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RESEARCH PAPER

Involvement of decreased muscarinic receptor function in prepulse inhibition deficits in mice reared in social isolation

K Koda[1], Y Ago[1], K Yano[1], M Nishimura[1], H Kobayashi[1], A Fukada[1], K Takuma[1] and T Matsuda[1,2,3]

[1]*Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, Yamada-oka, Suita, Osaka, Japan,* [2]*Department of Experimental Disease Model, The Osaka-Hamamatsu Joint Research Center For Child Mental Development, Graduate School of Medicine, Osaka University, Yamada-oka, Suita, Osaka, Japan, and* [3]*United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Osaka University, Yamada-oka, Suita Osaka, Japan*

Correspondence
Professor T Matsuda, Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan.
E-mail: matsuda@phs.osaka-u.ac.jp

K Koda and Y Ago contributed equally to this work.

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BACKGROUND AND PURPOSE

We have previously reported that galantamine, a weak acetylcholinesterase inhibitor, improves prepulse inhibition (PPI) deficits in mice reared in social isolation. ACh receptors are involved in the underlying mechanism of PPI, but whether rearing in social isolation causes dysfunction of the cholinergic system is unknown. In this study, we examined the involvement of muscarinic receptors in the improvement of PPI deficits induced by galantamine, and whether the cholinergic system is altered in mice reared in isolation.

EXPERIMENTAL APPROACH

Three-week-old male ddY mice were housed in isolated cages for 6 weeks before the initiation of experiments to create PPI deficits. Cholinergic functions were determined by measuring the behavioural and neurochemical responses to nicotinic and muscarinic receptor agonists.

KEY RESULTS

The improvement by galantamine of social isolation-induced PPI deficits was blocked by scopolamine, a non-selective muscarinic antagonist, and telenzepine, a preferential M_1 receptor antagonist. Activation of M_1 receptors improved social isolation-induced PPI deficits. Social isolation did not affect choline acetyltransferase and acetylcholinesterase activities in the prefrontal cortex and hippocampus, but it reduced the locomotor-suppressive response to muscarinic agonist oxotremorine, but not to nicotine. The isolation also attenuated the M_1 receptor agonist *N*-desmethylclozapine-induced increase in prefrontal dopamine release.

CONCLUSIONS AND IMPLICATIONS

Galantamine improves PPI deficits of mice reared in social isolation via activation of M_1 receptors. Social isolation reduces the muscarinic, especially M_1, receptor function and this is involved in PPI deficits.

Abbreviations

ISI, inter-stimulus interval; PPI, prepulse inhibition

Introduction

Galantamine and donepezil, acetylcholinesterase inhibitors, are used for the treatment of Alzheimer's disease, and attempts have been made to use it for negative and cognitive symptoms of schizophrenia. Clinical studies have shown that galantamine improves negative and cognitive symptoms in schizophrenia (Allen and McEvoy, 2002; Rosse and Deutsch, 2002; Bora *et al.*, 2005), while donepezil does not (Friedman *et al.*, 2003). The underlying mechanism for the difference is not known. Prepulse inhibition (PPI) is an operational measure of the pre-attentive filtering process known as sensorimotor gating. Although no reliable relationship between schizophrenia symptoms and PPI performance have been established so far, animal models of PPI deficits have been used to study the underlying pathological mechanisms involved in schizophrenia and to predict the antipsychotic potential of new compounds. We have previously examined the effects of galantamine and donepezil on PPI deficits in animal models induced by apomorphine, MK-801 and rearing in social isolation, and observed that only the isolation-reared model showed a difference in the effect on PPI deficits between galantamine and donepezil (Koda *et al.*, 2008). That is, galantamine improved PPI deficits of mice reared in social isolation, while donepezil did not. This finding suggests that the isolation-reared PPI deficit model is useful for studies on the mechanism of the clinical effects of these drugs.

Previous studies have demonstrated that PPI is disrupted by muscarinic antagonists (Wu *et al.*, 1993; Jones and Shannon, 2000b). The role of muscarinic receptors in PPI has also been demonstrated in receptor knockout mice (Thomsen *et al.*, 2007; 2010). Bosch and Schmid (2008) suggested that activation of midbrain neurones can inhibit startle signalling through a cholinergic mechanism. These findings suggest that the muscarinic receptor system is involved in the underlying mechanisms of PPI of the startle reflex. However, it is not known whether the cholinergic system is involved in isolated rearing-induced deficits of PPI, since the brain cholinergic system has not been extensively studied in rodents reared in isolation, compared with the dopaminergic and 5-hydroxytrptaminergic (serotonergic) systems (Ago *et al.*, 2002; 2008; Fone and Porkess, 2008).

Previously, we demonstrated that the improvement of PPI deficits by galantamine was not blocked by nicotinic antagonists in isolation-reared mice (Koda *et al.*, 2008). In contrast, we found that galantamine-induced improvement of PPI deficits of apomorphine-treated mice was blocked by muscarinic antagonists (Yano *et al.*, 2009). In the present study, we examined, first, whether muscarinic receptors are involved in the improvement of deficits of PPI in mice reared in social isolation and, second, the effect of isolation-rearing on the brain cholinergic system, so as to address the possibility that muscarinic receptor dysfunction is related to PPI deficits in these mice.

Methods

Animals

Procedures involving animals and their care were conducted according to Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society. Three-week-old male ddY mice (Japan SLC Inc., Hamamatsu, Japan) were commercially purchased and divided equally into isolation- and group-housed conditions at the same time. The mice for the isolation group were individually housed for 6 weeks in wire-topped opaque polypropylene cages (measuring $24 \times 17 \times 12$ cm^3), while the control group continued to be housed under normal group-housed conditions (five to six per cage) in same-sized wire-topped clear plastic cages (Sakaue *et al.*, 2003; Ago *et al.*, 2008; Koda *et al.*, 2008). Mice were kept in cages with fir-chip bedding (SHIMIZU Laboratory Supplies Co., Ltd, Kyoto, Japan) and the bedding was changed once a week. All mice were housed under a standard 12-h light/dark cycle (lights on at 8 h 00 min.) at a constant temperature of 22 ± 1°C with free access to food and water throughout the experiments. We used a total of 590 mice in all the experiments; different mice were used in each experiment.

Measurement of startle response and PPI

Acoustic startle responses were measured in a startle chamber (SR-LAB®; San Diego Instruments, San Diego, CA, USA) as described previously (Koda *et al.*, 2008; Yano *et al.*, 2009). Muscarinic receptors are important in rodents at interstimulus intervals (ISIs) of 100–1000 ms, but not at 30 ms (Jones and Shannon, 2000a). In addition, Ukai *et al.* (2004) reported that scopolamine affects PPI at ISI of 100 ms in the ddY strain of mouse. Although mice exhibit higher levels of PPI at shorter ISIs of 30–50 ms (Dirks *et al.*, 2002; Varty *et al.*, 2001; 2006), isolation-rearing induces deficits of PPI in 129T2 and C57 BL/6 mice at ISIs of 50, 100 and 200 ms (Varty *et al.*, 2006) and in ddY mice at ISI of 100 ms (Sakaue *et al.*, 2003; Koda *et al.*, 2008). Thus, this study used the common 100 ms ISI between prepulse and startle pulse. Each test session began by placing a mouse in the Plexiglas cylinder where it was left undisturbed. After a background noise of 65 dB had been presented for a 5 min acclimatization period, each mouse was exposed to four consecutive blocks (Blocks 1–4) with a total of 100 trials over the approximately 30 min test session. One block consisted of 25 trials including five different trial types: pulse-alone trials in which a 40 ms broadband 120 dB burst was presented; three different prepulse-pulse trials in which the onset of a 20 ms broadband noise preceded the onset of the 120 dB startle pulse by 100 ms (prepulse intensities 3, 6 and 9 dB above the 65 dB background noise were used); and no-stimulation trials in which only the background noise was presented. Trials were presented in a pseudo-random order separated by an average of 15 s (range 7–23 s). The startle response was recorded for 100 ms (measuring the response every 1 ms) starting at the onset of each startle stimulus. The maximum startle amplitude recorded during the 100 ms sampling window was used as the dependent variable. Baseline startle responses were calculated as the average response to the pulse-alone trials in Blocks 2–4 after the startle habituation. PPI was also calculated as a percentage score for each prepulse trial type using the data for Blocks 2–4. The following formula was used: %PPI = 100 – {[(startle response to prepulse-pulse trial)/(startle response to pulse-alone trial)] × 100}.

Measurement of spontaneous locomotor activity

The locomotor activity of mice was measured using a digital counting system with an infrared sensor (Supermex®, Muromachi Kikai Co., Ltd, Tokyo, Japan) (Ago *et al.*, 2008). Immediately after injection of nicotine (Figure 4A) or oxotremorine (Figure 5A), mice were placed individually in a novel clear plastic cage ($24 \times 17 \times 12$ cm^3), and then locomotor activity was recorded for 30 min.

Measurement of rectal body temperature

Rectal body temperature of mice was measured using a BAT-12 digital thermometer coupled with a RET-3 rectal probe (Physitemp Instruments Inc., CA, USA) (Kawasaki *et al.*, 2006). Rectal body temperature was measured under basal (no injection) conditions and at 15, 30, 45 and 60 min after injection of nicotine (Figure 4B) or oxotremorine (Figure 5B).

Measurement of acetylcholinesterase activity

Mice were killed by decapitation under anaesthesia. The prefrontal cortex and hippocampus were rapidly dissected on ice, and then homogenized with 50 volumes of ice-cold 0.1 M phosphate-buffer (pH 8.0). The homogenate was centrifuged at 1000× *g* for 10 min at 4°C, and the supernatant was used as the source of acetylcholinesterase. Acetylcholinesterase activity was determined according to the method of Ellman *et al.* (1961), and expressed as nmol of acetylthiocholine hydrolyzed min^{-1}·mg^{-1} protein.

Measurement of choline acetyltransferase activity

Mice were killed by decapitation under anaesthesia. The prefrontal cortex and hippocampus were rapidly dissected on ice, and then homogenates (5%, w·v^{-1}) were prepared in 10 mM phosphate-buffer (pH 7.4) containing 10 mM EDTA. Choline acetyltransferase activity was determined according to the method of Shiba *et al.* (2006), and expressed as c.p.m. of [^{3}H]-ACh synthesized min^{-1}·mg^{-1} protein.

Surgery and microdialysis procedures

One day before the completion of rearing in isolation or group for 6 weeks, mice were anaesthetized with sodium pentobarbital (40 mg·kg^{-1}, i.p.) and stereotaxically implanted with a guide cannula for a dialysis probe (Eicom, Kyoto, Japan) unilaterally in the prefrontal cortex (A + 1.9 mm, L – 0.5 mm, V – 3.8 mm, from the bregma and skull) or ventral hippocampus (A – 3.0 mm, L – 2.8 mm, V – 4.2 mm) (Franklin and Paxinos, 1997). The cannula was cemented in place with dental acrylic, and the animal was kept warm and allowed to recover from anaesthesia. Postoperative analgesia was induced with a single injection of buprenorphine (0.1 mg·kg^{-1}, i.p.) (Ago *et al.*, 2008; 2009). The actively probed membranes were 3 mm long in the prefrontal cortex and 2 mm long in the hippocampus of mice. Two days after surgery, the probe was perfused with Ringer's solution (147.2 mM NaCl, 4.0 mM KCl and 2.2 mM $CaCl_2$; Fuso Pharmaceutical Industries, Ltd, Osaka, Japan) at a constant flow rate of 2 μL·min^{-1} for the dopamine assay (Figure 6). For the ACh assay, the probe was perfused with Ringer's solution containing 10 nM neostigmine at a constant flow rate of 1 μL·min^{-1} (Table 2). A stabilization period of 3 h was allowed before the onset of the experiments. Microdialysis samples (20 μL) were collected every 10 min for the dopamine assay or 20 min for the ACh assay, and injected immediately onto a high-performance liquid chromatography column for detection of dopamine (Ago *et al.*, 2008; 2009) and ACh (Ago *et al.*, 2006; Sato *et al.*, 2007), as previously reported. After the experiments, Evans Blue dye was microinjected through the cannula to verify the position of the probe histologically, and only data from animals with correct probe placement were used in the analysis.

Data analysis

All data are expressed as the mean ± SEM. Testing for normality was performed with the Kolmogorov-Smirnov test and for equal variance with Bartlett's test. For the acoustic startle response profile, baseline startle responses were analysed using two- or three-way analysis of variance (ANOVA) for pretreatment or/and rearing condition and treatment as the intersubject factors. Data for PPI were analysed using three- or four-way ANOVA for pretreatment or/and rearing condition and treatment as the intersubject factors and repeated measures with prepulse intensity as the intrasubject factor. The *post hoc* individual comparisons were performed with the Fisher's PLSD test (Table 1 and Figure 1–3). Data from the 'no stim' trials are not included in the results because the values were negligible, relative to values on trials containing startle stimuli. Data for choline acetyltransferase and acetylcholinesterase activities and basal extracellular ACh levels were analysed using Student's *t*-test (Table 2). For the functional receptor study, data for locomotor activity were analysed using two-way ANOVA followed by the Fisher's PLSD test (Figures 4A and 5A). Analyses for body temperature were made using three-way ANOVA for rearing condition and treatment as the intersubject factors and repeated measures with time as the intrasubject factor (Figures 4B and 5B). For *in vivo* microdialysis studies, all data were calculated as percentage change from the dialysate basal concentrations, with 100% defined as the average of three fractions before administration. Analyses were made using two-way ANOVA for treatment as the intersubject factor and repeated measures with time as the intrasubject factor (Figure 6). Statistical analyses were made using a software package StatView® 5.0 for Windows (SAS Institute, Cary, NC). A value of $P < 0.05$ was considered statistically significant.

Drugs

The following drugs were used: galantamine (Janssen Pharmaceutical K.K., Tokyo, Japan); oxotremorine, *N*-desmethylclozapine, telenzepine and nicotine (Sigma, St Louis, MO, USA); scopolamine (Wako Pure Chemical Industries, Ltd, Osaka, Japan). All other commercially available chemicals used in the experiments were of superfine quality. Galantamine, oxotremorine, scopolamine, telenzepine and nicotine were dissolved in saline (0.9% solution of NaCl).

Table 1

Effects of galantamine, scopolamine, telenzepine, oxotremorine and *N*-desmethylclozapine on baseline startle responses of mice reared in groups or in isolation

Treatment	Baseline startle responses	
	Group-reared mice	Isolation-reared mice
Galantamine		
Saline + Vehicle	424 ± 61	658 ± 47**
Saline + Galantamine	450 ± 74	508 ± 39$^{\#}$
Scopolamine + Vehicle	241 ± 42$^{\#}$	516 ± 70**
Scopolamine + Galantamine	330 ± 49	656 ± 77**
Telenzepine + Vehicle	475 ± 59	730 ± 42**
Telenzepine + Galantamine	543 ± 125	905 ± 199$^{\#}$
Oxotremorine		
Vehicle	602 ± 82	450 ± 70
0.01 mg·kg^{-1}	326 ± 63$^{\#}$	706 ± 124*
0.03 mg·kg^{-1}	289 ± 49$^{\#\#}$	779 ± 104**,$^{\#}$
N-Desmethylclozapine		
Vehicle	539 ± 58	602 ± 94
5 mg·kg^{-1}	415 ± 129	539 ± 75
10 mg·kg^{-1}	410 ± 124	621 ± 68

Mice were housed either in groups of five or six per cage or isolated for 6 weeks before experiments. Vehicle, galantamine (3 mg·kg^{-1}, i.p.), oxotremorine (0.01, 0.03 mg·kg^{-1}, i.p.) or *N*-desmethylclozapine (5, 10 mg·kg^{-1}, i.p.) was injected 30 min before experiments. Saline, scopolamine (0.1 mg·kg^{-1}, s.c.) or telenzepine (3 mg·kg^{-1}, s.c.) was administered 30 min before galantamine treatment. Data were obtained from the same experiments as Figures 1–3.

*P < 0.05, **P < 0.01, compared with respective treatment group in group-reared mice; #P < 0.05, ##P < 0.01, compared with corresponding vehicle or saline treatment group in each rearing condition using Fisher's PLSD *post hoc* test. For galantamine (Figure 1), three-way ANOVA revealed the significant main effects of rearing ($F_{1,152}$ = 29.202, P < 0.0001) and pretreatment ($F_{2,152}$ = 7.873, P = 0.0006), but not of treatment ($F_{1,152}$ = 1.565, P > 0.05), and no significant interaction between rearing, pretreatment and treatment ($F_{2,152}$ = 0.893, P > 0.05). For oxotremorine, two-way ANOVA revealed the significant main effect of rearing ($F_{1,77}$ = 9.863, P = 0.0024), but not of treatment ($F_{2,77}$ = 0.017, P > 0.05), and there is the significant interaction between rearing and treatment ($F_{2,77}$ = 7.616, P = 0.0010). For *N*-desmethylclozapine, two-way ANOVA revealed no significant main effects of rearing ($F_{1,79}$ = 3.210, P > 0.05) and treatment ($F_{2,79}$ = 0.586, P > 0.05), and there is no significant interaction between rearing and treatment ($F_{2,79}$ = 0.343, P > 0.05).

N-Desmethylclozapine was dissolved in a small amount of 0.1 M tartaric acid and the pH adjusted to 6–7 with 0.1 N NaOH (Li *et al.*, 2005). Drugs were administered in a volume of 10 mL·kg^{-1} i.p. (galantamine, oxotremorine, *N*-desmethylclozapine) or s.c. (scopolamine, telenzepine, nicotine). The nomenclature used for the receptors, agonists and antagonists follows that of BJP's Guide to Receptors and Channels (Alexander *et al.*, 2009).

Results

Effects of muscarinic antagonists on galantamine-induced reversal of PPI deficits in isolation-reared mice

Social isolation caused a marked reduction in PPI of the acoustic startle response in mice. Galantamine (3 mg·kg^{-1}, i.p.) reversed the isolation-induced PPI deficits, as reported previously (Koda *et al.*, 2008). Scopolamine (0.1 mg·kg^{-1}, s.c.), a non-selective muscarinic antagonist, reduced PPI of group-reared mice at 74 dB prepulse intensity, whereas it did not affect that of isolation-reared mice. Telenzepine (3 mg·kg^{-1}, s.c.), a preferential M_1 receptor antagonist, did not affect PPI of either group- or isolation-reared mice. Under our conditions, galantamine-induced reversal of PPI deficits in isolation-reared mice was significantly attenuated by scopolamine and telenzepine (Figure 1). Scopolamine decreased the baseline startle responses of group-reared mice and telenzepine increased the baseline startle responses of galantamine-treated isolation-reared mice (Table 1).

Effects of muscarinic agonists on social isolation-induced deficits in PPI of the acoustic startle response

Social isolation-induced PPI deficits in mice were improved by oxotremorine (0.01, 0.03 mg·kg^{-1}, i.p.), a non-selective muscarinic agonist, and *N*-desmethylclozapine (5, 10 mg·kg^{-1}, i.p.), a preferential M_1 receptor agonist (Figures 2 and 3), whereas neither oxotremorine nor *N*-desmethylclozapine alone affected PPI of group-reared mice. Oxotremorine increased and decreased the baseline startle responses of isolation- and group-reared mice, respectively. *N*-Desmethylclozapine did not affect the baseline startle responses of either group (Table 1).

Choline acetyltransferase and acetylcholinesterase activities and basal extracellular ACh levels in the brain of isolation-reared mice

To evaluate the effect of social isolation on ACh metabolism in the brain, we determined the choline acetyltransferase and acetylcholinesterase activities and basal extracellular levels of ACh in the prefrontal cortex and hippocampus of group- and isolation-reared mice (Table 2). There were no significant differences in choline acetyltransferase and acetylcholinesterase activities and basal extracellular ACh levels between the groups.

Effect of social isolation on nicotine-induced hypolocomotion and hypothermia in mice

Nicotine induces hypolocomotion and hypothermia through nicotinic receptors (Zarrindast *et al.*, 2001; Weiss *et al.*, 2007). Nicotine (1, 3 mg·kg^{-1}, s.c.) dose-dependently decreased locomotor activity (Figure 4A) and body temperature

Table 2

Choline acetyltransferase and acetylcholinesterase activities and basal extracellular ACh levels in the brain of mice

Region	Rearing	Choline acetyltransferase c.p.m.·min^{-1}·mg^{-1}	Acetylcholinesterase nmol·min^{-1}·mg^{-1}	Basal ACh levels fmol
Prefrontal cortex	Group	409 ± 12	1.61 ± 0.09	210 ± 21
	Isolation	409 ± 16	1.73 ± 0.10	228 ± 59
Hippocampus	Group	373 ± 8	2.29 ± 0.20	130 ± 20
	Isolation	387 ± 12	2.25 ± 0.20	101 ± 4

Mice were housed either in groups of five or six per cage or isolated for 6 weeks before experiments. Basal extracellular levels of ACh in dialysate (not corrected for *in vitro* probe recovery) are expressed as fmol per fraction (20 µL). Data are expressed as the mean ± SEM from 3–6 mice. Choline acetyltransferase and acetylcholinesterase activities and basal extracellular ACh levels were not affected by social isolation ($P > 0.05$ using Student's *t*-test).

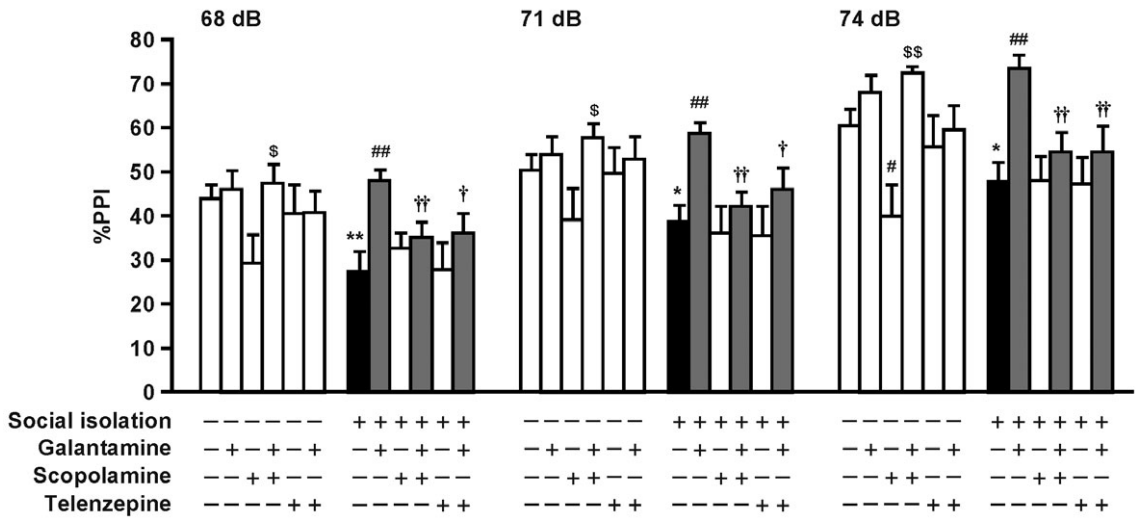

Figure 1

Effects of scopolamine and telenzepine on galantamine-induced reversal of PPI deficits in isolation-reared mice. Mice were housed either in groups of five or six per cage or isolated for 6 weeks before experiments. Vehicle or galantamine (3 mg·kg^{-1}, i.p.) was injected 30 min before experiments. Saline, scopolamine (0.1 mg·kg^{-1}, s.c.) or telenzepine (3 mg·kg^{-1}, s.c.) was administered 30 min before galantamine treatment. Data are expressed as the mean ± SEM from 10–16 mice. *$P < 0.05$, **$P < 0.01$, compared with vehicle alone treatment group of group-reared mice; $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, compared with scopolamine alone treatment group of group-reared mice; #$P < 0.05$, ##$P < 0.01$, compared with respective vehicle alone treatment group of isolation- and group-reared mice; †$P < 0.05$, ††$P < 0.01$, compared with galantamine alone treatment group of isolation-reared mice using Fisher's PLSD *post hoc* test, following repeated measures four-way ANOVA [significant main effects of prepulse intensity ($F_{2,304} = 181.430$, $P < 0.0001$), treatment (galantamine) ($F_{1,152} = 16.630$, $P < 0.0001$), rearing ($F_{1,152} = 5.881$, $P = 0.0165$), but not of pretreatment (muscarinic antagonists) ($F_{2,152} = 2.652$, $P > 0.05$); significant interaction between treatment, rearing and pretreatment ($F_{2,152} = 3.997$, $P = 0.0203$)].

(Figure 4B) in group- and isolation-reared mice, and these effects did not differ between the groups.

Effect of social isolation on oxotremorine-induced hypolocomotion and hypothermia in mice

Muscarinic agonists induce hypothermia via activation of M_2 and M_3 receptors (Sánchez and Lembøl, 1994; Gomeza *et al.*, 1999) and hypolocomotion possibly via activation of M_1/M_4 receptors (Sánchez *et al.*, 1998; Woolley *et al.*, 2009). Oxotremorine dose-dependently decreased locomotor activity in group- and isolation-reared mice, and the effect of oxotremorine at doses of 0.03 mg·kg^{-1} was less in isolation-reared mice than in group-reared mice (Figure 5A). On the other hand, oxotremorine (0.05, 0.1 mg·kg^{-1}, i.p.) significantly decreased body temperature in the group- and isolation-reared mice, but this effect did not differ between the groups (Figure 5B).

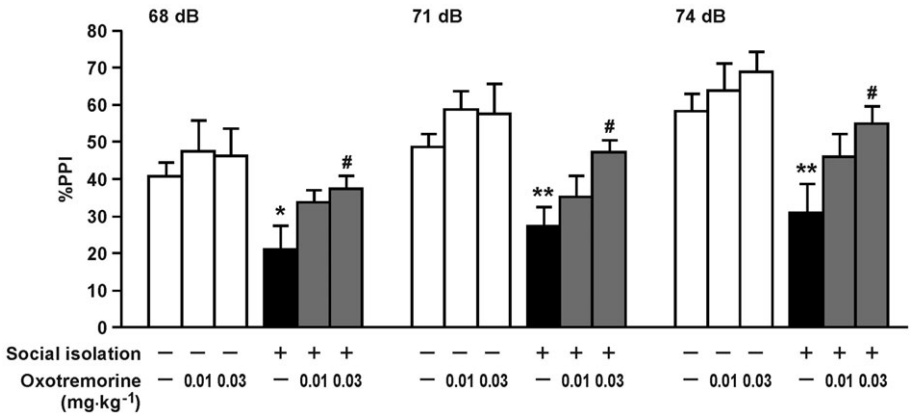

Figure 2

Effect of oxotremorine on social isolation-induced deficits in PPI of the acoustic startle response in mice. Mice were housed either in groups of five or six per cage or isolated for 6 weeks before experiments. Vehicle or oxotremorine (0.01, 0.03 mg·kg^{-1}, i.p.) was injected 30 min before experiments. Data are expressed as the mean ± SEM from 10–17 mice. *P < 0.05, **P < 0.01, compared with group-reared mice; #P < 0.05, compared with vehicle treatment group of isolation-reared mice using Fisher's PLSD *post hoc* test, following repeated measures three-way ANOVA [significant main effects of prepulse intensity ($F_{2,154}$ = 57.435, P < 0.0001), treatment ($F_{2,77}$ = 3.702, P = 0.0292) and rearing ($F_{1,77}$ = 14.504, P = 0.0003); no significant interaction between treatment and rearing ($F_{2,77}$ = 0.599, P > 0.05)].

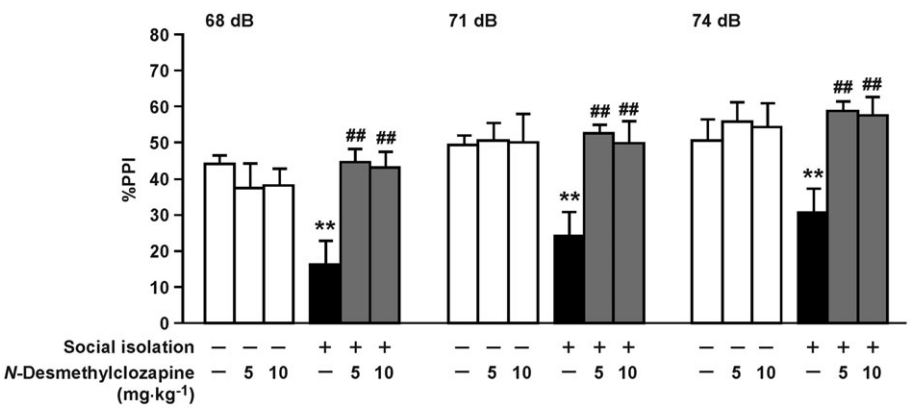

Figure 3

Effect of *N*-desmethylclozapine on social isolation-induced deficits in PPI of the acoustic startle response in mice. Mice were housed either in groups of five or six per cage or isolated for 6 weeks before experiments. Vehicle or *N*-desmethylclozapine (5, 10 mg·kg^{-1}, i.p.) was injected 30 min before the experiments. Data are expressed as the mean ± SEM from 10–17 mice. **P < 0.01, compared with group-reared mice; ##P < 0.01, compared with vehicle treatment group of isolation-reared mice using Fisher's PLSD *post hoc* test, following repeated measures three-way ANOVA [significant main effects of prepulse intensity ($F_{2,158}$ = 30.827, P < 0.0001) and treatment ($F_{2,79}$ = 5.676, P = 0.0050), but not of rearing ($F_{1,79}$ = 2.190, P > 0.05); significant interaction between treatment and rearing ($F_{2,79}$ = 5.887, P = 0.0041)].

Effect of social isolation on N-desmethylclozapine-induced increases in extracellular dopamine levels in the prefrontal cortex

N-Desmethylclozapine, an M_1 receptor agonist, increased extracellular dopamine levels in the prefrontal cortex of rats (Li *et al.*, 2005; 2009). *N*-Desmethylclozapine (5 mg·kg^{-1}, i.p.) caused robust increases in prefrontal dopamine levels of group-reared mice and this effect was blocked by telenzepine (1 mg·kg^{-1}, s.c.), suggesting the involvement of M_1 receptors in the dopamine release (Figure 6A). Under this condition, the effect of *N*-desmethylclozapine was less in isolation-reared mice than in group-reared mice (Figure 6B). Basal extracellular dopamine levels (not corrected for *in vitro* probe recovery) did not differ between the group- (2.45 ± 0.26 fmol 20 µL^{-1}; n = 20) and isolation-reared (2.19 ± 0.30 fmol 20 µL^{-1}; n = 10) mice (Figure 6A and B).

Discussion

A disruption of cerebral cholinergic pathways may contribute to the cognitive deficits of schizophrenia, and acetylcholinesterase inhibitors and ACh receptor agonists have a

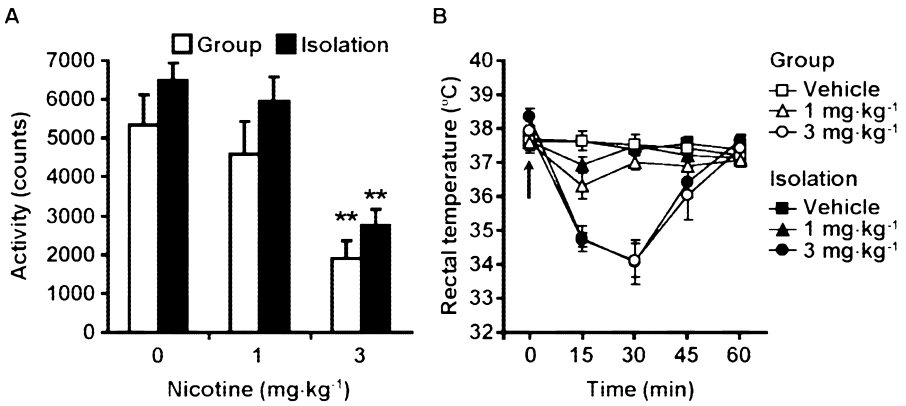

Figure 4

Effect of social isolation on nicotine-induced hypolocomotion and hypothermia in mice. Mice were housed either in groups of five or six per cage or isolated for 6 weeks before experiments. Total locomotor activity for 30 min (A) and rectal body temperature during 60 min (B) after s.c. injection of nicotine at indicated doses were measured in group- and isolation-reared mice. Data are expressed as the mean ± SEM from 6–12 (A) and 6–8 (B) mice. $^{**}P < 0.01$, compared with vehicle-treated mice using Fisher's PLSD *post hoc* test, following two-way ANOVA [main effects of rearing ($F_{1,45} = 5.056$, $P = 0.0295$) and treatment ($F_{2,45} = 22.227$, $P < 0.0001$); no significant interaction between rearing and treatment ($F_{2,45} = 0.101$, $P > 0.05$)] (A). Repeated measures three-way ANOVA indicated that nicotine induced significant decreases in rectal body temperature in group- and isolation-reared mice, but there was no difference between the two groups [significant main effects of treatment ($F_{2,38} = 15.017$, $P < 0.0001$), time ($F_{4,152} = 59.151$, $P < 0.0001$), but not of rearing ($F_{1,38} = 0.525$, $P > 0.05$); no significant interaction between treatment, rearing and time ($F_{8,152} = 0.485$, $P > 0.05$)] (B).

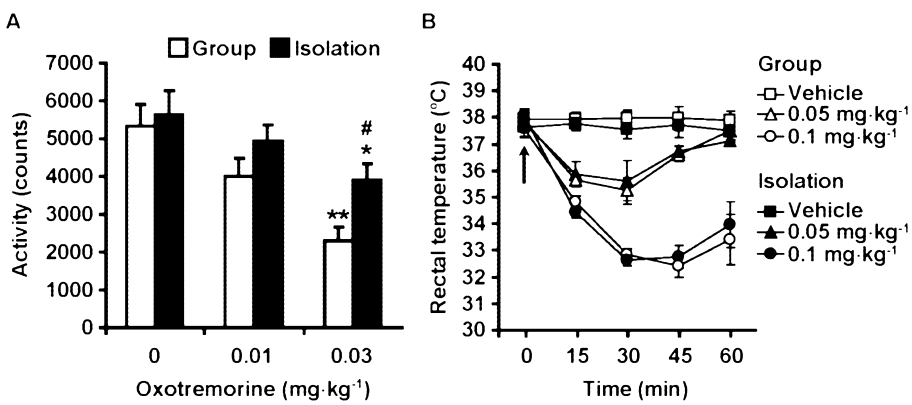

Figure 5

Effect of social isolation on oxotremorine-induced hypolocomotion and hypothermia in mice. Mice were housed either in groups of five or six per cage or isolated for 6 weeks before experiments. Total locomotor activity for 30 min (A) and rectal body temperature for 60 min (B) after i.p. injection of oxotremorine at the indicated doses were measured in group- and isolation-reared mice. Data are expressed as the mean ± SEM from 10–12 (A) and 5–6 (B) mice. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with vehicle-treated mice; $^{\#}P < 0.05$, compared with group-reared mice using Fisher's PLSD *post hoc* test, following two-way ANOVA [main effects of rearing ($F_{1,60} = 5.465$, $P = 0.0228$) and treatment ($F_{2,60} = 11.207$, $P < 0.0001$); no significant interaction between rearing and treatment ($F_{2,60} = 0.821$, $P > 0.05$)] (A). Repeated measures three-way ANOVA indicated that oxotremorine produced the significant decreases in rectal body temperature in group- and isolation-reared mice, but there was no difference between two groups [significant main effects of treatment ($F_{2,25} = 84.421$, $P < 0.0001$), time ($F_{4,100} = 54.052$, $P < 0.0001$), but not of rearing ($F_{1,25} = 0.035$, $P > 0.05$); no significant interaction between treatment, rearing and time ($F_{8,100} = 0.655$, $P > 0.05$)] (B).

therapeutic potential to improve such deficits (Friedman, 2004). We have recently reported that galantamine, but not donepezil, improved social isolation-induced PPI disruption in mice (Koda *et al.*, 2008). These drugs are acetylcholinesterase inhibitors, additionally galantamine is also an allosteric modulator of nicotinic receptor activity (Dajas-Bailador *et al.*, 2003; Samochocki *et al.*, 2003). However, in our previous study, we showed that the improvement of PPI deficits induced by galantamine was not blocked by nicotinic antagonists (mecamylamine and methyllycaconitine) (Koda *et al.*, 2008). In the present study, it was demonstrated that the muscarinic antagonists scopolamine and telenzepine attenuate galantamine-induced improvement of social isolation-induced PPI deficits. Scopolamine is a non-selective muscarinic antagonist, and telenzepine is a preferential M_1 receptor antagonist (Eltze *et al.*, 1985; Doods *et al.*, 1987;

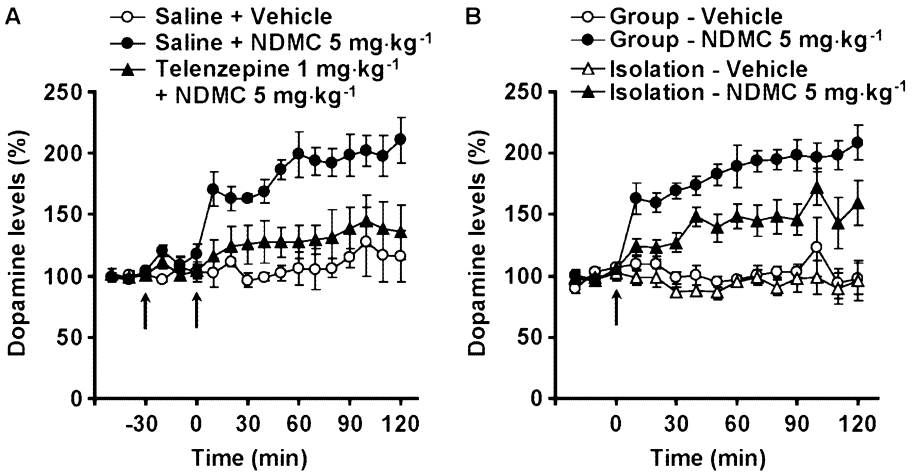

Figure 6

Effect of social isolation on *N*-desmethylclozapine-induced increases in prefrontal dopamine levels in mice. Mice were housed either in groups of five or six per cage or isolated for 6 weeks before experiments. (A) Vehicle or *N*-desmethylclozapine (NDMC; 5 mg·kg^{-1}) was injected i.p. in group-reared mice at 0 min (right arrow). Saline or telenzepine (1 mg·kg^{-1}, s.c.) was injected 30 min before NDMC treatment (left arrow). Data are expressed as the mean ± SEM from 3–4 mice. Repeated measures two-way ANOVA indicated that NDMC significantly increased dopamine levels [interaction (treatment × time): $F_{17,85} = 6.892$, $P < 0.0001$], and this effect was blocked by telenzepine ($F_{17,102} = 4.467$, $P < 0.0001$). (B) Vehicle or *N*-desmethylclozapine (NDMC; 5 mg·kg^{-1}) was injected i.p. in group- and isolation-reared mice at 0 min (arrow). Data are expressed as the mean ± SEM from 4 – 6 mice. Repeated measures two-way ANOVA indicated that NDMC produced the significant increases in dopamine levels in group- [interaction (treatment × time): $F_{14,98} = 13.798$, $P < 0.0001$] and isolation-reared ($F_{14,112} = 4.143$, $P < 0.0001$) mice, and this increasing effect was less in isolation-reared mice than in group-reared mice ($F_{14,126} = 2.992$, $P = 0.0006$).

Bymaster *et al.*, 1993). Therefore, it is likely that the effect of galantamine on the PPI deficits is mediated by M_1 receptors. In agreement with this suggestion, we found that the muscarinic agonists oxotremorine and *N*-desmethylclozapine, like galantamine, improved the isolation-induced PPI deficits in mice. Oxotremorine is a non-selective muscarinic agonist, and *N*-desmethylclozapine is a preferential M_1 receptor agonist (Sur *et al.*, 2003; Weiner *et al.*, 2004; Li *et al.*, 2005). A similar muscarinic receptor-mediated effect of galantamine was shown in apomorphine-induced PPI deficit mice (Yano *et al.*, 2009). Although galantamine is a weak acetylcholinesterase inhibitor, our microdialysis study demonstrated that systemic administration of galantamine increases the extracellular ACh levels in mouse cerebral cortex by a mechanism that is dependent on acetylcholinesterase inhibition and also an independent mechanism (Yano *et al.*, 2009). The ability of galantamine to increase extracellular ACh levels may contribute to its muscarinic mechanism.

Since the cholinergic system is involved in PPI, it is possible that the isolation-induced PPI deficits may be due to dysfunction of the cholinergic system in isolation-reared mice. To address this possibility, the present study examined the effect of isolation rearing on brain cholinergic functions. Choline acetyltransferase, a key enzyme in ACh synthesis, and acetylcholinesterase, an enzyme for ACh metabolism, are distributed extensively throughout the brain. These enzyme activities influence a wide range of cholinergic-dependent neurophysiological functions including cognitive performance and PPI (Beeri *et al.*, 1995; Sarter and Bruno, 1997; Ballmaier *et al.*, 2001; 2002; Wang *et al.*, 2009). However, the results of the present study show that there are no significant differences in the choline acetyltransferase and acetylcholinesterase activities and basal extracellular ACh levels between group- and isolation-reared mice. On the other hand, we found by measuring the cholinergic receptor agonist-induced behavioural responses that muscarinic receptor function is reduced in isolation-reared mice. Muscarinic agonists can cause hypolocomotion by activation of M_1 or/and M_4 receptors (Sánchez *et al.*, 1998; Woolley *et al.*, 2009), and hypothermia by activation of M_2 and M_3 receptors (Sánchez and Lembøl, 1994; Gomeza *et al.*, 1999). We showed that oxotremorine-induced hypolocomotion was reduced in social isolation-reared mice, while oxotremorine-induced hypothermia did not differ between group- and isolation-reared mice. Therefore, it is likely that the function of M_1 or/and M_4, but not M_2 and M_3, receptors is reduced in isolation-reared mice. We further examined, using a brain microdialysis technique, whether isolation rearing causes the dysfunction of M_1 receptors. Li *et al.* (2005) have reported that *N*-desmethylclozapine, a preferential M_1 receptor agonist, increases dopamine levels in the prefrontal cortex, and this increase is completely blocked preferentially by the M_1 receptor antagonist, telenzepine. Moreover, Li *et al.* (2009) have demonstrated that the effect of *N*-desmethylclozapine is blocked by local application of telenzepine, suggesting the involvement of M_1 receptors located in the prefrontal cortex. In the present study, we showed that a *N*-desmethylclozapine-induced increase in the prefrontal dopamine release was less in the isolation-reared than in control mice. In contrast to the response to the muscarinic agonist, nicotine-induced hypolocomotion and hypothermia were not altered in the socially isolated mice compared to the group-reared mice. These observations suggest that muscarinic, especially M_1,

but not nicotinic, receptor function is reduced in isolation-reared mice.

Galantamine and donepezil increase extracellular ACh levels in the brain (Yano *et al.*, 2009), but the former improves PPI deficits while the latter does not (Koda *et al.*, 2008). With regard to this difference, Snape *et al.* (1999) reported that donepezil also acts as an M_1 receptor antagonist. We found, in a separate study, that donepezil, but not galantamine, antagonized the M_1 receptor-mediated Ca^{2+} signal in SH-SY5Y cells *in vitro* and *N*-desmethylclozapine-induced increase in dopamine release *in vivo* (unpublished data). Therefore, it is possible that donepezil blocks the muscarinic receptors, which play a key role in the improvement of PPI deficits of isolation-reared mice and, hence, does not affect PPI.

In conclusion, we showed that galantamine improved PPI deficits in mice reared in social isolation in an M_1 receptor-dependent manner and a preferential M_1 receptor agonist also improved these PPI deficits. We also found that rearing mice in isolation decreased muscarinic, especially M_1, receptor function. These findings suggest that an M_1 receptor is involved in the effect of galantamine and that reduced muscarinic receptor function is involved in PPI deficits in mice reared in isolation.

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Conflicts of interest

The authors state no conflict of interest.

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