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Evidence from knockout mice that neuropeptide-Y Y2 and Y4 receptor signalling prevents long-term depression-like behaviour caused by immune challenge

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Abstract

Neuropeptide Y (NPY) participates in the acute behavioural responses to immune challenge, since Y2 receptor knockout (Y2–/–) mice are particularly sensitive to the short-term anxiogenic-like effect of bacterial lipopolysaccharide (LPS). The present exploratory study addressed the involvement of Y2 and Y4 receptors in the long-term behavioural responses to immune challenge. A single intraperitoneal injection of LPS (0.83 mg/kg) to control mice did not affect open field behaviour 3 h post-treatment but enhanced anxiety-like behaviour in Y2–/– as well as Y4–/– mice. Four weeks post-treatment this behavioural effect of LPS persisted in Y4–/– mice but had gone in Y2–/– mice. Depression-related behaviour in the forced swim test was enhanced 1 day post-LPS in control and Y2–/– mice, but not in Y4–/– mice. Four weeks post-treatment, the depressogenic-like effect of LPS had waned in control mice, persisted in Y2–/– mice and was first observed in Y4–/– mice. In summary, knockout of Y2 and/or Y4 receptors unmasks the ability of a single LPS injection to cause a delayed and prolonged increase in anxiety- and/or depression-like behaviour. These findings suggest that NPY acting via Y2 and Y4 receptors prevents the development of long-term anxiety- and depression-like behaviour caused by acute immune challenge.

Keywords

Neuropeptide-Y Y2 receptors; neuropeptide-Y Y4 receptors; bacterial lipopolysaccharide (endotoxin); anxiety-related behaviour; depression-like behaviour

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Introduction

Neuropeptide Y (NPY) is a messenger widely distributed in the peripheral and central nervous system. Its functional implications in the brain include the regulation of energy balance, cognition, mood, anxiety and stress sensitivity (Heilig, 2004; Karl and Herzog, 2007; Kask et al., 2002; Lin et al., 2004). Haplotype-driven expression of NPY in humans predicts brain responses to emotional and stress challenges and inversely correlates with trait anxiety (Zhou et al., 2008). The actions of NPY are mediated by several classes of NPY receptors, five of which (Y1, Y2, Y4, Y5 and y6) have been elucidated at the gene and protein level (Michel et al., 1998; Redrobe et al., 2004). Gene knockout studies have revealed that endogenous NPY acting via Y2 and Y4 receptors is involved in the regulation of anxiety and stress coping. Thus, anxiety- and depression-like behaviour is significantly reduced in Y2 receptor knockout (Y2−/−) mice (Carvajal et al., 2006; Redrobe et al., 2003; Tschenett et al., 2003), and a similar anxiolytic-like and antidepressant-like phenotype has been observed in Y4 receptor knockout (Y4−/−) mice (Painsipp et al., 2008b; Tasan et al., 2009).

Systemic immune challenge by intraperitoneal (IP) injection of bacterial lipopolysaccharide (LPS; endotoxin) causes a syndrome termed sickness behaviour which, among others, comprises fever and a decrease in social interaction (Goehler et al., 2000; Konsman et al., 2002; Romanovsky et al., 2005). NPY-expressing neurons in the arcuate and paraventricular nuclei of the hypothalamus have been implicated in the sickness response to LPS or proinflammatory cytokine administration (Konsman and Dantzer, 2001; McCarthy et al., 1995; McMahon et al., 1999; Romanovsky et al., 2005; Sonti et al., 1996). Animals deficient in Y2 receptors are particularly susceptible to the ability of LPS challenge to modify locomotor, social and anxiety-like behaviour, rectal temperature and circulating corticosterone levels as measured 3 h post-treatment (Painsipp et al., 2008a). Thus, Y2 receptors appear to play a regulatory role in the cerebral response to peripheral immune challenge.

Apart from eliciting a sickness response which peaks about 3 – 6 h post-treatment (Bluthé et al., 2000; Fishkin and Winslow, 1997), IP injection of LPS (0.83 mg/kg) to mice also induces a delayed increase in depression-like behaviour as observed 1 – 2 days post-treatment when the sickness behaviour has abated (Frenois et al., 2007). In addition, a single IP injection of LPS 1 (mg/kg) to rats can evoke long-term changes in neuroendocrine – immune interactions as observed 3 – 4 weeks post-treatment. Thus, adjuvant-induced arthritis is inhibited (Harbuz et al., 2002), an effect that appears to be related to desensitization of the hypothalamic-pituitary-adrenal (HPA) axis, increase in corticosterone secretion and suppression of proinflammatory cytokine formation (Richards et al., 2006; Vallès et al., 2002, 2003, 2005). Studies in humans indicate that the acute sickness response to cytokine therapy can be differentiated from a delayed increase in depression scores that are maintained for several weeks (Capuron et al., 2000, 2001).

Long-term changes in emotional-affective behaviour following a single LPS challenge to rodents have not yet been reported. We therefore carried out an exploratory study which pursued two specific aims. First, it was investigated whether a single IP injection of LPS

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would alter anxiety-like and depression-related behaviour as measured within 1 day post-treatment as well as after an interval of 4 weeks. The second aim was to explore whether Y2 and/or Y4 receptors contribute to the short- and long-term behavioural alterations evoked by a single IP injection of LPS. This goal was addressed by examining the short- and long-term effects of endotoxin in Y2–/– and Y4–/– mice, relative to control mice. The dose of LPS used here (0.83 mg/kg) was identical with that reported to induce a delayed depression-like behaviour in mice (Frenois et al., 2007). Our study was performed with female mice, because our previous studies on Y2–/– and Y4–/– mice had focused on female animals (Painsipp et al., 2008a, 2008b), given that affective disorders are more prevalent in women than in men (Gorman, 2006; Palanza, 2001; Simonds and Whiffen, 2003).

Methods and materials

Experimental animals

This study was carried out with age-matched adult female mice which were housed in groups of 3 – 4 per cage under controlled temperature (set point 22 °C), relative air humidity (set point 50 %) and light conditions (lights on at 6:00 h, lights off at 18:00 h, maximal intensity 150 lux). All experiments were approved by an ethical committee at the Federal Ministry of Science and Research of the Republic of Austria and conducted according to the Directive of the European Communities Council of 24 November 1986 (86/609/EEC). The experiments were designed in such a way that the number of animals used and their suffering was minimized.

The animals under study were germline Y2–/– and Y4–/– mice and non-induced conditional Y2 and Y4 receptor knockout (FY2 and FY4) mice which were bred in the Department of Pharmacology of the Medical University of Innsbruck (Innsbruck, Austria), while all experiments were carried out at the Medical University of Graz. The genetic design of these animals has been described previously (Sainsbury et al., 2002a, 2000b). Germline Y2–/– and Y4–/– mice were generated from the same founders on the same mixed C57BL/6 : 129/SvJ (50 % : 50 %) background as the conditional FY2 and FY4 knockout mice. Germline Y2–/– and Y4–/– mice were obtained by crossing chimeric mice carrying a Y2 floxed gene ($Y2^{lox/lox}$) or a Y4 floxed gene ($Y4^{lox/lox}$), respectively, with oocyte-specific Cre recombinase-expressing C57BL/6 mice (Sainsbury et al., 2002a, 2002b). Non-induced conditional FY2 and FY4 knockout mice were used as controls in all experiments and termed control mice throughout the paper. As demonstrated before, these non-induced conditional $Y2^{lox/lox}$ and $Y4^{lox/lox}$ mice do not differ from wild-type mice, as the level of expression of Y2 and Y4 receptors is not influenced by the introduction of the loxP sites (Sainsbury et al., 2002a, 2002b). The deletion or presence of Y2 and Y4 receptors in the germline and non-induced conditional knockout mice was verified by polymerase chain reaction using oligonucleotide primers recognizing DNA sequences adjacent to the loxP sites flanking the deleted or residing Y2 and Y4 receptor gene (Sainsbury et al., 2002a, 2002b).

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Experimental protocols

The animals were subjected to behavioural testing at short and long intervals after IP injection of LPS (0.83 mg/kg) or its vehicle. Each animal underwent two behavioural tests at an interval of 21 h, first the open field test (OFT) and then the forced swim test (FST). The study was conducted with a total of 30 control animals, 24 Y2–/– mice and 28 Y4–/– mice. Their allocation to the different treatment and test groups is shown in Table 1. In order to evaluate the short-term effects of LPS on emotional behaviour, anxiety-related behaviour in the OFT was evaluated 3 h after IP injection of LPS or vehicle (Painsipp et al., 2008a), while depression-like behaviour in the FST was determined 24 h post-treatment. Four weeks post-treatment, the long-term effects of LPS on behaviour were explored, with the OFT carried out 27 days and the FST carried out 28 days post-treatment.

Administration of lipopolysaccharide

LPS extracted from *E. coli* 0127:B8 (Sigma, Vienna, Austria) was dissolved in pyrogen-free sterile saline (0.9 % NaCl) at a concentration of 1 mg/ml. This stock solution was diluted with pyrogen-free sterile saline to yield an injection solution of 0.083 mg/ml LPS, which was injected IP at a volume of 0.01 ml/g, equivalent to a dose of 0.83 mg/kg LPS. Pyrogen-free sterile saline injected at the same volume was used as vehicle control.

Behavioural tests

General precautions—Prior to all behavioural tests, the mice were allowed to adapt to the test room (set points: 22 ^{o}C, 50 % relative air humidity, lights on at 6:00 h, lights off at 18:00 h, maximal light intensity 100 lux) for at least two days. The OFT and FST were performed during the period of 10:30 to 13:30 h.

Open field test—The open field consisted of a box (50 × 50 × 30 cm) that was made of opaque grey plastic and illuminated by 80 lux at floor level. The ground area of the box was divided into a 36 × 36 cm central area and the surrounding border zone. Mice were individually placed in a corner of the open field, and their behaviour during a 5 min test period was tracked by a video camera positioned above the center of the open field and recorded with the software VideoMot2 (TSE Systems, Bad Homburg, Germany). This software was used to evaluate the time spent in the central area, the number of entries into the central area and the total distance travelled in the open field.

Forced swim test—Each mouse was placed individually placed in a glass beaker (diameter: 13 cm, height: 23 cm) containing tap water at 25 °C. The water was 16 cm deep, which prevented the mice from touching the bottom of the beaker with their paws or the tail. Mice were tested for 6 min and their behavioural activity was scored by a trained observer. The times spent on climbing, swimming and immobility were recorded to determine active versus passive stress-coping performance. Mice were considered immobile when they floated passively in the water, performing only movements that enabled them to keep their heads above the water level (Cryan and Mombereau, 2004).

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Statistics

The results were analyzed on SPSS 14.0 (SPSS Inc., Chicago, Illinois, USA). Since we know from previous studies that control, Y2–/– and Y4–/– mice differ in their emotional behaviour and susceptibility to the acute behavioural effects of LPS (Painsipp et al., 2008a, 2008b), all data were analyzed by *planned comparisons* (Kirk, 1995) and one-way or two-way analysis of variance (ANOVA). If *planned comparisons* and ANOVA yielded the same results, only those obtained by ANOVA are reported. *Planned comparisons* were made with the *t*-test or one-way ANOVA. Whenever an ANOVA was performed, the homogeneity of variances was assessed with the Levene test. If a significant interaction between the test factors (genotype, treatment) was found, post-hoc analysis of group differences was made with the Tukey HSD (honestly significant difference) test or, in case of inhomogeneity of variances, with the Games-Howell test. Probability values   0.05 were regarded as statistically significant. All data are presented as means ± SEM, n referring to the number of mice in each group.

Results

General observations

As reported previously (Painsipp et al., 2008a), Y2–/– and Y4–/– mice did not have any gross abnormalities, did not exhibit any obvious signs of sensory deficits and appeared healthy. There was, however, a significant difference in the body weight between the three genotypes under study ($F_{(2,79)} = 29.80$, $P < 0.001$). Thus, both Y2–/– (24.5 ± 0.36 g, n = 24) and Y4–/– (23.5 ± 0.41 g, n = 28) animals had a lower body weight than control mice (28.1 ± 0.55 g, n = 30). In none of the experimental groups did the body weight change significantly (P > 0.05) during the course of the experiments.

Effect of LPS on behaviour in the OFT

The behaviour of control, Y2–/– and Y4–/– mice in the OFT was examined 3 h and 4 weeks after IP injection of vehicle or LPS. As can be seen from Figures 1 and 2, LPS had a time- and genotype-related effect on the behaviour in the OFT. In confirmation of previous observations (Painsipp et al., 2008a), vehicle-treated Y2–/– and Y4–/– mice spent nominally more time in the central area of the open field, entered the central area of the open field more often and travelled a longer distance in the open field than vehicle-treated control mice (Figures 1 and 2). This genotype-related difference in vehicle-treated animals was not further analyzed in the current study

OFT 3 h post-treatment—When examined 3 h post-treatment, LPS had a profound effect on all parameters of the OFT (Figure 1). Two-way ANOVA of the time spent in the central area of the open field revealed differences related to treatment ($F(1,35) = 11.59$, $P = 0.002$) but not genotype, with a significant interaction between these factors ($F(2,35) = 3.94$, $P = 0.03$). Specifically, LPS had no significant effect in control mice but shortened the time which Y2–/– and Y4–/– mice spent in the central area (Figure 1A). The effect of LPS was particularly pronounced in Y2–/– mice.

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A similar result was obtained with regard to the number of entries into the central area of the open field (Figure 1B). As shown by two-way ANOVA, the number of central area entries differed with treatment ($F(1,35) = 35.10$, $P < 0.0001$) and genotype ($F(2,35) = 6.16$, $P = 0.005$), and there was a significant interaction between these factors ($F(2,35) = 4.40$, $P = 0.02$). Post-hoc analysis revealed that the number of central area entries was left unaltered by LPS in control mice but significantly diminished in Y2–/– and Y4–/– mice, the effect in Y2–/– mice being particularly pronounced (Figure 1B).

The changes in central area entries observed 3 h post-treatment were paralleled by changes in the total distance travelled in the open field (Figure 1C). *Planned comparisons* indicated that LPS shortened the total distance travelled by Y2–/– and Y4–/– mice but not that travelled by control animals (Figure 1C). The change brought about by LPS was most conspicuous in Y2–/– mice. Two-way ANOVA confirmed that this parameter varied with treatment ($F(1,35) = 22.32$, $P < 0.0001$) and genotype ($F(2,35) = 8.03$, $P = 0.001$), without a significant interaction between these factors ($F(2,35) = 2.72$, $P = 0.08$).

OFT 4 weeks post-treatment—When the OFT was performed 4 weeks post-treatment, the genotype-related pattern in the effect of LPS differed from that seen 3 h post-treatment (Figure 2). *Planned comparisons* revealed that the central area time was shortened in LPS-treated Y4–/–mice, unchanged in LPS-treated Y2–/– mice and nominally, but not significantly, increased in LPS-treated control mice (Figure 2A). This outcome was confirmed by two-way ANOVA inasmuch as the time spent in the central area of the open field differed with genotype ($F(2,35) = 6.48$, $P = 0.004$) but not treatment, without a significant interaction between these factors.

A similar picture emerged with regard to the number of entries into the central area of the open field (Figure 2B). As shown by two-way ANOVA, the number of central area entries differed with treatment ($F(1,35) = 5.80$, $P = 0.021$) and genotype ($F(2,35) = 6.85$, $P = 0.003$), and there was a significant interaction between these factors ($F(2,35) = 8.88$, $P = 0.001$). Specifically, the number of entries into the central area of the open field was reduced in LPS-treated Y4–/–mice, unaltered in LPS-treated Y2–/– mice and nominally, but not significantly, increased in LPS-treated control mice (Figure 2B).

Two-way ANOVA of the total distance travelled in the open field (Figure 2C) indicated that this parameter varied with treatment ($F(1,35) = 6.19$, $P = 0.018$) and genotype ($F(2,35) = 5.79$, $P = 0.007$), and there was a significant interaction between these factors ($F(2,35) = 5.64$, $P = 0.008$). Post-hoc analysis indicated that the total distance travelled by LPS-treated Y4–/– mice was shorter than that travelled by vehicle-treated Y4–/– mice, whereas the distance travelled by control and Y2–/– mice was not affected by LPS (Figure 2C).

Effect of LPS on behaviour in the FST

The behaviour of control, Y2–/– and Y4–/– mice in the FST was determined 24 h and 4 weeks after IP injection of vehicle or LPS. As is depicted in Figures 3 and 4, LPS had a time- and genotype-related influence on the behaviour in the FST. In keeping with previous findings on the tail suspension test (Painsipp et al., 2008a), we found that the time which vehicle-treated Y2–/– and Y4–/– mice spent immobile in the FST was significantly less

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than that spent immobile by vehicle-treated control mice (Figures 3 and 4). This genotype-related difference in vehicle-treated animals was not further analyzed in the current study.

FST 24 h post-treatment—When evaluated 24 h post-treatment, LPS had an appreciable effect on the time of immobility and swimming in the FST (Figure 3). Two-way ANOVA of the time spent immobile disclosed differences related to treatment ($F(1,34) = 7.82$, $P = 0.008$) and genotype ($F(2,34) = 29.53$, $P < 0.0001$), with a significant interaction between these factors ($F(2,34) = 4.96$, $P = 0.013$). Specifically, LPS increased the time of immobility in control and Y2–/– mice, but not in Y4–/– animals (Figure 3A).

A mirror-like result was obtained with regard to the time of swimming (Figure 3B). *Planned comparisons* showed that LPS shortened this parameter in control mice, reduced it nominally, but not significantly, in Y2–/– mice, and did not alter it in Y4–/– animals (Figure 3B). Two-way ANOVA confirmed that the time of swimming differed with treatment ($F(1,34) = 5.08$, $P = 0.031$) and genotype ($F_{(2,34)} = 9.76$, $P < 0.0001$), without a significant interaction between these factors.

The third parameter analyzed in the FST was the time of climbing (Figure 3C). Planned comparisons disclosed that LPS shortened the time of climbing nominally, but not significantly, in all three genotypes under study (Figure 3C). However, two-way ANOVA of this parameter indicated differences with regard to treatment ($F_{(1,34)} = 7.31$, $P = 0.011$) and genotype ($F_{(2,34)} = 16.88$, $P < 0.0001$), without a significant interaction between these factors.

FST 4 weeks post-treatment—When the FST was performed 4 weeks post-treatment, the genotype-related pattern in the effect of LPS differed from that seen 1 day post-treatment (Figure 4). As shown by two-way ANOVA, the time of immobility differed with treatment ($F(1,34) = 13.39$, $P = 0.001$) and genotype ($F(2,34) = 5.20$, $P = 0.011$), and there was a significant interaction between these factors ($F(2,34) = 3.90$, $P = 0.03$). Post-hoc analysis disclosed that the time of immobility was enhanced in LPS-treated Y2–/– and Y4–/– animals, but unchanged in LPS-treated control mice (Figure 4A).

When the time of swimming (Figure 4B) was analyzed by two-way ANOVA, differences related to treatment ($F(1,34) = 8.32$, $P = 0.007$) and genotype ($F(2,34) = 4.38$, $P = 0.02$) and a significant interaction between these factors ($F(2,34) = 5.25$, $P = 0.01$) emerged. Specifically, the time of swimming was shortened in LPS-treated Y4–/– mice but left unaltered in LPS-treated control and Y2–/– mice (Figure 4B).

Analysis of the time of climbing (Figure 4C) with two-way ANOVA indicated differences with regard to treatment ($F(1,34) = 7.23$, $P = 0.011$) and genotype ($F(2,34) = 3.47$, $P = 0.043$) and a significant interaction between these factors ($F(2,34) = 3.98$, $P = 0.028$). Post-hoc analysis showed that the time of climbing was very much shortened in LPS-treated Y2–/– mice but unchanged in LPS-treated control and Y4–/– mice (Figure 4C).

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Discussion

The present data show that knockout of Y2 and Y4 receptors alters the effect of LPS-evoked immune stress on anxiety-like and depression-related behaviour not only in the short but also in the long term. It is of particular note that the behavioural changes seen within 1 day post-treatment and those observed 4 weeks post-treatment are profoundly different from each other, depending on the genotype. Specifically, a single challenge of Y4−/− mice with systemic LPS causes a persistent increase in anxiety-like behaviour and a decrease in locomotion, whereas no long-term change of these parameters is seen in control and Y2−/− mice. Conversely, depression-related behaviour is increased in both Y2−/− and Y4−/− mice 4 weeks post-treatment but is unchanged in control mice. It thus emerges that Y2 and Y4 receptors are highly relevant to the long-term impact of LPS on emotional-affective behaviour.

Knockout of the Y2 and Y4 receptor genes has previously been found to attenuate anxiety-related and depression-like behaviour and to increase locomotor activity (Carvajal et al., 2006; Painsipp et al., 2008b; Redrobe et al., 2003; Tasan et al. 2009; Tschenett et al., 2004). This behavioural phenotype of Y2−/− and Y4−/− mice was essentially confirmed in the present study, although the behavioural differences between control and knockout animals were less pronounced. This observation could be due to the fact that, unlike in the previous studies, all animals received an IP injection of vehicle or LPS before they were subjected to behavioural testing. It cannot be disregarded that this stress experience may have had a differential impact on the behavioural phenotype of control, Y2−/− and Y4−/− mice. Due to the limited supply of Y2−/− and Y4−/− mice, each animal was subjected to two behavioural tests at a 21 h interval. In this respect another potentially confounding factor need be considered, namely, that the stress associated with a behavioural test can influence the outcome of a subsequent behavioural test, as has been observed in Y1−/− mice (Painsipp et al., 2009).

To be consistent with our preceding studies on Y2−/− and Y4−/− mice (Painsipp et al., 2008a, 2008b), the current experiments were carried out with female animals. Although the estrus cycle was not determined, we consider it unlikely that our data were biased by this potentially confounding factor, as discussed previously (Painsipp et al., 2008b). We have found that anxiety-related behaviour does not vary significantly with the different phases of the estrus cycle which is synchronized not only among cage mates but also across cages (Painsipp et al., 2007).

The systemic effects of LPS are initiated by activation of Toll-like receptor-4, which triggers the release of interleukin-1β, interleukin-6 and tumor necrosis factor-α from phagocytic cells (Dantzer, 2006). These proinflammatory cytokines alter behaviour both via a humoral route and excitation of vagal afferent neurons (Goehler et al., 2000, 2007; Konsman et al., 2002), and the type, magnitude and mechanism of the behavioural reaction depends on the dose of LPS (Mormede et al., 2004; Romanovsky et al., 2005).

We have previously found that a dose of LPS (0.1 mg/kg) smaller than that used here attenuates central area time, central area entries and locomotion in Y2−/− mice subjected to

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the OFT within 3 h post-treatment, whereas the OFT parameters of control and Y4–/– animals remain unchanged by LPS (Painsipp et al., 2008a). From these data it has been concluded that Y2–/– mice are particularly susceptible to the behavioural effects of immune stress (Painsipp et al., 2008a). This conjecture was confirmed here with a higher dose of LPS (0.83 mg/kg) which also diminished the OFT parameters in Y4–/– mice, although to a lesser degree than in Y2–/–mice. Since the decrease in central area time and central area entries in Y2–/– and Y4–/– mice was associated with a decrease in locomotion, we cannot rule out that the apparently anxiogenic-like response to LPS reflected suppression of locomotor activity. The absence of any anxiogenic-like effect of LPS in our control mice, which have a mixed C57BL/6–129/SvJ background, is at variance with observations made in CD-1 mice, although the increase of anxiety-like behaviour seen in this mouse strain may also reflect attenuated locomotion rather than enhanced anxiety (Lacosta et al., 1999; Swiergiel and Dunn, 2007). It need be emphasized, however, that the control mice used in the present study show an acute sickness response to LPS as judged by an increase in rectal temperature and an attenuation of social interaction (Painsipp et al., 2008a).

The effect of LPS to reduce the OFT parameters in Y4–/– mice was of long duration and still evident 4 weeks post-treatment, at which time anxiety- and locomotion-related behaviour in LPS-treated Y2–/– mice was no longer different from that of vehicle-treated Y2–/– mice. Two important conclusions can be drawn from these findings. First, a single exposure to immune stress can lead to long-term alterations of behavioural parameters related to anxiety and locomotion. Second, these long-term alterations are seen only after Y4 receptors have been knocked out, which suggests that NPY acting via Y4 receptors normally suppresses the ability of immune stress to cause a long-term change of OFT parameters.

The observation that immobility in the FST was enhanced 1 day post-LPS in our control animals is in keeping with the effect of LPS, at the same dose, to increase the immobility of CD1 mice in both the FST and tail suspension test 24 h post-treatment (Frenois et al., 2007). An LPS-evoked increase in immobility of CD-1 mice in the FST and tail suspension test has also been reported by Jain et al. (2001) and Dunn and Swiergiel (2005). While the LPS-induced prolongation of immobility in the CD1 mice occurred at the expense of swimming time (Frenois et al., 2007), the LPS-induced increase in the immobility time of our control mice resulted from a decrease in both the swimming and climbing time. The short-term increase in immobility which LPS induced in the control animals was also seen in Y2–/– mice, but absent in Y4–/– mice. It would hence seem that the short term effect of LPS to enhance depression-like behaviour involves NPY acting via Y4 receptors. Deletion of these receptors abrogates the ability of immune stress to enhance the immobility time in the FST.

The action of LPS to increase depression-related behaviour in the control mice was gone 4 weeks post-treatment. At this time point, however, the immobility time in the FST was significantly enhanced in LPS-treated Y2–/– and Y4–/– mice. These findings prompt two important conclusions. First, a single exposure to immune stress can lead to a long-term enforcement of depression-related behaviour. Second, these long-term alterations are seen only after Y2 or Y4 receptors have been knocked out, which suggests that NPY acting via Y2 or Y4 receptors normally prevents the ability of immune stress to evoke a long-term change of FST parameters. It is worth noting that the pattern of FST parameter alterations

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seen 4 weeks post-treatment differed between LPS-treated Y2–/– and Y4–/– mice. While the increase in immobility of Y2–/–mice occurred at the expense of climbing, the immobility of Y4–/– mice was due to decreased swimming. We deduce from these observations that different neurochemical mechanisms underlie the long-term behavioural changes in LPS-treated Y2–/– and Y4–/– mice, and base this conclusion on an analogy with the behavioural effects of two classes of antidepressant drugs. Thus, antidepressants that act primarily by enforcing serotonergic transmission increase predominantly swimming behaviour, whereas antidepressants that act primarily by enforcing noradrenergic transmission increase predominantly climbing behaviour (Cryan et al., 2005; Detke et al., 1995).

There is ample evidence that short-term treatment of rodents with LPS or cytokines evokes a pattern of depression-like behaviour (Anisman et al., 2005; Dunn et al., 2005; Frenois et al., 2007; Tonelli et al., 2008). Cytokines are also known to induce a depression-like syndrome in humans that lasts for several weeks (Anisman et al., 2005; Capuron et al., 2000, 2001; Maes, 2008), and it has been speculated that increased translocation of LPS across the wall of the colon may contribute to the establishment of depressive symptoms (Maes, 2008). The current data show for the first time that a single challenge with LPS also leads to long-term changes in emotional-affective behaviour in mice and that these long-term changes are unmasked by deletion of Y2 and/or Y4 receptors. These findings raise the point that a single LPS injection in mice deficient in Y2 and/or Y4 receptors could be a valuable model to study long-term disturbances in anxiety and mood evoked by infection, inflammation or cytokine therapy. Based on our observation it may be speculated that the adverse effect of LPS administration and cytokine therapy to induce depression in some but not all subjects (Capuron et al., 2000, 2001; Maes, 2008; Reichenberg et al., 2001) is related to a dysfunction of the NPY system, given that the expression of NPY in humans is relevant to stress resilience (Zhou et al., 2008).

The ability of Y2 and Y4 knockout to unmask long-term changes in emotional-affective behaviour induced by LPS could reflect the presynaptic location of Y2, and possibly Y4, receptors in the brain. Knockout of presynaptic NPY receptors with a negative feedback role will disinhibit processes that normally are under negative control. Although the impact of immune challenge on central NPY neurons has not yet been studied, systemic LPS is known to activate neurons in the extended amygdala, hippocampus and hypothalamus (Frenois et al., 2007; Zhang et al., 2000). These brain regions not only control behaviour related to anxiety and depression, but also contain many NPY neurons and Y2 receptors with a predominantly presynaptic location (Fetissov et al., 2004; Gustafson et al., 1997; Parker and Herzog, 1999; Stanic et al., 2006). In contrast, Y4 receptors have a much more restricted distribution in the rodent brain than Y2 receptors. The role of Y4 receptors in the control of emotional-affective behaviour (Painsipp et al., 2008b; Tasan et al., 2009) may be related to an appreciable expression of Y4 receptors in amygdala, ventral tegmental area, hippocampus, hypothalamus, locus coeruleus and medullary brainstem (Campbell et al., 2003; Fetissov et al., 2004; Parker and Herzog, 1999; Whitcomb et al., 1997).

In summary, the current exploratory study shows that deletion of Y2 or Y4 receptors unveils different acute and long-term effects of a single immune challenge on locomotor, anxiety-

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and depression-related behaviour. Specifically, knockout of Y4 receptors unmasks the ability of a single LPS injection to cause a delayed and prolonged increase in anxiety- and depression-like behaviour. Although developmental compensations in germline gene knockout mice may mask the full implication of Y2 and Y4 receptors, our findings indicate that they were insufficient to balance the deficit in Y2 and Y4 receptors. Our results therefore underscore the conclusion that endogenous NPY has an important bearing on the long-term control of immune signalling to the brain. Specifically, NPY acting via Y2 and Y4 receptors prevents the development of long-term anxiety- and depression-like behaviour caused by acute immune challenge.

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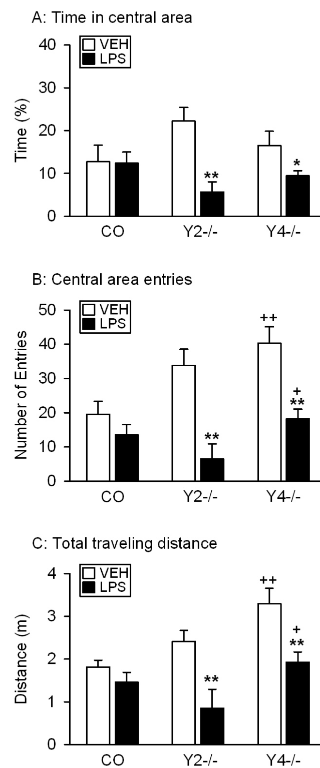

Figure 1.

Effect of LPS on the behaviour of control (CO), Y2–/– and Y4–/– mice in the OFT 3 h post-treatment. LPS (0.83 mg/kg) or vehicle (VEH, sterile saline) was injected IP 3 h before the behavioural test. The graphs show (A) the time spent in the central area, (B) the number of entries into the central area, and (C) the total distance travelled. The time spent in the central area (panel A) is expressed as a percentage of the total time spent in the OF. The values represent means ± SEM, n = 6 – 8. * P 0.05, ** $P < 0.01$ versus vehicle-treated mice of the same genotype, + P 0.05, ++ P 0.01 versus control mice with the same treatment.

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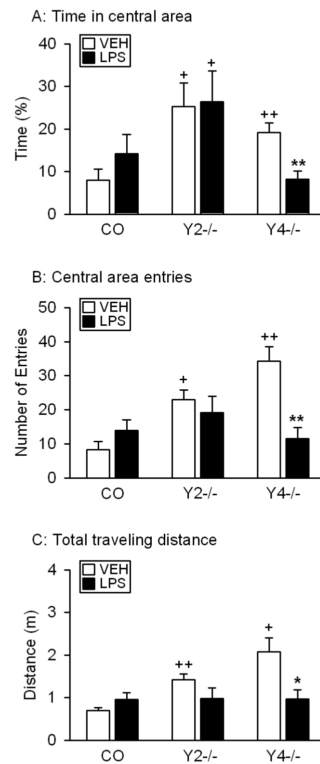

Figure 2.

Effect of LPS on the behaviour of control (CO), Y2–/– and Y4–/– mice in the OFT 4 weeks post-treatment. LPS (0.83 mg/kg) or vehicle (VEH, sterile saline) was injected IP 27 days before the behavioural test. The graphs show (A) the time spent in the central area, (B) the number of entries into the central area, and (C) the total distance travelled. The time spent in the central area (panel A) is expressed as a percentage of the total time spent in the OF. The values represent means ± SEM, n = 6 – 8. * *P*   0.05, ** *P* < 0.01 versus vehicle-treated mice of the same genotype, + *P*   0.05, ++ *P*   0.01 versus control mice with the same treatment.

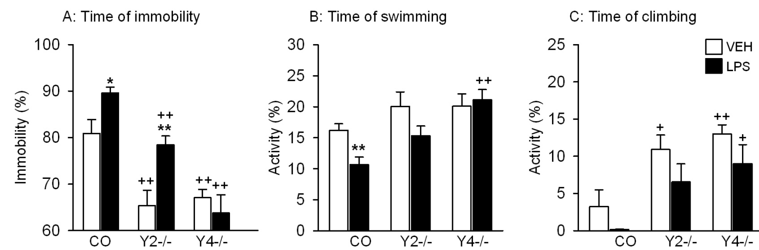

Figure 3.

Effect of LPS on the behaviour of control (CO), Y2−/− and Y4−/− mice in the FST 1 day post-treatment. LPS (0.83 mg/kg) or vehicle (VEH, sterile saline) was injected IP 24 h before the behavioural test. The graphs show (A) the time of immobility, (B) the time of swimming, and (C) the time of climbing, which are expressed as a percentage of the total test duration. The values represent means ± SEM, n = 6 – 8. * P 0.05, ** $P < 0.01$ versus vehicle-treated mice of the same genotype, + P 0.05, ++ P 0.01 versus control mice with the same treatment.

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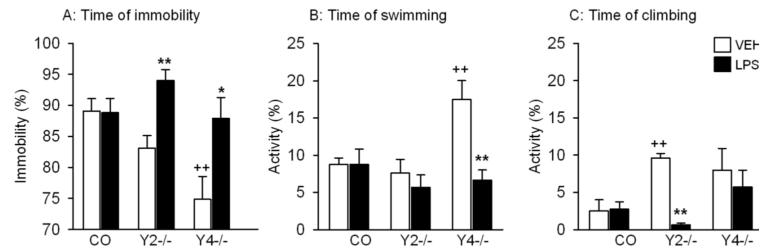

Figure 4.

Effect of LPS on the behaviour of control (CO), Y2–/– and Y4–/– mice in the FST 4 weeks post-treatment. LPS (0.83 mg/kg) or vehicle (VEH, sterile saline) was injected IP 28 days before the behavioural test. The graphs show (A) the time of immobility, (B) the time of swimming, and (C) the time of climbing, which are expressed as a percentage of the total test duration. The values represent means ± SEM, n = 6 – 7. * P 0.05, ** $P < 0.01$ versus vehicle-treated mice of the same genotype, ++ *P* 0.01 versus control mice with the same treatment.

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Table 1

Allocation of the experimental animals to the different treatment and test groups

Genotype	Intraperitoneal treatment	Behavioural testing
Control mice (30)	vehicle (7)	3 h (OFT) and 24 h (FST) post-treatment
	LPS (8)	3 h (OFT) and 24 h (FST) post-treatment
	vehicle (7) 27	d (OFT) and 28 d (FST) post-treatment
	LPS (8)	27 d (OFT) and 28 d (FST) post-treatment
Y2-/- mice (24)	vehicle (6)	3 h (OFT) and 24 h (FST) post-treatment
	LPS (6)	3 h (OFT) and 24 h (FST) post-treatment
	vehicle (6)	27 d (OFT) and 28 d (FST) post-treatment
	LPS (6)	27 d (OFT) and 28 d (FST) post-treatment
Y4-/- mice (28)	vehicle (7)	3 h (OFT) and 24 h (FST) post-treatment
	LPS (7)	3 h (OFT) and 24 h (FST) post-treatment
	vehicle (7)	27 d (OFT) and 28 d (FST) post-treatment
	LPS (7)	27 d (OFT) and 28 d (FST) post-treatment

The numbers in parentheses refer to the number of animals in each group.