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α7 nicotinic acetylcholine receptor activation ameliorates scopolamine-induced behavioural changes in a modified continuous Y-maze task in mice

John P. Redrobe *, Elsebet Ø. Nielsen, Jeppe K. Christensen, Dan Peters, Daniel B. Timmermann, Gunnar M. Olsen

Department of Affective Disorders, NeuroSearch A/S, Pederstrupvej 93, 2750 Ballerup, Denmark

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ABSTRACT

The alpha7 (α7) nicotinic acetylcholine receptor may represent a drug target for the treatment of disorders associated with working memory/attentional dysfunction. We investigated the effects of three distinct α7 nicotinic acetylcholine receptor agonists: 2-methyl-5-(6-phenyl-pyridazin-3-yl)-octahydro-pyrrolo[3,4-c] pyrrole (A-582941; 0.01–0.1 mg/kg), 4-bromophenyl 1,4-diazabicyclo(3.2.2) nonane-4-carboxylate (SSR180711; 0.3–3 mg/kg) and N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide (PNU-282987; 1–10 mg/kg), on scopolamine-induced deficits in a modified Y-maze procedure. Mice were forced to choose one of two visually distinct arms, and were confined there for a 5 min exploration period before being allowed to explore both arms for a 2 min test session, immediately thereafter. The time spent in each arm, entries and total distance travelled were recorded using an automated system. Characterisation experiments showed that scopolamine-treated (1 mg/kg) mice spent less time exploring the unfamiliar arm, when compared with vehicle-treated animals. Combination experiments showed that all three α7 agonists ameliorated scopolamine-induced changes in unfamiliar arm exploration. In conclusion, the present data support the idea that α7 nicotinic acetylcholine receptors may represent an interesting target for the treatment of conditions associated with attentional/working memory dysfunction.

1. Introduction

Exploratory behaviour is an important component of learning (Tolman, 1925) as it allows an organism to acquire information that may be crucial for its survival (Crusio and van Abeelen, 1986). Theories of exploration have postulated that, when given a choice, rodents generally prefer to explore novel environments (Syme and Syme, 1977), although for mice this may be strain-dependent (Griebel et al., 1993). Indeed, when placed in a T-, Y-, or radial-maze, a mouse or rat displays a strong propensity to alternate arm choices on successive trials, and this spontaneous alternation behaviour (SAB) is thought to reflect working memory performance (Lalonde, 2002; Hughes, 2004). The exploration of novel environmental stimuli is thought to be dependent on the integrity of limbic and non-limbic pathways, including the basal forebrain, the hippocampus, the thalamus, the prefrontal cortex, and the dorsal striatum, as well as the vestibular system and cerebellum (Lalonde, 2002). More specifically, cholinergic neurochemical pathways, among others, have been implicated in the modulation of exploratory activity in novel maze arms (Lalonde, 2002; Hughes, 2004).

The nicotinic acetylcholine receptor family includes genes that code for specific α and β subunits (α2-10, β2-4) within a general pentameric ion channel that is composed of five identical (homomer, α7) or five different (heteromer) α and β subunit combinations belonging to the family of cys-loop ligand-gated ion channels (Le Novere et al., 2002). The α7 nicotinic acetylcholine receptor is abundantly expressed in the brain, particularly in the cerebral cortex, the hippocampus, and limbic regions (Picciotto et al., 2001). The functional significance of this receptor is not only attributable to its modulatory properties on neuronal excitability and neurotransmitter release (Gotti et al., 2006; Barik and Wonnacott, 2006), but also to its high Ca^{2+} permeability and association with biochemical signaling pathways (for review, see Dajas-Bailador and Wonnacott, 2004). Nicotine is known to enhance cognitive and attentional function in both laboratory animals (Levin and Simon, 1998; Mirza and Stolerman, 1998) and in humans (Potter et al., 2006; Rezvani and Levin, 2001; Wilens and Decker, 2007), indicative of an important role for nicotinic acetylcholine receptors in these processes. Moreover, sensory gating (a neurophysiological phenomenon thought to be important for processing of sensory information) and performance in models of cognition and memory are known to be impaired in rodents with decreased expression or following pharmacological blockade of α7 nicotinic acetylcholine receptors (Stevens et al., 1996; Felix and Levin, 1997). Consistent with this finding, expression of α7 nicotinic acetylcholine receptor protein is reduced in the brains of patients suffering from Alzheimer's disease (Burghaus et al., 2000) and schizophrenia (Freedman et al., 1995). These and other observations

* Corresponding author. Tel.: +45 44 60 8232; fax: + 45 44 60 8080.
E-mail address: JPR@neurosearch.dk (J.P. Redrobe).

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have attracted considerable attention to the α7 nicotinic acetylcholine receptor as a drug target in recent years, the working hypothesis being that drugs capable of augmenting α7 receptor function may ameliorate the cognitive and mnemonic deficits of demented and/or schizophrenic patients. Indeed, a number of recently developed selective α7 agonists have been shown to enhance performance in various animal models thought to be associated with certain aspects of cognition, or to model some characteristics of cognitive impairments connected to neuropsychiatric disorders (for review, see Cincotta et al., 2008). However, these studies are most often from different laboratories, examining the effects of different α7 agonists in different animal models.

The experiments presented here employed a modified Y-maze procedure (termed V-maze) in mice. This automated continuous two-phase behavioural protocol facilitated the measurement of time spent in arm of choice (familiar versus unfamiliar, memory for and responsiveness to novelty), the number of arm entries and total distance travelled (locomotor activity). Characterisation experiments were performed using the muscarinic receptor antagonist, scopolamine (Rush, 1988); the non-subtype selective gamma-aminobutyric acid $GABA_A$ receptor modulator, chlordiazepoxide (Cash et al., 1997); the adenosine receptor antagonist caffeine (Ferré et al., 1997), and the sedative hypnotic $GABA_A$ receptor modulator zolpidem (Benavides et al., 1993), to investigate the influence of disrupted cholinergic neurotransmission, anxiety state, central stimulation and sedation, respectively, on behavioural readout from the test paradigm. Further studies outline the effects of three recently described α7 nicotinic acetylcholine receptor agonists: A-582941 (Bitner et al., 2007), SSR180711 (Biton et al., 2007) or PNU282987 (Hajos et al., 2005), on scopolamine-induced behavioural changes in V-maze performance.

2. Materials and methods

All experiments were carried out in accordance with the guidelines for care and use of laboratory animals as outlined by the Danish Committee for Experiments on Animals. Every effort was made to minimise animal suffering and the number of animals used (Animals were used only once: behavioural testing $n=8$; *in vitro* [^{3}H]α-bungarotoxin binding, $n=3$–4; *ex vivo* [^{3}H]α-bungarotoxin binding, 2 experiments of $n=3$.).

2.1. Animals and housing

Male C57BL/6J mice (22–25 g; Harlan, Netherlands), were used for all behavioural experiments and for *ex vivo* [^{3}H]α-bungarotoxin binding studies. Cerebral cortices and hippocampi from male Wistar rats (150–250 g; Taconic M & B, Ry, Denmark) were used for *in vitro* [^{3}H]α-bungarotoxin binding. All animals were allowed a minimum of 7 days acclimatisation to the laboratories before use. Mice were housed in groups of 8 and rats were housed in groups of 4 in Macrolon III cages contained in Scantainers (Scanbur A/S, Denmark) under a 12 h light/dark cycle (lights on: 0600 h) with free access to food (standard laboratory pellets) and tap water. Experiments were performed between 9:00 h and 16.00 h in temperature and humidity-regulated rooms (22–24 °C, relative humidity: 60–70%).

2.2. Chemicals, drugs and treatment

For *in vitro*/*ex vivo* binding studies, [^{3}H]α-bungarotoxin (60 Ci/mmol) was purchased from GE Healthcare UK Limited (Little Chalfont, UK). (−)-Nicotine was purchased from Sigma-Aldrich (Vallensbæk Strand, Denmark). All other chemicals were purchased from regular commercial sources and were of the purest grade available. For oocyte electrophysiology studies, collagenase Type 1A, gentamicin, tricaine (3-aminobenzoic acid ethyl ester methanesulfonate), and acetylcholine were obtained from Sigma-Aldrich (Vallensbæk Strand, Denmark). For *in vivo* behavioural studies, the following drugs were used: (−)-scopolamine hydrobromide and caffeine (as free base) were obtained from Sigma-Aldrich (Vallensbæk Strand, Denmark). Chlordiazepoxide hydrochloride, zolpidem tartrate, the selective α7 nicotinic acetylcholine receptor agonists: 2-methyl-5-(6-phenyl-pyridazin-3-yl)-octahydro-pyrrolo[3,4-c]pyrrole fumarate (A-582941), 4-bromophenyl 1,4diazabicyclo(3.2.2) nonane-4-carboxylate hydrochloride (SSR180711) and N-[(3R)-1-Azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide fumarate (PNU282987) were synthesised at NeuroSearch A/S. All compounds were dissolved in 0.9% saline, with the exception of zolpidem that was dissolved in 5% cremophor. All compounds were administered in an injection volume of 10 ml/kg and doses were calculated as the salt form, where salts were used. Scopolamine (0.25–1 mg/kg, subcutaneous, s.c.), chlordiazepoxide (10 and 20 mg/kg, intraperitoneal, i.p.), caffeine (5 and 10 mg/kg, i.p.) or zolpidem (1 and 3 mg/kg, i.p.) was administered 30 min before phase 1 testing. A-582941 (0.01–0.1 mg/kg, i.p.), SSR180711 (0.3–3 mg/kg, i.p.) or PNU282987 (1–10 mg/kg, i.p.) was administered 45 min prior to phase 1 testing.

2.3. In vitro [^{3}H]α-bungarotoxin assay

We decided to perform *in vitro* [^{3}H]α-bungarotoxin binding assays in order to confirm previous published data on SSR180711, A-582941 and PNU282987. In order to minimize the number of animals used due to the amount of tissue needed to perform the assay, cerebral cortices and hippocampi from Wistar rats (see Section 2.1) were homogenized for 10 s in 15 ml 50 mM Tris, HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2.5 mM $CaCl_2$ using an Ultra-Turrax homogenizer. All preparation procedures were performed at 0–4 °C unless otherwise indicated. The tissue suspension was centrifuged at 27,000 ×*g* for 10 min. The pellet was washed twice by centrifugation at 27,000 ×*g* for 10 min in 20 ml buffer, and the final pellet was re-suspended in buffer containing 0.01% bovine serum albumin (35 ml/g of original tissue) and used for binding assays.

[^{3}H]α-bungarotoxin binding was performed using the method described previously by Gopalakrishnan et al. (1995). In brief, the assay was performed at 1 nM [^{3}H]α-bungarotoxin (25 µl) in a final volume of 550 µl, mixed and incubated for 2 h at 37 °C in triplicate. Drugs (25 µl) were tested at concentrations ranging from 0.003 to 3 µM. Non-specific binding was determined in the presence of 1 mM (−)-nicotine.

Incubations were terminated by rapid vacuum filtration through Whatman GF/C glass fiber filters presoaked in 0.1% polyethyleneimine (PEI) for at least 20 min, and filters washed twice with 5 ml ice-cold Tris buffer containing 0.05% PEI. The amount of radioactivity on the filters was determined by conventional liquid scintillation counting using a Tri-Carb™ liquid scintillation counter (model 2800TR; PerkinElmer Life and Analytical Sciences, Downers Grove, IL).

2.4. Ex vivo [^{3}H]α-bungarotoxin assay

Groups of three male C57BL/6J mice (see Section 2.1) were injected i.p. with drug solutions prepared as described earlier. Three to four doses ranging from 0.3 to 30 mg/kg were tested for determination of ED_{50} values. Forty five minutes after injection mice were killed by decapitation, and the hippocampi were rapidly dissected on ice and the tissue weighed. Preparations were performed at 0–4 °C unless otherwise indicated. Each pair of hippocampi was homogenized for 10 s in 75 volumes of ice-cold 50 mM Tris, HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2.5 mM $CaCl_2$ containing 0.01% BSA using an Ultra-Turrax homogenizer, and the tissue suspension was used for binding assays. The remaining procedure was performed as described in Section 2.3. Groups of vehicle-treated mice served as controls for estimation of total and non-specific binding.

2.5. Xenopus oocyte preparation

Female *Xenopus laevis* frogs were obtained from Nasco (Fort Atkinson, Wisconsin, USA) and treated using standard protocols approved by NeuroSearch's Animal Care and Use Committee. Frogs were anesthetized with tricaine (0.28% in deionized water) and sections of one ovary (generally 3–4 lobes) were surgically removed and placed in low-Ca^{2+} Barth's solution (90 mM NaCl, 1 mM KCl, 0.66 mM $NaNO_3$, 2.4 mM $NaHCO_3$, 0.82 mM $MgCl_2$, 10 mM HEPES, and 100 μg/ml gentamicin, final pH 7.55). The anesthetized frog was sacrificed after the operation by decapitation. The isolated ovary sections were opened using blunt dissection and rinsed in low-Ca^{2+} Barth's solution. To remove the follicular layer, the ovary sections were incubated in collagenase (Company, 2 mg/ml in low-Ca^{2+} Barths' solution) for 1–2 h at room temperature. Oocytes not defolliculated by the collagenase treatment were manually defolliculated. Hereafter, the oocytes were maintained at 17 °C in normal Barth's solution (90 mM NaCl, 1 mM KCl, 0.66 mM $NaNO_3$, 2.40 mM $NaHCO_3$, 0.74 mM $CaCl_2$, 0.82 mM $MgCl_2$, 10 mM HEPES, and 100 μg/ml gentamicin, final pH 7.55). As an alternative source of oocytes, defolliculated oocytes were acquired and shipped directly from Ecocyte Bioscience (Castrop-Rauxel, Germany).

Cloning of the human nicotinic acetylcholine receptor α7 subunit and transcription into cRNA was performed as described previously (Timmermann et al., 2007). Defolliculated oocytes were injected with 40–50 nl (40–50 ng) of the α7 cRNA within 24–48 h of their preparation and were used 2–7 days after injection.

2.6. Electrophysiological recording conditions and experimental protocols

Recordings were made using two-electrode voltage clamp by using a Geneclamp 500B amplifier with a bath clamp configuration in combination with a Digidata 1322A interface (both Molecular Devices, Sunnyvale, CA, USA). Electrodes were made from bososilicate glass (1.5 mm O.D., 1.1 mm I.D.) and were filled with 3 M KCl. The impedance of the current-passing electrode was 0.5–2 MΩ. All experiments were conducted at a holding potential of −60 mV in an Oocyte Ringer's (OR) solution (90 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, final pH 7.4). Oocytes were placed in a custom made diamond-shaped recording chamber, which was continuously perfused at a rate of 1.5 ml/min with OR by using a peristaltic pump. Agonists, dissolved in OR, were applied through a glass capillary applicator (2.0 mm O.D./1.5 mm I.D.) placed in vicinity of the oocyte. Precise time controlled application was controlled by virtue of a Gilson 231 XL autosampler (Gilson, Inc., Middleton, WI) with the injection port connected to the applicator tube through teflon-tubing (1/16 in. O.D./0.25 mm I.D.), and the flow (2.5 ml/min) was created by the use of syringe pumps (Gilson 402; Gilson, Inc.). The timing of agonist applications was controlled by using the 735 Sampler Software (Gilson, Inc.), which was also used to trigger recording protocols defined by Clampex software (Molecular Devices, Sunnyvale, CA, USA). The duration of agonist exposure was 12 s and the interval between agonist applications was 5 min. Response stability was assessed through multiple applications of a saturating concentration of acetylcholine (10 mM) after which increasing concentrations of test agonists were applied. Responses of test agonists were quantified by measuring the amplitude of the response peak relative to the baseline current (Clampfit software, Molecular Devices, Sunnyvale, CA, USA) and were normalized to the response of saturating (10 mM) acetylcholine in the same oocyte.

2.7. Behavioural apparatus and procedure

Testing was carried out in a clear Plexiglass maze composed of 2 perpendicular arms connected to a runway. The 2 arms (available for exploration) and runway were 50 cm long and 8 cm wide, surrounded by clear Plexiglass walls 30 cm high. Each arm met at a central platform equipped with black removable partitions, enabling arms to be opened and closed as desired. The whole maze was enclosed in a triangular black Plexiglass box (1×1×1 m). The walls of this outer box surrounding each exploration arm were covered with distinct optical cues, e.g., white horizontal or vertical lines. The area surrounding the runway did not contain optical cues and was black in colour. Each arm of the maze was separated from another by an opaque partition, so a mouse on entering an arm could only see the distinct optical cues of that particular arm. The test consisted of two phases: In phase 1 (habituation), the mouse was placed at the end of the runway and was allowed access to one of the exploration arms by forced choice (i.e., the other arm was closed). After the mouse had entered the arm, access to the runway was blocked, and the mouse was allowed to explore the arm (termed familiar) for a period of 5 min. The familiar arm was alternated systematically to eliminate any place preference to confound the assay (Fig. 1). Immediately thereafter, in phase 2 (testing), the mouse was allowed to explore both the familiar and the unfamiliar exploration arms, but not the runway, for a period of 2 min (Fig. 1). The cumulative time spent in each arm, the number of entries and the total distance travelled were recorded during this test session by an automated video tracking system (ViewPoint, France). A discrimination index (DI) was calculated for each mouse, where "t_f" represented time exploring the familiar arm and "t_u" the time exploring the unfamiliar arm during phase 2 testing: $DI=(t_u-t_f)/(t_u+t_f)$ (Ennaceur and Delacour, 1988). We believe that the test is measuring attentional processing, particularly as the procedure did not incorporate a delay. There is a wealth of evidence in the literature suggesting modulation of cholinergic neurotransmission can affect attentional processing (see Introduction). However, we cannot rule out a working memory component as the procedure employed is a variant of the traditional "two-trial" spontaneous alternation task and the more recent "continous" task, thought to reflect working memory performance and be hippocampal-dependent (Lalonde, 2002; Gerlai, 1998).

2.8. Statistical analysis

For *in vitro*/*ex vivo* studies, compounds that displaced radioligand binding were tested over a wide range of concentrations, and IC_{50}/ED_{50} values were determined based on the equation $B=100-(100\cdot D^n/(IC_{50}^n+D^n))$, where B is the binding in percent of total specific binding; D the concentration/dose of test compound; and n the Hill coefficient. Estimates of binding parameters were calculated with the non-linear curve-fitting program GraphPad Prism™ (version 4.03; GraphPad Software, Inc., CA). K_i values were calculated from IC_{50}

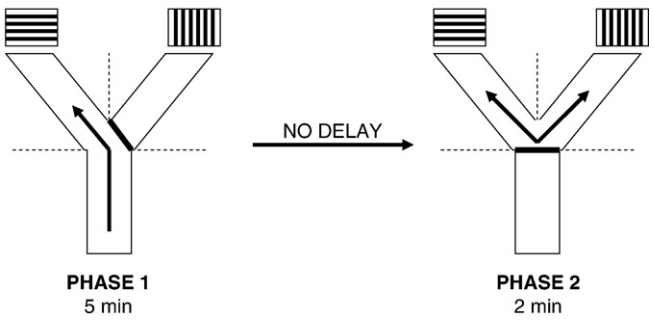

Fig. 1. V-maze apparatus and procedure. In phase 1 (habituation), the mouse was placed at the end of the runway and was allowed access to one of the exploration arms by forced choice for a period of 5 min. Immediately thereafter, in phase 2 (testing), the mouse was allowed to explore both the familiar and the unfamiliar exploration arms, but not the runway, for a period of 2 min. The cumulative time spent in each arm, the number of entries and the total distance travelled were recorded during this test session by an automated video tracking system (ViewPoint, France).

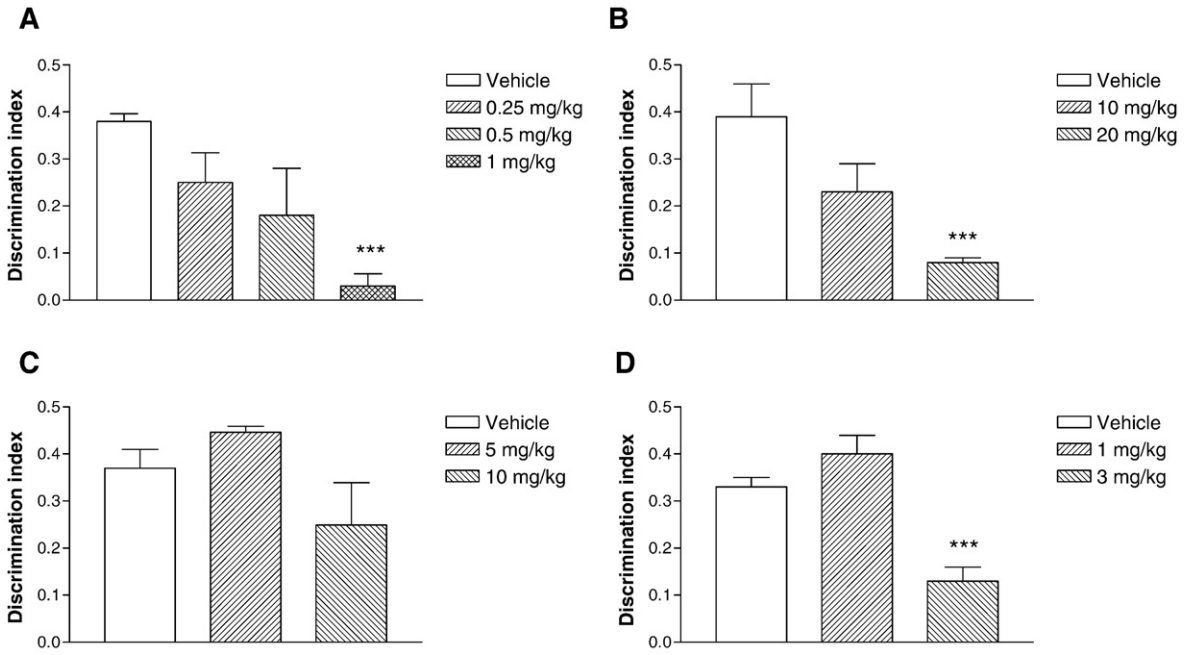

Fig. 2. The effect of (A) scopolamine, (B) chlordiazepoxide, (C) caffeine, and (D) zolpidem on discrimination index. Data expressed as mean±S.E.M. Mice were treated with scopolamine (0.25–1 mg/kg, s.c.), chlordiazepoxide (10 and 20 mg/kg, i.p.), caffeine (5 and 10 mg/kg, i.p.) or zolpidem (1 and 3 mg/kg, i.p.) 30 min before phase 1 testing. ***$P<0.001$ versus appropriate vehicle-treated control group (Dunnett's multiple comparison test preceded by one-way ANOVA; $n=8$; scopolamine: $F(3,28)=5.709$, $P<0.01$; chlordiazepoxide: $F(2,21)=8.384$, $P<0.01$; zolpidem: $F(2,21)=20.31$, $P<0.0001$).

values using the Cheng and Prusoff (1973) equation. All results are given as mean±S.E.M. For oocyte electrophysiology studies, concentration–response parameters were determined using the non-linear curve-fitting in the Prism software (GraphPad Software, La Jolla, CA, USA) and the built-in variable slope sigmoidal curve; fitting parameters were not constrained except that the bottom of the curve was set equal to 0. For behavioural studies, comparisons between groups were carried out by Student's *t*-test or analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests, where appropriate. Data were considered statistically significant when $P<0.05$ ($n=8$).

3. Results

3.1. Effects of scopolamine, chlordiazepoxide, caffeine and zolpidem on performance in the V-maze task

Scopolamine treatment (1 mg/kg, but not 0.25 or 0.5 mg/kg) significantly reduced time spent exploring the unfamiliar arm, and thus decreased the discrimination index in phase 2 testing, when compared to vehicle-treated animals ($P<0.001$; $n=8$; $F(3,28)=5.709$, Fig. 2A), without affecting the number of entries or total distance travelled (Table 1). Likewise, mice administered chlordiazepoxide (20 mg/kg, but not 10 mg/kg) or zolpidem (3 mg/kg, but not 1 mg/kg) spent less time exploring the unfamiliar arm ($P<0.001$ versus vehicle-treated group; $n=8$; $F(2,21)=8.384$ and $F(2,21)=20.31$, Fig. 2B,D, respectively), but also made fewer entries (chlordiazepoxide reduced unfamiliar arm entries, $P<0.001$ versus vehicle-treated group; $n=8$; $F(2,21)=6.658$; zolpidem decreased familiar, $F(2,21)=9.690$ and unfamiliar arm entries, $F(2,21)=9.209$, $P<0.001$ versus vehicle-treated group; $n=8$; Table 1) and travelled a shorter distance (zolpidem 1 mg/kg and 3 mg/kg reduced total distance, $P<0.05$ and $P<0.001$ versus vehicle-treated group, respectively; $F(2,21)=31.81$; Table 1). Caffeine (10 mg/kg, but not 5 mg/kg) administration increased entries into the familiar arm ($P<0.001$ versus vehicle-treated group; $n=8$; $F(2,21)=7.951$; Table 1) and total distance travelled ($P<0.001$ versus vehicle-treated group; $n=8$; $F(2,21)=11.87$; Table 1) but did not significantly affect unfamiliar arm exploration (Fig. 2C).

3.2. In vitro/ex vivo [^{3}H]α-bungarotoxin binding

A-582941, SSR180711 and PNU282987 were found to displace *in vitro* [^{3}H]α-bungarotoxin binding with K_i values of 75.2±6.3, 45.9±9.1 and 38.8±8.2 nM, respectively (Table 2). In contrast, scopolamine failed to affect *in vitro* [^{3}H]α-bungarotoxin binding at concentrations up to 30 μM. In *ex vivo* studies, A-582941, SSR180711 and PNU282987 were found to displace [^{3}H]α-bungarotoxin binding with ED_{50} values of 5.0 (4.5–5.5), 2.2 (1.8–2.6) and 12.0 (8.5–15.6) mg/kg, i.p., respectively (Table 2), indicating that these compounds were present

Table 1
The effect of scopolamine, chlordiazepoxide, caffeine and zolpidem on number of entries and total distance travelled

Compound	Dose	Entries		Distance (cm)
		Familiar	Unfamiliar	
Scopolamine	0	6.8±0.5	7.4±0.8	920±51
	0.25	6.5±0.8	6.6±0.4	803±48
	0.5	5.4±0.3	5.7±0.7	798±63
	1	7.5±0.7	6.4±0.4	787±22
Chlordiazepoxide	0	7.6±1.1	9.0±1.0	899±38
	10	7.9±1.3	7.4±1.0	943±110
	20	5.0±0.9	4.3±0.8[c]	789±121
Caffeine	0	6.9±0.1	7.9±0.9	895±28
	5	7.6±0.5	7.8±0.8	869±31
	10	9.5±0.7[c]	8.9±0.5	1039±18[c]
Zolpidem	0	6.4±0.7	7.1±0.7	887±46
	1	4.3±0.8	5.4±0.9	664±41[a]
	3	2.2±0.5[c]	2.3±0.8[c]	313±64[c]

Data expressed as mean±S.E.M. [a]$P<0.05$, [c]$P<0.001$ versus appropriate vehicle-control group (Dunnett's multiple comparison test preceded by one-way ANOVA, $n=8$; entries to familiar, caffeine: $F(2,21)=7.951$, $P<0.01$; zolpidem: $F(2,21)=9.690$, $P<0.01$; entries to unfamiliar, chlordiazepoxide: $F(2,21)=6.658$, $P<0.01$; zolpidem: $F(2,21)=9.209$, $P<0.01$; distance travelled, caffeine: $F(2,21)=11.87$, $P<0.001$; zolpidem: $F(2,21)=31.81$, $P<0.0001$).

Table 2
Inhibition of *in vitro* and *ex vivo* [3H]α-bungarotoxin binding by A-582941, SSR180711, PNU282987 and scopolamine

	K_i (nM)	ED_{50} (mg/kg, i.p.)
	In vitro [^{3}H]α-bungarotoxin	*Ex vivo* [^{3}H]α-bungarotoxin
A-582941	75.2±6.3	5.0 (4.5–5.5)
SSR180711	45.9±9.1	2.2 (1.8–2.6)
PNU282987	38.8±8.2	12.0 (8.5–15.6)
Scopolamine	>30,000	ND

In vitro (rat cortex and hippocampus, n=3–4, mean±S.E.M.) and *ex vivo* (mouse hippocampus, n=2 experiments with groups of 3 mice; the range shown in parentheses) potency of each compound for the nicotinic α7 receptor as labeled with [^{3}H]α-bungarotoxin. Binding assays were performed as described under Materials and methods. ND: not done.

in the brain at concentrations enabling measurable α7 receptor binding.

3.3. Concentration–response relationships for A-582941, SSR180711 and PNU282987 in oocytes expressing human α7 nicotinic receptors

To account for the variability in receptor expression among oocytes, the response amplitudes were all normalized to the response to 10 mM acetylcholine, determined in each of the same oocytes. The concentration–response relationships of A-582941, SSR180711 and PNU282987 are shown in Fig. 3. The calculated EC_{50} values were as follows: PNU282987: 0.63 μM (95% c.i., 0.47–0.83 μM, Hill coefficient=1.58), SSR180711: 1.39 μM (95% c.i., 1.1–1.8 μM, Hill coefficient=1.88) and A-582941: 2.45 μM (95% c.i., 1.7–3.5 μM, Hill coefficient=1.75). The fitted maximal response for PNU282987 relative to 10 mM acetylcholine was 99% (95% c.i., 91–107%). For SSR180711 and A-582941 the fitted maximal responses were 85% (95% c.i., 76–94%) and 72% (95% c.i., 62–82%), respectively. These data indicate that all three compounds acted as agonists at the human α7 receptor with largely equal potencies and efficacies.

3.4. Effects of A-582941, SSR180711 and PNU282987 on scopolamine-induced deficits in performance in the V-maze task

As observed in characterisation dose–response studies, scopolamine treatment (1 mg/kg) significantly reduced time spent exploring the unfamiliar arm, and thus decreased the discrimination index in phase 2 testing, when compared to vehicle-treated animals (P<0.05, A-582941 study; P<0.01, SSR180711 study; P<0.001, PNU282987 study; Student's t-test; Fig. 4), without affecting the number of entries or total distance travelled (Table 3). Pre-administration with A-582941 (0.03 and 1 mg/kg, but not 0.01 mg/kg), SSR180711 (3 mg/kg, but not 0.3 or 1 mg/kg), or PNU282987 (10 mg/kg, but not 1 or 3 mg/kg) attenuated scopolamine-induced changes in unfamiliar arm exploration (P<0.001, F(3,28)=9.282, Fig. 4A; P<0.05, F(3,28)=3.634, Fig. 4B; P<0.05, F(3,28)=3.449, Fig. 4C; versus scopolamine-treated group, respectively), without affecting the number of entries or total distance travelled (Table 3).

4. Discussion

Characterisation experiments in the V-maze paradigm showed that scopolamine-treated mice spent significantly less time exploring the unfamiliar arm, when compared to vehicle-treated animals, at a dose that consistently disrupts performance in SAB and passive avoidance paradigms (Hiramatsu and Inoue 1999, 2000; Hiramatsu et al., 2006). Although many studies presume that compromised cognitive performance following systemic treatment with scopolamine is due to memory impairments, there is some evidence to suggest that such changes may be a consequence of disrupted sensory/attentional processes (Warburton and Brown, 1971; Cheal, 1981) or performance deficits (Smith and Calhoun, 1972). In the present study, attentional/working memory deficits are most likely the source for the observed effects in the behavioural paradigm, particularly since the protocol used did not incorporate a delay between phase 1 and phase 2 testing. On the other hand, performance deficits are not likely, as entries and total distance travelled were not affected by scopolamine under the described testing conditions. Some studies have suggested that motivational factors, independent of central cholinergic activity, might also be involved since scopolamine has been shown to induce novelty avoidance (in the absence of effects on memory) possibly because of the drug's aversive peripheral action (Hughes, 1982; Horsburgh and Hughes, 1981). Drug-induced novelty avoidance is important regarding the interpretation of traditional SAB outcomes since the phenomenon is widely accepted to involve responsiveness to the more novel of two maze arms on any binary choice occasion (Hughes, 2004). In order to investigate this possibility further, and to assess the potential role of "anxiety state" in the test more generally, we decided to examine the effects of the anxiolytic chlordiazepoxide. Treatment with the full benzodiazepine receptor modulator reduced novel arm exploration to a similar degree to that of scopolamine, accompanied by a decrease in the number of visits to the unfamiliar arm, but did not change total distance travelled. These data suggest that the effects observed following scopolamine treatment may not involve avoidance of novelty, but be more related to its consequences on attentional/working memory performance. Chlordiazepoxide is well documented to increase exploration of novel environments and stimuli (Crawley, 1985). Indeed, chlordiazepoxide has been reported to reverse the preference of BALB/c mice, a mouse line which exhibits spontaneously elevated anxiety (Belzung and Griebel, 2001), for the familiar compartment towards the novel compartment in a free-exploration paradigm (Griebel et al., 1993). Moreover, chlordiazepoxide has been shown to compromise cholinergic neurotransmission and as a result induces deficits in SAB (Quintero et al., 1985) and passive avoidance acquisition when administered before training (Nabeshima et al., 1990).

In order to investigate the influence of increased/decreased motor activity on behavioural output from the test paradigm, we decided to test the central stimulant caffeine and the hypnotic zolpidem, respectively. The non-selective adenosine receptor antagonist caffeine significantly increased the number of arm entries and total distance travelled, but did not affect unfamiliar arm exploration. These data suggest that compounds inducing modest increases in motor activity do not influence performance of the task. Caffeine has been shown to improve cognitive performance in certain animal models (for review, see Takahashi et al., 2008), and humans (Ribeiro et al., 2002). However, caffeine has been reported to reduce performance in a traditional "two-trial" SAB procedure when administered alone

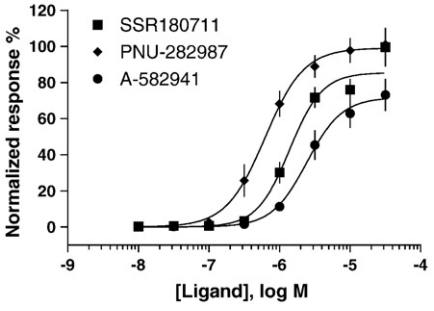

Fig. 3. Concentration–response relationships for A-582941, SSR180711 and PNU282987 in oocytes expressing human α7 nicotinic receptors. Each oocyte was exposed to a saturating concentration (10 mM) of acetylcholine and to multiple concentrations of one of the agonists. Agonist responses were normalized to the response induced by 10 mM acetylcholine. Data are shown as mean±S.E.M. and are from 4 oocytes for A-582941, 5 oocytes for PNU282987, and 8 oocytes for SSR180711.

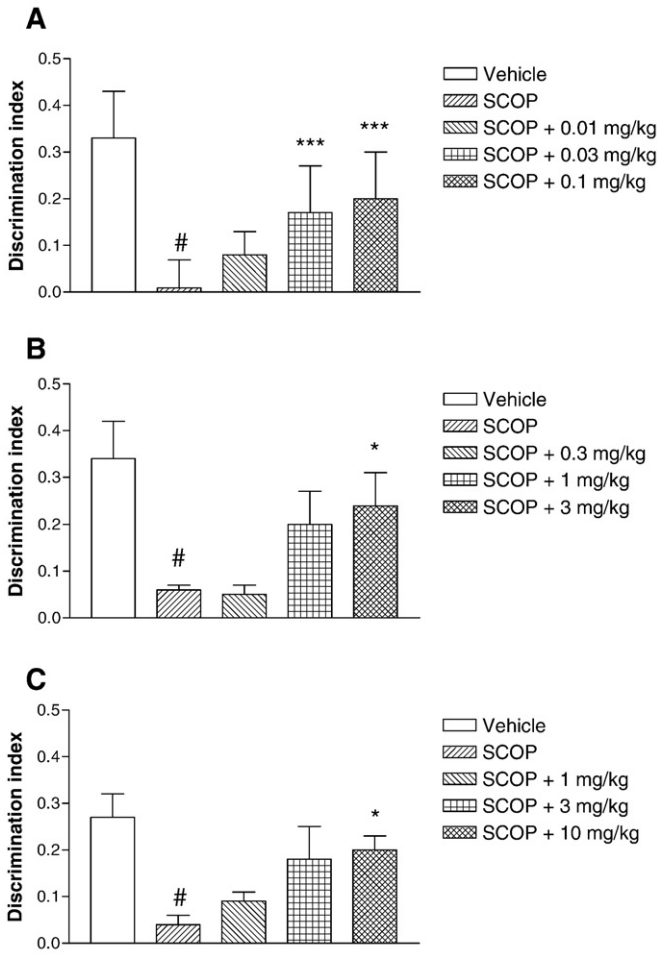

Fig. 4. The effect of (A) A-582941, (B) SSR180711 and (C) PNU282987 on scopolamine (SCOP)-induced changes in discrimination index. Data expressed as mean±S.E.M. Mice were pre-treated with: A-582941 (0.01–0.1 mg/kg, i.p.), SSR180711 (0.3–3 mg/kg, i.p.) or PNU282987 (1–10 mg/kg, i.p.), 15 min before scopolamine (SCOP, 1 mg/kg, s.c.), administered 30 min prior to phase 1 testing. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ versus appropriate vehicle-treated control group (Student's *t*-test); $^{*}P<0.05$, $^{***}P<0.001$ versus appropriate scopolamine-treated control group (Dunnett's multiple comparison test preceded by one-way ANOVA; $n=8$; A-582941: $F(3,28)=9.282$, $P<0.0001$; SSR180711: $F(3,28)=3.634$, $P<0.05$; PNU282987: $F(3,28)=3.449$, $P<0.05$).

(Hughes and Grieg, 1975). This, together with the observation that vehicle-treated animals spend a large proportion of the 2 min testing period exploring the unfamiliar arm, suggests the likelihood and window for detection of a "pro-cognitive" effect of caffeine, in particular, using the paradigm described here may be too small.

The $GABA_A$ receptor modulator zolpidem reduced the number of entries and total distance travelled, but only attenuated unfamiliar arm exploration at the higher dose. Again, this data suggests that performance of the task is not affected by modest changes in motor activity, and that it may be possible to distinguish and therefore dissociate non-specific effects on motility from cognitive performance using this procedure.

Investigations into the potential pro-cognitive effects of a test compound should ideally be performed in "normal" animals, and equally important in subjects who have received some sort of insult, whether it be pharmacological or lesion-based, in order to elucidate the effects of the compound under basal and compromised conditions (Decker, 2006). In the present study, we did not detect a pro-cognitive effect of caffeine when tested alone, for reasons discussed earlier. This led us to believe that a pharmacological deficit would therefore be necessary for further testing. Based on the results from characterisation studies, we decided to use scopolamine for further experiments as it induced changes in unfamiliar arm exploration without affecting measures that may have been indicative of non-specific effects on motility. Combination experiments showed that all three α7 nicotinic acetylcholine receptor agonists ameliorated scopolamine-induced changes in unfamiliar arm exploration. For SSR180711 and PNU282987, efficacy was apparent at doses largely corresponding to their respective *ex vivo* [^{3}H]α-bungarotoxin binding ED_{50} values (SSR180711: 3 mg/kg versus 2.2 mg/kg, respectively; PNU282987: 10 mg/kg versus 12.0 mg/kg, respectively). The *ex vivo* binding parameter is understood to be a measure of the ability of a compound to penetrate the blood-brain barrier and be present in concentrations that enable measurable receptor binding. A limitation of this procedure is that the test compounds may not have achieved the same concentrations at the receptor *in vivo* as they did following tissue processing in the *ex vivo* binding assay. However, as [^{3}H]α-bungarotoxin does not cross the blood-brain barrier, *in vivo* binding experiments were not possible. In contrast to SSR180711 and PNU282987, the minimal efficacious dose for A-582941 in the behavioural test (0.03 mg/kg) was markedly lower than the respective [^{3}H]α-bungarotoxin binding ED_{50} value (5.0 mg/kg). Importantly, the *in vivo* efficacious dose for A-582941 presented here is in good agreement with previous data reported from more traditional "memory" tests (Bitner et al., 2007). However, the discrepancy between the efficacious dose in the behavioural test and the binding assay remains unclear. Previous authors have suggested that, for this particular compound, pro-cognitive efficacy can be obtained at very low α7 receptor occupancy (Bitner et al., 2007). Of course, it is also possible that this is true for the other compounds, but that the *ex vivo* binding does not accurately represent the level of receptor occupancy achieved for the SSR and PNU compounds.

Electrophysiological studies in oocytes indicated that all three compounds increased the current flowing through α7 receptor with largely equal potencies and efficacies. The calculated EC_{50} value for A-582941 was in good agreement with previously reported data for this compound. However, the maximal efficacy of A-582941 was slightly higher than the efficacy reported by Bitner et al. (2007). For SSR180711, the calculated EC_{50} value was also in good agreement with previously reported data. However, in contrast to Biton et al. (2007), who reported SSR180711 to be a 51% partial agonist, the maximal efficacy of SSR180711 found in this study was 85%, indicating that this compound may act as a full agonist. Overall, the current data serves as confirmation that the compounds tested in the present study were present in the brain after systemic administration and were efficacious as α7 agonists.

Table 3
The effect of A-582941, SSR180711 and PNU282987 on scopolamine (SCOP)-induced changes in number of entries and total distance travelled

Compound	Dose	Entries		Distance (cm)
		Familiar	Unfamiliar	
A-582941	0	7.0±0.5	6.6±0.8	782±22
	SCOP	5.8±0.6	5.2±0.5	772±35
	0.01	6.9±0.5	4.9±0.9	833±31
	0.03	7.1±0.9	5.5±0.6	850±37
	0.1	8.1±1.2	7.3±0.9	859±51
SSR180711	0	7.8±0.6	7.4±0.9	999±21
	SCOP	8.2±0.8	6.1±0.7	927±33
	0.3	6.1±0.7	5.6±0.7	770±37
	1	6.9±0.8	7.0±0.6	970±41
	3	6.4±0.8	5.1±0.9	933±39
PNU282987	0	5.9±0.5	6.8±0.5	750±37
	SCOP	6.7±0.8	5.8±0.9	816±59
	1	5.6±0.8	5.5±0.3	797±39
	3	5.8±0.4	5.6±0.9	794±23
	10	7.9±0.9	6.9±0.8	869±35

Data expressed as mean±S.E.M. (one-way ANOVA, $n=8$).

Several studies have established an important role for cholinergic control of attentional and cognitive processes (Chen et al., 2004; Chiba et al., 1999; McGaughy et al., 2002; Mirza and Stolerman, 2000), while recent research has demonstrated that selective α7 nicotinic acetylcholine receptor agonists can enhance performance in animal models associated with diverse cognitive processes (for review, see Cincotta et al., 2008). Importantly, the doses found to be active in the present study are in good agreement with those reported for A-582941, SSR180711 and PNU-282987 to enhance various cognitive functions in both rats and mice (Bitner et al., 2007; Hajos et al., 2005; Pichat et al., 2007). Several investigations using α7 agonists have incorporated inter-trial delays or used aged animals in an attempt to detect improvements in performance in "normal" animals, whereas others used pharmacologically induced deficit approaches. As stated earlier, the behavioural paradigm described in the present study did not incorporate a delay between phase 1 and phase 2 testing. There are several important points for discussion regarding this modification to the traditional "two-trial" SAB procedure. It has been reported that, unlike rats, mice display an increase in fear-like behaviours when confronted by novel situations, including maze paradigms, and this may compromise performance of the task (Crusio et al., 1990; Dember, 1990). One of the major contributors to this fear response is thought to be handling of the test animals between trials, which often results in either passivity or freezing on the second trial (Gerlai, 1998). In addition, handling of subjects between trials may function as a negative reinforcer leading to avoidance learning, a behavioural phenomenon distinct from exploratory behaviour (Gerlai, 1998). The paradigm described here was designed in an attempt to minimize the risk of these problems compromising interpretation of the data or performance of the task. Importantly, performance of mice in a very similar paradigm that does not incorporate a delay between trials: the T-maze continuous alternation task (Gerlai, 1998), has been reported to be dependent on hippocampal functioning, a brain region implicitly involved in both working memory and attentional processing (for reviews, see Wall and Messier, 2001; Levin et al., 2006).

Attenuation of chemically induced cognitive effects by nicotinic ligands raises some questions with regard to the receptor mechanisms involved. Reversal of scopolamine-induced changes in performance in animal models indicative of cognitive ability has traditionally been considered to reflect muscarinic receptor function. Nevertheless, three chemically distinct α7 nicotinic acetylcholine receptor agonists have been shown to reverse scopolamine's muscarinic effects. One plausible explanation is that the cognitive improvements seen in the V-maze task may result from enhanced activation of postsynaptic α7 nicotinic acetylcholine receptors sufficient to overcome the effects of decreased muscarinic tone provoked by scopolamine. Alternatively, the α7 nicotinic acetylcholine receptor agonists may act indirectly to enhance ACh release by activating presynaptic receptors, which in turn would offset scopolamine-induced changes in cholinergic activity. This is consistent with the proposed role of presynaptic nicotinic acetylcholine receptors in modulating neurotransmitter release (Wonnacott et al., 1989). Indeed, SSR180711 has recently been shown to dose-dependently increase extracellular ACh levels in the hippocampus and prefrontal cortex of freely moving rats (Biton et al., 2007). In addition, the compound increased the spontaneous firing rate of retrosplenial cortex neurons and elevated extracellular dopamine levels in the prefrontal cortex of freely moving rats (Pichat et al., 2007), suggesting that dopaminergic transmission may play a part in the reported pro-cognitive effects. It is unlikely that the behavioural deficits observed in the present study were a result of a direct action of scopolamine on α7 nicotinic receptors, as this compound did not displace *in vitro* [^{3}H]α-bungarotoxin binding at concentrations up to 30 μM.

In conclusion, the present data support the idea that α7 nicotinic acetylcholine receptors may represent an interesting target for the treatment of conditions associated with working memory/attentional dysfunction.

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