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ARTICLE

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Human electrophysiological correlates of learned irrelevance: effects of the muscarinic M1 antagonist biperiden

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Abstract

Learned irrelevance (LIrr) refers to a reduction in associative learning after pre-exposure of the conditioned and unconditioned stimulus in a non-contingent fashion. This paradigm might serve as a translational model for (pre)attentive information processing deficits in schizophrenia. This is the first study to investigate the event-related potentials (ERPs) of a within-subject LIrr paradigm in humans. Furthermore, the effects of the muscarinic M1 antagonist biperiden on LIrr were assessed. As expected, LIrr was found to be intact in young healthy volunteers after placebo. Furthermore, in the placebo condition P3b latency was decreased for target stimuli, which were pre-cued. This suggests that the predictability of the occurrence of these stimuli is mainly reflected by this ERP component. Biperiden had no effect on the behavioural LIrr measures, although prolonged reaction times were evident. Biperiden increased the N1 amplitude of the pre-exposed predictor letters, suggesting an effect of this drug on early perceptual processing. In conclusion, the within-subject paradigm used in the current study in combination with electroencephalography can reveal brain mechanisms involved in LIrr. M1 antagonism did not affect LIrr performance but seemed to influence early information processing.

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Key words: Acetylcholine, attention, cognition, even-related potentials, schizophrenia.

Introduction

If a conditioned stimulus (CS) and an unconditioned stimulus (US) are repeatedly presented in a random, uncorrelated manner, it is believed that an animal or human learns that the occurrence of one stimulus is irrelevant to the occurrence of the other one (e.g. Baker, 1976; Mackintosh, 1973). Next, if the former inconsequential CS becomes paired with the US, the subsequent acquisition of the CS–US association is delayed compared to stimuli that are novel. This phenomenon, the retardation of associative CS–US learning or classical conditioning after prior non-contingent exposure to the conditioning stimuli, is called learned irrelevance (LIrr; Gal *et al.* 2005). Of note, presenting only the CS prior to subsequent conditioning also induces a delay in CS–US learning called latent inhibition (LI), which is viewed as being closely related to LIrr (Allen *et al.* 2002).

In acute, first-episode schizophrenia LIrr has been found to be reduced or fully disrupted; the acquisition of associations related to pre-exposed (PE) stimuli occurs faster in this group of patients compared with normal controls (Gal *et al.* 2005; Orosz *et al.* 2011; Young *et al.* 2005). This means that LIrr is attenuated or even absent and acute schizophrenics actually outperform healthy individuals on these tasks. Furthermore, in chronic schizophrenia patients a failure to learn the CS–US association has been reported (Gal *et al.* 2005; Young *et al.* 2005), indicative of a global learning deficit rather than impaired LIrr. High-risk individuals (i.e. showing prodromal signs of psychosis) and schizotypic people also show LIrr deficits, whereas asymptomatic first-degree relatives of schizophrenics are seemingly unaffected. Combined, these findings are indicative of LIrr being a state marker for

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psychosis but not a trait marker for a potential schizophrenia endophenotype (Orosz *et al.* 2011).

There is a great lack of electrophysiological [electroencephalography (EEG)] data on human LIrr, although there are a few studies on event-related potentials (ERPs) of LI (Guterman *et al.* 1996; Kathmann *et al.* 2000). Therefore, the present study measured EEG together with LIrr in healthy individuals; the main focus was on the N1 and P3 ERPs. The N1 peak is a negative component that occurs around 70–140 ms post-stimulus at frontal and central electrode channels. This component seems to be related to the visual properties of a stimulus but can be modulated by selective attention. More specifically, its amplitude has been shown to be larger (i.e. more negative) for attended than for unattended or divided-attention stimuli (García-Larrea *et al.* 1992; Golob *et al.* 2002; Jerger *et al.* 1992; Kho *et al.* 2003; Näätänen, 1990; Rockstroh *et al.* 1996; White & Yee, 1997). The N1 is also important for discrimination processes, as it is absent if participants merely have to detect the presence of a stimulus (Mangun & Hillyard, 1991). As LIrr might be interpreted as an attentional phenomenon (Lubow, 2005), we decided to focus on the N1 component in our analysis of the ERP data. The P3 peak is a large positive-going waveform occurring about 250–500 ms post-stimulus. The P3 can be divided into two subcomponents: an early occurring peak in response to novel or alerting stimuli – called the P3a – and a later component – dubbed the P3b – which occurs in most tasks requiring a decision process contingent on stimulus discrimination (Donchin, 1981).

There is a vast amount of literature on the role of the dopaminergic and glutaminergic neurotransmitter systems in schizophrenia. However, alterations of muscarinic signalling also appear to underlie the disorder (e.g. Brooks *et al.* 2011; Sarter *et al.* 2010; Tandon *et al.* 1991, 1999). Post-mortem and *in-vivo* imaging studies have shown a reduction of muscarinic receptors density or binding in certain brain regions of schizophrenia patients (but see Garcia-Rill *et al.* 1995; Owen *et al.* 1981; Watanabe *et al.* 1983) and data seem most consistent for the muscarinic M1 receptor subtype (Crook *et al.* 2000, 2001; Dean *et al.* 1996; Deng & Huang, 2005; Newell *et al.* 2007; Scarr *et al.* 2008; Zavitsanou *et al.* 2004; but see Dean *et al.* 2000; Scarr *et al.* 2007). However, most of these binding studies have used [^{3}H]pirenzepine as a radioligand, which also binds to the muscarinic M4 receptor subtype (Barak, 2009). Thus, differentiating between the muscarinic M1 and M4 receptor is not possible on the basis of these studies.

The current study is the first to assess the effects of the muscarinic M1 antagonist biperiden in healthy, young volunteers using a within-subject LIrr paradigm suitable for ERP measurement. We expected that our LIrr paradigm would be successful in inducing LIrr in the placebo condition. We expected that the LIrr effect would be disrupted after biperiden treatment. With regard to the ERPs, we hypothesized that the LIrr conditions would be most notably reflected in the N1 and P3a/P3b. After biperiden, LIrr would be disrupted as indicated by similar reaction times (RTs) to PE-cued and non-pre-exposed (NPE)-cued targets (i.e. no increase in RT due to pre-exposure). We expected that biperiden would most notably affect the N1 and P3a/P3b.

Methods

Participants

In total, 17 [seven male, 10 female; mean age 22.4 yr (S.D. = 3.0, range 19–29)] healthy volunteers were recruited from Maastricht University through poster advertisements. Participants were required to be aged between 18 and 35 yr. We decided on a restricted age range because EEG and ERPs can change with age and can be differentially sensitive to cholinergic modulation (Bennett *et al.* 2004; Fjell & Walhovd, 2004; Pekkonen *et al.* 2005). Participants were also required to have a body mass index of 18.5–30.0. They received an extensive medical screening before testing, consisting of a medical questionnaire, physical examination, measurement of blood pressure and pulse rate, blood samples for haematology and biochemistry, urine samples for drug screen and pregnancy test and a resting electrocardiogram. Exclusion criteria were past or current psychiatric, neurological, cardiac, gastrointestinal, haematological, hepatic, pulmonary or renal illness, pregnancy, lactation, excessive alcohol consumption (intake of >20 glasses/wk), use of any medication other than oral contraceptives, having a first-degree relative with a current or past psychiatric disorder and presence of other deficits that could be expected to influence performance. All subjects gave a signed informed consent before inclusion and were financially rewarded for their participation. The study was approved by the Medical Ethics Committee of Maastricht University.

Study design and procedures

The study was conducted according to a double-blind, placebo-controlled crossover design. In the course of the week before the actual test sessions, the

participants received a training session to minimize any possible learning effects. Subjects were not allowed to use any psychoactive medication within 5 d before drug intake. Participants were asked to abstain from alcohol on a testing day and 24 h before testing. They were also not allowed to smoke and were requested not to consume any caffeine-, teaine- or aspartame-containing beverages on a testing day. The participants were asked to fill out some questionnaires on mood and physical complaints (see below) 1 h before testing and were given a capsule containing either a placebo, or 2 mg biperiden hydrochloride (Akineton®, instant release; Laboratorio Farmaceutico SIT S.r.l., Italy). They were provided with lunch immediately afterwards: this was done in order to reduce the chances of participants developing any side-effects due to biperiden intake. Lunch consisted of a can of caffeine-free soda, gluten-free bread and sweet bread toppings. After drug intake EEG electrodes and a cap were placed. After about 1 h of testing, the participant had a short break, during which he or she was asked to fill out the same questionnaires again. The test session finished about 3 h after drug intake. We aimed to separate test sessions by about 7 d to ensure sufficient washout of biperiden (average number of washout days = 9.3).

Drug treatment

Biperiden is a muscarinic M1 antagonist approved for the treatment of Parkinson symptoms, which develop due to use of first-generation antipsychotics (e.g. Ogino *et al.* 2011). It has about 10-fold higher affinity for M1 as compared with M2–M5 receptors and is thus the most selective M1 antagonist available for use in human participants (Bolden *et al.* 1992; Katayama *et al.* 1990). Peak plasma concentrations are reached around 1–2 h after a single dose administration followed by a rapid initial decline to around 12% of the peak values at 6 h after intake. This is subsequently followed by a slow terminal elimination phase at 48 h (Hollman *et al.* 1984, 1987). The most common side-effects of biperiden on the central nervous system are drowsiness, vertigo, headache and dizziness. Peripheral side-effects consist of blurred vision, mydriasis, dry mouth, impaired sweating, abdominal discomfort and obstipation (e.g. Mintzer & Burns, 2000; Peters, 1989; Tune *et al.* 1992). We chose a dose of 2 mg as this lies well within the range of the therapeutically recommended doses for biperiden (1–4 mg). Moreover, oral treatment with 2 mg biperiden has been shown to impair cognitive performance in healthy elderly individuals (Wezenberg *et al.* 2005).

Sequence of blocks

Block	Letter sequence →
RAN1	E J Q Y E B B X B Q Y E J Y Q E X J Y J J X B Y X Q B Q X E
NPE1	Y G X J Y J Y Y Q Q E G X J G X E E G X Q E G X B J Q B B B
PE1	E B X J E Y Q J Y J B X J E Y B X E Y E B X Q B X J Q Y Q Q
RAN2	J X Y E X J Y Q B E B Y B Y X J J Q Y B B X Q Q E Q X E E J
PE2	J Q J Y E X B Q J Y J Q E X B Q B B J E X Y Y E X Q Y B E X
NPE2	B B Y J Y A X Y E J B E Y J Q A X E Q A X B J Q E A X Q
RAN3	E Q E E X E Q B J Y Q X J Y Y Q B E B J X B Y J Y X Q B X J
NPE3	E W X J J Q W X B W X Y B Y Y B E Q E W X B J J Q Q W X Y E
PE3	B J J Y Q X E E Q X B Y J Q X E Y Q X E Y E B Y J Q X B B J
NPE4	J E M X J J E M X Y B Y M X J M X Q Q B Q B E B Q M X Y Y E
PE4	Q E J Y X B B Q Y X E B E J J Q Y X E E B J J Q Y X B Y X Q
RAN4	Q X J E J Y X Q Y E Q J X Y Y B E B E X J Q B B X Y E J B Q
PE5	B B E J X Q B Y B J X Y J X Q E Q E E E Y J X Q Y B Y Q J X
RAN5	J B E J B J X B E Y X Q E J E B X B Y Y E X J Y Q X Q Q Q Y
NPE5	Y J T X Y J Y T X E B E E Q T X E B J B T X Y Q Q B T X J Q

Fig. 1. A schematic presentation of the learned irrelevance test set-up and the letter sequences of a test session. The order of blocks is the same for all test sessions and is shown in the first column. The horizontal lines show the letter sequence of the corresponding blocks in detail. Regardless of condition, each block contains 30 letters: five targets (in bold), five target predictors (in bold, preceding the target letter X) and 20 filler letters (PE letters: B, E, J, Q, Y). Adapted from Orosz *et al.* (2008).

Biperiden was purchased, blinded and labelled by the pharmacy of the University Hospital Maastricht according to the relevant good manufacturing practice guidelines.

LIrr task

The within-subject LIrr paradigm was based on the one developed by Young *et al.* (2005), which was further modified by Gal *et al.* (2005) and Orosz *et al.* (2007, 2008, 2011). The paradigm was presented as a visual target detection task using letter characters (see Fig. 1). Capital Latin letters were shown successively on the computer screen for 1 s each, with an inter-stimulus interval of 1 s. The letters were font size 250, coloured white [red, green, blue (RGB) colour model: 255-255-255] on a black background (RGB: 0-0-0) and appeared in the centre of the monitor. The participants were instructed to press the spacebar as soon as the target letter 'X' appeared on screen. In addition to the target, there were 10 other consonants and vowels presented during a single version of the task. These letters could either be preceding the 'X' – called predictor letters – or fill in the spaces between the predictor–target

combinations – called filler letters. During one test session, 375 non-target and 75 target letters were presented, which means that the test duration was approximately 15 min. The subjects were presented with a series of 15 blocks of 30 letters each. The blocks were divided into three different conditions (with each block/condition presented five times):

(1) NPE blocks – In NPE blocks the target 'X' was always predicted by the same letter, which was completely novel. Each NPE letter acted as a target predictor in one particular NPE block and was not presented before or in later test sessions. Thus, NPE cues reliably predicted the target and enabled full prediction of it.
(2) PE blocks – In a PE block the target was preceded five times by the same letter. However, this PE predictor letter had already been shown in previous blocks as a filler letter (i.e. uncorrelated to the target letter). In other words, whereas NPE predictors were completely novel, PE predictors were not. Therefore, prediction of the occurrence of the letter 'X' was partial in this condition.
(3) Random (RAN) blocks – Targets appeared randomly after different consonants or vowels, which also served as PE and filler letters. Prediction of the target letter was therefore zero in the RAN blocks.

According to the degree of prediction, the RT to target was expected to be the lowest for the NPE-, somewhat higher for the PE- and the highest for the RAN-cued targets. In cases of LIrr, the average RT to PE-cued targets is supposed to be significantly higher than in that of NPE-cued targets ($RT_{PE} > RT_{NPE}$, see Orosz *et al.* 2008). Our main outcome variables were RT (ms) to the target and a LIrr index, which was calculated as follows: $(RT_{PE}/RT_{RAN}) - (RT_{NPE}/RT_{RAN})$. A score greater than zero would be indicative of intact LIrr (Gal *et al.* 2005). In order to monitor whether participants had understood the instructions and were well motivated to perform the LIrr task, we also measured number of hits (actual target response), misses (failure of target response), false alarms (response to filler letters) and premature responses (response to predictors).

Questionnaires

To assess subjective mood changes, the Profile of Mood States (POMS) was used (McNair *et al.* 1971). This self-report questionnaire has 32 visual analogue scales, which measure the mood states tension–anxiety, depression–dejection, anger–hostility, fatigue–inertia and vigour–activity. We also used the Bond and Lader Mood Scale (Bond & Lader, 1974), which has 16 visual analogue scales that yield three mood factors: contented–discontented; alert–drowsy; calm–excited. For the POMS and Bond & Lader questionnaires, a difference score of mean $score_{T1}$ (1 h after drug intake) – mean $score_{T0}$ (baseline, before drug intake) was calculated and used for analysis. Physical or mental complaints were assessed with a self-report questionnaire consisting of 31 possible complaints to be rated on a 4-point scale.

EEG and ERPs

For the EEG recording, the Neuroscan Synamps system (Neuroscan, USA) was used with sample rate set at 1000 Hz. Data were filtered between 0.05–100 Hz. EEG was recorded from 32 electrodes placed on the scalp using an elastic cap (Electro-Cap International, USA) and positioned according to the 10–20 system (Jasper, 1958). The horizontal electro-oculogram was measured with two electrodes placed on the outer canthus of the left and right eye and two electrodes placed below and above the centre of the left eye recorded the vertical electro-oculogram (VEOG). Two electrodes behind the ears served as reference electrodes. Before placing the electrodes, all locations were cleaned with alcohol and gently scrubbed with a gel, to ensure good conduction of the signal. Impedance was kept $<5\ \Omega$. During the EEG recordings, the participants were sitting in an electrically shielded and sound-attenuated room with the lights dimmed.

All EEG data were analysed with Vision Analyzer 2.0. Before data analysis, the EEG data were visually inspected offline, to check for artefacts. The EEG signal was filtered with a high pass filter set at 1 Hz (12 dB slope) and a low pass filter set at 30 Hz (12 dB slope). The ERP epochs were set from 100 ms prior to stimulus onset to 900 ms after onset, using the 100 ms prestimulus as baseline. Eye movement artefacts were filtered out of the EEG using the data of the VEOG channel and the method developed by Semlitsch *et al.* (1986). In this way, every participant had about the same number of artefact-free trials, which were combined into the ERP averages. Separate averages were calculated for the predictors and the targets, as well as for the NPE, PE and RAN stimuli. For the predictor stimuli, N1 and P3a peaks were noted on the Fz, FCz and Cz channels. In the case of the targets, N1 and P3b peaks could be distinguished on the CPz, CP3, CP4, Pz, P3 and P4 electrodes. Please refer to Table 1 for the time windows chosen for ERP peak detection analysis. Peak windows were determined based on the grand averages. Peak amplitudes were calculated and

Table 1. Time windows (ms) used for ERP analyses

	Peaks		
Stimulus type	N1	P3a	P3b
Predictors	45–140	320–500	n.a.
Targets	35–125	n.a.	185–500

ERP, Event-related potential; n.a., not applicable.

latencies were computed based on when the peak was reached.

Statistical analysis

Only participants who had at least 80% target hits, i.e. 60 of the total of 75, were included in the behavioural and ERP analyses. This criterion was used to ensure that all participants understood and were able to follow the instructions. Statistical data were analysed using SPSS. For all analyses, significant interactions were examined in more detail by doing additional analyses of variance (ANOVAs) split for each level of one of the interaction variables. *Post-hoc* testing was performed with a least significant difference *post-hoc* test. For both the behavioural and ERP data, the first trial of a block was always excluded from analysis under the assumption that implicit associative learning will need at least one CS–US or predictor-target pairing in order to be expressed. Subsequently, behavioural and ERP data were collapsed across blocks.

To determine possible treatment effects on the LIrr index, behavioural data were analysed by parametric ANOVA (repeated measures ANOVA) with drug (two levels: placebo and biperiden) as within-subject variable. For the analyses of the RT data, LIrr condition (three levels: RAN, PE and NPE) was added as an additional within-subject variable. We then evaluated whether the LIrr conditions had a differential effect on the ERPs. Therefore, the placebo data were analysed using repeated measures ANOVAs with LIrr condition (three levels: RAN, PE and NPE) and electrode (three levels for predictors: Fz, FCz and Cz, two levels for targets: CPz, Pz). We determined the effects of biperiden on the processing of predictor and target letters by doing the same analyses with drug (two levels: placebo and biperiden) as an extra within-subject variable. ERP analyses were done separately for amplitude and latency, stimulus type (i.e. predictors and targets) and each of the ERP peaks.

Questionnaire data were analysed with paired samples *t* tests separately for each subscale. The POMS had five subscales, whereas for the Bond & Lader we only analysed the alert–drowsy subscale, as this one is the best validated. The self-report questionnaire consisted of 31 questions, but we only analysed those side-effects relevant for biperiden intake: sleepiness; dizziness; nausea; restlessness; heart palpitations; stomach ache; bloated stomach; decreased appetite; dry mouth; tiredness; blurred vision; drowsiness; loss of concentration; nervousness; apathy; inability to tolerate bright light.

Results

One participant did not meet the requirement of at least 80% target hits and was therefore excluded from behavioural and ERP analysis. Part of the behavioural data was missing for another subject; this person was also not included, leaving a total of 15 participants.

Behavioural data: LIrr effects

Figure 2 shows the effects of the LIrr conditions on RT to the target letters. In the analysis of the RT data, there was a main effect of LIrr condition on RT [$F(2, 28) = 21.73$, $p < 0.001$]. *Post-hoc* analysis showed that RTs to the target letters were faster for the NPE compared with the RAN ($p < 0.001$) and PE condition ($p < 0.001$).

ERP data: LIrr effects on predictor and target letters

In the placebo analyses of the predictor letters, there were no relevant task effects on N1 amplitude and latency (F's < 1.69, n.s.) or P3a amplitude and latency (F's < 2.50, n.s.). In the placebo analyses of the target letters, there were no relevant task effects on N1 amplitude and latency (F's < 1.58, n.s.) or on P3b amplitude (F's < 1.17, n.s.). The effect of LIrr condition on P3b latency did not vary per level of electrode [no LIrr condition × electrode interaction effects: $F(10\,130) = 0.44$, n.s.], yet P3b latency was found to differ between LIrr conditions [main effect of LIrr condition: $F(2, 26) = 11.99$, $p < 0.001$]; *post-hoc* analysis showed that the P3b latency of NPE-cued targets occurred earlier compared with the P3b latency of RAN-cued ($p < 0.001$) and PE-cued targets ($p < 0.05$). Figure 3 shows the effects of the LIrr conditions on the P3b component of the target letters in the placebo condition.

Behavioural data: effect of biperiden

Figures 2 and 4 show the effects of biperiden on RT for the target letters and the LIrr index, respectively. The effect of drug did not vary per level of LIrr

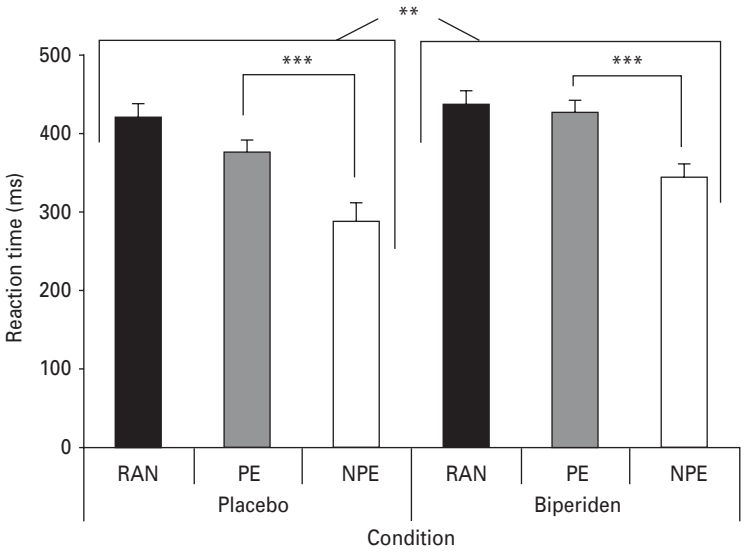

Fig. 2. Effects of the learned irrelevance (LIrr) conditions and biperiden on reaction time (RT) to the target letters (means + S.E.M., ** $p < 0.01$; *** $p < 0.001$). In the placebo condition, intact LIrr is present, reflected by larger RTs for pre-exposed (PE)- than for non-pre-exposed (NPE)-cued target letters. Biperiden prolonged RT compared to placebo, but did not affect LIrr. RAN, random.

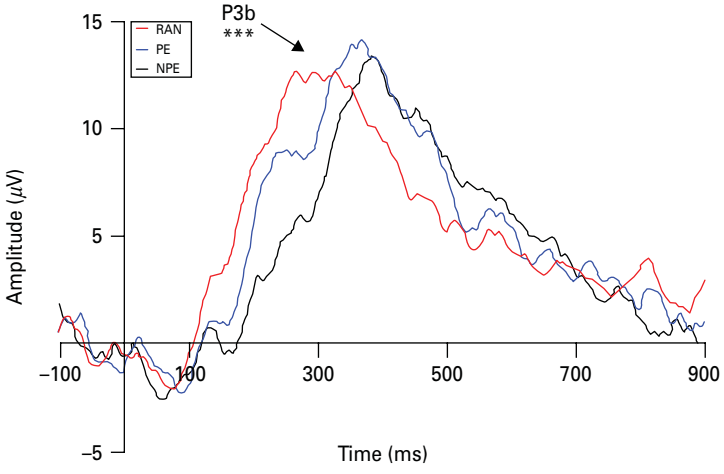

Fig. 3. Effects of the learned irrelevance (LIrr) conditions on the P3b component of the target letters in the placebo condition (Pz electrode channel). P3b latency is shortest in the non-pre-exposed (NPE) condition, intermediate for pre-exposed (PE)-cued targets and longest for targets belonging to the random (RAN) condition (*** $p < 0.001$).

condition [no drug × LIrr condition interaction effect: $F(2, 28) = 2.46$, n.s.]. RTs were increased after biperiden treatment [main effect of drug: $F(1, 14) = 12.43$, $p < 0.01$]. Biperiden had no effect on the LIrr index [no main effect of drug: $F(1, 14) = 0.06$, n.s.].

ERP data: effects of biperiden on predictor and target letters

Figure 5 shows the effects of biperiden on the N1 component of the PE predictor letters. The analysis of the amplitude of the N1 peak showed that the effect of biperiden varied per level of electrode [treatment × electrode interaction effect: $F(2, 26) = 7.54$, $p < 0.01$] and per level of LIrr condition [treatment × LIrr condition interaction effect: $F(2, 26) = 3.94$, $p < 0.05$]. Therefore, we decided to do separate repeated measures ANOVAs per LIrr condition. In the analyses of RAN and NPE predictors, there were no interaction or main effects of biperiden on N1 amplitude (F's < 1.98, n.s.). For the PE predictors, the effect of treatment on N1 amplitude varied per electrode [treatment × electrode interaction effect: $F(2, 26) = 7.42$,

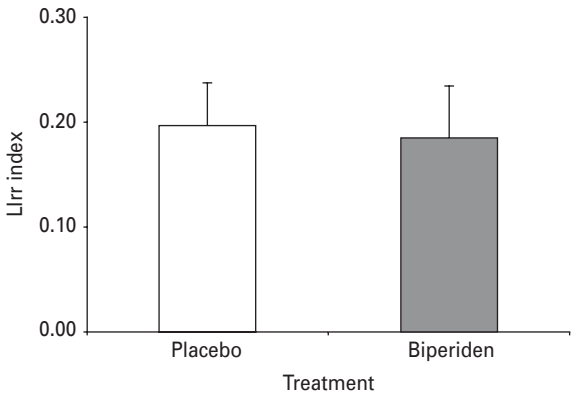

Fig. 4. Effects of biperiden on the learned irrelevance (LIrr) index (means + S.E.M.). In the placebo condition, intact LIrr is present, reflected by a LIrr index larger than zero. The same holds for the biperiden condition; in other words, biperiden failed to affect LIrr.

$p < 0.01$]; therefore we performed separate repeated measures ANOVAs per electrode. Biperiden was found to increase the N1 amplitude at Fz and FCz (F's > 5.77, $p < 0.05$). There were no relevant interaction effects or main effects of biperiden on N1 latency (F's < 2.40, n.s.), or on P3a amplitude or latency (F's < 2.25, n.s.) of the predictor letters. Furthermore, for the targets there were no effects of biperiden on N1 amplitude and latency (F's < 3.25, n.s.), or on P3b amplitude and latency (F's < 4.18, n.s.).

Questionnaire data

There were no effects of biperiden on the subscales of the self-report questionnaire (t's > -1.87, n.s.), the POMS (t's > -0.92, n.s.) or the Bond & Lader (t's > -1.52, n.s.).

Discussion

The goal of the current study was to develop a LIrr task suitable for ERP measurement and to examine the effects of the muscarinic M1 antagonist biperiden on behavioural performance and ERP correlates of LIrr in healthy young volunteers. The LIrr paradigm used in our current study was successful in inducing LIrr in healthy, young volunteers who received placebo medication (see Figs 2 and 4). For the behavioural results, LIrr was reflected in shorter RTs in relation to the degree of target predictability: fastest to NPE-cued targets, which were fully predictable, slower to PE-cued targets, which were partially predictable and slowest for RAN-cued targets, which yielded zero prediction. These findings are in accordance with earlier reports using within-subject LIrr-paradigms in humans (Gal *et al.* 2005; Orosz *et al.* 2007, 2008, 2011; Young *et al.* 2005). Moreover, our results are also in line with studies investigating the facilitating effect of stimulus predictability on RT (Barcelo & Knight, 2007; Fogelson *et al.* 2008; Suwazono *et al.* 2000). The LIrr index in our experiment was 0.2 after placebo, which is well over zero and therefore indicative of a robust LIrr effect of our task. In comparison, the LIrr index reported by Gal *et al.* (2005) in his group of healthy normal controls was about 0.1.

The three LIrr conditions (RAN, PE and NPE) affected P3b latency (but not amplitude) of the target letters as a function of the participant's degree of uncertainty about the occurrence of the target letter 'X' (see Fig. 3). The P3b occurred earliest after presentation of NPE-cued targets (averaged over all electrode channels), relatively later for PE-cued targets and was the slowest to occur for the RAN-cued targets. P3b latency is usually interpreted as an indication of stimulus evaluation speed, with shorter latencies indicative of superior cognitive performance (Polich & Criado, 2006). The traditional view states that the P3b starts to occur when stimulus evaluation processes are completed (Sutton *et al.* 1965, 1967). The finding that the P3b latency of a target stimulus is shortened by a predictor stimulus signalling subsequent target presentation is supported by evidence of earlier studies (Duncan-Johnson & Donchin, 1977, 1980; Fogelson *et al.* 2008), which showed that P3b latency is decreased for targets that are considered highly probable than those that are less probable. Furthermore, as already suggested by Fogelson *et al.* (2008), the enhancement of stimulus evaluation speed of predictable targets appears to be cognitive rather than perceptual. Our target stimuli were always similar (i.e. throughout the task the target stimulus was the letter X) and there was no influence of LIrr conditions on early perceptual ERP components (e.g. N1). In line with our findings, previous studies utilizing discrimination tasks in which participants are instructed to favour accuracy over speed have shown a positive correlation between P3b latency and RT performance (Kutas *et al.* 1977; but see Verleger, 1997). Taken together, the effects of the LIrr conditions on P3b but not N1 latency suggest that LIrr is more related to stimulus evaluation processes rather than early perceptual/attentional mechanisms.

A behavioural disruption of LIrr after biperiden treatment was expected to be reflected by similar RTs to NPE-cued and PE-cued targets (i.e. no increase in RT due to pre-exposure). However, biperiden did not affect LIrr as participants still showed faster RTs to NPE-cued as opposed to PE-cued target letters

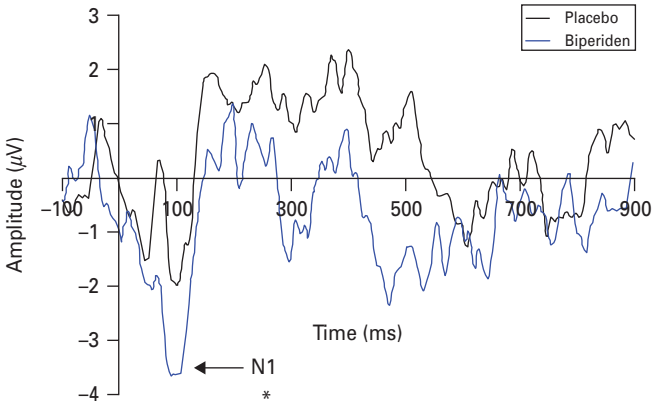

Fig. 5. Effects of biperiden on the N1 component of the pre-exposed predictor letters (Fz electrode channel). Biperiden increased N1 amplitude compared to placebo (* $p < 0.05$).

(see Fig. 2). Similarly, the LIrr index was not reduced (see Fig. 4) and participants did not report any side-effects on our questionnaires after biperiden. The only discernible effect of biperiden was an overall increase in RT. In line with our results, Wezenberg *et al.* (2005) reported a dose of 2 mg biperiden to increase movement time in a motor learning task. However, the authors interpreted this effect as being due to attentional deficits, rather than a general slowing of psychomotor performance.

Several studies have reported a detrimental effect of biperiden on memory processes. In the study of Guthrie *et al.* (2000), impairments were found in backward but not forward digit span. Both of these tasks are thought to tap into several cognitive processes, among which are memory and attention (Larrabee & Kane, 1986). In the same study, biperiden also affected verbal memory; on a selective reminding task participants recalled fewer words at both immediate and delayed recall. These results were replicated and extended by Wezenberg *et al.* (2005). These studies thus provide support for the view that the muscarinic M1 receptor is important for memory functions. Theories about LIrr have focused on behavioural switching (Weiner, 2003), attention (Lubow, 2005; Schmajuk, 2005) and inhibition of redundant stimuli (Gluck & Myers, 1993). Hence, LIrr is thought of as a paradigm that measures attentional rather than mnemonic abilities, which might explain why a memory-impairing drug such as biperiden failed to have an effect on our LIrr task.

Biperiden also increased the amplitude of the N1 component of the PE predictor letters – which were previously irrelevant but later on became partial predictors of the target stimulus (see Fig. 5). This was an unsuspected finding given our hypothesis of reduced amplitudes and/or increased latencies of the N1, P3a or P3b peaks after biperiden. It is unclear whether the biperiden-induced increment in N1 amplitude reflects cognitive/central or non-cognitive/peripheral processes. Biperiden has been known to cause blurred vision in healthy volunteers (e.g. Fleischhacker *et al.* 1987); however, impaired vision would be expected to reduce N1 amplitude rather than increase it. Moreover, our participants did not report visual impairments after biperiden compared to placebo. Third, blurred vision would be likely to globally influence N1 amplitude; that is, the N1 amplitude of RAN and NPE predictor letters would also be affected and not only that of the PE predictors. The only non-cognitive effect of biperiden we did find was a general slowing of psychomotor performance (see above). However, responding to a target stimulus takes place much later after presentation of that stimulus, whereas the N1 is an early perceptual component that is regarded as separate from response behaviour (Näätänen *et al.* 1988).

A cognitive explanation of the increment in N1 amplitude of PE predictor letters after biperiden intake is also not very straightforward. Given the finding that N1 amplitudes are larger for attended than for unattended stimuli (e.g. Haider *et al.* 1964), a drug that is known to disrupt cognition would be expected to reduce N1 amplitude rather than increase it. It might be the case that, after biperiden, there is an over-compensation of the irrelevant-to-relevant switch or, in other words, participants needed to allocate more attentional resources in order to successfully link the presentation of the previously irrelevant PE predictor with the subsequent occurrence of the target letter. Taken together, our results would argue for an attentional effect of biperiden.

Interestingly, a study examining LI and ERPs in acute and stable, partially remitted schizophrenia patients and healthy controls reported results that were comparable to our own (Kathmann *et al*. 2000). All participants exhibited robust LI; in other words, schizophrenics were not impaired in this regard. Schizophrenic patients did show slower RTs compared with the normal, healthy controls, which is in accordance with our biperiden data. In contrast to the behavioural results, the electrophysiological data did differentiate between diagnostic groups. Pre-exposure affected the N1 amplitudes to CS+ stimuli, which were irrelevant during pre-exposure but subsequently served as predictors during acquisition. Thus, these stimuli were conceptually similar to our PE predictor letters. Specifically, N1 amplitudes of CS+ stimuli were decreased in healthy controls, increased in acute schizophrenics and unchanged in partially remitted schizophrenics after pre-exposure. The authors interpreted this finding as an enhancement of allocation of attention to previously irrelevant stimuli or, alternatively, as a failure to inhibit previously irrelevant stimuli from gaining access to attentional processing in the acute schizophrenic patient group. Of note, all patients received neuroleptic medication, which means that an effect of these drugs on ERPs cannot be excluded.

In sum, our LIrr paradigm induced robust LIrr in healthy young volunteers. As for the ERP results, LIrr was reflected in shorter P3b latency (i.e. faster stimulus evaluation processing) in relation to the degree of target predictability. LIrr was not affected after biperiden, but the N1 amplitude of the PE predictor letters was increased (suggestive of increased allocation of attentional/behavioural switching resources). The present data are inconclusive as to the attentional or mnemonic effects of biperiden, although the ERP data suggest a role in early information processing. Changes in LIrr after drug intake might ultimately serve as a psychopharmacological model for neuropsychiatric disorders, such as schizophrenia. For translational purposes, it would be pertinent to also determine drug-induced alterations in ERP correlates of LIrr.

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Statement of Interest

None.

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