

Towards an animal model of an antipsychotic drug-resistant cognitive impairment in schizophrenia: scopolamine induces abnormally persistent latent inhibition, which can be reversed by cognitive enhancers but not by antipsychotic drugs

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Abstract

Schizophrenia symptoms segregate into positive, negative and cognitive, which exhibit differential sensitivity to drugs. Recent efforts to identify treatments targeting cognitive impairments in schizophrenia have directed attention to the cholinergic system for its well documented role in cognition. Relatedly, muscarinic antagonists (e.g. scopolamine) produce an 'antimuscarinic syndrome', characterized by psychosis and cognitive impairments. Latent inhibition (LI) is the poorer conditioning to a stimulus resulting from its non-reinforced pre-exposure. LI indexes the ability to ignore irrelevant stimuli and aberrations of this capacity produced by pro-psychotic agents (e.g. amphetamine, MK-801) are used extensively to model attentional impairments in schizophrenia. We recently showed that LI was disrupted by scopolamine at low doses, and this was reversed by typical and atypical antipsychotic drugs (APDs) and the acetylcholinesterase inhibitor physostigmine. Here, at a higher dose (1.5 mg/kg), scopolamine produced an opposite pole of attentional impairment, namely, attentional perseveration, whereby scopolamine-treated rats persisted in expressing LI under strong conditioning that prevented LI expression in controls. Scopolamine-induced persistent LI was reversed by cholinergic and glycinergic cognitive enhancers (physostigmine and glycine) but was resistant to both typical and atypical APDs (haloperidol and clozapine). The latter sets scopolamine-induced persistent LI apart from scopolamine- and amphetamine-induced disrupted LI, which are reversed by both typical and atypical APDs, as well as from other cases of abnormally persistent LI including MK-801-induced persistent LI, which is reversed by atypical APDs. Thus, scopolamine-induced persistent LI may provide a pharmacological LI model for screening cognitive enhancers that are efficient for the treatment of APD-resistant cognitive impairments in schizophrenia.

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Introduction

Schizophrenia symptoms are commonly divided into positive, negative and cognitive (Bell and Mishara, 2006; Tamminga et al., 1998). Two classes of psychotomimetics have been typically used to model these symptoms in animals: dopamine agonists like amphetamine, which produce and exacerbate positive

symptoms (Angrist et al., 1974; Snyder, 1973) and NMDA antagonists like phencyclidine or MK-801, which produce and exacerbate also negative and cognitive symptoms (Javitt and Zukin, 1991; Lahti et al., 2001). Although muscarinic antagonists (e.g. scopolamine, atropine) produce a schizophrenia-like syndrome ('antimuscarinic syndrome') in humans, which includes both positive symptoms and cognitive impairments (Clarke et al., 2004; Fisher, 1991; Marchlewski, 1994; Minzenberg et al., 2004; Perry et al., 1978; Yeomans, 1995), as well as psychotic-like effects in animal models of schizophrenia (Jones et al.,

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2005; Mathur et al., 1997; Shannon and Peters, 1990; Sipos et al., 1999; Ukai et al., 2004), the cholinergic system has received less attention in schizophrenia-related research. Recent efforts to identify treatments targeting cognitive impairments in schizophrenia (see Marder and Fenton, 2004) have directed attention to the cholinergic system because of its well known role in cognition (Dean et al., 2003; Everitt and Robbins, 1997; Kozak et al., 2007; Raedler et al., 2007; Sarter et al., 2003, 2005).

We have recently shown (Barak and Weiner, 2007) in rats that scopolamine disrupts the capacity to ignore irrelevant stimuli as manifested in disrupted latent inhibition (LI). LI is one of the best documented cross-species manifestations of attentional selectivity in associative learning (Lubow, 1989; Mackintosh, 1975), whereby repeated non-reinforced pre-exposure to the to-be-conditioned stimulus interferes with its subsequent efficacy to generate conditioned response. Amphetamine-induced disruption of LI and its reversal by typical and atypical antipsychotic drugs (APDs) is considered to model the inability to ignore irrelevant stimuli associated with positive symptoms of schizophrenia (Weiner, 2003). Conversely, MK-801- or phencyclidine-treated rodents persist in expressing LI under conditions that prevent/reduce LI expression in untreated rats (Gaisler-Salomon et al., 2008; Gaisler-Salomon and Weiner, 2003; Lipina et al., 2005; Palsson et al., 2005) and this has been suggested to model attentional perseveration associated with negative/cognitive symptoms of schizophrenia (Gaisler-Salomon et al., 2008). Persistent LI is reversed by atypical but not typical APDs and by glycinergic NMDA enhancers, consistent with the differential efficacy of these treatments in improving negative/cognitive symptoms (Harvey et al., 2005; Heresco-Levy et al., 2005; Lane et al., 2005; Moller, 2003).

We showed (Barak and Weiner, 2007) that low doses of scopolamine (0.15, 0.5 mg/kg) led to LI disruption, and this was reversed by both typical and atypical APDs and by the acetylcholinesterase (AChE) inhibitor physostigmine, as found with muscarinic antagonist-induced pro-psychotic effects in humans (Brown et al., 2004; Gopel et al., 2002; Granacher and Baldessarini, 1975) and animals (Hohnadel et al., 2007; Jones et al., 2005). Based on this behavioural and pharmacological profile, we suggested that scopolamine-induced LI disruption might model the positive spectrum of the antimuscarinic syndrome. In that same study (Barak and Weiner, 2007) we found that at a higher dose (1 mg/kg), scopolamine spared LI. Although this finding could simply suggest that LI is resistant to high doses of scopolamine, the fact that

scopolamine can produce perseverative behaviours (Chen et al., 2004; Ragazzino et al., 2002; Soffie and Lamberty, 1987) has led us to test the possibility that at higher doses of scopolamine, rats would not only show LI under conditions yielding LI in no-drug controls, but persist in showing LI under conditions preventing LI expression in controls. Of particular interest was the question of whether the pharmacological profile of such scopolamine-induced persistent LI would resemble that of scopolamine-induced disrupted LI, reflecting the common neurotransmitter dysfunction underlying these two LI aberrations, or that of MK-801-induced persistent LI, reflecting their common behavioural manifestation/cognitive deficit (persistent LI/attentional perseveration). We expected that scopolamine-induced persistent LI would be reversed by physostigmine like scopolamine-induced disrupted LI, but would exhibit a different profile with regard to APDs in that it would be reversed by clozapine but not by haloperidol, as has been shown for MK-801-induced persistent LI (Gaisler-Salomon and Weiner, 2003). In addition, it was also expected that scopolamine-induced persistent LI, like MK-801-induced persistent LI, would be reversed by glycine (Gaisler-Salomon et al., 2008). Finally, our previous studies showed that scopolamine and MK-801 acted to disrupt and induce persistent LI at different stages of the LI procedure, namely, pre-exposure and conditioning, respectively, presumably reflecting the targeting of different psychological processes (Barak and Weiner, 2007; Gaisler-Salomon et al., 2008). Therefore, in the present study we were also interested in determining whether scopolamine-induced persistent LI would stem from scopolamine action in the pre-exposure or the conditioning stage.

Specifically, we tested the effects of 1.5 mg/kg scopolamine on LI, using task parameters that led to LI (weak conditioning) or prevented the expression of LI (strong conditioning) in control rats, allowing the demonstration of LI disruption and LI persistence, respectively, in drug-treated rats. Having shown that scopolamine induces abnormally persistent LI, we tested whether this would be reversed by the typical APD haloperidol, the atypical APD clozapine, the NMDA co-agonist glycine, and the AChE inhibitor physostigmine.

Materials and methods

Subjects

Male Wistar rats aged 3–4 months and weighing 320–520 g (Tel-Aviv University Medical School,

Tel-Aviv), were housed four to a cage under a reversed 12-h light/dark cycle (lights on 19:00 hours) with food and water ad libitum except for the duration of the LI experiments. All experimental protocols conformed to the guidelines of the Institutional Animal Care and Use Committee of Tel-Aviv University, Israel, and to the guidelines of the NIH (animal welfare assurance no, A5010-01). All efforts were made to minimize the number of animals used and their suffering.

Apparatus and procedure

LI was measured in a thirst-motivated conditioned emotional response (CER) procedure using Campden Instruments (Loughborough, UK) rodent test chambers with a retractable bottle, each enclosed in a ventilated sound-attenuating chest. When the bottle was not present, the hole was covered with a metal lid. The pre-exposed to-be-conditioned stimulus was a 10 s, 80 dB, 2.8 kHz tone produced by a Sonalert module (model SC 628). Shock was supplied through the floor by a Campden Instruments shock generator and shock scrambler set at 0.5 mA intensity and 1 s duration. Licks were detected by a Campden Instruments drinkometer. Equipment programming and data recording were computer controlled.

Ten days prior to the beginning of the LI procedure, rats were put on a 23 h water restriction schedule and handled for about 2 min daily for 5 d. On the next 5 d, rats were trained to drink in the experimental chamber, 15 min/d. Water in the test apparatus was given in addition to the daily ration of 1 h given in the home cages. The LI procedure was conducted on days 11–14 and consisted of four stages given 24 h apart.

Pre-exposure

With the bottle removed, the pre-exposed (PE) rats received 40 tone presentations with an inter-stimulus interval of 40 s. The non-pre-exposed (NPE) rats were confined to the chamber for an identical period of time without receiving the tone.

Conditioning

With the bottle removed, rats received two (weak conditioning, expt 1) or five (strong conditioning, expts 1–7) tone-shock pairings given 5 min apart. Shock immediately followed tone termination.

Rebaseline

Rats were given a 15-min drinking session as in initial training.

Test

Each rat was placed in the chamber and allowed to drink from the bottle. When the rat completed 75 licks the tone was presented for 5 min. The following times were recorded: Time to first lick, time to complete licks 1–50, time to complete licks 51–75 (before tone onset) and time to complete licks 76–100 (after tone onset). Times to complete licks 76–100 were submitted to logarithmic transformation to allow parametric ANOVA. Longer log times indicate stronger suppression of drinking. LI is defined as significantly shorter log times to complete licks 76–100 of the PE compared NPE rats.

Drugs

All drugs were administered intraperitoneally in a volume of 1 ml/kg, except for glycine, which was administered in a volume of 3 ml/kg. Scopolamine HBr (1.5 mg/kg; Sigma, Israel), glycine (800 mg/kg; Sigma) and physostigmine (eserine) hemisulfate (0.05 or 0.15 mg/kg; Sigma) were diluted in saline. Haloperidol (0.1 or 0.2 mg/kg; Johnson & Johnson, Belgium) was prepared from an ampoule containing 5 mg haloperidol in 1 ml solvent containing 6 mg lactic acid and diluted with saline. Clozapine (5 or 10 mg/kg; Novartis, Switzerland) was dissolved in 1 N acetic acid (1.5 ml/10 mg) and diluted with saline (final pH 5.5). The dose of scopolamine was chosen on the basis of studies showing that it did not disrupt tone-shock conditioning (Anagnostaras et al., 1999). The doses of the other drugs were chosen based on our previous LI studies and other behavioural experiments with these drugs (haloperidol and clozapine: Barak and Weiner, 2007; Weiner et al., 1997; physostigmine: Barak and Weiner, 2007; Jones and Shannon, 2000; glycine: Gaisler-Salomon et al., 2008). In all the experiments, the drugs were injected prior to both the pre-exposure and conditioning stages, except for expt 2, in which scopolamine was administered either in the pre-exposure or in the conditioning stage and expt 5 in which clozapine was administered either in the pre-exposure or in the conditioning stage. Injection-session interval was 60 min for haloperidol and 30 min for all the other drugs. No-drug controls received the corresponding vehicle. In expts 3 and 4 about half of no-drug controls received the vehicle of clozapine and the other half the vehicle of haloperidol.

Data analysis

Times to complete licks 51–75 and mean log times to complete licks 76–100 were analysed using two-way

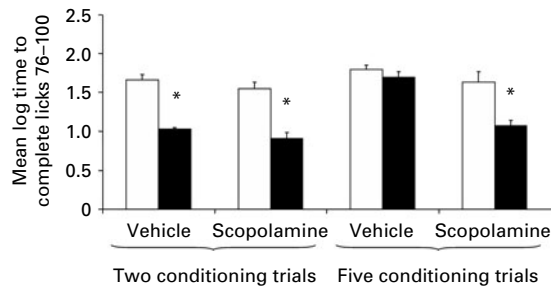


Figure 1. Effects of 1.5 mg/kg scopolamine on latent inhibition (LI) with weak or strong conditioning. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (■, PE) and non-pre-exposed (□, NPE) rats injected with vehicle or scopolamine (1.5 mg/kg) that underwent conditioning with two (weak conditioning) or five (strong conditioning) trials. Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

(expts 2 and 5) or three-way (expts 1, 3, 4, 6 and 7) ANOVAs with main factors of pre-exposure and drug condition/s (and number of tone-shock pairings in expt 1). LSD post-hoc comparisons were used to assess the difference between the PE and NPE groups within each treatment condition.

Results

Expt 1: effects of 1.5 mg/kg scopolamine on LI with weak or strong conditioning

The experiment included eight experimental groups in a $2 \times 2 \times 2$ design with main factors of pre-exposure (0, 40), number of conditioning trials (2, 5) and treatment (vehicle, 1.5 mg/kg scopolamine). There were six animals per group; data of one rat from the PE-vehicle group and one rat from PE-scopolamine group that received two conditioning trials were excluded from the analysis, leaving five animals in these two groups.

The eight experimental groups did not differ in their times to complete licks 51–75 before tone onset (A period: all p values > 0.05 , overall mean A period 8.13 s). Figure 1 presents the mean log times to complete licks 76–100 (after tone onset) of the different experimental groups. As can be seen, with two conditioning trials LI was present in both vehicle- and scopolamine-treated rats. In contrast, with five conditioning trials LI was absent in vehicle-treated rats, as expected under conditions of strong conditioning, but scopolamine-treated rats persisted in showing LI. ANOVA yielded significant main effects of pre-exposure ($F_{1,38} = 71.73$, $p < 0.0001$), number of conditioning trials ($F_{1,38} = 21.18$,

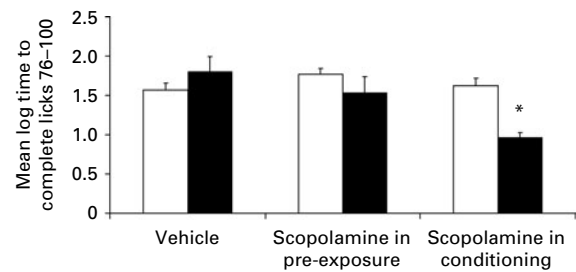


Figure 2. Effects of 1.5 mg/kg scopolamine administered in pre-exposure or conditioning on latent inhibition (LI) with strong conditioning. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (■, PE) and non-pre-exposed (□, NPE) vehicle- or scopolamine- (1.5 mg/kg) injected rats. Scopolamine was injected either in the pre-exposure stage or in the conditioning stage. Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

$p < 0.0001$), and treatment ($F_{1,38} = 19.85$, $p < 0.0001$), and significant interactions of pre-exposure \times number of conditioning trials ($F_{1,38} = 7.15$, $p < 0.05$), number of conditioning trials \times treatment ($F_{1,38} = 6.02$, $p < 0.05$) and pre-exposure \times number of conditioning trials \times treatment ($F_{1,38} = 4.21$, $p < 0.05$) as well as a nearly significant interaction of pre-exposure \times treatment ($F_{1,38} = 4.06$, $p = 0.051$). Post-hoc comparisons revealed a significant difference between the PE and NPE groups (i.e. presence of LI) in vehicle- and scopolamine-injected rats that received two conditioning trials (p values < 0.001), as well as in scopolamine-injected rats that received five conditioning trials ($p < 0.005$) but not in vehicle-treated rats that received five conditioning trials.

Expt 2: effects of 1.5 mg/kg scopolamine administered in pre-exposure or conditioning on LI with strong conditioning

The experiment included six experimental groups in a 2×3 design with main factors of pre-exposure (0, 40) and treatment (vehicle, scopolamine in pre-exposure, scopolamine in conditioning). There were eight animals per group except for NPE-scopolamine in pre-exposure group ($n = 7$), and the PE-scopolamine in pre-exposure and NPE-vehicle groups ($n = 6$).

The six experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p values > 0.05 , overall mean A period 7.33 s). Figure 2 presents the mean log times to complete licks 76–100 (after tone onset) of the different experimental groups. As can be seen, LI was absent in vehicle-treated rats as well as in rats that received scopolamine in the pre-exposure stage, but present in rats that received

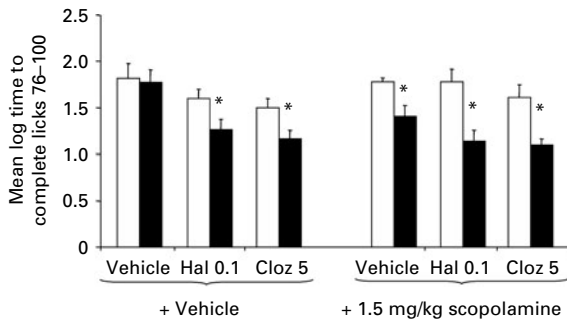


Figure 3. Effects of 0.1 mg/kg haloperidol or 5 mg/kg clozapine on scopolamine-induced latent inhibition (LI) persistence. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (■, PE) and non-pre-exposed (□, NPE) vehicle- or scopolamine- (1.5 mg/kg) injected rats, pre-treated with vehicle, haloperidol (Hal; 0.1 mg/kg) or clozapine (Cloz; 5 mg/kg). Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

scopolamine in the conditioning stage. ANOVA yielded a significant main effect of treatment ($F_{2,37} = 5.75$, $p < 0.01$) and a nearly significant main effect of pre-exposure ($F_{1,37} = 4.02$, $p = 0.052$), as well as a significant interaction pre-exposure \times treatment ($F_{2,37} = 5.96$, $p < 0.01$). Post-hoc comparisons revealed a significant difference between the PE and NPE groups (i.e. presence of LI) in rats that were injected with scopolamine in conditioning ($p < 0.001$), but not in rats that were treated with vehicle or scopolamine in pre-exposure.

Expt 3: effects of 0.1 mg/kg haloperidol or 5 mg/kg clozapine on scopolamine-induced LI persistence

The experiment included 12 experimental groups in a $2 \times 2 \times 3$ design with main factors of pre-exposure (0, 40), treatment (vehicle, 1.5 mg/kg scopolamine) and pre-treatment (vehicle, 0.1 mg/kg haloperidol, 5 mg/kg clozapine). There were eight animals per group except for the NPE-scopolamine-clozapine ($n = 7$) and PE-scopolamine-haloperidol ($n = 7$) groups.

The 12 experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p values > 0.05 , overall mean A period 6.86 s). Figure 3 presents the mean log times to complete licks 76–100 (after tone onset) of the different experimental groups. As expected with strong conditioning, LI was absent in vehicle-treated rats, but rats that received scopolamine persisted in showing LI, replicating the results of expt 1. Both haloperidol and clozapine led to LI in vehicle-treated rats, replicating the well documented LI potentiating effect of these drugs (Christison et al.,

1988; Dunn et al., 1993; Shadach et al., 2000; Weiner et al., 1987, 1997), but both drugs were without an effect in rats that received scopolamine; in other words, both APDs failed to reverse scopolamine-induced LI persistence. ANOVA yielded significant main effects of pre-exposure ($F_{1,82} = 31.93$, $p < 0.0001$), and pre-treatment ($F_{2,82} = 10.13$, $p < 0.0005$), and a significant interaction of pre-exposure \times treatment ($F_{1,82} = 4.22$, $p < 0.05$). Post-hoc comparisons revealed a significant difference between the PE and NPE groups (i.e. presence of LI) in rats that were injected with scopolamine, haloperidol or clozapine (p values < 0.05), scopolamine + clozapine ($p < 0.005$) and scopolamine + haloperidol ($p < 0.0005$), but not in vehicle-treated rats.

Experiment 4: effects of 0.2 mg/kg haloperidol or 10 mg/kg clozapine on scopolamine-induced LI persistence

Unlike MK-801-induced persistent LI which was shown to be reversible by clozapine but not haloperidol (Gaisler-Salomon and Weiner, 2003), scopolamine-induced persistent LI remained unaffected by both drugs. Consequently, in the present study we tried to obtain such differentiation by raising the dose of both APDs. The experiment included 12 experimental groups in a $2 \times 2 \times 3$ design with main factors of pre-exposure (0, 40), treatment (vehicle, 1.5 mg/kg scopolamine) and pre-treatment (vehicle, 0.2 mg/kg haloperidol, 10 mg/kg clozapine). There were eight animals per group except for the PE-vehicle-vehicle group ($n = 7$).

The 12 experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p values > 0.05 , overall mean A period 7.38 s). Figure 4 presents the mean log times to complete licks 76–100 (after tone onset) of the different experimental groups. As can be seen, LI was absent in vehicle-treated rats but present in rats that received scopolamine. Both haloperidol and clozapine led to LI in vehicle-treated rats, but clozapine reduced suppression in both the PE and the NPE rats and led to a much smaller LI effect. Scopolamine-treated rats given haloperidol continued to show LI, whereas no LI was seen in rats that received scopolamine and clozapine. However, loss of LI in the latter group was due to reduced suppression in the NPE group rather than better learning in the PE group as ANOVA yielded significant main effects of pre-exposure ($F_{1,83} = 46.86$, $p < 0.0001$), treatment ($F_{1,83} = 18.12$, $p < 0.0001$) and pre-treatment ($F_{2,83} = 37.06$, $p < 0.0001$), and significant interactions of pre-exposure \times pre-treatment ($F_{2,83} = 4.22$, $p < 0.01$) and pre-exposure \times treatment \times pre-treatment ($F_{2,83} = 3.54$,

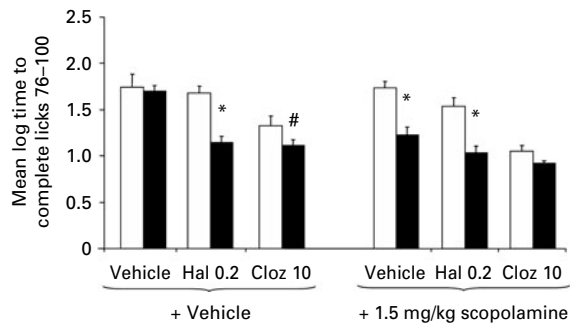


Figure 4. Effects of 0.2 mg/kg haloperidol or 10 mg/kg clozapine on scopolamine-induced latent inhibition (LI) persistence. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (■, PE) and non-pre-exposed (□, NPE) vehicle- or scopolamine- (1.5 mg/kg) injected rats, pre-treated with vehicle, haloperidol (Hal; 0.2 mg/kg) or clozapine (Cloz; 10 mg/kg). Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI; # indicates a marginally significant difference between the PE and NPE groups.

$p < 0.05$). Post-hoc comparisons revealed a significant difference between the PE and NPE groups (i.e. presence of LI) in rats that were injected with scopolamine, haloperidol and scopolamine + haloperidol (p values < 0.0001), and a marginal significance in clozapine-treated rats ($p = 0.068$), but not in rats that were treated with vehicle or scopolamine + clozapine.

Expt 5: effects of 5 mg/kg clozapine administered in pre-exposure or conditioning on scopolamine-induced LI persistence

We previously showed that reversal of MK-801-induced persistent LI by atypical APDs is due to their 5-HT_{2A} antagonistic action at the pre-exposure stage, and moreover, that these drugs are more effective if their administration is confined to pre-exposure (Gaisler-Salomon and Weiner, 2003). Therefore, this experiment tested whether clozapine would reverse scopolamine-induced persistent LI if injected only in the pre-exposure stage. We used the 5 mg/kg rather than the 10 mg/kg dose because the former was shown to be effective, when given in pre-exposure only, in reversing MK-801- and lesion-induced persistent LI (Gaisler-Salomon and Weiner, 2003; Schiller et al., 2006), and in terms of 5-HT_{2A} antagonism, they are equivalent (Schotte et al., 1996). The experiment included eight experimental groups in a 2×4 design with main factors of pre-exposure (0, 40), and treatment (vehicle, 1.5 mg/kg scopolamine, scopolamine + 5 mg/kg clozapine in pre-exposure, scopolamine +

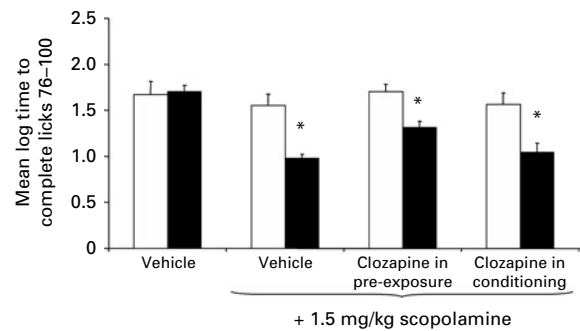


Figure 5. Effects of 5 mg/kg clozapine administered separately in pre-exposure or conditioning on scopolamine-induced abnormally persistent latent inhibition (LI). Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (■, PE) and non-pre-exposed (□, NPE) rats in four drug conditions: vehicle, scopolamine (1.5 mg/kg), scopolamine + clozapine (5 mg/kg) in pre-exposure, scopolamine + clozapine in conditioning. Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

clozapine in conditioning). There were six animals per group except for the PE-scopolamine-vehicle, the NPE-vehicle-vehicle and the NPE-scopolamine-clozapine in pre-exposure groups (all $n = 5$).

The eight experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p values > 0.05 , overall mean A period 7.59 s). Figure 5 presents the mean log times to complete licks 76–100 (after tone onset) of the different experimental groups. As can be seen, LI was absent in vehicle-treated rats, whereas rats that received scopolamine persisted in showing LI. Clozapine, regardless of the stage at which it was administered, failed to reverse scopolamine-induced persistent LI. ANOVA yielded a significant main effect of pre-exposure ($F_{1,37} = 27.18$, $p < 0.0001$) and treatment ($F_{3,37} = 7.74$, $p < 0.0005$), as well as a significant interaction pre-exposure \times treatment ($F_{1,37} = 3.87$, $p < 0.02$). Post-hoc comparisons revealed a significant difference between the PE and NPE groups (i.e. presence of LI) in rats that were injected with scopolamine ($p < 0.0005$), scopolamine + clozapine in pre-exposure ($p < 0.01$) and scopolamine + clozapine in conditioning ($p < 0.001$), but not in vehicle-treated rats.

Expt 6: effects of 800 mg/kg glycine on scopolamine-induced LI persistence

The experiment included eight experimental groups in a $2 \times 2 \times 2$ design with main factors of pre-exposure (0, 40), treatment (vehicle, 1.5 mg/kg scopolamine) and pre-treatment (vehicle, 800 mg/kg glycine). There

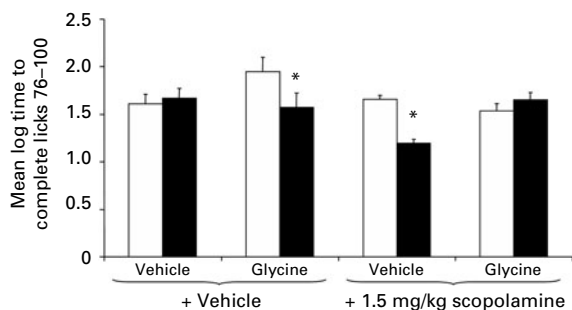


Figure 6. Effects of 800 mg/kg glycine on scopolamine-induced abnormally persistent latent inhibition (LI). Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (■, PE) and non-pre-exposed (□, NPE) vehicle- or scopolamine- (1.5 mg/kg) treated rats, pre-treated with glycine (800 mg/kg). Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

were six animals per group except for the PE-vehicle-glycine group ($n=5$).

The eight experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p values >0.05 , overall mean A period 6.91 s). Figure 6 presents the mean log times to complete licks 76–100 (after tone onset) of the different experimental groups. As can be seen, LI was absent in vehicle-treated rats, but present in rats that received scopolamine. LI was present in rats that received glycine on its own, as shown previously with glycinergic compounds (Lipina et al., 2005), but not in rats that were administered both scopolamine and glycine, i.e. glycine reversed scopolamine-induced LI persistence. ANOVA yielded a significant main effect of pre-treatment ($F_{1,39}=5.19$, $p<0.05$) and a nearly significant main effect of pre-exposure ($F_{1,39}=3.93$, $p=0.055$), as well as a significant interaction pre-exposure \times treatment \times pre-treatment ($F_{1,39}=9.06$, $p<0.005$). Post-hoc comparisons revealed a significant difference between the PE and NPE groups (i.e. presence of LI) in rats that were injected with scopolamine ($p<0.001$), and glycine ($p<0.05$), but not in rats that were treated with vehicle or scopolamine + glycine.

Expt 7: effects of 0.05 or 0.15 mg/kg physostigmine on scopolamine-induced LI persistence

The experiment included 12 experimental groups in a $2 \times 2 \times 3$ design with main factors of pre-exposure (0, 40), treatment (vehicle, 1.5 mg/kg scopolamine) and pre-treatment (0, 0.05, 0.15 mg/kg physostigmine), and was run in three replications. There were eight animals per group except for the PE-vehicle-0.05 mg/kg

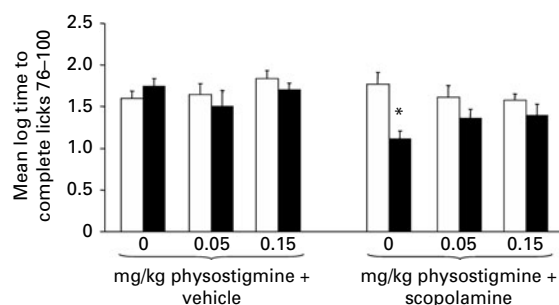


Figure 7. Effects of 0.05 or 0.15 mg/kg physostigmine on scopolamine-induced persistent latent inhibition (LI). Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (■, PE) and non-pre-exposed (□, NPE) vehicle- or scopolamine- (1.5 mg/kg) treated rats, pre-treated with physostigmine (0.05 mg/kg, 0.15 mg/kg). Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

physostigmine, the PE-vehicle-0.15 mg/kg physostigmine, the PE-scopolamine-0.05 mg/kg physostigmine, the NPE-vehicle-scopolamine and the NPE-vehicle-vehicle groups (all $n=7$).

The 12 experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p values >0.05 , overall mean A period 8.36 s). Figure 7 presents the mean log times to complete licks 76–100 (after tone onset) of the different experimental groups. As can be seen, LI was absent in vehicle-treated rats, whereas rats that received scopolamine persisted in showing LI. Physostigmine at both doses had no effect on its own, but reversed scopolamine-induced persistent LI. ANOVA yielded significant main effects of pre-exposure ($F_{1,79}=9.29$, $p<0.005$) and treatment ($F_{1,79}=8.79$, $p<0.005$), and a significant interaction of pre-exposure \times treatment ($F_{2,79}=5.61$, $p<0.025$), as well as a significant interaction of pre-exposure \times treatment \times pre-treatment ($F_{2,79}=3.15$, $p<0.05$). Post-hoc comparisons revealed a significant difference between the PE and NPE groups (i.e. presence of LI) in the rats that received scopolamine ($p<0.001$), but not in all the other groups.

Discussion

The present experiments demonstrated that the administration of scopolamine led to the persistence of LI under conditions that abolished its expression in non-treated animals. This LI aberration was reversed by cognitive enhancers, namely, the NMDA allosteric agonist glycine and the AChE inhibitor physostigmine, but was resistant to both the typical and the atypical APDs, haloperidol and clozapine,

respectively. This pharmacological profile sets scopolamine-induced LI persistence apart from both MK-801-induced LI persistence and scopolamine-induced LI disruption.

Expt 1 showed that under conditions that led to LI in controls (40 pre-exposures and two conditioning trials), rats treated with 1.5 mg/kg scopolamine showed intact LI, extending our previous finding of spared LI at 1 mg/kg scopolamine (Barak and Weiner, 2007). Furthermore, rats that were treated here with 1.5 mg/kg scopolamine persisted in expressing LI when the number of conditioning trials was raised to five. Thus, while vehicle-treated PE rats that received five tone-shock pairings following pre-exposure, showed levels of suppression comparable to those of their NPE counterparts, the administration of scopolamine led to the emergence of LI, i.e. lower suppression of the PE compared NPE rats. Importantly, scopolamine reduced suppression in the PE groups without concomitantly reducing suppression in the NPE groups, indicating that LI persistence was not due to impaired conditioning per se. Indeed, because scopolamine is known to impair associative learning (see Anagnostaras et al., 1995, 1999; Tinsley et al., 2004), doses of the drug that do not impair conditioning in the NPE animals are imperative for manifestation of persistent LI, since poorer conditioning of the PE compared to NPE rats cannot be manifested if the drug also reduces conditioning in the NPE group. Moreover, expt 2 showed that scopolamine was ineffective when given in pre-exposure but led to the emergence of LI when given in conditioning.

The present results, taken together with our previous findings that LI was disrupted by 0.15 and 0.5 mg/kg (Barak and Weiner, 2007) show that scopolamine can abolish LI or induce persistent LI as a function of dose. In terms of psychological processes underlying LI, it is believed that during pre-exposure, the acquisition of an association between the pre-exposed stimulus and the absence of a significant consequence results in the development of inattention to the stimulus, which inhibits the acquisition and/or the expression of the conditioned response (Bouton, 1993; Lubow, 1989; Lubow and Kaplan, 2005; Mackintosh, 1975; Weiner, 2003). Strong conditioning overrides the inhibitory influence of the inattentive response so that animals switch to respond according to the more recent stimulus-reinforcement relationship (Weiner, 1990, 2003). Thus, scopolamine produces opposite poles of impairment in attentional selectivity: at low doses it impairs the capacity to inattend to irrelevant stimuli, whereas at a higher dose it impairs the capacity to re-attend to irrelevant stimuli when

they become relevant through pairings with reinforcement. It should be evident, however, that both disruption and persistence of LI can stem from drug action in pre-exposure (impairment or facilitation, respectively, of learned inattention), or in conditioning (facilitation or impairment, respectively, of switching to respond according to stimulus-reinforcement association). We have previously shown, by confining drug administration to the pre-exposure or the conditioning stage, that LI disruption by low doses of scopolamine is due to the action of the drug in the pre-exposure stage, and thus presumably reflects impaired acquisition of inattention (Barak and Weiner, 2007). Conversely, in the present study we show that the higher dose acts to induce persistent LI in the conditioning stage. The fact that high scopolamine dose impaired performance selectively in the PE group, and that this action is exerted in the conditioning stage when the previously non-reinforced stimulus is followed by reinforcement, implies that this dose does not affect the acquisition of inattention in the pre-exposure stage but rather specifically impairs the process of updating/adjusting the response to the stimulus-reinforcement contingency in the conditioning stage. While conditioning based action of high scopolamine dose contrasts with site of action of low doses, it corresponds to the stage at which MK-801 produces persistent LI (Gaisler-Salomon and Weiner, 2003).

The neural mechanisms underlying the dose-dependent contrasting effects of scopolamine on LI remain to be determined, but they may stem from dose-dependent effects of scopolamine on cholinergic transmission and/or blockade of muscarinic receptors. Thus, scopolamine at a dose of 1.5 mg/kg has been shown to cause a threefold increase in ACh levels in the medial prefrontal cortex, whereas a dose of 0.16 mg/kg caused only a modest increase (Ichikawa et al., 2002), possibly by dose-dependent preferred effects on different muscarinic receptor subtypes, e.g. excitatory vs. inhibitory. Of particular relevance to the present context, Hasselmo and McGaughy (2004) have suggested that high levels of cortical ACh enhance attention to new external stimuli while concomitantly suppressing interference from internal signals, possibly reflecting previous experience with such stimuli, or other types of 'internal noise'. Enhanced attention is suggested to result from two simultaneous actions of ACh: augmentation of afferent cortical input through nicotinic receptors; and suppression of feedback from intrinsic cortical fibres by inhibition of glutamate release via muscarinic presynaptic receptor (Hasselmo, 2006). Extensive blockade of muscarinic receptors by scopolamine can be

expected to spare the nicotinic-mediated enhancement of attention, while disrupting the muscarinic-mediated suppression of interference by previous information. In LI this may lead to an augmented interference of previous experience with the stimulus, i.e. the stimulus–no event association acquired in pre-exposure with the subsequent expression/retrieval of the stimulus–reinforcement association, and thus lead to persistent LI. It is of note that enhanced LI was also found in muscarinic M₅ receptor mutant mice (Wang et al., 2004), raising the possibility that the above-mentioned muscarinic-based process is mediated via M₅ receptor blockade.

It should also be noted that nicotine and nicotinic agonists were reported to augment LI (Gould et al., 2001; Rochford et al., 1996), an effect that was reversed by nicotinic antagonists (Rochford et al., 1996), as well as to disrupt LI (Joseph et al., 1993; Moran et al., 1996; Rochford et al., 1996). While the latter effect is probably mediated via nicotinic-mediated enhancement of dopaminergic transmission akin to that of amphetamine (Joseph et al., 1993; Rochford et al., 1996), LI augmentation is believed to reflect a direct activation of nicotinic receptors (Rochford et al., 1996), or nicotinic modulation of other neurotransmitters (Gould et al., 2001). Importantly, the LI augmenting effect was found when nicotine was confined to the pre-exposure stage (Gould et al., 2001; Rochford et al., 1996), suggesting that nicotinic activation indeed facilitates the acquisition of inattention to irrelevant stimuli, as suggested here based on Hasselmo and McGaughy's model. More difficult to explain is the finding that nicotine can both disrupt and augment LI via conditioning (Rochford et al., 1996). Moreover, recently it has been argued that nicotinic effects on attention may be mediated via α_7 nicotinic receptors with particular relevance to the pathophysiology of schizophrenia (e.g. Levin et al., 2006; Martin and Freedman, 2007), and we have indeed found that α_7 nicotinic agonist induces persistent LI (S. Barak et al., unpublished observations), suggesting that the pro-attentional effects of nicotine on LI are mediated via the α_7 receptor. This was strengthened by the finding that knock-out mice lacking the β_2 subtype of nicotinic receptor showed intact LI (Caldarone et al., 2000).

As stated in the Introduction, we expected that scopolamine-induced persistent LI would share with scopolamine-induced LI disruption pharmacological sensitivity to physostigmine, but would resemble the profile of MK-801-induced persistent LI with regard to APDs and glycine, and thus be reversed by clozapine and glycine but not haloperidol (Gaisler-Salomon

et al., 2008; Gaisler-Salomon and Weiner, 2003). Scopolamine-induced persistent LI was indeed reversed by physostigmine, in line with our and others' reports of this drug's effectiveness in reversing cognitive deficits induced by muscarinic blockade (Barak and Weiner, 2007; Carnicella et al., 2005; Hironaka and Ando, 1996). Similarly, as expected, scopolamine-induced persistent LI was reversed by glycine. This is consistent with other reports that glycinergic agonists reverse scopolamine-induced cognitive impairments (Fishkin et al., 1993; Matsuoka and Aigner, 1996; Ohno and Watanabe, 1996; Sirvio et al., 1992; but see Viu et al., 2000). Interestingly, glycine administration in vehicle-treated animals led to the emergence of LI through acting on NPE animals, whereas in scopolamine-treated animals, glycine administration led to the disappearance of LI through acting on PE animals (see Figure 3). It thus appears that glycine treatment had opposite effects on associative learning (enhancement) and/or the capacity to ignore irrelevant stimuli (disruption) depending on the previous treatment history with scopolamine. However, it should be borne in mind that scopolamine-treated rats persist in ignoring stimuli under conditions in which normal rats treat them as relevant, and it is the latter capacity that is restored by glycine. Thus, glycine acts as a cognition-enhancing agent in both cases, improving the capacity to associate the stimulus with reinforcement in the control NPE condition and to dis-Ignore stimuli once they become relevant in the scopolamine-PE condition. This notion is in agreement with the capacity of NMDA function enhancers including glycinergic drugs to improve performance in a wide range of learning tasks (Aura and Riekkinen, 2000; Baxter et al., 1994; Billard and Rouaud, 2007; Ledgerwood et al., 2005; Monahan et al., 1989; Quartermain et al., 1994). The mechanism by which glycine reversed the effects of scopolamine may involve a direct interaction, as NMDA receptors are present on cholinergic neurons (Ransom and Deschenes, 1989). Since glycine enhances NMDA activity by increasing the frequency of opening of the associate ion channel (Monahan et al., 1989), glycine may increase ACh release, which competes with scopolamine at muscarinic receptor binding sites. Indeed, previous studies have shown that glutamate and glycine can enhance ACh release in certain neuronal populations (Nishimura and Boegman, 1990; Ransom and Deschenes, 1989; Scatton and Lehmann, 1982; Taylor et al., 1988). Our finding provides additional evidence for the capacity of glycinergic NMDA enhancers to reverse muscarinic antagonist-induced behavioural deficits and is the first such evidence in LI.

Our results with the two APDs, however, did not confirm our expectations as both haloperidol and clozapine failed to reverse scopolamine-induced persistent LI. This was seen in expt 3 with doses shown to produce a differential effect on MK-801-induced persistent LI, and increasing the doses of both APDs in expt 4 also failed to yield the expected differentiation. In fact, the higher dose of clozapine did lead to disappearance of scopolamine-induced persistent LI, but this was due to clozapine-induced impairment in fear conditioning (decrease of suppression) in the NPE group. This contrasts with the typical pattern of clozapine-induced reversal of persistent LI, as this is always attributable to the drug-induced increase of suppression in the PE group to a level comparable to that of the NPE group (Gaisler-Salomon and Weiner, 2003; Schiller et al., 2006). Indeed, it is evident from inspection of Figure 3 that although the administration of clozapine to scopolamine-treated rats led to disappearance of LI, the performance of these rats differed markedly from that of vehicle controls: whereas in vehicle controls, absence of LI was due to strong suppression in the PE group, which was as suppressed as their NPE counterparts, in the scopolamine + clozapine condition absence of LI was due to weak suppression in the NPE group, which performed as poorly as its PE counterpart. In other words, in vehicle rats LI absence was due to loss of effectiveness of pre-exposure, whereas in the scopolamine + clozapine rats it was due to an associative learning deficit in the NPE group. This finding may suggest that the combination of clozapine with antimuscarinic drugs, often used in the clinic for controlling extrapyramidal side-effects, may exacerbate learning deficits. It should be noted, however, that there remains a possibility that absence of LI in the scopolamine + clozapine condition was due to a floor effect in suppression level of the PE group. Finally, because previous studies have shown that clozapine acts to reverse persistent LI in the pre-exposure stage (Gaisler Salomon and Weiner, 2003) and that in fact its action in conditioning may interfere with its action in pre-exposure (Shadach et al., 2000), in expt 5 we confined clozapine administration to pre-exposure. This regime also failed to reverse scopolamine-induced persistent LI.

Both haloperidol and clozapine, at both doses used, were effective in potentiating LI in the vehicle controls, as expected from APDs under conditions not yielding LI in non-treated rats (Shadach et al., 2000; Weiner, 2003; Weiner et al., 1997, 2003). Unlike glycine, both APDs led to the emergence of LI reducing suppression of PE animals. The higher dose of clozapine given on its own also impaired conditioning per se, as

manifested in reduced suppression in the NPE group. This is in line with other reports that higher doses of clozapine impair learning (Hou et al., 2006; Levin and Christopher, 2006; Ninan and Kulkarni, 1996).

Clozapine inefficacy in reversing scopolamine-induced persistent LI might seem particularly puzzling because in the present study both glycine and clozapine induced persistent LI in vehicle-treated rats and both were shown previously to antagonize MK-801-induced persistent LI (Gaisler-Salomon et al., 2008; Gaisler-Salomon and Weiner, 2003; Lipina et al., 2005). However, previous data have indicated that the identical behavioural effects of the two compounds are apparently mediated via different mechanisms, which may explain their differential efficacy seen here. Clozapine-induced LI persistence (like that of other APDs), is conventionally attributed to its D_2 receptor antagonism (Weiner, 2003; Weiner and Feldon, 1997), whereas its antagonism of MK-801-induced persistence is probably due to its 5-HT_{2A/C} antagonism (Gaisler-Salomon et al., 2008; Gaisler-Salomon and Weiner, 2003). Furthermore, the former action of clozapine is exerted in conditioning (Weiner et al., 1997) whereas the latter is exerted in pre-exposure (Gaisler-Salomon et al., 2008; Gaisler-Salomon and Weiner, 2003). Glycine acts selectively by stimulating the glycine B site on the NMDA receptor, and this is the most likely mechanism by which this agent produces both LI persistence and antagonism of MK-801-induced LI persistence. Furthermore, both of these effects are exerted in conditioning (Gaisler-Salomon et al., 2008; Gaisler-Salomon and Weiner, 2003; A. De Levie and I. Weiner, unpublished observations). In view of the above, our present findings confirm the capacity of NMDA agonism to induce as well as reverse (scopolamine-induced) persistent LI, the latter presumably due to stimulation of NMDA receptors on cholinergic neurons. Regarding clozapine, it can be tentatively assumed that its failure to antagonize scopolamine-induced persistent LI is due to the insensitivity of this phenomenon to 5-HT_{2A/C} antagonism.

Importantly, similar effects exerted by clozapine and glycine in animal models (e.g. Gaisler-Salomon et al., 2008; Gaisler-Salomon and Weiner, 2003; Geyer et al., 2001; Karasawa et al., 2008; Le Pen et al., 2003; Lipina et al., 2005) as well as in counteracting negative/cognitive symptoms in schizophrenia and drug-induced psychotic-like symptoms in normal humans (e.g. Harvey et al., 2005; Heresco-Levy et al., 2005; Krystal et al., 2003; Lane et al., 2005; Lechner, 2006; Millan, 2005) have been taken to suggest that glycinergic drugs may possess properties of atypical APD (Gaisler-Salomon et al., 2008; see also Lane et al.,

2005; Lechner, 2006; Millan, 2005; Shim et al., 2008) or conversely, that the 'atypicality' of clozapine is due to its enhancing effects on NMDA transmission (Heresco-Levy, 2003; Javitt et al., 2005). Our findings indicate that these drugs are distinct and that scopolamine-induced persistent LI may have the capacity to distinguish between the two classes of drugs.

Finally, the failure of clozapine to reverse scopolamine-induced persistent LI is particularly noteworthy because pro-cognitive effects of clozapine have been attributed to the ability of its major metabolite, *N*-desmethyloclozapine, to increase cortical ACh levels via M₁ allosteric agonism (Davies et al., 2005; Li et al., 2005; Weiner et al., 2004). Although several M₁ agonists were shown to reverse scopolamine-induced behavioural and cognitive deficits (Bartolomeo et al., 2000; Espinosa-Raya et al., 2007; Jones et al., 2005) including scopolamine-induced persistent LI (S. Barak and I. Weiner, unpublished results), the present results suggest that the cholinergic effects of clozapine are insufficient to reverse the effects of high doses of scopolamine on LI.

Taken together, the present findings suggest that scopolamine-induced persistent LI may provide a novel LI model with a pharmacological profile that sets it apart from that of both scopolamine-induced disrupted LI and MK-801-induced persistent LI models, at least as has been shown with the representative drugs tested to date in these models. Specifically, while scopolamine-induced persistent LI shares with the other two models sensitivity to cognitive enhancers, it differs in its sensitivity to APDs as it is resistant to both typical and atypical APDs. It should be noted that scopolamine-induced persistent LI is the first instance of persistent LI that is insensitive to atypical APDs as reversal by clozapine has been shown for persistent LI caused by NMDA antagonists, lesions and neurodevelopmental manipulations (De Levie and Weiner, 2007; Gaisler-Salomon and Weiner, 2003; Schiller et al., 2006).

Clearly, additional studies are needed using a range of APDs and cognitive enhancers from different classes, to substantiate the selective sensitivity of scopolamine-induced persistent LI to cognitive enhancers, as well as the mechanisms of such selectivity. However, the pharmacological profile obtained in the present study provides preliminary evidence that scopolamine-induced persistent LI is an APD-resistant cognitive impairment, and thus may model APD-resistant cognitive impairments in schizophrenia. Furthermore, given its sensitivity to cognitive enhancers, scopolamine-induced persistent LI may have

considerable utility in detecting effective treatments for APD-resistant cognitive impairments in this disorder. Alternatively, given its insensitivity to APDs, abnormally persistent LI may represent a more general form of behavioural perseveration, which is common to a variety of neuropsychiatric disorders, including schizophrenia, autism, addictive behaviour and obsessive-compulsive disorders (Ridley, 1994); indeed, the latter has been shown to be associated with enhancement of LI (Kaplan et al., 2006; Swerdlow et al., 1999).

Attentional dysfunction has been considered central to schizophrenia ever since Emil Kraepelin (1919) described two poles of attentional impairment in schizophrenia patients: inability to fix attention on the one hand, and rigidity of attention on the other hand. As shown here and in our previous study (Barak and Weiner, 2007), muscarinic blockade can produce both these poles of attentional abnormality, as reflected in disruption and persistence of LI, respectively, suggesting that both abnormalities in schizophrenia may be related to dysfunction in cholinergic transmission. Moreover, our results suggest that rigidity of attention may constitute a fundamental treatment-resistant abnormality in schizophrenia which, however, might be responsive to cholinergic and glycinergic cognitive enhancers. Further investigation of the LI model of the antimuscarinic syndrome may provide useful insights into cholinergic-related psychosis and cognitive impairments and their treatment.

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Statement of Interest

None.

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