Y1 receptors regulate aggressive behavior by modulating serotonin pathways

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Neuropeptide Y (NPY) is pivotal in the coordinated regulation of food intake, growth, and reproduction, ensuring that procreation and growth occur only when food is abundant and allowing for energy conservation when food is scant. Although emotional and behavioral responses from the higher brain are known to be involved in all of these functions, understanding of the coordinated regulation of emotion/behavior and physiological functions is lacking. Here, we show that the NPY system plays a central role in this process because ablation of the Y1 receptor gene leads to a strong increase in territorial aggressive behavior. After exposure to the resident–intruder test, expression of c-fos mRNA in Y1-knockout mice is significantly increased in the medial amygdala, consistent with the activation of centers known to be important in regulating aggressive behavior. Expression of the serotonin [5-hydroxytryptamine (5-HT)] synthesis enzyme tryptophan hydroxylase is significantly reduced in Y1-deficient mice. Importantly, treatment with a 5-HT-1A agonist, (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide, abolished the aggressive behavior in Y1-knockout mice. These results suggest that NPY acting through Y1 receptors regulates the 5-HT system, thereby coordinately linking physiological survival mechanisms such as food intake with enabling territorial aggressive behavior.

Aggression is a fundamental behavior that has evolved to help organisms compete for limited resources and, therefore, to guarantee survival of the species. Behavioral, pharmacological, and genetic studies have shown that specific neuronal circuits and various neurotransmitters and hormones modulate impulsiveness and aggressiveness in animal models as well as humans (1, 2). In particular, brain areas such as the olfactory bulb, the prefrontal cortex, the amygdala, several hypothalamic structures, and the area of the periaqueductal gray have been implicated in the regulation of emotion- and aggression-related behavior (3).

Although science has begun to reveal these neuronal circuits involved in regulating aggression, little is known about the molecular mechanisms that link particular physiological events with appropriate aggressive behavior. For example, aggressive behavior could be advantageous for hunting or defending a territory, especially when an individual is hungry and there is competition for a limited food supply. Similarly, aggression in males of many species can be advantageous in attracting the fittest partner. However, the mechanisms by which aggression and appetite or aggression and sexual drive are coordinately regulated are unknown.

We hypothesized that the evolutionary conserved neuropeptide Y (NPY) and its Y-receptor system may fulfill this important role of integrating aggressive behavior, especially territorial aggressive behavior, with other survival-related physiological functions. NPY is widely expressed in the brain, including the cerebral cortex, the hippocampus, the hypothalamus, the septum, and the brainstem (4). Central NPY systems are known to be involved in the regulation of feeding and energy homeostasis, growth, reproductive behavior and physiology, circadian rhythms, gastrointestinal motility, memory, nociception, and blood pressure regulation (5–7). NPY is found in major brain structures implicated in the regulation of emotionality and aggression, including the prefrontal cortex, the amygdala, the medial hypothalamus, and midbrain regions such as the dorsal periaqueductal gray. NPY also is known to alter the expression and secretion of other neurotransmitters such as norepinephrine (8) and serotonin [5-hydroxytryptamine (5-HT)] (9), which have been implicated in aggressive behavior (10, 11). Particularly high concentrations of NPY are found in the olfactory bulb (12), and lesions within the olfactory bulb have been shown to lead to depression-related behaviors, strong aggression, and muricide (mouse-killing behavior) in rats (13). Interestingly, bulbectomy leads to a reduction in NPY levels in the medial amygdala, and injection of NPY, in combination with the maximal, noneffective dose of noradrenaline, into the amygdala can suppress muricide in 80% of the bulbectomized rats (14).

NPY's various functions are specifically mediated by the Y receptor gene family, consisting of at least five distinct members (Y1, Y2, Y4, Y5, and y6) (15), all of which are expressed differentially in the brain (16). The Y2 receptor has been shown to play a role in the regulation of anxiety-related behaviors (17, 18). However, there is also strong evidence implicating the Y1 receptor as the main mediator of anxiolytic actions of NPY (10, 19, 20). Lack of Y1 signaling, therefore, would predict an increase in anxiety and, associated with it, possibly lead to an increase in aggressive behavior. Because the Y1 receptor is also a major subtype involved in the stimulatory effect of NPY on food intake and an increase in aggressive behavior could be advantageous for the competition for food, the Y1 receptor seems to be the prime candidate to mediate such a coordinate regulatory function. To test this hypothesis, we investigated Y1-null mice generated in our laboratory in different behavioral models with regards to changes to aggression-related behaviors. To investigate possible neuronal mechanisms for any behavioral changes observed in Y1 receptor-knockout mice, we also analyzed potential alterations in the expression levels of key molecules involved in the regulation of aggressive behavior.

Methods

Animals. The generation of Y1-knockout mice is described in ref. 21. WT control and Y1-knockout ($Y1^{-/-}$) mice are both maintained on a mixed C57BL/6–129SvJ background. Adult male, standard opponent A/J mice (age- and weight-matched) were received from the Western Australia Animal Resources Center (Perth, Australia). All mice were maintained under standard laboratory conditions with a 12:12-h light:dark schedule. Animals were group-housed (two to three animals per cage) before the behavioral testing. The

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Abbreviations: CART, cocaine- and amphetamine-regulated transcript; 5-HT, 5-hydroxytryptamine (serotonin); GnRH, gonadotropin-releasing hormone; NPY, neuropeptide Y; 8-OH-DPAT, (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide; POMC, proopiomelanocortin; RI, resident–intruder; SA, spontaneous aggression; TPH, tryptophan hydroxylase.

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cages were equipped with environmental enrichment: a metal ring for climbing integrated in the cage lid, tubes or domes for hiding, and cellulose paper as nesting material. For habituation, all animals were transported to the testing room 1 h before behavioral testing. All research and animal care procedures were approved by the Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee and were in agreement with the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose.

General Health and Neurological Examination. All behavioral experiments were conducted during the light phase by using adult male WT and $Y1^{-/-}$ mice. General health, sensory abilities, and neurological reflexes that could influence behavioral testing were controlled and compared as described in refs. 22–24. Neuromuscular strength of the animals was tested in the wire-hang test. Furthermore, we used a range of basic tests including visual cliff behavior, olfaction, and motor reflexes (balance, righting, eye blink, ear twitch, and whisker orienting) to identify potential neurological dysfunctions, described in detail in ref. 22.

Determination of Motor Functions (Accelerod, Beam Walking, Pole, and Footprint Tests). ***Accelerod test.*** A computerized RotaRod, supplied by Technical & Scientific Equipment (Bad Homburg, Germany) was used. After 4 days of training (trial duration of 120 s twice daily with an intertrial interval of 90 min) at a constant speed of 14 rpm, animals were subjected to the apparatus for 4 days, and the rotation speed was constantly increased (4–40 rpm) over a 5-min time period. The latency to fall off the rod and the actual rotation speed were recorded (25).

Beam walking test. Motor coordination and balance were assessed by measuring the ability of the mice to traverse a graded series of five narrow beams (dimensions as described in ref. 22) to reach a safety platform (26). The latency to traverse each beam and the number of times a hind paw slipped off a beam were recorded for each trail.

Pole test. The task for the mice in this test is to turn around and climb down from the top of a vertical pole (dimensions as described in ref. 22) within 120 s. The latency to turn around and to reach the platform at the base of the pole was measured (27).

Footprint test. The footprint test evaluates the walking pattern of mice to detect gait abnormalities. The test was performed as described in ref. 28, with minor modifications. The footprint patterns were analyzed for the following three step parameters (26): stride length, hind- and front-base width, and distance from left or right front footprint to the ipsilateral hind footprint.

Motor Activity (Open Field). The open field apparatus used in this experiment consisted of an open-top, wooden box, painted black ($56 \times 56 \times 60$ cm). Animals were placed into the center of the highly illuminated arena. The behavior and the motor activity (traveled distance) were observed and recorded for 10 min, as described in ref. 22.

Aggression Paradigms (Spontaneous/Territorial Aggression). ***Spontaneous aggression (SA).*** In the SA test, the adult male, group-caged test animal was confronted with a lighter, group-housed standard opponent (A/J) in a neutral cage (29). The latency and frequency of the agonistic behaviors of tail rattling, aggressive grooming, and biting were measured during the next 10 min (30–32). In this task, animal's aggression is based exclusively on its spontaneous occurrence and not influenced by any territorial aspects. Additionally, the frequency and latency of behaviors such as nosing, following, anogenital sniffing, crawling over, and social grooming (33) were recorded. To avoid severe injuries experiments were stopped when escalated fighting occurred (10 bites against opponent or intense fighting for >5 s). In addition, the occurrence of fighting in the home cage after returning the test animal was recorded during the next 10 min.

Territorial aggression [resident–intruder (RI) test]. In the RI test, an individual test animal was confronted with a group-housed standard opponent (A/J) in its home cage, which induces strong territorial behavior in the residential animal (32, 34). One set of test animals was group-housed before the RI test and the second set of mice was individually housed 24 h before the RI test. The environmental enrichment, cage lid, and littermates (if present) were removed 10 min before the experiment. The procedure was the same as in the standard opponent test. Twenty minutes after the RI tests, the individually housed mice were killed by means of cervical dislocation and the brains were collected and frozen on dry ice.

Drug Treatment. The 5-HT-1A agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), purchased from Sigma, was dissolved in 0.9% saline solution. Animals were injected s.c. with saline or different doses of 8-OH-DPAT (0.1 mg/kg and 1.0 mg/kg of body weight) in a volume of 1 ml/100 g of body weight 25 min before RI testing (35). Adult male WT and $Y1^{-/-}$ mice received each agonist dose once (counterbalanced sequence) in a random order on a 4-day interval. All test animals were transferred to individual housing 5 days before first RI testing. Testing of drug-treated mice was performed after the procedure for territorial aggression described above.

Tissue Collection and Serum Analysis. At 16–18 weeks of age, male WT and $Y1^{-/-}$ mice were killed by cervical dislocation for collection of trunk blood, and brains were removed immediately and frozen on dry ice. The right testis and seminal vesicle were removed and weighed. Testosterone was determined by using a RIA kit from ICN.

Neurochemical Analyses. Coronal slices (20 μm) of the brains collected after RI testing were cut and thaw-mounted on charged slides. For *in situ* hybridization, DNA oligonucleotides complementary to mouse NPY (5′-GAGGGTCAGTCCACACAGCCCCATTCGCTTGTTACCTAGCAT-3′), proopiomelanocortin (POMC) (5′-TGGCTGCTCTCCAGGCACCAGCTCCACACATCTATGG AGG-3′), cocaine- and amphetamine-regulated transcript (CART) (5′-TCCTTCTCGTGGGACGCATCATCCACGGCAGAGTAGATGTCCAGG-3′), agouti-related protein (5′-AGCTTGCGGCAGTAGCAAAAGGCATTGAAGAAGCGGCAGTAGCAC-3′), corticotropin-releasing hormone (5′-CCGATAATCTCCATCAGTTTCCTGTTGCTGTGAGCTTGCTGAGCT), gonadotropin-releasing hormone (GnRH) (5′-CAAACACACAGTCAGCAGTAGAATGCCGGCCATCAG TTTGAGGATC-3′) and thyroid-stimulating hormone-releasing hormone (5′-AACCTTACTCCTCCAGAGGTTCCCTGACCCAGGCTTCCAGTTGTG-3′), c-fos (5′-CTTGGGCTCAGGGTCGTTGAGAAGGGGCAGGGTGAAGGCCTCCTCAGA-3′), and tryptophan hydroxylase (TPH) (5′-CACGCCTTGTCGGAAAGAGCATGCTTCAATTCTCCGATGGACG-3′) mRNAs were labeled with α-[^{35}S]thio-dATP (Amersham Pharmacia) by using terminal deoxynucleotidyltransferase (Roche). Matching sections from the same portion of the olfactory bulb, forebrain hypothalamus, or midbrain of knockout and control mice were analyzed as described in ref. 36.

Statistical Analysis. The various behavioral data, body weight, serum parameters, and mRNA expression levels were assessed with two-way (factor "genotype" and "treatment") and/or one-way (factor "genotype" split by "treatment") ANOVA followed by the Fisher probable least-squares difference test for post hoc comparisons, if appropriate. Differences were regarded as statistically significant if $P < 0.05$. Results presented are displayed as means ± SEM, and the appropriate P values of the post hoc tests are mentioned.

Table 1. Sensory abilities, neurological reflexes, neuromuscular strength, and motor functions of control and Y1$^{-/-}$ mice

Observation	Control	Y1$^{-/-}$
Sensory abilities		
Startle response	Normal	Normal
Visual cliff and strange stimulus	Normal	Normal
Olfaction (hidden cookie)	Normal	Normal
Neurological reflexes		
Balance ability and righting	Normal	Normal
Eye blink and ear twitch	Normal	Normal
Whisker-orienting	Normal	Normal
Constriction and dilatation	Normal	Normal
Neuromuscular strength		
Wire-hang test, s	58.3	47.4
Motor function tests		
Beam walking (different dimensions), s	74.0	52.5
Pole, s	83.7	79.2
Accelerod (day 1–4), s	224.0	263.4
Footprint		
Stride length, cm	5.7	5.7
Hind/front base width, cm	3.3	3.4
Distance front/hind footprint, cm	1.3	1.3

Existence/occurrence of sensory abilities/reflexes is defined as ''normal.'' For the different neuromuscular strength and motor functions tasks, means are presented. (n = 9–10 mice.)

Results

Y1 Receptor-Knockout Mice. The Y1$^{-/-}$ mice bred normally and had significantly larger litter sizes (Y1$^{-/-}$, 7.64 ± 0.51, and WT, 4.86 ± 0.32 offspring per litter; n = 18–24 breeding pairs). The body weight gain of male Y1$^{-/-}$ mice was not significantly different from that of control mice. Analysis of the sensory abilities and neurological reflexes as well as general motor functions (summarized in Table 1) did not reveal any significant differences between Y1$^{-/-}$ and control animals. Therefore, Y1$^{-/-}$ mice exhibit a WT-like phenotype in regard to these basic domains. However, increased motor activity for the male Y1$^{-/-}$ mice was evident when tested in the open field test, with knockout mice showing a significant higher number of square entries compared with control mice (Y1$^{-/-}$, 308 ± 40.8 vs. WT, 212 ± 18.6; n = 12 mice, $P < 0.05$). Increased aggressive behavior was noticed in these mice while handling and particularly after returning them to their home cage.

Aggressive Behavior of Y1$^{-/-}$ Mice. Two well established paradigms, the SA and territorial RI tests, were used to investigate changes in aggressive behavior in Y1$^{-/-}$ mice. In the SA test, neither the control nor the Y1$^{-/-}$ animals showed any spontaneous occurrence of agonistic behavior. However, a clear difference in aggressive behavior was observed after returning test animals to the home cage, with Y1$^{-/-}$ mice showing a 2.8-fold increase in the occurrence of territorial aggressive behavior compared with control animals. Furthermore, the frequency of anogenital sniffing was significantly increased in the Y1$^{-/-}$ animals (Y1$^{-/-}$, 8.6 ± 2.1 events per 10 min vs. WT, 2.7 ± 0.6 events per 10 min; n = 9–12 mice, $P < 0.01$).

The RI test was performed with group-housed as well as individually housed animals. Group-housed Y1$^{-/-}$ animals showed significantly increased frequencies of the agonistic behaviors tail rattling and biting (Fig. 1*a*). Additionally, the Y1$^{-/-}$ animals exhibited a significant increase in the frequency of anogenital sniffing (Y1$^{-/-}$, 10.3 ± 1.8 events per 10 min vs. WT, 5.9 ± 1.1

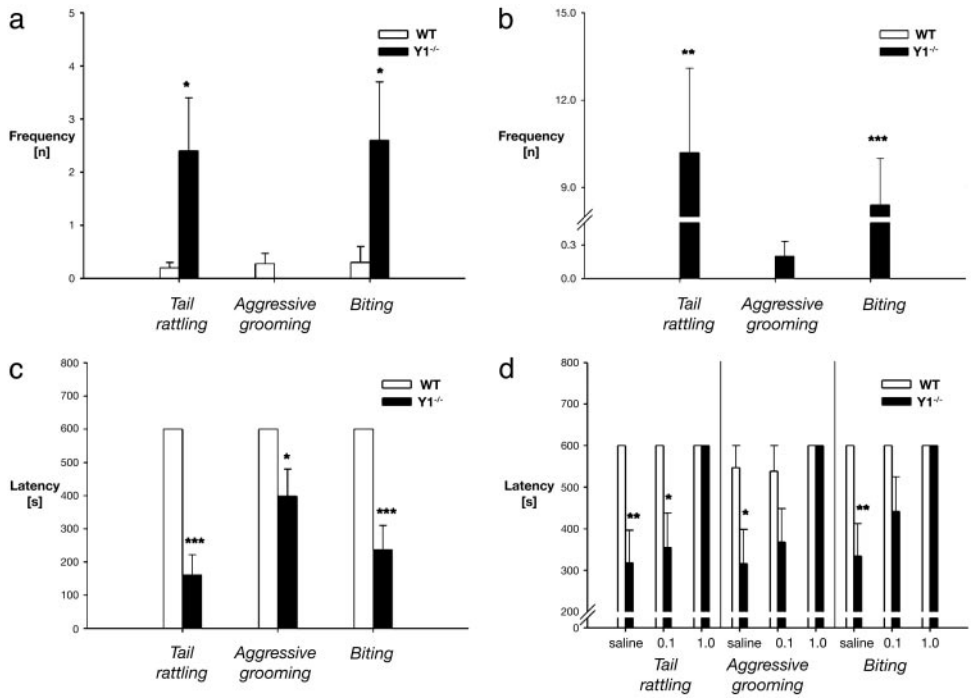

Fig. 1. Territorial aggression and its reduction after 5-HT1A agonist treatment. (*a* and *b*) Territorial aggression in group-housed (*a*) and individually housed (*b*) mice after being tested in the RI paradigm. Frequencies [*n*] of aggressive behaviors such as tail rattling, aggressive grooming, and biting in control and Y1$^{-/-}$ mice are shown. Data represent means ± SEM (n = 9–12 mice). Significant post hoc effects vs. control animals are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). (*c*) Territorial aggression in individually housed mice after being tested in the RI paradigm. Latencies [s] of aggressive behaviors such as tail rattling, aggressive grooming, and biting in control and Y1$^{-/-}$ mice are shown. Data represent means ± SEM (n = 10–12 mice). Significant post hoc effects vs. control animals are indicated by asterisks (*, $P < 0.05$; ***, $P < 0.001$). (*d*) Reduction in territorial aggressive behavior in the RI paradigm 25 min after s.c. treatment with different doses (saline, 0.1 or 1.0 mg/kg) of a 5HT-1A agonist (8-OH-DPAT). Latencies [s] of aggressive behaviors such as tail rattling, aggressive grooming, and biting in control and Y1$^{-/-}$ mice are shown, dependent on different drug doses (mg/kg of body weight). Data represent means ± SEM (n = 9–10 mice). Significant post hoc effects vs. control animals are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$).

Table 2. Alterations in neuropeptide expression in Y1$^{-/-}$ mice

Brain area	Neuropeptide	Control, %	Y1$^{-/-}$, %
Accessory olfactory bulb	NPY	100 ± 6.3 (5)	77 ± 4.7 (4)*
Medial amygdala	CART	100 ± 3.9 (5)	74 ± 7.0 (5)*
Central amygdala	CART	100 ± 2.3 (5)	103 ± 3.2 (5)
Arcuate nucleus	NPY	100 ± 4.2 (10)	86 ± 10.2 (4)
	AgRP	100 ± 3.3 (9)	91 ± 2.8 (4)
	CART	100 ± 2.7 (10)	76 ± 4.7 (5)***
	POMC	100 ± 2.7 (10)	79 ± 1.8 (5)***
Paraventricular nucleus	TRH	100 ± 2.6 (6)	111 ± 3.5 (5)*
	CRH	100 ± 2.1 (5)	91 ± 9.4 (5)
Scattered forebrain neurons	GnRH	100 ± 2.0 (5)	126 ± 3.0 (4)**

Data represent mean labeling intensity of neurons of the specified areas, given as percent of control ± SEM. The number of mice is shown in parentheses. AgRP, agouti-related protein; CRH, corticotropin-releasing hormone; TRH, thyroid-stimulating hormone-releasing hormone. Significant post hoc effects vs. controls are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

events per 10 min; $n = 9$–12 mice, $P < 0.05$). Similar data were recorded for animals that had been individually housed 24 h before the test. A clear increase in territorial aggression in the Y1-knockout mice was evident with significantly increased frequencies (Fig. 1*b*) and decreased latencies (Fig. 1*c*) of tail rattling and biting (Fig. 1*b*). Furthermore, the latencies for aggressive grooming (Fig. 1*c*) and latency to stop the agonistic encounters because of escalated fighting (Y1$^{-/-}$, 320.2 ± 68.4 s vs. WT, 600 ± 0.0 s; $n = 9$–12 mice, $P < 0.001$) were significantly decreased in Y1$^{-/-}$ mice.

Altered Neuropeptide mRNA Expression in Y1$^{-/-}$ Mice. To assess the consequences of Y1 receptor deletion on the central expression of peptides or their precursors known to play a role in anxiety-, aggressive-, and feeding-related behaviors, we performed *in situ* hybridization experiments on coronal brain sections obtained from male Y1$^{-/-}$ and control mice, employing specific radiolabeled antisense DNA oligonuclotides for the following mRNAs: NPY, agouti-related protein, proopiomelanocortin, thyroid-stimulating hormone-releasing hormone, CART, GnRH, and the rate-limiting enzyme for the synthesis of 5-HT, TPH (37). Deletion of the Y1-receptor gene caused marked alterations in neuropeptide mRNA expression throughout the brain (Table 2).

High concentrations of NPY are normally found in the olfactory bulb (12), and lesions within the olfactory bulb have been shown to lead to depression-related behaviors, strong aggression, and muricide in rats (13, 38). Interestingly, NPY mRNA levels are strongly reduced in the accessory olfactory bulb of Y1-knockout mice (Fig. 2 and Table 2). Furthermore, in the medial amygdala, a key area for the regulation of emotionality, the expression on CART mRNA was significantly reduced, whereas CART expression in the central amygdala was unaffected (Fig. 2 and Table 2). Interestingly, mRNA levels for CART and the colocalized POMC in the feeding-related arcuate nucleus of the hypothalamus were down-regulated (Fig. 2 and Table 2). We also investigated whether the increased aggression of Y1$^{-/-}$ mice was associated with altered function of the hypothalamo-pituitary-gonadotropic axis. Importantly, scattered neurons in the forebrain, including the medial septal nucleus and the medial preoptic area, displayed markedly augmented GnRH expression, suggesting an increased activity on the gonadotropin axis (Fig. 2 and Table 2). However, apart from increased litter size, this change did not translate to changes in serum testosterone levels (Y1$^{-/-}$, 4.5 ± 1.7 nmol/liter vs. WT, 6.0 ± 1.8 nmol/liter; $n = 22$–24 mice), testicular weight (Y1$^{-/-}$, 211 ± 5 mg vs. WT, 241 ± 4 mg), or seminal vesicle weight (Y1$^{-/-}$, 284 ± 18 mg vs. WT, 281 ± 10 mg; $n = 15$–25 mice).

Other Neurochemical Adaptations in Y1$^{-/-}$ Mice. Neuronal activity has been shown to be associated with increases in c-fos expression. Control and Y1$^{-/-}$ mice showed minimal basal c-fos expression throughout the brain, with the exception of augmentations in the motor cortex and parts of the striatum in the Y1$^{-/-}$ mice (data not

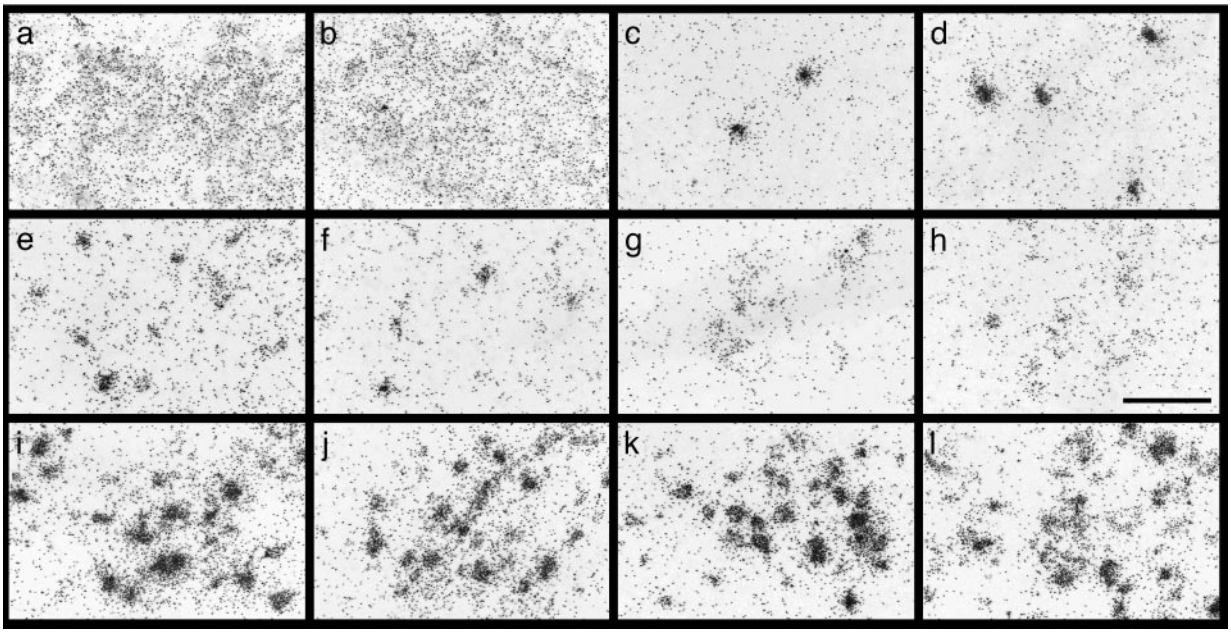

Fig. 2. Neuropeptide mRNA expression in the CNS. Altered neuropeptide mRNA levels in areas involved in aggression and feeding in Y1-deficient mice. High-power photomicrographs of photo emulsion-dipped sections obtained from control (*a*, *c*, *e*, *g*, *i*, and *k*) and Y1$^{-/-}$ (*b*, *d*, *f*, *h*, *j*, and *l*) mice after *in situ* hybridization for neuropeptide mRNAs. NPY in the accessory olfactory bulb (*a* and *b*), GnRH in scattered neurons of the medial septal nucleus (*c* and *d*), CART in the medial (*e* and *f*) and central (*g* and *h*) amygdaloid nucleus and the arcuate nucleus (*i* and *j*), and POMC in the arcuate nucleus (*k* and *l*) are depicted. (Scale bar = 100 μm.)

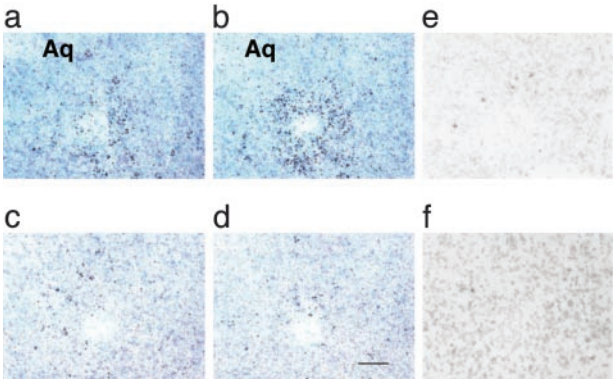

Fig. 3. TPH mRNA and c-fos mRNA expression in CNS. (*a–d*) Photomicrographs of photo emulsion-dipped sections obtained from $Y1^{-/-}$ (*a* and *c*) and control (*b* and *d*) mice after *in situ* hybridization for TPH mRNA. The dorsal raphe nucleus is depicted in *a* and *b*, and the pontine nucleus is shown in *c* and *d*. Aq, aqueduct. (*e* and *f*) c-fos mRNA expression in the central nucleus of the amygdala of $Y1^{-/-}$ and control mice, respectively, after exposure to the RI test. (Scale bar = 40 μm.)

shown). However, c-fos mRNA levels were increased in the accessory olfactory bulb and the medial amygdala in $Y1^{-/-}$ mice but not in the control animals 20 min after the RI test was performed (Fig. 3). This finding suggests that the lack of Y1 signaling is responsible for increased activity of neuronal populations important for mediating aggressive and emotional behaviors. This conclusion is also supported by the increase in motor activity and the frequency of anogenital sniffing, behaviors known to be linked to aggression.

These effects of altered Y1 signaling could be directly or indirectly mediated. NPY receptors, including Y2 and Y1, act in an inhibitory fashion by negatively regulating the accumulation of cAMP. As a consequence, the synthesis or release of other neurotransmitters can be reduced or inhibited. In this way, NPY regulates the action of many neurons, including 5-HT neurons (39, 40), which have been shown to play a role in the regulation of aggressive behavior when levels of this molecule are altered (41). To investigate whether deletion of the Y1 receptor causes alterations to the 5-HT system, we analyzed expression levels of TPH, the rate-limiting enzyme in 5-HT synthesis in the raphe nucleus. A significant decrease in TPH mRNA levels could be detected selectively in this nucleus but not in another 5-HT-producing center, the pontine nucleus, in $Y1^{-/-}$ mice compared with control mice (Fig. 3).

5-HT-1A Agonist Treatment in $Y1^{-/-}$ Mice. Because of this significant decrease in TPH mRNA levels in the raphe nucleus, we treated Y1-knockout mice with a 5-HT-1A agonist (8-OH-DPAT) to see whether increased activation of this receptor and its associated pathway can prevent the aggressive behavior observed in these mice (35). Indeed, injection of this agonist significantly reduced in a dose-dependent manner the aggressive behavior in Y1-knockout mice in the RI test (Fig. 1*d*), demonstrating a critical role of Y1 and 5-HT-1A receptor in integrating behavioral pathways to enabling aggression. No "5-HT syndrome-like" behaviors such as reciprocal forepaw treading, head-weaving, or flattened body posture (42) were observed in control or $Y1^{-/-}$ mice of both 0.1 and 1.0 mg/kg treatment groups.

Discussion

In this study, we provide evidence for a previously unknown role of NPY and specifically the Y1 receptor in territorial but not spontaneous aggressive behavior. Our experiments employing several well established behavioral test paradigms revealed robust phenotypic traits in Y1 receptor-deleted mice, suggesting an important role for the Y1 receptor in the regulation of territorial aggression. Because NPY is one of the most potent orexigenic agents known and is involved in reducing metabolic rate and switching off reproductive functions in times of famine or energy deficit, our findings suggest that NPY may act through the Y1 receptor as a molecular link in coordinating key physiological processes with conducive behaviors.

Lack of Y1 signaling is likely to change, either directly or indirectly, the effect of NPY on production or release of other neurotransmitters and subsequently lead to a decrease in their supply. Of particular interest is that NPY can inhibit the slow 5-HT-1A receptor-mediated inhibitory postsynaptic potential in the dorsal raphe nucleus by Y2 receptors (43), which might be compromised in the $Y1^{-/-}$ mice. Conversely, the 5-HT system is involved in the regulation of NPY release in the hypothalamus and the amygdala (44, 45), and 5-HT is thought to inhibit NPY's stimulatory effect on food intake and to regulate CART mRNA expression in the arcuate nucleus (46). Indeed, it is likely that reduced 5-HT production contributes to the territorial aggressive phenotype of $Y1^{-/-}$ mice, because these mice display a significant reduction in mRNA expression of the 5-HT synthesis-limiting enzyme TPH in the dorsal raphe nucleus compared with control mice. More importantly, treatment of $Y1^{-/-}$ mice with the 5-HT-1A agonist 8-OH-DPAT dose-dependently reduced the aggressive behavior in these mice already at the low, nonsedative acting dose of 0.1 mg, suggesting a specific, antiaggressive-like effect consistent with a deficient supply of 5-HT in $Y1^{-/-}$ mice. However, at the higher 1.0-mg dosage, an additive sedative-like effect of 8-OH-DPAT cannot be excluded (47).

The differences between spontaneous and territorial aggression in the test animals fits the hypothesis that a Y1 receptor-mediated increase in aggression could be advantageous for a mouse in situations of limited food supply within its territory. An increase of territorial aggression, but not SA, would increase the possibility to defend its territory and the food resources against competitors. This conclusion is further supported by an increase of home-cage aggression after the SA test. Furthermore, this difference between spontaneous and territorial aggression is indicative for a high degree of specificity in the regulation of context-dependent aggression and supports the hypothesis proposed in this paper that there is a functional connection specifically between territorial aggression and the Y1 receptor system.

It is not entirely clear whether the reduction of TPH mRNA levels in the dorsal raphe nucleus is a direct effect of the absence of Y1 signaling in these neurons or an indirect effect mediated by other pathways projecting to this nucleus. The latter is supported by the observed increase in c-fos mRNA expression in the accessory olfactory bulb and the medial nucleus of the amygdala in the $Y1^{-/-}$ mice shortly after the RI test. This observation also provides convincing evidence that these major centers and pathways, which are thought to mediate aggressive and emotional behaviors, are more strongly activated in $Y1^{-/-}$ mice. Furthermore, these brain regions in $Y1^{-/-}$ mice show alterations in other neurotransmitter mRNA levels, including a reduction of NPY mRNA levels in the accessory olfactory bulb and a reduced expression of CART mRNA in the medial nucleus of the amygdala. There is strong evidence that NPY exerts anxiolytic-like effects by action on Y1 receptors within the amygdala because selective Y1 agonists produce anxiolysis, and inhibition of amygdala Y1 receptor synthesis with antisense oligonucleotides blocks this anxiolytic action of NPY (20). Because the accessory olfactory bulb has projections to the amygdala and because olfactory bulbectomy is known to cause muricide, depression, and anxiety-like behavior as well as compensatory increases in NPY immunoreactivity in the medial amygdala (12), reduction of NPY expression in the accessory olfactory bulb in the $Y1^{-/-}$ mice might potentiate an anxiogenic as well as aggressive phenotype. This possible interaction be-

tween aggression and anxiety has been shown before (48). Both motor activity and the motivation to detect pheromones also are linked to aggression (49). Our data, which show an increase in motor activity during the open field test (confirmed by augmented basal c-fos levels in the motor cortex) and an increased frequency of anogenital sniffing in all aggression paradigms (supported by altered c-fos expression in olfactory bulbs) in the $Y1^{-/-}$ mice are consistent with these observations.

Besides effects on the behaviors of anxiety, aggression, and alcohol intake (50), the Y1 receptor also has been implicated in mediating the potent orexigenic effects of NPY within the hypothalamus. Appetite or food intake is dramatically increased in situations of increased hypothalamic NPY-ergic tonus, such as during fasting or in animals administered exogenous NPY. Y1 receptor deficiency has been shown to attenuate appetite in these situations (51). Moreover, pharmacological evidence indicates that Y-receptor agonists and antagonists stimulate or inhibit feeding in proportion to their specificity and affinity for the Y1 receptor (52). NPY neurons from the arcuate nucleus project not only to other feeding-related nuclei within the hypothalamus but also to the medial amygdala, where they have been shown to innervate CART-expressing neurons (53), potentially coordinating feeding-related behaviors with emotion- and aggression-related behaviors. Reduction of CART expression and signaling in this area of $Y1^{-/-}$ mice might be important in this process. By regulating both food intake and aggression, the Y1 receptor may provide a molecular link between the fundamental need for food and the necessary level of aggression required for attaining it.

The extensive network of NPY neurons seen in the mammalian brain connecting various important centers highlights NPY's potential to coordinately regulate different physiological and behavioral functions, including anxiety, aggression, and alcohol consumption. Because NPY is also one of the most potent orexigenic agents known and is involved in reducing metabolic rate and switching off reproductive functions in times of famine or energy deficit, our findings suggest that NPY may act as a molecular link in coordinating key physiological processes with conducive behaviors. This link also may have significant implications for the treatment of psychiatric disorders characterized by aggressiveness and impulsivity.

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