Review

Enhancement of latent inhibition in patients with chronic schizophrenia

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\textbf{Abstract}

\textbf{Objectives:} Latent inhibition (LI) refers to the retarding effects of inconsequential stimulus preexposure on subsequent conditioning to that stimulus, and reflects the organism’s capacity to ignore irrelevant stimuli. LI is disrupted in schizophrenia patients, due to faster learning of the association between the conditioned stimulus (CS) and an unconditioned stimulus (US). It was recently proposed that LI has an additional pole of abnormality indicated by LI persistence.

\textbf{Methods:} Two experiments were performed to test this hypothesis. Both experiments applied a new within-subject, visual recognition LI procedure in which the association between a cue (CS) and the target (US) is acquired. In Exp 1 the task was applied to healthy volunteers (\(n = 21\)). In Exp 2 chronic schizophrenia patients (\(n = 19\)) were compared to control subjects (\(n = 20\)).

\textbf{Results:} In Exp 1 the subjects showed LI in the initial trials of cue–target pairings, and an attenuation of the phenomenon at later trials. In Exp 2 control subjects showed a pattern of response comparable to the subjects of Exp 1, while the patients showed LI only on the later trials of the task.

\textbf{Conclusions:} This result suggests that patients with chronic schizophrenia showed LI persistence. The possible advantages of the new LI paradigm are discussed.

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1. Introduction

Latent inhibition (LI) is one of the most prominent models of the disruption in information processing in schizophrenia. First described by Lubow and Moore [1], LI refers to the retarding effects of inconsequential stimulus preexposure on subsequent conditioning to that stimulus, and reflects the organism's capacity to ignore irrelevant stimuli. The LI phenomenon has been widely studied, demonstrating its ubiquitous existence in a variety of species, including humans (for review see Refs. [2–5]).

Interest in the application of LI to the clinic increased after significant findings of LI disruption in acute schizophrenia patients [6–14] (but see Refs. [15,16]). In line with the dopamine (DA) hypothesis of schizophrenia this effect has been attributed to an increased DA activity in acute patients. This is supported by studies in both animals [17–19], and humans [20–22] in which the activity of DA has increased experimentally following the administration of the indirect DA agonist, amphetamine. Following a similar logic, it has been argued that the normalization of LI is due to the stabilization effect of the antipsychotic medications which block DAergic activity [7].

Recently, Weiner [5] proposed a novel conceptualization of LI as it is evidenced in schizophrenia patients. Based on animal studies, Weiner [5] argued that the LI phenomenon has a second anomalous expression, an enhanced LI, also termed an “abnormally persistent LI”. This enhancement is shown when LI is expressed under conditions which normally lead to a disruption of LI (e.g., increased number of conditioning trials, or context shift between the stages of preexposure and conditioning). While the disruption of LI is associated with increased activity of the DAergic system, the persistence of LI was attributed to the effects of glutamate antagonists at the N-methyl-D-aspartate (NMDA) receptor. Systemic administration of low doses of the NMDA blocker MK-801 leads to LI-enhancement [23]. This effect was shown to be reversed by the atypical antipsychotic clozapine [23] or by D-serine, a modulator of the glycine site on NMDA receptors [24]. Although the rationale for the LI attenuation is supported by substantial links with increased DA transmission, there is considerable evidence that schizophrenia is associated with deficits of glutamatergic mechanisms in general and NMDA receptors in particular. The latter may be related to the predominance of negative symptoms and cognitive dysfunction, mainly in the chronic course of illness (for review see Ref. [25]).

Therefore, while LI is expected to be disrupted in patients with schizophrenia during the acute and predominantly psychotic state, due to increased DA turnover, LI is also expected to be abnormally persistent in chronic patients with a predominant negative symptomatology due to the blockade of NMDA receptors. To date, only two studies of schizophrenia patients support this proposition and indicate that enhanced LI is present in chronic patients [11], or in adolescent patients with a high negative to positive symptoms ratio [26]. The main reason for the scarce evidence of LI-persistence is that it is difficult to unravel this phenomenon experimentally. Persistent LI appears as normal LI unless an appropriate procedure is used to detect its “excess”. Thus, the testing procedure should provide a scale which is broad enough to represent the continuum from the disruption through normal to a persistence of LI.

Traditional LI procedures apply between subjects designs with two groups: one group is given preexposure trials of the to-be-conditioned stimulus (CS), and the second group (the control group) is not-preexposed. In a second stage of the procedure, subjects of both groups are given a learning task which includes formation of new associations with preexposed (PE) CS, and the groups are compared in their learning of the new associations. However, in recent years these designs have been criticized, mainly in the context of clinical populations, due to the difficulty of matching patients of the PE and non-preexposed (NPE) conditions. Thus, it has become more common to apply within-subject designs [7,10,13,21]. In a within-subject design each subject is presented with both the PE-CSs and NPE-CSs (novel stimuli which were not formerly presented), and required to form new associations with each. The individual differences in the rate of association to PE and NPE stimuli serves as the measure of LI. In this way each subject is his own control for the formation of the CS–unconditioned stimulus (US) associations.

In the present study we applied a within-subject procedure which enabled testing of LI enhancement in chronic schizophrenia patients. LI is assessed according to the reaction time (RT) to a target stimulus (the US) which is predicted by a cue (the CS). This procedure is partially based on a learned irrelevance (LIirr) task previously used by us [27] and others [28,29]. The LIirr phenomenon is a preexposure effect in which uncorrelated presentations of the CS and the US retard a subsequent CS–US association. In LI procedures only the CS is PE. However, both LIirr and LI share the common feature of slowed learning as a consequence of stimulus preexposure.

It was expected that in the test phase healthy subjects would show LI during the early stages of the formation of the cue–target associations and that LI would be attenuated with further cue–target pairings, i.e., the response to PE cues would be as efficient as to NPE cues at later stages of the formation of the cue–target associations. Perseveration of LI in chronic schizophrenia patients was expected to be demonstrated as an enduring difference in response to PE as compared to NPE cues also at more advanced stages of the formation of the cue–target associations (where healthy subjects already show no such difference). In Exp 1, 21 healthy volunteers were tested to demonstrate the LI-effect as well as its attenuation following repeated cue–target pairings. In Exp 2, 19 patients suffering from chronic schizophrenia, and matched control subjects participated in the tasks in order to show the perseveration of LI in chronic schizophrenia patients.

3. Results

3.1. Experiment 1

3.1.1. Preexposure phase

3.1.2. Test phase

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3.2. Experiment 2

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3.3.1. Preexposure phase

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3.3.3. LI-index

4. Discussion

References
2. General methods

2.1. Latent inhibition

Apparatus and procedure: Programming and data recording were controlled by a portable PC with a 15'' VGA colored monitor (Dell, Inc.). The MATLAB 6.0 program was used (MathWorks, Inc.).

2.1.1. Methods

The LI procedure was implemented on a visual recognition task of letter characters. Latin letters were presented successively on the computer screen for 1 s each, with no inter-stimulus interval. The letters were upper case, 10 cm × 10 cm in size, with yellow text (RGB: 245–234–17) on a blue background (RGB: 41–14–244), which appeared in the center of the monitor.

Two conditioning sessions were used: a PE session and a test session.

Preexposure session: The session consisted of 180 letter presentations (overall duration of 3 min). The letter “Y” was defined as the target. The session was divided into 6 blocks of 30 letters each. The six blocks were composed of three random (non-cued) and three cued blocks. Random and cued blocks alternated, starting with a random block. In a random block vowel letters (A, E, I, O and U) and the target were presented in a pseudo-randomized order, with no more than three consecutive presentations of the same vowel letter occurring, and no consecutive presentations of the target (number of letters presented between two targets ranged from 2 to 12). In a cued block one consonant letter (J, C and P) was consistently presented prior to the target. In PE blocks the cue letters were vowels presentations (PE/NPE) during the test session were calculated for trials 1–5 and trials 6–10. Significant effects were further analyzed using the Duncan post hoc test. Analysis of the LI-index was performed using a paired t-test which compared between the two sets of 5-trials (trials 1–5 and trials 6–10). Response analysis of the LI data was performed using one-way ANOVAs with a repeated measures factor of preexposure (random, NPE and PE), and two sets of 5-trials (trials 1–5 and trials 6–10). Response analysis of the LI index was performed using one-way ANOVAs with a repeated measures factor of preexposure (random, NPE and PE) on hits, commissions, and total responses. Linear associations between different measures of the study were conducted by Pearson correlation coefficients.

2.2. Experiment I

2.2.1. Subjects

The study was held at the Academic College of Tel-Aviv-Yaffo, Israel. Twenty-one students aged 22–28 (mean 25.2, S.D. 2.3) participated in the study. There were 15 females and 6 males. The study was approved by the local ethical committee, and all subjects participating in the study had signed a written consent form.

One of the subjects, reported as using a psychiatric drug, was excluded from further data analysis.

2.2.2. Data analysis

For the analysis of the RT data during the prexposure session a paired t-test was performed, which compared the two target-presentation conditions (random and cued), collapsed over blocks. The data during the test session were analyzed using a 3 × 2 repeated measures ANOVA, with the within-subject factors of prexposure (random, NPE and PE), and two sets of 5-trials (trials 1–5 and trials 6–10). Significant effects were further analyzed using the Duncan post hoc test. Analysis of the LI-index was performed using a paired t-test which compared between the two sets of 5-trials (trials 1–5 and trials 6–10). Response analysis of the LI data was performed using one-way ANOVAs with a repeated measures factor of preexposure (random, NPE and PE) on hits, commissions, and total responses. Linear associations between different measures of the study were conducted by Pearson correlation coefficients.

2.3. Subjects

The study was held at the Shalvata Mental Health Center, School of Medicine, Tel-Aviv University, Israel. Nineteen patients suffering from schizophrenia participated in the study. The inclusion criteria were adult patients (18–65) suffering from chronic schizophrenia not experiencing an acute episode (documented either by their medical charts or psychiatrist). The patients were recruited from the hospital’s outpatients’ clinic. Diagnosis of all patients was done by two senior psychiatrists according to DSM-IV criteria. Exclusion criteria for all subjects were: substance and alcohol abuse, mental retardation, organic brain disease and/or head trauma. Twenty control subjects, members of the hospital’s personnel, were matched to the patients according to their level of education. Exclusion criteria were the same as the patients’, with the additional requirement that control subjects should not have suffered from any known mental condition or disease, or had not taken any neuro-psychiatric drug. The study was approved by the local institutional review board, and all subjects participating in the study have signed a written consent form.

2.3.2. Psychiatric scales

Data on the clinical state of the participants were collected on the day of testing for three scales: The positive and negative syndrome scale for schizophrenia (PANSS), clinical global impression (CGI), and the extrapyramidal symptoms rating scale (ESRS).

2.3.3. Procedure

Patients were off cholinergic drugs and benzodiazepines on the testing session day. The experimenter (YB) interviewed the patients and completed the psychiatric scales on the day of testing.

2.3.4. Background data

Data on sex, age, education level were collected (Table 1). A significant association was observed between group and age (t47) = 2.28, p < 0.05, due to the higher age of control subjects. A tendency to a group by sex association was also observed (χ2(3) = 3.14, p = 0.076).

Table 1

<table>
<thead>
<tr>
<th>Sex</th>
<th>Education</th>
<th>Age</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Control</td>
<td>48.3</td>
<td>8.0</td>
</tr>
<tr>
<td>F</td>
<td>Schizophrenia</td>
<td>41.8</td>
<td>9.6</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.D.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Education level: elementary – 8 years of schooling; partial high – up to 12 years, but did not accomplish the final examinations; full high – 12 years with final examinations accomplished; academic – more than 12 years of education.
Clinical data regarding age of onset, years of illness, number of previous hospitalizations, and drug therapy data, were collected from the patients' records (Table 2). Age of onset ranged from 12 to 45, with a minimal period from onset to the testing period of 5 years. Most of the patients were treated by atypical neuroleptics (10 patients, 52.6%), including risperidone, olanzapine, and clozapine. The other patients were treated by typical neuroleptics (5, 26.3%) including haloperidol, fluphenazine, zuclopenthixol or any combination thereof (4, 21.1%). Three patients received long acting injections of the drugs (fluphenazine and zuclopenthixol). In addition to neuroleptics the patients were treated by other psychiatric drugs: tranquilizers = 10, anti-depressants = 6, anticholinergic agents = 8, and mood-stabilizer = 1.

2.3.5. Data analysis
For the analysis of the RT data during the preexposure session a 2 × (2) repeated measures ANOVA was performed with the between subject factor of group (control and schizophrenia), and the within-subject factor of target presentation (random and cued). For the analysis of the LI RT data, a 2 × (3 × 2) repeated measures ANOVA was performed with the between subject factor of group (control and schizophrenia), and the within-subject factors of preexposure (random, NPE and PE), and two sets of 5-trials (trials 1–5 and trials 6–10). Significant effects were further analyzed using the Duncan post hoc test.

Response analysis of the LI data was performed by 2 × 3 ANOVAs with a main factor of group (control and schizophrenia) and a repeated measures factor of pre-exposure (random, NPE and PE) on hits, commissions, and total responses. Analysis of the LI-index was performed using grouped t-tests. LI-index was analyzed using grouped t-tests to compare the amount of LI between control and schizophrenia subjects. Paired t-tests were performed comparing between LI-indices of trials 1–5 and trials 6–10 within patients and controls. Linear associations between different measures of the study were conducted by Pearson correlation coefficients.

3. Results

3.1. Experiment 1

3.1.1. Preexposure phase
The RT data of the preexposure phase indicated lower RTs when the target was cued (mean 403.4, S.D. 116.1 ms) compared to random presentation (mean 493.2, S.D. 46.8 ms). A paired t-test performed on RTs revealed a significant difference between the presentation conditions (t = 3.21, d.f. = 19, p < 0.005).

Response analysis was performed on the hits and commissions to random or cued target presentation. The number of hits did not differ between presentation conditions (p > 0.38). However, more commissions were performed during the cued (mean 2.05, S.D. 1.54) compared to the random condition (mean 0.65, S.D. 0.67) (t = 4.63, d.f. = 19, p < 0.001).

3.1.2. Test phase
As shown in Fig. 1, LI was present during the first 5 target presentations and abolished with the continuation of the association trials. Importantly, the abolition of LI on trials 6–10 was due to change of the RTs to PE cues. On trials 1–5 (left) the RTs to random target presentation were higher compared to both PE and NPE cues. In addition, RTs to NPE cued targets were lower compared to PE cued targets, indicating the presence of LI. During the target presentations 6–10 (right) the RTs to random target presentation and NPE cued targets did not change compared to trials 1–5. However, RTs to PE cued targets were lower on trials 6–10 compared to trials 1–5.

This pattern was supported by a 3 × 2 ANOVA with the within-subject factors of preexposure and two 5-trials sets, performed on the RTs which revealed a significant preexposure effect (F = 15.49, d.f. = 2, 38, p < 0.001), as well as a significant preexposure by set interaction (F = 9.58, d.f. = 2, 38, p < 0.001). The preexposure by set interaction was further analyzed using Duncan's post hoc comparisons test which indicated a significant difference between RTs to random and both PE and NPE target presentations on trials 1–5 (both p's < 0.001), as well as a significant difference between RTs to PE and NPE (p < 0.001). On trials 6–10 a significant difference between RTs to random and both PE and NPE target presentations (both p's < 0.001), but no significant difference between PE and NPE (p = 0.87). In addition, a significant reduction of RTs to PE target presentations was observed between trials 1–5 and trials 6–10 (p < 0.001).

Response analysis was performed on the hits, commissions, and total responses performed in the three-target presentation conditions. No differences between conditions were observed on either measure. The average number of hits was high, ranging from 26.9 to 27.5, representing a hit ratio greater than 89%. The average number of commissions was low, ranging from 1.2 to 2.6 commissions. Total responses ranged from 28.1 to 29.0. Analyses were carried out using a 2 × 3 ANOVA with a within-subject factor of preexposure. No significant differences between preexposure conditions were observed (all p’s > 0.20).

3.1.3. LI-index
The LI-index indicated the existence of a robust LI at trials 1–5 (mean 1.29, S.D. 0.40) while no LI was seen at trials 6–10 (mean 0.96, S.D. 0.21). The difference between the two sets was statistically significant (t = 2.77, d.f. = 19, p = 0.012).

The LI-index of trials 1–5 and 6–10 did not differ between male and female subjects (p’s > 0.12). No associations with background variables of age or education were observed.

3.2. Experiment 2

3.2.1. Clinical state
The clinical state of the patients is presented in Table 3. Total PANSS values were high, as could be expected in patients suffering from chronic schizophrenia. The patients showed a predominantly high level of negative symptoms (average item score of 4 = moderate). The most prominent negative symptoms amongst the patients were stereotyped thinking (mean 5.1; 14 patients...
Table 3  
PANSS, CGI, and ESRS data of chronic schizophrenia patients

<table>
<thead>
<tr>
<th>Mean</th>
<th>S.D.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Negative</td>
<td>28.4</td>
<td>4.1</td>
</tr>
<tr>
<td>General</td>
<td>57.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Total</td>
<td>105.1</td>
<td>10.8</td>
</tr>
<tr>
<td>CGI</td>
<td>4.4</td>
<td>0.7</td>
</tr>
<tr>
<td>ESRS</td>
<td>7.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

PANSS, positive and negative syndrome scale for schizophrenia; CGI, clinical global impression; ESRS, extrapyramidal symptoms rating scale.

Table 4  
Mean and standard deviation (S.D.) of RTs (ms) for random and cued target presentation during the preexposure phase for schizophrenia patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Random</th>
<th>Cued</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Control</td>
<td>511</td>
<td>58</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>549</td>
<td>40</td>
</tr>
</tbody>
</table>

were scored moderate-severe to severe), and lack of spontaneity and flow of conversation (mean 4.7; 12 patients were scored moderate-severe to severe). CGI scores indicated that all the patients were rated moderately ill \( (n = 9, \text{score} = 4) \) or markedly ill \( (n = 10, \text{score} = 5) \). ESRS indicated that only five patients were relatively free of extrapyramidal symptoms (all items scored 0–2). Among other patients the dominant symptoms detected were gait and posture (mean 2.2; eight patients were scored moderate to severe), and bradykinesia-rigidity (mean 1.9; five patients were scored moderate to severe).

3.3. Latent inhibition

3.3.1. Preexposure phase

The RT data of the preexposure phase are presented in Table 4. As could be seen control subject showed lower RTs compared to patients. In addition, contrary to the findings of Exp 1, RTs of both groups tended to be higher when the target was cued compared to a random presentation. The ANOVA performed on RTs revealed a significant main effect of group \( (F = 4.63, \text{d.f.} = 1, 35, p = 0.038) \), as well as a cue effect which approached the acceptable significance level \( (F = 3.72, \text{d.f.} = 1, 35, p = 0.062) \).

Response analysis was performed on the hits and commissions to random or cued target presentation using \( 2 \times 2 \) ANOVAs (Table 5). The analysis of hits indicated that the number of hits did not differ between groups \( (p = 0.72) \) or presentation conditions \( (p = 0.63) \). The analysis of commissions indicated no difference between groups \( (p = 0.10) \). However, similar to Exp 1, subjects of both groups performed more commissions during the cued compared to random condition phases \( (F = 10.41, \text{d.f.} = 1, 35, p = 0.002) \).

Table 5  
Response data of control and schizophrenia patients during the preexposure phase, including mean (M) and standard deviation (S.D.) of hits, commissions (Com), and total responses of random, and cued blocks

<table>
<thead>
<tr>
<th></th>
<th>Random</th>
<th></th>
<th></th>
<th>Total</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Hits</td>
<td>S.D.</td>
<td>Com</td>
<td>S.D.</td>
<td>M</td>
<td>S.D.</td>
<td>Com</td>
<td>S.D.</td>
<td>M</td>
</tr>
<tr>
<td>Control</td>
<td>14.3</td>
<td>1.0</td>
<td>1.0</td>
<td>1.3</td>
<td>15.3</td>
<td>1.5</td>
<td>14.5</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>14.4</td>
<td>0.9</td>
<td>0.4</td>
<td>0.6</td>
<td>14.8</td>
<td>0.6</td>
<td>14.4</td>
<td>0.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

3.3.2. Test phase

As could be seen from Fig. 2, during target presentations 1–5 (plate A) control subjects showed lower RTs to PE- and NPE-cued compared to random target presentation. In addition, RTs to NPE-cued targets were lower compared to PE-cued targets, indicating the presence of LI. However, schizophrenia patients, while performing slower compared to controls, did not show a difference in RTs to the different conditions of target presentation, failing to show the LI phenomenon. During target presentations 6–10 (plate B) control subjects reduced the RTs mainly to PE cued targets, showing no difference between PE and NPE which indicated the attenuation of LI. Schizophrenia patients showed no change of RTs to random target presentation, while a decrease to NPE cued targets was present compared to trials 1–5. In contrast, RTs to PE cued targets were increased on trials 6–10 compared to trials 1–5. Thus, while control subjects showed LI attenuation on trials 6–10, among patients the LI phenomenon appeared on trials 6–10 only.

This pattern was supported by a \( 2 \times (3 \times 2) \) ANOVA with the between subjects factor of group and the within-subject factors of preexposure, and two 5-trials sets, performed on the RTs which revealed the significant main effects of group \( (F = 5.39, \text{d.f.} = 1, 35, p = 0.026) \), and preexposure effect \( (F = 14.81, \text{d.f.} = 2, 70, p < 0.001) \), as well as the significant interactions of preexposure by set \( (F = 6.16, \text{d.f.} = 2, 70, p = 0.003) \), and group by preexposure by set \( (F = 3.91, p = 0.05) \).
d.f. = 2, 70, p = 0.024). To assess differences between preexposure conditions the group by preexposure by set interaction was further analyzed using Duncan’s post hoc comparisons which revealed the following outcomes:

1. **Control subjects** showed on trials 1–5 a significant difference between RTs to random and both PE and NPE target presentations (p < 0.001), as well as a significant difference between PE and NPE target (p = 0.039). On trials 6–10 a significant difference was indicated between RTs to random and both PE and NPE target presentations (both p’s < 0.01), but not between PE and NPE (p = 0.14). The difference between RTs to PE target presentations on trials 1–5 and trials 6–10 approached the acceptable level of significance (p = 0.07).

2. **Schizophrenia patients** did not show RT differences between the three presentation conditions on trials 1–5 (all p’s > 0.12). On trials 6–10 RTs to NPE-cued targets were lower compared to random targets (p = 0.047). In addition RTs to PE-cued targets were higher compared to both random (p < 0.01) and NPE (p < 0.001).

Response analysis was performed on the hits, commissions, and total responses performed in the three target presentation conditions using a 2 × 3 ANOVA with a between subjects factor of group and a repeated measures factor of preexposure (Table 6). Differences between patients and control subjects were present on the hits and total response measures, but not on the commissions. The hits and total response differences were restricted to the NPE target presentation.

The analyses of the hits and total responses yielded significant group by preexposure interactions (F = 3.32, d.f. = 2, 70, p = 0.04; F = 6.32, d.f. = 2, 70, p = 0.002, respectively). Post hoc analysis revealed that control subjects showed fewer hits and total responses compared to the patients on the NPE target presentations only (both p’s < 0.01).

### 3.3.3. LI-index

The LI-index indicated that although control subjects showed the LI phenomenon on trials 1–5 it was rather weak and did not significantly differ from the pattern shown by the schizophrenia patients (t = 0.95, d.f. = 35, p = 0.35) (Fig. 3). In contrast, on trials 6–10 the schizophrenia patients showed a higher LI-index compared to control subjects (t = 2.55, d.f. = 35, p = 0.015). In addition, among schizophrenia patients a significant difference was present between the indices of trials 1–5 and 6–10 (t = 2.79, d.f. = 16, p = 0.013).

Associations between LI-index and gender, age, and years of education were calculated. Gender showed a tendency towards a significant difference: on trials 1–5 females showed higher values (mean = 1.07, S.D. = 0.11) compared to male subjects (mean = 1.0, S.D. = 0.12) (t = 1.87, d.f. = 35, p = 0.07). No associations with age or years of education were observed. The association with gender was further analyzed to test for possible interaction with group, using a 2 × 2 ANOVA. No significant group by gender interaction was indicated.

![Fig. 3. The LI-index of control and schizophrenia patients during trials 1–5 and trials 6–10. Bars represent 1 S.E. (*) Represents significant differences (p < 0.05) between control and schizophrenia patients and (†) represents significant difference between trials 1–5 and trials 6–10 among schizophrenia patients (p < 0.05).](image)

**Table 6** Response data of control and schizophrenia patients during the test phase, including mean (M) and standard deviation (S.D.) of hits, commissions (Com), and total responses of random, PE, and NPE blocks

<table>
<thead>
<tr>
<th></th>
<th>Hits</th>
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<th>Hits</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td>28.9</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td>28.6</td>
<td>1.3</td>
<td></td>
<td></td>
<td>28.0</td>
<td>1.7</td>
<td></td>
<td></td>
<td>28.6</td>
<td>1.8</td>
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<tr>
<td>Schizophrenia</td>
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<td>28.8</td>
<td>1.1</td>
<td></td>
<td></td>
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<td>29.0</td>
<td>0.9</td>
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</table>

**Table 7** Correlation coefficients between LI-indices of schizophrenia patients and clinical measures of PANSS, CGI, and ESRS

<table>
<thead>
<tr>
<th>Measure</th>
<th>PANSS-P</th>
<th>PANSS-N</th>
<th>PANSS-G</th>
<th>CGI</th>
<th>ESRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI-index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trials 1–5</td>
<td>0.35</td>
<td>−0.14</td>
<td>0.19</td>
<td>0.21</td>
<td>−0.02</td>
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<tr>
<td>LI-index</td>
<td>−0.18</td>
<td>0.21</td>
<td>−0.08</td>
<td>−0.04</td>
<td>0.03</td>
</tr>
</tbody>
</table>

For PANSS measures: P = positive; N = negative; G = general.

Correlations were calculated between LI index and clinical measures of PANSS, CGI, and ESRS. As can be seen in Table 7, no associations were found (all p’s > 0.16).

### 4. Discussion

The current study was composed of two experiments with the aim of testing the hypothesis that LI is abnormally persistent in chronic schizophrenia patients. This hypothesis [5], was suggested following animal studies where systemic administration of low doses of NMDA blockers, a model of the negative symptoms of schizophrenia [25], led to LI-enhancement.

In Experiment 1 healthy subjects showed a robust LI followed by a disruption of the phenomenon. The change in performance was clearly an outcome of the number of cue–target pairings. When the number of pairings was low (trials 1–5) the subjects showed the LI effect. As the RTs of the subjects were longer to targets cued by PE letters this effect could be attributed to a reduced associability, or learning of inattention of the PE letters, as could be expected in an LI procedure. However, when the number of cue–target pairings increased (trials 6–10) the healthy subjects demonstrated an ability to overcome the initial learning of inattention and acquired the cue–target association of PE letters similar to that of the NPE letters. Thus, we demonstrated two important features of this novel within-subject visual recognition task: (1) the applicability of the task as an LI procedure; and (2) the task includes the necessary features to serve as a test of LI perseveration.

The attenuation of LI following an increased number of CS–US pairings is well documented in the animal literature. For example,
in a conditioned emotional response procedure LI is routinely observed with two tone-shock pairings, but when the number of pairings is increased to five the LI-phenomenon is attenuated (for review see Ref. [5]). However, to the best of our knowledge the effect of increased number of CS–US pairings on LI was not yet shown in human studies. The reason for lack of findings in humans is twofold. First, many of the human LI studies have applied trials to criterion procedures (an all-or-none measure) which do not allow a continuous measure of the LI phenomenon [6–8,11,15,16,21,30–32]. Second, studies which applied continuous measures (e.g., RT and GSR) focused on the disruption of LI in the pattern shown by acute schizophrenia patients, and therefore used a restricted number of CS–US pairings [10,12,13,26].

The finding of LI attenuation in healthy subjects is in line with the common expectation that LI obtained under limited and specific environmental conditions. Outcomes of animal studies have shown that LI reflects a delicate balance between the conditions of preexposure and conditioning. It is only obtained with certain combinations of number of preexposures and number of conditioning trials, and disappears when this balance is disrupted, namely, when the number of preexposures is reduced [33,34] or when the number of conditioning pairings is increased [35]. The findings with healthy subjects are an important demonstration of this idea as the LI effect was diminished with the increase in conditioning pairings. Moreover, this finding indicates a major advantage of the new LI task as it can show the shift from the preexposure effect to its attenuation within the same session and in the same subject. To date, no other human-LI procedure was able to show such unique effects.

In Experiment 2 the same task was applied to patients with chronic schizophrenia and matched control subjects. While control subjects showed the LI effect in trials 1–5, the schizophrenia patients failed to show the phenomenon. However, this pattern was reversed in trials 6–10. Control subjects showed an attenuation of LI, due to slower RTs to targets cued by PE letters, while the schizophrenia patients showed the LI effect, with slower RTs to targets cued by NPE compared to PE letters. Importantly, in trials 6–10 the patients learned the cue–target association related to NPE letters, but failed to show any learning of the association between PE letters and the target. This was indicated by slower RTs to targets cued by PE letters in trials 6–10 compared to trials 1–5. An even better demonstration of the enhancement of LI in trials 6–10 was given by the LI-index which reflects the proportions between responses to targets cued by NPE to targets cued by PE letters. Therefore, Exp 2 demonstrated that chronic schizophrenia patients showed a persistence of the LI phenomenon, failing to learn the cue–target association of PE letters throughout the trials.

In contrast to control subjects the patients showed the LI effect in a delayed manner. While control subjects showed LI in trials 1–5 the patients failed to show LI at this stage. This finding is comparable to previous reports on the performance of chronic schizophrenia patients in an L1r task [27,29]. These studies reported that the patients failed to learn the cue–target associations for both the PE and the NPE letters. As in our former study we applied a procedure with five cue–target pairings per block, and suggested that L1r might be observed in chronic patients if the number of cue–target pairings were increased [27]. Indeed, this was the case in the current study. However, the acquired cue–target association was confined to the NPE-cue, while no association between the PE-cue and the target was seen. Moreover, while a reduced RT to targets cued by NPE letters was seen from the first to the second half, the RT to PE-cued targets increased in the second half, even when compared to random target presentations. This finding is difficult to interpret, but it can suggest that the PE letters were negatively associated with the target.

The outcome of LI perseveration in chronic schizophrenia patients is the first demonstration of the phenomenon in this subpopulation. As indicated in the introduction, two studies [11,26] reported on LI facilitation in schizophrenia patients. The first study of Rascle et al. [11] used a between subjects auditory LI procedure. They indicated that chronic schizophrenia patients who were preexposed showed a delay of learning which resulted in an enhancement of LI. The finding of delayed acquisition of the preexposed stimuli is similar to the current study. However, as the study applied a between subjects design it is difficult to compare between the findings. Rascle et al. [11] reported that more than 50% of the preexposed chronic patients failed to learn the association, compared to about 15% of the non-preexposed patients. However, it is possible that the patients allocated to the PE-group had poorer attention capacities or were lower in other general cognitive abilities compared to the other patients and this might have led to the lower rate of correct trials. In contrast, in the present study the within-subject procedure enabled us to show that the deficit of learning was restricted to PE cues, and the patients acquired the cue–target association of NPE letters, although in a delayed manner. This indeed is one important advantage of the within-subjects paradigms.

The study of Cohen et al. [26] attempted to associate the predominance of negative symptoms with LI perseveration in adolescent schizophrenia patients using a within-subject, visual search task. Although Weiner [5] suggested that LI perseveration is related to high level of negative symptoms, her model refers mainly to chronic patients. Since Cohen et al. [26] tested adolescent patients with an average of 13.9 months since the onset of the first episode, these patients could not be considered chronic patients. In comparison, in the current study an inclusion criterion was a minimum of 4 years since onset in order to meet the accepted clinical criterion of chronicity. Accordingly, in the current sample the average was 22 years since onset, and the patients showed high levels of negative symptoms (mean PANSS-negative of 28.4, compared to Cohen et al.: 18.3), as well as a high negative to positive symptoms ratio. Importantly, in the present study we were able to show an association between LI-performance and clinical condition (i.e., chronic schizophrenia), but not with the level of negative symptoms per-se.

One can speculate as to possible explanations for this finding. First, it is possible that the failure to find an association between the perseveration of LI and the negative symptoms was an artifact of the cutoff range in the symptoms level of the patients, as all the patients showed high levels of negative symptoms (PANSS-negative range 22–35). Second, in line with propositions that schizophrenia has a neurodegenerative course, some reports have indicated progressive deterioration of attentional set-shifting associated with illness duration [36,37]. Although LI is not considered as set-shifting task, in the current design an intra-dimensional shift could be assumed. Thus, in the test phase the rule regarding the preexposed letters is changed and the subject is required to shift between the acquired rule of novel letters to the rule of preexposed letters. Interestingly, a reduced set-shifting ability was associated in both humans and other species with a reduced fronto-striatal dopaminergic activity, and in patients with schizophrenia was interpreted as a likely outcome of drug therapy (for review see Ref. [38]).

Finally, the difference between control subjects in the two experiments reported here should be noted. The pattern of control subjects in Exps 1 and 2 resembled in trials 1–5, both that showing the LI effect. However, the attenuation of LI in trials 6–10 of control subjects in Exp 1 was not as robust as in Exp 1. Two aspects of the response pattern of the control subjects in Exp 2 contributed to this effect. First, in trials 6–10 the subjects showed faster RTs to targets cued by NPE letters compared to the RTs in trials 1–5. Second, the reduction of RTs to targets cued by PE letters was lesser com-
pared to control subjects in Exp 1. Both of these aspects could be attributed to slower learning of the association between NPE letters and the target and in control subjects in Exp 2 compared to the healthy volunteers in Exp 1. The slower learning led to further learning of the association between NPE letters and the target in trials 6–10, and this might have led to reduced attention span that could be directed to the association between PE letters and the target. This explanation is reasonable if we take into account the fact that Exp 1 used subjects as subjects, while in Exp 2 the control subjects were hospital personnel, groups that differ in important aspects such as age and education. In addition, it is possible that hospital’s personnel were less concentrated on the task because they were more preoccupied by their work.

In sum, the present study used a novel within-subject visual recognition LI procedure which was shown to be efficient in the testing of LI perseveration. Under conditions where control subjects attenuated their LI expression, chronic schizophrenia patients persistently showed LI. This difference was due to the acquisition of the association between PE cues and the target with the increased number of cue–target pairings among controls, while there was no such learning among patients. This implies that the new procedure is an efficient tool to investigate the perseveration of LI in chronic schizophrenia patients, as well as other clinical populations which might show a similar learning pattern. This is an important demonstration of the proposition that the abnormality of the LI effect in schizophrenia is related not only to the disruption of LI but also to the perseveration of the LI phenomenon.

References