The neuropeptide Y Y1 receptor subtype is necessary for the anxiolytic-like effects of neuropeptide Y, but not the antidepressant-like effects of fluoxetine, in mice

Rose-Marie Karlsson · Jessica S. Choe ·
Heather A. Cameron · Annika Thorsell ·
Jacqueline N. Crawley · Andrew Holmes ·
Markus Heilig

Abstract

Rationale Neuropeptide Y (NPY) is implicated in the pathophysiology of affective illness. Multiple receptor subtypes (Y1R, Y2R, and Y5R) have been suggested to contribute to NPY's effects on rodent anxiety and depression-related behaviors.
Objectives To further elucidate the role of Y1R in (1) NPY's anxiolytic-like effects and (2) fluoxetine's antidepressant-like and neurogenesis-inducing effects.
Methods Mice lacking Y1R were assessed for spontaneous anxiety-like behavior (open field, elevated plus-maze, and light/dark exploration test) and Pavlovian fear conditioning, and for the anxiolytic-like effects of intracerebroventricularly (icv)-administrated NPY (elevated plus-maze). Next, Y1R −/− were assessed for the antidepressant-like effects of acute fluoxetine in the forced swim test and chronic fluoxetine in the novelty-induced hypophagia test, as well as for chronic fluoxetine-induced hippocampal neurogenesis.
Results Y1R −/− exhibited largely normal baseline behavior as compared to +/+ littermate controls. Intraventricular administration of NPY in Y1R −/− mice failed to produce the normal anxiolytic-like effect in the elevated plus-maze test seen in +/+ mice. Y1R mutant mice showed higher immobility in the forced swim test and longer latencies in the novelty-induced hypophagia test. In addition, Y1R −/− mice responded normally to the acute and chronic effects of fluoxetine treatment in the forced swim test and the novelty-induced hypophagia test, respectively, as well as increased neuronal precursor cell proliferation in the hippocampus.
Conclusions These data demonstrate that Y1R is necessary for the anxiolytic-like effects of icv NPY, but not for the antidepressant-like or neurogenesis-inducing effects of fluoxetine. The present study supports targeting Y1R as a novel therapeutic target for anxiety disorders.

Keywords Neuropeptide Y · Y1 · Receptor · Knockout · Mouse · Fear · Anxiety · Depression · Neurogenesis

R.-M. Karlsson (✉) · A. Thorsell · M. Heilig
Laboratory of Clinical and Translational Studies, NIH,
National Institute of Alcohol Abuse and Alcoholism, NIH,
10 Center Drive, 1-15330, Bethesda, MD 20892-1375, USA
e-mail: karlssonr@mail.nih.gov

R.-M. Karlsson · M. Heilig
Section of Psychiatry, Department of Clinical Neuroscience,
Karolinska Institute, Stockholm, Sweden

R.-M. Karlsson · A. Holmes
Section of Behavioral Science and Genetics,
Laboratory of Integrative Neuroscience,
National Institute of Alcohol Abuse and Alcoholism, NIH,
Bethesda, MD, USA

J. S. Choe · H. A. Cameron
Unit of Neuroplasticity, Mood and Anxiety Disorders Program,
National Institute of Mental Health, NIH,
Bethesda, MD, USA

J. N. Crawley
Laboratory of Behavioral Neuroscience,
National Institute of Mental Health, NIH,
Bethesda, MD, USA

Introduction

Neuropeptide Y (NPY) is a highly conserved 36 amino acid neuropeptide, abundantly expressed in the central nervous sys-

tem of rats, mice, and humans (De Quidt and Emson 1986a, b). NPY acts as a major neuromodulator with important roles in various physiological functions including cardiovascular regulation, ingestive behavior, nociception, neuronal excitability, and cognition (Kask et al. 2002). Both human and animal studies also implicate NPY in the pathophysiology of affective illnesses such as anxiety disorders and depression (Redrobe et al. 2002b; Thorsell et al. 2006). For example, abnormally low levels of NPY in plasma and cerebrospinal fluid (CSF) have been found in patients with depression and anxiety disorders (Heilig et al. 2004; Rasmusson et al. 2000). Mutant mice lacking NPY show increased anxiety-like behavior on various tests (Bannon et al. 2000; Heilig et al. 2004) while, conversely, intracerebral administration of NPY or transgenic overexpression of NPY decreases measures of anxiety- and depression-like behavior in rats and mice (Broqua et al. 1995; Heilig et al. 1989; Karlsson et al. 2005; Thorsell et al. 2000). Together, these findings have stimulated considerable interest in the potential of targeting the NPY system as a novel treatment approach for affective illness (Heilig 2004; Holmes et al. 2003a).

NPY mediates its effects in brain via diverse receptor subtypes, all of which belong to the superfamily of G-protein coupled, seven transmembrane spanning domain receptors but differ in their ligand affinity profiles (Cabrele and Beck-Sickinger 2000; Wan and Lau 1995). NPY receptor subtypes, particularly Y1R, Y2R, and Y5R, exhibit dense and overlapping gene expression in brain regions implicated in anxiety and depression including the hippocampus, bed nucleus of stria terminalis, amygdala, and prefrontal cortex (Parker and Herzog 1999). Y5R and Y1R show similar patterns of expression in rat brain, although Y1R mRNA is expressed in some additional neuronal populations, while the profile of Y2R mRNA neural expression is distinct from that of Y1R and Y5R (Parker and Herzog 1999). These neuroanatomical profiles suggest that these receptor subtypes subserve critical, but potentially dissociable functional roles in mediating NPY's effects on behaviors including anxiety and depression.

Previous studies using rodent models have examined the contribution of Y1R, Y2R, and Y5R in the mediation of NPY's anxiolytic- and antidepressant-like effects. For example, intracerebroventricular (icv) administration of the Y1 antagonist, BIBP 3226, or antisense oligonucleotide knockdown of Y1R increased anxiety-like behavior in the rat elevated plus-maze test (Kask et al. 1996; Wahlestedt et al. 1993). Conversely, icv treatment with either the selective Y1R agonist, [D-His^{26}]NPY, or the selective Y5R agonist, [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP, produced anxiolytic-like effects in the rat elevated plus-maze and open field test (Sorensen et al. 2004). Similarly, the Y1/Y5 agonist [Leu^{31}-Pro^{34}]PYY produced antidepressant-like effect in the rat forced swim test while antagonism of the Y1R, through icv BIBP

3226 and BIBO 3304, did not alter the behavior, though both these compounds blocked the antidepressant-like effects of NPY (Redrobe et al. 2002a). So far, studies on Y1R KO mice have demonstrated a somewhat inconsistent anxiety-like phenotype, which has been shown to be dependent on task and circadian rhythm (Karl et al. 2006) while targeted mutation of the Y2R gene in mice produce anxiolytic- and antidepressant-like (Carvajal et al. 2006; Redrobe et al. 2003; Tschenett et al. 2003). Pharmacological studies with Y2R agonists have shown a more diverse role depending on task and region (Heilig et al. 1989; Heilig 1995; Kask et al. 1998). For instance, intra-amygdala administration of Y2-preferring agonist NPY3-36 was anxiolytic-like in the social interaction test, and these effects were blocked by the Y5R selective antagonist, CGP71683A, but not the Y1R selective antagonist, BIBO 3304 (Sajdyk et al. 2002). Taken together, the available evidence supports a potential involvement of Y1R, Y2R, and Y5R in mediating NPY's anxiolytic-like effects, while data seem more conclusive for the antidepressant-like effects of NPY being mediated through Y1R.

It has been postulated that hippocampal neurogenesis mediates the antidepressant actions of chronic treatment with serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Duman 2004; Santarelli et al. 2003 but see David et al. 2007; Holick et al. 2007; Meshi et al. 2006). It is interesting to note that, in this context, recent studies have shown that (1) antidepressant-like effects of icv NPY are attenuated by depletion of brain serotonin (Redrobe et al. 2005), and (2) that NPY stimulates hippocampal neurogenesis, and may do so in a Y1R-dependent manner (Howell et al. 2003, 2005, 2007). This raises the question of whether NPY, through actions at Y1R, contributes to the antidepressant-like effects of chronic SSRI treatment.

In the present study, we used mice lacking Y1R to further elucidate the role of Y1R in mediation of anxiolytic-like effects of NPY, the antidepressant-like effects of acute and chronic fluoxetine treatment and the neurogenesis-inducing effects of chronic fluoxetine treatment. We first examined spontaneous anxiety-like behavior using three separate tests (novel open field, elevated plus-maze, light/dark exploration test) and Pavlovian fear conditioning to study the possible role of endogenous NPY signaling through Y1R for these behaviors. We then tested whether the anxiolytic-like effects produced by icv-administration of NPY were lost in mice lacking Y1R. Next, baseline behavior and responses to acute fluoxetine treatment was assessed in the forced swim test. We employed the novelty-induced hypophagia test, which has been validated for chronic fluoxetine treatment (Cryan and Holmes 2005; Dulawa et al. 2004) to test antidepressant-like effects of this treatment. Finally, hippocampal neurogenesis after chronic fluoxetine treatment was measured.

Material and methods

Subjects

Mice lacking Y1R mice were generated as previously described (Naveilhan et al. 2001b). Briefly, exon 2 of the Y1 gene was partially deleted and replaced by an IRES-tau-lacZ cassette containing a neomycin-resistance gene driven by the PGK promoter and polyA. The targeting sequence was introduced into 129Sv embryonic stem cells by homologous recombination and transplanted into BALB/c blastocysts. For the current study, the mutation was backcrossed into C57BL/6 for seven generations. To avoid potential phenotypic abnormalities resulting from genotypic differences in maternal behavior and early life environment (Holmes et al. 2005), homozygous null mutant (Y1R −/−), heterozygous null mutant (Y1R +/−) and wild-type (+/+) mice were all generated from +/− × +/− matings. Both males and females were tested, unless otherwise indicated.

Mice were housed in groups of one to four per cage in a temperature and humidity controlled vivarium, under 12-h light/dark cycle (lights on 0600 h) and had ad libitum access to food and water in the home cage. Of the 281 mice used in the study, 11 +/+ and 13 −/− mice were single housed after weaning and throughout the study. Additional single housing has been specified further when applied. Testing and surgery commenced when mice were at least 8 weeks of age. On test days, mice were taken to the experimental room to acclimate for at least 1 h. The number of mice used for each experiment is given in the figure and table legends. Behavioral testing equipment was cleaned with 70% ethanol (v/v) solution between subjects where appropriate. The experimenter remained blind to the genotype during testing: mice were identified by subcutaneously implanted microchips (AVID MicroChip I.D., Folsom, LA, USA) or tail tattoo. All experimental procedures were approved by the National Institute of Alcohol Abuse and Alcoholism Animal Care and Use Committee and followed the NIH guidelines "Using Animals in Intramural Research".

Functional observation battery and spontaneous anxiety-like behaviors

Behavioral assessment was conducted in the following order, with at least 1 week between tests and the putatively more stressful tests later in the sequence: functional observation battery, novel open field, elevated plus-maze, and light/dark exploration test. Tests were performed as previously described (Boyce-Rustay and Holmes 2006). To exclude possible gross behavioral confounds affecting fear-, anxiety-, and depression-related behaviors, Y1R −/− mice were first evaluated for empty cage behaviors, physical health,

sensory, and neurological functions as previously described (Boyce-Rustay and Holmes 2006; Crawley 2007). Briefly, in the novel open field test the mouse was allowed to freely explore the apparatus (50 lux) for 15 min. Total distance traveled in the arena and time spent in the center (20×20 cm) was measured by the Ethovision videotracking system (Noldus Information Technology, Leesburg, VA, USA). In the elevated plus-maze, the mouse was placed in the center facing an open arm (90 lux) and allowed to freely explore the apparatus for 5 min. Time spent in the open arms and entries into the open and closed arms (arm entry was defined as all four paws into arm) were measured by the Ethovision videotracking system (Noldus Information Technology, Leesburg, VA, USA). In the light/dark exploration test (Boyce-Rustay and Holmes 2006; Crawley 1981), the mouse was placed into the dark compartment facing away from the partition, and allowed to freely explore the apparatus for 10 min. Time spent and full-body transitions between the light (40 lux) and dark compartment were measured by photocells connected to Med Associates software (Med Associates, Georgia, VT, USA).

Pavlovian fear conditioning

One week after testing in the light/dark exploration test, mice were assessed for cued and contextual fear conditioning as previously described (Hefner et al. 2007; Karlsson et al. 2005; Kim and Fanselow 1992). Briefly, the test was conducted in a Freeze Monitor system (San Diego Instruments, San Diego, CA, USA) scented with a 79.5% water/ 19.5% EtOH/1% vanilla extract solution, delivered a 30-s 80-dB tone and a 0.6-mA 2-s footshock. After a 120-s no-stimulus period, the mouse received three pairings of the tone and shock with the shock co-terminating with the tone, followed a 120-s no-stimulus period. Twenty-four hours later, tone-recall was tested in a novel environment and 48 h after conditioning context-recall was tested by placing the mouse in the training context. Freezing behavior was scored every 10 s, defined as the absence of any movement except respiration (Kim and Fanselow 1992). Data were calculated as the proportion of observations scored as freezing.

Anxiolytic-like effects of icv NPY

An experimentally naïve cohort of mice was tested for icv-administered effects of NPY in the open field and elevated plus-maze tests as previously described (Karlsson et al. 2005). Briefly, a 22-gauge stainless-steel cannula (Plastics One, Roanoke, VA, USA) was implanted using Stereotaxic Alignment System, SAS75 (Kopf, Tujunga, CA, USA) into the lateral ventricle, at coordinates 1.00 mm lateral, −0.05 mm anterior to bregma, and −2.60 mm ventral to the skull surface

(Paxinos and Franklin 2001). Mice were single housed post-surgery to prevent cage mate interference with the cannula and allowed 7 days to recover during which they were handled and injection-habituated for 3 days.

Vehicle (0), 0.5 or 1.0 nmol NPY was administered 15 min before testing via a stainless-steel injector (Plastics One, Roanoke, VA, USA), projecting 1 mm ventral from the tip of the guide cannula. Mice were tested on the novel open field, and 7 days later, the elevated plus-maze, as above. At the completion of testing, mice were euthanized via cervical dislocation and rapid decapitation to confirm cannula placement. Brains were immediately removed and placed in a 10% buffered neutral formalin solution (VWR International, West Chester, PA, USA). Tissue was sectioned at 50 μm, mounted on Superfrost/Plus Microslides (A. Daigger & Company, Vernin Hills, IL, USA). Sections were examined under stereoscopic light microscope to verify cannula placement: Five mice, which showed no clear cannula track into the lateral ventricle, were removed from the study.

Baseline behavior and response to acute fluoxetine in the forced swim test

Two weeks after fear conditioning, mice were assessed for baseline behavior in the forced swim test, conducted as previously described (Holmes et al. 2002; Porsolt et al. 1978). The apparatus was a transparent Plexiglas cylinder (20-cm diameter) filled halfway with water (24±1°C). The mouse was gently lowered into the water for 15 min on day 1 and 6 min on day 2. The presence/absence of immobility (cessation of limb movements except minor involuntary movements of the hind limb) was measured every 5 s during the last 4 min on day 2.

An experimentally-naïve cohort of Y1R −/− and +/+ mice was tested for antidepressant-related effects of acute fluoxetine treatment. Mice were injected intraperitoneally (ip) with vehicle (0), 7.5 or 15 mg/kg fluoxetine 30 min before testing on day 2, using the same procedure as for baseline testing.

Response to chronic fluoxetine in the novelty-induced hypophagia test

An experimentally naïve cohort of Y1R −/− and +/+ male mice was tested for antidepressant-related effects of chronic fluoxetine treatment. We employed the novelty-induced hypophagia test based on recent studies validating this test as an assay for chronic fluoxetine treatment (Dulawa et al. 2004). Mice were treated with fluoxetine (in drinking water) or tap water for 28 days. Because body weight differences between some cage mates necessitated administration of different concentrations of fluoxetine, 1 +/+ and 7 −/− were

single housed for treatment and testing. On day 22 of treatment mice were acclimated to a home cage for 2 days, and then presented with diluted sweetened condensed milk (1:3; milk/water) in 10 ml serological pipettes for 30 min each day for four consecutive days. Mice that did not drink were excluded. Drinking on the fourth day was taken as a measure of baseline drinking. Twenty-four hours later, mice were presented with milk in a novel, clean cage under 120 lux. The latency to begin drinking from the pipette (or from the floor if the milk dripped) was measured, with a 600-s maximum cutoff latency. Latencies greater than two standard deviations were excluded from the analysis.

Hippocampal neurogenesis after chronic fluoxetine

Twenty-four hours after novelty-induced hypophagia testing, mice were injected ip with 200 mg/kg body weight of the cell division marker bromodeoxyuridine (BrdU). Two hours later, mice were transcardially perfused with 4% paraformaldehyde (pH 7.4) under deep isoflurane anesthesia. Brains were removed, postfixed in 4% paraformaldehyde (pH 7.4) overnight, and cryoprotected in 20% sucrose. Coronal 40-μm sections through the entire hippocampus were cut on a sliding microtome. Series of every 12th section were mounted on slides and processed for BrdU immunohistochemistry using monoclonal anti-mouse anti-BrdU antibody (1:100, Becton-Dickinson, BD Biosciences, San Jose, CA, USA), biotinylated goat anti-mouse IgG (1:200, Sigma, St. Louis, MO, USA), avidin–biotin–horseradish peroxidase complex (Vector Labs, Vector Laboratories, Burlingame, CA, USA), and cobalt-enhanced DAB (Sigma Fast Tabs), according to a previously published protocol (Dayer et al. 2003). Sections were then counterstained using cresyl violet and coverslipped under Permount (Fisher Scientific, Pittsburgh, PA, USA). BrdU-labeled cells in the granule cell layer and hilus were counted at 400× on coded slides. Stereological counts were calculated by multiplying the total number of cells in the 1:12 series by 12. Only those mice providing behavioral data in the novelty-induced hypophagia test were analyzed for neurogenesis to permit comparison between the measures.

Drugs

NPY (American Peptide, Sunnyvale, CA, USA) was dissolved in deionized water and administered icv in a volume of 0.5 μl. Doses and injection-test interval was chosen based on our previous finding that these doses and this procedure produces robust anxiolytic-like effects in C57BL/6J mice (Karlsson et al. 2005). Fluoxetine hydrochloride (LKT Laboratories, St. Paul, MN, USA) was dissolved in 0.9% saline and injected in a volume of 10 ml/kg body weight for the acute experiment. Doses were chosen on the basis of previous data in mice

(Holmes et al. 2002). For chronic treatment, fluoxetine was made available ad libitum in the drinking water in bottles covered with aluminum foil to protect the drug from light. Drug concentration was determined from average daily water consumption and average body weight for each genotype to achieve the desired dose of 10 mg/kg/day (i.e., +/+= 100 mg/l, −/−=135 mg/l), which produces brain concentrations in the clinical range (Dulawa et al. 2004). 5′-bromo-2′-deoxyuridine (BrdU, 10 mg/ml in 0.007 N NaOH/0.9% saline) (Roche, Nutley, NJ, USA) was injected ip at a dose of 200 mg/kg body weight, based on previous studies in rats (Cameron and McKay 2001).

Statistical analysis

All datasets were checked for homogeneity of variance prior to parametric 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 StatView (SAS Institute, Cary, NC, USA). The threshold for statistical significance was set as $p<0.05$.

Results

Functional observation battery

As shown in Table 1, genotypes did not differ on various measures of physical health, neurological and sensory function, and gross behavioral parameters in the functional observation battery, with the exception that body weight was higher in Y1R −/− mice than +/+ (main effect of genotype: $F_{2,72}=3.31$, $p<0.05$).

Spontaneous anxiety-like behaviors

Novel open field

There was a significant effect of genotype for total distance traveled in the open field ($F_{2,72}=4.38$, $p<0.05$; Fig. 1a), but not percent time spent in center (Fig. 1b). Given the body weight difference between genotypes, distance traveled was reanalyzed with body weight as a covariate. Results showed that body weight significantly co-varied with distance traveled and that there was no longer an effect of genotype.

Elevated plus-maze

There was no significant effect of genotype for percent open arm time (Fig. 1c), percent open entries (Fig. 1d) or total entries (data not shown) in the plus-maze.

Table 1 Mice lacking Y1R show normal empty cage behaviors, physical health, sensory reflexes, and neurological functions

	+/+	+/−	−/−
Empty cage behaviors			
Freezing	0	0	0
Trembling	0	0	0
Sniffing	100	100	100
Licking	0	0	0
Rearing	100	100	100
Jumping	13	0	0
Seizure	0	0	0
Defecation	36	48	62
Urination	4	10	0
Head bobbing	0	0	0
Circling	0	0	0
Abnormal gait	0	0	0
Retropulsion	0	0	0
Physical health			
Missing whiskers	56	76	67
Bald patches	8	21	5
Exophthalmus	0	0	0
Straub tail	0	0	0
Kinked tail	0	0	0
Kyphosis	0	0	0
Lordosis	0	0	0
Body weight (g)	24.8±0.7	24.8±1.0	28.0±1.2*
Sensory reflexes			
Approach responses	100	100	100
Touch responses	100	100	100
Palpebral responses	100	97	100
Pinna reflex	100	97	100
Tail pinch response	28	17	29
Motor, neurological			
Splayed limbs	0	0	0
Forepaw clutch	0	0	0
Hindpaw clutch	0	0	0

Data are the percent number of animals observed, unless otherwise indicated in parenthesis. $n=21–29$/genotype.
*$p<0.05$ vs +/+

Light/dark exploration test

There was no significant effect of genotype for percent time spent in the light compartment (Fig. 1e) or light–dark transitions (Fig. 1f) in the light/dark test.

Pavlovian fear conditioning

There was no significant effect of genotype for percent freezing during conditioning (+/+: 24.0±8.0, +/−:29.3±6.7, −/−: 22.5±4.3), the auditory cue test (+/+: 37.1±8.3, +/−: 37.0±4.9, −/−: 52.3±6.4), or the context test (+/+: 8.5±1.9, +/−: 11.6±2.4, −/−:14.9±3.3).

Fig. 1 Spontaneous anxiety-related behaviors in mice lacking Y1R. Y1R −/− mice were less active than +/+ controls in the novel open field (**a**) but showed normal center time (**b**). Genotypes did not differ in percent open time (**c**) or percent open arm entries (**d**) in the elevated plus-maze test, or percent time spent in the light compartment (**e**) or light–dark compartment transitions (**f**) in the light/dark exploration test. $n=21$–25/genotype. ** $p<0.01$ vs +/+. Data in Figs 1, 2, 3 and 4 are means±SEM
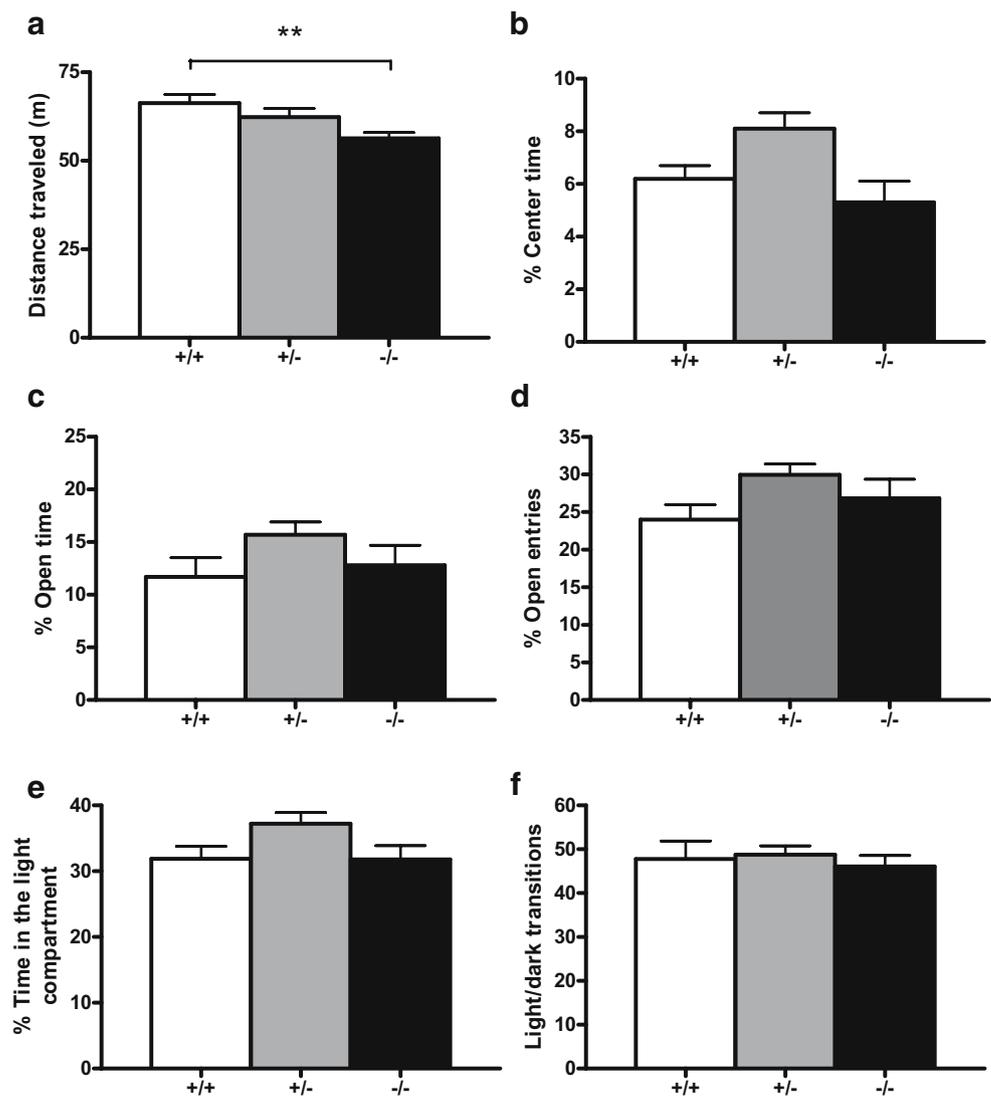
Anxiolytic-like effects of icv NPY

In the plus-maze, there was a significant effect of NPY on percent open arm time ($F_{2,82}=3.96$, $p<0.05$) and open arm entries ($F_{2,82}=4.28$, $p<0.05$) and a significant genotype × NPY interaction for percent open arm time ($F_{2,82}=3.51$, $p<0.05$) and open arm entries ($F_{2,82}=4.72$, $p<0.05$). 0.5 and 1 nmol doses of NPY increased percent open arm time (Fig. 2a) and open arm entries (Fig. 2b) in +/+ but not −/− mice. There were no main effects of genotype or NPY treatment or interactions for total arm entries (data not shown).

In the novel open field test, there was a significant main effect of genotype, but not of NPY and no genotype x NPY interaction for total distance traveled ($F_{1,84}=20.51$,
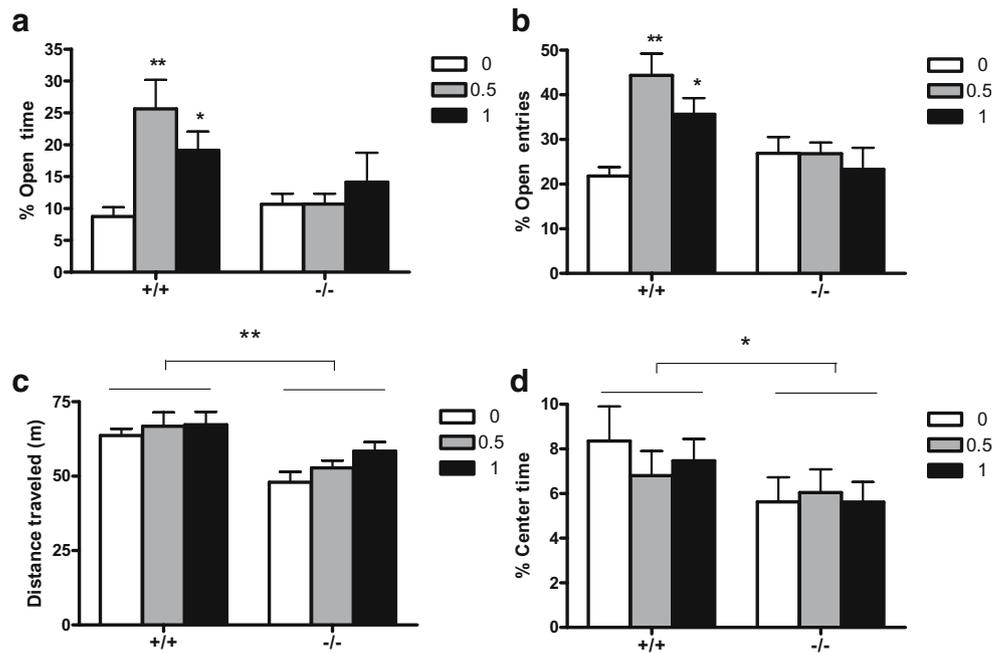
$p<0.01$, Fig. 2c) or percent center time ($F_{1,84}=3.61$, $p<0.05$, Fig. 2d).

Baseline behavior and response to acute fluoxetine in the forced swim test

There was a significant effect of genotype for percent immobility, due to greater immobility in Y1R−/− mice compared to +/+ controls ($F_{2,71}=6.92$, $p<0.01$; Fig. 3a).

After acute fluoxetine treatment there was a significant effect of genotype ($F_{1,66}=8.27$, $p<0.01$) and fluoxetine treatment ($F_{2,66}=4.46$, $p<0.05$) but no genotype × treatment interaction. Y1R −/− mice showed more immobility than +/+, while 15 mg/kg fluoxetine reduced immobility relative to vehicle, regardless of genotype (Fig. 3b).

Fig. 2 Anxiety-related effects of NPY in mice lacking Y1R. Intracerebroventricularly administered NPY significantly increased percent open arm time (a) and percent open arm entries (b) in +/+ mice treated, but not Y1R −/− mice. NPY did not alter total distance traveled (c) or percent center time (d) in the open field regardless of genotype. $n=13–17$/genotype per treatment. ** $p<0.01$, * $p<0.05$ vs +/+

Fig. 3 Baseline forced swim test behavior and antidepressant-related responses to acute treatment in mice lacking Y1R. Untreated Y1R −/− mice showed more immobility than +/+ controls in the forced swim test ($n=21–25$/genotype) (a). Acute treatment with fluoxetine decreased immobility in Y1R −/− mice and +/+ controls ($n=9–18$/genotype/dose) (b). ** $p<0.01$, * $p<0.05$ vs +/+

Response to chronic fluoxetine in the novelty-induced hypophagia test
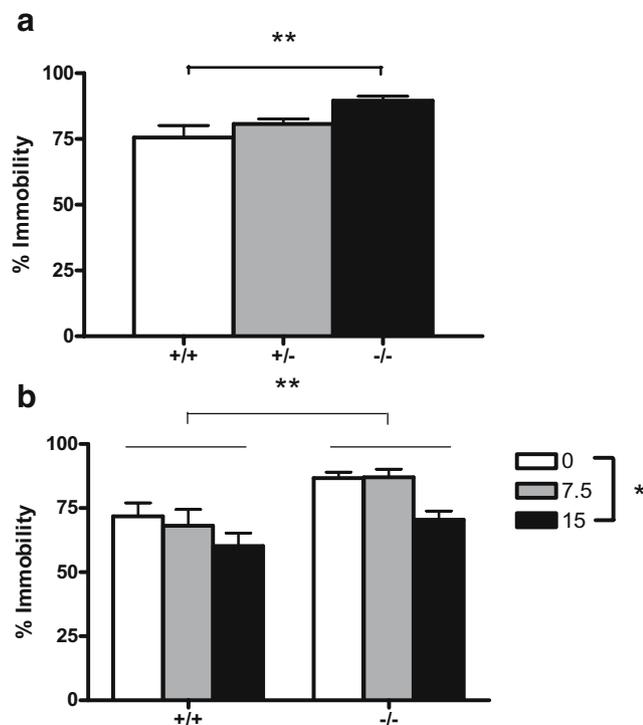
There was a significant effect of genotype ($F_{1,22}=6.15$, $p<0.05$) and fluoxetine treatment ($F_{1,22}=10.04$, $p<0.01$) but no genotype x treatment interaction for latency to drink in the novel cage. Fluoxetine decreased the latency to drink regardless of genotype, while Y1R −/− mice generally showed a longer latency than +/+ controls (Fig. 4a). Latency to drink in the home cage test was not altered by either genotype or treatment (Fig. 4a).

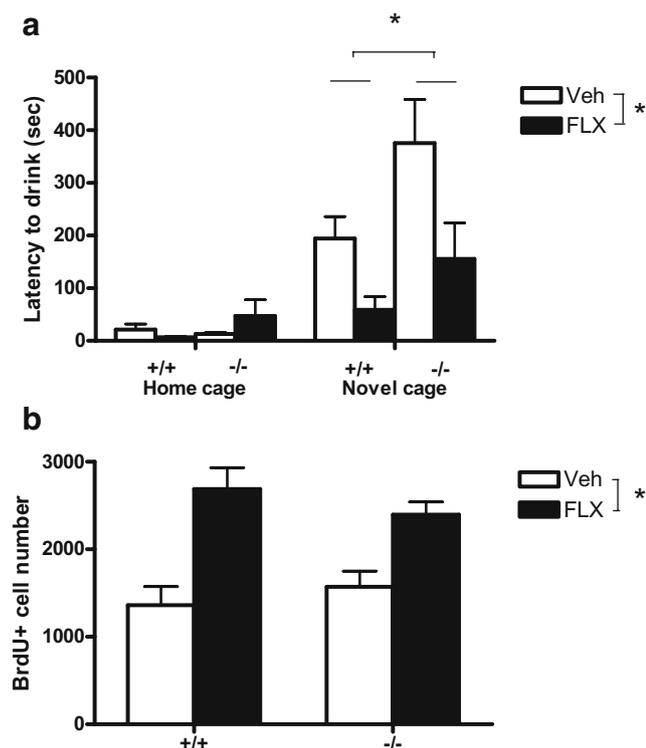## Hippocampal neurogenesis following chronic fluoxetine

There was a significant main effect of fluoxetine treatment ($F_{1,20}=19.37$, $p<0.001$) but not genotype and no genotype × treatment interaction for the number of BrdU-labeled cells measured in the dentate gyrus 2 h after BrdU injection (Fig. 4b). Fluoxetine treatment increased the number of BrdU-positive cells relative to vehicle regardless of genotype.

Discussion

The main findings of the present study were: First, mice lacking the NPY Y1R receptor subtype were largely normal on tests for spontaneous anxiety-related behavior, but failed to respond to the anxiolytic-like effects of NPY administered into the lateral ventricle as tested in the elevated plus-maze test. Second, mice lacking Y1R showed modestly

Fig. 4 Antidepressant-related responses to chronic fluoxetine treatment in the novelty-induced feeding test and fluoxetine-induced hippocampal neurogenesis in mice lacking Y1R. Mice treated chronically with fluoxetine treated mice showed decreased latency to first drink in the novel but not home cage condition relative to vehicle-treated controls, regardless of genotype (**a**). Y1R −/− mice were generally slower to first drink than +/+ controls. Chronically fluoxetine treated mice showed more BrdU-positive cells in the hippocampus than vehicle treated controls, regardless of genotype (**b**). $n=5–7$/genotype * $p<0.05$ vs +/+

elevated baseline immobility in the forced swim test, and responded normally to the acute "antidepressant-like" effects of fluoxetine in this test and the chronic "antidepressant-like" effects of fluoxetine in the novelty-induced hypophagia test. The ability of chronic fluoxetine to increase hippocampal neurogenesis was also unaltered in Y1R −/− mice.

Early experiments using [Leu^{31},Pro^{34}]NPY, considered at the time to be a selective Y1R agonist, suggested that this receptor subtype mediates anxiolytic-like actions of NPY, a notion supported by observations that Y1R antagonism produced anxiogenic-like behaviors (Heilig 1995; Kask et al. 1996; Sajdyk et al. 1999; Wahlestedt et al. 1993). After the cloning of Y5R (Gerald et al. 1996), however, it became clear that [Leu^{31},Pro^{34}]NPY does not distinguish between Y1R and Y5R. A contribution of Y5R to the anxiolytic-like actions of NPY has therefore remained a possibility, and has received some support from subsequent pharmacological studies. For example, systemic administration of the Y5R preferring antagonist CGP71683A, while devoid of anxiety-related effects under baseline conditions, was reported to be

anxiogenic-like under stressful conditions (Kask et al. 2001). CGP71683A administration into the basolateral amygdala also blocked anxiolytic-like effects of the mixed Y2R/Y5R agonist NPY3-36 (Sajdyk et al. 2002). Most recently, based on icv administration of a novel Y5R agonist, [cPP]hPP, it was proposed that anxiolytic-like effects of central NPY are mediated via both Y1R and Y5R, whereas sedation is mediated via Y5R (Sorensen et al. 2004). These pharmacological data do not appear consistent with studies in mutant mice, which found that sedative properties of high NPY doses were lost in Y1R KO mice (Naveilhan et al. 2001a).

Pharmacological data on the respective role of Y1R and Y5R in mediating anxiolytic-like actions have until now lacked confirmation from receptor mutants. As shown by the original [Leu^{31},Pro^{34}]NPY findings and more recent work with a ligand, GW438014A, putatively selective for Y5R (El Bahh et al. 2005), selectivity of pharmacological NPY receptor tools remains relative and incompletely understood. Similar to the prior study on mediation of sedative NPY actions (Naveilhan et al. 2001a), present data using Y1R −/− mice circumvent issues related to specificity and potential off-target actions of available pharmacological tools. Our finding that NPY's anxiolytic-like effects in the elevated plus-maze were lost in Y1R −/− mice therefore offers the most compelling evidence to date that this subtype is required for anxiolytic-like actions of NPY. The lack of behavioral effects of NPY in the open field test is consistent with previous data from our laboratory (Karlsson et al. 2005) and excludes nonspecific motor effects of NPY as a confounding influence in the plus-maze. This dissociation also suggests that, compared to the elevated plus-maze, the novel open field test is a relatively insensitive measure of anxiolytic-like effects of NPY, perhaps due to the latter test being relatively unstressful (Holmes et al. 2003b) (see also discussion below regarding stress recruitment of NPY).

While these data provide strong evidence that Y1R is necessary for central NPY to exert its anxiolytic-like effects, two potential caveats should be considered. First, the Y5R gene locus has been thought to overlap, in reverse orientation, with that of the Y1R gene, raising concerns that a mutation targeting the latter might also inactivate the former. This would clearly invalidate the posited Y1R specificity of the present dataset. However, recent resequencing data from 16 inbred mouse lines (http://mouse.perlegen.com/mouse/browser.html) clearly show that the two loci are separated by at least 16 kb, and the targeted mutation of exon 2 within the Y1R locus used in this study (Naveilhan et al. 2001a) does not disrupt the Y5R locus. It could be argued that, even in the absence of a Y5R locus disruption, the Y1R deletion could lead to neighborhood effects that quantitatively alter Y5R expression. However, GPCR systems typically have a large receptor reserve, and a near-complete loss of a receptor is required for major functional effects to be observed. There-

fore, in our view, the loss of anxiolytic-like NPY actions in Y1R −/− is most parsimoniously explained by Y1R mediation of these actions.

While present data demonstrate that Y1R is necessary for the anxiolytic-like actions of exogenous NPY, constitutive loss of Y1R did not affect spontaneous anxiety-related behaviors or fear learning, as some previous studies have indicated (Karl et al. 2004, 2006). The Y1R mutants did exhibit lower locomotor activity; these findings were related to increased body weight, previously reported in Y1R −/− (Pedrazzini et al. 1998; but see Karl et al. (2006)). The lack of spontaneous fear- or anxiety-like phenotypes observed here could conceivably be a false negative result caused by a relatively high level of anxiety-like behavior in +/+ control mice under our test conditions, creating a ceiling effect. A more likely explanation is that the endogenous NPY systems involved in regulation of emotionality are quiescent under our conditions for testing spontaneous anxiety-like behavior. Previous observations of altered anxiety-like behavior in Y1R −/− mice were shown to be highly dependent on task, time of testing within the circadian cycle, and prior stress (Karl et al. 2006). Central neuropeptide systems have been hypothesized to acts as "alarm systems" that are preferentially activated under conditions of high neuronal activity, e.g., high fear or chronic stress (Hokfelt et al. 1987; Karlsson and Holmes 2006). Also of note in this context is the finding that the genetic background used here, C57BL/6, has low levels of endogenous NPY in brain regions rich in Y1R (Hayes et al. 2005; Parker and Herzog 1999). Therefore, the release of endogenous NPY under mild test provocation may be particularly low in this genetic background, leading to low Y1R recruitment and negligible consequences of Y1R deletion.

A previous study found that depletion of serotonin attenuated the antidepressant-like behavior of NPY in the forced swim test and as well as significant increased in Y1R-, but only moderate elevation of the Y5R- (but not Y2R), binding sites at high-affinity state (Redrobe et al. 2002a), suggesting a relationship between serotonin and Y1R in mediating depression-related behavior in this test. In two tests of "depression-related" behaviors, mice lacking Y1R showed normal baseline phenotypic abnormalities. Specifically, Y1R −/− mice exhibited elevated immobility in the forced swim test and longer latencies to drink in the novelty-induced hypophagia test as compared to +/+ controls. These tests are validated for their sensitivity to the acute and chronic effects of antidepressants such as fluoxetine, respectively (Cryan and Holmes 2005; Dulawa et al. 2004), and to that extent suggest an increases in "depression-related" behavior in Y1R −/− mice. This effect of Y1R gene deletion is consistent with pharmacological studies showing antidepressant-like effects of NPY can be mimicked by the Y1R/Y5R agonist [Leu^{31}Pro^{34}]PYY and blocked by the Y1R

antagonists, BIBP3226 and BIBO3304 (Ishida et al. 2007; Redrobe et al. 2002a). A further novel finding was that the antidepressant-like effects of acute fluoxetine treatment in the forced swim test, and chronic fluoxetine in the novelty-induced hypophagia test were present in Y1R −/− mice. It should be noted that while novelty-induced hypophagia test has been validated for sensitivity to chronic, but not subchronic, antidepressant treatment it is also sensitive to acute administration of anxiolytics such as benzodiazepines (Bodnoff et al. 1988). Therefore, although we interpret the behavioral effects of chronic fluoxetine in Y1R −/− mice primarily as evidence of intact sensitivity to the antidepressant properties of the drug, we do not discount the possibility that these effects may also reflect an extant anxiolytic-like component in the Y1R −/− mice to chronic fluoxetine.

Hippocampal neurogenesis has been proposed as a necessary mechanistic step underlying the behavioral effects of antidepressants including fluoxetine (Santarelli et al. 2003). While Howell et al. (2005, 2007) recently demonstrated a possible requirement of NPY and Y1R in hippocampal neurogenesis both in vitro and in vivo, with a decrease in cell proliferation in the Y1R −/− mice our present data detected no effect of loss of Y1R on baseline cell proliferation or on the increase in granule cell precursor proliferation produced by chronic fluoxetine treatment in vivo. The apparent discrepancy between studies is somewhat puzzling. One salient factor may again be genetic background. In contrast to the C57BL/6 background used in this study, studies reporting changes in cell proliferation used a mixed C57BL/6 × 129SvJ background (Howell et al. 2005, 2007; Karl et al. 2006). Hippocampal cell proliferation is greater in C57BL/6 mice compared to 129Sv/J (Kempermann et al. 1997) and it is possible that an epistatic interaction with C57BL/6 background genes negated the Y1R effect. The recent resequencing of the mouse genome cited above has identified considerable genetic variation between inbred mouse lines at the NPY locus. Several single nucleotide polymorphisms (SNPs) are located in the promoter region, first intron and 3′-UTR region, respectively, all of which might contribute to differential preproNPY expression between lines as a substrate for the variability in neurogenesis across backgrounds (Kempermann et al. 1997). More generally, it should be noted that recent work has found that fluoxetine alters behavior in the forced swim and novelty-induced hypophagia tests in a manner independent of neurogenesis (Holick et al. 2007). Similarly, behavioral effects of environmental enrichment (Meshi et al. 2006) and the melatonin concentrating hormone receptor antagonist SNAP 94847 (David et al. 2007) have also been shown to be neurogenesis-independent. Thus, the interrelationship between hippocampal neurogenesis and antidepressant-related behavioral effects remains to be fully elucidated, and further studies will be needed to clarify the role of Y1R in neurogenesis.

In summary, using NPY Y1R null mutant mice, we show that the Y1R subtype is necessary for the anxiolytic-like effects of icv-administered NPY, but not for the antidepressant-like or neurogenesis-stimulating effects of fluoxetine. These data reinforce and extend the understanding of the Y1R subtype for the neuropeptide Y neurotransmission, and the Y1R as a potential target for novel anxiolytic medication.

References

Bannon AW, Seda J, Carmouche M, Francis JM, Norman MH, Karbon B, Mccaleb ML (2000) Behavioral characterization of neuropeptide Y knockout mice. Brain Res 868:79–87

Bodnoff SR, Suranyi-Cadotte B, Aitken DH, Quirion R, Meaney MJ (1988) The effects of chronic antidepressant treatment in an animal model of anxiety. Psychopharmacology (Berl) 95:298–302

Boyce-Rustay JM, Holmes A (2006) Genetic inactivation of the NMDA receptor NR2A subunit has anxiolytic- and antidepressant-like effects in mice. Neuropsychopharmacology 31:2405–2414

Broqua P, Wettstein JG, Rocher MN, Gauthier-Martin B, Junien JL (1995) Behavioral effects of neuropeptide receptor agonists in the elevated plus-maze and fear-potentiated startle procedure. Behav Pharmacol 6:215–222

Cabrele C, Beck-Sickinger AG (2000) Molecular characterization of the ligand-receptor interaction of the neuropeptide Y family. J Pept Sci 6:97–122

Cameron HA, McKay RD (2001) Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 435:406–417

Carvajal C, Dumont Y, Herzog H, Quirion R (2006) Emotional behavior in aged neuropeptide Y (NPY) Y2 knockout mice. J Mol Neurosci 28:239–245

Crawley JN (1981) Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol Biochem Behav 15:695–699

Crawley J (2007) What's wrong with my mouse? Behavioral phenotyping of transgenic and knockout mice, 2nd edn. Wiley, Hoboken, NJ

Cryan JF, Holmes A (2005) The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4:775–790

David DJ, Klemenhagen KC, Holick KA, Saxe MD, Mendez I, Santarelli L, Craig DA, Zhong H, Swanson CJ, Hegde LG, Ping XI, Dong D, Marzabadi MR, Gerald CP, Hen R (2007) Efficacy of the MCHR1 antagonist N-[3-(1-{[4-(3,4-difluorophenoxy)phenyl]methyl}(4-piperidyl))-4-methylphen yl]-2-methylpropanamide (SNAP 94847) in mouse models of anxiety and depression following acute and chronic administration is independent of hippocampal neurogenesis. J Pharmacol Exp Ther 321:237–248

Dayer AG, Ford AA, Cleaver KM, Yassaee M, Cameron HA (2003) Short-term and long-term survival of new neurons in the rat dentate gyrus. J Comp Neurol 460:563–572

De Quidt ME, Emson PC (1986a) Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system—I. Radio-immunoassay and chromatographic characterisation. Neuroscience 18:527–543

De Quidt ME, Emson PC (1986b) Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system—II. Immunohistochemical analysis. Neuroscience 18:545–618

Dulawa SC, Holick KA, Gundersen B, Hen R (2004) Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology 29:1321–1330

Duman RS (2004) Depression: a case of neuronal life and death? Biol Psychiatry 56:140–145

El Bahh B, Balosso S, Hamilton T, Herzog H, Beck-Sickinger AG, Sperk G, Gehlert DR, Vezzani A, Colmers WF (2005) The anti-epileptic actions of neuropeptide Y in the hippocampus are mediated by Y and not Y receptors. Euro J Neurosci 22:1417–1430

Gerald C, Walker MW, Criscione L, Gustafson EL, Batzlhartmann C, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Whitebread S, Hofbauer KG, Taber RI, Branchek TA, Weinshank RL (1996) A receptor subtype involved in neuropeptide-Y-induced food intake. Nature 382:168–171

Hayes DM, Knapp DJ, Breese GR, Thiele TE (2005) Comparison of basal neuropeptide Y and corticotropin releasing factor levels between the high ethanol drinking C57BL/6J and low ethanol drinking DBA/2J inbred mouse strains. Alcohol Clin Exp Res 29:721–729

Hefner K, Cameron HA, Karlsson RM, Holmes A (2007) Short-term and long-term effects of postnatal exposure to an adult male in C57BL/6J mice. Behav Brain Res 182:344–348

Heilig M (1995) Antisense inhibition of neuropeptide Y (NPY)-Y1 receptor expression blocks the anxiolytic like action of NPY in amygdala and paradoxically increases feeding. Regul Pept 59:201–205

Heilig M (2004) The NPY system in stress, anxiety and depression. Neuropeptides 38:213–224

Heilig M, Söderpalm B, Engel JA, Widerlöv E (1989) Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. Psychopharmacology (Berl) 98:524–529

Heilig M, Zachrisson O, Thorsell A, Ehnvall A, Mottagui-Tabar S, Sjogren M, Asberg M, Ekman R, Wahlestedt C, Agren H (2004) Decreased cerebrospinal fluid neuropeptide Y (NPY) in patients with treatment refractory unipolar major depression: preliminary evidence for association with preproNPY gene polymorphism. J Psychiatr Res 38:113–121

Hokfelt T, Millhorn D, Seroogy K, Tsuruo Y, Ceccatelli S, Lindh B, Meister B, Melander T, Schalling M, Bartfai T (1987) Coexistence of peptides with classical neurotransmitters. Experientia 43:768–780

Holick KA, Lee DC, Hen R, Dulawa SC (2007) Behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the serotonin 1A receptor. Neuropsychopharmacology, Epub ahead of print

Holmes A, Yang RJ, Murphy DL, Crawley JN (2002) Evaluation of antidepressant-related behavioral responses in mice lacking the serotonin transporter. Neuropsychopharmacology 27:914–923

Holmes A, Heilig M, Rupniak NMJ, Steckler T, Griebel G (2003a) Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol Sci 24:580–588

Holmes A, Kinney JW, Wrenn CC, Li Q, Yang RJ, Ma L, Vishwanath J, Saavedra MC, Innerfield CE, Jacoby AS, Shine J, Iismaa TP, Crawley JN (2003b) Galanin GAL-R1 receptor null mutant mice display increased anxiety-like behavior specific to the elevated plus-maze. Neuropsychopharmacology 28:1031–1044

Holmes A, le Guisquet AM, Vogel E, Millstein RA, Leman S, Belzung C (2005) Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. Neurosci Biobehav Rev 29:1335–1346

Howell OW, Scharfman HE, Herzog H, Sundstrom LE, Beck-Sickinger A, Gray WP (2003) Neuropeptide Y is neuroproliferative for post-natal hippocampal precursor cells. J Neurochem 86:646–659

Howell OW, Doyle K, Goodman JH, Scharfman HE, Herzog H, Pringle A, Beck-Sickinger AG, Gray WP (2005) Neuropeptide Y stimulates neuronal precursor proliferation in the post-natal and adult dentate gyrus. J Neurochem 93:560–570

Howell OW, Silva S, Scharfman HE, Sosunov AA, Zaben M, Shatya A, McKhann G, Herzog H, Laskowski A, Gray WP (2007) Neuropeptide Y is important for basal and seizure-induced precursor cell proliferation in the hippocampus. Neurobiol Dis 26:174–188

Ishida H, Shirayama Y, Iwata M, Katayama S, Yamamoto A, Kawahara R, Nakagome K (2007) Infusion of neuropeptide Y into CA3 region of hippocampus produces antidepressant-like effect via Y1 receptor. Hippocampus 17:271–280

Karl T, Lin S, Schwarzer C, Sainsbury A, Couzens M, Wittmann W, Boey D, von HS, Herzog H (2004) Y1 receptors regulate aggressive behavior by modulating serotonin pathways. Proc Natl Acad Sci USA 101:12742–12747

Karl T, Burne TH, Herzog H (2006) Effect of Y1 receptor deficiency on motor activity, exploration, and anxiety. Behav Brain Res 167:87–93

Karlsson RM, Holmes A (2006) Galanin as a modulator of anxiety and depression and a therapeutic target for affective disease. Amino Acids 31:231–239

Karlsson RM, Holmes A, Heilig M, Crawley JN (2005) Anxiolytic-like actions of centrally-administered neuropeptide Y, but not galanin, in C57BL/6J mice. Pharmacol Biochem Behav 80:427–436

Kask A, Rago L, Harro J (1996) Anxiogenic-like effect of the neuropeptide Y Y-1 receptor antagonist BIBP3226—antagonism with diazepam. Euro J Pharmacol 317:R3–R4

Kask A, Rago L, Harro J (1998) Anxiolytic-like effect of neuropeptide Y (NPY) and NPY13-36 microinjected into vicinity of locus coeruleus in rats. Brain Res 788:345–348

Kask A, Vasar E, Heidmets LT, Allikmets L, Wikberg JE (2001) Neuropeptide Y Y(5) receptor antagonist CGP71683A: the effects on food intake and anxiety-related behavior in the rat. Eur J Pharmacol 414:215–224

Kask A, Harro J, von Horsten S, Redrobe JP, Dumont Y, Quirion R (2002) The neurocircuitry and receptor subtypes mediating anxiolytic-like effects of neuropeptide Y. Neurosci Biobehav Rev 26:259–283

Kempermann G, Kuhn HG, Gage FH (1997) Genetic influence on neurogenesis in the dentate gyrus of adult mice. Proc Natl Acad Sci USA 94:10409–10414

Kim JJ, Fanselow MS (1992) Modality-specific retrograde amnesia of fear. Science 256:675–677

Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L, Malapani C, Moore H, Hen R (2006) Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. Nat Neurosci 9:729–731

Naveilhan P, Canals JM, Valjakka A, Vartiainen J, Arenas E, Ernfors P (2001a) Neuropeptide Y alters sedation through a hypothalamic Y1-mediated mechanism. Eur J Neurosci 13:2241–2246

Naveilhan P, Hassani H, Lucas G, Blakeman KH, Hao JX, Xu XJ, Wiesenfeld-Hallin Z, Thoren P, Ernfors P (2001b) Reduced antinociception and plasma extravasation in mice lacking a neuropeptide Y receptor. Nature 409:513–517

Parker RM, Herzog H (1999) Regional distribution of Y-receptor subtype mRNAs in rat brain. Eur J Neurosci 11:1431–1448

Paxinos G, Franklin KBJ (2001) The mouse brain in stereotaxic coordinates, 2nd edn. Academic Press, London

Pedrazzini T, Seydoux J, Kunstner P, Aubert JF, Grouzmann E, Beermann F, Brunner HR (1998) Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor (see comments). Nat Med 4:722–726

Porsolt RD, Bertin A, Jalfre M (1978) "Behavioural despair" in rats and mice: strain differences and the effects of imipramine. Eur J Pharmacol 51:291–294

Rasmusson AM, Hauger RL, Morgan CA, Bremner JD, Charney DS, Southwick SM (2000) Low baseline and yohimbine-stimulated plasma neuropeptide Y (NPY) levels in combat-related PTSD. Biol Psychiatry 47:526–539

Redrobe JP, Dumont Y, Fournier A, Quirion R (2002a) The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. Neuropsychopharmacology 26:615–624

Redrobe JP, Dumont Y, Quirion R (2002b) Neuropeptide Y (NPY) and depression: from animal studies to the human condition. Life Sci 71:2921–2937

Redrobe JP, Dumont Y, Herzog H, Quirion R (2003) Neuropeptide Y (NPY) Y2 receptors mediate behaviour in two animal models of anxiety: evidence from Y2 receptor knockout mice. Behav Brain Res 141:251–255

Redrobe JP, Dumont Y, Fournier A, Baker GB, Quirion R (2005) Role of serotonin (5-HT) in the antidepressant-like properties of neuropeptide Y (NPY) in the mouse forced swim test. Peptides 26:1394–1400

Sajdyk TJ, Vandergriff MG, Gehlert DR (1999) Amygdalar neuropeptide Y Y-1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test. Eur J Pharmacol 368:143–147

Sajdyk TJ, Schober DA, Gehlert DR (2002) Neuropeptide Y receptor subtypes in the basolateral nucleus of the amygdala modulate anxiogenic responses in rats. Neuropharmacology 43:1165–1172

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805–809

Sorensen G, Lindberg C, Wortwein G, Bolwig TG, Woldbye DP (2004) Differential roles for neuropeptide Y Y1 and Y5 receptors in anxiety and sedation. J Neurosci Res 77:723–729

Thorsell A, Michalkiewicz M, Dumont Y, Quirion R, Caberlotto L, Rimondini R, Mathe AA, Heilig M (2000) Behavioral insensitivity to restraint stress, absent fear suppression of behavior and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression. Proc Natl Acad Sci USA 97:12852–12857

Thorsell A, Karlsson RM, Heilig M (2006) NPY in alcoholism and psychiatric disorders. In: Zukowska Z, Feuerslein GZ (eds) NPY family of peptides in tneurobiology, cardiovascular and metabolic disorders: from genes to therapeutics. Birkhauser Verlag, Switzerland, pp 183–192

Tschenett A, Singewald N, Carli M, Balducci C, Salchner P, Vezzani A, Herzog H, Sperk G (2003) Reduced anxiety and improved stress coping ability in mice lacking NPY-Y2 receptors. Eur J Neurosci 18:143–148

Wahlestedt C, Pich EM, Koob GF, Yee F, Heilig M (1993) Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligodeoxynucleotides. Science 259:528–531

Wan CP, Lau BH (1995) Neuropeptide Y receptor subtypes. Life Sci 56:1055–1064