

Differential Role of Muscarinic Transmission within the Entorhinal Cortex and Basolateral Amygdala in the Processing of Irrelevant Stimuli

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Cholinergic projections to the entorhinal cortex (EC) and basolateral amygdala (BLA) mediate distinct cognitive processes through muscarinic acetylcholine receptors (mAChRs). In this study, we sought to further differentiate the role of muscarinic transmission in these regions in cognition, using the latent inhibition (LI) phenomenon. LI is a cross-species phenomenon manifested as poorer conditioning to a stimulus experienced as irrelevant during an earlier stage of repeated non-reinforced pre-exposure to that stimulus, and is considered to index the ability to ignore, or to in-attend to, irrelevant stimuli. Given our recent findings that systemic administration of the mAChR antagonist scopolamine can produce two contrasting LI abnormalities in rats, ie, abolish LI under conditions yielding LI in non-treated controls, or produce abnormally persistent LI under conditions preventing its expression in non-treated controls, we tested whether mAChR blockade in the EC and BLA would induce LI abolition and persistence, respectively. We found that intra-EC scopolamine infusion (1, 10 µg per hemisphere) abolished LI when infused in pre-exposure or both pre-exposure and conditioning, but not in conditioning alone, whereas intra-BLA scopolamine infusion led to persistent LI when infused in conditioning or both stages, but not in pre-exposure alone. Although cholinergic innervation of the EC and BLA has long been implicated in attention to novel stimuli and in processing of motivationally significant stimuli, respectively, our results provide evidence that EC mAChRs also have a role in the development of inattention to stimuli, whereas BLA mAChRs have a role in re-attending to previously irrelevant stimuli that became motivationally relevant.

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INTRODUCTION

Cholinergic projections from the basal forebrain (BF) have a fundamental role in cognitive processes, and abnormalities of the cholinergic system are implicated in cognitive dysfunctions associated with several neurological/neuropsychiatric disorders (for reviews, see Everitt and Robbins, 1997; Fibiger, 1991; Hasselmo, 2006; Power *et al*, 2003; Sarter *et al*, 2003). Blockade of muscarinic acetylcholine receptors (mAChRs) has been shown to induce memory and attentional deficits in both animals and humans (Blokland, 1995; Ebert and Kirch, 1998; Everitt and Robbins, 1997; Green *et al*, 2005). Two of the cholinergic projections from the BF, those to the entorhinal cortex (EC) and the

basolateral amygdala (BLA), have been shown to mediate distinct cognitive processes through mAChRs (Hasselmo, 2006; Hasselmo and McGaughy, 2004; Ingles *et al*, 1993; Power, 2004). This study sought to further differentiate the role of muscarinic transmission in these two regions in cognition, using the latent inhibition (LI) phenomenon.

LI is one of the best documented cross-species manifestations of attentional selectivity in associative learning (Lubow, 1989; Lubow and Weiner, 2009). It is manifested when repeated non-reinforced pre-exposure to the to-be-conditioned stimulus reduces the efficacy of this stimulus to acquire and/or express conditioned responses when paired with reinforcement. We have recently shown that systemic administration of the mAChR antagonist scopolamine in rats can produce two contrasting LI abnormalities as a function of dose and stage of administration. At low doses, scopolamine abolishes LI, whereas rats treated with higher doses persist in expressing LI under conditions that prevent/reduce LI expression in untreated rats. Scopolamine-induced LI abolition is due to the action of the drug in the pre-exposure stage (Barak and Weiner, 2007), whereas scopolamine-induced LI persistence is due to its action in

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the conditioning stage (Barak and Weiner, 2009). Thus, scopolamine produces contrast poles of impairment in attentional selectivity: at low doses it impairs the capacity to inattent 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. By extension, these findings suggest that normal muscarinic transmission is required for the acquisition of inattention to irrelevant stimuli as well as for the adjustment of responding based on changes in the relevance of stimuli (Barak, 2009).

It has been previously shown that lesions to the EC and BLA produce LI abolition and persistence, respectively (Coutureau *et al*, 1999, 2000; Schiller and Weiner, 2004, 2005; Schiller *et al*, 2006; Shohamy *et al*, 2000; Yee *et al*, 1995) (but see Coutureau *et al*, 2001). Moreover, reversible EC inactivation, similar to our systemic scopolamine injections, were effective in pre-exposure but not conditioning (Jeanblanc *et al*, 2004; Lewis and Gould, 2007a; Seillier *et al*, 2007). No stage selectivity has been tested for BLA lesions. In this study, we tested the hypothesis that LI abolition and persistence produced by EC and BLA lesions are mediated by impaired muscarinic transmission in these regions. More specifically, we expected two outcomes. First, that blockade of muscarinic transmission in the EC and BLA will result in LI loss and persistence, respectively. Second, that these effects will show stage specificity, namely, that muscarinic blockade within the EC will abolish LI by action in pre-exposure, whereas muscarinic blockade within the BLA will produce persistent LI by action in conditioning. For this aim, we tested the effects of scopolamine infusion into EC or BLA before pre-exposure and/or before conditioning using experimental protocols that produce LI or prevent its expression in control rats, therefore allowing demonstration of LI abolition or persistence, respectively, in treated rats.

MATERIALS AND METHODS

Subjects

Male Wistar rats (Tel Aviv University Medical School, Tel Aviv, Israel), approximately 3 months old and weighing 300–460 g at the time of surgery, were housed individually under reversed cycle lighting (lights on: 1900–0700 h) with *ad lib* food and water except for the duration of the LI experiments (see below). 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 30/09/2011). All efforts were made to minimize the number of animals used and their suffering.

Surgery

Rats were given an i.p. injection of diazepam (0.6 mg/kg) and 5 min later were anesthetized with an i.p. injection of avertin (10 ml/kg). They were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and an incision was made into the scalp to expose the skull. The vertical coordinates of bregma and lambda were measured in order to align them in the same (level head) plane. Holes were drilled and rats were implanted bilaterally with 23-gauge

indwelling stainless steel guide cannulae (Plastic Ones, USA) aimed 1 mm above the EC or the BLA according to the following coordinates, measured relative to the skull at bregma (Paxinos and Watson, 1998): EC: 7.1 mm posterior to bregma, 5 mm lateral to the midline, 8.0 mm ventral to the skull; BLA: 2.8 mm posterior to bregma, 5 mm lateral to the midline, 8.5 mm ventral to the skull. Thus, the guide cannulae were aimed just dorsal to the main target area. The cannulae were anchored to the skull with three stainless steel screws and dental cement. Stainless steel stylets (27G) cut to extend 1.0 mm beyond the tip of the guide cannulae were placed into the cannulae to prevent occlusion. After surgery, rats were returned to their home cages and allowed 7–12 days of recovery before the initiation of behavioral testing. During recovery, rats were given daily health checks in which the experimenter weighted them and replaced missing stylets.

Behavioral Apparatus and Procedure

LI was measured in a thirst-motivated conditioned emotional response (CER) procedure by comparing the suppression of drinking to a tone previously paired with a footshock in rats that received non-reinforced exposure to the tone before conditioning (pre-exposed, PE) and in rats for whom the tone was novel (non-pre-exposed, NPE).

Rats were tested in Campden Instruments rodent test chambers (model 410) with a retractable bottle. When the bottle was not present, the hole was covered by a metal lid. Licks were detected by a Campden Instruments drinkometer (model 435). The PE 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 (model 521/C) and shock scrambler (model 521/S) set at 0.5 mA and 1 s duration. Equipment programming and data recording were computer controlled.

Before surgery, rats were put on a 23 h water restriction schedule for 8 days. They were handled for about 2 min daily on the first 5 days and trained to drink in the experimental chamber for 15 min daily for the next 3 days. Water in the test apparatus was given in addition to the daily ration of 1 h given in the home cages. After recovery from surgery, water restriction schedule was re-instated. Rats were handled daily for 3 days, and on the next 3 days, re-trained to drink in the experimental chamber for 15 min/day. On the next 4 days, the LI procedure was conducted, consisting of the following stages:

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

Conditioning. With the bottle removed, rats received 2 (weak conditioning; Experiments 1–3) or 5 (strong conditioning; Experiments 3 and 4) tone-shock pairings given 5 min apart. Shock immediately followed tone termination. The first tone-shock pairing was given 5 min after the start of the session. After the last pairing, rats were left in the experimental chamber for an additional 5 min.

The two (weak and strong) conditioning protocols are routinely used by us to demonstrate LI abolition and persistence in the present CER procedure. Specifically, weak conditioning produces LI in non-treated controls and thus allows the demonstration of treatment-induced LI abolition. Conversely, strong conditioning prevents LI in non-treated controls and thus allows the demonstration of treatment-induced abnormally persistent LI.

Rebaseline. Rats were given a 15 min drinking session as in initial training. Data of rats that failed to complete 600 licks were dropped from the analysis.

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 analysis of variance. 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 with NPE rats.

Drugs and Infusions

Rats received bilateral infusions of either physiological saline (0.9% sodium chloride, pH 7.0) or scopolamine hydrobromide (1 or 10 µg/0.5 µl in physiological saline; Sigma, Israel). The doses of scopolamine were based on similar behavioral studies that used scopolamine infusion into cortical and/or hippocampal formation regions (Barros *et al*, 2001; Herremans *et al*, 1996; Warburton *et al*, 2003; Winters *et al*, 2006). Importantly, these doses were lower than the doses shown before to impair classical conditioning when infused to BLA (See *et al*, 2003), because impairment of conditioning *per se* (impaired conditioning in the NPE groups) masks the effects of experimental manipulations on LI. Infusion volume was based on a routinely used volume in similar behavioral studies ((Barros *et al*, 2001; Lewis and Gould, 2007a, b; Roesler *et al*, 2002; Seillier *et al*, 2007) for EC; (Barros *et al*, 2001; Chhatwal *et al*, 2009; Milton *et al*, 2008; See *et al*, 2001, 2003; Zhao *et al*, 2009) for BLA). Infusions were given in a different room from the behavioral testing area. Animals were gently restrained by the experimenter throughout the infusion process. Stylets were removed, and the stainless steel 28-gauge injection cannulae, which extended 1.00 mm beyond the tip of the guide cannulae, were inserted into the guide cannulae. The injection cannulae were attached to tubing attached to 5 µl microsyringes mounted on a motorized syringe pump (model WPI SP200). Bilateral infusions were performed simultaneously. A total of 0.5 µl per hemisphere was delivered over 120 s. After the pump was shut off, injection cannulae were kept in place for additional 90 s to allow for diffusion of the infusate. Injectors were then removed, stylets were replaced, and animals were placed into the test chambers. On the last day of handling and the second day of drinking training (see above in section Behavioral Apparatus and Procedure), rats experienced a

'mock' infusion identical in all respects to the procedure described above except that the injection cannulae contained no liquid and the tubes were unconnected to the pump. This was done to habituate the animals to the general protocol of the infusion procedure, including insertion of the infusion cannulae and the sound of the pump.

The EC and BLA infusions were given before the pre-exposure and/or the conditioning stages depending on the aim of the experiment (see Table 1).

Histology

After the completion of behavioral testing, rats were anesthetized with an overdose of pentobarbital (60 mg/ml) and perfused intracardially with physiological saline, followed by 10% formalin. Their brains were removed from the skulls and stored in 10% formalin for at least 24 h, and then in 30% sucrose solution for at least 24 h before being sectioned in the coronal plane at 40 µm thickness. Sections were mounted on a gelatinized glass slide and stained with thionin blue for histological examination. Verification of cannulae placements used the atlas of Paxinos and Watson (1998).

Data Analysis

Times to complete licks 51–75 and mean log times to complete licks 76–100 were analyzed using a two- or three-way ANOVAs with main factors of pre-exposure, intracerebral treatment (Experiments 1–4), and number of tone-shock pairings (Experiment 3). LSD *post hoc* comparisons were used to assess the difference between the PE and NPE groups within each treatment condition.

Experimental Design

Experiments 1 and 2 tested the effects of intra-EC scopolamine infusion on LI, and Experiments 3 and 4 tested the effects of intra-BLA scopolamine infusion on LI. The region- and stage-specific effects of intra-EC and intra-BLA scopolamine infusion were determined by combining weak and/or strong conditioning protocols (see Behavioral Apparatus and Procedure section) and drug infusion in the pre-exposure and/or conditioning stage (see Table 1 and below).

RESULTS

Histological Assessment

A schematic reconstruction of cannulae placement in the EC and BLA is presented in the Supplementary Figure S1a and b, respectively. Data from rats in which cannulae tips were located outside the EC (two in Experiment 1; two in Experiment 2) or the BLA (one in Experiment 3; three in Experiment 4) were excluded from the statistical analysis.

Behavior

Experiment 1—Effects of intra-EC scopolamine infusion (1 or 10 µg per hemisphere) before pre-exposure on LI with weak conditioning. As systemic scopolamine

Table 1 Experimental Design of the Five Experiments

	Tone-shock pairings	Treatment group	Infusion		Results
			Pre-exposure	Conditioning	
Experiment 1—EC	2	Control	Vehicle	Vehicle	LI
		Scop in prex	Scop 1 µg	Vehicle	No LI
		Scop in prex	Scop 10 µg	Vehicle	No LI
Experiment 2—EC	2	Control	Vehicle	Vehicle	LI
		Scop in cond	Vehicle	Scop 10 µg	LI
		Scop in both stages	Scop 10 µg	Scop 10 µg	No LI
Experiment 3—BLA	2	Control	Vehicle	Vehicle	LI
		Scop in both stages	Scop 10 µg	Scop 10 µg	LI
	5	Control	Vehicle	Vehicle	No LI
Experiment 4—BLA	5	Scop in both stages	Scop 10 µg	Scop 10 µg	LI
		Control	Vehicle	Vehicle	No LI
		Scop in prex	Scop 10 µg	Vehicle	No LI
		Scop in cond	Vehicle	Scop 10 µg	LI

Abbreviations: BLA, basolateral amygdala; EC, entorhinal cortex; LI, latent inhibition; prex, pre-exposure; scop, scopolamine; cond, conditioning.

Summary of the parametric manipulation (number of tone-shock pairings) and regime of scopolamine infusion in the experimental groups (rows) in Experiments 1–4.

abolishes LI when administered in pre-exposure but not in conditioning (Barak and Weiner, 2007), and there is extensive evidence that inactivation of the EC during pre-exposure abolishes LI (Jeanblanc *et al*, 2004; Lewis and Gould, 2007a; Seillier *et al*, 2007), we had a strong prediction that intra-EC scopolamine infusion before pre-exposure would abolish LI. Therefore, here we confined the infusion to the pre-exposure stage. Weak conditioning that leads to LI in non-treated controls and allows demonstration of LI abolition was used.

The experiment included six experimental groups (n per group = 5–6) in a 2×3 design with main factors of pre-exposure (NPE, PE) and intra-EC treatment (vehicle, 1, 10 µg scopolamine). 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 = 9.10 s). Figure 1 presents the mean log times to complete licks 76–100 (after tone onset) of the six experimental groups. As can be seen, LI was present in vehicle-infused rats, but was dose-dependently abolished by intra-EC infusion of scopolamine. ANOVA yielded main effects of pre-exposure ($F_{(1,26)} = 6.57, p < 0.02$) and an interaction of pre-exposure \times intra-EC treatment ($F_{(2,26)} = 5.858, p < 0.01$). *Post hoc* comparisons revealed a significant difference between the PE and NPE groups, ie, presence of LI in rats that received intra-EC infusion of vehicle ($p < 0.001$), but not in rats that received intra-EC infusion of 1 or 10 µg scopolamine.

Experiment 2—Effects of intra-EC scopolamine infusion (10 µg per hemisphere) before conditioning or both stages on LI with weak conditioning. Having shown in Experiment 1 that LI was abolished, as predicted, by intra-EC scopolamine infusion in the pre-exposure stage, this

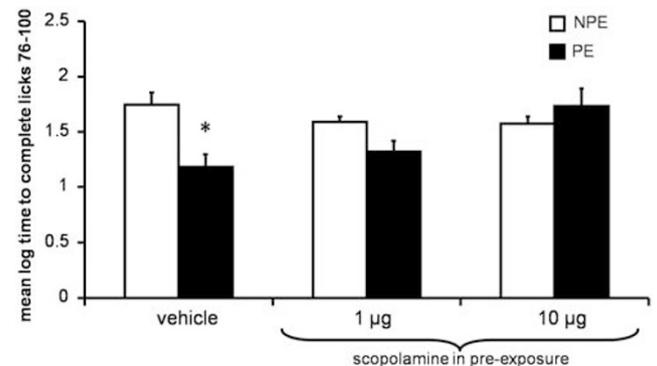


Figure 1 Effects of intra-entorhinal cortex scopolamine infusion (1 or 10 µg per hemisphere) before pre-exposure on LI with weak conditioning. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and non-pre-exposed (NPE) rats infused with vehicle or scopolamine (1, 10 µg) into the entorhinal cortex. Scopolamine was infused in the pre-exposure stage. Forty pre-exposures and two conditioning trials (weak conditioning) were used. Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

experiment sought to show that scopolamine will be ineffective if given only before conditioning, but will be effective if given before both pre-exposure and conditioning stages due to its action in pre-exposure. Weak conditioning was used to allow demonstration of LI abolition.

The experiment included six experimental groups (n per group = 6–7) in a 2×3 design with main factors of pre-exposure (NPE, PE) and intra-EC treatment (vehicle, 10 µg scopolamine in conditioning, scopolamine in both stages). 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 = 9.38 s). Figure 2 presents the mean log times to complete licks 76–100 (after tone onset) of the six experimental groups. As can be seen, LI was present in vehicle-infused rats as well as in rats infused with scopolamine before conditioning, but not in rats that had scopolamine infused before both stages. ANOVA yielded main effects of pre-exposure ($F_{(1,34)} = 24.00, p < 0.0001$) and intra-EC treatment ($F_{(1,34)} = 4.14, p < 0.025$) and an interaction of pre-exposure \times intra-EC treatment ($F_{(2,34)} = 7.57, p < 0.002$). *Post hoc* comparisons confirmed presence of LI in rats that received intra-EC infusion of vehicle ($p < 0.001$) or scopolamine before conditioning ($p < 0.0001$), but not in rats that received intra-EC scopolamine infusion before both stages.

Experiment 3—Effects of intra-BLA scopolamine infusion ($10 \mu\text{g}$ per hemisphere) before pre-exposure and conditioning on LI with weak and strong conditioning. Because we showed that BLA lesion leads to abnormally persistent LI (Schiller and Weiner, 2004, 2005; Schiller *et al.*, 2006), we hypothesized that conditioning-based persistent LI produced by scopolamine is mediated by its effects in BLA. However, because some studies found that BLA perturbations disrupt LI (Coutureau *et al.*, 2001; Schauz and Koch, 2000), we first tested whether scopolamine infusion would indeed produce persistent LI. For this, we used both the weak and the strong conditioning protocol to demonstrate that intra-BLA scopolamine spares LI under the former conditions, and causes abnormally persistent LI under the latter conditions. Scopolamine was infused in both pre-exposure and conditioning.

The experiment included eight experimental groups (n per group = 5–6) in a $2 \times 2 \times 2$ design with main factors of pre-exposure (NPE, PE), number of conditioning trials (two, five), and intra-BLA treatment (vehicle, $10 \mu\text{g}$ scopolamine). 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 = 7.93 s). Figure 3

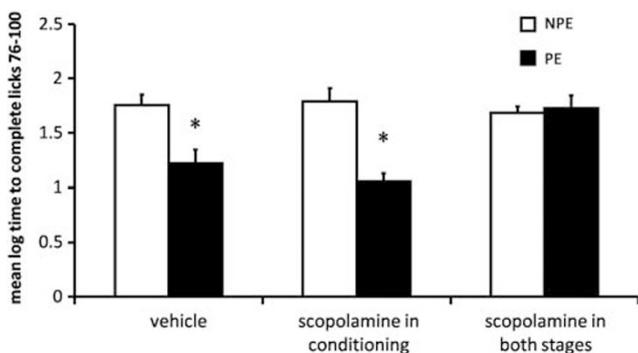


Figure 2 Effects of intra-entorhinal cortex scopolamine infusion ($10 \mu\text{g}$ per hemisphere) before conditioning or both stages on LI with weak conditioning. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and non-pre-exposed (NPE) rats infused with vehicle or scopolamine ($10 \mu\text{g}$) into the entorhinal cortex. Scopolamine was infused in the conditioning stage or in both pre-exposure and conditioning. Forty pre-exposures and two conditioning trials (weak conditioning) were used. Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

presents the mean log times to complete licks 76–100 (after tone onset) of the eight experimental groups. As can be seen, with two conditioning trials LI was present in both vehicle- and scopolamine-infused rats. In contrast, with five conditioning trials LI was absent in vehicle-infused rats, as expected under conditions of strong conditioning, but rats that received intra-BLA scopolamine infusion persisted in expressing LI. ANOVA yielded main effects of pre-exposure ($F_{(1,39)} = 37.50, p < 0.0001$), number of conditioning trials ($F_{(1,39)} = 5.97, p < 0.02$), and interactions of pre-exposure \times number of conditioning trials ($F_{(1,39)} = 6.08, p < 0.02$) and pre-exposure \times intra-BLA treatment ($F_{(1,39)} = 5.75, p < 0.025$), but the pre-exposure \times number of conditioning trials \times intra-BLA treatment interaction did not reach significance ($F_{(1,39)} = 3.44, p = 0.071$). *Post hoc* comparisons confirmed presence of LI in vehicle- and scopolamine-infused rats that received two conditioning trials (p -values < 0.0005), as well as in scopolamine-infused rats that received five conditioning trials ($p < 0.0005$), but not in vehicle-infused rats that received five conditioning trials.

Experiment 4—Effects of intra-BLA scopolamine infusion ($10 \mu\text{g}$ per hemisphere) before pre-exposure or conditioning on LI with strong conditioning. Having shown that scopolamine induces abnormally persistent LI when infused in both pre-exposure and conditioning, we tested at which of the stages this drug acts in the BLA to induce LI persistence. Given that systemic scopolamine induces LI persistence when administered in conditioning but not in pre-exposure (Barak and Weiner, 2009), we expected that intra-BLA scopolamine infusion would also induce LI persistence when infused immediately before conditioning. The strong conditioning protocol was used to allow demonstration of LI persistence.

The experiment included six experimental groups (n per group = 6–7) in a 2×3 design with main factors of pre-exposure (NPE, PE) and intra-BLA treatment (vehicle, $10 \mu\text{g}$ scopolamine in pre-exposure, scopolamine in conditioning). 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 = 10.32 s). Figure 4 presents

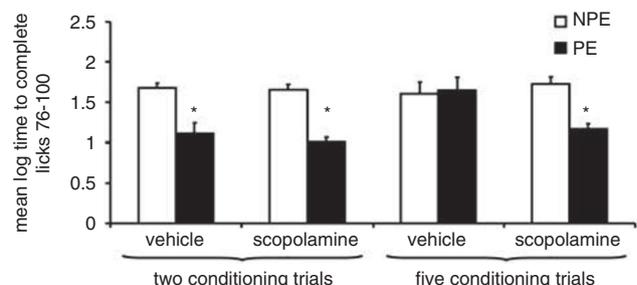


Figure 3 Effects of intra-basolateral amygdala scopolamine infusion ($10 \mu\text{g}$ per hemisphere) on LI with weak or strong conditioning. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and non-pre-exposed (NPE) rats infused with vehicle or scopolamine ($10 \mu\text{g}$) into the basolateral amygdala that underwent conditioning with two (weak conditioning) or five (strong conditioning) trials. Forty pre-exposures were used. Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

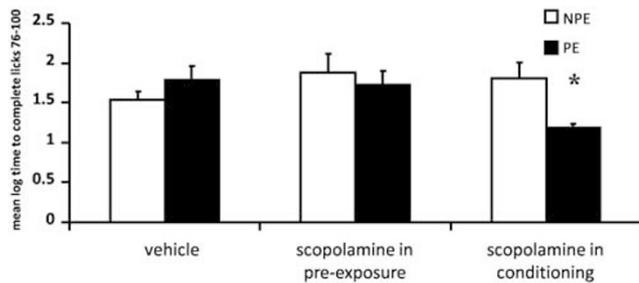


Figure 4 Effects of intra-basolateral amygdala scopolamine infusion ($10\mu\text{g}$ per hemisphere) before pre-exposure or conditioning on LI with strong conditioning. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and non-pre-exposed (NPE) rats infused with vehicle or scopolamine ($10\mu\text{g}$) into the basolateral amygdala. Scopolamine was infused either in the pre-exposure stage or in the conditioning stage. Forty pre-exposures and five conditioning trials (strong conditioning) were used. Asterisk indicates a significant difference between the PE and NPE groups, namely, presence of LI.

the mean log times to complete licks 76–100 (after tone onset) of the six experimental groups. As can be seen, LI was absent in vehicle-infused rats and in rats that received scopolamine in the pre-exposure stage, but present in rats that received scopolamine in the conditioning stage. ANOVA yielded a pre-exposure \times intra-BLA treatment interaction ($F_{(2,32)} = 3.53$, $p < 0.05$). *Post hoc* comparisons confirmed presence of LI in rats infused with scopolamine in conditioning ($p < 0.02$), but not in rats infused with vehicle or scopolamine in pre-exposure.

DISCUSSION

Using two LI protocols, with two or five conditioning trials after 40 stimulus pre-exposures, this study demonstrated a dissociation between the role of muscarinic transmission in the EC and BLA, in the regulation of cognitive processes underlying LI. Vehicle-infused rats showed LI with weak conditioning, but LI was lost with strong conditioning. Intra-EC scopolamine-infused rats failed to show LI with weak conditioning. In contrast, intra-BLA scopolamine-infused rats showed LI with weak and strong conditioning. The effects of intra-BLA and intra-EC scopolamine infusion were further differentiated by the stage at which they acted to produce these LI aberrations. Thus, intra-EC scopolamine abolished LI if infused before pre-exposure or before pre-exposure and conditioning, but not if confined to conditioning. In contrast, intra-BLA scopolamine led to persistent LI if infused before conditioning or before both stages, but not if confined to pre-exposure. These results are consistent with our previous results with systemic scopolamine showing that this drug abolishes LI when injected before pre-exposure (Barak and Weiner, 2007), but induces persistent LI when injected before conditioning (Barak and Weiner, 2009). Our present results suggest that the capacity of systemic scopolamine to induce these contrasting effects on LI may result from its preferred activity at these brain regions during the two stages of the LI procedure. Our results are also consistent with lesion results, showing that EC lesions or its temporary inactivation during

pre-exposure abolish LI (Coutureau *et al*, 1999; Jeanblanc *et al*, 2004; Lewis and Gould, 2007a; Seillier *et al*, 2007; Shohamy *et al*, 2000; Yee *et al*, 1995), whereas BLA lesions lead to abnormally persistent LI (Schiller and Weiner, 2004, 2005; Schiller *et al*, 2006; but see Coutureau *et al*, 2001).

In terms of psychological processes underlying LI, it is believed that during pre-exposure, the acquisition of an association between the PE stimulus and the absence of a significant consequence results in the development of inattention to the stimulus, which inhibits the acquisition and/or the performance of the conditioned response (Bouton, 1993; Lubow and Kaplan, 2005; Weiner, 1990, 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), leading to loss of LI.

Our finding that intra-EC scopolamine infusion abolishes LI when infused before pre-exposure implies that interference with muscarinic neurotransmission in the EC prevents the acquisition of inattention to irrelevant stimuli, and by extension, that muscarinic transmission in the EC is required for this process. This finding is consistent with other findings and models suggesting a crucial role of cholinergic innervation of the EC in attentional processes in general, and in the acquisition/encoding of associations with novel stimuli in particular (Hasselmo, 2006; Hasselmo and McGaughy, 2004; Hasselmo and Stern, 2006; McGaughy *et al*, 2005; Ramirez *et al*, 2007). Notably, although cholinergic innervation of the EC has long been postulated to be involved in the attention to, and encoding of, novel stimuli, our results provide the first evidence that it also has a crucial role in the development of inattention to stimuli. The latter may specifically involve EC-mesolimbic dopamine interactions, as Jeanblanc *et al* (2004) found that blockade of the EC with tetrodotoxin before pre-exposure disrupted normal accumbal DA responses in the PE condition, in parallel to the disruption of the behavioral expression of LI in these rats.

Scopolamine infusion into the EC before conditioning spared LI, suggesting that muscarinic transmission in this region does not have a role in the expression of the stimulus-no outcome contingency acquired in pre-exposure. Lewis and Gould (2007a) recently reported the same outcome using reversible inactivation of EC by the GABA agonist muscimol in a similar CER procedure. Muscimol infusion during pre-exposure disrupted LI, whereas inactivation during conditioning had no effect. Taken together, these and our results imply that the EC may be the site where the stimulus-no outcome association is formed during pre-exposure, but that this region is not involved in processes occurring in conditioning to both the PE and the novel stimulus. In addition, Lewis and Gould (2007a) found that inactivation of the EC during the test stage also disrupted LI, suggesting that the EC has a role in both the formation and the retrieval of the stimulus-no outcome contingency acquired in pre-exposure. As the present study has not tested the effects of muscarinic blockade during the test stage, we cannot extend this interpretation to the cholinergic innervation of the EC.

The finding that intra-BLA scopolamine produced persistent LI by action in conditioning implies that

impaired muscarinic transmission within the BLA does not affect the acquisition of the stimulus–no event association. In fact, animals with impaired muscarinic BLA transmission continue to behave according to the stimulus–no event association under conditions that normally do not support LI. Furthermore, although pharmacological and lesion-induced BLA perturbations, including intra-BLA scopolamine infusion (See *et al*, 2003), are known to impair conditioning (Cousens and Otto, 1998; Laurent and Westbrook, 2009; Schoenbaum *et al*, 2003; See *et al*, 2001), the dose of scopolamine used here did not impair conditioning in the NPE groups conditioned with either two or five trials. It should be noted that there was no behavioral difference between conditioning with two or five trials in the NPE groups suggesting a ceiling effect on performance, but in both cases suppression level was high leaving plenty of room for reduction by intra-BLA muscarinic blockade. In contrast to its lack of effect on conditioning in the NPE groups, intra-BLA scopolamine infusion reduced suppression in the PE group conditioned with five trials. Taken together, these outcomes suggest that muscarinic blockade within the BLA affects conditioning differentially to a PE and a novel stimulus, consistent with our previous results with BLA lesion (Schiller and Weiner 2004). Indeed, as can be seen in Figure 4, intra-BLA scopolamine infusion in conditioning led to the emergence of persistent LI by reducing conditioning in the PE group, while at the same time having no effect on the NPE group, although both groups had the same level of suppression in the vehicle condition.

The fact that intra-BLA scopolamine infusion impairs performance selectively in the PE group, and that this action is exerted in the conditioning stage, implies that muscarinic blockade in this region specifically impairs the capacity of the PE rats to switch responding from the stimulus–no outcome association acquired in pre-exposure to the stimulus–reinforcement association introduced in conditioning, under conditions triggering such a shift in controls. In other words, although vehicle-infused PE rats conditioned with five trials switch to respond according to the stimulus–reinforcement contingency acquired in conditioning, PE BLA scopolamine-infused rats are apparently unable to adjust their responding to the changed stimulus–reinforcement association in conditioning. This outcome is consistent with numerous findings and theories emphasizing the central role of BLA in the capacity to alter responding based upon changes in stimulus–outcomes relationships (Baxter *et al*, 2000; Corbit and Balleine, 2005; Hatfield *et al*, 1996; Helms and Mitchell, 2008; Malkova *et al*, 1997; Schoenbaum *et al*, 2003), and suggests that muscarinic transmission in the BLA contributes to cognitive flexibility related to outcome significance.

The above explanation is based on the view of LI as a performance/expression deficit resulting from a competition between the stimulus–no event and stimulus–reinforcement associations in conditioning (Weiner 1990, 2003; Bouton 1993). An alternative explanation could be derived from the more traditional attentional models of LI, which view LI as an acquisition deficit, namely, a failure to acquire the CS–reinforcement association as a consequence of the non-reinforced pre-exposure decreasing the attention to, or the associability, of the CS (Lubow, 1989; Lubow and

Kaplan, 2005; Mackintosh, 1975). According to these theories, effective conditioning in normal animals after pre-exposure reflects restored attention to, or associability of, the stimulus, which was reduced by its non-reinforced pre-exposure. Viewed in this light, muscarinic blockade in conditioning can be said to impair the capacity to re-attend to a previously irrelevant stimulus that regains relevance through pairings with reinforcement.

Whatever the precise cognitive mechanisms underlying the recently observed effects, the fact that in rats with impaired muscarinic transmission in the BLA, LI continues to be manifested also when the impact of conditioning increases to a level disrupting LI in normal rats, implies that in the intact brain muscarinic transmission in the BLA is necessary for neither the acquisition nor the expression of LI, but rather for the prevention of its expression (ie, disruption of LI) when the impact of conditioning increases. This stands in marked contrast to the role of the muscarinic transmission of the EC, which is required for the acquisition of LI. It is to be noted that given the anatomical proximity of the BLA to other amygdalar nuclei (ie, the central and lateral nuclei), we cannot completely rule out the possibility that scopolamine diffused to adjacent nuclei. However, as the central nucleus of the amygdala is involved in the expression of fear (LeDoux *et al*, 1988), and its lesions disrupt fear conditioning (Goosens and Maren, 2001), diffusion to this nucleus would be expected to disrupt fear conditioning; however, we did not observe such a disruption. Although we believe that the effects we observed resulted from muscarinic blockade in the BLA, if there indeed were diffusion to adjacent nuclei, this would imply that not only muscarinic transmission in the BLA but in the entire amygdala is unnecessary for the acquisition/expression LI, supporting the proposed amygdala–EC dissociation.

Figure 5a depicts a scheme of cholinergic modulation of LI expression, based on a model of brain circuitry regulating LI (Weiner, 1990, 2003), and models of cholinergic-related circuitries mediating attentional processing (Hasselmo and McGaughy, 2004; Sarter *et al*, 2005; Tzavara *et al*, 2004; Yeomans, 1995). In the model, LI expression depends on the nucleus accumbens (NAC) and its inputs from the EC, insular cortex (IC), BLA, and prefrontal cortex (PFC). Specifically, switching to respond according to the stimulus–reinforcement contingency (LI abolition) is subserved by increased dopamine (DA) release in the core, which can be inhibited by the shell through its control of DA input to the core from the ventral tegmental area (VTA). The critical input that subserves the inhibition of core switching, namely, the ‘CS–no event’ information, is relayed to the core by the shell, from the EC and the IC. BLA and PFC relay to the core the information on the motivational value of the CS, which promotes switching to respond according to the CS–reinforcement contingency. Inputs from the PFC and BLA to NAC core, and from the EC and IC to NAC shell (Brog *et al*, 1993; Chikama *et al*, 1997; Weiner, 2003; Weiner and Feldon, 1997; Jeanblanc *et al*, 2004), compete to enhance and reduce, respectively, DA release in the NAC core. BLA, EC, IC, and PFC receive cholinergic afferents from the BF, providing the substrate for cholinergically mediated modulation of these regions’ inputs to the NAC. Specifically, muscarinic blockade in the

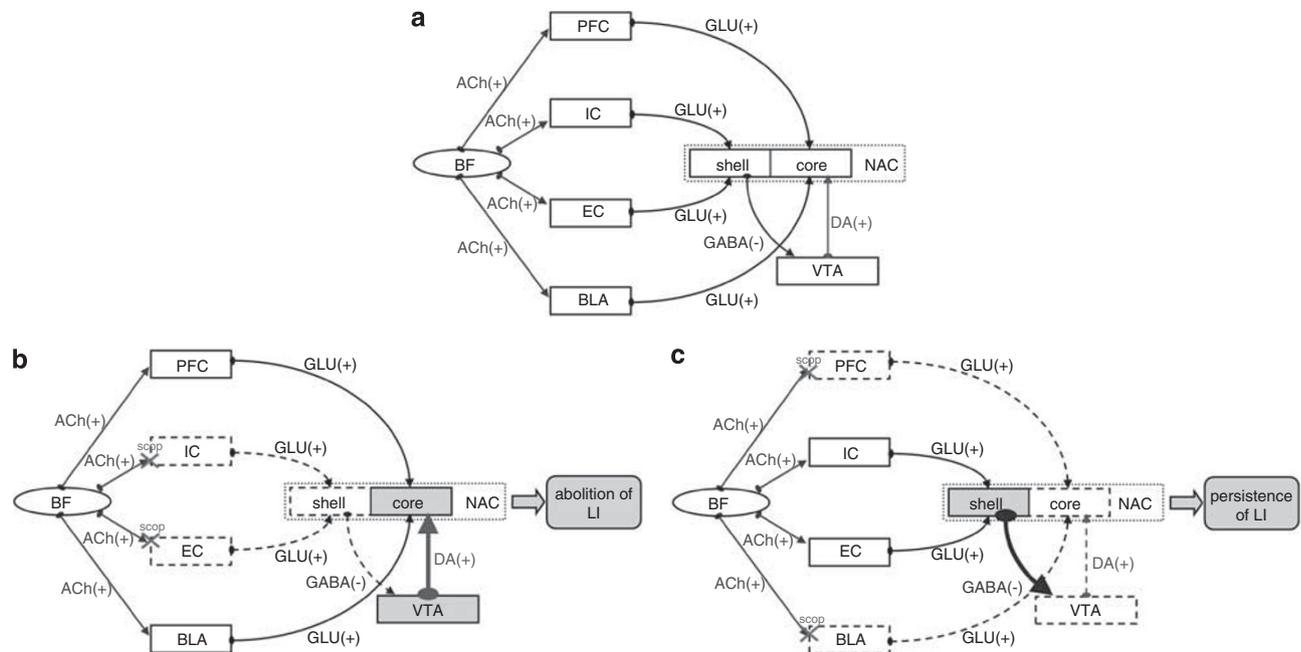


Figure 5 Neural circuitry through which cholinergic projections modulate the expression of latent inhibition. ACh, acetylcholine; BF, basal forebrain; BLA, basolateral amygdala; DA, dopamine; EC, entorhinal cortex; GLU, glutamate; IC, insular cortex; LI, latent inhibition; NAC, nucleus accumbens; PFC, prefrontal cortex; SCOP, scopolamine; VTA, ventral tegmental area. (a) The PFC, EC, IC, and BLA receive cholinergic afferents from the BF. Inputs from the PFC and the BLA to the NAC core, and from the EC and IC to the NAC shell enhance and reduce, respectively, DA release from the VTA to the NAC core. Increased and decreased DA levels in the NAC core are associated with LI abolition and persistence, respectively. (b) Muscarinic blockade in the EC or IC inhibits the inputs of these regions to the NAC shell, causing disinhibition of the VTA and enhancing DA release in the NAC core, and leading to abolition of LI. (c) Muscarinic blockade in the BLA inhibits its inputs to the NAC core. Concurrently, the NAC shell, which receives excitatory inputs from the IC and EC, sends inhibitory inputs to the VTA, reducing DA release in the NAC core. Both of these effects lead to LI persistence. Intra-PFC scopolamine infusion is expected to affect LI similarly. This model is based on the switching model of LI (Weiner, 1990, 2003; Weiner and Feldon, 1997), models of cholinergic-related circuitries mediating attentional processing (Hasselmo and McGaughy, 2004; Sarter *et al.*, 2005; Yeomans, 1995), and LI studies using muscarinic antagonists (Barak, 2009). Figure 5 is adapted from Barak (2009).

EC or IC inhibits the inputs of these regions to the NAC shell, causing disinhibition of the VTA and enhanced DA release in the NAC core, thus leading to LI abolition (Miranda and Bermudez-Rattoni, 2007; Naor and Dudai, 1996). The latter is further facilitated by concurrent excitatory inputs from the PFC and BLA to NAC core (see Figure 5b). Conversely, muscarinic blockade in the BLA inhibits the inputs of this brain region to the NAC core; concurrently, NAC shell, which receives excitatory inputs from the IC and EC, sends inhibitory inputs to the VTA, reducing DA release in the core. Both of these effects lead to LI persistence (see Figure 5c). Intra-PFC scopolamine is expected to affect LI similarly.

This scheme implies that muscarinic transmission in the regions described above mediate attentional processes underlying LI, and by extension, normal attentional processing. Conversely, abnormalities in muscarinic cholinergic transmission in these brain regions may underlie two poles of attentional aberrations manifested in abolished and persistent LI: distractibility caused by impaired ability to in-attend to irrelevant stimuli, mediated by muscarinic transmission in the EC and/or IC; and cognitive rigidity caused by impaired ability re-attend to stimuli that regain relevance, mediated by muscarinic transmission in the BLA and/or the PFC.

Disrupted and persistent LI are considered to model domains of pathology in schizophrenia (Weiner, 2003;

Weiner and Arad, 2009). In particular, scopolamine-induced disrupted LI is considered to model cholinergic-related positive symptoms (Barak, 2009; Barak and Weiner, 2007; Weiner and Arad, 2009) and scopolamine-induced persistent LI is considered to model antipsychotic drug-resistant cognitive impairments of schizophrenia (Barak, 2009; Barak and Weiner, 2009; Weiner and Arad, 2009). Abnormalities of the BLA and the EC have been documented in schizophrenia (Aleman and Kahn, 2005; Arnold, 2000; Benes and Berretta, 2000; Grace, 2000; Haber and Fudge, 1997). Thus, muscarinic transmission in these regions may have a critical role in these abnormalities.

In summary, our results suggest that in the intact brain, muscarinic transmission in the EC and in the BLA have contrasting roles in the regulation of LI expression. Muscarinic transmission in the EC is required for the acquisition of in-attention during pre-exposure, and is therefore essential for the formation of LI. In contrast, muscarinic transmission in the BLA is required for flexible re-attending to stimuli that signal different outcomes than previously experienced, and is therefore essential for LI abolition. These findings suggest that abnormalities in muscarinic cholinergic transmission in these brain regions may have a key role in the cognitive lability and cognitive rigidity observed in schizophrenia and other neuropsychiatric disorders.

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DISCLOSURE

The authors declare no conflict of interest.

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