Neuropeptides 44 (2010) 233–239

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Acoustic startle response and sensorimotor gating in a genetic mouse model for the Y_1 receptor

T. Karl [a,b,c,*,1], R. Chesworth [a,b,c,1], L. Duffy [a,b], H. Herzog [a]

[a] Neuroscience Research Program, Garvan Institute of Medical Research, Sydney, NSW 2010, Australia
[b] Schizophrenia Research Institute (SRI), Sydney, NSW 2010, Australia
[c] Prince of Wales Medical Research Institute, Sydney, NSW 2031, Australia

ARTICLE INFO

Article history:
Received 30 June 2009
Accepted 11 December 2009
Available online 25 January 2010

Keywords:
Neuropeptide Y
Y1 receptor knockout mouse
Startle habituation
Sensorimotor gating
Prepulse inhibition
Schizophrenia

ABSTRACT

Recent research has highlighted a potential role for neuropeptide Y (NPY) and its Y_1 receptor in the development of schizophrenia. Genetic as well as molecular biological studies have demonstrated reduced levels of NPY in schizophrenia patients. Importantly, Y_1 receptors may mediate some of the potential effects of NPY on schizophrenia, as decreased Y_1 receptor expression has been found in the lymphocytes of schizophrenia patients. To clarify NPY's role in schizophrenia, we investigated a genetic animal model for Y_1 deficiency in regard to (i) acoustic startle response (ASR), (ii) habituation to ASR and (iii) sensorimotor gating [i.e. prepulse inhibition (PPI)] using two different PPI protocols. Mutant and wild type-like mice were screened for baseline behaviours and after pharmacological challenge with the psychotropic drugs dexamphetamine (DEX) and MK-801. Y_1 knockout mice ($Y_1^{-/-}$) showed a moderate reduction of the ASR and an impaired ASR habituation at baseline and after DEX treatment. The baseline PPI performance of Y_1 mutant mice was unaltered their response to DEX and MK-801 challenge was moderately different compared to control mice, which was dependent on the PPI protocol used. MK-801 challenge had a protocol-dependent differential effect in $Y_1^{-/-}$ mice and DEX a more pronounced impact at the highest prepulse intensities. In conclusion, it appears that the Y_1 receptor influences the acoustic startle response and its habituation but does not play a major role in sensorimotor gating. Further explorations into the effects of Y_1 deficiency seem valid.

1. Introduction

Recent research has highlighted a potential role for neuropeptide Y (NPY) in the development of schizophrenia (SCZ). A single nucleotide polymorphism in NPY resulting in decreased neural NPY expression seems associated with an increased susceptibility to SCZ (Itokawa et al., 2003). Furthermore, patients with SCZ exhibit (i) a reduced number of cortical NPY neurons (Ikeda et al., 2004), (ii) lower NPY mRNA levels in the frontal cortex (Kuromitsu et al., 2001) and (iii) decreased NPY protein expression in the cerebral cortex (Gabriel et al., 1996) compared to healthy subjects. NPY expression was also found to be down regulated in the post-mortem tissue of patients with psychosis (Choi et al., 2008). Despite this evidence, the role of NPY in SCZ is controversial: unaltered NPY-like immunoreactivity has been found in the hippocampus and amygdala of SCZ patients (Beal et al., 1987) and no association could be established between a polymorphism in the promoter region of NPY and this illness (Lindberg et al., 2006). Thus, the evidence linking altered NPY expression with SCZ demands further exploration.

The postsynaptically located Y_1 receptor may mediate some of the potential effects of NPY on SCZ, as it is highly expressed in schizophrenia-relevant brain areas [i.e. dentate gyrus and medial amygdala (Kishi et al., 2005)] and decreased Y_1 receptor expression has been found in the lymphocytes of SCZ patients (Vawter et al., 2004). Interestingly, a genetic mouse model for Y_1 receptor deficiency displays – circadian rhythm- and stress-dependent hyperlocomotion (Karl et al., 2006) and increased aggression (Karl et al., 2004). Hyperactivity is a classic feature of animal models of SCZ and can be indicative of exaggerated dopaminergic function, which has been linked to psychosis. Aggression also appears to be associated with SCZ (Sachs, 2006). However, as these behavioural characteristics are not specific for this illness, our study aimed to further examine the relevance of this classic germline Y_1 knockout model for SCZ research.

Prepulse inhibition (PPI) describes the reduction of the startle response to an (acoustic) startle stimulus caused by a preceding

* Corresponding author. Address: Prince of Wales Medical Research Institute, Corner Barker Street and Easy Street, Randwick (Sydney), NSW 2031, Australia. Tel./fax: +61 2 9399 1005.
E-mail addresses: t.karl@schizophreniaresearch.org.au, t.karl@powmri.edu.au (T. Karl).
[1] Authors contributed equally to this work.

0143-4179/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.npep.2009.12.008

prepulse and is a measure of sensorimotor gating. PPI has face, construct and predictive validity for SCZ research: (i) PPI is impaired in SCZ patients (Braff et al., 2001), (ii) drugs stimulating dopaminergic transmission and inhibiting glutamatergic NMDA (*N*-methyl-D-aspartic acid) receptor function impair PPI [in accordance with the dopaminergic hyperstimulation and glutamatergic hypofunction theories of SCZ (Geyer et al., 2001; Varty et al., 2001)] and (iii) PPI can be facilitated by antipsychotic treatment (Geyer et al., 2001). Importantly, laboratory rodents are tested in a similar manner to humans, suggesting a high level of comparability. PPI testing also incorporates measurement of habituation to the acoustic startle response (ASR), which has been shown to be diminished in SCZ patients (Geyer and Braff, 1982). Thus, PPI is suitable to further evaluate the potential of our Y_1 mouse model in SCZ research. We investigated sensorimotor gating of Y_1 depleted mice at baseline as well as after acute pharmacological challenge with the non-competitive NMDA antagonist MK-801 and the catecholaminergic stimulant dexamphetamine as these drugs are known to induce or enhance psychotic symptoms in humans with SCZ, and also impair PPI in rodents (Braff et al., 2001). Our study applied two different PPI protocols, using a fixed and a variable interstimulus interval (ISI), to avoid false positive/negative results as studies have shown a clear impact of the interstimulus interval length and its variation on the prepulse inhibition performance (Swerdlow et al., 2000; Varty et al., 2001; Wang et al., 2003).

2. Methods

2.1. Animals

Germline Y_1 receptor knockout mice were generated as described earlier (Howell et al., 2003). In short, a targeting vector for the Y_1 receptor gene was designed and generated (based on 129/SvJ mouse genomic BAC library), which allowed the production of both conditional and germline ($Y_1^{-/-}$) knockout mice. Chimeras carrying the Y_1 floxed gene were crossed with oozyte-specific Cre-recombinase-expressing C57BL/6 Arc mice in order to obtain heterozygotes carrying the Cre-recombinase gene and having the floxed gene already deleted (germline $Y_1^{-/+}$). Absence of the Y_1 gene in homozygote germline $Y_1^{-/-}$ mice was confirmed by Southern analysis employing a Y_1 receptor coding sequence-specific DNA fragment and PCR. All further mice generated were maintained on the mixed C57BL/6-129/SvJ background. For the germline Y_1 receptor knockouts, animals no longer carrying the Cre-transgene were selected. Consistency of various phenotypes has been confirmed in Y_1 knockout and wild type-like animals over more than 10 generations.

Age-matched male mice were group housed in polysulfone cages (Tecniplast, Rydalmere, Australia) under a 12:12 h white light:red light cycle (light phase: 80 l× – red phase: <2 l×). Cages were equipped with a metal ring in the lid (3 cm in diameter) and cellulose paper for nesting material. Different age-matched cohorts of adult wild type-like (WT) bred from the same colony as Y_1 knockout mice and germline Y_1 knockout mice ($Y_1^{-/-}$) were tested. All research and animal care procedures were approved by the "Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee" and in accordance with the "Australian Code of Practice for the Care and Use of Animals for Scientific Purposes".

2.2. Drugs

Dexamphetamine (DEX) and MK-801 (Sigma–Aldrich, Sydney, Australia) were dissolved in 0.9% saline solution. Doses tested were 5 mg/kg for DEX and 0.5 mg/kg for MK-801 respectively, as these doses have been reported to induce PPI deficits in mice in other laboratories [DEX: (van den Buuse et al., 2005); MK-801: (Yee et al., 2004)]. 0.9% saline was used as vehicle. Injection volume was 1 ml/100 g administered intraperitoneally 15 min prior to testing.

2.3. Sensorimotor gating (prepulse inhibition, PPI)

PPI was tested during the light phase using an automated startle system (SR-Lab: San Diego Instruments, San Diego, USA). Following habituation to the device and animal enclosure (5 min for three days), animals were tested using an inter-session interval of seven days. The animal enclosures were cleaned with 70% ethanol between animals. Two different sets of test mice were used in the two PPI protocols (both including a 5 min acclimatisation period with a 70 dB background noise: protocol A: $n = 9$–11 per genotype – protocol B: $n = 12$–15). Mice of both genotypes were tested in a quasi-randomized order on each test day.

(A) Fixed ISI (i.e. 80 ms): pseudo-randomized order of 10 × 90 dB acoustic startle response (ASR) trials, 18 × 120 dB ASR trials, two prepulse alone trials (prepulse intensities of 74/78/82/86 dB), eight PPI response trials [prepulse followed 80 ms later by 120 dB startle pulse], and eight no pulse trials (background noise only). Mice received saline treatment in the first session followed by 0.5 mg/kg MK-801 seven days later and 5 mg/kg DEX treatment in the last session.

(B) Variable ISI (i.e. 32–512 ms): the protocol started with five 120 dB startle pulses after which four startle pulses (70/80/100/120 dB) were presented five times each in a pseudo-randomized order. Afterwards 75 PPI response trials (prepulse intensities of 74/82/86 dB followed by a 120 dB startle pulse) were presented five times in a quasi-randomized order employing five different ISIs (32/64/128/256/512 ms) followed by a final five 120 dB startle pulses. Mice in protocol B were tested in a counterbalanced manner: genotypes were represented equally in each treatment condition during all three test sessions (inter-session interval of at least seven days).

For both protocols, startle response was measured as the average amplitude of the startle. The intertrial interval was 15 s (averaged over 10–20 s); the duration of the prepulse was 20 ms and 40 ms for the startle. Percentage of PPI (%PPI) was calculated as [(ASR 120 dB – prepulse response) × 100/ASR 120 dB]. The %PPI for the different prepulse intensities (protocol B) was calculated. Habituation to the 120 dB startle pulse was assessed for protocol B by comparing the first five 120 dB ASRs with the middle and last five ASRs.

2.4. Statistical analyses

Data were analysed using four-way, three-way or two-way repeated measures ANOVAs (software: SPSS 16.0). The within-group repeated measures factors were 'treatment' (saline versus DEX versus MK-801), 'prepulse intensity' (74–86 dB), 'interstimulus interval' (for protocol B: 32–512 ms) and 'time' (for startle habituation in protocol B: averaged first versus middle versus last ASR five startle responses). The between-group factor was 'genotype' (WT versus $Y_1^{-/-}$). Where appropriate, one-way ANOVA split by corresponding factors followed. Bonferroni-corrected simple contrasts were used to compare the specific effects of DEX or MK-801 versus saline treatment. Differences were regarded as statistically significant if $p < 0.05$ ($p < 0.025$ for Bonferroni-corrected simple contrasts). Data are presented as means + SEM. Significant genotype effects are indicated by asterisks (versus WT; $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$). Significant treatment effects are

indicated by "#" (versus saline; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$), trends are indicated by "∼" ($\sim p = 0.03$).

3. Results

3.1. Startle response

Two-way repeated measures (RM) ANOVA (factors: 'genotype' and 'treatment') on the overall acoustic startle response (ASR) in protocol A revealed a significant main effect for the factor 'treatment' [$F(2,38) = 16.2$, $p < 0.0001$]. This was confirmed using one-way ANOVA split by 'genotype' for both WT mice [$F(2,16) = 6.7$, $p = 0.008$] and $Y_1^{-/-}$ mice [$F(2,22) = 12.1$, $p < 0.0001$]. However, Bonferroni-corrected simple contrasts revealed that only MK-801 but not DEX treatment increased ASR significantly in both genotypes (Fig. 1A). A significant main effect for the factor 'genotype' [two-way RM ANOVA: $F(1,19) = 9.4$, $p = 0.006$] was confirmed using one-way ANOVA split by 'treatment' [saline: $F(1,19) = 9.5$, $p = 0.006$ – DEX: $F(1,19) = 11.9$, $p = 0.003$ – MK-801: $F(1,19) = 6.1$, $p = 0.02$] with $Y_1^{-/-}$ mice exhibiting significantly decreased ASR (Fig. 1A).

Using protocol B, two-way RM ANOVA revealed a significant main effect for 'treatment' [$F(2,44) = 24.9$, $p < 0.0001$], which was evident in both genotypes [one-way ANOVAs split by 'genotype': WT: $F(2,16) = 20.8$, $p < 0.0001$ – $Y_1^{-/-}$: $F(2,28) = 8.9$, $p = 0.001$]. Similar to protocol A, Bonferroni-corrected simple contrasts revealed that only MK-801 significantly increased ASR performance (Fig. 1B). There was a trend for a main effect of 'genotype' on ASR [two-way RM ANOVA: $F(1,22) = 4.9$, $p = 0.06$], with $Y_1^{-/-}$ mutant mice showing a reduced ASR (Fig. 1B).

3.2. Startle habituation

All mice habituated to the startle stimulus [three-way RM ANOVA – factor 'time': $F(2,44) = 23.4$, $p < 0.0001$] regardless of the factors 'genotype' and 'treatment'. Two-way RM ANOVA split by the factor 'genotype' confirmed that habituation had occurred in both WT [$F(2,16) = 11.9$, $p = 0.001$] and $Y_1^{-/-}$ mice [$F(2,28) = 8.4$,

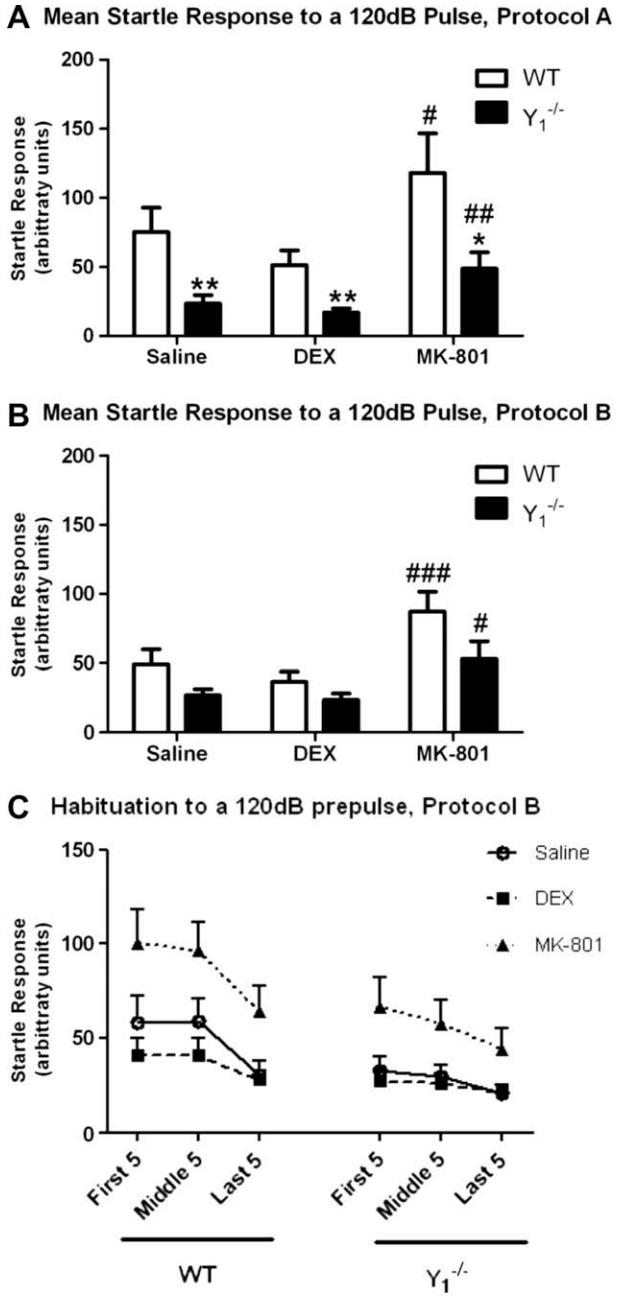

Fig. 1. (A–C) The acoustic startle response (ASR: averaged startle amplitude in arbitrary units) and the habituation to ASR: (A) ASR to a 120 dB pulse using protocol A (fixed interstimulus intervals, ISI), (B) ASR to a 120 dB pulse using protocol B (variable ISI) and (C) habituation to the 120 dB startle stimulus using protocol B ['time' by 'genotype' interaction – one-way ANOVAs split by the factors 'genotype' and 'treatment' revealed a significant habituation of the acoustic startle response for WT mice (all treatment groups) and $Y_1^{-/-}$ after MK-801 treatment only]. Data are presented as means + SEM. Significant genotype effects are indicated by asterisks (versus WT; $^{*}p < 0.05$ and $^{**}p < 0.01$). Significant treatment effects are indicated by "#" (versus saline; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$).

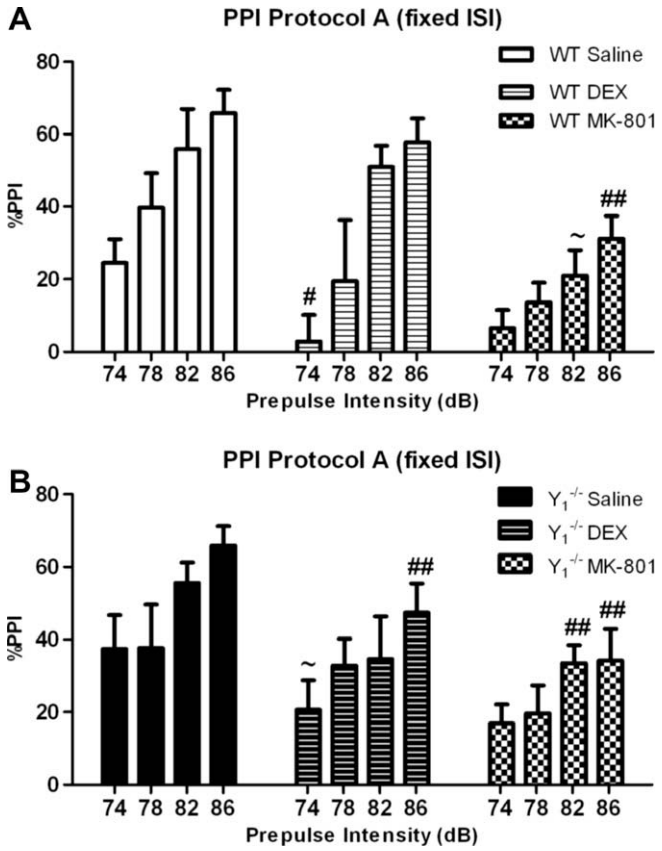

Fig. 2. (A and B) Percentage prepulse inhibition (%PPI) for different prepulse intensities (74–86 dB) of WT (A) and $Y_1^{-/-}$ (B) mice for protocol A. Data are presented as means + SEM. Significant Bonferroni-corrected simple contrast effects are indicated by "#" (versus saline; $^{\#}p < 0.025$ and $^{\#\#}p < 0.01$), trends are represented by "∼" ($\sim p = 0.03$).

$p = 0.001$]. Drug treatment impacted significantly on the habituation to ASR [three-way RM ANOVA main effect of 'treatment': $F(2,44) = 24.9$, $p < 0.0001$] and this was evident in both genotypes [two-way RM ANOVAs split by 'genotype': WT: $F(2,16) = 20.8$, $p < 0.0001$ – $Y_1^{-/-}$: $F(2,28) = 8.7$, $p = 0.001$]. Importantly, three-way RM ANOVA also detected a significant 'time' by 'genotype' interaction [$F(2,44) = 3.5$, $p = 0.04$] (Fig. 1C). Whereas WT mice habituated over time under all treatment conditions [RM ANOVA split by 'genotype' and 'treatment': saline: $F(2,18) = 7.9$, $p = 0.003$ – DEX: $F(2,20) = 5.2$, $p = 0.02$ – MK-801: $F(2,20) = 6.9$, $p = 0.007$], the habituation of $Y_1^{-/-}$ mice to ASR was disrupted under saline and DEX conditions [RM ANOVA split by 'genotype' and 'treatment': saline: $F(2,28) = 3.0$, $p = 0.07$ – DEX: $F(2,28) = 1.9$, $p = 0.2$ – MK-801: $F(2,28) = 10.8$, $p < 0.0001$] (Fig. 1C).

3.3. Sensorimotor gating (PPI)

3.3.1. Effects of the factor 'prepulse intensity' on %PPI

In protocol A, three-way RM ANOVA (factors: 'genotype', 'treatment' and 'prepulse intensity') found a significant main effect for the factor 'prepulse intensity' [$F(3,57) = 53.5$, $p < 0.0001$], which was evident in both genotypes [two-way RM ANOVA split by 'genotype': WT: $F(3,24) = 21.4$, $p < 0.0001$ – $Y_1^{-/-}$: $F(3,33) = 36.7$, $p < 0.0001$]. As expected, increased prepulse intensities were associated with elevations in %PPI (Fig. 2A and B). No interactions were found.

Protocol B confirmed these findings: there was a significant main effect for 'prepulse intensity' [four-way RM ANOVA: $F(2,48) = 91.6$, $p < 0.0001$], as increasing prepulse intensities were

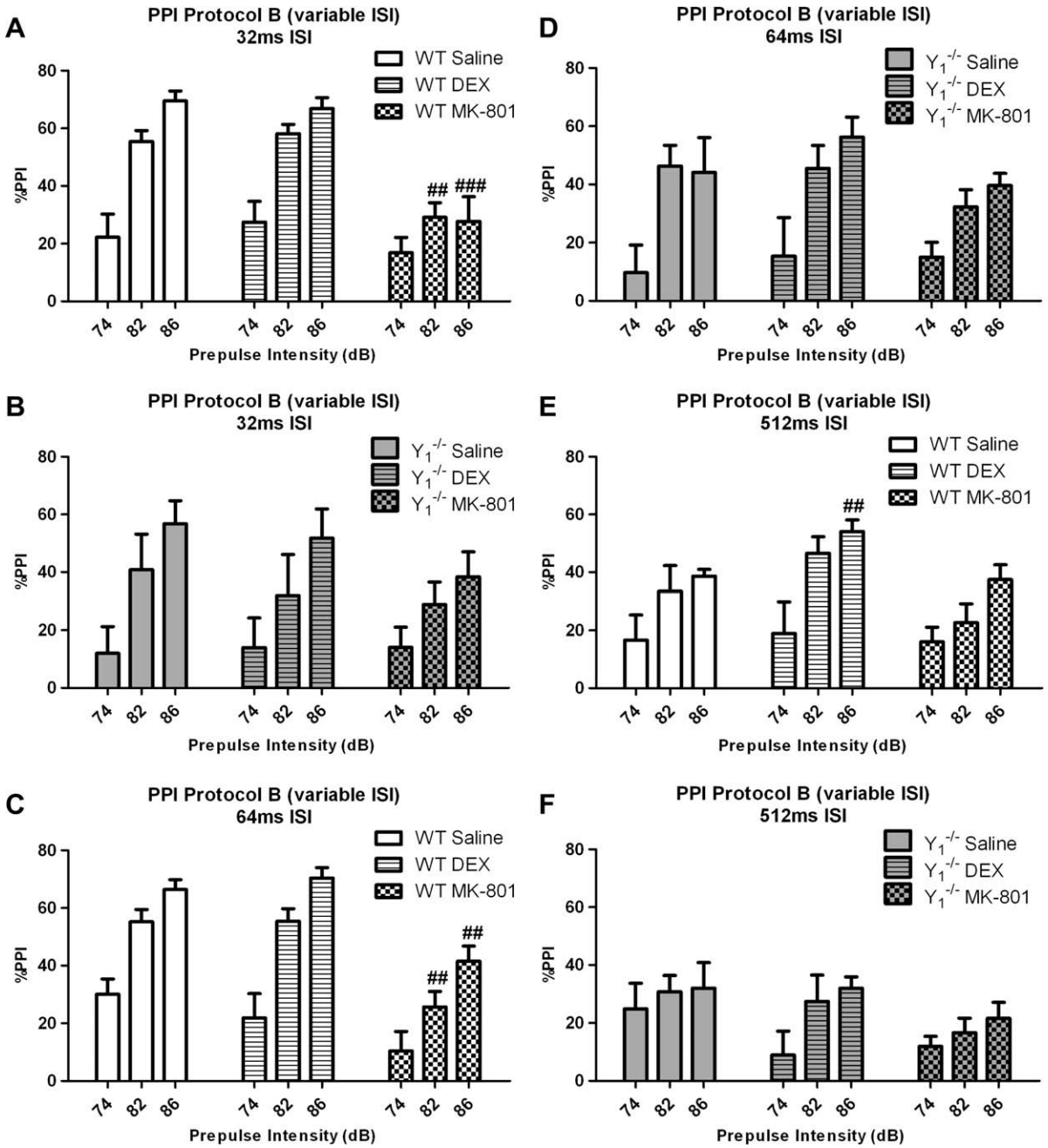

Fig. 3. (A–F) Percentage prepulse inhibition (%PPI) of WT (A, C, and E) and $Y_1^{-/-}$ (B, D, and F) mice for protocol B – displayed for interstimulus intervals of 32 ms (A and B), 64 ms (C and D) and 512 ms (E and F). Data are presented as means + SEM. Significant Bonferroni-corrected simple contrast effects are indicated by "#" (versus saline; $^{\#}p < 0.025$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$), trends are represented by "∼" ($\sim p = 0.03$).

correlated with augmented %PPI. Three-way RM ANOVA split by 'genotype' confirmed that this effect was independent of the genotype [WT: $F(2,16) = 46.1$, $p < 0.0001$ – $Y_1^{-/-}$: $F(2,28) = 51.3$, $p < 0.0001$] and also detected a significant 'prepulse intensity' by 'treatment' interaction for WT mice [$F(4,32) = 4.5$, $p = 0.006$]. Two-way RM ANOVA split by 'genotype' and 'treatment' confirmed a significant effect of 'prepulse intensity' under all conditions (all $p < 0.0001$). The effect of 'prepulse intensity' was also independent of the ISI used [three-way RM ANOVA split by 'ISI': 32 ms: $F(2,44) = 42.2$, $p < 0.0001$ – 64 ms: $F(2,44) = 44.2$, $p < 0.0001$ – 128 ms: $F(2,44) = 19.7$, $p < 0.0001$ – 256 ms: $F(2,44) = 17.0$, $p < 0.0001$ – 512 ms: $F(2,44) = 16.7$, $p < 0.0001$] (partially shown in Fig. 3A–F).

3.3.2. Effects of the factor 'interstimulus interval' (ISI) on %PPI using protocol B

In protocol B, four-way RM ANOVA (factors 'interstimulus interval', 'prepulse intensity', 'treatment' and 'genotype') detected a significant main effect of 'ISI' [$F(4,96) = 9.6$, $p < 0.0001$] on sensorimotor gating of mice and a significant interaction between 'ISI' and 'prepulse intensity' [$F(8,176) = 3.7$, $p < 0.0001$] as well as a trend for an 'ISI' and 'genotype' interaction [$F(4,88) = 2.2$, $p = 0.08$]. Three-way RM ANOVA split by the factor 'prepulse intensity' found a significant impact of 'ISI' on %PPI [82 dB: $F(4,88) = 7.8$, $p < 0.0001$ – 86 dB: $F(4,88) = 10.4$, $p < 0.0001$]. Longer ISIs produced lower %PPI compared to shorter ISIs of 32–64 ms, which generated the most potent PPI performance in mice (data are shown for saline condition – Table 1).

3.3.3. Effects of the factors 'genotype' and 'treatment' on %PPI

In protocol A, three-way RM ANOVA (factors 'genotype', 'treatment' and 'prepulse intensity') did not reveal a significant main effect of the factor 'genotype' [$F(1,19) = 0.2$, $p = 0.7$]. However, drug treatment influenced PPI performance [three-way RM ANOVA for the factor 'treatment': $F(2,38) = 17.1$, $p < 0.0001$]. This significant main effect of 'treatment' was detected in both genotypes [two-way RM ANOVA split by 'genotype': WT: $F(2,16) = 10.4$, $p = 0.001$ – $Y_1^{-/-}$: $F(2,22) = 7.5$, $p = 0.003$]. Two-way ANOVA split by 'prepulse intensity' exhibited a significant 'treatment' effect at all prepulse intensities [74 dB: $F(2,38) = 6.5$, $p = 0.004$ – 78 dB: $F(2,38) = 4.5$, $p = 0.02$ – 82 dB: $F(2,38) = 8.9$, $p = 0.001$ – 86 dB: $F(2,38) = 21.7$, $p < 0.0001$]. One-way ANOVAs split by the factors 'genotype' and 'prepulse intensity' revealed significant PPI-inhibiting treatment effects at prepulse intensities of 74 dB [WT: $F(2,16) = 4.2$, $p = 0.03$ – trend for $Y_1^{-/-}$: $F(2,22) = 3.1$, $p = 0.07$], 82 dB [WT: $F(2,16) = 6.9$, $p = 0.007$ – $Y_1^{-/-}$: $F(2,22) = 3.9$, $p = 0.04$] and 86 dB [WT: $F(2,16) = 13.6$, $p < 0.0001$ – $Y_1^{-/-}$: $F(2,22) = 10.2$, $p = 0.001$]. Bonferroni-corrected simple contrasts indicated that, in WT mice, DEX was only effective at reducing %PPI at 74 dB whereas MK-801 showed its strongest impact at 82 and 86 dB (Fig. 2A). Importantly, $Y_1^{-/-}$ mice appeared more susceptible to drug-induced impairment of sensorimotor gating as both DEX and MK-801 were potent %PPI inhibitors at the higher prepulse intensities of 82 (MK-801) and 86 dB (DEX and MK-801) (Fig. 2B).

Using protocol B, four-way RM ANOVA for factors 'genotype', 'treatment', 'prepulse intensity' and 'interstimulus interval' detected no main effect of 'genotype' on the PPI performance of mice [$F(1,22) = 0.4$, $p = 0.5$] (Fig. 3A–F). However, the factor 'treatment' had a significant main effect on %PPI [$F(2,48) = 3.9$, $p = 0.03$]. Three-way RM ANOVA split by 'prepulse intensity' confirmed an effect of 'treatment' for the higher prepulse intensities of 82 dB [$F(2,44) = 4.6$, $p = 0.02$] and 86 dB [$F(2,44) = 7.6$, $p = 0.001$] but not 74 dB [$F(2,44) = 0.7$, $p = 0.5$]. Furthermore, drug treatment was only effective in WT control mice [three-way ANOVA split by 'genotype': WT: $F(2,16) = 8.5$, $p = 0.003$ – $Y_1^{-/-}$: $F(2,28) = 1.2$, $p = 0.3$]. Two-way RM ANOVAs split by 'prepulse intensity' and 'genotype' confirmed significant drug effects for WT mice at 82 dB [$F(2,16) = 7.8$, $p = 0.004$] and 86 dB [$F(2,16) = 18.1$, $p < 0.0001$]. One-way RM ANOVA for 'treatment' split by all other factors revealed that 'treatment' had a significant effect in WT mice at ISIs of 32 ms [82 dB: $F(2,16) = 21.5$, $p < 0.0001$ – 86 dB: $F(2,16) = 16.6$, $p < 0.0001$], 64 ms [82 dB: $F(2,16) = 18.6$, $p < 0.0001$ – 86 dB: $F(2,16) = 20.2$, $p < 0.0001$] and 512 ms [82 dB: $F(2,16) = 4.0$, $p = 0.04$ – 86 dB: $F(2,16) = 9.1$, $p < 0.002$] (Fig. 3A–F). Bonferroni-corrected simple contrasts showed that MK-801 exhibited a potent PPI-inhibiting potential in WT mice whereas DEX improved PPI performance of control mice under one particular condition (i.e. at 86 dB using an ISI of 512 ms) (Fig. 3A–F).

Table 1
Percentage prepulse inhibition for different interstimulus intervals.

Prepulse intensity (dB)	ISI (ms)	WT (%PPI)	$Y_1^{-/-}$ (%PPI)
74	32	22.3 + 8.0	11.9 + 9.3
	64	30 + 5.3	9.7 + 9.5
	128	8.4 + 11.8	23.6 + 7.2
	256	18.5 + 6.2	19.9 + 7.3
	512	16.7 + 8.6	24.9 + 8.9
82	32	55.4 + 3.8	40.9 + 12.3
	64	55.2 + 4.3	46.3 + 7.1
	128	35.6 + 7.5	50.6 + 4.9
	256	25.8 + 10.3	24.5 + 7.4
	512	33.4 + 8.9	30.8 + 5.6
86	32	69.5 + 3.5	56.8 + 8.0
	64	66.5 + 3.4	44.1 + 11.9
	128	47.1 + 6.1	49.0 + 6.0
	256	41.5 + 5.4	42.7 + 5.8
	512	38.6 + 2.3	32.0 + 8.8

Percentage prepulse inhibition (%PPI) for the various interstimulus intervals and prepulse intensities (protocol B) tested under saline conditions. Data are presented as means + SEM.

4. Discussion

Results from our experiment demonstrate that $Y_1^{-/-}$ mice exhibit a reduced acoustic startle response to a 120 dB startle stimulus and deficits in habituation to this stimulus compared to WT mice. However, Y_1 deficient mice show unaltered sensorimotor gating at baseline and a PPI protocol-dependent differential response to the effects of DEX and MK-801 compared to controls.

We assessed the ASR and its habituation in $Y_1^{-/-}$ mice, as patients with SCZ have demonstrated startle habituation deficits with no alterations to baseline startle (Geyer and Braff, 1982). Overall, $Y_1^{-/-}$ mice showed impaired ASR habituation compared to WT mice but exhibited a similarly increased ASR response to MK-801. Mutant mice exhibited startle habituation deficits at baseline, i.e. after saline treatment, and after treatment with DEX. The lack of habituation to DEX-induced ASR of Y_1 deficient mice was not affected by genotype-specific differences in DEX-induced ASR. However, since $Y_1^{-/-}$ mice exhibited a lower overall startle response than WT mice, it is possible that floor effects have masked the habituation to the acoustic stimulus at baseline. Furthermore, $Y_1^{-/-}$ mice demonstrated startle habituation after administration of MK-801, which increased ASR, confirming that low ASR levels may have impacted on ASR habituation differences. Thus, it is unclear whether $Y_1^{-/-}$ mice model the startle habituation deficits in patients with SCZ. It is possible that the decreased ASR in $Y_1^{-/-}$ mice tested in protocol A reflects anxiolytic-like behaviour [i.e. ASR is modified by aversive events and administration of anxiogenic/anxiolytic drugs (Koch, 1999)] thereby confirming the circadian rhythm-dependent anxiolytic-like phenotype described for Y_1 depleted mice during the light phase (Karl et al., 2006). Different

knockout models for NPY itself and its two main receptors Y_1 and Y_2 have been investigated regarding their potential for anxiety research (Bannon et al., 2000; Karl et al., 2006; Tschenett et al., 2003). Out of these the Y_2 knockout model appears the most relevant for anxiety research as it does not show any context-dependency nor corresponding changes to motor activity. Previous research has also shown compensatory changes in Y_2 receptor expression being induced by germline deletion of the Y_1 receptor in structures like the amygdala and hippocampus (Wittmann et al., 2005). It is possible that such alterations of Y_2 receptor expression in the Y_1 knockout model may influence some of the behavioural aspects of these mice, particularly in anxiety related domains. Further studies using conditional Y_1 knockout strategies will help to address this issue.

As expected, increasing prepulse intensities and shorter interstimulus intervals resulted in stronger prepulse inhibition, confirming that both PPI protocols worked well. Sensorimotor gating deficits have been demonstrated in patients with SCZ (but not exclusively). It was hypothesized that the Y_1 receptor might modulate sensorimotor gating, in accordance with its role in the modulation of SCZ-relevant behaviours such as aggressive behaviour and hyperlocomotion (Karl et al., 2004, 2006). However, our results suggest that Y_1 deletion in mice does not alter sensorimotor gating since $Y_1^{-/-}$ mice showed no differences in baseline PPI performance compared to WT mice. This observation is consistent with earlier research, which has found the NPY system not to be directly implicated in sensorimotor gating [e.g. (Husum et al., 2002)].

Our results suggest $Y_1^{-/-}$ mice may not be a suitable model of the sensorimotor gating deficits noted in patients with SCZ. However, given the evidence for the role of NPY and Y_1 receptors in SCZ (see Introduction) it was also hypothesized that PPI of $Y_1^{-/-}$ mice might be differentially affected by a psychotropic drug challenge in comparison to WT control mice. This would reflect the increased susceptibility to psychostimulants and psychomimetics observed in patients with SCZ (Curran et al., 2004). The study intended to screen for a subthreshold phenotype potentially evident after psychotropic drug administration and therefore used commonly accepted psychotropic drugs at well-accepted doses. The characteristics of DEX and MK-801 challenge on the prepulse inhibition performance of mice were dependent on the PPI protocol used. MK-801 impaired PPI at higher prepulse intensities when using a fixed ISI (protocol A). MK-801's drug effect seemed stronger compared to DEX's effects. Interestingly, $Y_1^{-/-}$ mice appeared more sensitive to the drug challenge at prepulse intensities of 82 dB (MK-801) and 86 dB (DEX). Using a variable ISI (protocol B), mice of either genotype did not exhibit gross impairments of PPI after DEX treatment. Only when using a interstimulus interval of 512 ms at a prepulse intensity of 86 dB did WT control mice show a DEX-induced alteration of PPI. Interestingly, MK-801 suppressed the PPI performance at the two highest prepulse intensities and did so only in wild type-like mice confirming the relevance of PPI protocol specifications (e.g. fixed versus variable ISI) for PPI performances. It is important to note that ISI had an impact on the effects of MK-801, as no PPI-impairing characteristics of MK-801 were seen for ISIs of 128 ms and 256 ms. It is possible that Y_1 receptor deletion modulates the response to dopaminergic agonists and NMDA antagonists via altered expression of the relevant receptor systems, thereby explaining the reduced effectiveness of MK-801 in protocol B in the mutant mice. In this line, Naveilhan and coworkers (Naveilhan et al., 1998) demonstrated that mice lacking the Y_1 receptor were insensitive to the behavioural effects of ketamine, which is another NMDA antagonist. The effects of MK-801 on sensorimotor gating have to be interpreted with caution as all mice showed a significantly increased acoustic startle response after MK-801 treatment. These differences between MK-801-induced ASR and baseline ASR might influence the prepulse inhibition performance (Csomor et al., 2008). Further research is needed to clarify whether there are alterations to the dopaminergic and glutamatergic system of Y_1 depleted mice.

It is likely that differences in the design and drug regime of the two PPI protocols used account for the protocol-specific effects highlighted above. While PPI protocols share similar general principles (i.e. a weak prepulse followed by a louder startle pulse decreases ASR relative to the ASR induced by the startle pulse alone), protocols vary widely between laboratories (e.g. in regard to prepulse intensities, ISI, number of trials etc.). For example, depending on the PPI protocol and mouse strain used, PPI can be greatest at 50–100 ms ISI, exhibiting a U-shaped curve with increasing prepulse intensities (Swerdlow et al., 2000; Varty et al., 2001; Wang et al., 2003). Importantly, the current study reports differential drug effects on the sensorimotor gating performance of mice, which are dependent on the type of PPI protocol used. This finding is relevant for other researchers working within the field.

In summary, it appears that the Y_1 receptor influences the acoustic startle response and its habituation but is not involved in shaping sensorimotor gating *per se*. As MK-801 challenge had a protocol-dependent differential effect in $Y_1^{-/-}$ mice and DEX a pronounced impact at higher prepulse intensities, further explorations into the effects of Y_1 deficiency – in particular employing conditional knockout approaches – seem valid.

5. Funding

The study was supported by Schizophrenia Research Institute (SRI), utilizing infrastructure funding from NSW Health and the Baxter Charitable Foundation and the Alma Hazel Eddy Trust; TK is supported by a project grant (493301) and a career development award (568752) of the National Health and Medical Research Council (NHMRC) and by the National Alliance for Research on Schizophrenia and Depression (Young Investigator Award). HH is supported by a grant from the NHMRC and a NHMRC Fellowship.

Acknowledgements

We thank the biological testing facility staff for their support. The critical comments by Jerry Tanda on the manuscript are gratefully acknowledged.

References

Bannon, A.W., Seda, J., Carmouche, M., Francis, J.M., Norman, M.H., Karbon, B., McCaleb, M.L., 2000. Behavioural characterization of neuropeptide Y knockout mice. Brain Res. 868, 79–87.

Beal, M.F., Svendsen, C.N., Bird, E.D., Martin, J.B., 1987. Somatostatin and neuropeptide Y are unaltered in the amygdala in schizophrenia. Neurochem. Pathol. 6, 169–176.

Braff, D.L., Geyer, M.A., Swerdlow, N.R., 2001. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 156, 234–258.

Choi, K.H., Elashoff, M., Higgs, B.W., Song, J., Kim, S., Sabunciyan, S., Diglisic, S., Yolken, R.H., Knable, M.B., Torrey, E.F., Webster, M.J., 2008. Putative psychosis genes in the prefrontal cortex: combined analysis of gene expression microarrays. BMC Psychiatry 8, 87.

Csomor, P.A., Yee, B.K., Vollenweider, F.X., Feldon, J., Nicolet, T., Quednow, B.B., 2008. On the influence of baseline startle reactivity on the indexation of prepulse inhibition. Behav. Neurosci. 122, 885–900.

Curran, C., Byrappa, N., McBride, A., 2004. Stimulant psychosis: systematic review. Br. J. Psychiatry 185, 196–204.

Gabriel, S.M., Davidson, M., Haroutunian, V., Powchik, P., Bierer, L.M., Purohit, D.P., Perl, D.P., Davis, K.L., 1996. Neuropeptide deficits in schizophrenia vs. Alzheimer's disease cerebral cortex. Biol. Psychiatry 39, 82–91.

Geyer, M.A., Braff, D.L., 1982. Habituation of the blink reflex in normals and schizophrenic patients. Psychophysiology 19, 1–6.

Geyer, M.A., Krebs-Thomson, K., Braff, D.L., Swerdlow, N.R., 2001. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 156, 117–154.

Howell, O.W., Scharfman, H.E., Herzog, H., Sundstrom, L.E., Beck-Sickinger, A., Gray, W.P., 2003. Neuropeptide Y is neuroproliferative for post-natal hippocampal precursor cells. J. Neurochem. 86, 646–659.

Husum, H., Termeer, E., Mathe, A.A., Bolwig, T.G., Ellenbroek, B.A., 2002. Early maternal deprivation alters hippocampal levels of neuropeptide Y and calcitonin-gene related peptide in adult rats. Neuropharmacology 42, 798–806.

Ikeda, K., Ikeda, K., Iritani, S., Ueno, H., Niizato, K., 2004. Distribution of neuropeptide Y interneurons in the dorsal prefrontal cortex of schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 28, 379–383.

Itokawa, M., Arai, M., Kato, S., Ogata, Y., Furukawa, A., Haga, S., Ujike, H., Sora, I., Ikeda, K., Yoshikawa, T., 2003. Association between a novel polymorphism in the promoter region of the neuropeptide Y gene and schizophrenia in humans. Neurosci. Lett. 347, 202–204.

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

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

Kishi, T., Aschkenasi, C.J., Choi, B.J., Lopez, M.E., Lee, C.E., Liu, H., Hollenberg, A.N., Friedman, J.M., Elmquist, J.K., 2005. Neuropeptide Y Y1 receptor mRNA in rodent brain: distribution and colocalization with melanocortin-4 receptor. J. Comp. Neurol. 482, 217–243.

Koch, M., 1999. The neurobiology of startle. Prog. Neurobiol. 59, 107–128.

Kuromitsu, J., Yokoi, A., Kawai, T., Nagasu, T., Aizawa, T., Haga, S., Ikeda, K., 2001. Reduced neuropeptide Y mRNA levels in the frontal cortex of people with schizophrenia and bipolar disorder. Gene Expr. Patterns 1, 17–21.

Lindberg, C., Koefoed, P., Hansen, E.S., Bolwig, T.G., Rehfeld, J.F., Mellerup, E., Jorgensen, O.S., Kessing, L.V., Werge, T., Haugbol, S., Wang, A.G., Woldbye, D.P., 2006. No association between the $-399\,^{\circ}C > T$ polymorphism of the neuropeptide Y gene and schizophrenia, unipolar depression or panic disorder in a Danish population. Acta Psychiatr. Scand. 113, 54–58.

Naveilhan, P., Neveu, I., Arenas, E., Ernfors, P., 1998. Complementary and overlapping expression of Y1, Y2 and Y5 receptors in the developing and adult mouse nervous system. Neuroscience 87, 289–302.

Sachs, G.S., 2006. A review of agitation in mental illness: burden of illness and underlying pathology. J. Clin. Psychiatry 67 (Suppl. 10), 5–12.

Swerdlow, N.R., Braff, D.L., Geyer, M.A., 2000. Animal models of deficient sensorimotor gating: what we know, what we think we now, and what we hope to know soon. Behav. Pharmacol. 11, 185–204.

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-Y_2 receptors. Eur. J. Neurosci. 18, 143–148.

van den Buuse, M., Garner, B., Gogos, A., Kusljic, S., 2005. Importance of animal models in schizophrenia research. Aust. N. Z. J. Psychiatry 39, 550–557.

Varty, G.B., Walters, N., Cohen-Williams, M., Carey, G.J., 2001. Comparison of apomorphine, amphetamine and dizocilpine disruptions of prepulse inhibition in inbred and outbred mice strains. Eur. J. Pharmacol. 424, 27–36.

Vawter, M.P., Ferran, E., Galke, B., Cooper, K., Bunney, W.E., Byerley, W., 2004. Microarray screening of lymphocyte gene expression differences in a multiplex schizophrenia pedigree. Schizophr. Res. 67, 41–52.

Wang, J.H., Short, J., Ledent, C., Lawrence, A.J., van den Buuse, M., 2003. Reduced startle habituation and prepulse inhibition in mice lacking the adenosine A2A receptor. Behav. Brain Res. 143, 201–207.

Wittmann, W., Loacker, S., Kapeller, I., Herzog, H., Schwarzer, C., 2005. Y1-receptors regulate the expression of Y2-receptors in distinct mouse forebrain areas. Neuroscience 136, 241–250.

Yee, B.K., Chang, D.L., Feldon, J., 2004. The effects of dizocilpine and phencyclidine on prepulse inhibition of the acoustic startle reflex and on prepulse-elicited reactivity in C57BL6 mice. Neuropsychopharmacology 29, 1865–1877.