Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2009 | 109 | 744–754 doi: 10.1111/j.1471-4159.2009.06000.x

Anti-amnesic properties of (±)-PPCC, a novel sigma receptor ligand, on cognitive dysfunction induced by selective cholinergic lesion in rats

Vuokko Antonini,* Orazio Prezzavento,† Marino Coradazzi,* Agostino Marrazzo,† Simone Ronsisvalle,† Emanuela Arena† and Giampiero Leanza*

*B.R.A.I.N. Centre for Neuroscience, Department of Life Sciences, University of Trieste, Trieste, Italy

†Department of Pharmaceutical Sciences, University of Catania, Catania, Italy

Abstract

Previous studies have reported that selective sigma-1 agonists may improve cognitive abilities in experimental animals possibly via a cholinergic mechanism. However, the issue of a direct action on to sigma-1 receptors in memory-related brain areas has been much less investigated. The newly synthetised compound methyl(1*R*,2*S*/1*S*,2*R*)-2-[4-hydroxy-4-phenylpiperidin-1-yl)methyl]-1-(4-methylphenyl) cyclopropanecarboxylate [(±)-PPCC] has recently been shown to possess high affinity for the sigma-1 receptor where it specifically acts as an agonist. Here, the functional effects of (±)-PPCC were investigated in rat models of mild or severe cognitive dysfunction based on a sub-total (≤ 70–80%) or complete (≥ 90–95%) central cholinergic depletion induced by different doses of the selective immunotoxin 192 IgG-saporin injected intraventricularly. At 5–6 weeks post-surgery, the lesioned animals exhibited dose-dependent deficits in reference memory, as assessed using the Morris water maze task, whereas working memory abilities, evaluated using the radial arm water maze task, appeared equally impaired in the two dose groups. Daily treatment with (±)-PPCC significantly improved both reference and working memory performance in all lesioned animals but it did not affect intact or sham-lesioned subjects. In a separate test, treatment with (±)-PPCC reversed the learning deficits induced by the muscarinic receptor antagonist atropine sulphate in both control and mild-lesioned rats. The effect was blocked in lesioned, but not normal animals by pre-treatment with the sigma-1 antagonist *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine. The results suggest that (±)-PPCC may efficiently ameliorate perturbed cognitive abilities, and that these anti-amnesic effects most probably occur via a direct interaction of the compound with sigma-1 receptors.

Keywords: acetylcholine, Alzheimer's disease, immunotoxin, rat, sigma-1 receptors, spatial learning.

J. Neurochem. (2009) **109**, 744–754.

Degeneration of acetylcholine (ACh)-producing neurons in the basal forebrain nuclei, and loss of cholinergic neurotransmission in neocortical and hippocampal target regions, represent consistent features of Alzheimer's disease (AD), and have been proposed to account for the progressive cognitive decline in AD patients (Whitehouse *et al.* 1981; Bartus *et al.* 1982; Coyle *et al.* 1983; Pappas *et al.* 2000; see Mesulam 2004 for review). These findings, together with evidence of improved cognitive performance following treatment with cholinomimetic drugs in both animal and clinical studies (e.g. Collerton 1986; Becker *et al.* 1988) have encouraged the use of cholinergic agents to treat AD (e.g. Summers *et al.* 1986; Baskin *et al.* 1999; Minger *et al.* 2000; Doody *et al.* 2001). Pharmacological strategies presently adopted to treat cognitive disturbances in AD, mainly aim at attenuating central cholinergic or glutamatergic dysfunctions. Thus, reducing ACh enzymatic breakdown with acetylcholinesterase inhibitors (e.g. Rogers *et al.* 1998; Cummings

Received December 15, 2008; revised manuscript received January 24, 2009; accepted February 11, 2009.

Address correspondence and reprint requests to Giampiero Leanza, B.R.A.I.N. Centre for Neuroscience, Department of Life Sciences, University of Trieste, Via Fleming 22, 34127, Trieste, Italy.
E-mail: gileanza@dfp.units.it

Abbreviations used: (±)-PPCC, methyl(1*R*,2*S*/1*S*,2*R*)-2-[4-hydroxy-4-phenylpiperidin-1-yl)methyl]-1-(4-methylphenyl) cyclopropanecarboxylate; ACh, acetylcholine; AD, Alzheimer's disease; BD1047, *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine; HPC, hippocampus; PBS, phosphate-buffered saline; RAWM, radial arm water maze; SW, southwest.

et al. 2000; Tariot *et al.* 2000) or, more recently, blocking excessive activation of NMDA receptors with non-competitive antagonists (Winblad and Poritis 1999; Reisberg *et al.* 2003) have been shown to yield beneficial effects on either early or late stages of the disease (see Scarpini *et al.* 2003, for review). Notwithstanding, none of the above strategies significantly slows or prevents the progression of AD. As a result, novel compounds enabling effective treatment of age-associated learning deficits, and/or protection of degenerating neurons are still actively searched for.

Owing to their powerful anti-amnesic and neuroprotective effects, as well as a series of actions on mood, depression, response to stress or psychotomimetic states (reviewed in Guitart *et al.* 2004; Maurice 2007), ligands for the sigma receptors have attracted particular attention as possible therapeutic tools.

Sigma binding sites were first described as an opiate receptor subtype (Martin *et al.* 1976). More recently, however, these sites have been observed to possess unique features (such as the lack of aminoacid sequence homology, the ability to efficiently modulate the functioning of various transmitter receptors and ion channels or their participation in intracellular calcium trafficking) that make them distinct from other known ionotropic or metabotropic trasmitter receptors. At least two distinct receptor subtypes, termed sigma-1 and sigma-2, have been demonstrated each with its own regional distribution, subcellular localization and physiological function (Hellewell and Bowen 1990; Quirion *et al.* 1992; Bouchard and Quirion 1997). Sigma-2 receptor sites exhibit a relatively restricted distribution in brain areas mainly implicated in the control of posture and movement (Walker *et al.* 1990, 1994). By contrast, the sigma-1 receptors (the best characterized so far, see Hayashi and Su 2005 for a recent review) appear to be more widely distributed in the central nervous system, and are particularly abundant in the olfactory bulb, the dentate gyrus of the hippocampus (HPC), superficial layer of the cortex, amygdala and the basal forebrain nuclei (Alonso *et al.* 2000) i.e. regions prominently associated with learning and memory.

Based on the observation that sigma-1 receptor agonists enhance ACh release both *in vitro* (Junien *et al.* 1991), and *in vivo* (Matsuno *et al.* 1993, 1995), several studies addressing the pro-cognitive actions of sigma-1 ligands, have proposed that some of the effects of these compounds may result from the reinstatement of a cholinergic modulatory control onto the cortical and hippocampal areas involved in cognitive processing. Thus, the anti-amnesic efficacy of sigma-1 ligands have been investigated following cholinergic hypofunction induced by either pharmacological cholinergic receptor blockade (Earley *et al.* 1991; Matsuno *et al.* 1994; Tottori *et al.* 2002) or central administration of neurotoxic agents (Maurice *et al.* 1996, 1998; Senda *et al.* 1998). However, none of the above procedures has proven to be selective for cholinergic neurons in the basal forebrain, nor do they enable the possibility to produce lesions with varying degrees of severity. These issues are of importance, when restorative strategies based on drug-induced neuroprotection or functional replacement are to be tested and possible mechanisms of action are to be inferred.

The compound methyl(1*R*,2*S*/1*S*,2*R*)-2-[4-hydroxy-4-phenylpiperidin-1-yl)methyl]-1-(4-methylphenyl) cyclopropanecarboxylate [(±)-PPCC] is a selective sigma ligand with negligible affinity towards several other receptor systems. This compound interacts mainly with sigma-1 receptors (K_i = 1.5 nM) and shows a 34-fold higher selectivity than over sigma-2 receptors. Conversely, it exhibits negligible affinity for NMDA, dopaminergic, cholinergic muscarinic, histaminergic, or serotonergic receptors (Prezzavento *et al.* 2007). On the basis of its antiopioid action *in vivo*, (±)-PPCC has recently been proposed to act as a sigma-1 agonist (Prezzavento *et al.* 2008), however, no additional data are presently available concerning its functional activity. In the present study, the effects of (±)-PPCC, on different aspects of learning and memory were investigated in rats with selective cholinergic depletions induced by a powerful immunotoxin, 192 IgG-saporin (Wiley *et al.* 1991). This well-characterized lesioning agent is composed by a ribosome-inactivating protein, saporin, coupled with a monoclonal antibody, 192 IgG, specific for the low affinity nerve growth factor receptor. Upon binding of the antibody moiety with the receptor, that is specifically expressed by cholinergic neurons in the basal forebrain, the toxin conjugate is internalized, retrogradely transported and accumulated in the cell body, where the saporin moiety blocks protein synthesis, thereby inducing cell death (see Wrenn and Wiley 1998; Wiley and Kline 2000 for reviews). Here, the immunotoxin was administered at doses producing either mild or severe cognitive impairments associated with moderate (≤ 70–80%) or complete (≥ 90–95%) central cholinergic depletion (Leanza *et al.* 1995). Moreover, the direct involvement of the sigma-1 receptor in the functional effects of the (±)-PPCC compound was addressed in a separate test where the animals were pre-treated with the sigma-1 receptor antagonist *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine (BD1047) prior to being subjected to central cholinergic muscarinic receptor blockade with atropine.

Methods

Subjects and design

All experiments involving laboratory animals were conducted following the Italian Guidelines for Animal Care (D.L. 116/92), which were also in accordance with the European Communities Council Directives (86/609/EEC) and were approved by the Ethical Committee at the University of Trieste. Sixty-six young female Sprague–Dawley rats (provided by the animal facility at the University of Trieste) weighing 200–250 g at the time of surgery,

housed under 12 h light/12 h dark conditions with free access to food and water were used. The animals were randomly allocated into groups receiving bilateral intraventricular injections of a low (lesioned, low; $n = 20$) or high dose (lesioned, high; $n = 20$) 192 IgG-saporin or phosphate-buffered saline (PBS) alone (sham-lesioned, $n = 13$), whereas the remaining animals (intact, $n = 13$) were not injected and served as unoperated controls. Behavioral analyses were begun at 3–4 weeks post-lesion, and consisted in the sequential administration of tests specifically designed to evaluate sensory-motor performance, as well as reference and working memory abilities. At 5–6 weeks post-lesion, prior to being cognitively tested, half of the animals in each group were randomly assigned to groups treated with either (±)-PPCC or saline. Thus, the following groups were generated: intact, (±)-PPCC-treated ($n = 7$); intact, saline-treated ($n = 6$); sham-lesioned (±)-PPCC-treated ($n = 7$); sham-lesioned, saline-treated ($n = 6$); lesioned, low PPCC-treated ($n = 10$), lesioned, low saline-treated ($n = 10$); lesioned, high (±)-PPCC-treated ($n = 10$); lesioned, high saline-treated ($n = 10$). Upon completion of the last testing session, at about 8 weeks post-lesion (Fig. 1), the animals were perfused and the brains processed for quantitative histo- and immunohistochemistry (relevant data provided as Supporting information).

Lesion surgery

All surgical procedures were carried out on deeply anesthetized animals (sodium pentobarbital, 5 mg/100 g i.p.). Intraventricular administration of 192 IgG-saporin (purchased from Advanced Targeting System, San Diego, CA, USA) was performed as previously described (Leanza *et al.* 1995, 1998), the doses being carefully selected in pilot experiments so as to produce a ≈70–80% or a ≈90–95% cholinergic depletion (see also Leanza *et al.* 1995; Waite *et al.* 1995). Briefly, 1.5 μg or 2.5 μg of the immunotoxin were dissolved in 7 μL of sterile PBS and injected into the lateral ventricle of each side (each rat thus receiving a total of 3.0 or 5.0 μg in a volume of 7 + 7 μL vehicle) at the following coordinates (in mm, according to Paxinos and Watson 1986): $A = -0.6$ (from bregma); $L = \pm 1.5$ (from the midline); $V = -4.5$ (from the skull bone) with the incisor bar set at the level of the interaural line. The toxin conjugate was injected as a bolus (at a speed of 1 μL/s) with a 10 μL Hamilton microsyringe, allowing 1 min for diffusion before withdrawal. For sham lesions, sterile PBS was injected using the same coordinates, volume, and speed.

Drugs

The compound (±)-PPCC, (1 mg/kg/mL) was prepared as described previously (Prezzavento *et al.* 2007). The sigma-1 receptor antagonist BD1047 (1 mg/kg/mL) was purchased from Tocris (Bristol, UK). Atropine sulphate (50 mg/kg/mL) was purchased from Sigma (Milan, Italy).

Drugs were all freshly dissolved in sterile saline solution, and injected i.p. to the animals 30–45 min before the beginning of each relevant testing session. Doses were chosen on the basis of pilot studies, or according to previously established administration protocols.

Behavioral tests

All testing was consistently carried out between 9:00 AM and 3:00 PM. In order to assess unspecific motor disturbances possibly induced by the immunotoxin (Berger-Sweeney *et al.* 1994; Waite *et al.* 1995), simple motor tests of limb strength and coordination were administered to all animals at 3–4 weeks post-lesion, as previously described (Leanza *et al.* 1998). Briefly, locomotive form and support were assessed after placing the rat onto a wooden ramp 80 cm long, which was connected to the animal's home cage and was maintained either horizontal or inclined at a 45° angle. An inclined (75°) 80 × 30 cm framed grid made of coarse-mesh chicken wire was also used, where the rats were placed head-down, being requested to reverse the direction and climb onto it.

Morris water maze

Spatial learning was tested using the Morris water maze (Morris 1984). The apparatus consisted in a circular pool, 140 cm in diameter and 50 cm deep filled to a depth of 35 cm with room temperature (20°C) water. Four equally spaced points (conventionally indicated as North, South, East, and West) served as start locations, also dividing the tank into four quadrants, the tank was located in a corner of a room containing many external cues that could be used by the animals for orientation. A circular platform (10 cm diameter) was anchored to the bottom of the pool in the southwest (SW) (training) quadrant with its top 2 cm below (and thus invisible from) the water surface, onto which the animal could climb to escape. Four annuli were defined as a circular area in the middle of each quadrant, corresponding to the site where the platform would have been, if placed in that quadrant. Starting from about 6 weeks post-lesion, the rats were trained over seven consecutive days using a four trials a day schedule, with a 30 s inter-trial interval. On each trial, the rat was placed into the water facing the wall of the tank at one of the starting positions, and given 60 s to find the submerged platform and climb onto it. Once the rat had reached the platform, it was allowed to rest for the subsequent 30 s, before being picked up and placed in the next pre-determined position. The latency to find the hidden platform, the distance swum and the swim speed were recorded by a computer-based video tracking system. On the final day of testing (day 7), after the last trial, the platform was removed and a fifth spatial probe trial was administered, in which the rat was allowed to swim freely for 60 s. The swim path was plotted, and the distance swum and the number of annulus crossings in each quadrant were recorded.

The following day, a separate 4-day experiment commenced where the performance of animals randomly selected from the intact ($n = 6$), sham-lesioned ($n = 6$) and lesioned, low ($n = 8$) groups was evaluated in the Morris water maze under central cholinergic muscarinic receptor blockade. On each day, animals were given four trials (with the escape platform placed in the SW quadrant) followed

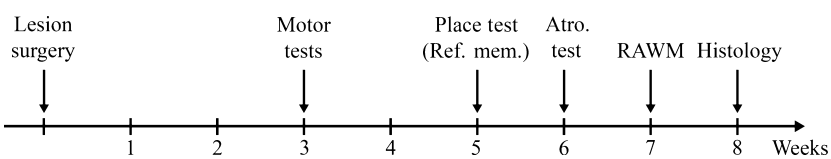

Fig. 1 General experimental design showing the temporal sequence of surgery and tests, and the intervals between them.

by a fifth spatial probe trial. On the first day, the rats were tested without any drug treatment, receiving only a single i.p. injection of sterile saline (1 mL/kg). On the second day, 50 mg/kg atropine sulphate in saline was injected i.p. about 45 min before the beginning of the test. On the third day, atropine sulphate was administered about 15 min after the i.p. injection of (±)-PPCC (1 mg/kg in saline). On the fourth day, the sigma receptor antagonist BD1047, (±)-PPCC (both at a dose of 1 mg/kg in saline) and atropine sulphate, were injected sequentially to each rat at intervals of about 15 min from one another. The test was then started about 30 min after the last injection. The latency to find the platform, the distance swum and the number of annulus crossings in the training (SW) quadrant, as well as the actual swim paths were recorded as above.

Radial arm water maze

Spatial working memory was studied using a radial arm water maze (RAWM) procedure modified from Diamond *et al.* (1999) and Arendash *et al.* (2001). The apparatus consisted in the same circular pool, placed in the same cued room and filled with water as above. Twelve Plexiglas walls (50 cm length × 50 cm height) were taped together and positioned within the pool so as to produce six swim alleys or arms (50 cm length × 20 cm wide) radiating out of a open central area. Approximately at the end of one of the arm (referred to as the goal arm) was a circular platform (10 cm in diameter) located 2.0 cm below the surface onto which the animals could escape. The platform remained in the same arm for the four trials given within a day, and moved pseudorandomly to a new arm on each of five consecutive testing days. For any given trial, the animal was placed into the designated start arm, and given up to 60 s to locate the hidden platform with a 30 s inter-trial time. If the platform was not located within the given time, the animal was guided to the goal arm by the experimenter and allowed to remain on the platform for 30 s prior to being placed at the next start position. Entering an incorrect arm (i.e. an arm that did not contain the platform) was counted as an entry error. For each trial, the latency to reach the platform and the number of arm selection errors prior to locating the goal arm were recorded. Moreover, the difference between latency or error scores on trial 1 and 2, expressed as a percentage of the respective scores recorded on trial 1, provided an additional measure of working memory performance indicated as 'savings' (Netto *et al.* 1993).

Results

General observations

One intact and one sham-lesioned animal from the (±)-PPCC-treated groups, three lesioned, low rats [one saline-treated and two (±)-PPCC-treated] and three lesioned, high rats [two saline-treated and one (±)-PPCC-treated) were lost during testing. Thus, the groups resulted as follows : intact, saline-treated (n = 6); sham-lesioned, saline-treated (n = 6); intact, (±)-PPCC-treated (n = 6); sham-lesioned (±)-PPCC-treated (n = 6); lesioned, low saline-treated (n = 9); lesioned, low (±)-PPCC-treated (n = 8) lesioned, high saline-treated (n = 8); lesioned, high (±)-PPCC-treated (n = 9). Sham-lesioned and unoperated animals did not differ on any of the behavioral or morphometrical parameters analyzed, and were thus combined into single normal groups treated with either saline (n = 12) or (±)-PPCC (n = 12).

Table 1 Motor performance

	Equilibrium time on ramp (%)	Latency to cross ramp (s)	Latency to reverse on grids (s)	Number of falls in grids
Normal	97.6 ± 9.7	6.5 ± 0.4	6.2 ± 0.7	2.3 ± 0.4
Lesion low	96.4 ± 5.7	6.8 ± 0.4	7.0 ± 0.7	2.3 ± 0.5
Lesion low PPCC	98.2 ± 7.5	6.8 ± 0.5	6.6 ± 0.8	2.8 ± 0.7
Lesion high	97.4 ± 8.3	7.0 ± 0.4	5.9 ± 0.6	2.1 ± 0.5
Lesion high PPCC	97.7 ± 12.7	6.6 ± 0.4	6.5 ± 1.1	2.2 ± 0.6

Simple motor tests were administered all animals starting from 3 to 4 weeks post-lesion, and consisted in the evaluation of postural and locomotive form onto a 80 cm-long wooden ramp (maintained either horizontal or with a 45° inclination), or an inclined (75°) grid, both connected to the animals' home cage. Parameters to be analyzed were the % balance time onto the ramp and the time required to cross it, as well as the latency to reverse direction and the number of falls when placed onto a grid. Numbers represent the mean of four determinations ±SEM. PPCC, methyl(1*R*,2*S*/1*S*,2*R*)-2-[4-hydroxy-4-phenylpiperidin-1-yl)methyl]-1-(4-methylphenyl) cyclopropanecarboxylate.

At about 3 weeks post-lesion, when first tested for motor performance, all lesioned and control rats, irrespective of the (±)-PPCC treatment, exhibited fairly normal sensory-motor functioning and did not differ from each other, as seen in the bridge and grid tests (Table 1). These observations, together with the finding of no differences in swim speed among the groups (see below) indicated that changes in motor activity could not account for water maze performance (note that data and illustrations from morphometrical analyses are reported separately as Supporting information).

Behavioral tests

Morris water maze

Average latencies and swim distances on successive days of training in the reference memory version of the water maze task are shown in Fig. 2(a and b). All animals initially required approximately 32–48 s and 8–15 m to locate the submerged platform and improved significantly thereafter (repeated measure ANOVA, effect of day on latency: $F_{6,312} = 48.6$; on distance: $F_{6,312} = 33.7$, in both cases $p < 0.001$). However, whereas saline- and (±)-PPCC-treated normal animals, as well as lesioned-low and lesioned-high animals treated with (±)-PPCC learned rapidly to locate the platform and did not differ from each other, lesioned rats treated with saline exhibited significant acquisition deficits which appeared particularly severe in the high dose group

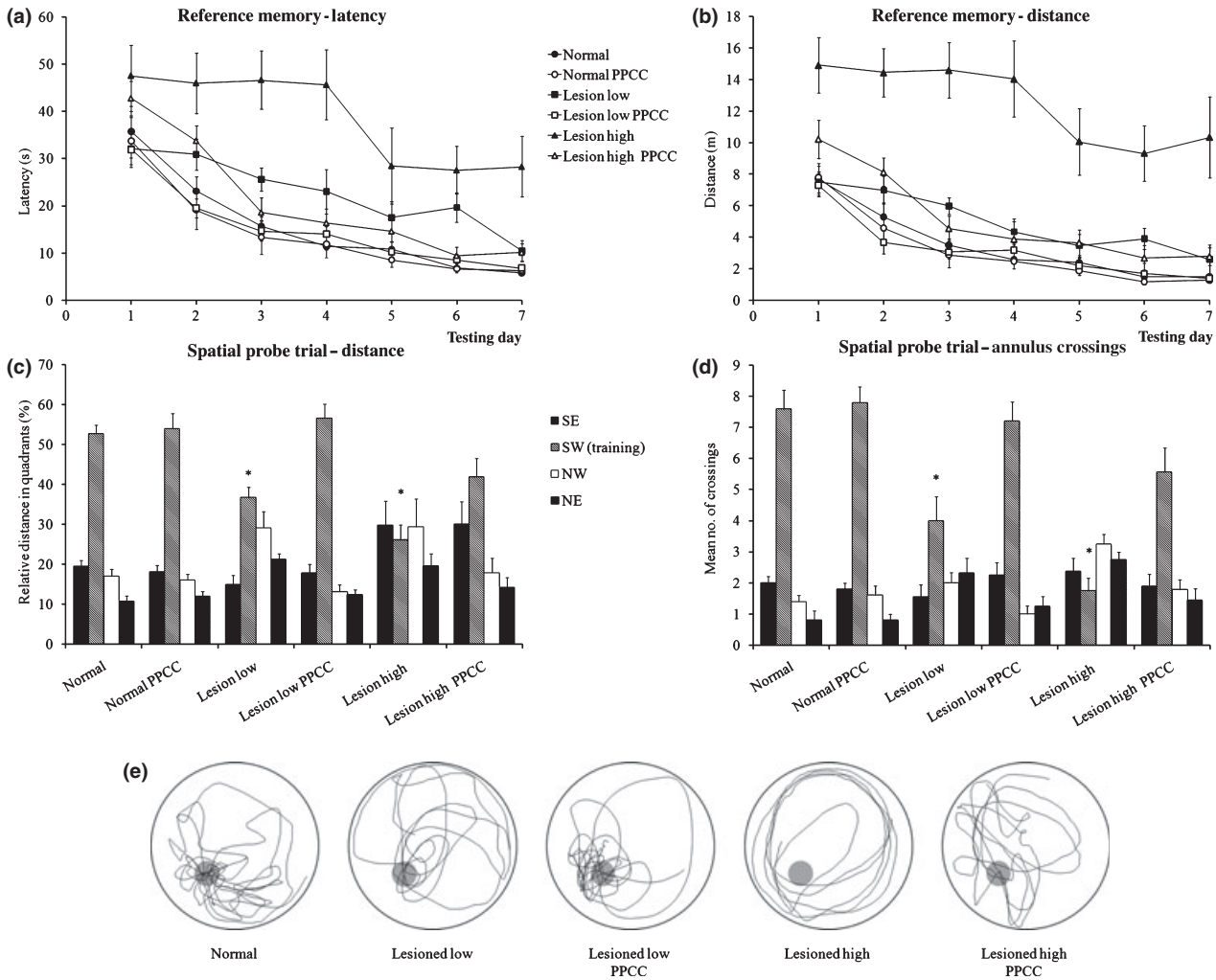

Fig. 2 Reference memory test. Average latency (a) and swim distance (b) required to locate the submerged platform during the acquisition phase of the spatial navigation task. Each sample point represents the mean value ± SEM for the block of four trials on each of the seven consecutive days of testing. Lower diagrams illustrate the mean relative distance swum (c) and the average number of annulus crossings in each quadrant (d) during the spatial probe trial, upon removal of the escape platform from the SW (training) quadrant. In (e) the actual swim paths taken by representative animals from the different groups are illustrated. Note the varying severity of the deficits induced by the 192 IgG-saporin immunotoxin at the low (3.0 μg) or high (5.0 μg) doses and their amelioration following administration of the (±)-PPCC compound (1.0 mg/kg). Asterisks indicate significant difference from the normal group at $p < 0.05$.

(group effect on latency: $F_{5,52} = 32.2$; on distance: $F_{5,52} = 84.3$, in both cases $p < 0.001$, no group × day interaction).

In the spatial probe trial, on day 7, when the platform was removed and the animals were allowed to swim freely, the normal groups and the lesioned animals in both dose-groups treated with (±)-PPCC swam primarily in the training quadrant. By contrast, the saline-treated lesioned animals in both low- and high-dose groups showed a poorly focused search behavior, with the latter distributing their swim over the four quadrants (Fig. 2c and d). Statistical analysis on the distance and annulus crossing measures revealed a significant effect of quadrant (for distance: $F_{3,156} = 95.4$; for annulus crossings: $F_{3,156} = 145.2$; in both cases $p < 0.001$), as well as a group x quadrant interaction (for distance: $F_{15,156} = 6.5$; for annulus crossings: $F_{15,156} = 12.1$; in both cases $p < 0.001$). Subsequent analyses (one-way ANOVA + Fisher's protected least significant difference, *post hoc* test) as well as inspection of the actual swim paths (Fig. 2e) confirmed that the saline-treated lesioned animals in both low- and high-dose groups swam significantly less in the training quadrant than those in the other groups (for distance: $F_{5,52} = 13.0$; for annulus crossings: $F_{5,52} = 13.8$; in both cases $p < 0.001$). No difference between groups was observed in the total number of annulus crossings ($F_{5,52} = 1.4$, $p > 0.2$, NS) or swim speed ($F_{5,52} = 1.9$,

$p > 0.1$, NS) suggesting an active, albeit not equally effective, search behavior in all animals.

Atropine test

After completion of the previous testing session, a separate 4-day atropine test was given to 12 normal and eight lesioned-low animals, in order to evaluate the effects of (±)-PPCC upon spatial navigation under central cholinergic muscarinic receptor blockade. As shown in Fig. 3(a–d), atropine sulphate severely impaired performance in all measures analyzed, compared to the no-drug (saline) condition. Thus, both groups exhibited increased latencies and distances

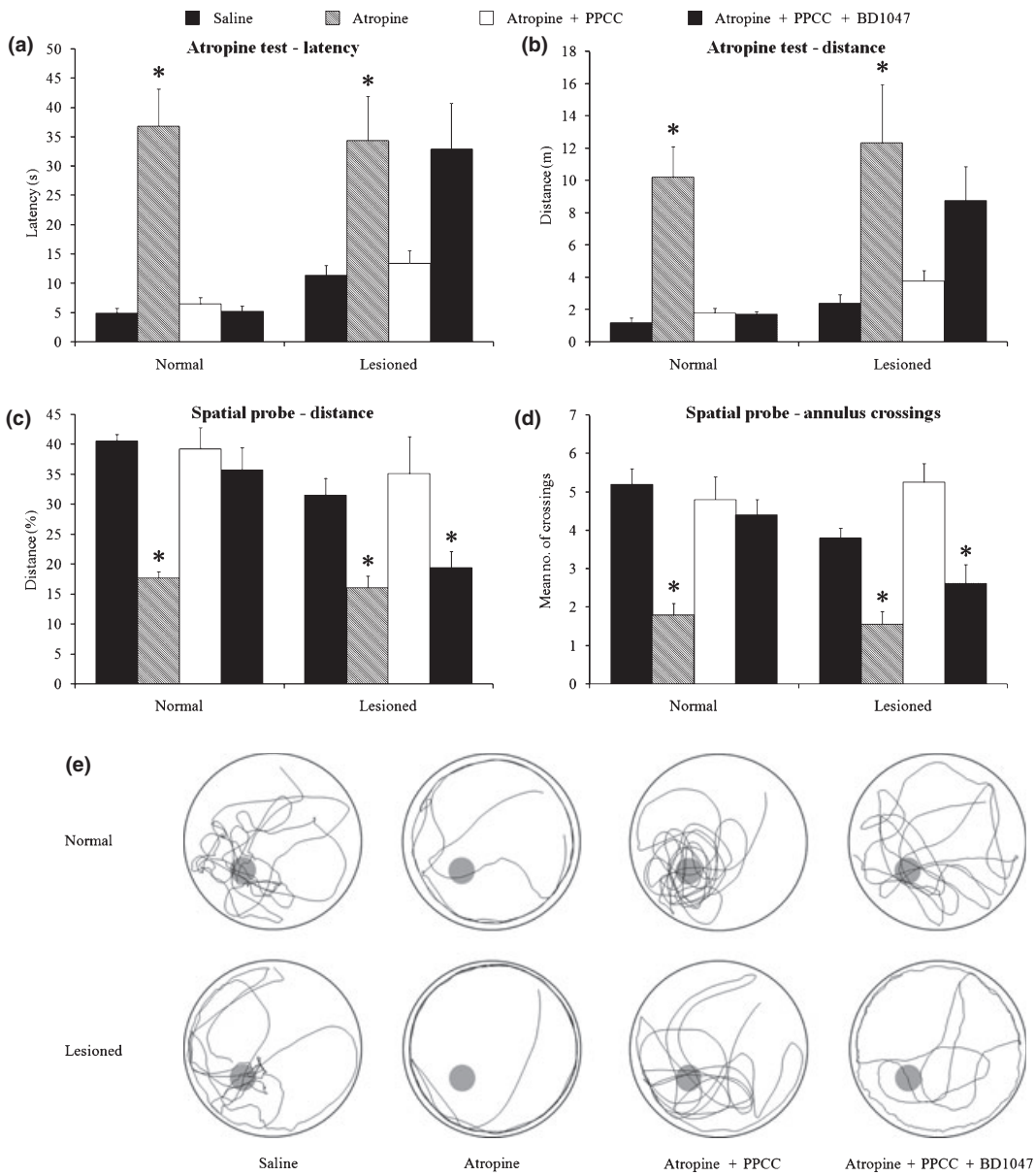

Fig. 3 Atropine test. Average latency (a) and distance swum (b) required to locate the platform during the 4-day atropine test administered to randomly selected animals from the normal, sham-lesioned, and lesioned low groups. The histograms represent the mean value ± SEM for the block of four trials on each testing day. Lower diagrams illustrate the mean relative distance swum (c) and the average number of annulus crossings (d) in the SW (training) quadrant during the spatial probe trial administered after the fourth trial on each day. In (e) the actual swim paths taken by representative animals from the various groups are illustrated. Note the worsened performance of both groups during atropine (50 mg/kg) and its remarkable amelioration by pre-treatment with (±)-PPCC (1.0 mg/kg), which was abolished in lesioned, but not normal animals by pre-treatment with the sigma-1 receptor antagonist BD1047 (1.0 mg/kg). Asterisks indicate significant difference from the normal group at $p < 0.05$.

(repeated measures ANOVA, respectively $F_{1,18} = 29.4$ and $F_{1,18} = 22.6$; both $p < 0.001$) as well as a decreased rate of distance swum and annulus crossings in the training (SW) quadrant during the spatial probe test (repeated measures ANOVA, respectively $F_{1,18} = 131.9$ and $F_{1,18} = 117.4$; both $p < 0.001$). Notably, groups performance returned to pre-atropine levels during (±)-PPCC ($p < 0.001$ vs. atropine sulphate), and no main group effect nor group × drug interaction was observed during atropine or (±)-PPCC, suggesting that both groups responded similarly to the drug challenge. However, this was not the case on the last day of the test, when the sigma antagonist BD1047 was administered together with atropine and (±)-PPCC. In fact, whereas in the lesioned animals BD1047 abolished the effects of (±)-PPCC, resulting in a worsened performance as during atropine, it produced no detectable changes in normal animals. Repeated measures ANOVA on latency and distance revealed a significant group effect (respectively, $F_{1,18} = 29.3$ and $F_{1,18} = 29.8$; both $p < 0.001$), as well as a group × drug interaction (respectively, $F_{1,18} = 8.6$ and $F_{1,18} = 6.8$; both $p < 0.05$). A similar dissociation was seen also during the probe trial, where the groups responded differently to the sigma antagonist (repeated measures ANOVA, groups × drug for distance, $F_{1,18} = 7.4$; for annulus crossings, $F_{1,18} = 6.4$; both $p < 0.05$). Swim speed, monitored as a general measure of rats' motor performance was not affected by any drug treatment, and averaged 0.2–0.3 m/s throughout the four testing days.

Inspection of the actual swim paths (Fig. 3e) confirmed that all animals tended to swim in wide circles and predominantly along the edge of the pool under the influence of atropine. Such pattern of poorly focussed search behavior was abolished by pre-treatment with (±)-PPCC in both groups, but only in the lesioned animals was the sigma antagonist BD1047 seen to dramatically reverse the effect of (±)-PPCC, whereas the normal animals did not respond to the challenge. Similar results were obtained in separate experiments where scopolamine (1.0 mg/kg i.p.) was used to induce muscarinic receptor blockade (data not shown).

Radial arm water maze

The performances of the groups in the RAWM testing are shown in Fig. 4. In this task, the platform was moved to a new quadrant every day, and the animals had to re-learn its position within the four trials of each testing day. Since analysis of both latency and errors to locate the platform produced very similar results, only data concerning the error measure will be reported here. In general, all animals exhibited a relatively high number of arm selection errors (i.e. entering an arm not containing the platform) during the first trial of each day, but they progressively reduced the number of entry errors thereafter, reaching an asymptotic performance between the third and fifth trial (repeated measures ANOVA, effect of trial, $F_{4,208} = 77.0$; $p < 0.001$).

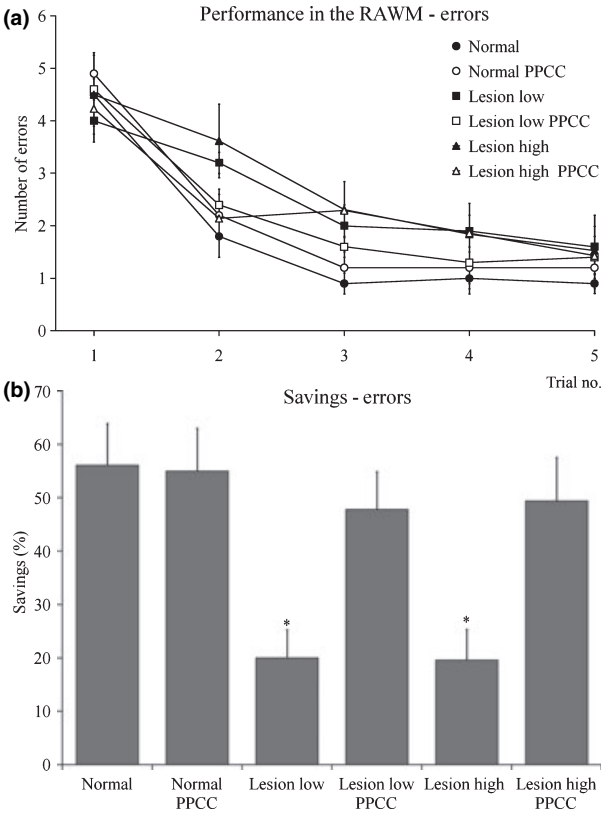

Fig. 4 Working memory test. Average number of entry errors (a) done by the animals in the radial arm water maze task. Each sample point represents the mean errors recorded during each 60 s trial over five consecutive testing days ± SEM. Note the rapid improvement in the normal and (±)-PPCC-treated lesioned animals. In the lower diagram (b) the percent improvement (savings) between trials 1 and 2 is illustrated. A very similar profile was observed also for escape latency measure. Asterisks indicate significant difference from the normal group at $p < 0.01$.

There was a significant difference among groups (repeated measures ANOVA, main group effect, $F_{5,52} = 3.6$; $p < 0.01$) suggesting that the animals differed in the number of entry errors across the trials (Fig. 4a). Closer inspection of data revealed that the saline- and (±)-PPCC-treated normal animals, as well as the (±)-PPCC-treated lesioned animals from both dose groups rapidly learned to locate the platform and markedly reduced the number of errors between the first and the second trial. By contrast, rats lesioned with a low or high dose of immunotoxin and treated with saline did not exhibit such improvements and appeared equally impaired. In order to provide a measure of the learning efficiency in this task, the percentage improvement between trials 1 and 2 were analyzed and plotted in terms of savings. Under these conditions, saline- and (±)-PPCC-treated normal, and (±)-PPCC-treated lesioned animals from both dose-groups reduced their entry errors by about 50% and did not differ from each other. By contrast, the percent improvement

exhibited by the saline-treated lesioned animals in both dose-groups was significantly lower than normal ($p < 0.01$) and never exceeded 15–20% (Fig. 4b).

Discussion

In the present series of experiments, the anti-amnesic properties of a novel sigma-1 receptor agonist, (±)-PPCC (Prezzavento *et al.* 2007, 2008), and their possible dependence upon either a cholinergic mechanism or a direct interaction with sigma-1 receptor were studied in the 192 IgG-saporin-lesioned rat model. The rationale for the study was twofold: first, the peculiar distribution of sigma-1 binding sites in neocortical and hippocampal areas classically associated with learning and memory processes (Alonso *et al.* 2000); secondly, the well-known effects of sigma receptor ligands upon cholinergic neurotransmission both *in vitro* and *in vivo*, as seen respectively in hippocampal slice preparations (Junien *et al.* 1991), and following placement of microdialysis probes in the neocortex or HPC (Matsuno *et al.* 1993, 1995). Thus, the effects of the compound were investigated under relatively moderate or severe cholinergic depletion and spatial learning impairments induced by different doses of the toxin conjugate.

Effects of the lesion

The selective immunotoxin 192 IgG-saporin, able to efficiently target cholinergic neurons in the basal forebrain (Wiley *et al.* 1991) represents a powerful tool to investigate the role played by this system in learning and memory (see e.g. Parent and Baxter 2004; Mesulam 2004 for recent reviews). Consistent with previous reports (e.g. Heckers *et al.* 1994; Leanza *et al.* 1995, 1998), the toxin conjugate produced an evident loss of choline acetyl transferase-immunoreactive neurons throughout the basal forebrain, associated to a marked depletion of acetylcholinesterase-positive fibers innervating the neocortex and the HPC, whereas non-cholinergic, GABAergic neurons in the septum/vertical limb of the diagonal band of Broca were unaffected. Notably, the lesioning paradigms adopted in the present study, whereby either a relatively low (3.0 μg), or a high dose (5.0 μg) of toxin was delivered to the lateral ventricles, enabled the production of graded, dose-dependent cholinergic depletions (estimated to range from ≤ 70–80% to ≥ 90–95%) resulting in mild-to-severe deficits in reference memory abilities (see e.g. Leanza *et al.* 1995; Waite *et al.* 1995). Working memory performance, as monitored here using the RAWM task, was also affected by the immunotoxin treatment. This is consistent with previous findings using delayed matching and non-matching to position tasks (Torres *et al.* 1994; Steckler *et al.* 1995; Leanza *et al.* 1996, 1998) and points to a role for ascending basal forebrain cholinergic afferents in the regulation of short-term memory. It should be noted, however, that the deficits observed here appeared to be less sensitive to the dose of toxin injected, as they were of similar magnitude in both dose-groups. The lack of dose-dependent impairments in working memory may reflect a poor sensitivity of the RAWM procedure in detecting subtle differences in the acquisiton of a very complex task and will require further investigation. In any event, the possibility to produce mild lesions is of importance when experimental strategies aimed at protecting and/or sustaining transmitter activity in residual neurons are to be tested. Likewise, by completely removing cholinergic afferents to neocortex and HPC it is possible to address the efficacy of restorative treatments without the confounding presence of spared elements that may interefere with a correct interpretation of the observed effects. Taken together, therefore, the results highlight the usefulness of the 192 IgG-saporin lesion approach to address issues of drug-induced recovery of cognitive functions in models of either mild or severe loss of regulatory cholinergic inputs.

Effects of (±)-PPCC

The results of the present study indicate that the novel sigma ligand (±)-PPCC is able to reverse the impairments in both reference and working memory induced by selective cholinergic lesions. Remarkably, the efficacy of the (±)-PPCC compound was observed not only when the cholinergic depletion and the resulting cognitive impairments were very mild, but also when the deficits were highly severe and the cholinergic loss was virtually complete. Conversely, no clear-cut learning facilitation was observed in control animals treated with (±)-PPCC, nor was the sigma-1 antagonist BD1047 seen to efficiently block in these animals the effects of the (±)-PPCC compound during the atropine test. In keeping with previous evidence (reviewed in Maurice 2007) the present data, therefore, seem to suggest that sigma receptors are marginally involved in normal cognitive processing, and that their ligands (acting as either agonists or antagonists) would exert their action only when cholinergic neurotransmission is perturbed.

The issue of a cholinergic involvement in the anti-amnesic actions of sigma-1 agonists has been addressed in a number of previous studies (e.g. Earley *et al.* 1991; Matsuno *et al.* 1994; Maurice *et al.* 1996, 1998; Senda *et al.* 1998; Tottori *et al.* 2002; see also Maurice 2007 for a recent review). Notably, however, several observations strongly suggests that the effects reported here may take place independently of a cholinergic mechanism, but most probably require a direct interaction with sigma-1 receptors. First, the anti-amnesic effects of (±)-PPCC are still detected following complete cholinergic depletion, i.e. a condition where virtually no neurons are left spared in the basal forebrain nuclei. Although an interaction of (±)-PPCC with cholinergic receptors onto target neurons cannot completely be ruled out, this seem unlikely, in light of the very low or no affinity exhibited by the compound for muscarinic receptors [K_i > 10 000 nM,

Prezzavento *et al.* 2007; the affinity of the (±)-PPCC compound for nicotinic receptor subtypes is currently being investigated]. Second, perhaps more importantly, the (±)-PPCC-induced effects are dramatically blocked by pre-treatment with BD1047 in animals later subjected to cholinergic muscarinic receptor blockade with atropine. This finding suggests that the (±)-PPCC compound does not simply act as a cholinomimetic drug, but may possess a very specific mode of action directly onto sigma-1 receptors densely expressed in the same cortical and hippocampal regions denervated by the present lesioning procedure (Alonso *et al.* 2000). Although this notion cannot be directly inferred from the present data, the fact that (±)-PPCC has recently been shown to exhibit one of the best sigma-1 agonist profile among the various putative ligands tested [$K_i(\sigma_1)$ = 1.5 nM; Prezzavento *et al.* 2007] argues that this may be the case.

The mechanism whereby sigma-1 receptor agonists exert their physiological actions is still elusive. One interesting possibility is that activated sigma-1 receptors may participate to the modulation of inositol 1,4,5-triphosphate receptor-mediated Ca^{2+}signalling and activation of phospholipase C/protein kinase C pathways (Morin-Surun *et al.* 1999; Hayashi *et al.* 2000). Very recently, the activated receptors have also been proposed to prolong Ca^{2+} signalling from the endoplasmic reticulum into mitochondria by stabilizing inositol 1,4,5-triphosphate, thus attenuating the consequences of biological insults imposed to the cells (Hayashi and Su 2007). This mechanism has recently been proposed to account for the marked restorative and neuroprotective effects of donezepil reported *in vitro* (Ishima *et al.* 2008) and it is likely to take place also *in vivo*, following administration to mice with learning impairments induced by either carbon monoxide or amyloid β25–35 (Meunier *et al.* 2006a,b). To the best of our knowledge, no study to date has investigated the anti-amnesic actions of sigma-1 receptor agonists following selective cholinergic dysfunction. Never the less, in keeping with those observations, it is plausible that a direct activation of sigma-1 receptors and the downstream functional consequences possibly promoted by (±)-PPCC may have well taken place also in the present experimental conditions. An intriguing possibility, in this respect, will be to investigate the possible effects of these compounds on other histopathological aspects of the disease known to be replicated in the 192 IgG-saporin rat model (Leanza 1998; Lin *et al.* 1998) and normalized following muscarinic receptor activation (Lin *et al.* 1999) or cholinergic cell replacement (Aztiria *et al.* 2008). Studies are in fact underway to address this issue (Antonini *et al.* 2007).

In conclusion, the present findings show that the putative sigma-1 receptor agonist (±)-PPCC has powerful anti-amnesic actions when administered to animals with either mild or severe cognitive impairments, and that these effects most probably occur via a direct interaction of the compound with sigma-1 receptors onto denervated neurons. Thus, further developments of sigma-1 agonists may represent viable tools to ameliorate cognitive performance and warrant further investigation on the possible use of these compounds as supporting strategies in experimental studies aimed at cholinergic cell protection or replacement.

Acknowledgements

This work was supported by the FIRB 2003 (Grant No. RBNE03FH5Y) and PRIN 2005 (Grant No. 2005032713) programs from MIUR (Rome) and the Kathleen Foreman-Casali Foundation, Trieste, Italy. The valuable assistance of Dr M. Stebel and his staff at the CSPA facility for animal care, University of Trieste, is gratefully acknowledged.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Representative examples of choline acetyltransferase (ChAT) immunostaining in coronal sections from the septum/vDBB (a–c) and NBM (d–f).

Fig. S2 Representative examples of acetylcholinesterase (AChE) histochemistry showing, on the coronal plane, the extent of cholinergic denervation after intraventricular administration of vehicle (sterile PBS, a), or 192 IgG-saporin at low (3.0 μg, b) or high doses (5.0 μg, c).

Table S1 Morphometric analyses.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

Alonso G., Phan V., Guillemain I., Saunier M., Legrand A., Anoal M. and Maurice T. (2000) Immunocytochemical localization of the sigma(1) receptor in the adult rat central nervous system. *Neuroscience* **97**, 155–170.

Antonini V., Marrazzo A., Boffo S., Aniskovich N., Ronsisvalle S., Prezzavento O., Ronsisvalle G. and Leanza G. (2007) MR22, a novel sigma-1 receptor agonist: effects on lesion-induced memory dysfunction and amyloid deposition in rats. *Front. CNS Oncol. Med. Chem.* **41**, 56.

Arendash G. W., King D. L., Gordon M. N., Morgan D., Hatcher J. M., Hope C. E. and Diamond D. M. (2001) Progressive, age-related behavioral impairments in transgenic mice carrying both mutant amyloid precursor protein and presenilin-1 transgenes. *Brain Res.* **891**, 42–53.

Aztiria E., Cataudella T., Spampinato S. and Leanza G. (2008) Septal grafts restore cognitive abilities and amyloid precursor protein metabolism. *Neurobiol. Aging* [Epub ahead of print] doi: 10.1016/j.neurobiolaging.2007.12.018.

Bartus R. T., Dean R. L. 3rd, Beer B. and Lippa A. S. (1982) The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408–414.

Baskin D. S., Browning J. L., Pirozzolo F. J., Korporaal S., Baskin J. A. and Appel S. H. (1999) Brain choline acetyltransferase and

mental function in Alzheimer disease. *Arch. Neurol.* **56**, 1121–1123.

Becker R., Giacobini E., Elble R., McIlhany M. and Sherman K. (1988) Potential pharmacotherapy of Alzheimer disease. a comparison of various forms of physostigmine administration. *Acta Neurol. Scand.* **116**, 19–32.

Berger-Sweeney J., Heckers S., Mesulam M. M., Wiley R. G., Lappi D. A. and Sharma M. (1994) Differential effects on spatial navigation of immunotoxin-induced cholinergic lesions of the medial septal area and nucleus basalis magnocellularis. *J. Neurosci.* **14**, 4507–4519.

Bouchard P. and Quirion R. (1997) [3H]1,3-di(2-tolyl)guanidine and [3H](+)pentazocine binding sites in the rat brain: autoradiographic visualization of the putative sigma1 and sigma2 receptor subtypes. *Neuroscience* **76**, 467–477.

Collerton D. (1986) Cholinergic function and intellectual decline in Alzheimer's disease. *Neuroscience* **19**, 1–28.

Coyle J. T., Price D. L. and DeLong M. R. (1983) Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* **219**, 1184–1190.

Cummings J. L., Donohue J. A. and Brooks R. L. (2000) The relationship between donepezil and behavioral disturbances in patients with Alzheimer's disease. *Am. J. Geriatr. Psychiatry* **8**, 134–140.

Diamond D. M., Park C. R., Heman K. L. and Rose G. M. (1999) Exposing rats to a predator impairs spatial working memory in the radial arm water maze. *Hippocampus* **9**, 542–552.

Doody R. S., Dunn J. K., Clark C. M., Farlow M., Foster N. L., Liao T., Gonzales N., Lai E. and Massman P. (2001) Chronic donepezil treatment is associated with slowed cognitive decline in Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* **12**, 295–300.

Earley B., Burke M., Leonard B. E., Gouret C. J. and Junien J. L. (1991) Evidence for an anti-amnesic effect of JO 1784 in the rat: a potent and selective ligand for the sigma receptor. *Brain Res.* **546**, 282–286.

Guitart X., Codony X. and Monroy X. (2004) Sigma receptors: biology and therapeutic potential. *Psychopharmacology* **174**, 301–319.

Hayashi T. and Su T.-P. (2005) The sigma receptor: evolution of the concept in neuropsychopharmacology. *Curr. Neuropharmacol.* **3**, 267–280.

Hayashi T. and Su T.-P. (2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca^{2+} signalling and cell survival. *Cell* **131**, 596–610.

Hayashi T., Maurice T. and Su T. P. (2000) Ca(2+) signaling via sigma(1)-receptors: novel regulatory mechanism affecting intracellular Ca(2+) concentration. *J. Pharmacol. Exp. Ther.* **293**, 788–798.

Heckers S., Ohtake T., Wiley R. G., Lappi D. A., Geula C. and Mesulam M. M. (1994) Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. *J. Neurosci.* **14**, 1271–1289.

Hellewell S. B. and Bowen W. D. (1990) A sigma-like binding site in rat pheochromocytoma (PC12) cells: decreased affinity for (+)-benzomorphans and lower molecular weight suggest a different sigma receptor form from that of guinea pig brain. *Brain Res.* **527**, 244–253.

Ishima T., Nishimura T., Iyo M. and Hashimoto K. (2008) Potentiation of nerve growth factor-induced neurite outgrowth in PC12 cells by donepezil: role of sigma-1 receptors and IP(3) receptors. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **32**, 1656–1659.

Junien J. L., Roman F. J., Brunelle G. and Pascaud X. (1991) JO1784, a novel sigma ligand, potentiates [3H]acetylcholine release from rat hippocampal slices. *Eur. J. Pharmacol.* **200**, 343–345.

Leanza G. (1998) Chronic elevation of amyloid precursor protein expression in the neocortex and hippocampus of rats with selective cholinergic lesions. *Neurosci. Lett.* **257**, 53–56.

Leanza G., Nilsson O. G., Wiley R. G. and Bjorklund A. (1995) Selective lesioning of the basal forebrain cholinergic system by intraventricular 192 IgG-saporin: behavioural, biochemical and stereological studies in the rat. *Eur. J. Neurosci.* **7**, 329–343.

Leanza G., Muir J., Nilsson O. G., Wiley R. G., Dunnett S. B. and Bjorklund A. (1996) Selective immunolesioning of the basal forebrain cholinergic system disrupts short-term memory in rats. *Eur. J. Neurosci.* **8**, 1535–1544.

Leanza G., Martinez-Serrano A. and Bjorklund A. (1998) Amelioration of spatial navigation and short-term memory deficits by grafts of foetal basal forebrain tissue placed into the hippocampus and cortex of rats with selective cholinergic lesions. *Eur. J. Neurosci.* **10**, 2353–2370.

Lin L., LeBlanc C. J., Deacon T. W. and Isacson O. (1998) Chronic cognitive deficits and amyloid precursor protein elevation after selective immunotoxin lesions of the basal forebrain cholinergic system. *Neuroreport* **9**, 547–552.

Lin L., Georgievska B., Mattsson A. and Isacson O. (1999) Cognitive changes and modified processing of amyloid precursor protein in the cortical and hippocampal system after cholinergic synapsed loss and muscarinic receptor activation. *Proc. Natl Acad. Sci. USA* **96**, 12108–12113.

Martin W. R., Eades C. G., Thompson J. A., Huppler R. E. and Gilbert P. E. (1976) The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther.* **197**, 517–532.

Matsuno K., Matsunaga K., Senda T. and Mita S. (1993) Increase in extracellular acetylcholine level by sigma ligands in rat frontal cortex. *J. Pharmacol. Exp. Ther.* **265**, 851–859.

Matsuno K., Senda T., Matsunaga K. and Mita S. (1994) Ameliorating effects of sigma receptor ligands on the impairment of passive avoidance tasks in mice: involvement in the central acetylcholinergic system. *Eur. J. Pharmacol.* **261**, 221–224.

Matsuno K., Senda T., Kobayashi T. and Mita S. (1995) Involvement of sigma1 receptor in (+)-N-allylnormetazocine-stimulated hippocampal cholinergic functions in rats. *Brain Res.* **690**, 200–206.

Maurice T. (2007) Cognitive effects of sigma receptor ligands, in *Sigma Receptors. Chemistry, Cell Biology and Clinical Implications* (Matsumoto R. R., Bowen W. D. and Su T.-P., eds), pp. 237–271. Springer, New York.

Maurice T., Lockhart B. P. and Privat A. (1996) Amnesia induced in mice by centrally administered β-amyloid involves cholinergic dysfunction. *Brain Res.* **706**, 181–193.

Maurice T., Su T. P. and Privat A. (1998) Sigma1 (sigma 1) receptor agonists and neurosteroids attenuate β25–35-amyloid peptide-induced amnesia in mice through a common mechanism. *Neuroscience* **83**, 413–428.

Mesulam M. (2004) The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? *Learn Mem.* **11**, 43–49.

Meunier J., Ieni J. and Maurice T. (2006a) Antiamnesic and neuroprotective effects of donepezil against learning impairments induced in mice by exposure to carbon monoxide gas. *J. Pharmacol. Exp. Ther.* **317**, 1307–1319.

Meunier J., Ieni J. and Maurice T. (2006b) The anti-amnesic and neuroprotective effects of donepezil against amyloid beta25–35 peptide-induced toxicity in mice involve an interaction with the sigma1 receptor. *Br. J. Pharmacol.* **149**, 998–1012.

Minger S. L., Esiri M. M., McDonald B., Keene J., Carter J., Hope T. and Francis P. T. (2000) Cholinergic deficits contribute to behavioral disturbance in patients with dementia. *Neurology* **55**, 1460–1467.

Morin-Surun M. P., Collin T., Denavit-Saubie M., Baulieu E. E. and Monnet F. P. (1999) Intracellular sigma1 receptor modulates phospholipase C and protein kinase C activities in the brainstem. *Proc. Natl Acad. Sci. USA* **96**, 8196–8199.

Morris R. (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* **11**, 47–60.

Netto C. A., Hodges H., Sinden J. D., Le Peillet E., Kershaw T., Sowinski P., Meldrum B. S. and Gray J. A. (1993) Effects of fetal hippocampal field grafts on ischaemic-induced deficits in spatial navigation in the water maze. *Neuroscience* **54**, 69–92.

Pappas B. A., Bayley P. J., Bui B. K., Hansen L. A. and Thal L. J. (2000) Choline acetyltransferase activity and cognitive domain scores of Alzheimer's patients. *Neurobiol. Aging* **21**, 11–17.

Parent M. B. and Baxter M. G. (2004) Septohippocampal acetylcholine: involved in but not necessary for learning and memory? *Learn Mem.* **11**, 9–20.

Paxinos G. and Watson C. (1986) *The Rat Brain in Stereotaxic Coordinates*, 2nd edn. Academic Press, San Diego.

Prezzavento O., Campisi A., Ronsisvalle S. *et al.* (2007) Novel sigma receptor ligands: synthesis and biological profile. *J. Med. Chem.* **50**, 951–961.

Prezzavento O., Parenti C., Marrazzo A., Ronsisvalle S., Vittorio F., Arico G., Scoto G. M. and Ronsisvalle G. (2008) A new sigma ligand, (+/−)-PPCC, antagonizes kappa opioid receptor-mediated antinociceptive effect. *Life Sci.* **82**, 549–553.

Quirion R., Bowen W. D., Itzhak Y., Junien J. L., Musacchio J. M., Rothman R. B., Su T. P., Tam S. W. and Taylor D. P. (1992) A proposal for the classification of sigma binding sites. *Trends Pharmacol. Sci.* **13**, 85–86.

Reisberg B., Doody R., Stoffler A., Schmitt F., Ferris S. and Mobius H. J. (2003) Memantine in moderate-to-severe Alzheimer's disease. *N. Engl. J. Med.* **348**, 1333–1341.

Rogers S. L., Farlow M. R., Doody R. S., Mohs R. and Friedhoff L. T. (1998) A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. Donepezil Study Group. *Neurology* **50**, 136–145.

Scarpini E., Scheltens P. and Feldman H. (2003) Treatment of Alzheimer's disease: current status and new perspectives. *Lancet Neurol.* **2**, 539–547.

Senda T., Matsuno K., Kobayashi T., Nakazawa M., Nikata K. and Mita S. (1998) Ameliorative effect of SA4503, a novel cognitive enhancer on the basal forebrain lesion-induced impairment of the spatial learning performance in rats. *Pharmacol. Biochem. Behav.* **59**, 129–134.

Steckler T., Keith A. B., Wiley R. G. and Sahgal A. (1995) Cholinergic lesions by 192 IgG-saporin and short-term recognition memory: role of the septohippocampal projection. *Neuroscience* **66**, 101–114.

Summers W. K., Majovski L. V., Marsh G. M., Tachiki K. and Kling A. (1986) Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. *N. Engl. J. Med.* **315**, 1241–1245.

Tariot P. N., Solomon P. R., Morris J. C., Kershaw P., Lilienfeld S. and Ding C. (2000) A 5-month, randomized, placebo-controlled trial of galantamine in AD. The galantamine USA-10 Study Group. *Neurology* **54**, 2269–2276.

Torres E. M., Perry T. A., Blockland A., Wilkinson L. S., Wiley R. G., Lappi D. A. and Dunnet S. B. (1994) Behavioural, histochemical and biochemical consequences of selective immunolesions in discrete regions of the basal forebrain cholinergic system. *Neuroscience* **63**, 95–122.

Tottori K., Nakai M., Uwahodo Y., Miwa T., Yamada S., Oshiro Y., Kikuchi T. and Altar C. A. (2002) Attenuation of scopolamine-induced and age-associated memory impairments by the sigma and 5-hydroxytryptamine(1A) receptor agonist OPC-14523 (1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2[1H]-quinolinone monomethanesulfonate). *J. Pharmacol. Exp. Ther.* **301**, 249–257.

Waite J. J., Chen A. D., Wardlow M. L., Wiley R. G., Lappi D. A. and Thal L. J. (1995) 192 immunoglobulin G-saporin produces graded behavioral and biochemical changes accompanying the loss of cholinergic neurons of the basal forebrain and cerebellar Purkinje cells. *Neuroscience* **65**, 463–476.

Walker J. M., Bowen W. D., Walker F. O., Matsumoto R. R., De Costa B. and Rice K. C. (1990) Sigma receptors: biology and function. *Pharmacol. Rev.* **42**, 355–402.

Walker J. M., Martin W. J., Hohmann A. G., Hemstreet M. K., Roth J. S., Leitner M. S., Weised S. D., Patrick S. L., Patrick R. L. and Matsumoto R. R. (1994) Role of sigma receptors in brain mechanisms of movement. in *Sigma Receptors* (Itzhak Y., ed.), pp. 205–224. Academic Press, San Diego.

Whitehouse P. J., Price D. L., Clark A. W., Coyle J. T. and DeLong M. R. (1981) Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* **10**, 122–126.

Wiley R. G. and Kline R. H. I. V. (2000) Neuronal lesioning with axonally transported toxins. *J. Neurosci. Methods* **103**, 73–82.

Wiley R. G., Oeltmann T. N. and Lappi D. A. (1991) Immunolesioning: selective destruction of neurons using immunotoxin to rat NGF receptor. *Brain Res.* **562**, 149–153.

Winblad B. and Poritis N. (1999) Memantine in severe dementia: results of the 9M-Best Study (Benefit and efficacy in severely demented patients during treatment with memantine). *Int. J. Geriatr. Psychiatry* **14**, 135–146.

Wrenn C. C. and Wiley R. G. (1998) The behavioral functions of the cholinergic basal forebrain: lessons from 192 IgG-saporin. *Int. J. Dev. Neurosci.* **16**, 595–602.

