

The M₁/M₄ preferring agonist xanomeline reverses amphetamine-, MK801- and scopolamine-induced abnormalities of latent inhibition: putative efficacy against positive, negative and cognitive symptoms in schizophrenia

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Abstract

A major challenge in developing schizophrenia pharmacotherapy is treating the different symptoms of this disorder, typically divided into positive, negative and cognitive symptoms. M₁/M₄ muscarinic acetylcholine receptor (mAChR) agonists have emerged as a promising therapeutic target, particularly for positive and cognitive symptoms. Here, we examined the activity of the M₁/M₄ mAChR-preferring agonist xanomeline in four pharmacological latent inhibition (LI) models. LI is the poorer conditioning to a stimulus previously experienced as irrelevant during repeated non-reinforced pre-exposure to that stimulus. No-drug controls displayed LI if non-reinforced pre-exposure to a tone was followed by weak, but not strong, conditioning (2 vs. 5 tone-shock pairings). Amphetamine (1 mg/kg)- or scopolamine (0.15 mg/kg)-treated rats failed to show LI with weak conditioning, whereas MK801 (0.05 mg/kg)- or scopolamine (1.5 mg/kg)-treated rats persisted in displaying LI with strong conditioning. Xanomeline (5 mg/kg, 15 mg/kg) reversed amphetamine- and scopolamine-induced LI disruption, effects considered predictive of activity against positive symptoms of schizophrenia. In addition, xanomeline alleviated MK801-induced abnormally persistent LI. Activity of xanomeline on NMDA antagonist-induced behaviour was demonstrated here for the first time and suggests that the drug is effective against negative/cognitive symptoms. Finally, xanomeline alleviated abnormally persistent LI induced by scopolamine, which was suggested to model antipsychotic drug-resistant cognitive impairments, providing further evidence for the cognition-enhancing capacity of xanomeline. Although the use of xanomeline in schizophrenia was discontinued due to cholinergic-related side-effects, our findings suggest that M₁/M₄ mAChR agonism should be an important target in drug development in schizophrenia, potentially beneficial for treatment of positive, negative and cognitive symptoms.

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Introduction

Schizophrenia symptoms segregate into positive, negative and cognitive symptoms. Antipsychotic drugs (APDs), while effective in ameliorating positive

symptoms, have limited efficacy in improving negative/cognitive symptoms (Buchanan *et al.* 2007; Miyamoto *et al.* 2005). In recent years, therapeutic strategies have focused on enhancing the function of the cholinergic system, because of its central role in cognition and evidence of cholinergic dysfunction in schizophrenia (Friedman, 2004; Raedler *et al.* 2007).

Among cholinergic function enhancers, M₁ and M₄ muscarinic acetylcholine (ACh) receptor (mAChR) agonists have emerged as particularly promising

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(Barak, 2009; Chan *et al.* 2008; Felder *et al.* 2001; Raedler *et al.* 2007). M_1 mAChR agonists have been shown to facilitate cognitive function in rodents and humans (Fisher, 2000; Friedman, 2004; Raedler *et al.* 2007). Relatedly, decreased M_1/M_4 mAChR density was found in the brains of schizophrenia patients (for review, see Scarr & Dean, 2008). Thus, M_1 , but not M_4 mAChR levels are decreased in the cortex of schizophrenia patients (Dean *et al.* 2002), whereas M_4 , but not M_1 levels are reduced in the hippocampus of schizophrenia patients (Scarr *et al.* 2007). These region-specific changes are consistent with the notion that deficits in cortical M_1 and hippocampal M_4 mAChR are associated with cognitive deficits and psychosis generation, respectively, in schizophrenia (Barak, 2009; Raedler *et al.* 2007; Scarr & Dean, 2008; Scarr *et al.* 2007). These data are supported by findings that $M_4^{-/-}$ knockout mice show phenotypes expected in a mouse model of psychosis (Wess *et al.* 2007), including elevated accumbal dopamine levels (Zhang *et al.* 2002) and increased spontaneous and dopamine agonist-induced locomotor activity (Gomez *et al.* 1999), whereas $M_1^{-/-}$ knockout mice have selective deficits in cortical-related cognition and memory (Anagnostaras *et al.* 2003; Wess *et al.* 2007), although they also show other schizophrenia-relevant abnormalities like elevated striatal dopamine levels and increased spontaneous and amphetamine-induced locomotor hyperactivity (Gerber *et al.* 2001; Miyakawa *et al.* 2001). Taken together, these findings suggest that stimulation of M_1 and M_4 mAChRs may be beneficial for schizophrenia symptoms, including cognitive impairments.

Xanomeline, an M_1/M_4 mAChR-preferring agonist (Shannon *et al.* 1994), exhibits antipsychotic-like effects in dopamine- and antimuscarinic-dependent rodent models such as amphetamine- and scopolamine-induced hyperactivity (Andersen *et al.* 2003; Stanhope *et al.* 2001; Woolley *et al.* 2009) and prepulse inhibition (PPI) deficit (Bymaster *et al.* 2002; Jones *et al.* 2005; Stanhope *et al.* 2001). In humans, xanomeline ameliorates cognitive impairments in Alzheimer's disease patients (Bodick *et al.* 1997a,b) and may be beneficial against positive, negative and cognitive symptoms in schizophrenia (Shekhar *et al.* 2008). Here, the activity of xanomeline was evaluated in the latent inhibition (LI) model of schizophrenia (Weiner, 2003; Weiner & Arad, 2009).

LI is the poorer conditioning to a previously exposed, inconsequential stimulus, compared to a novel stimulus. LI is a phenomenon of selective attention in the sense that it reflects a modulating effect of past experience on current performance, whereby organisms

ignore stimuli that had been irrelevant in the past in spite of their current relationship with a reinforcer. Since selective attention deficit is a core cognitive dysfunction of schizophrenia (Luck & Gold, 2008), LI abnormalities induced by psycho/schizo-mimetics in rodents are considered to model selective attention deficits associated with this disorder (Kilts, 2001; Lubow, 2005; Powell & Miyakawa, 2006; Weiner, 2003). The link between LI and schizophrenia is supported by the presence of LI abnormalities in schizophrenia patients (Cohen *et al.* 2004; Gray *et al.* 1992; Rasclé *et al.* 2001b; Salgado *et al.* 2000a; Thornton *et al.* 1996).

Previous studies have shown that LI abnormalities produced by different psycho/schizo-mimetics are distinguishable in terms of their behavioural manifestation (disrupted or abnormally persistent LI) and their amelioration by APDs and other schizophrenia-relevant treatments (see Weiner, 2003; Weiner & Arad, 2009). Briefly, the dopamine releaser amphetamine, which produces and exacerbates positive (psychotic) symptoms (Meltzer & Stahl, 1976), *disrupts LI* in rodents (Warburton *et al.* 1994; Weiner, 2003; Weiner *et al.* 1988), and this is paralleled by the capacity of this drug to disrupt LI in normal humans (Gray *et al.* 1992; Salgado *et al.* 2000b; Swerdlow *et al.* 2003; Thornton *et al.* 1996). Amphetamine-induced LI disruption in rodents is reversed by both typical and atypical APDs, consistent with their efficacy against positive symptoms (Moser *et al.* 2000; Weiner, 2003). LI disruption by amphetamine is a well established model of positive symptoms, and restoration of LI in amphetamine-treated rodents as well as LI potentiation, are widely used to evaluate antipsychotic properties (Gray *et al.* 1991; Moser *et al.* 2000; Weiner, 2003; Weiner & Arad, 2009). In contrast to amphetamine, NMDA antagonists (e.g. MK801, PCP, ketamine), which produce and exacerbate negative and cognitive symptoms (Krystal *et al.* 1994, 2003), produce an *abnormally persistent LI*, manifested under conditions preventing LI expression in non-manipulated controls (Barak *et al.* 2009; Black *et al.* 2008; Gaisler-Salomon & Weiner, 2003; Gaisler-Salomon *et al.* 2008; Lipina *et al.* 2005). MK801-induced persistent LI is reversed by atypical but not typical APDs (Gaisler-Salomon & Weiner, 2003), as well as by glycine enhancers and nicotinic or non-specific cholinomimetics (Barak & Weiner, 2006; Barak *et al.* 2009; Black *et al.* 2008; Gaisler-Salomon *et al.* 2008; Lipina *et al.* 2005). MK801-induced persistent LI is considered to model the negative/cognitive spectrum of schizophrenia symptoms (Gaisler-Salomon & Weiner, 2003; Gaisler-Salomon *et al.* 2008; Weiner & Arad, 2009), and this has been supported by demonstrations

of excessively strong LI in schizophrenia patients, which is positively correlated with negative symptoms severity (Cohen *et al.* 2004; Gal *et al.* 2009; Raschle *et al.* 2001*a*). Finally, the mAChR antagonist scopolamine, which produces psychotic and cognitive symptoms (Barak, 2009; Yeomans, 1995), produces both LI disruption and persistence at low and high doses, respectively (Barak, 2009; Barak & Weiner, 2007, 2009, 2010*b*). Scopolamine-induced disrupted LI, like amphetamine-induced disrupted LI, is reversed by both typical and atypical APDs, but scopolamine-induced persistent LI is resistant to both classes of APD; both scopolamine-induced abnormalities are reversed by the cholinergic and glycinergic cognitive enhancers, physostigmine and glycine (Barak & Weiner, 2007, 2009, 2010*b*). Consistent with these distinct pharmacological profiles, we have recently suggested that scopolamine-induced LI disruption models cholinergic-related positive symptoms, whereas scopolamine-induced LI persistence models APD-resistant cognitive impairments in schizophrenia (Barak, 2009; Barak & Weiner, 2007, 2009, 2010*b*). The present study tested the effects of xanomeline in these four LI models.

Materials and methods

Subjects

Male Wistar rats (Tel Aviv University Medical School, Israel) aged 3–4 months and weighing 340–510 g, were housed four per cage under a reversed 12-h light cycle (lights on 19:00 hours) with food and water available *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 number A5010–01, expiry date 30 September 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 (CER) procedure using Campden Instruments (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. 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. Over 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) or five (strong conditioning) tone-shock pairings given 5 min apart. Shock immediately followed tone termination. Weak conditioning produces LI in non-treated controls and thus allows the demonstration of treatment-induced LI disruption. This level of conditioning was therefore used with amphetamine and low scopolamine (expt 1). Conversely, strong conditioning prevents LI in non-treated controls and thus allows the demonstration of treatment-induced abnormally persistent LI. This level of conditioning was used with MK801 and high scopolamine treatments (expts 2 and 3).

Rebaseline

Rats were given a 15-min drinking session as 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 [see Supplementary Table 1 (available online) for raw data]. Time to complete licks 76–100 reflects the degree of suppression in drinking (reflecting rats' freezing) in response to tone presentation and is the measure of

rats' fear of the tone. If the animal is poorly conditioned (as normal PE rats), it does not fear the tone and therefore drinks licks 76–100 in a relatively short time, whereas if the rat is well conditioned (as normal NPE rats) it takes a longer time to complete licks 76–100. LI, namely, the poorer conditioning of PE compared to NPE rats, is manifested in significantly shorter log times to complete licks 76–100 by PE compared to NPE rats.

Drugs

Scopolamine HBr (0.15 and 1.5 mg/kg; Sigma, Israel), amphetamine (1 mg/kg; Sigma, Israel), MK801 (dizocilpine, 0.05 mg/kg; Sigma, Israel) and xanomeline tartrate (5 and 15 mg/kg; Lilly Research Laboratories, USA) were dissolved in saline. All drugs were administered in a volume of 1 ml/kg, 30 min prior to both pre-exposure and conditioning stages. The doses of scopolamine, amphetamine and MK801 were based on previous LI studies in our laboratory (e.g. Barak & Weiner, 2007, 2009, 2010*b*; Barak *et al.* 2009; Gaisler-Salomon & Weiner, 2003); the doses of xanomeline were based on previous behavioural studies with this drug (Carnicella *et al.* 2005; Jones *et al.* 2005). No-drug controls received the corresponding vehicle. All drugs were administered intraperitoneally, except for xanomeline, which was administered subcutaneously, as instructed by Eli Lilly.

Experimental design

Although all drugs were administered at the same time prior to the behavioural stages, for the ANOVA factors we denote the psycho/schizo-mimetic drugs as 'treatment' and the APDs as 'pre-treatment'.

Expt 1 tested the effects of xanomeline on scopolamine- and amphetamine-induced disrupted LI. The experiment included 18 groups in a $2 \times 3 \times 3$ design with main factors of pre-exposure (PE, NPE), treatment (vehicle, 0.15 mg/kg scopolamine, 1 mg/kg amphetamine), and pre-treatment (0, 5, 15 mg/kg xanomeline).

Expt 2 tested the effects of xanomeline on scopolamine-induced persistent LI. The experiment included 12 groups in a $2 \times 2 \times 3$ design with main factors of pre-exposure (PE, NPE), treatment (vehicle, scopolamine), and pre-treatment (0, 5, 15 mg/kg xanomeline).

Expt 3 tested the effects of xanomeline on MK801-induced persistent LI. The experiment included 12 groups in a $2 \times 2 \times 3$ design with main factors of pre-exposure (PE, NPE), treatment (vehicle, MK801), and pre-treatment (0, 5, 15 mg/kg xanomeline).

Since expts 2 and 3 used strong conditioning, the effects of xanomeline on the non-treated controls allowed the demonstration of xanomeline-induced LI potentiation.

Data analysis

Times to complete licks 51–75 and mean log times to complete licks 76–100 were analysed using a three-way ANOVA with main factors of pre-exposure, treatment (pro-psychotic drug) and pre-treatment (xanomeline doses). 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 xanomeline on amphetamine- and low scopolamine-induced disrupted LI

Since xanomeline was shown to have antipsychotic properties, here we tested its activity in the two LI models predictive of efficacy against positive symptoms, namely, amphetamine-induced LI disruption (Moser *et al.* 2000; Warburton *et al.* 1994; Weiner, 2003; Weiner & Arad, 2009), and low scopolamine-induced LI disruption (Barak, 2009; Barak & Weiner, 2007, 2009). Weak conditioning (two tone-shock pairings), which yields LI in no-drug animals, was used in this experiment to allow demonstration of LI disruption in amphetamine- or scopolamine-treated animals.

The experiment included 101 rats (run in two replications, $n=5-6$ per group). The 18 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 = 7.92 s; see Supplementary Table 1 for A period data by group). Figure 1 presents the mean log times to complete licks 76–100 (after tone onset) of the 18 experimental groups. As expected with weak conditioning, vehicle-injected rats showed LI, but scopolamine, as well as amphetamine, led to LI disruption. Xanomeline on its own did not affect LI, but reversed both scopolamine- and amphetamine-induced LI disruptions at both doses tested.

Three-way ANOVA with main factors of pre-exposure, treatment, and pre-treatment yielded main effects of pre-exposure [$F(1, 83)=85.45$, $p<0.0001$], treatment [$F(2, 83)=4.21$, $p<0.05$], and pre-treatment [$F(2, 83)=6.22$, $p<0.005$], as well as interactions of pre-exposure \times treatment [$F(2, 83)=5.41$, $p<0.01$], and pre-exposure \times pre-treatment [$F(2, 83)=8.44$, $p<0.001$]. *Post-hoc* comparisons revealed a significant difference between the PE and NPE groups in the vehicle-vehicle and all xanomeline-treated conditions

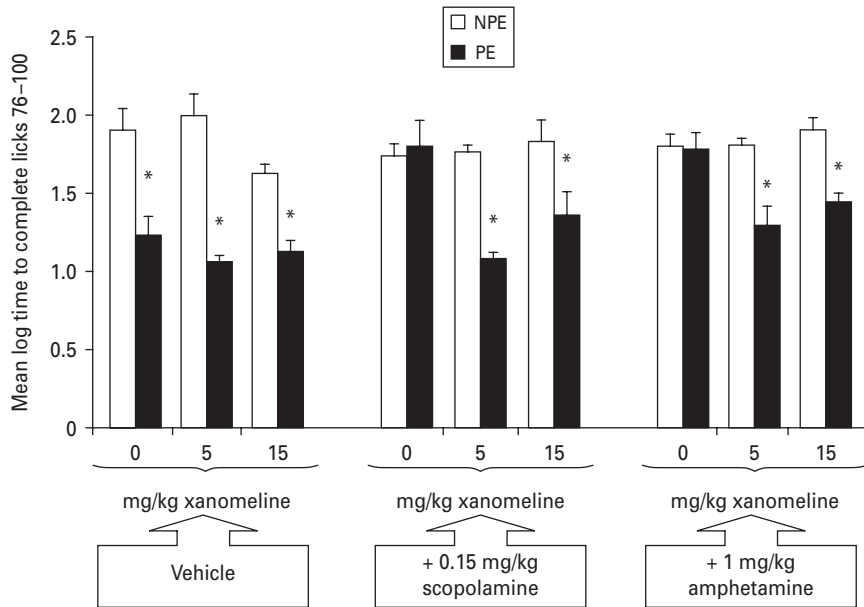


Fig. 1. Effects of xanomeline on scopolamine- and amphetamine-induced latent inhibition (LI) disruption. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and non-pre-exposed (NPE) vehicle-, scopolamine (0.15 mg/kg)-, or amphetamine (1 mg/kg)-treated rats, pre-treated with xanomeline (5 mg/kg, 15 mg/kg). Asterisk (*) indicates a significant difference between the PE and NPE groups, namely, presence of LI.

(p values <0.005), but not in conditions that received scopolamine or amphetamine alone.

Expt 2: effects of xanomeline on high scopolamine-induced persistent LI

Muscarinic cholinergic blockade has been used for decades to model cognitive impairments (for review see Klinkenberg & Blokland, 2010). We have recently shown that strong conditioning (five tone-shock pairings) prevents the expression of LI in no-drug animals, and a moderate-to-high dose of scopolamine induces persistent LI (Barak & Weiner, 2009). This attentional perseveration was resistant to typical and atypical APDs but was reversed by glycinergic and cholinergic cognitive enhancers (Barak, 2009; Barak & Weiner, 2009, 2010b). Here we tested whether xanomeline would reverse LI persistence induced by scopolamine, an effect that would indicate cognition-enhancing properties of this drug.

The experiment included 93 rats (run in two replications, $n=7-8$ per group). 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 = 7.25 s; see Supplementary Table 1). Figure 2 presents the mean log times to complete licks 76–100 (after tone onset) of the 12 experimental groups. As expected with strong conditioning, LI was absent in vehicle-treated rats, but rats that received scopolamine

persisted in expressing LI. Xanomeline on its own potentiated LI at 15 mg/kg but not at 5 mg/kg. In addition, xanomeline reversed scopolamine-induced LI persistence at 15 mg/kg, but not at 5 mg/kg.

Three-way ANOVA with main factors of pre-exposure, treatment, and pre-treatment yielded main effects of pre-exposure [$F(1, 81)=12.72$, $p<0.001$] and treatment [$F(1, 81)=13.32$, $p<0.0005$], as well as significant interactions of treatment \times pre-treatment [$F(2, 81)=4.09$, $p<0.025$], and pre-exposure \times treatment \times pre-treatment [$F(2, 81)=3.76$, $p<0.03$]. *Post-hoc* comparisons confirmed a significant difference between the PE and NPE groups in the scopolamine + vehicle ($p<0.05$), scopolamine + 5 mg/kg xanomeline ($p<0.005$) and vehicle + 15 mg/kg xanomeline conditions ($p<0.02$), but not in the remaining conditions.

Expt 3: effects of xanomeline on MK801-induced persistent LI

Thus far, to the best of our knowledge, xanomeline has not been shown to reverse NMDA antagonist-induced behavioural deficits, considered predictive of activity against negative/cognitive symptoms. Here we tested whether xanomeline would reverse MK801-induced persistent LI, indicating for the first time its efficacy against negative/cognitive symptoms.

The experiment included 72 rats (run in two replications, $n=6$ per group). The 12 experimental

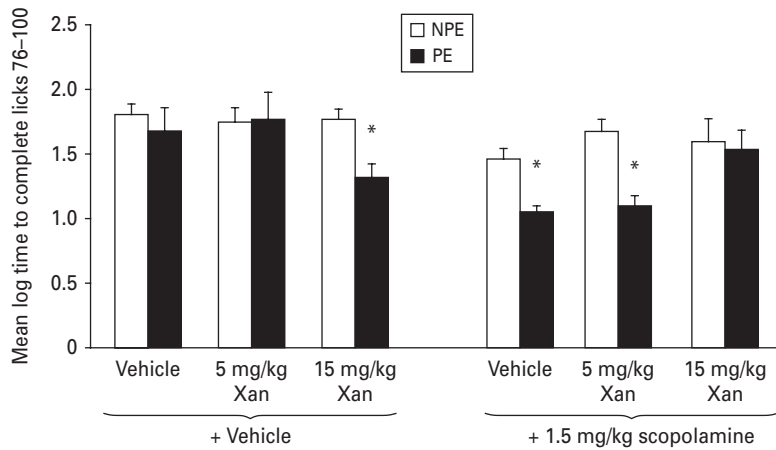


Fig. 2. Effects of xanomeline on scopolamine-induced persistent latent inhibition (LI). Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and non-pre-exposed (NPE) vehicle- or scopolamine (1.5 mg/kg)-treated rats, pre-treated with xanomeline (Xan; 5 mg/kg, 15 mg/kg). Asterisk (*) indicates a significant difference between the PE and NPE groups, namely, presence of LI.

groups did not differ in their times to complete licks 51–75 before tone onset (all p values >0.05 , overall mean A period = 7.09 s; see Supplementary Table 1). Figure 3 presents the mean log times to complete licks 76–100 (after tone onset) of the 12 experimental groups. LI was absent in vehicle-treated rats, but rats that received MK801 persisted in showing LI. Xanomeline on its own potentiated LI at 15 mg/kg but not at 5 mg/kg. In addition, xanomeline reversed MK801-induced LI persistence at 5 mg/kg but not at 15 mg/kg.

Three-way ANOVA with main factors of pre-exposure, treatment, and pre-treatment, yielded main effects of pre-exposure [$F(1, 60) = 14.71$, $p < 0.003$], as well as an interaction of pre-exposure \times treatment \times pre-treatment [$F(2, 60) = 3.43$, $p < 0.05$]. *Post-hoc* comparisons confirmed a significant difference between the PE and NPE groups in the MK801 + vehicle MK801 + 15 mg/kg xanomeline and vehicle + 15 mg/kg xanomeline conditions (p values < 0.05), but not in the remaining conditions.

Discussion

The aim of the present experiments was to profile the M_1/M_4 -preferring agonist xanomeline in four acute pharmacological models of LI. We show that xanomeline reversed amphetamine- and scopolamine-induced LI disruption. These models are considered predictive of activity against positive symptoms of schizophrenia (Barak, 2009; Barak & Weiner, 2007; Gray *et al.* 1991; Kilts, 2001; Lipska, 2004; Lipska & Weinberger, 2000; Moser *et al.* 2000; Powell &

Miyakawa, 2006; Smith *et al.* 2007; Weiner, 1990; Weiner, 2003; Weiner & Arad, 2009) and activity here was consistent with previous findings with xanomeline (Andersen *et al.* 2003; Bymaster *et al.* 2002; Carnicella *et al.* 2005; Mirza *et al.* 2003; Shannon *et al.* 2000; Stanhope *et al.* 2001). Xanomeline was also able to alleviate abnormally persistent LI produced by MK801 and scopolamine; these models are believed to model negative and cognitive aspects of schizophrenia (Barak *et al.* 2009; Barak & Weiner, 2009; Gaisler-Salomon & Weiner, 2003; Gaisler-Salomon *et al.* 2008; Lipina *et al.* 2005; Weiner, 2003; Weiner & Arad, 2009) and activity here is demonstrated for the first time.

Reversal of disrupted LI: efficacy for positive symptoms

Amphetamine-induced LI disruption and its reversal by both typical and atypical APDs is a long-standing model of positive symptoms (Warburton *et al.* 1994; Weiner, 2003; Weiner & Arad, 2009). We have recently shown that scopolamine-induced disrupted LI also qualifies to model positive symptoms because it is reversed by both typical and atypical APDs (Barak & Weiner, 2007), as has been shown for other scopolamine-induced psychosis-like deficits, e.g. disrupted PPI and locomotor hyperactivity (Jones *et al.* 2005; Shannon & Peters, 1990). Given the above, the finding that xanomeline reversed amphetamine- and scopolamine-induced disrupted LI indicates that this agent possesses antipsychotic properties. The latter conclusion is in line with previous findings showing that xanomeline can reverse psychosis-mimicking

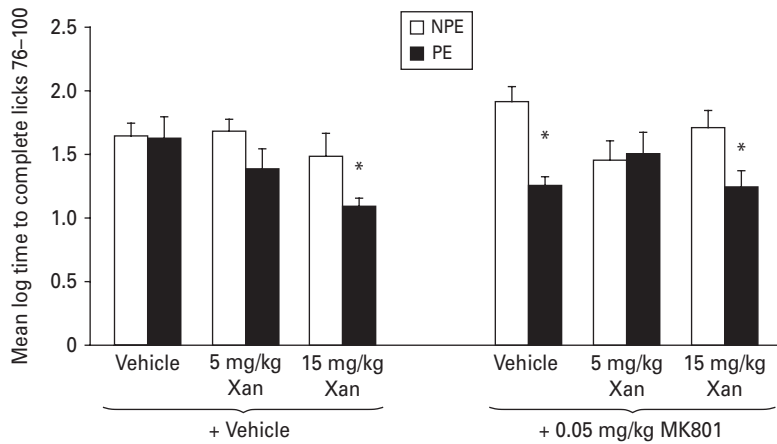


Fig. 3. Effects of xanomeline on MK801-induced persistent latent inhibition (LI). Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and non-pre-exposed (NPE) vehicle- or MK801 (0.05 mg/kg)-treated rats, pre-treated with xanomeline (Xan; 5 mg/kg, 15 mg/kg). Asterisk (*) indicates a significant difference between the PE and NPE groups, namely, presence of LI.

abnormalities induced by dopamine agonists and scopolamine in rodents, such as hyperactivity (Andersen *et al.* 2003; Stanhope *et al.* 2001; Woolley *et al.* 2009) and disrupted PPI (Bymaster *et al.* 2002; Jones *et al.* 2005; Stanhope *et al.* 2001).

The efficacy of xanomeline in reversing amphetamine- and scopolamine-induced disrupted LI sets it apart from the AChE inhibitor physostigmine, which is ineffective in the amphetamine model (Barak & Weiner, 2007). Since physostigmine is a non-specific, indirect cholinergic agonist, our results indicate that specific activation of M_1/M_4 mAChRs is more effective than an increase in ACh levels in reversing amphetamine-induced disrupted LI, and by extension, more effective in the treatment of dopamine-mediated positive symptoms.

Amphetamine-induced LI disruption is mediated by increased dopamine release in the nucleus accumbens (NAc) at the time of conditioning (Gray *et al.* 1997; Joseph *et al.* 2000). Therefore, reversal of this abnormality by xanomeline may be due to its capacity to inhibit dopaminergic cell activity in the ventral tegmental area (VTA) via its action at the mAChRs (Shannon *et al.* 2000). The capacity of xanomeline to increase prefrontal dopamine levels (Li *et al.* 2008; Stanhope *et al.* 2001) could be another mechanism underlying reversal of amphetamine-induced disruption of LI, since it would be expected to reduce mesolimbic dopamine function and thus block the behavioural effects of amphetamine (Goto & Grace, 2005, 2007; Grace, 1991; Jackson & Moghaddam, 2001).

Although scopolamine also increases NAc dopamine levels (Ichikawa *et al.* 2002; Yeomans, 1995), the

mechanisms suggested above as underlying xanomeline action in the amphetamine model cannot explain its action in the scopolamine model, because unlike amphetamine, scopolamine disrupts LI at the time of *pre-exposure* and not at *conditioning* (Barak & Weiner, 2007). We have recently shown, using intra-entorhinal cortex scopolamine infusion, that pre-exposure-based scopolamine-induced LI disruption is mediated by mAChRs blockade in the entorhinal cortex (Barak, 2009; Barak & Weiner, 2010a). Thus, it is possible that xanomeline competes with scopolamine at mAChRs in the entorhinal cortex during pre-exposure to prevent muscarinic blockade and reverse scopolamine's LI-disruptive effects. Of particular relevance to this possibility, xanomeline has been shown to exhibit a lengthy receptor-occupancy property (Jakubik *et al.* 2004), which is likely to contribute to its competition with scopolamine at mAChRs. Although it is not surprising that xanomeline, a muscarinic agonist, reverses the effects of scopolamine, it should be borne in mind that scopolamine is a non-specific muscarinic blocker, whereas xanomeline is an M_1/M_4 -preferring agonist. Therefore, the fact that xanomeline antagonized the behavioural effects of scopolamine suggests that these mAChR subtypes play a role in LI abnormalities induced by scopolamine.

Reversal of abnormally persistent LI: putative efficacy for negative and APD-resistant cognitive symptoms

As shown by us previously (Barak *et al.* 2009; Black *et al.* 2008; Gaisler-Salomon & Weiner, 2003;

Gaisler-Salomon *et al.* 2008; Lipina *et al.* 2005), here normal rats pre-exposed to 40 tones and conditioned with five trials did not show LI, but MK801-treated rats persisted in displaying LI. In other words, whereas PE rats treated with vehicle switched in the conditioning stage to respond according to the stimulus-reinforcement contingency, MK801-treated PE rats perseverated in responding according to the stimulus-no event contingency acquired in pre-exposure in spite of the repeated pairings of the stimulus with reinforcement. This outcome is consistent with findings showing that NMDA receptor (NMDAR) blockade induces behavioural and cognitive inflexibility (Carlsson & Carlsson, 1990; Jentsch & Taylor, 2001; Moghaddam *et al.* 1997; Svensson, 2000; van der Meulen *et al.* 2003). In the present study, we show that MK801-induced cognitive inflexibility was ameliorated by xanomeline.

NMDA antagonist-induced behavioural effects are typically reversed selectively by atypical APDs, and the latter are considered to imply efficacy in the treatment of negative/cognitive symptoms (Javitt & Zukin, 1991; Moghaddam & Jackson, 2003). Accordingly, MK801-induced persistent LI is reversed by atypical but not typical APDs (Gaisler-Salomon & Weiner, 2003; Weiner & Arad, 2009). In addition, MK801-induced persistent LI is reversed by a wide variety of agents enhancing NMDAR function (Black *et al.* 2008; Gaisler-Salomon *et al.* 2008) as well as by physostigmine and the α_7 nicotinic agonist SSR180711 (Barak *et al.* 2009; Barak & Weiner, 2006). To the best of our knowledge, we provide here the first evidence that behavioural deficits induced by NMDA antagonists can be reversed by xanomeline. Since NMDA antagonist behavioural abnormalities are considered to model negative/cognitive symptoms, this finding suggests that xanomeline has the capacity to ameliorate negative symptoms and cognitive abnormalities.

Given the recent reports that M_1 mAChR agonism can enhance NMDA function (Jones *et al.* 2008; Marino *et al.* 1998; Sur *et al.* 2003), the effectiveness of xanomeline in the MK801 model is likely to be mediated by its capacity to enhance NMDAR activity through activation of M_1 mAChRs in regions such as the prefrontal cortex (PFC), the hippocampus and the amygdala. This action of xanomeline is likely to be further facilitated by its capacity to enhance extracellular ACh levels in the medial PFC (Li *et al.* 2008), that would also activate M_1 mAChRs and enhance NMDAR activity. Interestingly, the fact that xanomeline reversed MK801-induced persistent LI at 5 mg/kg but not at 15 mg/kg, is consistent with the finding that the capacity of the M_1 agonist *N*-desmethylclozapine to

potentiate NMDAR shows an inverse dose-response curve (Sur *et al.* 2003). Another possibility is that xanomeline reversed MK801-induced persistent LI via its ability to bind to serotonergic receptors (Watson *et al.* 1998). In particular, xanomeline has been reported to possess 5-HT₂ antagonism (Watson *et al.* 1998). Such antagonism, e.g. by the selective 5-HT_{2A} antagonist M100907, reverses MK801-induced persistent LI (Gaisler-Salomon *et al.* 2008).

Perseveration in ignoring irrelevant stimuli akin to that produced by MK801 is also produced by high scopolamine, consistent with studies showing that this drug can induce perseveration (Chen *et al.* 2004; Ragazzino *et al.* 2002; Soffie & Lamberty, 1987). However, we have recently shown that the pharmacological profile of this LI abnormality differs from that of MK801-induced persistent LI as well as from that of low scopolamine-induced disrupted LI, since high scopolamine-induced persistent LI is resistant to both atypical and typical APDs (Barak & Weiner, 2009). We proposed that persistent LI induced by scopolamine represents an APD-resistant cognitive impairment, and as such may model cognitive deficits in schizophrenia, which are commonly resistant to APDs (Barak, 2009; Barak & Weiner, 2009; Weiner & Arad, 2009). The fact that xanomeline reverses high scopolamine-induced persistent LI supports the notion that this drug has the capacity to ameliorate cognitive deficits and indeed it may be effective in the treatment of APD-resistant cognitive impairments in schizophrenia. While the evidence for the cognition-enhancing capacity of xanomeline in animal studies is limited (Bymaster *et al.* 2002), our findings are in line with several reports of studies in human patients showing that xanomeline is effective in alleviating cognitive impairments in Alzheimer's disease and schizophrenia (Bodick *et al.* 1997a,b; Shekhar *et al.* 2008). Recently, we have shown that scopolamine-induced LI persistence is mediated by mAChR blockade in the basolateral amygdala (Barak & Weiner, 2010a). This could be one region where xanomeline acts to reverse scopolamine-induced persistent LI.

Xanomeline-induced persistent LI

Xanomeline given on its own produced LI persistence like high scopolamine and MK801. This outcome is puzzling and raises a question as to how LI persistence can model negative/cognitive symptoms on the one hand and predict an antipsychotic/anti-schizophrenia action on the other (Weiner & Arad, 2009). One accepted answer to this question is that an animal model can only be identified adequately by specifying both

the behavioural manifestation and the manipulation (such as the drug) used to induce the behavioural abnormalities (Geyer, 2008; Swerdlow *et al.* 2008; Weiner & Arad, 2009). Therefore, persistent LI induced by xanomeline, which improves schizophrenia symptoms and cognitive deficits in humans (Bodick *et al.* 1997*a,b*; Shekhar *et al.* 2008), is likely to be predictive of antipsychotic activity. Conversely, LI persistence induced by scopolamine, which is a pro-psychotic and induces cognitive impairment in humans, is likely to model schizophrenia-like cognitive impairment (Barak, 2009; Barak & Weiner, 2009). This conceptual differentiation assumes different mechanisms of action for the pro- and antipsychotic effects. Indeed, although the behavioural manifestation is the same, the mechanisms mediating xanomeline-induced persistent LI are unlikely to be similar to scopolamine- and MK801-induced persistent LI since the latter two are *antagonized* by xanomeline, suggesting that they actually exert opposing actions. Also in behavioural/performance terms, on its own xanomeline strengthens LI, whereas when given with scopolamine or MK801, it disrupts LI.

Behavioural and psychological profile of xanomeline

While the precise mechanisms underlying the effects of xanomeline seen here remain to be investigated, our results demonstrate that in the LI model this agent possesses a broad behavioural profile which consists of LI potentiation when given on its own, a reversal of amphetamine-induced and scopolamine-induced disrupted LI and a reversal of MK801-induced and high scopolamine-induced persistent LI. While it has been suggested that xanomeline possesses a profile of atypical APDs (Shannon *et al.* 2000), our results indicate that xanomeline is more effective than atypical APDs which do not reverse scopolamine-induced persistent LI. In fact, xanomeline is the most effective compound tested to date in the LI model (see Table 1), although it could reflect the fact that other promising compounds such as α_7 agonists have not been tested in all of the LI models.

It is important to note that in all of the models, xanomeline exerted its action selectively on the PE groups, without having any effects on conditioning *per se* in the NPE groups. Thus, this compound targets selectively the processes responsible for attentional selectivity without affecting associative capacity. This selectivity was further underscored by the fact that xanomeline exerts distinct and in fact opposite actions on LI: it *restores* LI in the amphetamine and low-scopolamine models, and *abolishes* LI in the

Table 1. The efficacy of xanomeline, antipsychotic drugs and other schizophrenia-relevant drug treatments in the amphetamine (Amph), low scopolamine (Low Scop), MK801 and high scopolamine (High Scop) latent inhibition (LI) models. Xanomeline is the only drug to date possessing effectiveness in all four models

Model ...	Disrupted LI		Persistent LI	
	Amph	Low Scop	MK801	High Scop
Symptom domain	Pos.	Pos.	Negative/ cognitive	Cognitive
Haloperidol	+	+	–	–
Clozapine	+	+	+	–
Glycine	–	+	+	+
Physostigmine	–	+	+	+
Xanomeline	+	+	+	+

Pos., Positive.

MK801 and high-scopolamine models. In psychological terms, reversal of disrupted and persistent LI can be seen as normalization of two poles of dysfunctional attentional control. On the one hand, xanomeline strengthens/restores the capacity to ignore irrelevant stimuli in amphetamine- and scopolamine-treated rats. On the other hand, xanomeline strengthens/restores the capacity to ‘dis-ignore’ irrelevant stimuli when they become relevant, enabling flexible re-deployment of attentional resources according to current situational demands, in MK801- and high-scopolamine-treated rats. The former would be beneficial in normalizing aberrantly increased salience perception and distractibility that are associated with psychotic symptoms (Gray *et al.* 1991; Kapur, 2003; Smith *et al.* 2006; Swerdlow & Koob, 1987; Weiner & Joel, 2002), whereas the latter would be beneficial in reducing cognitive inflexibility and inattention that are associated with negative/cognitive symptoms (Carlsson & Carlsson, 1990; Krystal *et al.* 2003; Moghaddam *et al.* 1997; Weiner, 2003).

The dual effect of xanomeline exerted on disrupted and persistent LI is particularly marked in that in terms of effects on performance, the drug influenced the pre-exposed MK801 and high-scopolamine groups in opposite direction from that of the pre-exposed amphetamine and low-scopolamine group, namely, improved conditioning in the two former groups and impaired conditioning in the latter two groups. Thus, the action of xanomeline may be seen as reflecting optimal cognitive enhancement, namely, optimization/

normalization of cognitive performance irrespective of the overt behavioural manifestation associated with such improvement.

In summary, based on its action in the four LI models tested here xanomeline emerges as a highly effective compound potentially beneficial for treatment of positive and negative symptoms as well as cognitive impairments in schizophrenia. Unfortunately, clinical studies with xanomeline have found side-effects associated with hyperactivation of peripheral cholinergic systems, which caused discontinuation of the treatment among patients, therefore critically limiting the clinical use of this drug (Bodick *et al.* 1997a; Mirza *et al.* 2003). However, given that abnormalities in M₁ and M₄ mAChRs have been demonstrated in schizophrenia, and agonism of M₁/M₄ mAChRs have been suggested to be beneficial for positive and cognitive symptoms (Raedler *et al.* 2007; Scarr & Dean, 2008), taken together with the fact that M₁ agonism was suggested as a potential target for cognition enhancement in schizophrenia by the NIH – Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) consensus committee (Marder, 2006) our results suggest that targeting these receptors may be a highly beneficial strategy for drug development in positive, negative and cognitive symptoms of schizophrenia.

Importantly, the fact that abnormalities in mAChRs in schizophrenia patients were shown to be region-specific might also have implications for the putative site of action of xanomeline. Thus, region-specific reduction in M₁ (Dean *et al.* 2002) and M₄ (Scarr *et al.* 2007) receptor levels was found in the PFC and hippocampus, respectively, of schizophrenia patients and has been associated with cognitive impairments and psychosis, respectively (Raedler *et al.* 2007; Scarr & Dean, 2008; Scarr *et al.* 2007). This raises the possibility that xanomeline might enhance cognitive function through activation of M₁ mAChRs in the PFC, and act as an APD through ligation to hippocampal M₄ mAChRs. However, this possibility also suggests that schizophrenia patients who lost a great majority of their cortical M₁ mAChRs would be less likely to benefit from pro-cognitive treatment with mAChR agonists such as xanomeline. In this respect, as we suggested above, xanomeline may act via multiple mechanisms and at different brain regions to ameliorate schizophrenia symptoms. These may include increasing dopamine and ACh levels in the cortex through action in cortex-projecting brain regions, blockade of 5-HT₂ receptors, and increasing NMDAR function in other brain regions through M₁ mAChRs. Thus, even with cortical mAChR deficiency, patients

are likely to benefit from treatment with xanomeline, and with M₁ agonists in general.

It has been suggested that the future of pharmacological treatment of schizophrenia will be characterized by the use of ‘selectively nonselective single compounds that can target multiple domains at once’ (Gray & Roth, 2007). Our study provides further evidence for the notion that M₁/M₄ muscarinic agonism may provide a potential pharmacological strategy for treating the wide spectrum of schizophrenia symptoms if new compound development can overcome the side-effects associated with cholinomimetic pharmacotherapy.

Note

Supplementary material accompanies this paper on the Journal’s website (<http://journals.cambridge.org/pnp>).

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Statement of Interest

None.

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