of studies on stress- and anxiety-like behavior in GAL mutant mice.

The behavioral effects of NPY and GAL were replicated in another well-validated test for anxiety-like behavior, the light↔dark exploration test. NPY increased time spent in the aversive, light, compartment and the number of transitions between the light and dark compartments, relative to vehicle-treated controls. These parameters are sensitive to anxiolytics such as benzodiazepines (Crawley, 1981; Mathis et al., 1994) confirming a robust anxiolytic-like effect of intraventricularly administered NPY in mice. Present findings further demonstrate that NPY reduced risk assessment behaviors (i.e., scanning the light compartment from the safety of the dark compartment). Previous studies have shown that risk assessment behaviors in this test provides a

valuable measure of the anxiety-related effects of both classical and novel anxiolytics (Griebel et al., 1998). Thus, taken together, present results establish robust anxiolytic-like effects of icv NPY in mice, as measured across multiple behavioral measures and tasks. In contrast, and in agreement with the absence of GAL effects in the plus-maze, GAL did not alter behavior in the light↔dark exploration.

NPY treatment produced signs of sedation in a small subset of animals tested on our various fear- and anxiety-related tasks. Sedative effects have been commonly observed at high doses of NPY administered systemically and icv (Heilig and Murison, 1987; Heilig et al., 1989, 1991; Redrobe et al., 2000; Naveilhan et al., 2001). Data from these mice were excluded from the present analyses to avoid confounding proper interpretation of anxiety-like behavior. The reason why only one or two animals exhibited sedation in each experiment remains unclear. Histological analysis found no evidence of misplaced cannula in these mice. Interestingly, the lower dose of NPY (0.5 nmol) tested produced a significant increase in open field locomotor activity. This profile may further reflect the anxiolytic-like effects of NPY given the aversive, brightly-lit open field test that was presently used. However, the fact that, in contrast to profiles on the elevated plus-maze and light↔dark exploration test, effects were only seen at one dose of NPY and only on one behavioral measure likely reflects the relative insensitivity of the open field as a measure of anxiety-like behavior.

Cued and contextual fear conditioning is a widely-used paradigm for studying emotional learning (LeDoux, 1998). Mice treated with either dose of NPY prior to training showed significantly less freezing than vehicle-treated controls on re-exposure to the training context one day after training, while the higher dose reduced freezing to the conditioned auditory cue two days after training. These findings suggest an NPY-induced impairment in the acquisition of both cued and contextual conditioning. While NPY treatment did not significantly alter freezing during the 120 sec period after delivery of the final US–CS pairing during training, there were trends for reduced freezing in NPY-treated mice. To exclude the possibility that reductions in freezing during training in NPY-treated mice were caused by antinociceptive effects, the effects of NPY were assessed in the hot plate test. The dose of 0.5 nmol (but not 1.0 nmol) NPY reduced response latencies in the hot plate, suggesting an increase in pain sensitivity and contrasting with the hyperalgesic effects of icv NPY previously observed in mice (Mellado et al., 1996). Nonetheless, these data indicate that reduced pain sensitivity is unlikely to account for acquisition deficits following NPY treatment. Rather, reduced freezing during training is likely to reflect the anxiolytic-like effects of NPY. To our knowledge, present findings provide the first demonstration that NPY mediates conditioned fear behaviors.

Icv GAL treatment significantly reduced context freezing, but did not alter cued conditioning. However, these effects were seen at 0.5 nmol, but not 1.0 nmol GAL. GAL produced no confounding effects on pain perception as measured by the hot plate test. Specific deficits in contextual conditioning suggest an effect of GAL in the hippocampal formation, a brain region believed to be integral to context learning (LeDoux, 1998). This would be consistent with previous evidence that icv GAL impairs a hippocampal-dependent form of cued ("trace") fear conditioning in rats and mice (Kinney et al., 2002). However, because this earlier work did not reveal a significant impairment in standard contextual conditioning, present results await further replication.

In summary, the results of the present study confirm the potent anxiolytic-like effects of centrally-administered NPY, and extend these findings to mice. NPY administered icv produced robust anti-anxiety effects in two well-validated anxiety-related tasks. A novel finding was that NPY also impaired acquisition of learned fear behaviors in the cued and contextual fear conditioning paradigm. Contrariwise, icv GAL failed to alter anxiety-related behaviors at the doses tested. The absence of GAL effects provides further support to the hypothesis that GAL's effects on fear and anxiety-like behaviors are preferentially recruited under conditions of high or chronic stress. Together, these results provide a basis to elucidate the anatomical sites, neural pathways and receptor subtypes mediating the effects of these neuropeptides on emotionality, using genetically modified mice and other emerging techniques.

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