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Research report

Schizophrenia-relevant behaviours in a genetic mouse model for Y2 deficiency

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ABSTRACT

Expression levels of neuropeptide Y (NPY) are changed in schizophrenia patients. However, the direction of changes to NPY expression and the mechanisms behind NPY's impact on the development of the illness is not understood in detail. Here we investigated whether alterations in Y2 activity may be involved in the development of schizophrenia-related behaviours. We examined NPY Y2 receptor deficient male mice in behavioural domains relevant for the illness: locomotion, learning and memory, social interaction and sensorimotor gating (baseline and after acute challenge with psychotropic drugs) and the most relevant tasks were also completed in female Y2 mutants. Our investigations confirmed a hyper-locomotive phenotype for Y2 deficient male mice and no alterations in working and reference memory performance. Mutant males exhibited an increase in social interaction and moderately improved sensorimotor gating. The psychotropic drugs dexamphetamine and MK-801 affected prepulse inhibition similarly, whereas MK-801 appeared to be a slightly more potent stimulant for the acoustic startle response (ASR). Female Y2 deficient mice showed wild type-like performances in social interaction, working memory and prepulse inhibition. However, Y2 mutant females exhibited a moderately increased ASR compared to control mice.

Taken together, lack of Y2 signalling in mice not only leads to altered locomotion but also changes social behaviours and affects sensorimotor gating. Thus, Y2 depletion influences a range of behaviours, which are potentially relevant for schizophrenia-related research.

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1. Introduction

Neuropeptide Y (NPY) has been suggested as a potential key molecule for schizophrenia (SCZ). Indeed, reduced NPY levels have been found in the frontal cortex [30] and in the dorsal prefrontal cortex [24] of schizophrenia patients. Furthermore, antipsychotic treatment increases NPY levels [36], and a polymorphism in the promoter region of *NPY* has been associated with schizophrenia [25]. *NPY* gene expression is also down regulated in the post-mortem tissue of patients with psychosis [8]. A more recent study, however, failed to establish an association between a polymorphism in the *NPY* gene and SCZ [31], confirming that the role of NPY in this disorder might be more complex and demands further exploration.

A genetic animal model for Y1, the main NPY receptor, exhibits behavioural features that are relevant for schizophrenia-related animal research, including hyperlocomotion and increased aggression [26,27]. Another NPY receptor, the Y2 receptor, is expressed abundantly in the hippocampus and the amygdala [16] and seems to be involved in the inhibitory regulation of glutamate, dopamine and gamma-aminobutyric acid (GABA) release [9,10,46]. Importantly, glutamatergic hypofunction is believed to be a key mechanism underlying the development of both positive and negative symptoms of SCZ. The genetic mouse model for Y2 displayed a hyperlocomotive and anxiolytic-like phenotype [42,43] and showed deficits in attention [23]. One study also found learning and memory impairments in these mice [39].

Because the Y2 receptor affects both neurophysiological and behavioural processes involved in schizophrenia, we hypothesized that the mouse model would be an interesting target for further, more schizophrenia-specific analyses. The paradigms commonly used to detect schizophrenia-like behaviours in animal models are not specific to this neuropsychiatric disorder. Due to this lack of specificity, a comprehensive, multi-tiered phenotyping strategy is required, using tasks that map directly or indirectly to a variety of behaviours, which can then be related to human symptoms of schizophrenia. Our study therefore deployed tests for positive (hyperactivity), negative (social withdrawal) and cognitive (learning and memory) symptoms as well as sensorimotor gating in male mice [38]. We investigated sensorimotor gating not only at baseline

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but also after acute challenge with the psychotomimetic drugs dexamphetamine (DEX; a catecholaminergic stimulant) and MK-801 [a non-competitive N-methyl-D-aspartic acid (NMDA) antagonist]. These drugs are known to induce or enhance psychotic symptoms in schizophrenia patients [33] and also impair sensorimotor gating in rodents [5,44]. This strategy of combining a genetic risk factor with an environmental one (i.e. drugs of abuse) is in accordance with the "two-hit hypothesis" of schizophrenia, which states that in addition to genetic predisposition for the disease (first hit), subsequent exposure to environmental risk factors (second hit) is necessary for schizophrenia to develop [3]. To complement the initial findings in male mice we also tested female Y2 receptor mutant mice in selected (i.e. most relevant) test paradigms as sex-specific differences in the severity of schizophrenia symptoms have been described for patients.

2. Materials and methods

2.1. Experimental animals

Germline Y2 knockout (Y2 KO or Y2$^{-/-}$) and wild type-like (WT) control mice from a colony maintained at the Biological Testing Facility of the Garvan Institute of Medical Research were used for the experiments (mixed C57BL/6J-129/SvJ background; for generation of Y2 KO mice see [2]). Two sets of male, adult, age-matched (±10 days) mice (set 1: WT = 10 vs. Y2 KO = 10; set 2: WT = 7 vs. Y2 KO = 6) and – for the follow-up experiments – one set of female, adult, age-matched (±14) mice (WT = 11 vs. Y2 KO = 8) of various litters were used. Mice of the same genotype were pair-housed in Polysulfone cages (Type 1144-B.00SU: Tecniplast, Rydalmere, Australia), which were provided with cellulose paper as nesting material, and received food and water *ad libitum*. Same-sex, adult, age-matched (±7 days), group-housed A/J mice (Animal Resources Centre, Canning Vale, Australia) were used as standard opponents in the social interaction test. A/J mice were kept in identical cages and within the same holding room as the test mice. Cages were held in a temperature- and humidity-controlled room (22 °C; 55–60% relative humidity) with a 12:12 h light:dark cycle (light phase: ~70 lx white light, dark phase: <2 lx red light). Microbiological monitoring revealed no infection of the SPF facility, with the exception of the pathogens commonly found in commercial and research facilities, *Pasteurella pneumotropica* and *Helicobacter* spp. Mice were transferred to the experimental room one hour prior to testing to allow habituation. Equipment was cleaned with 30% ethanol solution before each animal was tested. Unless otherwise stated, testing was commenced one hour after onset of light phase.

Male test order: i) physical exam, ii) open field, iii) Y maze, iv) social interaction, v) passive avoidance (only set 1 was tested) and vi) prepulse inhibition (PPI).

Female test order: i) social interaction, ii) Y maze and iii) prepulse inhibition.

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 Purposes.

2.1.1. Drug treatment

Dexamphetamine (DEX) and MK-801 (Sigma, Sydney, Australia) were dissolved in saline. Male mice of both genotypes received intraperitoneal (i.p.) injections of saline, DEX [5 mg/kg body weight (BW)] or MK-801 (0.5 mg/kg BW) 15 min prior to PPI testing. All mice underwent all treatments using a quasi-randomized design with an inter-test interval of at least five days. The doses used are well known to induce impairment in sensorimotor gating. 0.5 mg/kg BW MK-801 results in *popping* behaviour, which could be observed when animals were placed back into the home cage after completion of the PPI protocol. All drugs were injected at a volume of 10 ml/kg body weight. Female mice were treated with saline and MK-801 only using a quasi-randomized design.

2.2. Physical exam (PE)

General health, sensory abilities, neurological motor reflexes, and motor function/coordination have a huge impact on animals' behavioural performance and aberrations in these basic functions can be misinterpreted as alterations in more complex behavioural domains [13,14]. A wide range of basic tasks were used to evaluate all mice for such basic sensory and motor abilities including the accelerod test (for details of test paradigms see [28]).

2.3. Open field (OF)

In this test, the conflict between the drive to explore a new environment and a natural aversion to illuminated open areas is used to examine both anxiety and motor activity [12,15]. Mice were tested in an automated, photobeam-controlled open field, 43.2 cm × 43.2 cm (Med Associates Inc., Vermont, USA). The arena was divided into a central and peripheral zone (central zone photobeam coordinates 3/3, 3/13, 13/3, 13/13). Mice were placed in a corner of the arena (illumination level: 20 lx) and were allowed to explore the arena for the following 10 min, while their activity was measured automatically (software settings: box size: 4; ambulatory trigger: 2; resting delay: 1500 ms). Overall distance travelled, frequency of ambulation and the number of ambulatory episodes were used as measures of motor activity.

2.4. Y maze (YM)

Mice have a tendency to explore novel environments. We tested the ability of mice to remember familiar arms of a YM by comparing the level of locomotion/exploration and of time spent in a novel, unexplored arm and two familiar arms [22]. Mice were placed into the centre of a grey Perspex Y-shaped maze (30 cm × 10 cm × 17 cm arms joined by a triangular centre section) and allowed to explore two arms freely for 10 min (third arm was blocked by a grey Perspex insert; trainings trial). Different striped patterns on the arm walls provided intra-maze directionality cues while the test room provided extra-maze directionality cues. After an intertrial interval of 1 h mice were replaced into the centre of the YM for 5 min but this time all three arms were accessible (test trial). We manually recorded the number of arm entries (entry: mouse inside the arm with at least 50% of its body), time spent in the arms as well as the *rearing* frequency for each individual arm. Working memory scores were calculated for each parameter (e.g. novel arm entries as percentage of total arm entries).

2.5. Passive avoidance (PA)

In this basic hippocampus-dependent learning test, the avoidance of a naturally less aversive dark compartment after it is paired with an electrical foot shock indicates the retention of this memory [4]. The behavioural performance of rodents in this task is also influenced by their general stress response (i.e. fear of highly illuminated areas and aversive stimuli such as electrical foot shock) and nociception. In the training session mice were placed in a highly illuminated compartment (illumination: 70 lx; Shuttle Box System—TSE Systems, Bad Homburg, Germany). After 10 s, the door to a dark chamber was opened. Once the mouse moved into the dark chamber (illumination: <2 lx), the door was closed and a single foot shock (0.4 mA for 2 s) was delivered. Mice were kept in the dark chamber for another 60 s to allow the formation of an association between the location and the foot shock. In the retention session (test trial) 24 h later mice were again placed in the light compartment and 10 s later the door connecting light and dark chambers was opened. The latency to enter the dark chamber (light–dark transition time) on training and test trial was recorded as a measure of reference memory performance: increased entry latency on the second day (test trial) indicated memory of the aversive stimulus experienced on training day.

2.6. Social interaction (SI)

The SI model is widely used to measure anxiety-like behaviours as well as social withdrawal [18,29,38]. Test animals were placed together with a same sex A/J standard opponent into opposite corners of a grey Perspex open field activity test chamber (35 cm × 35 cm × 30 cm), where they were allowed to explore the arena and each other freely for 10 min. The behaviour of the test mouse was recorded online. Frequency and total duration (so-called active social interaction time) of the active socio-positive behaviours *general sniffing*, *anogenital sniffing*, *allogrooming*, *following* and *crawling over*/*under* were recorded.

2.7. Sensorimotor gating measured by prepulse inhibition (PPI)

Patients with schizophrenia show impaired sensorimotor gating. PPI is an operational measure of sensorimotor gating, in which a weak pre-stimulus (prepulse) attenuates the startle response to a sudden loud noise [47]. PPI was tested in two startle chambers (SR-Lab: San Diego Instruments, San Diego, USA). Animals were habituated to the test device for three consecutive days (2 × 2 min per day) before being tested. The protocol used was adapted from methods developed by Geyer and Swerdlow [21] using a variable interstimulus (prepulse-pulse) interval (ISI). Briefly, after a 5 min acclimation period with a 70 dB background noise the test session began (105 trials in a pseudorandom order) with five 120 dB startle pulses were presented followed by four startle pulses (70, 80, 100, 120 dB) presented five times each in a pseudo-randomised order. Afterwards 75 PPI response trials (prepulse intensities of 74, 82, and 86 dB followed by a 120 dB startle pulse) were run employing five different ISIs (32, 64, 128, 256 and 512 ms). PPI response trials were repeated five times in a pseudo-randomised order. The session ended after a final five 120 dB startle pulses.

The intertrial interval varied randomly from 10 to 20 s (average of 15 s), the prepulse duration was 20 ms and startle duration was 40 ms. Responses to each trial were calculated as the average mean amplitude detected by the accelerometer. Percentage PPI (%PPI) was calculated as [(startle response (120 dB) – PPI response)/startle response (120 dB)] × 100. %PPI was averaged across ISIs to produce mean %PPI for each prepulse intensity. Mice were tested for baseline PPI (saline injection) and drug-induced PPI using the psychotropic drugs DEX and MK-801.

2.7.1. Statistical analysis

Results were analysed for each sex using two-way ANOVA [between factor: 'genotype' – repeated factor: 'drug treatment' and 'startle stimulus intensity' (PPI) as well as 'latency to enter' (PA)] and one-way (main factor: 'genotype') analysis of variance (ANOVA). Simple contrasts with a Bonferroni correction were applied, when appropriate. Differences were regarded as significant if the p-value was <.05. All data are presented as means ± standard error of the mean (SEM). Figures show means + SEM and significant genotype effects *versus* WT mice) are indicated by asterisks ($^{*}p < .05$, $^{**}p < .01$ and $^{***}p < .001$) whereas treatment effects *versus* saline injection, as well as repeated testing effects (PA) are shown by "#" ($^{\#}p < .05$, $^{\#\#}p < .01$ and $^{\#\#\#}p < .001$).

3. Results

The physical examination and accelerod testing confirmed that all male mice, independent of genotype, were in good general health, with intact sensory and motor functions/coordination, neurological reflexes and neuromuscular strength (data not shown).

3.1. Locomotion (OF)

The open field testing confirmed the previously described hyperlocomotive phenotype of Y2-deficent mice. One-way ANOVA revealed a significant increase in motor activity (i.e. overall distance travelled) over the 10 min test period [factor genotype: $F(1,31) = 8.0$, $p = .008$; WT = 7231.9 ± 561.5 vs. Y2 KO = 9540.2 ± 593.1]. The phenotype was also evident in the behavioural measures frequency of ambulation [one-way ANOVA: $F(1,31) = 8.6$, $p = .006$; WT = 4434.9 ± 374.0 vs. Y2 KO = 6177.3 ± 464.4] and number of ambulatory episodes [one-way ANOVA: $F(1,31) = 5.3$, $p = .03$; WT = 440.1 ± 29.8 vs. Y2 KO = 530.3 ± 25.2]. Other behavioural paradigms such as the Y maze test confirmed the hyper-active phenotype: Y2 KO mice entered significantly more arms during the test session than WT mice [one-way ANOVA: $F(1,31) = 5.4$, $p = .03$; WT = 17.5 ± 1.1 vs. Y2 KO = 23.9 ± 2.6].

3.2. Learning and memory (YM and PA)

Testing Y2-deficient and WT control mice in behavioural paradigms for working and reference memory revealed no significant alterations to the learning and memory abilities of mutant mice.

3.2.1. Working memory (YM)

In the Y maze, mice of both genotypes showed a moderate preference for the novel arm as confirmed by the percentage of entries into the unfamiliar compartment of the Y maze. However, they did not spend significant more time in the novel arm than in the familiar one (Fig. 1A). One-way ANOVA detected no significant differences between WT and Y2 KO mice regarding their preference for the novel arm in the test trial. Both genotypes showed similar percentage of novel arm entries [one-way ANOVA: $F(1,31) = 0.2$, $p = .7$] and time spent in the novel arm [one-way ANOVA: $F(1,31) = 1.7$, $p = .2$] (Fig. 1A).

3.2.2. Reference memory (PA)

The passive avoidance task measures the long term memory of an aversive foot shock in test animals (i.e. after a 24 h delay). All mice associated the dark chamber with the electrical foot shock received in this location during training, as repeated measures (RM) ANOVA confirmed a significant increase in the latency to enter the dark compartment for all mice [$F(1,18) = 59.7$, $p < .0001$; Fig. 1B]. Importantly, the learned avoidance of the dark chamber evident in the test session was similar in both wild type-like and mutant animals [$F(1,18) = 2.3$, $p = .2$], revealing no differences in reference memory.

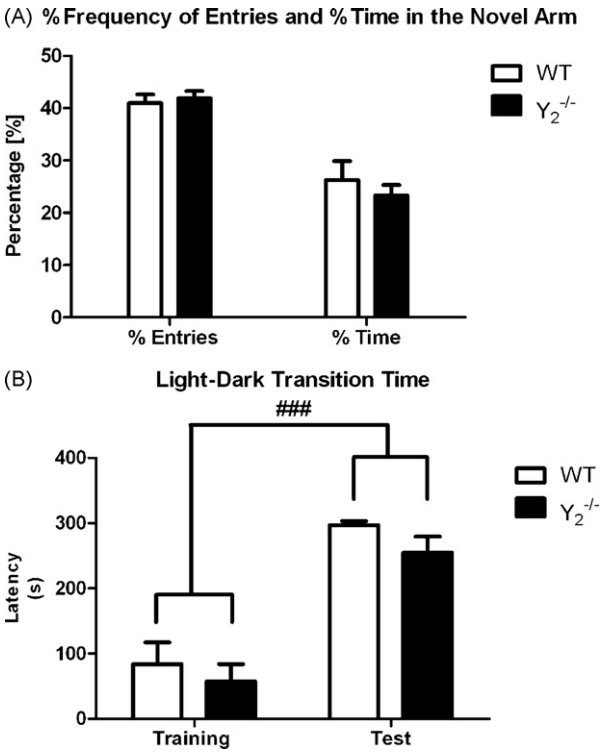

Fig. 1. (A and B) Cognition: (A) Working memory: percentage [%] of time spent/arm entries in(to) the novel arm compared to overall performance in the Y maze. (B) Reference memory: light–dark transition time [s] in the passive avoidance task (PA). Data are shown as means + SEM for male WT and Y2 KO mice. Repeated measures ANOVA effects between training and test session in the PA are indicated by hashes ($^{\#\#\#}p < .001$).

3.3. Social interaction (SI)

In addition to differences in the social behavior of mice, the SI task also provides an indication of their anxiety response. One-way ANOVA revealed a significant increase in active social interaction time for Y2 KO mice [$F(1,31) = 6.9$, $p < .05$; Fig. 2]. This increase in SI time was associated with an elevated duration of behaviours such as *nosing* [$F(1,31) = 4.1$, $p < .05$; WT = 35.9 ± 2.0 vs. Y2 KO = 41.4 ± 1.7], *anogenital sniffing* [$F(1,31) = 14.3$, $p < .001$; WT = 4.0 ± 0.6 vs. Y2 KO = 8.9 ± 1.2] and *following* [$F(1,31) = 4.5$, $p < .05$; WT = 0.5 ± 0.2 vs. Y2 KO = 1.3 ± 0.3] (Fig. 2).

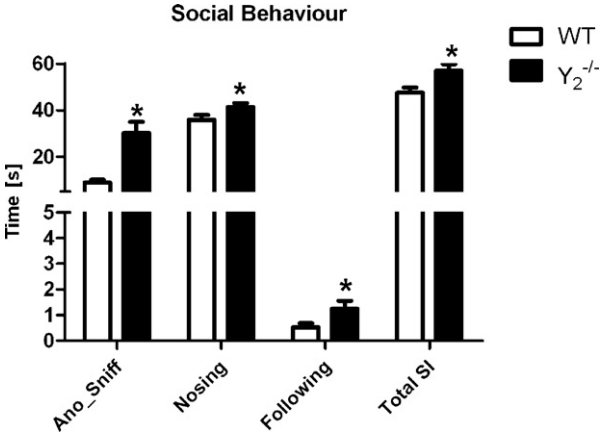

Fig. 2. Social withdrawal: Total time spent in *nosing*, *anogenital sniffing* (*ano_sniff*), *following* and active social interaction (total SI) [s] in the social interaction task. Data are shown as means + SEM. Significant genotype effects *versus* WT mice are indicated by asterisks ($^{*}p < .05$).

Table 1
Acoustic startle response to a 70–120 dB stimulus [measured as average mean amplitude detected by the accelerometer]. Data are shown as means ± SEM separated for male and female WT and Y2 KO mice and for the different treatment groups. Significant genotype effects *versus* WT are indicated by asterisks ($^{*}p < .05$) and trends are indicated by "^" ($^{\wedge}p = .05–.08$). Significant treatment effects *versus* saline are indicated by hashes ($^{\#}p < .05$, $^{\#\#}p < .01$ and $^{\#\#\#}p < .001$); trends are indicated by "~", $^{\sim}p = .05–.08$.

Sex	Startle stimulus	Treatment	WT	Y2 KO
Male	70 dB	Saline	3.9 ± 1.0	2.9 ± 0.5
		DEX	27.3 ± 7.9$^{\#\#}$	21.3 ± 7.2$^{\#}$
		MK-801	7.6 ± 0.8$^{\#\#}$	7.1 ± 0.7$^{\#\#\#}$
	80 dB	Saline	3.7 ± 1.0	2.9 ± 0.8
		DEX	27.4 ± 7.8$^{\#}$	20.5 ± 7.0$^{\sim}$
		MK-801	6.8 ± 0.9	5.3 ± 0.7$^{\#\#}$
	100 dB	Saline	6.5 ± 1.8	9.5 ± 2.2
		DEX	31.7 ± 7.6$^{\#\#}$	26.3 ± 7.8
		MK-801	12.9 ± 2.4$^{\#\#}$	13.7 ± 1.9
	120 dB	Saline	45 ± 7.4	62.8 ± 8.7
		DEX	54.8 + 11.5	54.1 ± 9.6
		MK-801	82.4 + 9.6$^{\#\#\#}$	110 ± 12.5$^{\#\#\#}$
Female	70 dB	Saline	4.3 ± 1	3.9 ± 0.8
		MK-801	7.5 ± 0.7$^{\#}$	7.4 ± 1.2$^{\#}$
	80 dB	Saline	5.1 ± 1	2.1 ± 0.5*
		MK-801	7.1 ± 0.8	4.8 ± 0.6$^{*\#\#}$
	100 dB	Saline	27.9 ± 4.7	11.3 ± 2*
		MK-801	35.9 ± 6.8	18.2 ± 6.3$^{\wedge}$
	120 dB	Saline	77.1 ± 9.5	50.1 ± 9.6
		MK-801	85.6 ± 12.8	63.5 ± 13.5

3.4. Acoustic startle response and sensorimotor gating

3.4.1. Acoustic startle response (ASR)

Analysis of the acoustic startle response of mutant and control mice to a 120 dB stimulus revealed no significant genotype-specific differences (Table 1). As expected, an increase in the sound pressure level of the acoustic startle stimulus (70–120 dB) was accompanied by an elevation of the ASR (RM ANOVA: $F(3,90) = 110.8$, $p < .0001$). RM ANOVA also detected a significant increasing effect of drug treatment [70 dB $F(2,60) = 12.7$, $p < .0001$; 80 dB $F(2,60) = 13.3$, $p < .0001$; 100 dB $F(2,60) = 12.5$, $p < .0001$; 120 dB: $F(2,60) = 27.8$, $p < .0001$] on ASR, which was confirmed by one-way ANOVAs split by genotype [WT: 70 dB $F(2,32) = 7.5$, $p = .002$; 80 dB $F(2,32) = 8.0$, $p = .002$; 100 dB $F(2,32)$–10.6, $p < .0001$; 120 dB: $F(2,32) = 14.7$, $p < .0001$; Y2KO: 70 dB $F(2,28) = 5.5$, $p = .01$; 80 dB $F(2,28) = 5.7$, $p = .008$; 100 dB $F(2,28) = 3.4$, $p < .05$; 120 dB: $F(2,28) = 14.3$, $p < .0001$]. Simple contrasts with a Bonferroni correction detected that MK-801 was more effective than DEX at enhancing the startle response at 120 dB (Table 1). We also found a trend for a genotype by sound pressure level interaction [RM ANOVA: $F(3,90) = 2.6$, $p = .06$].

3.4.2. Sensorimotor gating measured by prepulse inhibition (PPI)

Mean %PPI was calculated separately for the three prepulse intensities (Fig. 3A–C). Repeated measures (RM) ANOVA detected a clear treatment effect for all prepulse intensities [74 dB: $F(2,62) = 15.5$, $p < .001$; 82 dB: $F(2,62) = 10.6$, $p < .001$; 86 dB: $F(2,62) = 8.7$, $p < .001$] and one-way ANOVAs confirmed the significant PPI-suppressing effect of DEX and MK-801 treatment in both WT [74 dB: $F(2,32) = 8.2$, $p = .001$; 82 dB: $F(2,32) = 45.0$, $p = .02$; 86 dB: $F(2,32) = 4.5$, $p = .02$] and Y2 KO [74 dB: $F(2,30) = 7.5$, $p = .002$; 82 dB: $F(2,30) = 8.2$, $p = .001$; 86 dB: $F(2,30) = 5.6$, $p = .008$] mice. Simple contrast with a Bonferroni correction revealed that MK-801 impaired PPI at all prepulse intensities whereas DEX exhibited less prominent effects (Fig. 3A–C). RM ANOVA also found a moderate main effect of genotype on prepulse inhibition at prepulse intensities of 74 dB [trend: $F(1,31) = 3.3$, $p = .08$] and 86 dB [$F(1,31) = 5.0$, $p = .03$]. Y2 KO mice showed an overall improved PPI performance compared to WT mice (Fig. 3A and C).

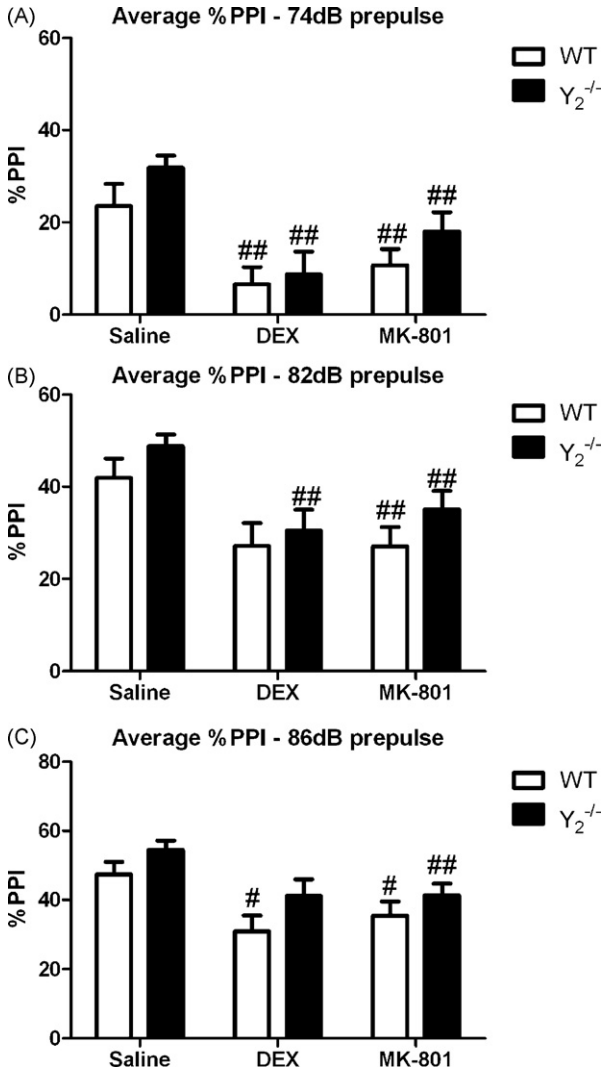

Fig. 3. (A and B) Sensorimotor gating of male mice: Mean %PPI is shown for the various prepulse intensities (A: 74 dB, B: 82 dB, C: 86 dB) for the different treatment groups. All data are averaged for the different ISIs. Mean + SEM are shown. Significant treatment effects (i.e. DEX or MK-801) *versus* saline are represented by hashes ($^{\#}p < .05$ and $^{\#\#}p < .01$).

3.5. Characterisation of female Y2 KO mice

Female Y2 mutant and wild type-like mice were tested for social interaction, working memory (i.e. Y maze) and sensorimotor gating (i.e. prepulse inhibition). No significant behavioural differences were detected for the factor 'genotype' in the social interaction test (Fig. 4A). In the Y maze, female Y2 mutant mice exhibited significantly reduced locomotion [entries into arms: $F(1,17) = 6.9$, $p = .02$; WT = 32.9 ± 4.9 vs. Y2 KO = 17.5 ± 1.5] and exploration [number of *rearings*: $F(1,17) = 9.5$, $p = .007$; WT = 30.4 ± 3.0 vs. Y2 KO = 18.25 ± 2.0] but performed WT-like in memory-related parameters (no significant genotype effects; Fig. 4B). Similar to the males, increasing sound pressure levels of the startle stimulus (70–120 dB) were accompanied by an elevation of the ASR (RM ANOVA: $F(3,51) = 60.4$, $p < .0001$). RM ANOVA detected a significant ASR increasing effect of drug treatment for lower sound pressure levels [70 dB $F(1,17) = 15.5$, $p = .001$; 80 dB $F(1,17) = 7.8$, $p = .01$; 100 dB $F(1,17) = 4.2$, $p = .06$; 120 dB: $F(1,17) = .2$, non significant; Table 1]. Furthermore, genotype differences were detected for the ASR of female mice [RM ANOVA: 80 dB $F(1,17) = 10.5$, $p = .005$; 100 dB $F(1,17) = 5.9$, $p = .03$; for one-way ANOVA split by drug

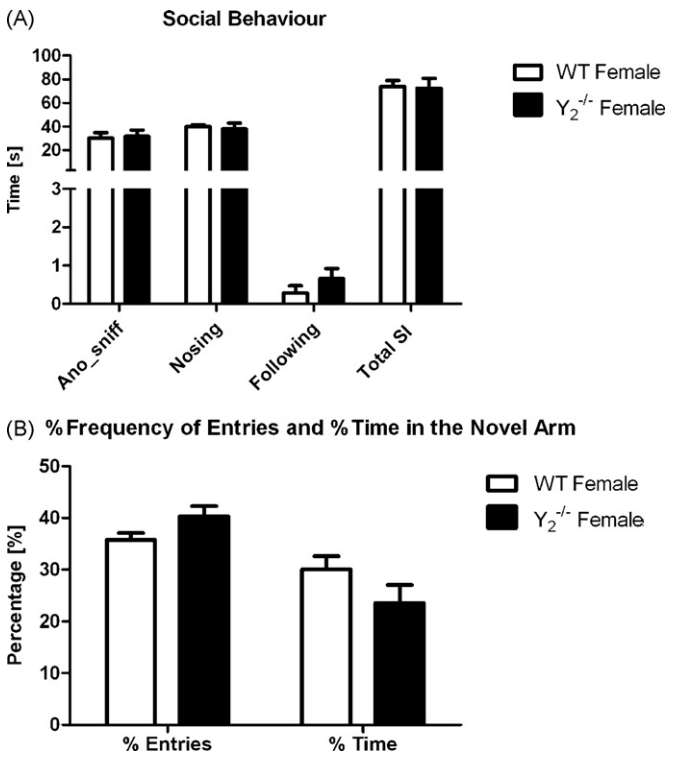

Fig. 4. (A and B) Social withdrawal and cognition of female WT and Y2 KO mice. (A) Social withdrawal: Total time spent in *nosing*, *anogenital sniffing* (*ano_sniff*), *following* and active social interaction (total SI) [s] in the social interaction task. (B) Working memory: Percentage [%] of time spent/arm entries in(to) the novel arm compared to overall performance in the Y maze. Data are shown as means + SEM.

treatment see Table 1] with mutant females exhibiting a lower ASR compared to WT mice. Baseline and MK-801-induced PPI performance of female Y2 KO mice were wild type-like (Fig. 5).

4. Discussion

This study demonstrates that lack of Y2 signalling in mice causes alterations to a variety of behaviours; some of which possess relevance for schizophrenia-related behaviours. Male Y2 deficient mice exhibited an increase in locomotor activity and social interaction, whereas their working and reference memory was unaltered. Y2 KO males also demonstrated moderately improved sensorimotor gating. However, their acoustic startle response and their prepulse inhibition in response to psychotropic substances were not significantly affected by Y2 depletion. The reported changes to the phenotype of Y2 knockout males were sex-specific as female mutants exhibited wild type-like social interaction, working memory abilities, and prepulse inhibition.

Hyperlocomotion is a classic feature of animal models of SCZ, as it can be indicative of exaggerated dopaminergic function, which has been linked to psychosis [1]. However, enhanced locomotor activity can be induced by a wide range of neurotransmitter manipulations, such as glutamatergic inhibition [7] or serotonin re-uptake inhibitors [6], either directly or via modulation of dopaminergic activity. Furthermore, changes to open field locomotion can be stress-related. Thus, the increased levels of motor activity observed in Y2 deficient males could be caused by alterations to a range of different neurotransmitter systems or could be linked to their anxiolytic-like phenotype [42,43].

Interestingly, depletion of Y2 receptors (Y2R) induced a sex-specific increase in social interaction. Y2 knockout males spent more time investigating a standard opponent mouse, including behaviours such as *nosing*, *anogenital sniffing* and *following*. This

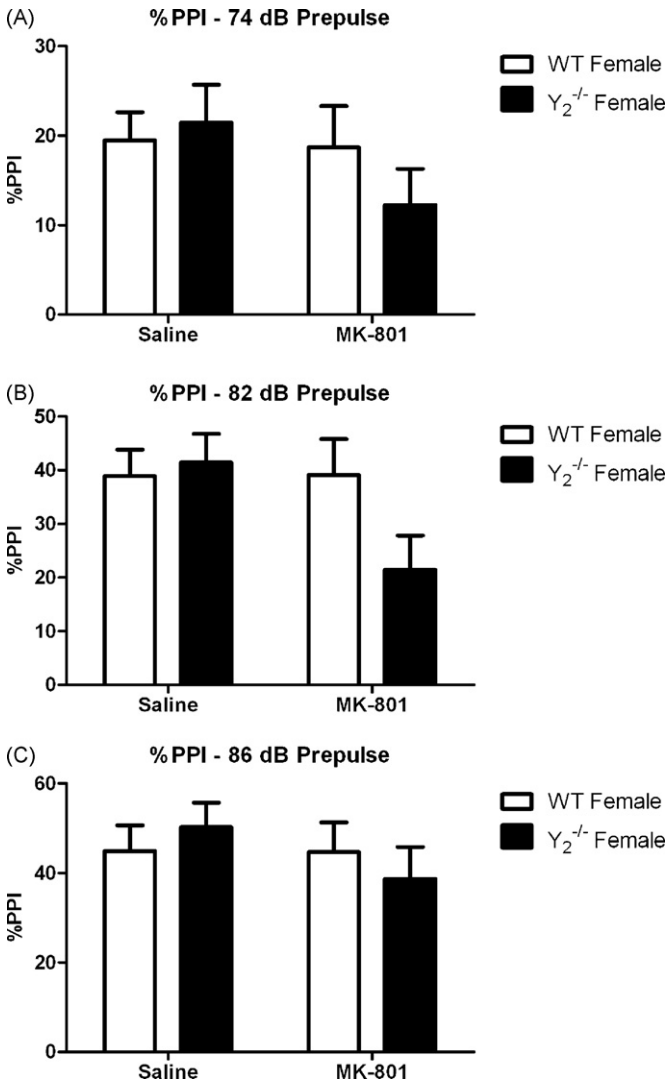

Fig. 5. (A–C) Sensorimotor gating of female mice: Mean %PPI is shown for the various prepulse intensities (A: 74 dB, B: 82 dB, C: 86 dB) for the different treatment groups. All data are averaged for the different ISIs. Mean + SEM are shown.

is particularly interesting, as the SI test has been pharmacologically validated for screening rodents in behaviours corresponding to negative symptoms of schizophrenia (i.e. social withdrawal—see [34,35]) as well as anxiety [19]. Indeed, antipsychotics increase social interaction time [11] whereas treatment with the psychotropic NMDA antagonist phencyclidine induces social isolation (reversed by clozapine [40]). Importantly, we confirmed wild type-like levels of social interaction in Y2 knockout females, as reported previously [37]. Further investigations will be necessary to clarify whether increased social interaction of mutant Y2 males is a proxy for changes to anxiety- or schizophrenia-relevant pathways.

NPY and its Y2 receptor have been suggested to be involved in learning and memory processes. However, no differences in the learning and memory abilities of control and Y2 knockout mice of either sex were revealed in our paradigms. Another study [39] described moderate impairments in the memory abilities of Y2 mutant males in a novel object recognition task; however, these deficits were only evident after an extended delay between the training and test trials. In the same study, Y2 deficient mice showed no learning and memory difficulties when repeatedly locating a hidden platform in the Morris water maze. Although the Y2 knockout mice subsequently showed a reduced preference for a previously learned target location, this could also be due to a more

general effect of the Y2 depletion on cognitive aspects rather than memory per se (i.e. extra-maze cue association or path integration). More comprehensive testing might clarify the role of the Y2 receptor in learning and memory. The decreased locomotion rates of Y2 females indicate that the hyperlocomotive phenotype reported earlier for these mice might be dependent on test conditions [37].

Prepulse inhibition (PPI) is a measure of sensorimotor gating with face, construct and predictive validity for SCZ research [17] as (i) SCZ patients exhibit deficits in PPI [5], (ii) dopaminergic agonists and glutamatergic antagonists both reduce PPI, as suggested by the dopaminergic hyperstimulation and glutamatergic hypofunction theories of SCZ [20,45] and (iii) antipsychotic treatment facilitates PPI [41]. Control and Y2 mutant males were tested for their baseline and drug-induced PPI response (i.e. impairment of sensorimotor gating by drug treatment) after challenge with established doses of DEX and MK-801. Male Y2 KOs exhibited overall improved PPI performance compared to WT mice. The response of control and mutant males to the PPI-suppressing psychotropic drugs revealed no abnormalities for either genotype. We chose behaviourally active doses of DEX and MK-801 to guarantee a reduction in PPI performance of male WT mice. The baseline (i.e. non-drugged) increase in PPI in Y2 KO males could be related to the inhibitory regulation of NMDA release by the Y2 receptor [10]. Y2R's inhibitory effect on NMDA might be rather moderate in this mouse model resulting in subtle changes to PPI. Therefore a broad dose-range for MK-801 would have to be employed to discover potential differences in the sensitivity to this psychotropic drug between control and mutant mice. Dysregulation of the NMDA system and its inhibition by Y2R would have to be expected in brain areas relevant for sensorimotor gating (e.g. limbic cortex, striatum and pallidum [41]). Future behavioural studies will focus on the glutamatergic system in Y2 deficient mice and its effect on sensorimotor gating. The prepulse inhibition task also measured the acoustic startle response to a 120 dB tone stimulus. Male Y2 knockout mice did not exhibit any gross irregularities in their ASR although increasing sound pressure levels seemed to elevate the ASR of Y2 mutants more than control mice. ASR was positively correlated to sound pressure levels of the startle stimulus and drug treatment (with MK-801 having more pronounced effects than DEX). Female mutants showed an ASR phenotype similarly affected by sound pressure levels and MK-801 treatment. Interestingly, Y2 deficiency in female mice seemed to have a moderate inhibiting impact on ASR. Females also exhibited a diminished susceptibility to MK-801, which demands further investigation. It is established that blockade of NMDA receptors causes dose- and strain-dependent increase of the ASR in mice [32,47] and that dopaminergic stimulation does not necessarily alter ASR [45,47].

These experiments aimed to characterise a genetic mouse model of the Y2 receptor for a variety of behaviours, of which some are discussed regarding their relevance for schizophrenia-related research. In summary, it appears that Y2 receptor signalling is sex-specifically involved in mediating locomotion, the anxiety response to novelty, and social behaviours. Furthermore, Y2 signalling appears to influence sensorimotor gating to a certain degree. However, learning and memory do not appear to be strongly affected by Y2 depletion. In conclusion, Y2 receptor knockout mice exhibit a complex, sex-specific phenotype that might be linked to alterations to the glutamatergic system.

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