International Journal of Neuropsychopharmacology, Page 1 of 14. © CINP 2012
doi:10.1017/S1461145711001787

ARTICLE

CINP

Sensorimotor gating and D_2 receptor signalling: evidence from a molecular genetic approach

Christoph Völter[1,2], Michael Riedel[2,3], Nicola Wöstmann[2], Désirée S. Aichert[2], Sarah Lobo[4], Anna Costa[2], Anne Schmechtig[5], David A. Collier[6], Annette M. Hartmann[2], Ina Giegling[2], Hans-Jürgen Möller[2], Boris B. Quednow[7], Dan Rujescu[2], Veena Kumari[4,8]* and Ulrich Ettinger[1,2]*

[1] *Department of Psychology, University of Munich, Germany*
[2] *Department of Psychiatry, University of Munich, Germany*
[3] *Klinik für Psychiatrie, Psychotherapie, Gerontopsychiatrie und Neurologie, Rottweil, Germany*
[4] *Department of Psychology, Institute of Psychiatry, King's College London, UK*
[5] *Department of Neuroimaging, Institute of Psychiatry, King's College London, UK*
[6] *Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, UK*
[7] *University Hospital of Psychiatry, Clinical and Experimental Pharmacopsychology, University of Zurich, Switzerland*
[8] *NIHR Biomedical Research Centre for Mental Health, South London and Maudsley NHS Foundation Trust, London, UK*

Abstract

Converging evidence from pharmacological investigations, genetic association studies and schizophrenia research indicates an important influence of the dopamine system on sensorimotor gating as measured by prepulse inhibition (PPI) of the acoustic startle response. In particular, D_2 receptor agonists have been shown to disrupt PPI in humans and rodents. In the present study, we investigated the associations of two functional *DRD2* related single nucleotide polymorphisms (rs4648317 and rs1800497, the latter also known as *DRD2/ANKK1* Taq1A) with PPI in two independent healthy human samples (overall $n=197$; Munich $n=101$; London $n=96$). Taq1A is a prominent marker of striatal D_2 receptor signalling and was therefore hypothesized to impact on PPI. In line with our hypothesis, we report here reduced PPI levels in individuals with higher striatal D_2 receptor signalling as indicated by the Taq1A genotype. Meta-analysis across both samples confirmed this finding. In contrast, an association between rs4648317 and PPI found in the Munich sample could not be confirmed in the London sample. Overall, the present study helps to bridge the gap between pharmacological manipulations of PPI and molecular genetics of the dopaminergic system.

Received 2 May 2011; Reviewed 26 July 2011; Revised 15 November 2011; Accepted 16 November 2011

Key words: Acoustic startle response, dopamine, *DRD2*, prepulse inhibition, Taq1a.

Introduction

Prepulse inhibition (PPI) of the acoustic startle response (ASR) is a widely used psychophysiological measure of sensorimotor gating (Braff *et al.* 1978; Geyer *et al.* 1990; Swerdlow & Geyer, 1998), which helps to avoid sensory information overflow (Braff *et al.* 1999). In this paradigm, in humans, first described by Krauter *et al.* (1973) and systematically investigated by Graham (1975), a loud noise is used to produce a startle reflex that is commonly measured electromyographically above the musculus orbicularis oculi as an eyeblink reflex. Graham (1975) found that a non-startling pre-stimulus preceding the startling stimulus by 30–500 ms reduces the eyeblink reflex of human subjects.

PPI is reduced in people with schizophrenia (e.g. Braff *et al.* 1978; Kumari *et al.* 2008; Swerdlow *et al.* 2006), in unaffected first-degree relatives of schizophrenia patients (Cadenhead *et al.* 2000; Kumari *et al.* 2005*b*), in the prodromal stage of schizophrenia (Quednow *et al.* 2008) and in schizotypal personality disorder (Cadenhead *et al.* 1993, 2000). PPI is partially heritable (Anokhin *et al.* 2003; Dulawa & Geyer, 2000;

Address for correspondence: Dr U. Ettinger, Department of Psychiatry, University of Munich, Nussbaumstr. 7, 80336 Munich, Germany.
Tel: +49 89 5160 3499 *Fax*: +49 89 5160 5789
Email: ulrich.ettinger@med.uni-muenchen.de
* These authors served as Joint Senior authors.

Greenwood *et al.* 2007; Willott *et al.* 2003), with heritability estimates ranging from 32 to >50%. Together, these studies indicate that PPI may represent a schizophrenia endophenotype (Gottesman & Gould, 2003).

The neural basis of PPI in rodents lies in the cortico-pallido-striatal-thalamic and pontine circuitry, with evidence for involvement of the prefrontal cortex, thalamus, hippocampus, nucleus accumbens (NAc), striatum, ventral pallidum (VP), globus pallidus and subpallidal efferents to the pedunculopontine nucleus (PPTg) (for a review, see Swerdlow *et al.* 2008). Functional (Campbell *et al.* 2007; Kumari *et al.* 2003, 2007; Neuner *et al.* 2010) and structural (Kumari *et al.* 2005*a*) neuroimaging studies agree with involvement of these regions in human PPI.

Important clues in the ongoing investigation of the neural control of PPI come from pharmacological studies. Specifically, there is evidence for an important role of the D_2 dopamine receptor (DRD2). For example, it has been shown that DRD2 agonists in rats disrupt PPI (Peng *et al.* 1990; Swerdlow *et al.* 2002*b*). Furthermore, PPI deficits induced by apomorphine (a D_1 and D_2 receptor agonist) in rats can be reversed by haloperidol (which acts as a D_2 antagonist) exhibiting a dose–response relation that is highly correlated with its antipsychotic potency in schizophrenia patients (Martinez *et al.* 2000; Swerdlow & Geyer, 1993; Swerdlow *et al.* 1994). These disruptive effects of D_2 receptor agonists on PPI are thought to be mediated by dopaminergic activity in the NAc (for a review, see Swerdlow *et al.* 2001): increased dopaminergic signalling in the NAc presumably reduces activity of GABAergic projections to the VP (Kretschmer & Koch, 1998). VP activity, in turn, is assumed to cause a tonic regulation of the PPTg, which is thought to mediate PPI via innervations of the nucleus reticularis pontis caudalis (for a review, see Fendt *et al.* 2001). Irrespective of NAc activity, the caudate nucleus also seems to play a role in the dopaminergic regulation of PPI (Hart *et al.* 1998).

In contrast to rats, in humans, the effects of D_2 receptor agonists such as bromocriptine are less clear: whereas some studies found a disruptive effect of bromocriptine on PPI (Abduljawad *et al.* 1998, 1999), others did not (Swerdlow *et al.* 2002*a*, *b*). In the context of schizophrenia, it has been shown that antipsychotic-induced improvement of PPI is likely due to D_2 receptor antagonism (Quednow *et al.* 2006).

Complementary to pharmacological manipulation studies, the investigation of functional genetic polymorphisms offers another way to elucidate the role of specific neurotransmitter systems in human PPI. An advantage of this approach is that naturally occurring variation in the expression level or functional properties of a protein is used without exogenous manipulations as in pharmacological investigations. Focusing on dopaminergic influences on sensorimotor gating, such studies have established associations between a number of genetic polymorphisms and PPI. Among the most prominent single nucleotide polymorphisms (SNPs) showing association with PPI are the catechol-*O*-methyltransferase (*COMT*) $Val^{158}Met$ SNP (Quednow *et al.* 2009, 2010; Roussos *et al.* 2008*b*) and the dopamine receptor D_3 (*DRD3*) $Ser^{9}Gly$ SNP (Roussos *et al.* 2008*a*). These molecular genetic findings strengthen the evidence for a modulatory role of dopamine in PPI.

The present study aims to clarify the influence of D_2 receptor signalling on sensorimotor gating by investigating associations of *DRD2* SNPs with PPI. Specifically, two functional *DRD2* related SNPs, rs7122454/rs4648317 and rs1800497 (*DRD2/ANKK1* Taq1A), are examined. While the present study was in progress, three studies have been published that investigated associations of PPI with SNPs in the D_2 dopamine receptor gene (*DRD2*) (Hokyo *et al.* 2010; Montag *et al.* 2008; Roussos *et al.* 2008*b*). Hokyo *et al.* (2010) investigated interaction effects in Japanese schizophrenia patients and healthy controls on the association between PPI and *DRD2* rs1801028 and rs6277, respectively. Montag *et al.* (2008) and Roussos *et al.* (2008*b*) examined the *DRD2/ANKK1* Taq1A (rs1800497) SNP in healthy, Caucasian subjects. None of these studies obtained a significant association between the *DRD2* SNPs and PPI. However, the two studies of the *DRD2/ANKK1* Taq1A SNP (Montag *et al.* 2008; Roussos *et al.* 2008*b*), a polymorphism widely studied in the context of striatal dopamine signalling, used somewhat uncommon startle parameters and experimental variations of prepulse intensity, respectively. Importantly, small changes in stimulus characteristics can considerably affect PPI (e.g. Csomor *et al.* 2006, 2008; Hsieh *et al.* 2006). Therefore, the generalizability of those initial *DRD2/ANKK1* Taq1A SNP findings might be limited. Additionally, Montag *et al.* (2008) sampled only female subjects, whereas Roussos *et al.* (2008*b*) included only male subjects.

Given the role of the *DRD2/ANKK1* Taq1A SNP in striatal dopamine signalling (Hirvonen *et al.* 2009; Jönsson *et al.* 1999; Pohjalainen *et al.* 1998*a*; Ritchie & Noble, 2003; Thompson *et al.* 1997), it is important to further examine this matter using established stimulus characteristics with known sensitivity to schizophrenia (Kumari & Ettinger, 2005).

The *DRD2/ANKK1* Taq1A polymorphism is located 9.5 kb downstream from the *DRD2* gene (Grandy *et al.* 1993) in the ankyrin repeat and kinase domain containing 1 (*ANKK1*) gene. The *ANKK1* and *DRD2* genes overlap and share haplotype blocks. The Taq1A SNP leads to a substitution of an acidic amino acid for a basic amino acid ($Glu^{713}Lys$), which potentially might lead to a change in protein function. The mutated A1 allele has a prevalence of 19.5%, the homozygous A1A1 genotype of 5.3% in healthy humans of Caucasian descent (The International HapMap Consortium, 2003). Therefore, the A1 allele carriers are often contrasted with carriers without the A1 allele, i.e. A2 allele homozygotes. Crucially, several positron emission tomography and autoradiographic studies have shown that the minor A1 allele is associated with reduced striatal D_2 receptor density (up to 30%) and receptor binding compared to the A2A2 allele status in humans (Hirvonen *et al.* 2009; Jönsson *et al.* 1999; Pohjalainen *et al.* 1998*a*; Ritchie & Noble, 2003; Thompson *et al.* 1997). In addition, the A1 allele has been associated with reduced substantia nigra volume (Cerasa *et al.* 2009), a key dopaminergic area. The role of the Taq1A polymorphism in the aetiology of schizophrenia is controversial: whereas some case–control studies found an association of the A1 allele with schizophrenia (Comings *et al.* 1991; Monakhov *et al.* 2008), other studies provide evidence for an increased frequency of the A2 allele in schizophrenia patients (Dubertret *et al.* 2001, 2004, 2010; Vijayan *et al.* 2007). Golimbet *et al.* (2003) found that schizophrenia-concordant sibling pairs had significantly more often common alleles at the Taq1A locus.

The second SNP investigated in this study is rs4648317/rs7122454, two SNPs in complete linkage disequilibrium (LD). This SNP (unlike Taq1A) has been associated in females with prolactin concentrations (Fukui *et al.* 2010). Prolactin secretion is inhibited by the hypothalamic dopamine transmission via the D_2 receptor (Grattan & Kokay, 2008). Specifically, the T allele was associated with increased prolactin concentration, presumably caused by a reduced D_2 receptor transmission. This SNP is located in intron 1 of *DRD2* and alters two potential transcription factor binding sites (Laucht *et al.* 2008), which might indicate an effect on *DRD2* expression. Laucht *et al.* (2008) also reported an association between rs4648317 and nicotine dependence in adolescents, with the T allele carrier status related to higher nicotine dependence. As nicotine causes striatal dopamine release in rats (e.g. Kaiser *et al.* 1998; Wonnacott *et al.* 2000) and in humans (Brody *et al.* 2004), Laucht *et al.* (2008) suggested that the association between *DRD2* SNPs and nicotine dependence might rely on changes in striatal *DRD2* expression levels that influence, in turn, nicotine-induced reward mediated by dopamine release. If this is true, we would also expect an association of this SNP with PPI.

In addition, Hamidovic *et al.* (2009) found an association between rs4648317 genotype and inhibitory performance in the stop signal task as well as self-report impulsivity (Zuckerman–Kuhlman Personality Questionnaire, Impulsivity subscale). Both measures indicated lower impulsivity of T allele carriers compared to CC carriers.

In summary, we investigated the association with PPI of two *DRD2* SNPs thought to influence striatal dopamine signalling. Given the importance of replication in molecular genetics (Chanock *et al.* 2007), we investigate this relationship in two independent samples. Based on previous molecular studies, we hypothesized that Taq1A A2A2 allele carriers, who exhibit higher striatal *DRD2* levels compared to A1 carriers, would show reduced PPI. Considering rs4648317/rs7122454, we had no directed hypothesis. However, based on previous research on nicotine dependence we expected an association between this SNP and PPI.

Method and materials

Participants

Munich sample

Altogether, 118 healthy, unrelated volunteers of Caucasian descent were recruited through local advertisements in Munich, Germany. Exclusion criteria consisted of: (1) any current psychiatric diagnosis (using the German version of the Mini International Neuropsychiatric Interview; Ackenheil *et al.* 1999); (2) a history of neurological complications; (3) a history of psychosis or attention deficit hyperactivity disorder in first-degree relatives; (4) any current physical condition; (5) any current consumption of over-the-counter or prescription medication (except for contraceptives); (6) any hearing impairments or tinnitus. Participants provided demographic information and data on smoking status and severity (using the Fagerström test for nicotine dependence; Heatherton *et al.* 1991). Subjects scoring >0 on this test were considered smokers.

London sample

Altogether, 106 healthy Caucasian volunteers were recruited through local advertisements in South

London, UK. Exclusion criteria consisted of: (1) DSM-IV Axis I disorders using the Structured Clinical Interview for DSM-IV disorders for healthy subjects; (2) a history of neurological complications, substance abuse or dependence or hearing impairments; (3) a history of psychosis in first-degree relatives; (4) any current physical condition or medication. The assessment of smoking behaviour was identical to the Munich sample.

For both samples, the study had approval from the local ethics committees. Subjects provided written informed consent and were reimbursed for their participation.

Genotyping

For the Munich sample, DNA was extracted from 3 ml saliva samples using QIAamp DNA-Blood-Midi-Kit (Qiagen, Germany). For the London sample, DNA was extracted from cheek swabs or venous blood. Genotyping was based on matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry (Haff & Smirnov, 1997). Therefore, genomic sequences flanking the SNPs of interest of the extracted DNA samples were first amplified using a multiplex polymerase chain reaction (PCR), otherwise called SNP capture reaction (Oeth *et al.* 2005). The primers for the multiplex PCR were as follows: for *ANKK1* Taq1A (rs1800497) the forward primer: 5′-XTG TGC AGC TCA CTC CAT CCT-3′ and the reverse primer: 5′-XTC AAG GGC AAC ACA GCC ATC-3′; for *DRD2* rs7122454 the forward primer: 5′-XTA AAA TGG TGG TGG CCA GAG-3′ and the reverse primer: 5′-XGT CTT GAA ACA CTG AAG CTC-3′ (X = ACGTTGGATG; mass tag).

MassExtend primers that were hybridizing at the 5′ end directly in front of the polymorphic locus of the PCR products were used for genotyping. These MassExtend primers (including possible extensions) were as follows: for *ANKK1* Taq1A (rs1800497): 5′-CAT CCT CAA AGT GCT GGT C(A/G)-3′; for *DRD2* rs7122454: 5′-ACG AAT TCT CCA TGT TAC T(C/G)-3′. In the cyclic iPLEX reaction, the MassExtend primers were extended by a single base extension (SBE) that was complementary to the base at the polymorphic locus. These SBE products were then analysed by MALDI-TOF mass spectrometry (Oeth *et al.* 2005). Thereby, the SNP genotype could be identified.

Genotyping was successful in 98.1% of subjects for both investigated SNPs in the Munich sample. For the London sample, genotyping was successful in 97.1% of subjects for the rs1800497 SNP and 94.2% of subjects for the rs7122454 SNP.

Startle response measurement

The ASR was measured with a commercially available electromyographic (EMG) startle reflex system (SR-Lab, USA). The system generates and delivers the acoustic stimuli and records and scores EMG activity for a period of 250 ms (sample interval = 1 ms) beginning at the onset of the acoustic stimulus. The stimuli were presented binaurally to the subjects via headphones while they were sitting comfortably in a moderately lit laboratory room. The startle response was recorded from the right orbicularis oculi muscle with two (6 mm) silver/silver chloride electrodes filled with electrode gel. The electrodes were placed approximately 1 cm below the pupil and 1 cm below the outer corner of the right eye. The ground electrode was located behind the left ear above the mastoid. Previously, the skin at these positions was cleaned with sterile alcohol swabs.

Each session started with a 4-min acclimatization period consisting of 70 dB white noise that was present throughout the session. Subjects were then presented with 49 white-noise sound pulses. Each pulse-alone (PA) stimulus had an intensity of 110 dB in the Munich sample and 115 dB in the London sample, which was presented for a period of 40 ms. The inter-trial intervals varied between 9 and 23 s (mean = 15 s). In 36 of these trials, there was an 85-dB white noise prepulse presented for a period of 20 ms preceding the pulse with a stimulus-onset asynchrony (SOA) of 30, 60 or 120 ms (12 trials for each SOA). The first PA trial was not used in the statistical analysis. The order of the remaining 48 trials was pseudo-randomized. The whole session lasted approximately 16 min.

Each trial was inspected offline in order to exclude unusable trials, i.e. trials with unstable or noisy data. Scoring was performed by the analytic SR-Lab program (SRRED2). The criterion for the onset latency (ms) was a shift of six digital units from the baseline value occurring in a time window of 21–120 ms after the pulse. The baseline was determined by the mean amplitude (arbitrary analogue-to-digital units; 1 unit = 2.62 μV) in the first 20 ms after the pulse stimulus. The peak latency (ms) was defined as the point in time of maximal amplitude occurring within 150 ms after the acoustic stimulus. Exclusion criteria for single trials were periods of >95 ms between the onset and peak latencies and onset latency >100 ms. For all subjects, the amplifier gain control was kept constant. A 50-Hz band-pass filter was applied to eliminate interference from the power mains. Subjects with response rejections $>30\%$ in PA trials were

excluded from further analysis. Smoking before the assessment was not restricted to prevent effects of smoking withdrawal (Kumari *et al.* 1996).

Statistical analysis

Statistical analysis using SPSS 17.0 (SPSS Inc., USA) was identical in both samples. The significance level was set at $p<0.05$. p values between 0.1 and 0.05 were considered trends towards significance. In order to eliminate individual differences in startle responsivity, %PPI was calculated as follows:

$$\%\mathrm{PPI} = 100 \times \frac{\begin{pmatrix}\text{amplitude of}\\ \text{PA trial}\end{pmatrix} - \begin{pmatrix}\text{amplitude of}\\ \text{prepulse trial}\end{pmatrix}}{\text{amplitude of PA trial}}$$

PPI was computed for each SOA and subject separately. Previous research has shown that gender (Aasen *et al.* 2005; Swerdlow *et al.* 1993) and smoking status (Swerdlow *et al.* 2006) influences PPI. Therefore, %PPI was analysed by analysis of covariance (ANCOVA) using the factors SOA and genotype of both SNPs and the covariates gender and smoking status.

For the analysis of the habituation of the ASR, PA trials were divided into four blocks. Percent habituation was calculated as percent reduction in startle magnitude between the first and second block as well as between the first and last block of the PA trials. Startle reactivity was measured by calculating the mean values of PA trials in the first block and across all four blocks. Moreover, the mean amplitudes of prepulse trials and latencies to onset and peak of the ASR in PA trials were analysed.

The analyses of genotype associations with demographic data and psychophysiological measures (habituation, startle reactivity and latency to onset) were conducted using one-way analyses of variance (ANOVAs) for continuous measures and χ^2 tests for frequency data. Greenhouse–Geisser correction of degrees of freedom was applied to ANOVAs if the assumption of sphericity was violated, as assessed by Mauchly's test. On the basis of significant interactions *post-hoc* pairwise t tests using Bonferroni correction were conducted. As the distribution of startle amplitudes significantly deviated from normal distribution, a square root transformation was applied to normalize the data. It should be noted that the pattern of significant findings was independent of whether square root transformed or untransformed variables were used.

To estimate effect sizes for between-group differences, we used Cohen's d. These calculations are based on pooled SOA conditions. To estimate effect sizes for differences between more than two groups, η^2_P values were calculated (Pierce *et al.* 2004).

Finally, in order to provide summary statistics of genotype associations across the two samples in this study, meta-analysis was carried out to assess genotype associations with PPI across both samples by using Stouffer's z-trend method and Fisher's trend to combine p values (Whitlock, 2005). For the analysis of effect sized across samples, a random effects model was used according to Hedges & Olkin (1985).

Results

Munich sample

Fifteen subjects were startle non-responders, as defined by response rejections in $>30\%$ of PA trials and were excluded from further analysis, leaving a total sample of $n=101$.

As expected, rs7122454 and rs4648317 were in complete LD ($r^2=1.0$, $d'=1.0$). *ANKK1* Taq1A (rs1800497) and rs7122454/rs4648317 polymorphisms were not in strong LD ($r^2=0.01$, $d'=0.57$). For *ANKK1* Taq1A [$\chi^2(1)=0.48$, $p=0.49$] and rs7122454/rs4648317 [$\chi^2(1)=0.67$, $p=0.41$] genotype frequencies were distributed in accordance with the Hardy–Weinberg Equilibrium (HWE).

Genetic associations with PPI

Demographic and startle data of the Munich sample and their distribution across genotype groups are shown in Table 1 (for habituation and latency data, see Supplementary Table S1). The *ANKK1* Taq1A genotype groups differed in the gender distribution. However, startle amplitudes did not differ significantly between genotype groups.

PPI was affected by genotype: a $3\times2\times2$ (SOA condition $\times$ *DRD2* rs7122454 SNP $\times$ *ANKK1* Taq1A SNP) repeated measures ANCOVA with the covariates gender and smoking status revealed a significant main effect of SOA ($F_{2,190}=12.8$, $p<0.001$, $\eta^2_P=0.12$) and a trend-level effect of the covariate gender reflecting stronger PPI in males than females ($F_{1,95}=3.4$, $p=0.07$, $\eta^2_P=0.04$). Moreover, there was a SOA $\times$ *ANKK1* Taq1A genotype interaction ($F_{2,190}=5.9$, $p<0.01$, $\eta^2_P=0.06$) and a significant main effect of Taq1A genotype ($F_{1,95}=4.3$, $p<0.05$, $\eta^2_P=0.04$, $d=0.236$), reflecting reduced PPI for A2A2 allele carriers compared to A1 allele carriers. In addition, there was a trend towards significance for rs7122454 ($F_{1,95}=3.5$, $p=0.07$, $\eta^2_P=0.04$, $d=0.660$) pointing to lower PPI in CG/GG allele carriers compared to CC allele carriers. Finally, a significant interaction between the two SNPs was found

Table 1. Demographic and psychophysiological data of the Munich sample grouped according to *ANKK1* Taq1A and *DRD2* rs7122454 genotype

ANKK1 Taq1A	A2A2		A1			*ANKK1* Taq1A		*DRD2* rs7122454		*ANKK1* Taq1A × *DRD2* rs7122454		
DRD2 rs7122454	CC	CG/GG	CC	CG/GG	Total	F/χ^2	p	F/χ^2	p	F/χ^2	p	df/df_{er}
n	49(49%)	26(25%)	18(18%)	8(8%)	101(100%)							
Age, yr	24.3±4.7	26.3±6.3	26.1±6.3	23.8±3.2	24.9±5.4	0.47	0.50	0.08	0.79	1.61	0.21	1/97
Men, %	34.7	42.3	11.1	12.5	30.7	6.04	0.03*	0.51	0.50			1
Smokers, %	8.0	3.8	11.1	0.0	6.9	0.04	1.00	1.23	0.42			1
Years of education	15.6±1.6	16.9±2.6	15.6±2.0	15.9±2.0	16.0±2.0	1.04	0.31	2.71	0.10	0.76	0.39	1/95
First block, amplitude of PA trials (arbitrary units)	197.9±132.3	214.2±129.3	237.6±133.6	263.2±189.8	214.3±136.2	1.76	0.19	0.39	0.53	0.02	0.89	1/97
Mean amplitude of PA trials (arbitrary units)	143.3±101.8	152.5±95.5	180.8±107.1	190.9±120.0	156.2±102.5	2.20	0.14	0.10	0.75	0.00	0.95	1/97
Mean amplitude of PP30 trials (arbitrary units)	99.4±81.7	119.2±96.6	145.1±99.5	157.0±144.4	117.2±95.4	2.82	0.10(*)	0.26	0.61	0.17	0.69	1/97
Mean amplitude of PP60 trials (arbitrary units)	57.1±40.2	93.2±82.4	86.7±74.4	94.5±90.3	74.6±65.4	0.73	0.39	1.64	0.20	1.25	0.27	1/97
Mean amplitude of PP120 trials (arbitrary units)	49.9±43.8	78.8±64.2	53.6±59.1	57.0±40.8	58.6±53.0	0.49	0.49	2.13	0.15	0.69	0.41	1/97
Mean %PPI across three SOA conditions	50.4±14.4	30.8±28.4	47.9±17.4	49.3±18.4	44.8±21.1	4.29	0.04*	3.47	0.07(*)	5.30	0.02*	1/95

PA, Pulse-alone; PP, prepulse; SOA, stimulus onset asynchrony.

Values are shown as mean (±S.D.).

Univariate analyses of variance [for % prepulse inhibition (PPI): analysis of covariance with gender and smoking status as covariates] and for the frequency data χ^2 tests were computed revealing potential differences in the distribution of the shown variables between the genotype groups: $^*p<0.05$, $^{(*)}p<0.1$.

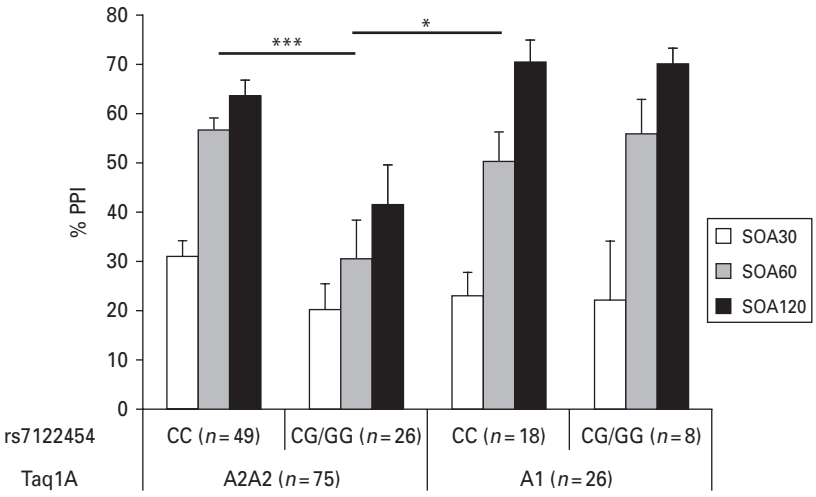

Fig. 1. The association of genotype with prepulse inhibition (PPI) in the Munich sample. Three different stimulus-onset asynchronies (SOAs) between prepulse and pulse were used: 30, 60 and 120 ms. Mean PPI (+S.E.M.) across all participants of the Munich sample and grouped according to the *DRD2* rs7122454 SNP/*ANKK1* Taq1A genotype is depicted (*post-hoc t* tests between genotype groups: * $p<0.05$; *** $p<0.001$; Bonferroni-corrected). Rs7122454 genotype is shown nested within rs1800497 genotype groups.

($F_{1,95}=5.3$, $p<0.05$, $\eta^2_P=0.05$) (see Fig. 1). Bonferroni-corrected *t* tests showed that subjects with the combination A2A2/rs7122454 G had significantly reduced PPI compared to A2A2/rs7122454 CC ($p<0.001$, $d=0.872$) and to A1/rs7122454 G ($p<0.05$, $d=0.775$) carriers. Furthermore, *post-hoc* tests on the basis of the significant interaction between SOA and *ANKK1* Taq1A genotype revealed that A2A2 carriers had reduced PPI only at SOA 120 ms ($p<0.01$, $d=0.590$) and by trend at SOA 60 ms ($p=0.06$, $d=0.162$). At SOA 30 ms, there was no significant difference between the Taq1A genotype groups ($p>0.5$).

Finally, the effects of gender on the main effects of the two polymorphisms were investigated. When restricting the analysis to female subjects, we observed a trend towards a main effect of Taq1A ($F_{1,65}=3.8$, $p=0.056$, $d=0.264$, $\eta^2_P=0.06$) and a significant effect of rs7122454 ($F_{1,65}=4.5$, $p<0.05$, $d=0.726$, $\eta^2_P=0.06$). In contrast, when analysing only male subjects, there was no significant association between PPI and Taq1A ($F_{1,26}=1.0$, $p=0.32$, $d=0.786$, $\eta^2_P=0.04$) or rs7122454 ($F_{1,26}=0.2$, $p=0.63$, $d=0.562$, $\eta^2_P=0.01$).

London sample

Four subjects were startle non-responders and excluded from further analysis, leaving a final sample of $n=96$.

As expected, rs7122454 and rs4648317 were in complete LD ($r^2=1.0$, $d'=1.0$). Again, *ANKK1* Taq1A (rs1800497) and rs7122454/rs4648317 were in weak LD ($r^2=0.002$, $d'=0.30$). For *ANKK1* Taq1A [$\chi^2(1)=0.12$, $p=0.73$] and rs7122454/rs4648317 [$\chi^2(1)=0.06$, $p=0.80$] genotype frequencies were distributed in accordance to HWE.

Genetic associations with PPI

Demographic and startle data of the London sample and their distribution across genotype groups are shown in Table 2 (for habituation and latency data see Supplementary Table S2). The *DRD2* rs7122454 genotype groups differed in their gender distribution. However, startle amplitudes did not differ significantly between genotype groups.

PPI was associated with one of the genotypes: a $3\times2\times2$ (SOA condition $\times$ *DRD2* rs7122454 SNP $\times$ *ANKK1* Taq1A SNP) repeated measures ANCOVA with the covariates gender and smoking status revealed a significant main effect of Taq1A genotype ($F_{1,90}=7.2$, $p<0.01$, $\eta^2_P=0.07$, $d=0.752$). This finding reflects reduced PPI for A2A2 allele carriers compared to A1 allele carriers (see Fig. 2). In contrast, there was no effect of rs7122454 genotype ($F_{1,90}=0.6$, $p=0.44$, $\eta^2_P=0.01$, $d=0.002$) or an interaction between the two SNPs ($F_{1,90}=0.1$, $p=0.80$, $\eta^2_P=0.001$). Additionally, there was the expected significant main effect of SOA ($F_{2,180}=7.7$, $p<0.01$, $\eta^2_P=0.08$). Finally, there was a significant effect of the covariate gender ($F_{1,90}=12.3$, $p<0.01$, $\eta^2_P=0.12$) indicating stronger PPI in males than females.

Next, potential effects of gender on the Taq1A main effect were investigated. When restricting the analysis of the current data to female subjects, we still observed

Table 2. Demographic and psychophysiological data of the London sample grouped according to ANKK1 Taq1A and DRD2 rs7122454 genotype

ANKK1 Taq1A	A2A2		A1			*ANKK1 Taq1A*		*DRD2* rs7122454		*ANKK1* Taq1A × *DRD2* rs7122454		
DRD2 rs7122454	CC	CG/GG	CC	CG/GG	Total	F/χ^2	p	F/χ^2	p	F/χ^2	p	df/df$_{er}$
n	48 (50%)	15 (16%)	27 (28%)	6 (6%)	96 (100%)							
Age, yr	26.4±6.0	25.0±6.0	26.7±6.3	25.0±1.9	26.2±5.8	0.01	0.92	0.91	0.34	0.01	0.92	1/93
Men, %	45.8	66.7	39.3	66.7	48.5	0.49	0.53	4.48	0.05*			1
Smokers, %	16.7	33.3	33.3	33.3	25.0	2.29	0.14	0.46	0.56			1
Years of education	16.9±3.4	17.5±3.3	17.5±4.2	19.0±2.8	17.3±3.6	1.12	0.29	1.12	0.29	0.23	0.63	1/93
First block, amplitude of PA trials (arbitrary units)	670.9±371.4	876.2±523.1	691.6±369.0	583.3±273.1	703.2±394.7	1.65	0.20	0.21	0.65	2.20	0.14	1/93
Mean amplitude of PA trials (arbitrary units)	528.2±346.2	710.4±447.2	584.6±353.9	488.5±180.4	570.2±359.4	0.23	0.64	0.24	0.63	1.27	0.26	1/93
Mean amplitude of PP30 trials (arbitrary units)	472.9±332.2	687.0±526.4	484.1±322.9	399.3±131.3	495.3±352.1	0.97	0.33	0.37	0.55	1.29	0.26	1/93
Mean amplitude of PP60 trials (arbitrary units)	343.6±218.1	504.3±394.3	322.2±226.3	264.1±132.5	350.0±247.1	2.62	0.11	0.31	0.58	1.61	0.21	1/93
Mean amplitude of PP120 trials (arbitrary units)	288.8±202.8	425.3±327.7	312.1±249.0	208.4±80.2	303.4±224.5	1.71	0.19	0.06	0.80	2.15	0.15	1/93
Mean %PPI across three SOA conditions	26.2±19.3	26.8±16.0	38.0±13.2	39.5±11.4	30.4±17.6	7.24	0.01*	0.61	0.44	0.07	0.80	1/90

PA, Pulse-alone; PP, prepulse; SOA, stimulus onset asynchrony.

Values are shown as mean (±S.D.).

Univariate analyses of variance [for prepulse inhibition (PPI): analysis of covariance with gender and smoking status as covariates] and for the frequency data χ^2 tests were computed revealing potential differences in the distribution of the shown variables between the genotype groups: * $p<0.05$.

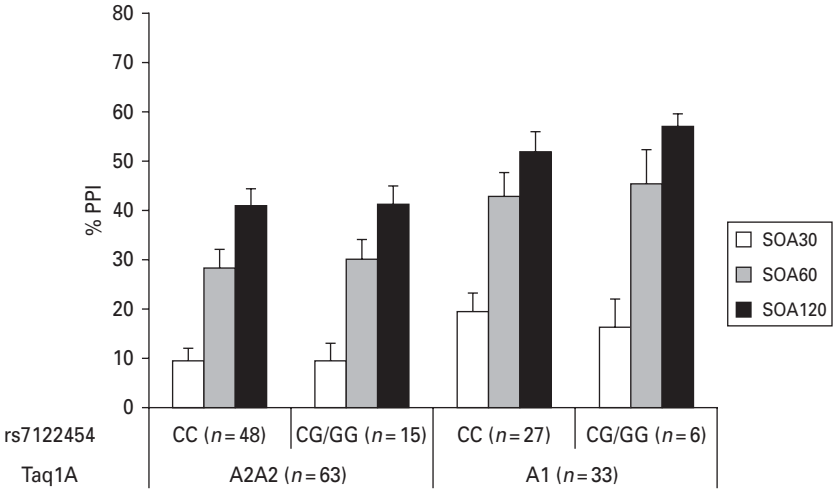

Fig. 2. The association of genotype with prepulse inhibition (PPI) in the London sample. Three different stimulus-onset asynchronies (SOAs) between prepulse and pulse were used: 30, 60 and 120 ms. Mean PPI (+S.E.M.) across all participants of the London sample and separated according to the *DRD2* rs7122454 SNP/*ANKK1* Taq1A genotype is shown. Rs7122454 genotype is shown nested withinTaq1A genotype groups. Taq1A A2A2 allele carrier exhibit significantly reduced PPI compared to A1 allele carriers ($p < 0.01$).

a significant main effect of Taq1A ($F_{1,42} = 4.6$, $p < 0.05$, $d = 0.704$, $\eta^2_P = 0.06$). In contrast, when analysing only male subjects, there was no significant association between PPI and Taq1A ($F_{1,44} = 2.9$, $p = 0.10$, $d = 0.508$, $\eta^2_P = 0.06$).

Meta-analysis

Meta-analysis across the two samples confirmed a significant effect of Taq1A ($p < 0.01$). Using the random effects model, the combined effect size was $d = 0.49$.

For rs7122454, the combined p value was not statistically significant ($p > 0.1$). Using Fisher's trend method the meta-analysis of the interaction between both investigated SNPs reveals a trend towards significance ($p = 0.06$) across samples.

Discussion

In the present study, we aimed to investigate the relationship between sensorimotor gating and striatal D_2 receptor signalling via two functional *DRD2* related polymorphisms. Our results indicate an influence of *DRD2* genotype on PPI. In the Munich sample, both SNPs were associated with PPI. Considering the Taq1A SNP, the A2A2 allele status was associated with reduced PPI. This effect was replicated in the London sample. With regard to rs7122454 (rs4648317), G (T) allele carriers showed reduced PPI in the Munich sample. However, this effect was not confirmed by the data obtained from the London sample. Meta-analysis across both samples indicated a significant association of Taq1A but not of rs4648317/rs7122454 with PPI. In addition, we found a trend for an interaction between both SNPs across the two samples.

Dopaminergic neurotransmission plays a major role within the striatum (e.g. Schultz, 2002) and there is consistent evidence of striatal involvement in PPI modulation (for a review, see Swerdlow *et al.* 2008). Some human pharmacological studies, too, as noted earlier, reveal the impact of striatal, dopaminergic neurotransmission on PPI (Abduljawad *et al.* 1998, 1999). In rats, increased dopaminergic neurotransmission in the NAc disrupts PPI via projections from the VP to the PPTg (Kretschmer & Koch, 1998). The present results are in line with these findings. The Taq1A A2A2 allele status that has previously been associated with increased striatal D_2 receptor availability (Jönsson *et al.* 1999; Pohjalainen *et al.* 1998*a*; Ritchie & Noble, 2003; Thompson *et al.* 1997) shows an association with reduced PPI in our sample. The association of the Taq1A SNP with PPI is likely due to increased striatal D_2 receptor availability in A2A2 allele carriers compared to A1 allele carriers.

The present findings can also be reconciled with evidence from dopaminergic disorders such as schizophrenia. Interestingly, it has been shown that in schizophrenia patients there is an increased baseline occupancy of striatal D_2 receptors (Abi-Dargham *et al.* 2000). Moreover, unaffected monozygotic twins of

schizophrenia patients exhibit increased D_2 receptor density in caudate compared with unaffected dizygotic twins and healthy controls (Hirvonen *et al.* 2005). Together with the current results, these findings suggest that increased striatal D_2 dependent dopamine signalling might be at least partially responsible for PPI deficits in schizophrenia patients and their relatives. Future studies combining molecular imaging of striatal D_2 receptor signalling, PPI recordings and molecular genetics will allow this hypothesis to be critically evaluated.

In the Munich sample, the association of Taq1A with PPI was present for the SOAs of 60 and 120 ms but not for 30 ms. At longer SOAs attentional processes can modulate PPI (Filion *et al.* 1993). As these modulatory influences are not present at very short SOAs, D_2 receptor dependent neurotransmission might not affect PPI at those SOAs, which would explain the present findings. Earlier, Kumari *et al.* (1999) had suggested a particularly strong influence of dopaminergic (D_2) status on PPI at longer SOAs based on their data showing normal range 120 ms PPI in patients medicated with typical or atypical antipsychotics, whereas only clozapine appeared to be effective at shorter SOAs.

In contrast to the present results, Montag *et al.* (2008) and Roussos *et al.* (2008*b*) did not find associations of Taq1A SNP with PPI. The discrepancy of our findings and these previous results might be due to differences in the PPI measurement and sample characteristics. PPI was measured by Montag and colleagues using intensities of background noise, prepulse and pulses of 55, 74 and 106 dB, respectively. In contrast, in the current study, the intensity of background noise, prepulse and pulse was at 70, 85 and 110/115 dB, respectively. We chose these parameters because of their proven sensitivity to schizophrenia in many previous studies (Kumari & Ettinger, 2005). It has already been shown that stimulus characteristics (e.g. ambient 54 dB background noise *vs.* experimentally delivered 70 dB background noise) dramatically alter PPI in healthy people (Hsieh *et al.* 2006) and can produce divergent results in comparisons of schizophrenia *vs.* healthy populations (Braff *et al.* 2001). It is likely that stimulus characteristics are also a source of variance in genetic studies of human PPI. The sample of Roussos *et al.* included only young males and the startle paradigm used an experimental variation of the loudness of the prepulse. The two samples used in the current study were of mixed gender and demographically more heterogeneous. However, when we analysed the gene effects for each gender separately, we still found an association between Taq1A and PPI in female subjects. In contrast, no such effect was found in male subjects. This suggests that the association found in the current study is mainly driven by female subjects, which could explain the lack of an association of the Taq1A polymorphism with PPI in the Roussos study. It is likely that the absence of an association between Taq1A and PPI in the Munich sample is due to the small number of male subjects and to the unexpected interaction between gender and Taq1A genotype (the reasons for which are unclear), yielding only very few male subjects with A1 genotype ($n=3$). However, in the London sample, there are 18 male subjects with A1 genotype (and 15 female A1 subjects) and still there was no significant effect of Taq1A on PPI in male subjects. Whereas previous imaging studies on the effects of Taq1A on striatal D_2 receptor signalling have not found gender differences (Jönsson *et al.* 1999; Pohjalainen *et al.* 1998*a*), the present results might be due to the generally lower striatal D_2 receptor affinity found in women compared to men (Pohjalainen *et al.* 1998*b*). Higher D_2 receptor affinity in men might mask the effects of Taq1A via changes in the D_2 receptor density on PPI.

Finally, on a descriptive level, Montag *et al.* (2008) also found lower levels of PPI for female A2A2 allele carriers compared to A1 carriers.

Considering the second SNP investigated here, rs7122454 (rs4648317), the results of the Munich sample indicated that the G(T) allele status might be associated with reduced PPI. Previous research has shown that the G(T) allele status might also be related to increased nicotine dependence (Laucht *et al.* 2008). Laucht *et al.* suggested that this finding might rely on changes in striatal *DRD2* expression, which affects, in turn, the nicotine-induced reward. Interestingly, high levels of nicotine dependence have also been shown to disrupt PPI under smoking withdrawal (Kumari & Gray, 1999). The present relation might therefore be due to increased striatal D_2 receptor availability for the rs7122454G/rs4648317T genotype. However, since this effect could not be replicated in the London sample and imaging data are missing for this SNP, this conclusion remains speculative and requires further investigation.

The differences in %PPI between the current samples require further discussion. Apart from population differences between London and Munich, there are differences in stimulus characteristics across samples. In the Munich sample, we used a pulse intensity of 110 db, in contrast to 115 db in the London sample. Recently, it has been shown that PPI magnitude decreases with increasingly intense pulse stimuli (Csomor *et al.* 2006, 2008; Yee *et al.* 2005). The current

results confirm these findings. The mean %PPI level (across different SOAs) was significantly lower in the London sample (30.3% ± 17.6) than in the Munich sample (44.8% ± 21.1) [$t(195) = 5.2$; $p < 0.001$]. As already stated above, such differences in stimulus characteristics are a source of additional variance that exacerbates the replication of genetic association studies. This additional variance might also be a reason for the failure to replicate the association between rs7122454 and PPI in the London sample.

In summary, the present results indicate an association of *DRD2* genotype with PPI in healthy humans. Specifically, increased striatal receptor availability as indicated by the Taq1A A2A2 genotype was associated with reduced PPI. This effect was replicated in an independent sample. However, the present findings must be considered within the broader context of previous studies, which did not find such an association. Overall, the molecular genetic approach may provide an important contribution to the characterization of the neural mechanisms of PPI by bridging the gap between evidence from pharmacological studies, imaging studies and molecular genetics.

Note

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/pnp).

Acknowledgement

This work was funded by the Deutsche Forschungsgemeinschaft (ET 31/2-1). Dr Quednow is supported by the Swiss National Science Foundation (Grant No. PP00P1_123516).

Statement of Interest

None.

References

Aasen I, Kolli L, Kumari V (2005). Sex effects in prepulse inhibition and facilitation of the acoustic startle response: implications for pharmacological and treatment studies. *Journal of Psychopharmacology* **19**, 39–45.

Abduljawad KAJ, Langley RW, Bradshaw CM, Szabadi E (1998). Effects of bromocriptine and haloperidol on prepulse inhibition of the acoustic startle response in man. *Journal of Psychopharmacology* **12**, 239–245.

Abduljawad KAJ, Langley RW, Bradshaw CM, Szabadi E (1999). Effects of bromocriptine and haloperidol on prepulse inhibition: comparison of the acoustic startle eyeblink response and the N1/P2 auditory-evoked response in man. *Journal of Psychopharmacology* **13**, 3–9.

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, *et al.* (2000). Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proceedings of the National Academy of Sciences USA* **97**, 8104–8109.

Ackenheil M, Stotz G, Dietz-Bauer R, Vossen A (1999). *Mini International Interview – German version 5.0.0*. München: Psychiatrische Universitätsklinik München.

Anokhin AP, Heath AC, Myers E, Ralano A, *et al.* (2003). Genetic influences on prepulse inhibition of startle reflex in humans. *Neuroscience Letters* **353**, 45–48.

Braff DL, Geyer MA, Light GA, Sprock J, *et al.* (2001). Impact of prepulse characteristics on the detection of sensorimotor gating deficits in schizophrenia. *Schizophrenia Research* **49**, 171–178.

Braff DL, Stone C, Callaway E, Geyer M, *et al.* (1978). Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology* **15**, 339–343.

Braff DL, Swerdlow NR, Geyer MA (1999). Symptom correlates of prepulse inhibition deficits in male schizophrenic patients. *American Journal of Psychiatry* **156**, 596–602.

Brody AL, Olmstead RE, London ED, Farahi J, *et al.* (2004). Smoking-induced ventral striatum dopamine release. *American Journal of Psychiatry* **161**, 1211–1218.

Cadenhead KS, Geyer MA, Braff DL (1993). Impaired startle prepulse inhibition and habituation in patients with schizotypal personality disorder. *American Journal of Psychiatry* **150**, 1862–1867.

Cadenhead KS, Swerdlow NR, Shafer KM, Diaz M, *et al.* (2000). Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. *American Journal of Psychiatry* **157**, 1660–1668.

Campbell LE, Hughes M, Budd TW, Cooper G, *et al.* (2007). Primary and secondary neural networks of auditory prepulse inhibition: a functional magnetic resonance imaging study of sensorimotor gating of the human acoustic startle response. *European Journal of Neuroscience* **26**, 2327–2333.

Cerasa A, Gioia MC, Tarantino P, Labate A, *et al.* (2009). The DRD2 TaqIA polymorphism associated with changed midbrain volumes in healthy individuals. *Genes, Brain and Behavior* **8**, 459–463.

Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, *et al.* (2007). Replicating genotype-phenotype associations. *Nature* **447**, 655–660.

Comings DE, Comings BG, Muhleman D, Dietz G, *et al.* (1991). The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. *Journal of the American Medical Association* **266**, 1793–1800.

Csomor PA, Yee BK, Quednow BB, Stadler RR, *et al.* (2006). The monotonic dependency of prepulse inhibition of the acoustic startle reflex on the intensity of the startle-eliciting stimulus. *Behavioural Brain Research* **174**, 143–150.

Csomor PA, Yee BK, Vollenweider FX, Feldon J, *et al.* (2008). On the influence of baseline startle reactivity on the indexation of prepulse inhibition. *Behavioral Neuroscience* **122**, 885–900.

Dubertret C, Bardel C, Ramoz N, Martin PM, *et al.* (2010). A genetic schizophrenia-susceptibility region located between the ANKK1 and DRD2 genes. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **34**, 492–499.

Dubertret C, Gorwood P, Gouya L, Deybach JC, *et al.* (2001). Association and excess of transmission of a DRD2 haplotype in a sample of French schizophrenic patients. *Schizophrenia Research* **49**, 203–212.

Dubertret C, Gouya L, Hanoun N, Deybach JC, *et al.* (2004). The 3′ region of the DRD2 gene is involved in genetic susceptibility to schizophrenia. *Schizophrenia Research* **67**, 75–85.

Dulawa SC, Geyer MA (2000). Effects of strain and serotonergic agents on prepulse inhibition and habituation in mice. *Neuropharmacology* **39**, 2170–2179.

Fendt M, Li L, Yeomans JS (2001). Brain stem circuits mediating prepulse inhibition of the startle reflex. *Psychopharmacology* **156**, 216–224.

Filion DL, Dawson ME, Schell AM (1993). Modification of the acoustic startle-reflex eyeblink: a tool for investigating early and late attentional processes. *Biological Psychology* **35**, 185–200.

Fukui N, Suzuki Y, Sugai T, Watanabe J, *et al.* (2010). Exploring functional polymorphisms in the dopamine receptor D2 gene using prolactin concentration in healthy subjects. *Molecular Psychiatry* **16**, 356–358.

Geyer MA, Swerdlow NR, Mansbach RS, Braff DL (1990). Startle response models of sensorimotor gating and habituation deficits in schizophrenia. *Brain Research Bulletin* **25**, 485–498.

Golimbet VE, Aksenova MG, Nosikov VV, Orlova VA, *et al.* (2003). Analysis of the linkage of the Taq1A and Taq1B loci of the dopamine D2 receptor gene with schizophrenia in patients and their siblings. *Neuroscience and Behavioral Physiology* **33**, 223–225.

Gottesman II, Gould TD (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *American Journal of Psychiatry* **160**, 636–645.

Graham FK (1975). The more or less startling effects of weak prestimulation. *Psychophysiology* **12**, 238–248.

Grandy DK, Zhang Y, Civelli O (1993). PCR detection of the TaqA RFLP at the DRD2 locus. *Human Molecular Genetics* **2**, 2197.

Grattan DR, Kokay IC (2008). Prolactin: a pleiotropic neuroendocrine hormone. *Journal of Neuroendocrinology* **20**, 752–763.

Greenwood TA, Braff DL, Light GA, Cadenhead KS, *et al.* (2007). Initial heritability analyses of endophenotypic measures for schizophrenia: the consortium on the genetics of schizophrenia. *Archives of General Psychiatry* **64**, 1242–1250.

Haff LA, Smirnov IP (1997). Single-nucleotide polymorphism identification assays using a thermostable DNA polymerase and delayed extraction MALDI-TOF mass spectrometry. *Genome Research* **7**, 378–388.

Hamidovic A, Dlugos A, Skol A, Palmer AA, *et al.* (2009). Evaluation of genetic variability in the dopamine receptor D2 in relation to behavioral inhibition and impulsivity/sensation seeking: an exploratory study with d-amphetamine in healthy participants. *Experimental and Clinical Psychopharmacology* **17**, 374–383.

Hart S, Zreik M, Carper R, Swerdlow NR (1998). Localizing haloperidol effects on sensorimotor gating in a predictive model of antipsychotic potency. *Pharmacology Biochemistry and Behavior* **61**, 113–119.

Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO (1991). The fagerström test for nicotine dependence: a revision of the fagerström tolerance questionnaire. *British Journal of Addiction* **86**, 1119–1127.

Hedges LV, Olkin I (1985). *Statistical Methods for Meta-Analysis*. Orlando, FL: Academic Press.

Hirvonen J, van Erp TGM, Huttunen J, Aalto S, *et al.* (2005). Increased caudate dopamine D2 receptor availability as a genetic marker for schizophrenia. *Archives of General Psychiatry* **62**, 371–378.

Hirvonen MM, Laakso A, Nögren K, Rinne JO, *et al.* (2009). C957T polymorphism of dopamine D2 receptor gene affects striatal DRD2 in vivo availability by changing the receptor affinity. *Synapse* **63**, 907–912.

Hokyo A, Kanazawa T, Uenishi H, Tsutsumi A, *et al.* (2010). Habituation in prepulse inhibition is affected by a polymorphism on the NMDA receptor 2B subunit gene (GRIN2B). *Psychiatric Genetics* **20**, 191–198.

Hsieh MH, Swerdlow NR, Braff DL (2006). Effects of background and prepulse characteristics on prepulse inhibition and facilitation: implications for neuropsychiatric research. *Biological Psychiatry* **59**, 555–559.

Jönsson EG, Nöthen MM, Grünhage F, Farde L, *et al.* (1999). Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Molecular Psychiatry* **4**, 290–296.

Kaiser SA, Soliakov L, Harvey SC, Luetje CW, *et al.* (1998). Differential inhibition by conotoxin MII of the nicotinic stimulation of [^{3}H] dopamine release from rat striatal synaptosomes and slices. *Journal of Neurochemistry* **70**, 1069–1076.

Krauter EE, Leonard DW, Ison JR (1973). Inhibition of human eye blink by brief acoustic stimulus. *Journal of Comparative and Physiological Psychology* **84**, 246–251.

Kretschmer BD, Koch M (1998). The ventral pallidum mediates disruption of prepulse inhibition of the acoustic startle response induced by dopamine agonists, but not by NMDA antagonists. *Brain Research* **798**, 204–210.

Kumari V, Antonova E, Geyer MA, Ffytche D, *et al.* (2007). A fMRI investigation of startle gating deficits in schizophrenia patients treated with typical or atypical antipsychotics. *International Journal of Neuropsychopharmacology* **10**, 463–477.

Kumari V, Antonova E, Zachariah E, Galea A, *et al.* (2005*a*). Structural brain correlates of prepulse inhibition of the

acoustic startle response in healthy humans. *Neuroimage* **26**, 1052–1058.

Kumari V, Checkley SA, Gray JA (1996). Effect of cigarette smoking on prepulse inhibition of the acoustic startle reflex in healthy male smokers. *Psychopharmacology* **128**, 54–60.

Kumari V, Das M, Zachariah E, Ettinger U, *et al.* (2005*b*). Reduced prepulse inhibition in unaffected siblings of schizophrenia patients. *Psychophysiology* **42**, 588–594.

Kumari V, Ettinger U (2005). Prepulse inhibition deficits in schizophrenia: static or amenable to treatment? *Trends in Schizophrenia Research* 95–117.

Kumari V, Gray JA (1999). Smoking withdrawal, nicotine dependence and prepulse inhibition of the acoustic startle reflex. *Psychopharmacology* **141**, 11–15.

Kumari V, Gray JA, Geyer MA, Ffytche DH, *et al.* (2003). Neural correlates of tactile prepulse inhibition: a functional MRI study in normal and schizophrenic subjects. *Psychiatry Research* **122**, 99–113.

Kumari V, Peters ER, Fannon D, Premkumar P, *et al.* (2008). Uncontrollable voices and their relationship to gating deficits in schizophrenia. *Schizophrenia Research* **101**, 185.

Kumari V, Soni W, Sharma T (1999). Normalization of information processing deficits in schizophrenia with clozapine. *American Journal of Psychiatry* **156**, 1046–1051.

Laucht M, Becker K, Frank J, Schmidt MH, *et al.* (2008). Genetic variation in dopamine pathways differentially associated with smoking progression in adolescence. *Journal of the American Academy of Child & Adolescent Psychiatry* **47**, 673–681.

Martinez ZA, Oostwegel J, Geyer MA, Ellison GD, *et al.* (2000). 'Early' and 'Late' effects of sustained haloperidol on apomorphine- and phencyclidine-induced sensorimotor gating deficits. *Neuropsychopharmacology* **23**, 517–527.

Monakhov M, Golimbet V, Abramova L, Kaleda V, *et al.* (2008). Association study of three polymorphisms in the dopamine D2 receptor gene and schizophrenia in the Russian population. *Schizophrenia Research* **100**, 302–307.

Montag C, Hartmann P, Merz M, Burk C, *et al.* (2008). D2 receptor density and prepulse inhibition in humans: negative findings from a molecular genetic approach. *Behavioural Brain Research* **187**, 428–432.

Neuner I, Stöcker T, Kellermann T, Ermer V, *et al.* (2010). Electrophysiology meets fMRI: neural correlates of the startle reflex assessed by simultaneous EMG-fMRI data acquisition. *Human Brain Mapping* **31**, 1675–1685.

Oeth P, Beaulieu M, Park C, Kosman D, *et al.* (2005). iPLEX assay: increased plexing efficiency and flexibility for MassARRAY system through single base primer extension with mass-modified terminators (www.sequenom.com; http://128.135.75.36/iPLEXAppNote.pdf). Accessed 10 November 2006.

Peng RY, Mansbach RS, Braff DL, Geyer MA (1990). A D-sub-2 dopamine receptor agonist disrupts sensorimotor gating in rats: implications for dopaminergic abnormalities in schizophrenia. *Neuropsychopharmacology* **3**, 211–218.

Pierce CA, Block RA, Aguinis H (2004). Cautionary note on reporting eta-squared values from multifactor ANOVA designs. *Educational and Psychological Measurement* **64**, 916–924.

Pohjalainen T, Rinne JO, Nagren K, Lehikoinen P, *et al.* (1998*a*). The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Molecular Psychiatry* **3**, 256–260.

Pohjalainen T, Rinne JO, Nagren K, SyvAlahti E, *et al.* (1998*b*). Sex differences in the striatal dopamine D2 receptor binding characteristics in vivo. *American Journal of Psychiatry* **155**, 768–773.

Quednow BB, Wagner M, Westheide J, Beckmann K, *et al.* (2006). Sensorimotor gating and habituation of the startle response in schizophrenic patients randomly treated with amisulpride or olanzapine. *Biological Psychiatry* **59**, 536.

Quednow BB, Frommann I, Berning J, Kühn KU, *et al.* (2008). Impaired sensorimotor gating of the acoustic startle response in the prodrome of schizophrenia. *Biological Psychiatry* **64**, 766–773.

Quednow BB, Schmechtig A, Ettinger U, Petrovsky N, *et al.* (2009). Sensorimotor gating depends on polymorphisms of the serotonin-2A receptor and catechol-O-methyltransferase, but not on neuregulin-1 Arg38Gln genotype: a replication study. *Biological Psychiatry* **66**, 614–620.

Quednow BB, Wagner M, Mossner R, Maier W, *et al.* (2010). Sensorimotor gating of schizophrenia patients depends on catechol O-Methyltransferase Val158Met polymorphism. *Schizophrenia Bulletin* **36**, 341–346.

Ritchie T, Noble EP (2003). Association of seven polymorphisms of the D2 dopamine receptor gene with brain receptor-binding characteristics. *Neurochemical Research* **28**, 73–82.

Roussos P, Giakoumaki SG, Bitsios P (2008*a*). The dopamine D3 receptor Ser9Gly polymorphism modulates prepulse inhibition of the acoustic startle reflex. *Biological Psychiatry* **64**, 235–240.

Roussos P, Giakoumaki SG, Rogdaki M, Pavlakis S, *et al.* (2008*b*). Prepulse inhibition of the startle reflex depends on the catechol O-methyltransferase Val158Met gene polymorphism. *Psychological Medicine* **38**, 1651–1658.

Schultz W (2002). Getting formal with dopamine and reward. *Neuron* **36**, 241–263.

Swerdlow NR, Auerbach P, Monroe SM, Hartston H, *et al.* (1993). Men are more inhibited than women by weak prepulses. *Biological Psychiatry* **34**, 253–260.

Swerdlow NR, Braff DL, Taaid N, Geyer MA (1994). Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Archives of General Psychiatry* **51**, 139–154.

Swerdlow NR, Eastvold A, Karban B, Ploum Y, *et al.* (2002*a*). Dopamine agonist effects on startle and sensorimotor gating in normal male subjects: time course studies. *Psychopharmacology* **161**, 189–201.

Swerdlow NR, Geyer MA (1993). Clozapine and haloperidol in an animal model of sensorimotor gating deficits in schizophrenia. *Pharmacology Biochemistry and Behavior* **44**, 741–744.

Swerdlow NR, Geyer MA (1998). Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophrenia Bulletin* **24**, 285–301.

Swerdlow NR, Geyer MA, Braff DL (2001). Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology* **156**, 194–215.

Swerdlow NR, Light GA, Cadenhead KS, Sprock J, *et al.* (2006). Startle gating deficits in a large cohort of patients with schizophrenia: relationship to medications, symptoms, neurocognition, and level of function. *Archives of General Psychiatry* **63**, 1325–1335.

Swerdlow NR, Stephany N, Shoemaker JM, Ross L, *et al.* (2002*b*). Effects of amantadine and bromocriptine on startle and sensorimotor gating: parametric studies and cross-species comparisons. *Psychopharmacology* **164**, 82–92.

Swerdlow NR, Weber M, Qu Y, Light GA, *et al.* (2008). Realistic expectations of prepulse inhibition in translational models for schizophrenia research. *Psychopharmacology* **199**, 331–388.

The International HapMap Consortium (2003). The international hapmap project. *Nature* **426**, 789–796.

Thompson J, Thomas N, Singleton A, Piggot M, *et al.* (1997). D2 dopamine receptor gene (DRD2) Taql A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics and Genomics* **7**, 479–484.

Vijayan NN, Bhaskaran S, Koshy LV, Natarajan C, *et al.* (2007). Association of dopamine receptor polymorphisms with schizophrenia and antipsychotic response in a South Indian population. *Behavioral and Brain Functions* **3**, 34–46.

Whitlock MC (2005). Combining probability from independent tests: the weighted Z method is superior to Fisher's approach. *Journal of Evolutionary Biology* **18**, 1368–1373.

Willott JF, Tanner L, O'Steen J, Johnson KR, *et al.* (2003). Acoustic startle and prepulse inhibition in 40 inbred strains of mice. *Behavioral Neuroscience* **117**, 716–726.

Wonnacott S, Kaiser S, Mogg A, Soliakov L, *et al.* (2000). Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. *European Journal of Pharmacology* **393**, 51–58.

Yee BK, Chang T, Pietropaolo S, Feldon J (2005). The expression of prepulse inhibition of the acoustic startle reflex as a function of three pulse stimulus intensities, three prepulse stimulus intensities, and three levels of startle responsiveness in C57BL6/J mice. *Behavioural Brain Research* **163**, 265–276.