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Enhanced effect of dopaminergic stimulation on prepulse inhibition in mice deficient in the alpha subunit of G_z

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Abstract *Rationale:* G_z is a member of the G_i G protein family associated with dopamine D2-like receptors; however, its functions remain relatively unknown. The aim of the present study was to investigate prepulse inhibition (PPI) of acoustic startle, locomotor hyperactivity and dopamine D2 receptor binding in mice deficient in the α subunit of G_z. *Methods:* We used automated startle boxes to assess startle and PPI after treatment with saline, amphetamine, apomorphine or MK-801. We used photocell cages to quantitate locomotor activity after amphetamine treatment. Dopamine D2 receptor density was determined by autoradiography. *Results:* Startle responses and baseline PPI were not different between the G α_z knockout mice and wild-type controls (average PPI 46±4 vs 49±3%, respectively). Amphetamine treatment caused a marked disruption of PPI in G α_z knockouts (average PPI 22±2%), but less so in controls (average PPI 42±3%). Similar genotype-dependent responses were seen after apomorphine treatment (average PPI 23±3% vs 40±3%), but not after MK-801 treatment (average PPI 29±5 vs 33±2%). Amphetamine-induced locomotor hyperactivity was great-

er in G α_z knockouts than in controls. There was no difference in the density of dopamine D2 receptors in nucleus accumbens. *Conclusions:* Mice deficient in the α subunit of G_z show enhanced sensitivity to the disruption of PPI and locomotor hyperactivity caused by dopaminergic stimulation. These results suggest a possible role for G_z in neuropsychiatric illnesses with presumed dopaminergic hyperactivity, such as schizophrenia.

Keywords Dopamine · Prepulse inhibition · G protein · Knockout mice · Nucleus accumbens · G_z

Introduction

Prepulse inhibition (PPI) of the startle reflex is a widely used animal behavioural model of sensorimotor gating and sensory information processing (Geyer and Markou 1995). PPI is defined as a reduction in the reflex response when a startle-producing stimulus is preceded by a weak prepulse. PPI is disrupted in a number of mental illnesses, such as schizophrenia (Braff et al. 1995; Geyer and Markou 1995), post-traumatic stress disorder (Grillon et al. 1996), Huntington's disease (Swerdlow et al. 1995), Tourette's syndrome (Castellanos et al. 1996) and obsessive-compulsive disorder (Swerdlow et al. 1993). In experimental animals and humans, PPI disruption can be induced by treatment with a variety of drugs, such as the dopamine-releasing drug amphetamine, the dopamine receptor agonist apomorphine or the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Geyer et al. 2001; Swerdlow et al. 2002). Drug-induced disruptions in PPI in rats and mice have been used as models of the disruptions seen in patients with psychiatric illnesses (Geyer et al. 2002; Swerdlow and Geyer 1998; van den Buuse et al. 2005a).

Previous studies have shown that the dopamine D2 receptor is a key modulator of PPI. In dopamine D2 receptor knockout mice, treatment with amphetamine failed to disrupt PPI, whereas in D3 or D4 receptor knockout mice, the amphetamine response was intact (Ralph et al. 1999). In contrast, in dopamine D2 receptor

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knockout mice, apomorphine treatment caused the expected disruption of PPI; however, in D1 receptor knockout mice, this disruption was absent (Ralph-Williams et al. 2002). On the other hand, antagonism of dopamine D2 receptors will reverse apomorphine-induced disruption of PPI, while direct stimulation of D2 receptors with highly selective ligands produces disruptions in PPI (Geyer et al. 2001). Therefore, receptor studies alone have not provided a clear idea of the role of dopamine receptors in the regulation of PPI.

There are four major families of G proteins, G_i , G_s , G_q and G_{12} (Hendry et al. 2000; Simon et al. 1991). G_z is a member of the G_i family based on their α subunit amino acid sequence homology (Casey et al. 1990). G_z is the only member of the G_i family that is resistant to pertussis toxin, and it has a particularly slow rate of GTP hydrolysis (Casey et al. 1990). This slow rate means that once it becomes activated, it can stay in that state for a long time. This unusual property of G_{α_z} makes it a possible candidate for mediating signal transduction over relatively long time periods (Casey et al. 1990). There are a number of receptors that couple to G_z , including α_2 -adrenergic, dopamine D2 and muscarinic m2 receptors (Casey et al. 1990; Obadiah et al. 1999; Wong et al. 1992). Of the dopamine receptors of the D2-like family, the D2S and D2L form couple to G_z , whereas the D3 and D4 receptors are less efficient (Obadiah et al. 1999). G_z gene expression is high in striatum, which is also particularly rich in dopamine receptors (Friberg et al. 1998). G_{α_z} also plays a role in transducing the effects of morphine receptor activation in the brain through its signaling pathway (Hendry et al. 2000; Leck 2002).

Recently, mice that are deficient in the α subunit of G_z have become available, enabling *in vivo* studies on the role of G_{α_z} (Hendry et al. 2000; Yang et al. 2000). These G_{α_z} knockout mice show enhanced locomotor activity responses to treatment with cocaine (Yang et al. 2000), amphetamine and morphine (Hendry et al. 2000; Leck 2002, 2004), but not to treatment with the dopamine D1 receptor agonist SKF 38393 (Leck 2002). While treatment with the dopamine D2/D3 receptor agonist quinpirole reduced locomotor activity in both genotypes, mice lacking G_{α_z} showed a reduced effect of this drug compared with wild-type controls (Leck 2002). In addition, chronoamperometric measurement of extracellular dopamine levels showed that these mice display reduced inhibition of dopamine release after treatment with quinpirole (Leck 2002). Because of the pivotal role of central dopamine (D2) receptors in PPI, the aim of the present study was to investigate whether G_{α_z} knockout mice have (1) differences in baseline PPI and startle habituation compared with wild-type controls; (2) enhanced sensitivity to drug treatments that disrupt PPI by directly or indirectly modulating dopaminergic activity; (3) enhanced sensitivity to amphetamine in another behaviour, locomotor activity; (4) altered dopamine receptor density.

Methods

Animals

Male G_{α_z} knockout mice and wild-type controls 8 weeks of age were obtained from a breeding colony at the Division of Neuroscience, Australian National University, Canberra, which was originally established by gene targeting in C57Bl/6 mice (Hendry et al. 2000). The mice weighed between 22 and 30 g at the start of the experiments and were kept at the Mental Health Research Institute animal facility in groups of 2–4 in standard plastic mouse boxes, with pellet food and tap water available *ad libitum*. The animals were regularly handled and weighed before experiments. All experiments were done within the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, as published by the National Health and Medical Research Council, the Commonwealth Scientific and Industrial Research Organization, and the Australian Agricultural Council.

PPI of the acoustic startle response

PPI experiments were done on 18 wild-type controls and 16 G_{α_z} knockout mice using a six-unit automated SRLab startle system (San Diego Instruments, San Diego, CA, USA). Each unit consisted of a small Plexiglas cylinder on a platform under which a sensitive piezoelectric sensor was mounted. The cylinders were 5 cm in diameter and closed on both ends. During the sessions, the animals remained in the cylinders within a sound-attenuating cabinet where a 70-dB white background noise was delivered through speakers in the ceiling of the box. Stimuli were delivered and startle responses were measured by the SRLab software (San Diego Instruments) running on a PC in an adjacent room.

For all experiments, an identical PPI protocol was used, as published previously (van den Buuse et al. 2003, 2005b; Wang et al. 2003). A total of 100 trials was delivered, with an average (but not constant) interval of 25 s. The first and last ten trials consisted of single 40-ms 115-dB pulse-alone startle stimuli. These two groups of ten stimuli and the middle two groups of ten pulse-alone stimuli (see below) were used to obtain a measure of response habituation in response to repeated delivery of the startling stimuli. The middle 80 trials consisted of random delivery of twenty 115-dB pulse-alone trials, fifty prepulse trials and ten no stimulus (NOSTIM) trials. Prepulse trials consisted of a single 115-dB pulse preceded by 100 ms by a 20-ms prepulse of 2, 4, 8, 12 or 16 dB over baseline (i.e. 72, 74, 78, 82 or 86 dB). The same session definition was used for all experiments in this study.

Experimental protocol

First, all mice were tested without any treatment in order to provide baseline data for startle, startle habituation and PPI

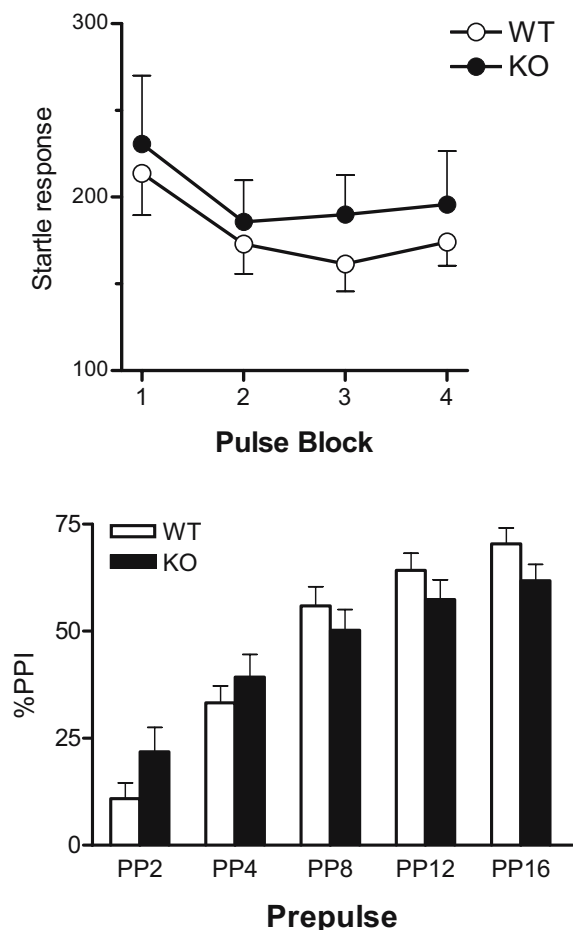


Fig. 1 Startle amplitude and startle habituation (*top panel*) and PPI of startle (*bottom panel*) of untreated $G\alpha_z$ knockout mice (*KO*, $n=16$, *closed symbols*) and wild-type control mice (*WT*, $n=18$, *open symbols*). There were no significant differences between the genotypes

(Fig. 1). A randomized treatment protocol was then used to assess the effect of treatment with saline (10 ml/kg), amphetamine (5 mg/kg, Sigma), apomorphine (5 mg/kg, Sigma) and MK-801 (0.25 mg/kg, Sigma). Drugs were dissolved in saline and injected subcutaneously (s.c.) 15 min prior to the test. All drugs were administered in a volume of 0.1 ml per 10 g body weight. Drug tests were done in 3- to 4-day intervals to allow drug clearance. The sequence of drug treatment was randomized, reducing the risk of between-drug interactions and allowing within-animal comparison. The choice of drugs and drug doses was based upon the literature (Geyer et al. 2001, 2002; Ralph and Caine 2005; Ralph-Williams et al. 2002; van den Buuse et al. 2005b; Wang et al. 2003) and preliminary experiments.

Locomotor hyperactivity

A separate cohort of mice was accustomed to the testing room for 2 h prior to the start of the experiment. Locomotor activity measurement commenced between 1330 and

1530 hours. After the injection of saline vehicle ($n=10$ per genotype) or 1 ($n=8$) or 3 mg/kg of amphetamine ($n=8$), each mouse was placed immediately into a cage (29 cm \times 18 cm) fitted with two pairs of infrared photocells positioned 1.5 cm above the floor and spaced 10 cm apart. The cage had sawdust on its floor, and food and water were available to the mice. Eight mice (four wild type and four $G\alpha_z$ knockouts) were tested simultaneously in eight testing cages. The total number of beam breaks was obtained every 15 min starting from the fourth hour after injection.

Tissue preparation

Another separate group of mice ($n=8$ per genotype) was decapitated, and the brains were removed quickly, frozen on dry ice and stored at -80°C until receptor assay. Coronal sections (20 μl) of mice striatum were cut at -12°C using a cryostat. After the sections were cut, they were directly thaw-mounted onto gelatin-coated microscope slides and stored at -20°C prior to use. Adjacent sections were stained with Cresyl violet and examined with a light microscope. Immediately prior to incubation, all the tissue sections were removed from -20°C storage and air-dried at room temperature for 60 min.

Measurement of D2 receptors and image analysis

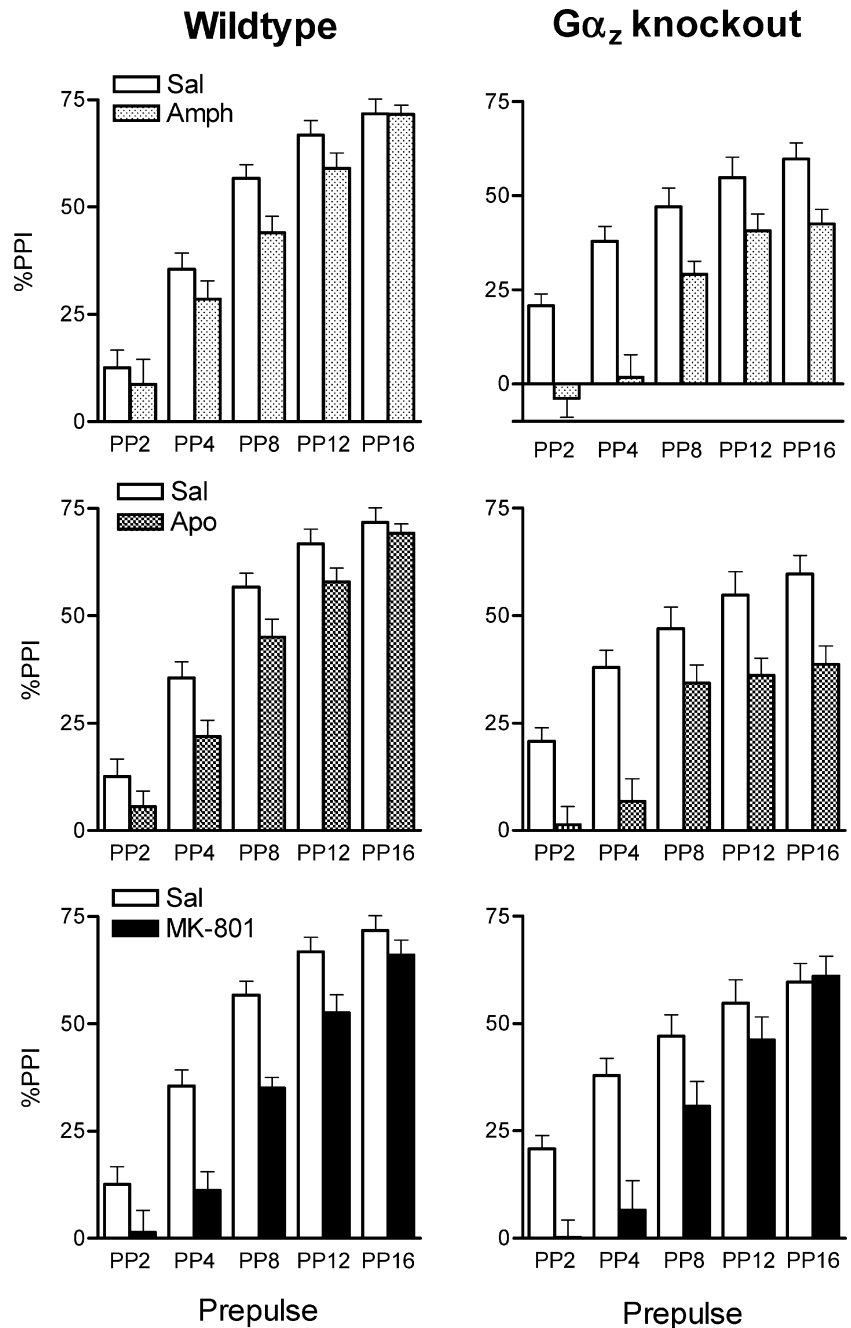
Autoradiographic distribution of dopamine D₂ receptors was performed according to the method of Tarazi et al. (1997), with slight modifications. Coronal sections of the mice brain were pre-incubated for 30 min at room temperature in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂ and 2 mM CaCl₂. The sections were then dipped in ice-cold distilled water and dried with a cool stream of air. Sections were then incubated at room temperature for 60 min in fresh buffer with 1.0 nM [³H] YM-09151-2, in the presence of 1,3-Di-

Table 1 Startle responses in mice at baseline and after treatment with saline, amphetamine (5.0 mg/kg), apomorphine (5.0 mg/kg) and MK-801 (0.25 mg/kg)

Strain/treatment	Block 1	Block 2	Block 3	Block 4
Wild type				
Saline	188±23	190±29	153±17	170±19
Amphetamine	196±21	227±23	233±28	203±18
Apomorphine	148±19	155±23	174±38	166±24
MK-801	337±44	315±47	223±24	207±16
$G\alpha_z$ knockout				
Saline	209±40	199±39	195±33	207±37
Amphetamine	183±14	217±20	212±22	226±22
Apomorphine	260±35	226±28	222±31	251±41
MK-801	330±63	279±37	262±27	256±33

Data are mean \pm SEM of wild-type ($n=18$) and $G\alpha_z$ knockout mice ($n=16$). For statistical analysis, see text

Fig. 2 PPI of acoustic startle in $G\alpha_z$ knockout mice ($n=16$, right panels) and wild-type control mice ($n=18$, left panels) after treatment with 5 mg/kg of amphetamine (*Amph*, top panels), 5 mg/kg of apomorphine (*Apo*, middle panels) or 0.25 mg/kg of MK-801 (*bottom panels*). PPI was disrupted by all drugs; however, the effect of amphetamine and apomorphine was significantly greater in $G\alpha_z$ knockout mice than in controls. For details on statistical analysis, see text



o-tolylguanidine (0.5 μ M) and pindolol (0.1 μ M) to mask sigma sites and serotonin 5-HT_{1A} receptors, respectively. Non-specific binding was determined with 10 μ M *S*(-)-sulpiride. After incubation, the sections were rinsed in fresh ice-cold buffer twice (5 min), dipped in ice-cold distilled water and dried in a cool stream of air.

Sections were partially fixed in paraformaldehyde vapour overnight. Both radiolabelled slides and [³H] microscales were apposed to a BAS-TR2025 phosphoimaging plate (Fuji Imaging Plates, Berthold, Australia) for 6 days at room temperature. Images were quantified using the Analytical Imaging Station (AIS) image analysis software (Imaging Research Inc., Ontario, Canada), where the den-

sity of the image was compared to the standard curve of the microscales, with the results expressed as disintegration per minute per milligram (dpm/mg) estimated tissue equivalent (ETE; wet weight). This was converted to femtomole per milligram ETE using the specific activity and decay factor of the ligand.

Statistical comparison

For PPI experiments, we used analysis of variance (ANOVA) with repeated measures where appropriate. Within-animal factors were pulse block, drug treatment or prepulse intensity.

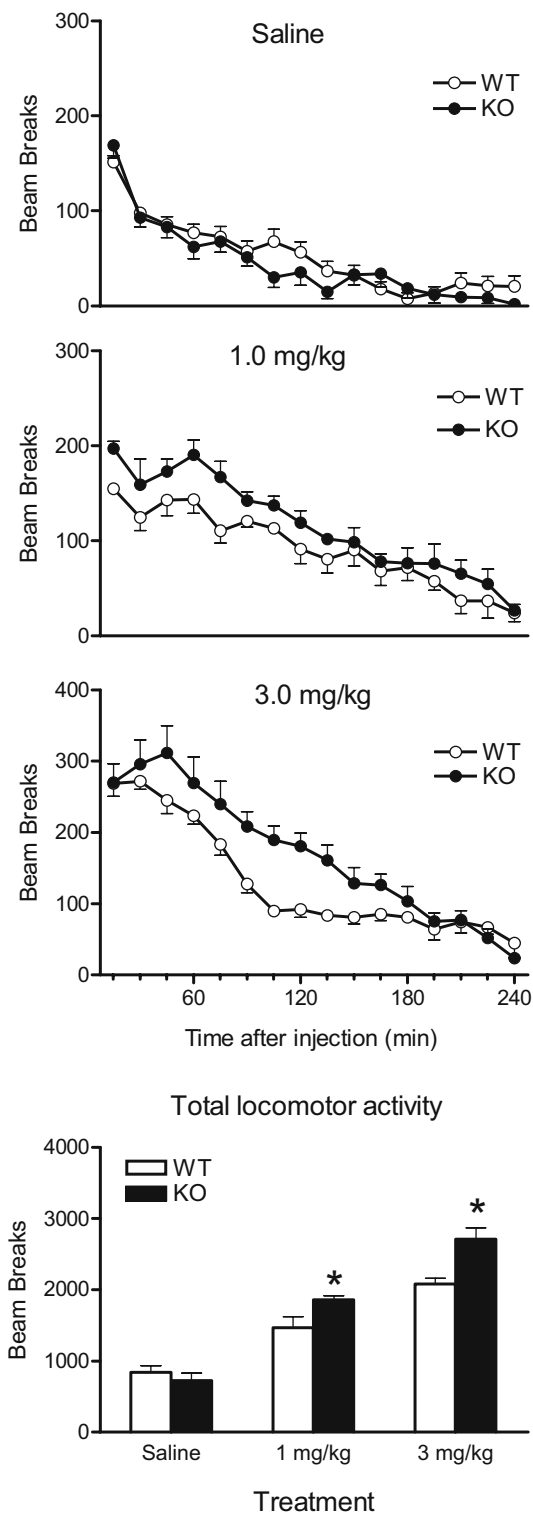


Fig. 3 Locomotor activity of $G\alpha_z$ knockout mice (closed circles) and wild-type control mice (open circles) after treatment with saline (top panel) or 1 (second panel) or 3 mg/kg of amphetamine (third panel). Bottom panel depicts total locomotor activity over the 4-h observation time. Amphetamine treatment increased locomotor activity, and its effect was significantly greater in $G\alpha_z$ knockout mice compared with that in controls. Locomotor activity was measured as the number of beam breaks in 15 min or 4 h \pm SEM. WT Wild-type control mice, KO $G\alpha_z$ knockout mice, * p <0.05 for difference with controls

The between-group factor was genotype. To limit the number of statistical results, post hoc ANOVAs were only done to further analyse genotype differences. For locomotor activity experiments, we similarly used ANOVA with repeated measures, with time after injection being the within-animal factor and genotype being the between-group factor. For receptor autoradiography of different brain regions, one-way ANOVA was used to assess differences between the groups. When P <0.05, differences were considered statistically significant.

Results

Baseline startle and PPI in wild-type and $G\alpha_z$ knockout mice

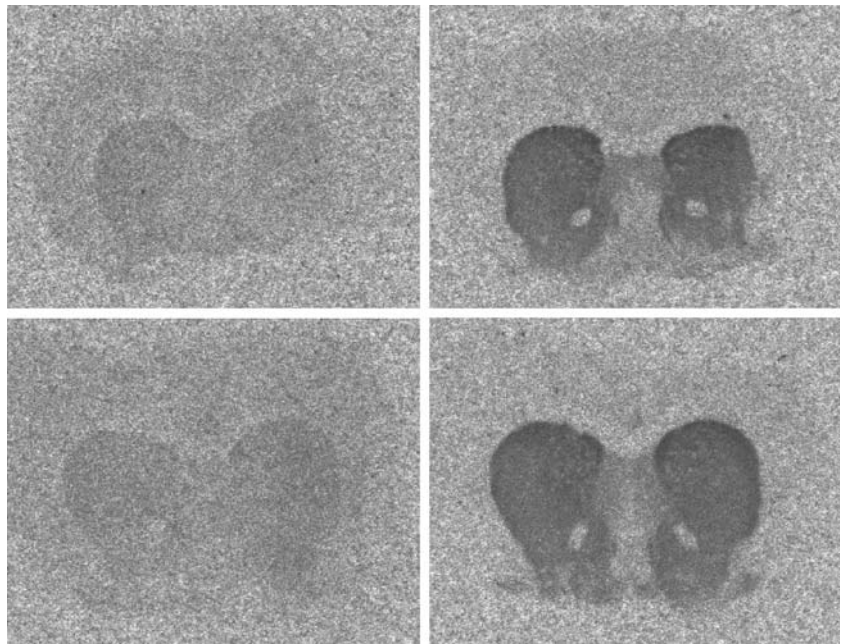
There were no obvious differences between $G\alpha_z$ knockout mice and wild-type controls with respect to appearance, size, and general motor and exploratory behaviours. Comparison of startle, startle habituation and PPI of untreated wild-type mice and $G\alpha_z$ knockouts showed no genotype effect on startle (Fig. 1). Furthermore, while there was significant habituation of startle over the test session [$F_{(3,93)}=7.0$, P <0.001], this habituation did not differ between groups (Fig. 1). Similarly, while there was a significant main effect of prepulse intensity level [$F_{(4,124)}=94.9$, P <0.001], there was no difference between the groups in PPI (Fig. 1). A genotype \times prepulse interaction reflected the tendency for $G\alpha_z$ knockout mice to show slightly higher PPI than controls at low prepulse intensities and slightly lower PPI at high prepulse intensities (Fig. 1). However, analysis of the individual prepulse intensities failed to find significant differences between the groups. Average PPI was $47\pm 3\%$ in wild-type controls and $46\pm 4\%$ in $G\alpha_z$ knockout mice.

Effect of amphetamine, apomorphine and MK-801 treatment on startle

When all treatment data were included, analysis of startle responses to four blocks of pulse-alone stimuli (Table 1) revealed a main effect of treatment [$F_{(4,93)}=9.9$, P <0.001]; however, the lack of a main effect of genotype or a treatment \times genotype interaction indicated that there was no difference in the effect of treatment on startle between wild-type and knockout mice. There was also the expected main effect of block [$F_{(3,93)}=3.3$, $P=0.033$], reflecting habituation of the startle response; however, while this was differentially affected by the treatments [block \times treatment interaction, $F_{(9,279)}=5.7$, P <0.001], habituation did not differ between the genotypes (Table 1).

Further pairwise analysis was done on data from different treatments vs saline. Amphetamine treatment had no effect on startle, although a small effect on habituation was found [treatment \times block interaction, $F_{(3,93)}=3.9$, $P=0.012$]. Apomorphine treatment had no effect on startle or habituation. In contrast, MK-801 treatment significantly increased startle

Fig. 4 Representative dopamine D2 receptor autoradiograms of a wild-type control mouse (*top panels*) and a $G\alpha_z$ knockout mouse (*bottom panels*). *Left panels* show non-specific binding, whereas *right panels* show total binding of [3 H] YM-09151-2. For group averages of specific binding densities, see Table 1



responses [$F_{(1,31)}=20.1$, $P<0.001$] and enhanced habituation [main effect of block, $F_{(3,93)}=8.4$, $P<0.001$; block \times treatment interaction, $F_{(3,93)}=5.5$, $P=0.002$]. In all of these cases, responses were not different between wild-type controls and $G\alpha_z$ knockout mice (Table 1).

Effect of amphetamine, apomorphine and MK-801 treatment on PPI

When all treatment data were included (Fig. 2), analysis of PPI revealed main effects of genotype [$F_{(1,31)}=15.4$, $P<0.001$], of treatment [$F_{(3,93)}=15.0$, $P<0.001$] and of prepulse [$F_{(4,124)}=284.9$, $P<0.001$], as well as genotype \times treatment [$F_{(3,93)}=4.6$, $P=0.005$], genotype \times prepulse [$F_{(4,124)}=5.2$, $P=0.001$] and treatment \times prepulse interactions [$F_{(12,372)}=3.6$, $P<0.001$].

Further analysis of the effect of the individual drugs compared to saline revealed that the effect of amphetamine on PPI was greater in $G\alpha_z$ knockout mice than in controls [main effect of treatment, $F_{(1,31)}=11.8$, $P=0.002$; treatment \times genotype interaction, $F_{(1,31)}=14.7$, $P=0.001$]. Amphetamine treatment markedly reduced PPI in $G\alpha_z$ knockout mice [average PPI reduced from 44 ± 4 to $22\pm 2\%$, $F_{(1,14)}=61.5$, $P<0.001$]. In contrast, the effect of amphetamine was of borderline significance in wild-type controls [average PPI reduced from 49 ± 3 to $42\pm 3\%$, $F_{(1,17)}=4.7$, $P=0.044$]. Unlike in saline treatment, there was a significant difference in the level of PPI between the genotypes after amphetamine treatment [$F_{(1,31)}=30.3$, $P<0.001$].

Furthermore, the effect of apomorphine compared to saline was greater in $G\alpha_z$ knockout mice than in controls [main effect of treatment, $F_{(1,31)}=28.7$, $P<0.001$; treatment \times genotype interaction, $F_{(1,31)}=4.7$, $P=0.038$]. The effect of apomorphine on PPI was significant in both genotypes, although it was greater in $G\alpha_z$ knockout mice [average PPI

reduced from 44 ± 4 to $23\pm 3\%$, $F_{(1,14)}=17.8$, $P=0.001$] than in wild-type controls [average PPI reduced from 49 ± 3 to $40\pm 2\%$, $F_{(1,17)}=9.0$, $P=0.008$]. As with amphetamine treatment, there was a significant difference in the level of PPI between the genotypes after apomorphine treatment [$F_{(1,31)}=14.7$, $P=0.001$].

In contrast to amphetamine and apomorphine, the effect of MK-801 on PPI was not different between $G\alpha_z$ knockout mice and controls [main effect of treatment only, $F_{(1,31)}=21.1$, $P<0.001$]. Average PPI was reduced from 44 ± 4 to $29\pm 5\%$ in $G\alpha_z$ knockout mice and from 49 ± 3 to $33\pm 2\%$ in wild-type controls.

Analysis of individual treatment effects also showed the expected main effects of prepulse and treatment \times prepulse interactions, reflecting that drug effects were greatest at lower prepulse intensities (not shown).

Effect of amphetamine treatment on locomotor activity

Analysis of locomotor activity data from all treatment groups showed the expected main effect of dose [$F_{(2,43)}=106.5$, $p<0.001$], confirming the locomotor hyperactivity induced by amphetamine treatment (Fig. 3). This effect was greater in $G\alpha_z$ knockout mice than in wild-type control mice [main effect of genotype, $F_{(1,43)}=10.3$, $p=0.003$; genotype \times dose interaction, $F_{(2,43)}=6.0$, $p=0.005$]. There were also main effects of time [$F_{(15,645)}=107.0$, $p<0.001$] and time \times dose [$F_{(30,346)}=9.9$, $p<0.001$] and time \times dose \times genotype interactions [$F_{(30,645)}=1.9$, $p=0.002$].

When analysing data after saline treatment, there was no difference between $G\alpha_z$ knockout mice and controls. Only a significant effect of time was found [$F_{(15,270)}=51.4$, $p<0.001$], illustrating the fall in spontaneous activity over time in both groups (Fig. 3). When analysing data after treatment with 1 mg/kg of amphetamine, it was found that

Table 2 Dopamine D2 receptor binding densities in the forebrain of $G\alpha_z$ knockout mice compared to wild-type controls, as assessed by [3H] YM-09151-2 autoradiography

Brain region	Wild-type controls	$G\alpha_z$ knockout mice
Nucleus accumbens shell	55.4±4.8	53.7±2.5
Nucleus accumbens core	59.1±3.4	60.2±2.9
Prelimbic cortex	1.28±0.39	1.69±0.44
Caudate nucleus	68.4±6.3	74.3±3.2

Data are mean±SEM of eight mice per group

$G\alpha_z$ knockout mice displayed slightly, but significantly, greater locomotor hyperactivity than controls (Fig. 3), with the same time-course [main effect of genotype, $F_{(1,12)}=5.7$, $p=0.034$; main effect of time, $F_{(15,180)}=28.9$, $p<0.001$]. When analysing data after treatment with 3 mg/kg of amphetamine, the genotype difference was more pronounced [Fig. 3, main effect of genotype, $F_{(1,13)}=11.8$, $p=0.004$; main effect of time, $F_{(15,195)}=39.3$, $p<0.001$; genotype × time interaction, $F_{(15,195)}=2.0$, $p=0.017$]. In addition, the total number of beam breaks in the 4-h observation time was significantly greater in $G\alpha_z$ knockout mice than in controls (Fig. 3).

Autoradiography analysis

When comparing $G\alpha_z$ knockout mice and wild-type control mice across the three different brain regions, there was a main effect of region [main effect of region, $F_{(2,26)}=17.0$, $p<0.001$], reflecting the higher binding in the striatum compared with the nucleus accumbens core and shell (Fig. 4). In contrast, there was no main effect of genotype or any interaction between genotype and regions, indicating that the binding densities were similar in $G\alpha_z$ knockout mice and controls (Table 2).

Discussion

This study examined the effect of the loss of $G\alpha_z$ on startle responses and PPI. $G\alpha_z$ knockout mice were also tested for the effect of amphetamine, apomorphine and MK-801 on PPI and of amphetamine on locomotor activity. Finally, we assessed if $G\alpha_z$ knockout mice showed changes in dopamine D2 receptor density. The main finding of this study was that $G\alpha_z$ knockout mice showed enhanced sensitivity to the disruption of PPI induced by treatment with amphetamine and apomorphine but not MK-801 and, similarly, enhanced sensitivity to the locomotor activity stimulating action of amphetamine. On the other hand, dopamine D2 receptor densities were not altered in the nucleus accumbens or caudate nucleus of these mice, showing that the behavioural results could not simply be explained by an up-regulation of D2 receptor levels in the knockout mice.

Amphetamine is an indirect dopaminergic stimulant, acting on the dopamine transporter to increase extracellular

dopamine levels, which then stimulates pre- and postsynaptic dopamine receptors. Amphetamine is not specific for dopamine systems, acting on noradrenaline and, to some extent, serotonin systems as well. However, the effect of amphetamine on PPI in rats has been shown to be mediated by dopamine release in the nucleus accumbens, as 6-hydroxydopamine-induced dopamine depletion in this nucleus reverses the effect (Swerdlow et al. 1990). Similar dopamine depletion in the nucleus accumbens inhibited the effect of amphetamine on locomotor activity (Creese and Iversen 1975; Kelly et al. 1975). The action of amphetamine on PPI was not seen in genetically modified mice lacking the dopamine D2 receptors, although mice lacking the D3 and D4 receptors displayed normal disruption of PPI after amphetamine treatment (Ralph et al. 1999). Taken together, it is likely that the enhanced effect of amphetamine in $G\alpha_z$ knockout mice in the present study is mediated by enhanced dopamine release acting on dopamine D2 receptors in the nucleus accumbens.

If it is assumed that G_z is coupled particularly to presynaptic dopamine D2 receptor function (see "Introduction"), we can explain the enhanced effect of amphetamine on PPI and on locomotor activity in $G\alpha_z$ knockout mice. Amphetamine-induced dopamine release is normally limited via the stimulation of presynaptic dopamine D2 receptors. If this presynaptic inhibition is reduced due to the lack of $G\alpha_z$, dopamine release and the resulting activation of postsynaptic dopamine D2 receptors would be enhanced, leading to a greater disruption of PPI and stimulation of locomotor activity. Clearly, this hypothetical model needs to be tested, for example by microdialysis experiments, to confirm the enhanced increase in extracellular levels of dopamine levels after amphetamine treatment in $G\alpha_z$ knockout mice.

Other studies have also found that presynaptic regulation of dopamine release plays an important role in PPI. Previous studies in the $G\alpha_z$ knockout mouse found enhanced locomotor hyperactivity after treatment with cocaine (Yang et al. 2000). In rats, when a group of animals was divided into those with high PPI and those with low PPI, the high-PPI group showed significantly lower presynaptic inhibition of dopamine release in vitro than the low-PPI group (Yamada et al. 1998). It is tempting to speculate that these natural variations in presynaptic control of PPI are mediated by different levels of G_z activity. Thus, the reduced presynaptic inhibition of dopamine release seen in the high-PPI group of rats (Yamada et al. 1998) would be comparable with the reduced presynaptic inhibition of dopamine release and enhanced disruption of PPI in the amphetamine-treated $G\alpha_z$ knockout mice in the present study.

Apomorphine is a dopamine receptor agonist that stimulates both D1-like receptors and D2-like receptors. There is some controversy as to the exact mechanism of action of apomorphine on PPI. In rats, systemic administration of the dopamine D2/D3 receptor agonist quinpirole, but not the dopamine D1 receptor agonist SK&F 38393, disrupts PPI (Wan and Swerdlow 1993). This effect was also seen when quinpirole was infused into the nucleus

accumbens (Wan and Swerdlow 1993). These data would suggest that dopamine D2/D3 receptor stimulation in the nucleus accumbens plays an important role in the regulation of PPI. In support of this, the disruption of PPI caused by apomorphine treatment in rats can be blocked by pretreatment with haloperidol and other drugs with dopamine D2 receptor blocking activity (Geyer et al. 2001). However, the disruption of PPI caused by apomorphine treatment could not be completely prevented by microinjection of haloperidol into the nucleus accumbens (Hart et al. 1998). Furthermore, in contrast to rats, in mice, the effect of apomorphine on PPI appears to be mediated predominantly by dopamine D1 receptors (Ralph-Williams et al. 2003). The action of apomorphine was not altered in dopamine D2 receptor knockout mice, while the effect was eliminated in dopamine D1 knockout mice (Ralph-Williams et al. 2002). Moreover, treatment with the selective dopamine D1 receptor agonist SK&F 82958, but not quinpirole, caused disruption of PPI in mice (Ralph-Williams et al. 2003).

Dopamine D1 receptors are known to couple to G_s , leading to the activation of adenylate cyclase activity upon stimulation of the receptors (Jackson and Westlind-Danielsson 1994). It is known that dopamine D1 receptors do not couple to G_z (Obadiah et al. 1999; Sidhu et al. 1998). On the other hand, dopamine D5 receptors do interact with G_z (Sidhu et al. 1998), which could be a target for apomorphine's action on PPI. It should be noted, however, that mice deficient in dopamine D5 receptors showed little change in startle responses or PPI compared to wild-type controls (Holmes et al. 2001), although the action of apomorphine was not tested in these animals.

Another possibility to explain the effect of loss of G_{α_z} function on the D1-mediated action of apomorphine could be the interaction of D1 and D2 receptor function. It has been shown in rats that the effects of dopamine D2 receptor stimulation on PPI require concomitant stimulation of D1 receptors (Schwarzkopf et al. 1993; Wan et al. 1996). For example, in rats, the effect of apomorphine treatment on PPI can be blocked by pretreatment with a D1 receptor antagonist (Wan et al. 1996). In mice, a similar interaction could occur (Ralph and Caine 2005). Apomorphine treatment will lead to stimulation of pre- and postsynaptic dopamine D2 receptors, as well as postsynaptic D1 receptors. The effect of apomorphine is then a complex interaction between presynaptic inhibition of endogenous dopamine release and direct stimulation of both D1 and D2 receptors. Reduced G_{α_z} function of (presumably) presynaptic D2 receptors could then lead to an altered balance between pre- and postsynaptic effects of apomorphine, leading to an apparently higher sensitivity to the PPI disruption caused by this treatment.

A third possibility to explain the enhancement of the effect of apomorphine could be to postulate postsynaptic alterations in dopamine D2 receptor functioning, in addition to presynaptic effects, which could influence the apomorphine effect by an alteration of postsynaptic D1/D2 in-

teractions. Either of these hypothetical explanations would need to be investigated in further experiments.

The effect of MK-801 treatment on PPI was not significantly different between G_{α_z} knockout mice and their controls. The NMDA receptor, which is blocked by MK-801, is an ion channel receptor with no association with G_z (Casey et al. 1990; Obadiah et al. 1999; Wong et al. 1992). Thus, it is not surprising that deficiency of G_{α_z} appeared to be without effect. However, some of the effects of NMDA receptor antagonism involve changes in dopamine release and dopamine receptor activation in the nucleus accumbens. For example, stimulation of dopamine D1 receptors, but not D2 receptors, potentiates the disruption of PPI caused by treatment with MK-801 in rats (Bortolato et al. 2005). Unlike the effect of apomorphine, this interaction is apparently not influenced by G_z .

If it is accepted that presynaptic control of dopamine release is altered in the G_{α_z} knockout mice, it could be seen as surprising that (postsynaptic) dopamine D2 receptor densities were not altered. This lack of changes most likely reflects compensations to the mutation during development of the animals. In the mature mice, baseline PPI and locomotor activity were not different, reflecting normal baseline dopaminergic regulation. However, when phasic stimulations occur, such as amphetamine treatment in our experiments, the deficit becomes clear. These phasic deficits are not enough to cause a constitutive upregulation of postsynaptic receptors.

PPI is widely used as a model of sensorimotor gating, and deficiencies of PPI are observed in mental illnesses such as schizophrenia (see "Introduction"). Our results could then have implications for these illnesses. It has been shown by imaging studies that amphetamine treatment in humans leads to enhanced dopamine release in the ventral striatum (nucleus accumbens) more than the dorsal striatum (Drevets et al. 2001). Amphetamine-induced dopamine release was significantly enhanced in patients with schizophrenia (Abi-Dargham et al. 1998; Laruelle et al. 1996). It is tempting to speculate on a role of G_{α_z} in these illness-related changes in dopamine release, i.e. reduced G_{α_z} function may result in reduced presynaptic control of dopamine release, leading to exaggerated release upon stimulation. While this is purely speculative at this point in time, it could make G_z a target for future drug development or, at the very least, a candidate for further study in schizophrenia and related psychiatric illnesses.

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All experiments described in this paper comply with the current laws of Australia.

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