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ORIGINAL INVESTIGATION

Dissociating scopolamine-induced disrupted and persistent latent inhibition: stage-dependent effects of glycine and physostigmine

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Abstract
Rationale Latent inhibition (LI) is the poorer conditioning to a stimulus seen when conditioning is preceded by repeated non-reinforced pre-exposure to the stimulus. LI indexes the ability to ignore irrelevant stimuli and is used extensively to model attentional impairments in schizophrenia. We showed that the pro-psychotic muscarinic antagonist scopolamine can produce LI disruption or LI persistence depending on dose and stage of administration: low doses disrupt LI acting in the pre-exposure stage of the LI procedure, whereas higher dose produces abnormally persistent LI via action in the conditioning stage. The two LI abnormalities show distinct response to antipsychotic drugs (APDs), with LI disruption, but not LI persistence, reversed by APDs.
Objectives The objective of this study is to show that both LI abnormalities will be reversed by the cognitive enhancers, glycine and physostigmine, in a stage-specific manner, reversing each abnormality via the stage at which it is induced by scopolamine.
Methods LI was measured in a conditioned emotional response procedure. Scopolamine, physostigmine, and glycine were administered in pre-exposure and/or in conditioning.
Results Scopolamine (0.15 mg/kg)-induced disrupted LI was reversed by glycine (800 mg/kg) and physostigmine (0.15 mg/kg) via action in pre-exposure, whereas scopolamine (1.5 mg/kg)-induced persistent LI was reversed by these compounds via action in conditioning. In addition, glycine reversed scopolamine-induced disrupted LI via action in conditioning. Finally, glycine failed to reverse amphetamine-induced disrupted LI.
Conclusions These results extend the pharmacological differentiation between scopolamine-induced disrupted and persistent LI and indicate that the scopolamine LI model may have a unique capacity to discriminate between typical APDs, atypical APDs, and cognitive enhancers.

Keywords Latent inhibition · Schizophrenia · Scopolamine · Acetylcholinesterase inhibitors · Glycine · Attention · Muscarinic · Animal model

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Introduction

The dopamine (DA) and NMDA hypotheses of schizophrenia have been complemented during the last decade by the cholinergic hypothesis, kindled by the fact that muscarinic antagonists such as scopolamine provoke schizophrenia-like positive and cognitive symptoms in humans and exacerbate symptoms in schizophrenia patients (Barak 2009; Yeomans 1995). This hypothesis has been further supported by findings of abnormalities of cholinergic neurotransmission in schizophrenia patients (Barak 2009; Raedler et al. 2007; Ripoll et al. 2004; Scarr and Dean 2008) and has led to expectations that cholinergic compounds may be beneficial in the treatment of cognitive impairments of schizophrenia (Friedman 2004; Levin and Rezvani 2007; Sellin et al. 2008). Despite the increased

interest in the cholinergic underpinnings of schizophrenia, the use of muscarinic acetylcholine receptor (mAChR) antagonists in animal modeling of this disorder has been limited compared to DA agonists and NMDA antagonists (Barak 2009).

Recently, we have shown that scopolamine can produce both psychotic-like and cognitive-like effects in the latent inhibition (LI) model of schizophrenia (Barak 2009; Barak and Weiner 2007, 2009). LI refers to the poorer conditioning of a previously non-reinforced stimulus, and LI abnormalities in rodents are considered to model selective attention deficits associated with schizophrenia (Kilts 2001; Lipska and Weinberger 2000; Powell and Miyakawa 2006; Weiner 1990, 2003). Pro-psychotic drugs from the DA agonist and NMDA antagonist classes produce opposite poles of abnormality in LI: The psychosis-inducing DA agonist amphetamine *disrupts* LI whereas NMDA antagonists such as MK-801 or ketamine that evoke negative and cognitive symptoms produce *abnormally persistent* LI. In other words, amphetamine-treated rats fail to ignore irrelevant stimuli, whereas NMDA antagonist-treated rats perseverate in ignoring the pre-exposed stimulus under conditions in which normal animals shift to treating it as relevant. These two effects on LI have been suggested to model attentional overswitching associated with psychosis, and attentional perseveration (or impaired set shifting) associated with the negative/cognitive symptoms of schizophrenia (Gaisler-Salomon and Weiner 2003; Weiner 2003; Weiner and Arad 2009).

We have found that scopolamine can produce *both* disrupted and persistent LI depending on the dose and the stage of administration. Thus, low scopolamine doses (0.15 or 0.5 mg/kg) disrupted LI by an action in the pre-exposure stage, whereas a higher dose (1.5 mg/kg) produced abnormally persistent LI via action in the conditioning stage (Barak 2009; Barak and Weiner 2007, 2009). Stage-specific action on LI was also obtained with intracerebral scopolamine infusion, with infusion into the entorhinal cortex before pre-exposure but not conditioning disrupting LI and infusion into the basolateral amygdala before conditioning but not pre-exposure inducing LI persistence (Barak 2009; Barak and Weiner 2010). Pharmacologically, the distinction between the two LI abnormalities was supported by their distinct response to antipsychotic drugs (APDs), with LI disruption reversed by the typical APD haloperidol and the atypical APD clozapine and LI persistence being *resistant* to both of these APDs (Barak 2009; Barak and Weiner 2007, 2009). We have suggested that scopolamine-induced LI disruption may model the positive spectrum of the antimuscarinic psychosis, whereas scopolamine-induced persistent LI may model APD-resistant cognitive impairments in this disorder (Barak 2009; Barak and Weiner 2007, 2009).

The present experiments were designed to provide further pharmacological differentiation between the two scopolamine-induced LI abnormalities. Specifically, we sought to show that pharmacological treatments that are effective in both models will exert their action in a stage-specific manner, reversing each abnormality via the stage at which it is induced by scopolamine. In recent years, new therapeutic strategies for schizophrenia have been proposed, including enhancement of NMDA transmission via the glycineB modulatory site on the NMDA receptor, either directly by agonists such as glycine (Heresco-Levy and Javitt 2004) and D-serine (Heresco-Levy and Javitt 2004; Heresco-Levy et al. 2005; Lane et al. 2005) or indirectly by inhibiting the glycine transporter (GlyT1) (Javitt 2008; Javitt et al. 2005; Lane et al. 2005); and enhancement of cholinergic transmission using acetylcholinesterase inhibitors (AChE-Is) such as physostigmine (Barak 2009; Friedman 2004), mAChR agonists such as xanomeline (Barak 2009; Bymaster et al. 2002; Sellin et al. 2008), and alpha7 nicotinic receptor agonists (Hashimoto et al. 2005). We previously showed that both scopolamine-induced LI disruption and persistence were reversed by physostigmine and that LI persistence was reversed by glycine (Barak and Weiner 2007, 2009). Here, we focused on these two compounds, with the expectation that both would reverse scopolamine-induced disrupted LI via pre-exposure and persistent LI via conditioning, reflecting direct interaction with scopolamine.

Material and methods

Subjects

Male Wistar rats (Tel Aviv University Medical School, Tel Aviv) 3–5 months old and weighing 330–540 g, were housed four to a cage under reversed cycle lighting (lights on: 1900–0700) with ad lib food and water 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 number A5010-01, expires on 09/30/2011). 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 procedure using Campden Instruments 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. 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 days. On the next 5 days, rats were trained to drink in the experimental chamber, 15 min/day. 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) or five (strong conditioning) tone-shock pairings given 5 min apart. Shock immediately followed tone termination. Weak conditioning produces LI in nontreated controls and thus allows the demonstration of treatment-induced LI disruption. This level of conditioning was, therefore, used with low scopolamine (Experiments 1–3). Conversely, strong conditioning prevents LI in nontreated controls and thus allows the demonstration of treatment-induced abnormally persistent LI. This level of conditioning was used with high scopolamine (Experiments 4 and 5).

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 30 min prior to the pre-exposure and/or the conditioning stage, in a volume of 1 ml/kg, except for glycine, which was administered in a volume of 3 ml/kg. Scopolamine HBr (0.15 or 1.5 mg/kg; Sigma, Israel), *d*-amphetamine (1 mg/kg; Sigma, Switzerland), glycine (800 mg/kg; Sigma, Israel), and physostigmine (eserine) hemisulfate (0.15 mg/kg; Sigma, Israel) were dissolved in saline. The doses of all drugs were chosen on the basis of previous LI studies in our laboratory (Barak and Weiner 2007, 2009). No-drug controls received the corresponding vehicle.

Experimental design

Experiments 1–3 were conducted with 40 tone pre-exposure followed by two tone-shock pairings, whereas Experiments 4 and 5 used five tone-shock pairings (see "Apparatus and procedure" above); the former set of parameters produces LI in nontreated controls, therefore, allowing demonstration of LI disruption, whereas the latter set of parameters abolishes LI in nontreated controls, therefore, allowing demonstration of LI persistence (see Table 1). Experiment 1 tested whether glycine would reverse scopolamine- and amphetamine-induced disrupted LI. The experiment included 12 groups in a 2×3×2 design with main factors of pre-exposure (NPE, PE), pro-psychotic drug (vehicle, 0.15 mg/kg scopolamine, 1 mg/kg amphetamine), and treatment (vehicle, 800 mg/kg glycine). Experiment 2 tested whether physostigmine would reverse scopolamine-induced disrupted LI by acting in the pre-exposure or the conditioning stage of the LI procedure. The experiment included eight groups in a 2×4 design with main factors of pre-exposure (NPE, PE) and treatment (vehicle, 0.15 mg/kg scopolamine, scopolamine + 0.15 mg/kg physostigmine in pre-exposure, scopolamine + physostigmine in conditioning). Experiment 3 tested whether glycine would reverse scopolamine-induced disrupted LI by acting in the pre-exposure or the conditioning stage of the LI procedure. The experiment included eight groups in a 2×4 design with main factors of pre-exposure (NPE, PE) and treatment (vehicle, 0.15 mg/kg scopolamine, scopolamine + 800 mg/kg glycine in pre-exposure, scopolamine + glycine in conditioning). Experiment 4 tested whether physostigmine would reverse scopolamine-induced persistent LI by acting in the pre-exposure or the conditioning stage of the LI procedure. The experiment included eight groups in a 2×4 design with main factors of pre-exposure

Table 1 Experimental design of the five experiments

	Tone-shock pairings	Pro-psychotic drug (both stages)	Treatment/stage
Experiment 1	2	vehicle low scop amph	veh/gly in both stages
Experiment 2	2	vehicle	vehicle
		low scop	veh/physo in prex or cond
Experiment 3		vehicle	vehicle
		low scop	veh/gly in prex or cond
Experiment 4	5	vehicle	vehicle
		high scop	veh/physo in prex or cond
Experiment 5		vehicle	vehicle
		high scop	veh/gly in prex or cond

amph amphetamine; *cond* conditioning; *gly* glycine; *prex* pre-exposure; *scop* scopolamine; *veh* vehicle

(NPE, PE) and treatment (vehicle, 1.5 mg/kg scopolamine, scopolamine + 0.15 mg/kg physostigmine in pre-exposure, scopolamine + physostigmine in conditioning). Experiment 5 tested whether glycine would reverse scopolamine-induced persistent LI by acting in the pre-exposure or the conditioning stage of the LI procedure. The experiment included eight groups in a 2×4 design with main factors of pre-exposure (NPE, PE) and treatment (vehicle, 1.5 mg/kg scopolamine, scopolamine + 800 mg/kg glycine in pre-exposure, scopolamine + glycine in conditioning).

Data analysis

Times to complete licks 51–75 and mean log times to complete licks 76–100 were analyzed using three-way (Experiment 1) or two-way (Experiments 2–5) ANOVAs. LSD post hoc comparisons were used to assess the difference between the PE and NPE groups within each treatment condition.

Experiment 1 Effects of glycine administered in both pre-exposure and conditioning on scopolamine- and amphetamine-induced LI disruption

We previously showed that scopolamine-induced persistent LI was reversed by glycine (Barak and Weiner 2009) but did not test the effects of glycine on scopolamine-induced disrupted LI. Therefore, the first experiment sought to show that glycine reverses also scopolamine-induced disrupted LI. Because scopolamine-induced disrupted LI is a model of positive symptoms, it is important to characterize its pharmacology in comparison to amphetamine-induced disrupted LI. We previously showed that both LI disruptions were reversed by the typical APD haloperidol as well as the atypical APD clozapine, but only scopolamine-induced disrupted LI was reversed by physostigmine. Since it is not known whether amphetamine-induced LI disruption is affected by glycine, we included this model for purposes of comparison.

The experiment included 93 rats (run in two replications; n per group=8 except for the NPE-amphetamine + glycine, NPE-amphetamine + vehicle and PE-vehicle + vehicle groups, for which n=7). The 12 experimental groups did not differ in their times to complete licks 51–75 before tone onset (all $p>0.05$; overall mean A period=7.01 s). Figure 1 presents the mean log times to complete licks 76–100 (after tone onset) of the experimental groups. As can be seen, vehicle-treated rats showed LI, which was disrupted by scopolamine as well as by amphetamine. Glycine reversed scopolamine-induced LI disruption, so that rats that received scopolamine + glycine showed LI but failed to reverse amphetamine-induced LI disruption. Glycine did not affect LI when given on its own. ANOVA yielded main effects of pre-exposure ($F_{(1,81)}=24.82$, $p<0.0001$) and pro-psychotic drug ($F_{(2,81)}=5.20$, $p<0.0075$) as well as interactions of pre-exposure × pro-psychotic drug ($F_{(2,81)}=4.86$, $p<0.02$), pro-psychotic drug × treatment ($F_{(2,81)}=7.48$, $p<0.002$) and pre-exposure × pro-psychotic drug × treatment ($F_{(2,81)}=5.03$, $p<0.01$). Post hoc comparisons confirmed a significant difference between the pre-exposed and non-pre-exposed groups (i.e., presence of LI) in the vehicle, vehicle + glycine, and scopolamine + glycine conditions (all $p<0.005$), but not in the scopolamine + vehicle condition.

Experiment 2 Effects of physostigmine administered in pre-exposure or conditioning on scopolamine-induced LI disruption

The experiment included 46 rats (n per group was 6 except for the NPE and PE scopolamine-vehicle groups, for which n=5). The eight experimental groups did not differ in their times to complete licks 51–75 before tone onset (all $p>0.05$; overall mean A period=7.02 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 present in vehicle-treated rats but was disrupted in scopolamine-treated rats. Administration of physostigmine in pre-exposure, but not in conditioning, restored LI in scopolamine-injected

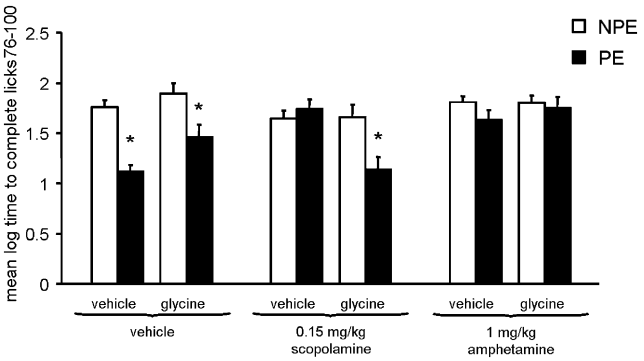

Fig. 1 Effects of glycine on scopolamine- and amphetamine-induced LI disruption. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the *PE* and *NPE* vehicle-, scopolamine (0.15 mg/kg)-, or amphetamine (1 mg/kg)-treated rats, pretreated with vehicle or glycine (800 mg/kg). Forty pre-exposures and two conditioning trials were used. *Asterisk* indicates a significant difference between the PE and NPE groups, namely, presence of LI

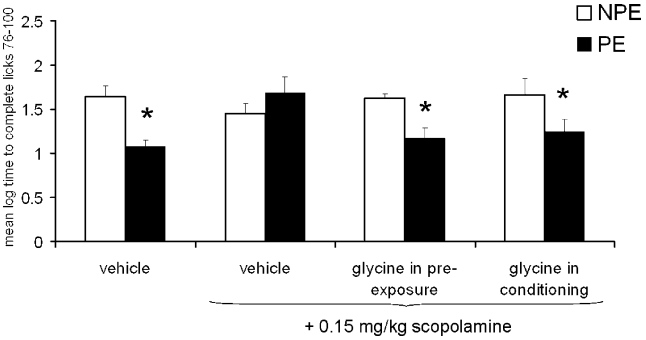

Fig. 3 Effects glycine administered in pre-exposure or conditioning on scopolamine-induced LI disruption. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the *PE* and *NPE* vehicle- or scopolamine (0.15 mg/kg)-treated rats, pretreated with vehicle, glycine (800 mg/kg) in pre-exposure or glycine in conditioning. Forty pre-exposures and two conditioning trials were used. *Asterisk* indicates a significant difference between the PE and NPE groups, namely, presence of LI

rats. ANOVA yielded main effects of pre-exposure ($F_{(1,38)}$= 15.43, p<0.0005) and treatment ($F_{(3,38)}$=3.40, p<0.03), as well as a pre-exposure × treatment interaction ($F_{(3,38)}$=2.27, p< 0.04). Post hoc comparisons confirmed a significant difference between the pre-exposed and non-pre-exposed groups in the vehicle and scopolamine + physostigmine in pre-exposure conditions (all p<0.002), but not in the remaining conditions.

Experiment 3 Effects of glycine administered in pre-exposure or conditioning on scopolamine-induced LI disruption

The experiment included 47 rats (n per group was 6 except for the NPE scopolamine + glycine in pre-exposure group, for which n=5). The eight experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p>0.05; overall mean A period=7.40 s). Figure 3 presents the mean log times to complete licks 76–100 (after tone onset) of the different experimental groups. As can be seen, vehicle-treated but not scopolamine-treated rats showed LI. Glycine reversed scopolamine-induced LI disruption regardless of the stage at which it was administered. ANOVA yielded a main effect of pre-exposure ($F_{(1,39)}$=10.14, p<0.003), as well as pre-exposure × treatment interaction ($F_{(3,39)}$=3.69, p<0.02). Post hoc comparisons confirmed presence of LI in the vehicle, scopolamine + glycine in pre-exposure, and scopolamine + glycine in conditioning conditions (all p<0.05), but not in the scopolamine-alone condition.

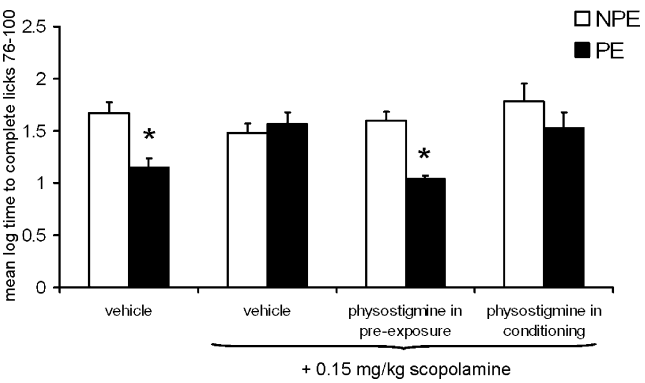

Fig. 2 Effects of physostigmine administered in pre-exposure or conditioning on scopolamine-induced LI disruption. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the *PE* and *NPE* vehicle- or scopolamine (0.15 mg/kg)-treated rats, pretreated with vehicle, physostigmine (0.15 mg/kg) in pre-exposure or physostigmine in conditioning. Forty pre-exposures and two conditioning trials were used. *Asterisk* indicates a significant difference between the PE and NPE groups, namely, presence of LI

Experiment 4 Effects of physostigmine administered in pre-exposure or conditioning on scopolamine-induced LI persistence

The experiment included 47 rats (n per group was 6 except for the NPE scopolamine–vehicle group, for which n=5). The eight experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p> 0.05; overall mean A period=7.63 s). Figure 4 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, whereas rats that received scopolamine persisted in showing LI. Administration of physostigmine in conditioning, but not in pre-exposure, reversed scopolamine-induced persistent LI. ANOVA yielded main effects of pre-exposure ($F_{(1,39)}$=5.81, p<0.025) and treatment ($F_{(3,39)}$= 5.76, p<0.003), as well as an interaction of pre-exposure ×

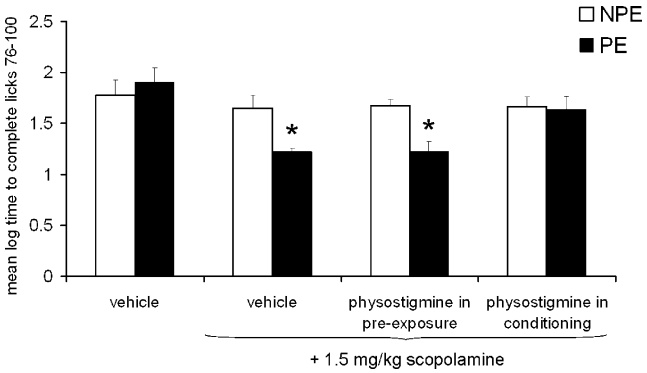

Fig. 4 Effects physostigmine administered in pre-exposure or conditioning on scopolamine-induced LI persistence. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the *PE* and *NPE* vehicle- or scopolamine (0.15 mg/kg)-treated rats, pretreated with vehicle, physostigmine (0.15 mg/kg) in pre-exposure or physostigmine in conditioning. Forty pre-exposures and five conditioning trials were used. *Asterisk* indicates a significant difference between the PE and NPE groups, namely, presence of LI

treatment ($F_{(3,39)}$=3.40, p<0.03). Post hoc comparisons confirmed a significant difference between the pre-exposed and non-pre-exposed groups in the scopolamine and scopolamine + physostigmine in pre-exposure conditions (all p<0.02), but not in the remaining conditions.

Experiment 5 Effects of glycine administered in pre-exposure or conditioning on scopolamine-induced LI persistence

The experiment included 48 rats (n per group was 6). The eight experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p>0.05; overall mean A period=7.29 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. Administration of glycine in conditioning, but not in pre-exposure, reversed scopolamine-induced persistent LI. ANOVA yielded main effects of pre-exposure ($F_{(1,40)}$=39.43, p<0.0001) and treatment ($F_{(3,40)}$= 12.06, p<0.0001), as well as pre-exposure × treatment interaction ($F_{(3,40)}$=17.44, p<0.0001). Post hoc comparisons confirmed presence of LI in the scopolamine and scopolamine + glycine in pre-exposure conditions (all p<0.0001), but not in the remaining conditions.

Discussion

The present results replicated and extended our previous findings by showing that scopolamine can induce both disrupted and persistent LI and that both these LI abnormalities are reversed by physostigmine and glycine in a stage-specific manner. As shown by us previously (Barak and Weiner 2007, 2009), here, normal rats pre-exposed to 40 tones and conditioned with *two* tone-shock pairings showed LI, whereas low scopolamine (0.15 mg/kg)-treated rats failed to show LI. In contrast, with *five* tone-shock pairings, control rats failed to show LI, but rats that were treated with a high dose of scopolamine (1.5 mg/kg) persisted in displaying LI.

Both disrupted and persistent LI were reversed by physostigmine as well as glycine. These outcomes are consistent with our previous results (Barak and Weiner 2007, 2009), as well as with other data on the effectiveness of AChE-Is (Barak 2009; Carnicella et al. 2005; Hironaka and Ando 1996; Hohnadel et al. 2007; Shannon and Peters 1990) and glycine (Fishkin et al. 1993; Matsuoka and Aigner 1996; Ohno and Watanabe 1996; Sirvio et al. 1992; but see Viu et al. 2000) in reversing psychotomimetic effects and cognitive deficits induced by muscarinic blockade. Here, we show that both physostigmine and glycine reversed the effects of scopolamine on LI at the stage which scopolamine affected LI, reversing disrupted LI via action in pre-exposure and reversing persistent LI via action in conditioning. Such specific "targeting" is not surprising for physostigmine since increased synaptic levels of acetylcholine produced by this compound would presumably overcome any of the behavioral effects of scopolamine antagonism at mAChRs. As for the mechanism by which glycine reversed the effects of scopolamine, this could also involve a direct interaction as NMDA receptors are present on cholinergic neurons (Aigner 1995; Bloomfield et al. 2007; Ransom and Deschenes 1989).

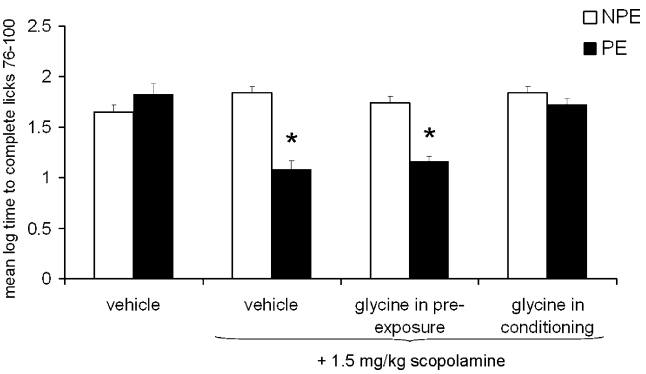

Fig. 5 Effects glycine administered in pre-exposure or conditioning on scopolamine-induced LI persistence. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the *PE* and *NPE* vehicle- or scopolamine (0.15 mg/kg)-treated rats, pretreated with vehicle, glycine (800 mg/kg) in pre-exposure or glycine in conditioning. Forty pre-exposures and five conditioning trials were used. *Asterisk* indicates a significant difference between the PE and NPE groups, namely, presence of LI

Thus, glycine may increase ACh release, which competes with scopolamine at mAChR-binding sites. Indeed, glutamate and glycine have been shown ex vivo and in vivo to enhance striatal, hippocampal, and cortical ACh release (Depoortere et al. 2005; Hernandes et al. 2007; Nishimura and Boegman 1990; Ransom and Deschenes 1989; Scatton and Lehmann 1982; Taylor et al. 1988).

As noted in the "Introduction", to date, the low and high scopolamine LI models have been dissociated psychopharmacologically at two levels: stage of action of scopolamine, with low doses acting in pre-exposure and high doses acting in conditioning; and response to APDs, with scopolamine-induced LI disruption reversed by both the typical and atypical APDs haloperidol and clozapine, respectively, and scopolamine-induced LI persistence resistant to both the APDs from both classes. Stage-dependent reversal of these abnormalities by cognitive enhancers found here reveals additional dissociation between low and high scopolamine-induced LI abnormalities. It should be noted that had we used only administration in both pre-exposure and conditioning as routinely done, the fact that both LI abnormalities induced by scopolamine were reversed by physostigmine and glycine would be interpreted as showing that these LI aberrations did not differ in their responsiveness to these cognitive enhancers.

In addition to further highlighting the dissociation between scopolamine-induced disruption and persistence of LI, stage-based action of physostigmine and glycine allows the characterization of the cognitive processes presumably targeted by these compounds. 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/performance of the conditioned response (Bouton 1993; Lubow 1989, 2005; Lubow and Kaplan 2005; Lubow and Weiner 2010; Mackintosh 1975; Weiner 1990, 2003). Strong conditioning overrides the inhibitory influence of the inattentional response so that animals switch to respond according to the more recent stimulus-reinforcement relationship (Weiner 1990, 2003). It can thus be suggested that low doses of scopolamine apparently act by attenuating or preventing the normal loss of attention to the stimulus occurring during non-reinforced pre-exposure. This action of scopolamine on LI is in line with extensive evidence implicating the cholinergic system in attentional processes (Everitt and Robbins 1997; Hasselmo and McGaughy 2004; Sarter et al. 2005). Moreover, it suggests that muscarinic blockade can lead to abnormally enhanced stimulus salience, a process often suggested as giving rise to positive symptoms but conventionally attributed to dopaminergic agonism (Kapur 2003). Given the above, the fact that physostigmine and glycine reverse scopolamine-induced disrupted LI due to their action in pre-exposure implies that in psychological terms, these compounds restore animals' capacity to inattend to inconsequential stimuli. Such effect would be beneficial in normalizing aberrantly increased salience perception and distractibility that are associated with psychotic symptoms (Kapur 2003; Smith et al. 2006; Weiner and Joel 2002).

High doses of scopolamine presumably weaken the normal ability to re-attend the previously irrelevant stimuli when they become relevant through pairing with reinforcement. In other words, scopolamine-treated animals perseverate in ignoring the pre-exposed stimulus under conditions in which normal animals switch to respond according to the current stimulus-reinforcement contingency (Barak 2009; Barak and Weiner 2009), consistent with other demonstrations that scopolamine can induce perseveration (Chen et al. 2004; Ragozzino et al. 2002; Soffie and Lamberty 1987). In psychological terms, then, the fact that physostigmine- and glycine-produced reversal of persistent LI is due to their action in conditioning implies that these compounds enable flexible redeployment of attentional resources and readjustment of responding according to current situational demands. Such action would be beneficial in reducing cognitive inflexibility and inattention that are associated with negative or cognitive symptoms (Carlsson and Carlsson 1990; Krystal et al. 2003; Moghaddam et al. 1997; Weiner 2003). It should be noted that both physostigmine and glycine reverse also MK-801-induced persistent LI via conditioning (Barak and Weiner, unpublished observations; Gaisler-Solomon et al. 2008), suggesting that they reduce behavioral inflexibility regardless of its underlying neural dysfunction.

Rather remarkably, glycine reversed scopolamine-induced LI disruption also when given only in conditioning. This action cannot be explained in terms of direct interaction, since scopolamine-induced LI deficit and its reversal by glycine in conditioning were generated in different stages taking place 24 h apart. Such an action could reflect some complex interactions within the brain circuitry that modulates the expression of LI (Weiner 2003), whereby glycine exerts in conditioning some compensatory action that is independent of but interact with brain substrates that regulate LI in pre-exposure (Barak 2009; Weiner 1990, 2003). Irrespective of the mechanisms, these findings suggest that within the realm of antimuscarinic psychosis, low scopolamine-induced disrupted LI model may allow the detection of antipsychotic drug action that is independent of the mechanism of action of the pro-psychotic drug, allowing identification of agents acting through novel mechanisms.

Glycine failed to affect amphetamine-induced LI disruption as was found previously for physostigmine (Barak and

Weiner 2007), supporting previous data that distinguished between antimuscarinic and dopamine agonist LI disruption models (also see Barak 2009; Weiner and Arad 2009). The two models are distinct in several respects. First, amphetamine disrupts LI via effects exerted at the conditioning stage and spares LI if it is given only before pre-exposure, whereas scopolamine disrupts LI via effects exerted at the pre-exposure stage and spares LI when given only in conditioning (Barak and Weiner 2007). Second, although the APDs haloperidol and clozapine reverse both abnormalities, in the case of amphetamine-induced LI disruption, the pro-psychotic and the antipsychotic actions are exerted at the same stage of the LI procedure (conditioning) and thus reflect a direct interaction, whereas in the case of scopolamine-induced LI disruption, the pro-psychotic and antipsychotic actions are generated in different stages of the procedure (pre-exposure and conditioning, respectively) and thus mediated by distinct mechanisms (Barak and Weiner 2007). Third, scopolamine-induced, but not amphetamine-induced, LI disruption, is reversed by physostigmine (Barak and Weiner 2007). The inefficacy of glycine would further support the pharmacological distinction between the two abnormalities in the realm of glutamatergic transmission. However, since amphetamine-induced LI disruption is reversed by the novel Glyt1 inhibitors SSR103800 (Black et al. 2008), which represents a more efficient strategy of activating the glycineB site, the two deficits may merely differ in their sensitivity to glycinergic enhancers.

Summary and conclusion

The present demonstration of the stage-dependent reversal of scopolamine-induced disrupted and persistent LI by physostigmine and glycine, taken together with our previous findings with APDs, suggests that the scopolamine LI model allows a double differentiation between atypical APDs and cognitive enhancers from the glycinergic and cholinergic classes. These classes of drugs are discriminated at two levels: efficacy vs inefficacy in the high scopolamine model (cognitive enhancers effective, atypical APDs ineffective) and stage-specific efficacy in the low-scopolamine model (cognitive enhancers active in pre-exposure, APDs active in conditioning). Further studies with additional atypical APDs are needed to evaluate the generality of such a discrimination, as studies using intradimensional/extradimensional set shifting revealed discrimination from cognitive enhancers with some atypical APDs but not others (Broberg et al. 2009; Goetghebeur and Dias 2009; Rodefer et al. 2008).

Given the continuing debate on the capacity of typical APDs to enhance cognition on the one hand (Buchanan et al. 2007; Miyamoto et al. 2005; Sarter et al. 2008) and the search for cognition enhancing agents for schizophrenia (Buchanan et al. 2007; Marder 2006; Marder and Fenton 2004; Stip et al. 2005) on the other hand, a model that can distinguish between atypical APDs and cognitive enhancers may have great utility. Our data suggest that at least with regard to some psychotic and cognitive deficits, namely, those that are muscarinic-dependent, cognitive enhancers may offer a better treatment alternative or a useful addition to APDs. Individualized pharmacotherapy combined with polypharmacy and augmentation strategies aimed at treating the multiple symptom domains of schizophrenia would, therefore, be a best option given the heterogeneity of neurotransmitter dysfunctions implicated in schizophrenia (Carpenter and Koenig 2008; Gray and Roth 2007).

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