Stability of prepulse inhibition and habituation of the startle reflex in schizophrenia: a 6-year follow-up study of initially antipsychotic-naive, first-episode schizophrenia patients

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Abstract

Deficits in information processing appear to be core features in the pathogenesis of schizophrenia. Prepulse inhibition (PPI) and habituation of the startle reflex are operational measures of early information processing. Impaired PPI in schizophrenia has been replicated in many studies and is regarded as an endophenotype for schizophrenia. However, reports on the stability of PPI over a longer period of time are lacking, both for patients with schizophrenia and for healthy subjects. The current study examined 25 initially drug-naive, first-episode schizophrenia patients and 23 healthy matched controls. Three PPI measures [stimulus onset asynchrony (SOA) 30, 60, 120 ms] and habituation were assessed at baseline, and again after 6 yr. Sixteen patients and 17 healthy controls completed the study, and 13 patients and 17 healthy controls were included in the final analysis. The schizophrenia patients had PPI deficits compared to controls at baseline. After 6 yr, no significant group differences were found. PPI had increased significantly in the patients and had decreased significantly in controls. In addition, patients showed significantly less habituation than controls while habituation did not change in patients or controls. The present results show that PPI in drug-naive, first-episode schizophrenia patients can improve significantly over time. As PPI increased in patients over the same period that it decreased in controls, it is likely that the increase was caused by disease-related factors such as disease process, clinical state, or medication.

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Key words: Antipsychotic naive, follow-up study, habituation, PPI, schizophrenia.

Introduction

Disturbances in attention and information processing are proposed to be fundamental causes of symptoms in schizophrenia (Braff & Geyer, 1990; McGhie & Chapman, 1961) describing how schizophrenia patients are unable to focus their attention and are distracted by irrelevant stimuli. This reduced capacity to filter out irrelevant stimuli is hypothesized to cause an overload of sensory information, which may in part underlie positive symptoms (Braff & Geyer, 1990).

Prepulse inhibition (PPI) of the startle reflex paradigm is an operational measure of sensorimotor gating. The PPI paradigm is based on an individual’s startle reflex, i.e. the response to a sudden and intense stimulus. In human studies, the startle reflex is usually measured as the electromyographic (EMG) response of m. orbicularis oculi. If the startle-eliciting stimulus is preceded (30–500 ms) by a weak stimulus (prepulse) the startle response is usually reduced (Graham, 1975). Habituation is another measure of the plasticity of the startle response. Habituation is the reduction in magnitude of the startle response due to repeated presentations of the same, initially novel, stimulus over a test session. Habituation is believed to be the simplest form of learning (Rankin et al. 2009).

Deficient PPI is found in all stages and states of schizophrenia (Braff et al. 1978; Ludewig et al. 2003a;...
Mackeprang et al. 2002; Meincke et al. 2004b; Parwani et al. 2000; Quednow et al. 2008), in schizotypal disorder (Cadenhead et al. 1993, 2000), psychosis-prone subjects (Swerdlow et al. 1995), and clinically unaffected relatives of schizophrenia patients (Cadenhead et al. 2000; Kumari et al. 2005). Therefore these deficits are regarded as fundamental, stable, or at least intermediate phenotypic markers of schizophrenia, related to neurodevelopmental and/or genetic factors (Braff, 1993; Bredgaard & Glenthoj, 2000; Cadenhead et al. 2000; Turetsky et al. 2007).

Until now, the impact of time-dependent factors, like treatment effects, disease progress and age on PPI has mainly been deduced from cross-sectional studies (Kumari & Sharma, 2002; Kumari et al. 2002; Oranje et al. 2002b; Swerdlow et al. 2006) and some short duration longitudinal studies (Aggernaes et al. 2010; Duncan et al. 2003a; Mackeprang et al. 2002; Martinez-Gras et al. 2009; Minassian et al. 2007; Quednow et al. 2006b; Wynn et al. 2007). Comparative studies suggest that second-generation antipsychotics (SGAs) increase PPI and that first-generation antipsychotics (FGAs) have a smaller or no effect on PPI (Kumari & Sharma, 2002; Kumari et al. 2002; Oranje et al. 2002b; Swerdlow et al. 2006) whereas the results with regard to the limited number of short duration follow-up studies are contradictory (see below).

To our knowledge, nine longitudinal studies on PPI in schizophrenia patients have been published (Aggernaes et al. 2010; Duncan et al. 2003b; Ludewig et al. 2002; Mackeprang et al. 2002; Martinez-Gras et al. 2009; Meincke et al. 2004b; Minassian et al. 2007; Quednow et al. 2006b; Wynn et al. 2007) and all except two (Aggernaes et al. 2010; Mackeprang et al. 2002), were performed on chronic patients many of whom had been treated with FGAs prior to the study. Five studies examined the effect of antipsychotics on PPI: none of the studies found that FGAs increased PPI (Duncan et al. 2003a; Mackeprang et al. 2002; Wynn et al. 2007), two studies reported that SGAs with low \( D_2 \) affinity increased/normalized PPI (Quednow et al. 2006b; Wynn et al. 2007), whereas the effects of SGAs with a high \( D_2 \) affinity, were inconsistent (Csomor et al. 2009; Mackeprang et al. 2002; Martinez-Gras et al. 2009; Quednow et al. 2006b; Wynn et al. 2007). [It should be noted that the results from the study by Quednow et al. (2006b) are difficult to interpret because the reported improvement in PPI in patients compared to controls appeared to be driven by a reduction in PPI of the healthy controls from baseline to follow-up, while the PPI of patients seemed to remain stable over that same time period.] It has therefore been suggested that only antipsychotics with a low \( D_2 \) affinity can increase PPI (Oranje et al. 2002b), which is in agreement with recent data from our group (Aggernaes et al. 2010).

The follow-up periods for the previous longitudinal studies were a maximum of 6 months, i.e. too short a period to show effects of age. The results of cross-sectional studies on age and PPI in healthy subjects are contradictory: PPI has been found to be unaffected by age (Ludewig et al. 2003b; Swerdlow et al. 2006), to decrease with age (Kumari et al. 2008a; McDowd et al. 1993), and to be less at younger and older ages and to peak at intermediate ages (Ellwanger et al. 2003).

Studies on habituation and schizophrenia are scarce and the results are less consistent than for PPI. Some studies find habituation deficits in patients (Geyer & Braff, 1982; Ludewig et al. 2003a; Meincke et al. 2004a; Takahashi et al. 2008) while others do not (Kumari et al. 2007; Mackeprang et al. 2002; Quednow et al. 2006b, 2008; Wynn et al. 2007). An altered sensitization process in schizophrenia patients has been suggested to confound comparisons of habituation between patients and healthy controls (Meincke et al. 2004a).

Eight shorter duration longitudinal studies reported on habituation in schizophrenia; none of the studies found an effect of time (Ludewig et al. 2002), symptomatic state (Meincke et al. 2004a) or antipsychotic treatment (Aggernaes et al. 2010; Duncan et al. 2003b; Mackeprang et al. 2002; Meincke et al. 2004a; Quednow et al. 2006b; Wynn et al. 2007). A lack of medication effects is, however, not surprising since none of the medication studies found a habituation deficit in the patients at baseline. Increased age has been associated with increased habituation in healthy subjects in some cross-sectional studies (Cadenhead et al. 2000; Ludewig et al. 2003b).

The current study reports on the second re-examination of a cohort of antipsychotic-naive, first-episode patients and matched healthy controls. The aim of the present study was to examine PPI and habituation in these patients and matched controls over an extended period of time, i.e. 6 yr. Due to the long follow-up period the study had a naturalistic design; i.e. there was no control exerted over the type of antipsychotic medication.

**Materials and methods**

**Participants**

The ethical committees of Copenhagen and Frederiksborg approved the study (KF 01-078/97 +01-012/98), and written informed consent was obtained from participants before inclusion.
Baseline
Twenty-five patients diagnosed with schizophrenia according to ICD-10 criteria (WHO), and 23 healthy controls matched for gender, age and socioeconomic status of their parents, participated in the project. Patients were admitted to treatment for the first time and had never received any antipsychotic medication. They were recruited from hospitals in the catchment area of Copenhagen (both in-patients and outpatients) and diagnosed with schizophrenia by the referring psychiatrists. The diagnosis was confirmed by a trained psychiatrist using SCAN 2.0 (Wing et al. 1990). Psychopathology was assessed with the Positive and Negative Syndrome Scale (PANSS; Kay et al. 1987). Patients abusing alcohol or cannabis at inclusion were not excluded, but urine was analysed for drugs of abuse. Healthy controls were excluded if they had any history of psychiatric disorder in first-degree relatives, somatic disorder, or a history of drug or alcohol abuse. Controls were screened using SCAN 2.0 to exclude major psychopathology.

The subjects were examined comprehensively with measures of psychopathology, extrapyramidal side-effects, sensorimotor gating (Mackeprang et al. 2002), cognitive functioning (Fagerlund et al. 2004), SPECT (Glenthoj et al. 2006) and MRI (Glenthoj et al. 2007). The current paper will only report on sensorimotor gating and habituation.

Patients were examined before and after 3 months of treatment with risperidone or zuclopenthixol and the healthy controls at inclusion only. The results of the 3-month follow-up were reported by Mackeprang et al. (2002).

Follow-up
On average, 6 yr (4.9–7.1 yr) after the baseline assessments all subjects were asked to participate in a follow-up study. Of the initial 25 patients, 16 agreed to participate; three had died during the follow-up period, and six declined further participation. Seventeen of the initial 23 healthy controls agreed to participate. SCAN 2.1 was used to re-diagnose the patients and to screen the controls for major psychopathology. All patients still met ICD-10 diagnostic criteria for schizophrenia at follow-up. One patient was excluded from analyses at follow-up due to severe substance abuse (heroin) during the follow-up period, and for drinking alcohol on the day of PPI assessment. Two patients were non-responders [i.e. mean startle amplitude of pulse-alone trials in block 1 was less than 10 digital units (7.8 µV)] and were excluded.

Therefore, 13 patients and 17 healthy controls were included in the final analyses. Demographic data and PANSS ratings on patients and healthy controls are listed in Table 1. No significant group differences in the demographic data were found. Significantly more patients than healthy controls were smokers.

Substance abuse and medication
Substance abuse during the follow-up period was estimated via interviews with the patients and based on medical records from hospitals, outpatient clinics and psychiatrists in private practice. Apart from the patient excluded with severe heroin and alcohol abuse, five patients at baseline and four patients at follow-up were diagnosed with psychoactive substance abuse (ICD-10: F10-F19) of alcohol (baseline: n = 3; follow-up: n = 2) and/or other psychoactive substances (baseline: n = 3; follow-up: n = 4). One patient abused cocaine at follow-up. None of the controls had a psychoactive substance abuse diagnosis. We were unable to acquire the smoking status on two patients and all the healthy subjects at baseline. At follow-up significantly more patients (n = 10) than healthy controls (n = 2) were smokers.

Current medical status and estimation of the amount and type of medication taken during the follow-up period, was based on patient interviews and prescriptions in the patients’ medical records. Two patients had taken benzodiazepines on the day of PPI assessment and one of these was later excluded because he was a non-responder. Data on pharmacological treatment are listed in Table 2.

### Table 1. Demographic data and psychopathology (PANSS)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>(n=13)</td>
<td>(n=17)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>30.0(6.6)</td>
<td>28.6(6.0)</td>
</tr>
<tr>
<td>PANSS score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19.9(3.4)</td>
<td>13.7(5.0)</td>
</tr>
<tr>
<td>Negative</td>
<td>20.6(5.5)</td>
<td>16.0(4.9)</td>
</tr>
<tr>
<td>General</td>
<td>28.8(5.0)</td>
<td>25.9(5.3)</td>
</tr>
<tr>
<td>Total</td>
<td>69.4(12.4)</td>
<td>55.6(9.6)</td>
</tr>
</tbody>
</table>

Values are mean (s.d.).

*Psychopathology was significantly reduced compared to baseline, p < 0.05.
The experimental procedures at baseline and follow-up were identical, and have been described by Mackeprang et al. (2002). Briefly, subjects were requested to abstain from smoking or drinking any caffeine-containing beverages 1 h prior to testing. Assessment of the acoustic startle response was performed with a commercial system from San Diego Instruments (SR-Lab, USA). All participants were screened for hearing deficits with 40 dB(A) stimuli at 500, 1000 and 6000 Hz (no participants were excluded). The eye-blink component of the acoustic startle response was measured by recording EMG activity from the right m. orbicularis oculi. Stimuli were presented binaurally through earphones (Maico, USA). The test session started with a 5-min acclimation period consisting of 70 dB(A) broad-frequency spectrum noise which continued as a background noise throughout the whole experimental session. This acclimation period was followed by the PPI paradigm, which consisted of 73 trials: pulse-alone (PA) trials or prepulse-pulse trials. PA stimuli were 40 ms of 116 dB(A) broad-frequency spectrum noise. Prepulse-pulse trials were PA trials preceded by a prepulse stimulus [20 ms, 85 dB(A) broad-frequency spectrum noise]. The prepulse stimulus was presented 30, 60 or 120 ms [stimulus onset asynchrony (SOA)] prior to onset of the startle pulse. The first trial in the PPI paradigm was a PA trial which was followed by six blocks of 12 trials. Each block consisted of three PA trials and three of each prepulse-pulse trial type (PPI30, SOA = 30 ms; PPI60, SOA = 60 ms; PPI120, SOA = 120 ms) which were pseudo-randomly intermixed, i.e. two identical trial types never followed each other.

Startle reactivity was assessed as the mean startle amplitude of PA trials in block 1. Habituation was defined as the linear gradient coefficient \( b \). The linear gradient coefficient \( b \) represents a measure for the rate of fall (negative ascending slope) of the habituation curve (Geyer & Braff, 1982) and has been found to be more reliable than reduction of startle amplitude as expressed in percentages (Quednow et al. 2006a). Coefficient \( b \) was calculated across the PA trials starting from PA trial 4 to the end (trial 19) within each subject:

\[
b = \frac{\sum xy - (\sum x)(\sum y)}{\sum x^2 - (\sum x)^2},
\]

where \( x = \) trial number, \( y = \) startle amplitude PA trials. PA trials 2 and 3 were excluded from the calculation of habituation because of possible confounding effects of sensitization (Meincke et al. 2004a). PPI was expressed as

\[
100\% \times \frac{\text{mean startle amplitude to prepulse-pulse trials}}{\text{mean startle amplitude to PA trials}} - 1
\]

### Table 2. Medication data

<table>
<thead>
<tr>
<th></th>
<th>Baseline (( n = 13 ))</th>
<th>During follow-up period (( n = 13 )) (numbers in parentheses are accumulated haloperidol equivalents)</th>
<th>At follow-up (( n = 13 )) (numbers in parentheses are daily dose in mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No antipsychotic medication</td>
<td>13</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Atypical antipsychotic medication</td>
<td>0</td>
<td>5 (10004, s.d. = 12147)(^a)</td>
<td>2 Olanzapine (12.5 mg, s.d. = 10.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 Risperidone (2.7 mg, s.d. = 2.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 Ziprasidone (160 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 Quetiapine (125 mg)</td>
</tr>
<tr>
<td>Typical antipsychotic medication</td>
<td>0</td>
<td>1 (2292)</td>
<td>1 Chlorprothixine (1, 3)</td>
</tr>
<tr>
<td>Typical + atypical</td>
<td>0</td>
<td>7 (8835, s.d. = 2799)(^b)</td>
<td>1 Ziprasidone + zuclopenthixol + chlorprothixene (320 mg + 20 mg + 100 mg, respectively)</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>0</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\) Information on medication regimen was insufficient to make a reliable estimate of haloperidol equivalents in two patients.

\(^b\) Information on medication regimen was insufficient to make a reliable estimate of haloperidol equivalents in three patients.
(note the first PA trial was not included in assessment of PPI).

**Statistical analysis**

Statistical analyses were performed with SPSS version 11.0 (SPSS Inc., USA). Data on PPI60 and startle amplitude to PA trials in block 1 were skewed because of one outlier in each dataset (both outliers were patients). Outlying values in these datasets were therefore winsorized to values corresponding to 2 S.D. from the mean (Dixon & Tukey, 1968). All data were normally distributed (Kolmogorov–Smirnov test), therefore all data were analysed with parametric statistics.

Startle reactivity was analysed as the startle amplitude of PA trials in block 1 by ANOVA with between-factor group (schizophrenia patient or matched healthy control), and within-factor time (baseline or follow-up).

Habituation was analysed by ANCOVA with between-factor group and within-factor time. Habituation is known to be affected by startle reactivity and mean startle amplitude to PA trials 4–6 was therefore used as covariate in the analysis.

PPI of the startle reflex was analysed by ANCOVA with between-factor group and within-factors time and SOA (30, 60, 120 ms). Startle amplitude to all PA trials (except the first one) was used as covariate in the analysis. To avoid alpha inflation, post-hoc analyses were only performed when the ANOVAs indicated significant results ($p < 0.05$).

The effect of time (baseline to follow-up) on psychopathology (positive, negative, general and total PANSS scores) was analysed using paired-samples Student’s $t$ tests.

Correlations between PPI and symptomatology were explored by means of Pearson’s correlation coefficient. Similarly, correlations between amount of antipsychotic medication during the follow-up period (measured in haloperidol equivalents) and change in PPI were explored with Pearson’s correlation coefficient.

Since it is known that gender, smoking, age, type of current antipsychotic medication, use of benzodiazepines and substance abuse might influence our dependent variables, they were used as covariates: baseline data for the covariates for the baseline analysis, follow-up data for the covariates for the follow-up analysis and both baseline and follow-up data for the covariates in the ANCOVA analysis which included the factor time. If their influence was non-significant they were removed from the analysis. The patient who used cocaine was included and also excluded from the analysis to examine if the results were affected. The results did not change and the data of this patient were included in the final analysis.

The reliability of the different parameters over time (baseline to 6 yr) was investigated using the intraclass correlation coefficient (ICC) model (3,k) (McGraw & Wong, 1996) (which is equivalent to Cronbach’s $\alpha$ coefficient of internal consistency). The coefficient estimates the proportion of total between-subject variability due to ‘error-free’ between-subject differences.

**Results**

**General**

None of the possible confounding measures (gender, smoking, age, drug abuse, type of current antipsychotic medication, use of benzodiazepines) covaried significantly with startle reactivity, PPI or habituation. There was no significant difference in age, PPI, habituation, startle magnitude or PANSS scores at baseline between patients who participated and patients who did not participate at follow-up.

**Startle magnitude**

The ANOVA on startle reactivity of PA trials in block 1 showed an interaction effect of time $\times$ group [$F(1,28) = 4.776, p = 0.037$]. Further analysis of this time $\times$ group interaction effect showed a trend towards less startle reactivity in patients than in healthy controls at baseline [$t(28) = -1.9, p = 0.068$] but not at follow-up ($p = 0.9$). Post-hoc paired-samples Student’s $t$ tests of the group $\times$ time effect showed a significant reduction in startle amplitude in the controls from baseline to follow-up [$t(16) = 2.379, p = 0.030$], with no significant changes over time in the patient group (see Fig. 1). Startle amplitude was reliable in the whole sample with ICC of 0.82 ($p < 0.000$) and in patients with ICC of 0.92 ($p = 0.000$) for baseline to 6 yr. The startle amplitude was not reliable in healthy controls (ICC = 0.048, $p = 0.1$).

**Habituation**

The ANCOVA on habituation only showed a main effect of group [$F(1,28) = 6.675, p = 0.015$] (see Figs 2 and 3). This significant group effect was driven by non-significant group differences at baseline [$t(28) = 1.725, p = 0.084$], and at follow-up [$t(28) = 1.274, p = 0.213$]. The data were additionally analysed with change of mean startle amplitude of PA trials 4–6 between baseline and follow-up as covariate (the covariate was non-significant). The main effect of group remained...
significant ($p=0.035$). We found a significant negative correlation between the change in habituation and the change in mean startle amplitude of PA trials 4–6 (Pearson’s correlation $r=-0.66$, $p=0.000$). ICC for habituation was non-significant for the whole sample, for patients and for healthy controls.

**PPI**

The ANOVA on group, time and SOA indicated a significant interaction effects of time $\times$ group [$F(1, 28)=5.247$, $p=0.030$] and time $\times$ SOA $\times$ group [$F(2, 56)=3.737$, $p=0.036$]. Gender and change in mean responses to all PA trials (baseline to follow-up) were introduced as covariates; both were non-significant ($p>0.62$). The time $\times$ group interaction remained significant after introduction of gender as covariate. Introduction of the change in mean startle response reduced the time $\times$ SOA $\times$ group interaction to a trend level ($p=0.087$).

Post-hoc analyses showed significantly less PPI60 in patients compared to healthy controls at baseline [$t(28)=-2.819$, $p=0.009$], which did not change when controlling for change in mean response to all PA trials, and gender. There were no significant group difference in the PPI30 ($p=0.389$) or the PPI120 ($p=0.155$) trials at baseline. PPI in patients did not differ significantly from PPI in controls in any SOA condition at follow-up ($p>0.9$). In fact, in patients, PPI60 increased significantly from baseline to follow-up [$t(12)=-2.307$, $p=0.040$], while the PPI60 in the controls decreased significantly [$t(16)=2.183$, $p=0.044$]. The increase in PPI60 in patients did not reflect significant changes in the submeasures from which PPI is derived, i.e. mean startle amplitude to prepulse-pulse trials ($p=0.736$) and mean PA amplitude to PA trials ($p=0.62$). The decrease in PPI60 in controls was caused by a significant decrease in mean PA amplitude to PA trials ($p=0.037$). Results on PPI are presented in Figs. 4 and 5. Note that in our total baseline cohort (20 patients, 23 controls) we found significantly...
reduced PPI in patients, compared to controls in all three SOA conditions (Mackeprang et al. 2002).

PPI30 showed no significant ICCs. However, PPI60 and PPI120 measures were reliable over time, both in the whole sample as well as in patients and controls: ICC of PPI60 for the whole sample was 0.70 ($p<0.001$), for patients 0.79 ($p=0.006$) and for controls 0.70 ($p=0.01$). ICC of PPI120 was 0.69 for the whole sample ($p<0.001$) and 0.70 for both patients ($p=0.02$) and controls ($p=0.023$).

**Psychopathology and correlations with PPI**

All PANSS scores improved significantly from baseline to follow-up (see Table 1). There were no significant correlations between PANSS scores and PPI scores (PPI30, PPI60, PPI120). The change over time in PPI did not correlate with changes over time in PANSS scores (positive, negative, general, total). There were no significant correlations between any of the PPI scores (at baseline, at follow-up and their change...
score) and any of the measures of antipsychotic medication (dose at follow-up and accumulated dose during the follow-up period).

Discussion

The present study is the first to follow PPI, startle reactivity and habituation in initially antipsychotic-naive, first-episode schizophrenia patients and matched healthy controls over 6 yr; and therefore the first to investigate reliability over time and time-dependent factors in this long-term perspective.

The main findings were that PPI increased in schizophrenia patients during the 6-yr period between baseline and follow-up. In contrast, PPI in healthy controls decreased over the same period. Even though PPI changed it showed a relatively high ICC, indicating that the significant change of PPI over time may be considered a reliable and consistent phenomenon. Startle reactivity to PA trials was stable in patients but decreased in controls over the 6 yr. Habituation was deficient in patients and did not change over time in either patients or healthy controls. However, habituation did not show significant ICC, indicating that it was not consistent on an individual level and therefore not reliable across the test sessions.

The effect of increasing age on PPI has until now been deduced from cross-sectional studies (Cadenhead et al. 2000; Ellwanger et al. 2003; Kumari et al. 2008a; Ludewig et al. 2003b). Our longitudinal finding in healthy subjects is in accordance with cross-sectional studies that found a negative correlation between age and PPI (Kumari et al. 2008a; McDowd et al. 1993), but contrasts with two studies that primarily addressed the effect of age; they found either no effect of age (2003b) or an inverted U-curved relationship such that PPI was highest in middle age (2003). The authors of the last study noted that the gender distribution of their age groups was not even, i.e. their results could have been confounded by gender effects. We found no significant correlations between age and PPI; such a correlation would have supported the assumption that PPI is affected by age in healthy controls. However, it cannot be ruled out that this lack of correlation in our data could be due to a type II error or a nonlinear relationship between age and PPI. Alternative explanations for our significant PPI reduction over time could have been a retest effect. Although retest effects have been found in other studies with much shorter test intervals (e.g. Quednow et al. 2006a) we regard it unlikely that it had an impact on our results, given the long interval (6 yr) between tests.

The present study is the first longitudinal study to show that PPI in drug-naive, first-episode patients can increase significantly over time. Since PPI decreased in healthy controls it is likely that the increase seen in patients was caused by disease-related factors. The two other published longitudinal studies to report on PPI in drug-naive, first-episode patients are also from our laboratory (Aggernaes et al. 2010; Mackeprang et al. 2002). The earliest paper reported the 3-month follow-up of the cohort from the present study (Mackeprang et al. 2002). No significant change in patients’ PPI was found after 3 months of randomized treatment with either the FGA zuclopenthixol or the SGA risperidone. The lack of increase in the risperidone group was unexpected since cross-sectional studies had reported superiority of SGAs vs. FGAs in reversing PPI deficits (Kumari et al. 1999, 2000, 2002; Leumann et al. 2002; Oranje et al. 2002b; Swerdlow et al. 2006). However, although risperidone is a SGA, it does have a high D2 receptor affinity. High D2 receptor affinity FGAs disrupt PPI in healthy controls (Abduljawad et al. 1998; Kumari et al. 1998; Oranje et al. 2002a, 2004) and it has been suggested that only antipsychotics with a low D2 receptor affinity will increase PPI (Oranje et al. 2002b). At the present 6 yr follow-up, only one third of the patients were treated with high D2 affinity antagonists. Therefore, changes in the pharmacological treatment could explain why PPI had increased significantly after 6 yr, but not after 3 months. This is in accord with the findings of Aggernaes et al. (2010) that 6 months of treatment with the atypical compound quetiapine reduced the PPI difference between initially antipsychotic-naive schizophrenic patients and healthy matched controls.

A longitudinal study on the effect of antipsychotic medication in chronic patients found that the SGA olanzapine (which has a low D2 affinity) but neither risperidone nor the FGA haloperidol increased PPI (Wynn et al. 2007). This result is at odds with two other studies, one longitudinal and one cross-sectional, stating that risperidone was superior to FGAs in restoring PPI deficits in patients (Kumari et al. 2002; Martinez-Gras et al. 2009). However, in those studies, risperidone was compared to FGAs and not to placebo or untreated subjects and therefore an alternative interpretation of the results could be that FGAs, in contrast to risperidone, cause disturbances in PPI. This interpretation is plausible since risperidone has a higher affinity for the 5-HT2A receptor than the D2 receptor (Schotte et al. 1996). The 5-HT2A receptor blockade has been suggested to increase dopamine release in the prefrontal cortex (PFC), thereby either
preventing or compensating for a hypodopaminergic state in the PFC, caused by dopamine blockade or disease-related processes, respectively (Glenthoj et al. 1999; Meltzer et al. 2003). A hypodopaminergic state in the PFC decreases PPI in rats (Ellenbroek et al. 1996; Shoemaker et al. 2005) and it might be the reason why high D2 receptor affinity FGAs decrease PPI in healthy subjects (Abduljawad et al. 1998; Ellenbroek et al. 1996; Kumari et al. 1998; Oranje et al. 2002a, 2004; Shoemaker et al. 2005). An alternative explanation for our different results in PPI at the 3-month and the 6-yr follow-up, could be that it takes longer than 3 months to increase PPI in antipsychotic-naive, first-episode patients.

It may be argued that the increase in PPI as found in the current study could have been caused by the changed symptomatic state of the patients, i.e. most patients went from being first-episode psychotic patients to being chronic patients. However, this explanation is unlikely since numerous studies have found PPI deficits in the chronic state of schizophrenia (Braff, 1993; Braff et al. 1978, 1992; Duncan et al. 2006; Kumari et al. 2000; Kunugi et al. 2007; Oranje et al. 2002b; Perry et al. 2002; Swerdlow et al. 2006; Wynn et al. 2007).

Even though PPI changed in both patients and healthy controls it was found to be reliable across the test sessions, as was indicated by a significant and relatively high ICC (note that PPI30 was not reliable, p > 0.05). This shows that the change of PPI over time was a consistent phenomenon, and is in agreement with the results of Quednow et al. (2006a) who found PPI in healthy subjects to decrease across test sessions (baseline–4 wk–8 wk) but still to be reliable.

The present study found no correlation between PPI and symptoms at baseline, at follow-up or change in PPI from baseline to follow-up. This lack of correlation could be due to the small sample size. Results from other studies are inconsistent, some finding correlations (Braff et al. 1999; Duncan et al. 2006; Kumari et al. 2008b; Martinez-Gras et al. 2009; Meincke et al. 2004b; Minassian et al. 2007; Perry & Braff, 1994) while others do not (Kumari et al. 2000; Mackeprang et al. 2002; Parwani et al. 2000; Quednow et al. 2008; Swerdlow et al. 2006). A plausible, although speculative, explanation for the inconsistent findings, is that the current rating scales, e.g. PANSS, Scale for the Assessment of Negative Symptoms and Scale for the Assessment of Positive Symptoms do not quantify symptoms in a manner that captures PPI deficit-induced symptoms (Light & Braff, 2000). Kumari et al. for example found self-perceived lack of control over hearing voices to be significantly associated with PPI, which was not the case for any of the symptom dimensions of the PANSS (Kumari et al. 2008b).

Some studies find that schizophrenia patients have a startle reactivity deficit compared to healthy controls (Meincke et al. 2004a; Quednow et al. 2006b, 2008). This is in line with our results at baseline, where we found a strong trend towards a startle reactivity deficit in patients. The deficit was not found at follow-up, which was mostly due to a decrease in startle reactivity in healthy controls. This decrease in startle reactivity is consistent with cross-sectional studies that have found startle reactivity to correlate negatively with age (Cadenhead et al. 2000; Ellwanger et al. 2003; Ford et al. 1995; Ison et al. 1991; Ludewig et al. 2003b; Varty et al. 1998). It may be argued that the decreased startle reactivity is related to a change in threshold hearing acuity; however, older individuals show less startle reactivity, even when the intensity of the startle stimulus is individually adjusted to control for differences in hearing thresholds (Ford et al. 1995) and alterations in central information processing rather than in primary auditory mechanisms are suggested to be responsible for the age-related decline of startle reactivity (Ludewig et al. 2003b). In contrast to healthy controls, startle reactivity was stable over time in the patients of the current study. SGAs have been suggested to increase startle reactivity (Quednow et al. 2006b, 2008) and in the present study SGAs might have prevented a time-dependent decrease in patients.

Habituation deficits in schizophrenia patients have been found in some studies (Akdag et al. 2003; Duncan et al. 2003b; Geyer & Braff, 1982; Kumari et al. 2007; Ludewig et al. 2003a; Meincke et al. 2004a; Perry et al. 2002), but not in all (Mackeprang et al. 2002; Oranje et al. 2002b; Quednow et al. 2006b; Swerdlow et al. 2006; Wynn et al. 2007). It has been suggested that some of these inconsistencies may be due to abnormal initial sensitization effects to startle-elicitng stimuli in patients (Meincke et al. 2004a) or due to startle amplitude deficits in patients (Quednow et al. 2008). The present study found impaired habituation of the acoustic startle in schizophrenia patients, at a time when the influence of sensitization was decreased; correcting for startle reactivity did not change this finding. The average level of habituation was stable over time, both in patients and in healthy controls. However, ICC for habituation was non-significant indicating that the stability was only present at the group level and not at the level of the individual. This could have been a consequence of the paradigm that was used. Calculating habituation from PA trials that were intermixed with prepulse-pulse trials might not be the optimal way to assess habituation.
Increasing age has been associated with increasing habituation (Cadenhead et al. 2000; Ludewig et al. 2003b; Rinaldi & Thompson, 1985; Rogozea & Florea-Ciocoiu, 1988). The present study did not support this association and is in line with other studies finding no effect of age on habituation (Ellwanger et al. 2003; Varty et al. 1998). Nevertheless, even though this is a longitudinal study with the longest interval between tests in this field, the present 6-yr follow-up period could have been too short to detect age-induced changes in habituation.

Several factors may have influenced the present results and limit their interpretation. The sample size was small and increased the risk of type II errors. Most likely, lack of statistical power explains why the PPI30 and PPI120 deficit in patients at baseline was insignificant in the present study but significant in our total baseline cohort (Mackeprang et al. 2002), since none of the PPI results of the current sample differed significantly from that of the original (larger) sample. Furthermore, some of our results only reached trend levels of significance and should be confirmed in larger subject populations. Due to the naturalistic design of the study there were possible confounding factors for which we could not control. Nevertheless, because of its naturalistic design, this study provides information for future studies on chronic patients and for the prospective use of the startle reflex and its plasticity in clinical settings. The total examination programme was extensive, which caused a risk of selection bias, where the patients who participated at follow-up might differ from the patients who did not. However, there were no significant differences in age, PPI, habituation, startle magnitude or PANSS scores at baseline between patients who participated and patients who did not participate at follow-up. The clinical presentation of the patients at follow-up varied between patients being in remission and patients having severe delusions and hallucinations, i.e. the patients re-examined at the 6-yr follow-up were not a homogeneous group. Smoking may increase PPI (George et al. 2006; Postma et al. 2006; Swerdlow et al. 2006; Woznica et al. 2009). A change in smoking status from baseline to follow-up could therefore theoretically have contributed to the increase in PPI in the patients of the present study; however, the number of patients smoking had at most gone up by one at follow-up. We did not register the participants’ exact smoking patterns, neither at baseline nor at follow-up. However, although we tried to avoid the effect of acute smoking as well as its withdrawal symptoms by requesting subjects not to smoke 1 h prior to PPI assessment, we cannot rule out that a major change in smoking pattern in patients or healthy controls, between baseline and follow-up, could have contributed to the PPI changes.

Conclusion
In conclusion, this study is the first to follow startle reactivity, habituation and PPI in antipsychotic-naive, first-episode schizophrenia patients and healthy matched controls over a 6-yr period, and the first to show that PPI in antipsychotic-naive schizophrenia patients can increase significantly over time. The increase was most likely due to medical treatment or other disease-related factors since PPI in healthy controls decreased significantly over the same period. Startle reactivity decreased in the healthy controls, which supports the idea that startle reactivity decreases with normal ageing. Habituation did not change over time, and was deficient in patients compared to healthy controls both at baseline and follow-up. However, habituation was not reliable on an individual level.

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Statement of Interest
None.

References


