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SSR180711, a Novel Selective α7 Nicotinic Receptor Partial Agonist: (II) Efficacy in Experimental Models Predictive of Activity Against Cognitive Symptoms of Schizophrenia

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SSR180711 (4-bromophenyl 1,4diazabicyclo(3.2.2) nonane-4-carboxylate, monohydrochloride) is a selective α7 nicotinic receptor (n-AChR) partial agonist. Based on the purported implication of this receptor in cognitive deficits associated with schizophrenia, the present study assessed efficacy of SSR180711 (i.p. and p.o.) in different types of learning and memory involved in this pathology. SSR180711 enhanced episodic memory in the object recognition task in rats and mice (MED: 0.3 mg/kg), an effect mediated by the α7 n-AChR, as it was no longer seen in mice lacking this receptor. Efficacy was retained after repeated treatment (eight administrations over 5 days, 1 mg/kg), indicating lack of tachyphylaxia. SSR180711 also reversed (MED: 0.3 mg/kg) MK-801-induced deficits in retention of episodic memory in rats (object recognition). The drug reversed (MED: 0.3 mg/kg) selective attention impaired by neonatal phencyclidine (PCP) treatment and restored MK-801- or PCP-induced memory deficits in the Morris or linear maze (MED: 1–3 mg/kg). In neurochemical and electrophysiological correlates of antipsychotic drug action, SSR180711 increased extracellular levels of dopamine in the prefrontal cortex (MED: 1 mg/kg) and enhanced (3 mg/kg) spontaneous firing of retrosplenial cortex neurons in rats. Selectivity of SSR180711 was confirmed as these effects were abolished by methyllycaconitine (3 mg/kg, i.p. and 1 mg/kg, i.v., respectively), a selective α7 n-AChR antagonist. Additional antidepressant-like properties of SSR180711 were demonstrated in the forced-swimming test in rats (MED: 1 mg/kg), the maternal separation-induced ultrasonic vocalization paradigm in rat pups (MED: 3 mg/kg) and the chronic mild stress procedure in mice (10 mg/kg o.d. for 3 weeks). Taken together, these findings characterize SSR180711 as a promising new agent for the treatment of cognitive symptoms of schizophrenia. The antidepressant-like properties of SSR180711 are of added interest, considering the high prevalence of depressive symptoms in schizophrenic patients.
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INTRODUCTION

Nicotine, the prototypical agonist for nicotinic acetylcholine receptors (n-AChRs), has long been known to possess procognitive activity. Experiments emphasizing its ability to enhance cognitive functions, including attention, learning, and retention, were shown, both in laboratory animals and humans, to result from activation of brain nicotinic acetylcholine receptors (Levin and Simon, 1998; Levin and Rezvani, 2002). However, the side-effects profile of nicotine (see below for details) precludes the use of this compound or of its analogs in the clinic. The reduction or elimination of undesirable effects at peripheral sites, particularly cardiovascular sites, and the development of improved, selective nicotinic receptor ligands, could provide an innovative approach for the development of therapies for central nervous system (CNS) diseases characterized by cognitive impairment (for a review, see Gotti *et al*, 1997).

Molecular biology techniques have contributed to the extension of knowledge on n-AChRs by showing that these receptors are homo- or heteropentameric ligand-gated ion channels. Differential association of the various types of subunits gives rise to a multitude of subtypes (for a review, see Buccafusco, 2004). Nicotine is a nonselective agonist for all these subtypes, so that selective targeting of some of them might give rise to the sought-after therapeutic activity, while avoiding the emergence of unwanted side effects. Two

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subtypes mainly expressed in the brain are of major interest in this respect: the heteromeric $\alpha4\beta2$ and the homomeric $\alpha7$. Evidence from neuroanatomical, electrophysiological, and behavioral studies supports a role for these two receptor subtypes in processes of learning and memory. Selective agonists for the $\alpha7$ n-AChR should also offer, at least, additional advantage of presenting less side effects presumably associated with the activation of the central $\alpha3\beta4$ and $\alpha4\beta4$ (possibly mediating the addictive, convulsive, and emetic activities of nicotine) and peripheral $\alpha3\beta2$, $\alpha3\beta4$, and $\alpha1\beta1\gamma\delta$ (thought to be responsible for its gastro-intestinal, vaso-constrictive, and respiratory side effects) (Martin *et al*, 2004). The $\alpha7$ n-AChR, because of its high density in structures such as the hippocampus and the cerebral cortex, strongly implicated in cognition and memory, and its role in learning and memory processes (Paterson and Nordberg, 2000; Levin and Rezvani, 2002), might represent the target of choice.

As a consequence, activation of the $\alpha7$ n-AChR might be particularly suitable for therapeutic application in devastating psychiatric conditions characterized by marked cognitive deterioration, such as schizophrenia. Interestingly, diminished expression of the $\alpha7$ n-AChR occurs in selected brains regions of patients with schizophrenia (Freedman *et al*, 2000), which may account for pathophysiological abnormalities such as deficits in attention and information processing. Schizophrenic patients suffer from a myriad of signs and symptoms: one of them, deficit of auditory gating (ie diminished suppression of the P50 response following auditory stimulation), has recently captured the interest of the scientific community. This gating abnormality has been suggested to be linked to the $\alpha7$ n-AChR (for a review, see Martin *et al*, 2004), and might be due to diminished density of this receptor subtype in the hippocampus (Adler *et al*, 1998). Improving these deficits in auditory (and other modalities) sensory gating might be of great benefit to patients, as they are considered by some authors to be conducive to sensory 'flooding', and one step further to general cognitive impairment and hallucinations (Adler *et al*, 1998).

Furthermore, activation of presynaptic $\alpha7$ n-AChRs present on glutamatergic neurons has been shown to increase levels and exocytosis of glutamate (Gray *et al*, 1996). In the context of the hypoglutamatergic hypothesis of schizophrenia (Javitt and Zukin, 1991; Olney and Farber, 1995), activation of these receptors (ie by use of an agonist) should provide a mean of alleviating cognitive deficits and possibly negative symptoms, two aspects that are considered to form the core of the pathology and that are poorly responsive to current antipsychotics. In addition, it has also been suggested that the high prevalence and the heavy pattern of intake of tobacco smoking (ie nicotine) among schizophrenic patients might stem from a self-medication strategy aimed, among other things, at alleviating negative symptomatology (Svensson *et al*, 1990; Smith *et al*, 2002) and/or deficits in cognitive processes (Taiminen *et al*, 1998), in particular sensory gating (Adler *et al*, 1993). It is well known that the $\alpha7$ n-AChRs rapidly desensitizes after its activation by selective agonists. However, such an effect was demonstrated mainly in *in vitro* experiments, and very little is known about the behavioral consequences of desensitization of the $\alpha7$ n-AChRs by selective agonists. For example, there is one study (Stevens *et al*, 1998) showing that the $\alpha7$ n-AChR, GTS-21, produced no tolerance to its effects on auditory gating in DBA/2 mice after repeated administration, unlike nicotine, which produced clear tachyphyllaxia to these effects after protracted treatment.

Several (more or less selective) $\alpha7$ n-AChR agonists have been described in the literature: two of them, GTS-21 and AR-R17779, have been tested in various animal models of memory, another more recent one (PNU-282987) having been used, to the best of our knowledge, only in a model of auditory gating deficit (Hajos *et al*, 2005). GTS-21 has been shown to display memory-enhancing properties in passive and active avoidance tests, in the Morris and radial mazes in rats (Arendash *et al*, 1995; Meyer *et al*, 1998; Kem, 2000). It has even proven to have favorable effects on cognitive function in humans (Kitagawa *et al*, 2003) especially on attention, working memory, and episodic secondary memory. However, this compound is not selective for the human $\alpha7$ n-AChR, as it is also functionally active at $\alpha4\beta2$ n-AChR (Briggs *et al*, 1997). The more selective compound, AR-R17779, has been less extensively investigated: it improved learning and memory of a long-term win-shift acquisition in an eight-arm radial maze and social recognition memory in rats (Levin *et al*, 1999; Van Kampen *et al*, 2004). However, pharmacological activity of these compounds appears not to extend across all learning and memory tests, as they failed to reverse a scopolamine-induced deficit in spatial memory in a Morris water maze or in attention tasks (Grottick and Higgins, 2000; Hahn *et al*, 2001).

In a companion paper (Biton *et al*, submitted), we have shown that SSR180711 is a selective agonist at $\alpha7$ n-AChRs (K_i of 22 ± 4 and 14 ± 1 nM for rat and human receptors, respectively), with no significant binding and/or functional activity at human $\alpha4\beta2$, $\alpha3\beta4$, $\alpha3\beta2$, and $\alpha1\beta1\gamma\delta$, n-AChRs. Furthermore, SSR180711 was characterized as a partial agonist at human $\alpha7$ n-AChRs transiently expressed either in GH4C1 or xenopus oocytes (intrinsic activities of 39 and 51% that of acetylcholine, respectively), with weak desensitizing properties. SSR180711 also potentiated the amplitude of electrically evoked glutamate excitatory postsynaptic currents recorded in rat CA1 hippocampal pyramidal-like cells. Finally, the compound increased long-term potentiation (LTP) of synaptic transmission induced *in vitro* by tetanic stimulation of the CA1 hippocampal field, and increased extracellular levels of acetylcholine in the hippocampus and the cortex of freely moving rats (Biton *et al*, submitted). Consequently, SSR180711 may be useful for the treatment of the cognitive symptoms associated with schizophrenia, in which a deficit in glutamatergic neurotransmission (Javitt and Zukin, 1991) and also possibly an hypoactive muscarinic cholinergic transmission (Bymaster *et al*, 1999; Hyde and Crook, 2001) may underlie the pathology.

This report describes a series of experiments aimed at profiling SSR180711 in neurochemical, electrophysiological, and cognitive tests that address various aspects of learning and memory deficits observed in schizophrenia. Based on the disruptive effects of some of the noncompetitive antagonists at the NMDA receptor (phencyclidine (PCP), MK-801) that bear a resemblance to a broad range of symptoms associated with schizophrenia in human, SSR180711 was evaluated (1) for its potential to antagonize

MK-801- or PCP-induced deficits of spatial working, episodic and sequential memory tasks (water maze in mice, object recognition and linear maze in rats); (2) for its activity against a deficit of selective attention in a social recognition test in adult rats treated at the neonatal stage with high doses of PCP (Terranova *et al*, 2005); (3) for its ability to enhance extracellular levels of dopamine (DA) in the prefrontal cortex (PFC) (as low levels of this neurotransmitter are posited to mediate negative symptoms and cognitive deficits of schizophrenia: Kapur and Remington, 1996), and (4) for its potential to increase spontaneous firing rate neurons in the rat retrosplenial cortex, a brain structure that is reciprocally connected with the hippocampal formation and anterior thalamic nuclei. These brain structures are known to mediate several types of memory formation, including episodic memory and spatial learning in animals (Aggleton and Brown, 1999; Aggleton and Pearce, 2001; Lukoyanov *et al*, 2005), and are suspected to be hypoactive in schizophrenic patients (Harrison and Lewis, 2003; Tendolkar *et al*, 2004). Moreover, as symptoms of depression are often associated with schizophrenia, we additionally evaluated the activity of SSR180711 in rodent models of depression. Lastly, interaction studies with the α7 nAChR antagonist methyllycaconitine (MLA) or mice lacking the α7 n-AChR were undertaken to ascertain the involvement of α7 n-AChRs in the effects of SSR180711.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley or Wistar rats (Iffa Credo, L'Arbresle; Charles-River, Saint Aubin-lès-Elbeuf; and CERJ, Le Genest-Saint-Isle, France), weighing 180–350 g at the time of testing, were used in the object recognition, Morris water maze, linear maze, forced-swimming tests, and in electrophysiological and microdialysis studies. They were housed in groups of two to five per cage. Moreover, adult female and male juvenile (3–7 days old) Sprague–Dawley (Iffa Credo) and Wistar (CERJ) rats were used for the vocalization and novelty discrimination tests. Male C57Bl/6j (10-week old, object recognition) and BALB/c (6-week old, chronic mild stress (CMS) procedure) mice, weighing 17–32 g at the time of testing, were supplied by Iffa Credo. Mice were grouped in cages of five except in the CMS procedure, where they were housed singly. All animals were maintained under standard laboratory conditions and kept in temperature- and humidity-controlled rooms ($22 \pm 1^{\circ}$C, 50%) with lights on from 0700 to 1900 hours, and water and food available *ad libitum* (except when indicated otherwise). Mice (from a C57Bl/6j background) lacking the α7-nAChR nicotinic receptor were provided by the Baylor College of Medicine, (Huston, USA). The mutation deletion concerned the last three exons (8–10) of the α7 locus as described elsewhere (Orr-Urtreger *et al*, 1997). Mice were group housed in cages of five under standard animal conditions and maintained for 1 week before behavioral testing. Different groups of male α7-nAChR knockout (KO) mice and wild-type (WT) littermates were used: (20–32 weeks old) weighing 32–35 g at the start of testing. All procedures have been approved by the 'Animal Care and Use of Laboratory Animals' (National Institutes of Health) and were approved by the Animal Ethics Committee of Sanofi-Aventis on research involving laboratory animals.

Drugs

SSR180711 and PCP were synthesized by the CNS Medicinal Chemistry Department of Sanofi-Aventis (Bagneux, France). MK-801 and MLA citrate were obtained from Sigma Aldrich (Saint Quentin Fallavier, France) or Bio-rad Laboratories (Life Science Group, Marnes-la-Coquette, France). Drugs were dissolved in saline or distilled water alone or with a few drops of Tween 80, unless specified otherwise. Doses refer to the weight of the free base, except when indicated otherwise; all drug solutions were prepared daily and injected i.p. or s.c. 30 min pre-test (10 or 20 ml/kg in mice, 2 or 5 ml/kg in rats, 0.1 ml in rat pups) or i.v. (1 ml/kg in rats). For p.o. administrations (60 min pre-test), SSR180711 was suspended in distilled water with a few drops of Tween 80, and the volume of administration was 10 ml/kg in mice and 5 ml/kg in rats.

Effect of Acute (Alone or Coadministered with MLA) or Subchronic Treatment with SSR180711 in the Object Recognition Task in Rats (Long-Term Episodic Memory Procedure)

The object recognition task was similar to that described by Ennaceur and Delacour (1988) in young rats. The apparatus consisted of a uniformly lit (100 lux) wooden enclosure ($65 \times 45 \times 45$ high cm) with a video camera positioned 160 cm above the bench. The observer was located in an adjacent room fitted with a video-monitoring system. Each separate experiment consisted of three sessions: during the first session (context habituation), the subjects ($N = 10$–12 animals per group) were allowed 2 min to become acquainted to the apparatus. Locomotor activity was manually recorded with a precision of ± 1 s. The animals were again placed in the enclosure 24 h thereafter for the second (acquisition) session, during which they were exposed to a pair of identical objects (either $7 \times 3 \times 8$ cm metal triangles or $9 \times 3 \times 7$ cm plastic pyramids) placed 10 cm away from the two opposite corners of the back wall. They were left in the enclosure for the amount of time necessary to spend at most 20 s exploring these two objects, with a limit of 3 min. Animals were removed from the cage once they have reached the 20 s exploration time. Exploration of an object was defined as the rat having its head within 2 cm of the object while looking at it, sniffing it, or touching it. Any rat spending less than 20 s exploring the two objects within 3 min was eliminated from the study. Two different identical sets of objects were used to allow for cleaning between one rat and the next, to minimize the possibility that olfactory cues left by the preceding rat might bias the behavior of the following one. Combinations of orders of presentation and locations of objects were balanced to reduce potential biases owing to spatial or objects preferences.

During the third (recall) session, rats were exposed to the familiar (ie presented during the acquisition session) and to the novel (ie never presented before) objects for 3 min, and the time spent exploring each object was recorded (precision ± 1 s). Any animal spending less than 3 s

exploring both objects was discarded from the study. This third session took place 24 h after the second session: At this intersession interval, there is virtually no discrimination between the two objects, that is the rat spends an equal amount of time exploring the familiar and the novel object. This indicates that the rat has lost its ability to discriminate between the two objects, suggestive of an impairment of this type of episodic memory. This interval was used to evaluate a possible improvement of performance following acute treatment with SSR180711 (or vehicle: distilled water, as a control) administered p.o. 60 min before each of the three sessions.

In a separate experiment, the α7 n-AChRs antagonist MLA (3 mg/kg i.p.) or its vehicle (NaCl, 0.9%) was administered simultaneously with SSR180711 (0.3 mg/kg i.p.) or its vehicle (NaCl 0.9%).

In addition, because of the propensity of α7 n-AChRs to desensitize following activation by full agonists, we verified that owing to the *in vitro* partial agonist profile of SSR180711 (Biton *et al*, submitted), there would be no tolerance (ie no tachyphylaxia) to the effects of SSR180711 administered subchronically. To that end, rats were treated b.i.d. during the 3 days preceding the first session. The protocol of administration was thereafter similar to that described above for administration of acute SSR180711 alone.

Data (time exploring each of the two objects, in seconds) were analyzed with a two-way ANOVA, with the treatment and the object as the between factors, followed by a Winer analysis for comparing the time spent exploring the familiar *vs* the novel object for each treatment. This analysis, and all subsequent ones, was performed using the SAS software (SAS Institute Inc., Cary, NC, USA).

Effect of Acute Treatment with SSR180711 in the Object Recognition Task in Mice

All separate studies using C57Bl/6j or KO and WT mice were adapted from those described in rats. Studies were performed in a PVC open-field box (51 cm wide × 51 cm long × 58 cm high) with light gray vertical walls and floor. The light intensity in the middle of the field was 30 lux. The objects to be discriminated were metal triangles (3 cm high, object A) and plastic pyramids (3 cm high, object B). Mice were allowed individually to habituate to the open field for 5 min. The next day, they were submitted to an acquisition session in order to achieve 10 min of object exploration, during which they were placed in the open field in the presence of two objects A placed 5 cm away from the two opposite corners of the back wall. Time that animals took to explore the two objects A (animal's snout direct toward the object at a distance <1 cm) was recorded. They were left in the enclosure for the amount of time necessary to spend at most 10 s exploring these two objects, with a limit of 5 min. Animals were removed from the apparatus once they have reached the 10 s exploration time. A 5-min retention trial (recall phase) occurred 1 h after to establish the effect on short-term memory for the KO and WT animals or 48 h later (long-term memory) for the C57Bl/6j mouse strain. Previous studies demonstrated that this 48 h interval was necessary for this strain of mice, to loose their ability to discriminate between objects suggestive of a spontaneous long-term episodic memory impairment (data not shown). During this recall session, the objects A and B were placed in the open field, and the time that animal took to explore the familiar object A (*F*) and the novel object B (*N*) were recorded. A recognition index was defined as the ratio ($N/(N+F)$). In order to determine the suitability of the C57Bl/6j mouse strain as transgenic background strains for the KO mice, we first investigated the dose relationship improvement of SSR180711 performance in object recognition performance task (or vehicle: distilled water, as a control) administered p.o. 60 min before each of the three sessions ($N=8$–20 animals per group). In separate experiments, KO mice and their corresponding WT littermates ($N=8$–10 animals per group) were used to establish their abilities to modify short-term episodic memory in object recognition task and further to determine the specificity of the effects of SSR180711 in this task in improving long-term episodic memory.

Effect of SSR180711, Alone or Coadministered with MLA, on MK-801-Induced Deficits in the Object Recognition Task in Rats (Short-Term Episodic Memory Procedure)

All procedures in rats were similar to those described in the paragraph above, except for the following two points: (1) the third (recall) session was performed 1 h after the second session: at this intersession interval, the rat spends more time exploring the novel than the familiar object. This indicates that the rat can discriminate between the two objects, an index of a preserved episodic memory capacity. As a consequence, this interval was used to assess the efficacy of SSR180711 to reverse a deficit induced by MK-801 (0.1 mg/kg i.p., $N=9$–12 animals per group). In a separate experiment, MLA, 3 mg/kg i.p. was coadministered simultaneously with SSR180711 (0.3 mg/kg i.p.) and MK-801 (0.1 mg/kg i.p., $N=12$–15 animals per group).

Effect of SSR180711 on MK-801-Induced Deficits in the Water Maze Task in Rats (Working Memory Procedure)

The Morris water maze apparatus consisted of a PVC pool (1.20 m diameter × 0.60 m high), filled with thermostated water ($23\pm2^{\circ}$C) to a depth of 35 cm, with the addition of milk to render the water opalescent. A Plexiglas escape platform (12 cm diameter) was placed into the pool, 1 cm below the water surface and 10 cm from the wall. The test room contained several permanent extra-maze cues such as posters, flag, etc. on walls. A video-tracking camera (placed 200 cm above the center of the pool surface) monitored the trajectory of the rat (male Wistar strain, 250–300 g at the start of the study, $N=12$ animals per group) and the video signal was transmitted to a computer in an adjacent room and analyzed using the VIDEOTRACK© system (View Point Ltd, Champagne au Mont d'Or, France). The platform was placed at one of four possible cardinal locations NW, SE, NE, and SW, and NW for learning session 1, 2, 3, 4, and 5, respectively. Each learning session consisted of four trials, with a maximal duration of 120 s and an intertrial interval of 30 s. Latency times (in seconds) to find the hidden platform were recorded during each trial of each learning session. If the rat located the platform within the maximum time

allowed (120 s), it was left on the platform for 30 s. If the rat did not locate the platform within the time limit, it was gently placed on it for a 30-s period.

At the start of each trial of each day, the rat was gently placed at the periphery of the maze, opposite of the platform (ie for the first trial E, for the NW quadrant). For each subsequent learning session, a similar cardinal rule was applied (ie N, W, N, and E for the SE, NE and SW, and NW quadrant, respectively).

Effects of i.p. treatment (30 min before each training day) with SSR180711 or its vehicle (NaCl 0.9%) were assessed on a deficit of performance induced by MK-801 (0.075 mg/kg i.p., 30 min before each training day). The dose of MK-801 was chosen because it does not induce detectable locomotor adverse effects in rats, which could have contaminated its cognitive effects. Absolute control consisted of a double injection with vehicle (NaCl 0.9%). Data (latency times to reach the platform, expressed as the mean of each collapsed daily trial (1–4) over all five learning session per animal were analyzed with a two-way ANOVA, with the treatment as the between factor, and the training day as the within factor. *Post hoc* analysis was performed with Dunn's *post hoc* tests taking the vehicle/MK-801 group as the comparator.

Effect of SSR180711 on the Deficit in the Object Recognition Task in Rats Sensitized to PCP (Short-Term Episodic Memory Procedure)

Behavioral sensitization was induced by daily injections with PCP (10 mg/kg i.p.) or its vehicle (distilled water + Tween 80) for five consecutive days. Rats ($N = 10$–12 animals per group) were then left unused for 6 days before starting the experiment. On the 11th day, they were submitted to a protocol inspired from that used for inducing deficits with MK-801. Rats were first exposed to the context habituation session and 1 h later to the acquisition session. They were injected with the challenge dose of PCP (1 mg/kg i.p.) or vehicle (saline) and with vehicle or SSR180711 (1 mg/kg p.o.) right after the end of the acquisition session. They were thereafter subjected to the recall session 90 min later.

Data (time exploring each of the two objects) in seconds were analyzed with a two-way ANOVA, with the treatment and the object as the between factors, followed by a Winer analysis for comparing the time spent exploring the familiar *vs* the novel object for each treatment.

Effect of SSR180711 on the Impairment of Novelty Discrimination in Adult Rats Treated with PCP at the Neonatal Stage

Female Wistar Han rats were obtained with 10 male pups on postnatal day 3 (PN 3). Pups were treated on PN 7, 9, and 11 with 10 mg/kg of PCP (s.c. administration, 1 ml/100 g body weight) or its vehicle (NaCl 0.9% + Tween 80). Pups from the same litter received an identical treatment. The mother and pups were housed together until weaning at PN 21, at which stage pups where housed five per cage until 2 weeks before the beginning of behavioral experiments, when they were housed individually. Behavioral experiments were performed once they reached the adult stage (between PN 118 and PN 142).

Juvenile male Wistar rats (3-week old, 45–50 g on arrival, $N = 5$ animals per group) were housed five per cage. They were left alone for 1 week, before the beginning of experiments (presentation to the adult rats treated at the neonatal stage: see above) that lasted for a week. Each juvenile was used only once a day, and was chosen at random as first or second for presentation to the adult. All animals (mothers, pups, adult, and juvenile rats) were kept on a reversed light–dark cycle (light on from 1900 to 0700 hours).

Experiments were performed during the dark phase, under infrared illumination (15 lux). Juvenile rats were isolated 30 min before being placed into the home cage of an adult rat. The cage was placed underneath a video camera, the mesh top removed and replaced by a Plexiglas cover. A first (familiar) juvenile was placed inside the home cage containing one adult rat for a period of 30 min. A second (novel) juvenile was introduced at the end of this period. Durations of investigation behavior (nosing, sniffing, grooming, close chase of the juvenile rat) between the adult rat and each of the two juveniles were recorded manually for a period of 5 min following the introduction of the novel juvenile, by an observer located in an adjacent room fitted with a video monitor. SSR180711 or its vehicle (NaCl 0.9%) was administered p.o. to the adult rat 60 min before exposure to the first juvenile. Each adult rat was subjected to five treatments: one vehicle and four doses of SSR180711, with 1 or 2 days between each treatment.

In a separate experiment, the α7 nAChR receptor antagonist MLA or its vehicle (NaCl 0.9%) was injected s.c. (3 mg/kg) 55 min after SSR180711 (3 mg/kg i.p.) or vehicle (NaCl 0.9%).

Data are expressed as the mean of a novelty discrimination index (NDI), which was calculated as the ratio of the time spent investigating the novel juvenile divided by the time spent investigating the familiar juvenile. NDI's were first log-transformed because of the limited number of subjects and the lack of homogeneity of variances between groups. Statistical analysis was carried out using a two-way ANOVA, with treatment at the neonatal stage as the between-subjects factor, and acute treatment at the adult stage as the within-subjects factor, followed by appropriate *post hoc* tests.

Effect of SSR180711 on a PCP-Induced Deficit of Sequential Memory in Acquisition of a Linear Maze Task in Rats

At 1 week before the beginning of the experiments, water was freely available but rats were kept on a daily schedule of food deprivation of 15 g of standard chow per rat per day that was maintained until the end of the study.

The linear maze ($290 \times 50 \times 35$ high cm) was made of beige PVC, and consisted of a start box ($35 \times 35 \times 35$ cm), six choice units ($40 \times 50 \times 35$ cm with a 10 cm partition disposed lengthwise in the middle of each unit) with 10 cm wide openings between each of them, and a goal box ($25 \times 25 \times 35$ cm). Start and goal boxes were separated from the first and sixth choice units, respectively, by sliding doors that could be operated manually through a system of

pulleys and strings. The configuration of the maze was operated by disposing vertical Plexiglas barriers inside each of the choice units, so as to create either an alley to have access to the next choice unit, or to a 'blind alley'. The task required rats to learn a sequence of six successive left–right choices to reach the goal box and obtain food reinforcement. Six different maze configurations were used and randomized between animals: LRRLLR, LRLLRR, RRLLRL, RLRRLL, RLLRRL, LLRRLR (R: right, L: left). For example, with the LRRLLR configuration, the rat was required to go to the left of the partition in the first choice unit to have access to the second choice unit, in which it had to go to the right to have access to the third choice unit, and so on. The reader is referred to Figure 1 of the Sara's publication (1985) for a graphic representation of this type of maze.

Illumination was provided by six 60-W lamps placed 2 m above each choice unit to avoid shadows in the apparatus. Two video cameras, positioned 2 m above the middle of the second and fifth choice units, were connected to a video recorder and monitor, located behind a curtain.

During the week before the learning phase, rats ($N=20$ per group) were handled, weighed, and habituated to become progressively familiar to the environmental context, and to associate reinforcement (Kellogg's, Coco pops®) with the goal box. Animals were provided with about 10 g of Coco pops® in their home cage during the first day. During the following days, rats were placed in the start box and allowed to freely explore the maze (no barriers) to reach the goal box baited with about 10 g Coco pops placed in a cup. They were also habituated from the fourth day to drug administration procedure by being treated with saline (i.p. or p.o.).

During the learning phase, each rat was subjected to one session per day for five consecutive days. For each session, the rat was first placed in the start box, the sliding door was raised and the animal was allowed to reach the baited goal box (maximum 10 min). Each rat was required to learn one of the six configurations (see above) to reach the goal box (rats were exposed to the same configuration for the entire week). An error was defined as an entry with four paws into a 'blind alley'. Once the animal entered the goal box, the sliding door was lowered and the rat left to consume the reinforcement for 2 min before being removed and returned to its home cage. SSR180711 or its vehicle (NaCl, 0.9%) was administered p.o. 45 min before each daily learning session, followed 15 min later by PCP (1.5 mg/kg i.p.) or its vehicle (NaCl 0.9%).

Data (number of errors) were recorded for each trial for each day and were first transformed ($y=$ square root (number of errors + 0.5)). Data were then analyzed using a two-way ANOVA for repeated measures, with treatment as the between-subjects factor, and learning sessions as the within-subjects factor. Data were also cumulated across all five learning sessions for analysis with a one-way ANOVA, with treatment as the between-subjects factor, followed by Dunnett's *post hoc* tests.

Effect of SSR180711 on the Spontaneous Firing Rate of Retrosplenial Cortex Neurons of Rats

Rats ($N=3$–6 per group) were placed in a stereotaxic frame (David-Kopf Instruments, Tujunga, CA, USA). The lateral tail vein was catheterized to allow for slow infusion (microinfusion pump, Harvard, Edenbridge, UK) of supplementary doses of chloral hydrate (120 mg/kg/h) as well as bolus injection of test compounds. Body temperature was maintained at $37\pm0.1^{\circ}C$ using a temperature-controlled heating pad (Harvard Apparatus, Edenbridge, UK). After resection of the scalp and underlying tissues, a 2 mm burr hole was drilled into the skull above the areas to be explored (see below). Extracellular recordings were made with pulled (PE-2 puller, Narishige, Tokyo, Japan) glass micropipettes whose tips were broken under microscopic control so as to obtain an outside diameter of approximately 1 μm. The micropipettes were filled with 1 M NaCl or 0.5 M sodium acetate containing 2% (w/v) pontamine sky blue (impedance 5–10 MΩ at 100 Hz). Micropipettes were lowered *in situ* with an hydraulic microdrive (MMO-203, Narishige, Tokyo, Japan) to target the retrosplenial cortex (5.8 mm posterior and 0.8 mm lateral to bregma, 1.2–2 mm below the cortical surface). Stereotaxic coordinates were determined according to the atlas of Paxinos and Watson (1998).

Action potentials (spikes) were amplified, filtered (400–4000 Hz), and monitored on both a digital oscilloscope and an audio amplifier. The spikes were also fed into a window discriminator that delivered TTL pulses to a 'μ1401 Intelligent Laboratory Interface' connected to a PC running the CED 'Spike2' software (Cambridge Electronic Design, Cambridge, UK) for on- and off-line construction and analysis of firing rate histograms. After detection of their spontaneous activity, cells were recorded in a continuous mode for at least 40 min. A single cell was recorded per rat.

SSR180711 or its vehicle (distilled water) was administered i.v. (1 ml/kg body weight, delivered over a duration of 1 min) after a 10–15 min period of stabilization of firing rate. In a separate experiment, MLA or its vehicle (distilled water) was administered i.v. (1 mg/kg) 15 min before SSR180711.

Data (mean firing rate, in spikes/s) were calculated on-line every 5 min. Data are expressed as a percentage change compared with baseline activity (firing rate collected 5 min before i.v. injection of SSR180711 or vehicle). For each treatment, raw data were analyzed by means of a one-way ANOVA for repeated measures, followed by a Duncan's *post hoc* test.

Effect of SSR180711 on Extracellular Levels of Dopamine in the Prefrontal Cortex of Freely Moving Rats

Rats ($N=7$–9 per group) were anesthetized with chloral hydrate (400 mg/kg, i.p.), placed in a stereotaxic frame (David Kop Instruments, Tujunga, CA, USA) and a guide cannula was stereotaxically implanted above the medial prefrontal cortex (3.5 mm anterior to bregma, 0.5 mm lateral to bregma: Paxinos and Watson, 1998). At 24 h after surgery, a microdialysis probe (Carnegie Medicine, Stockholm, Sweden), 500 μm in diameter with an exposed membrane length of 4 mm, was positioned within the guide cannula (vertical coordinates: 5 mm under the cortical surface) and perfused (CMA/100 pump, Carnegie Medicine) at a flow rate of 2 μl/min with artificial cerebrospinal fluid (mM): NaCl (147), KCl (4), $CaCl_2$ (1.2), $MgCl_2$ (1). The animals were left for at least 3 h to allow the system to equilibrate, then dialysate samples were collected every

20 min and analyzed using HPLC with electrochemical detection (details of the methodology in Curet *et al*, 1996). The changes in dopamine levels are expressed as a percentage of the mean value ± SEM of the four stable basal samples before i.p. treatment with SSR180711 or its vehicle (distilled water + Tween 80). Statistical analysis was carried out by a two-way ANOVA, with time as the within factor, and treatment as the between factor, followed by one-way ANOVA's at each time, with Dunnett's *post hoc* tests.

In a separate experiment, MLA (3 mg/kg, i.p.) or its vehicle (distilled water + Tween 80) was administered 1 h before SSR180711 at 1 mg/kg, i.p. Control rats were treated with a double i.p. injection of vehicle. Data (mean area under the curve ± SEM for the 120 min period after the injection of SSR180711 or vehicle) were analyzed with a one-way ANOVA followed by a Dunnett's *post hoc* test.

Effect of SSR180711 in the Forced-Swimming Test in Rats

The procedure was a modification of the technique described by Porsolt (1977). Two swimming sessions were conducted (an initial 15-min pre-test followed 24 h later by a 6-min test). Rats ($N = 7$–8 per group) were placed in individual glass cylinders containing water (23 ± 1°C), and the total duration of immobility was measured for a 6-min period. An animal was considered as immobile whenever it remained floating passively in the water. SSR180711 or its vehicle (distilled water + Tween 80) was administered p.o. 15 min after the pre-test, and 60 min before the test session. Data (time spent in immobility, in seconds) were analyzed with a one-way ANOVA followed by a Dunnett's *post hoc* test.

Effect of SSR180711 on Ultrasonic Distress Vocalizations in Rat Pups

Female Sprague–Dawley rats were obtained with 10 male pups on PN 3–4. The procedure, adapted from the one described by Gardner (1985), was as follows: each 7-day-old pup was first separated from its mother and littermates, for s.c. injections ($N = 7$–8 animals per group) of SSR180711 or vehicle (NaCl 0.9% with Tween 80), and returned to its mother. After 30 min, the pup was placed in a soundproof cage. The Ultravox® system (Noldus, Wageningen, The Netherlands) was used to record ultrasonic vocalizations (in the 40 kHz range). A modified ultrasound detector (Mini-3 bat model) connected to a microphone (positioned next to the pup) was first used to transform ultrasonic sound into audible sound. The signal was then filtered (user-defined frequency range and amplitude threshold) and sent to a computer for analysis. The UltraVox software recorded each bout of ultrasonic vocalizations that lasted more than 10 ms during the 3 min test session.

Data are presented as the mean number of ultrasonic calls emitted during a 3-min separation period and were analyzed with a Kruskal–Wallis test, followed by a Dunn's *post hoc* test.

Effect of SSR180711 in a CMS Procedure in Mice

The CMS protocol, originally described by Willner *et al* (1992) for rats, was adapted from the one described by Griebel *et al* (2002) for mice. It consisted in the sequential application of a variety of mild stressors, including restraint, forced swimming, water and/or food deprivation, pairing with another stressed animal, each applied for a period ranging from 2 to 24 h, in a schedule lasting 36 days.

Administrations of SSR180711 (10 mg/kg, i.p.), fluoxetine (10 mg/kg, i.p.), or vehicle (NaCl 0.9%) were started 14 days after the beginning of the CMS. The dose of SSR180711 was chosen on the basis of its activity in *ex-vivo* bindings studies in mice (Biton *et al*, submitted). Animals ($N = 17$–20 per group) were injected i.p. once a day during 22 days. Parallels between depression in humans and the behavior of chronically stressed animals have been drawn on the difficulty of the patient to accomplish even the smallest tasks (eg washing and dressing in the morning), leading to the inability to maintain minimal personal hygiene, and the decrease in grooming behavior seen in chronically stressed animals. In this latter case, there is a degradation of the physical state of the coat, consisting mainly in dirtying and/or loss of fur. Based on these observations, we measured physical state of the coat once a week on five occasions over the entire 35-day CMS period and on the day after (day 36) for the last measure using the following quotation scale:

3.0 clean and well groomed coat
2.5 disorganized (poorly groomed) coat on the neck
2.0 disorganized (poorly groomed) coat on the neck and on the back
1.5 dirty coat with or without loss of patches of fur on the flanks
1.0 dirty coat with loss of patches of fur on the flanks and on the belly.

Data are presented as the mean physical state scores, and were analyzed with a Kruskal–Wallis test for each day of measure, followed by Dunn's *post hoc* tests.

RESULTS

Acute Administration of SSR180711 Facilitated Episodic Memory in the Object Recognition Task in Rats (Long-Term Episodic Memory Procedure)

Under control (vehicle) conditions, rats spent an equivalent amount of time investigating the novel and the familiar object (9.0 ± 1.6 *vs* 10.6 ± 1.6 s), 24 h after exposure to the familiar object (Figure 1). This indicates that rats lost their ability to discriminate between the two objects, indicative of a physiological forgetting delay of episodic memory for this strain of rat. SSR180711 (0.3–10 mg/kg p.o.) significantly increased the amount of time preferentially spent investigating the novel object (Winer analysis comparing the time spent investigating the novel *vs* the familiar object, following significant object, and treatment × object interaction effects, two-way ANOVA: $F(1,39) = 83.9$, $P < 0.0001$ and $F(4,39) = 3.9$, $P < 0.01$, respectively).

Neither the locomotor activity recorded during the context habituation session, nor the total time spent in exploring both objects during the acquisition and the recall sessions was significantly modified by the treatment (data not shown). Thus, the effects of SSR180711 did not result from nonspecific biases such as sedation and/or motor effects such as ataxia.

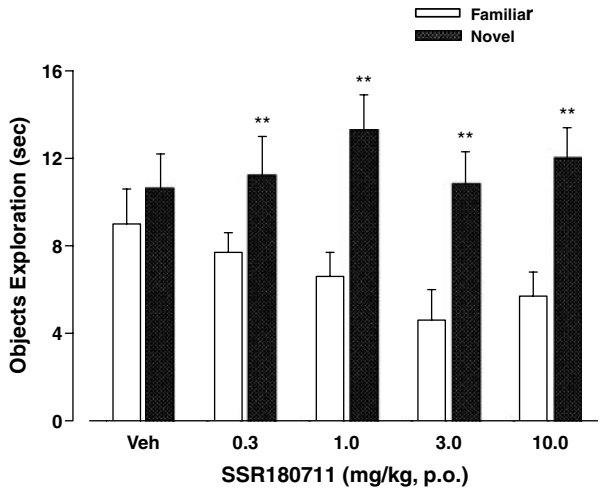

Figure 1 Acute treatment with SSR180711 enhanced episodic memory in an object recognition task in rats. Each bar represents the average (+SEM) time spent exploring a novel or a familiar object. The interval between the acquisition and the recall session was 24 h. *Post hoc* analyses following a two-way ANOVA: $^{**}P<0.01$, novel *vs* familiar object at the concerned dose of SSR180711. $N=12$ rats per group.

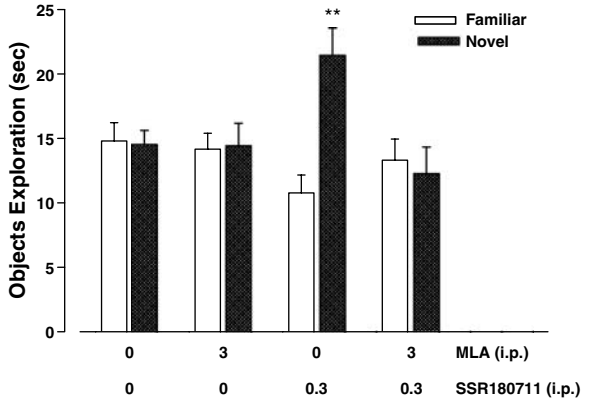

Figure 2 Enhancement by acute treatment with SSR180711 of episodic memory in an object recognition task in rats is reversed by the α7 n-AChR antagonist MLA. Refer to legend of Figure 1 for details. *Post hoc* analyses following a two-way ANOVA: $^{**}P<0.01$, novel *vs* familiar object at the considered treatment condition. $N=11$–12 rats per group.

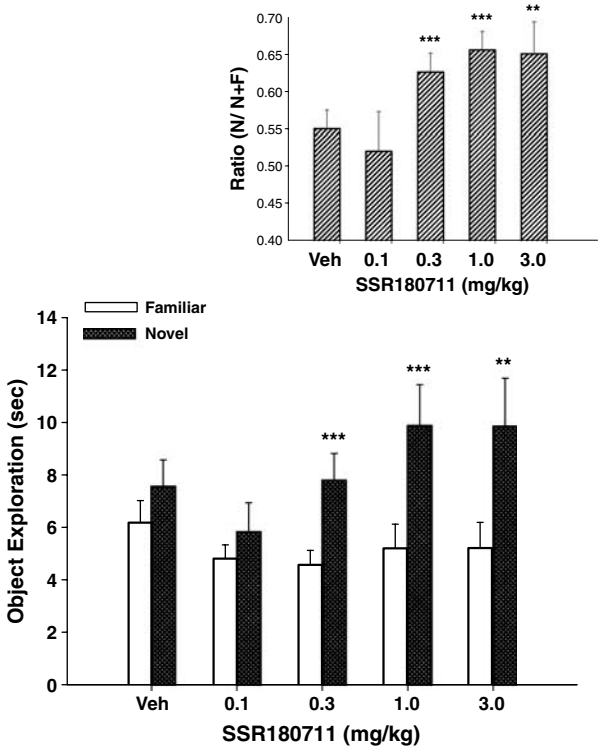

Figure 3 Dose relationship of the enhancement by SSR180711 of episodic memory in an object recognition task in mice. Each bar represents the average (+SEM) time spent exploring a novel or a familiar object. The interval between the acquisition and the recall session was 48 h. *Post hoc* analyses following a two-way ANOVA: $^{**}P<0.01$, $^{***}P<0.001$ novel *vs* familiar object at the concerned dose of SSR180711. $N=8$–20 rats per group.

MLA Abolished the Facilitatory Effect of Acute SSR180711 in the Object Recognition Task in Rats (Long-Term Episodic Memory Procedure)

Given by itself, MLA (3 mg/kg i.p.) did not significantly modify the ratio of the time spent investigating either one of the two objects (Figure 2). When coadministered with 0.3 mg/kg i.p. of SSR180711, MLA abolished the enhancement of time spent preferentially investigating the novel object produced by SSR180711 alone (Figure 2). This was supported by the Winer analysis (comparing the time spent investigating the novel *vs* the familiar object, following significant object and treatment × object interaction effects, two-way ANOVA: $F(1,43)=5.20$, $P<0.05$ and $F(3,43)=7.07$, $P<0.001$, respectively).

Dose-Relationship Facilitatory Effect of SSR180711 in the Object Recognition Task in Mice (Long-Term Memory Procedure)

Previously, data from the laboratory have shown that in this strain of mice, the ability to discriminate between a familiar and novel object was obtained at a longer retention time compared to that of rats. A 48 h retention time was used for a complete physiological forgetting delay of episodic memory. In the present study, under control (vehicle) conditions, mice spent an equivalent amount of time exploring the novel object than the familiar object, with similar levels of motivation and short-term memory (Figure 3). SSR180711, tested within the dose range of 0.1–3 mg/kg, p.o., significantly increased the amount of time spent investigating the novel *vs* the familiar object from 0.3 mg/kg (Winer analysis comparing the time spent investigating the novel *vs* the familiar object following significant object: two-way ANOVA: $F(1,71)=35.22$, $P<0.0001$ and $F(4,71)=2.63$ $P<0.05$, respectively). In addition, at a 48 h delay, the relative time spent exploring the novel object (recognition index expressed as the ratio $N/N+F$) was calculated for each mouse (Figure 3, insert) and treatment with SSR180711 differed significantly from chance level (0.5) from 0.3 mg/kg p.o. This difference compared to control group indicated that SSR180711 improved long-term recognition memory in a dose-dependent manner, an effect being significant at 0.3, 1, and 3 mg/kg (*post hoc* comparisons of calculated ratio to the

theoretical chance level of 0.5 performed using univariate *t* tests (all *P*'s <0.05 respectively, following a significant treatment effect ($F(4,62)=3.12$, $P<0.05$) from the one-way ANOVA).

Effects of SSR180711 in the Object Recognition Task in α7 n-AChR KO Mice

Two-way ANOVA ($F(1,16)=65.62$, $P<0.0001$) for factors object and genotype ($F(1,16)=0.015$, $P>0.05$) indicated that memory performances were similar between α7 n-AChR KO and WT control animals when a 1-h delay was used between both sessions. Both groups of mice exhibited similar object recognition behavior 1 h after the exploration training of object A, suggesting a similar ability for short-term recognition memory and no discriminatory or motivational deficit, once a novel object was introduced (see Figure 4, insert).

When a long-term forgetting delay (ie 48 h) was used, α7-nAChR KO mice and their WT counterparts spent an equivalent amount of time investigating the novel and the familiar object. Treatment with SSR180711 (see Figure 4), at 0.3 mg/kg, i.p., significantly improved the amount of time spent investigating the novel object in WT, but not in α7 nAChR KO mice.

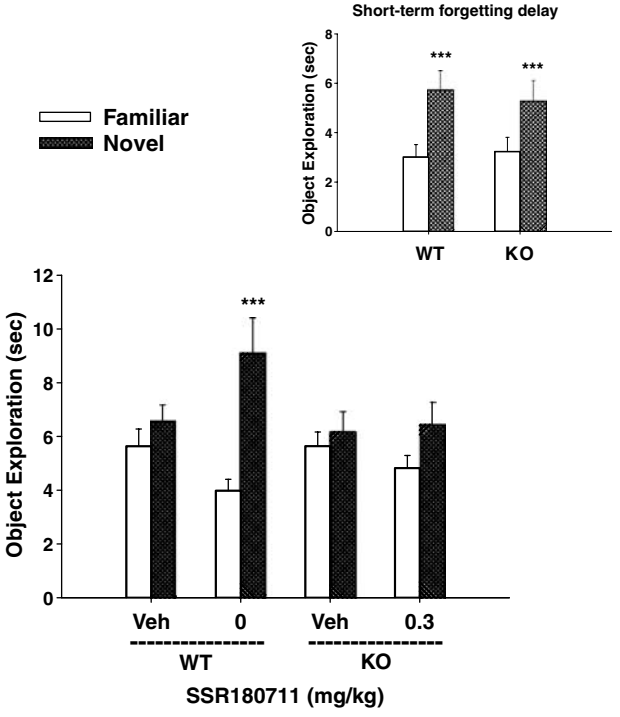

Figure 4 Facilitatory effect of SSR180711 in the object recognition task was abolished in α7-nAChR knockout KO mice (long-term memory procedure). Each bar represents the average (+SEM) time spent exploring a novel or a familiar object in α7-nAChR knockout KO and WT treated animals. The interval between the acquisition and the recall session was 48 h. *Post hoc* analyses following a two-way ANOVA: ***$P<0.001$ novel *vs* familiar object at the concerned dose of SSR180711, $N=8$–10 mice per group. In insert, each bar represents the average (+SEM) time spent exploring a novel or a familiar object in α7-nAChR knockout and WT control animals. The interval between the acquisition and the recall session was 1 h (short-term memory). *Post hoc* analyses following a two-way ANOVA: ***$P<0.001$ novel *vs* familiar object at the concerned animals genotype, $N=8$–10 mice per group.

Facilitatory Effect of SSR180711 in the Object Recognition Task in Rats (Long-Term Episodic Memory Procedure) was Preserved Following Repeated Treatment (Eight Administrations)

Treatment with SSR180711, 1 mg/kg p.o. (Figure 5) eight administrations over 5 days did not significantly affect the ability of an subacute challenge (three administrations over 3 days) with SSR180711 to enhance the amount of time spent investigating preferentially the novel object ($P>0.05$, *post hoc* test, comparing the time spent investigating the novel *vs* the familiar object, following significant object and treatment × object interaction effects, two-way ANOVA: $F(1,29)=26.76$, $P<0.0001$ and $F(2,29)=7.68$, $P<0.001$, respectively). This suggests that there was no tolerance (ie no tachyphylaxia) to the beneficial effect of SSR180711 on this type of episodic memory (Figure 5).

SSR180711 Reversed MK-801-Induced Deficits in the Object Recognition Task in Rats (Short-Term Episodic Memory Procedure)

Using the protocol where both objects were presented 1 h after exposure to the familiar object, control rats spent a greater amount of time investigating the novel one (19.8 ± 1.2 *vs* 5.5 ± 1.1 s: first pair of bars, starting from the left, Figure 6). This preferential investigation of the novel object was abolished (9.7 ± 1.5 *vs* 10.5 ± 2.0 s) by administration of 0.1 mg/kg i.p. of MK-801 immediately after presentation of the familiar object. SSR180711, at 0.3 and 1 mg/kg p.o., when coadministered with MK-801, significantly restored this preferential investigation (Winer analysis comparing the time spent investigating the novel *vs* the familiar object, following significant treatment and treatment × object interaction effects, two-way ANOVA: $F(4,44)=9.34$, $P<0.0001$ and $F(4,44)=18.35$, $P<0.0001$, respectively).

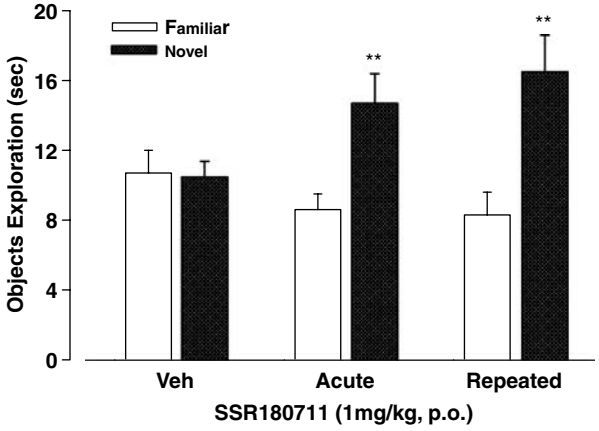

Figure 5 Enhancement by SSR180711 of episodic memory in an object recognition task in rats is maintained upon repeated administration. Refer to legend of Figure 1 for details. *Post hoc* analyses following a two-way ANOVA: **$P<0.01$, novel *vs* familiar object at the considered treatment condition. $N=10$–11 rats per group.

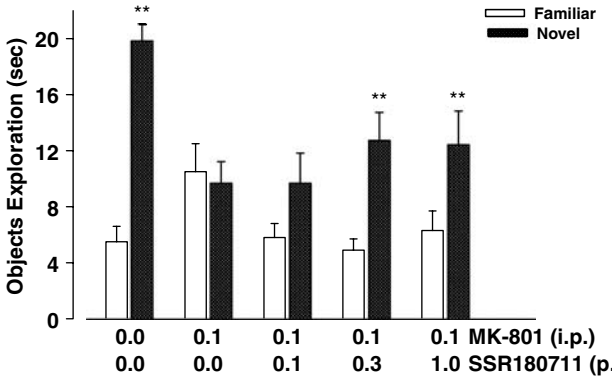

Figure 6 Reversal by SSR180711 of an MK-801-induced deficit of episodic memory in an object recognition task in rats. Each bar represents the average (+SEM) time spent exploring a novel or a familiar object. The delay between the acquisition and the recall session was 1 h. *Post hoc* analyses following a two-way ANOVA: $^{**}P<0.01$, novel *vs* familiar object at the considered treatment condition. $N=9$–12 rats per group.

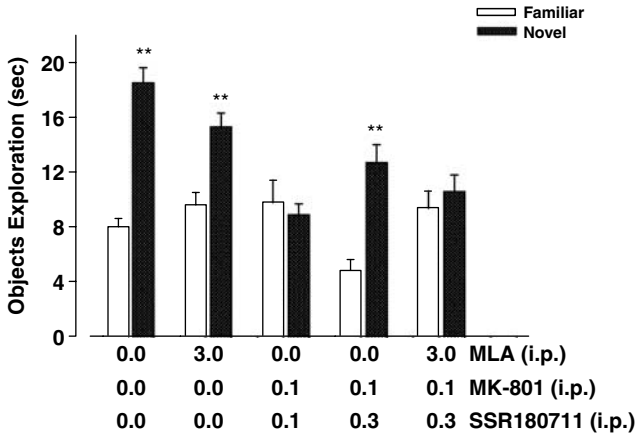

Figure 7 Reversal by SSR180711 of an MK-801-induced deficit of episodic memory in an object recognition task in rats is antagonized by the α7 n-AchR antagonist MLA. Refer to legend of Figure 4 for details. *Post hoc* analyses following a two-way ANOVA: $^{**}P<0.01$, novel *vs* familiar object at the considered treatment condition. $N=12$–15 rats per group.

MLA Abolished the Reversing Effect of SSR180711 on MK-801-Induced Deficits in the Object Recognition Task in Rats (Short-Term Episodic Memory Procedure)

The preferential investigation towards the novel object displayed by rats treated with vehicle was not modified following the administration of MLA alone (3 mg/kg i.p.) (Figure 7). Similarly to what was observed in the experiment above, treatment with SSR180711 (0.3 mg/kg i.p.) restored the deficit of preferential investigation produced by MK-801 (third and fourth pairs of bars, from left). The beneficial effect of SSR180711 was, however, completely abolished by coadministration of MLA (Figure 7) (Winer analysis comparing the time spent investigating the novel *vs* the familiar object, following significant treatment, object and treatment × object interaction effects, two-way ANOVA: $F(4,59)=3.92$, $P<0.01$, $F(1,59)=69.72$, $P<0.0001$ and $F(4,59)=12.62$, $P<0.0001$, respectively).

SSR180711 Reversed MK-801-Induced Deficits in the Water Maze Task in Rats (Spatial Working Memory Procedure)

With respect to controls, MK-801 robustly increased the time to reach the platform across all four learning sessions. The mean overall swim speed for MK-801-treated control animals was not modified over the five training days: ranging from 15.1 ± 1.0 to 18.8 ± 1.7 and 14.2 ± 0.9 to 19.5 ± 1.4 (in cm/s ± SEM) for the vehicle/vehicle and the vehicle/MK-801 group, respectively. SSR180711 dose-dependently attenuated the deleterious effect of MK-801 in this model of working memory (Figure 8).

Mean overall times to reach the platform for each treatment for all four learning sessions were: 95.21 ± 2.80, 80.34 ± 3.15, 73.76 ± 3.17, and 58.26 ± 3.12 (in seconds ± SEM) for the vehicle/MK-801, vehicle/SSR 3 mg/kg, i.p., vehicle/SSR 10 mg/kg, i.p., and the vehicle/vehicle groups, respectively. Statistical analysis of these means confirmed the effects of SSR180711, with the vehicle/SSR 3 mg/kg, vehicle/SSR 10 mg/kg, and the vehicle/vehicle groups being significantly different from the vehicle/MK-801 group

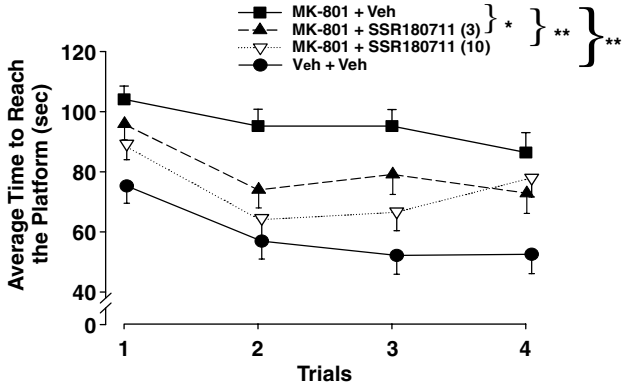

Figure 8 Reversal by SSR180711 of MK-801-induced deficits in the water maze task in a spatial working memory procedure in the rat. Each line represents the average (+SEM) latency to reach the platform. Numbers in parentheses in the graph legend indicate the dose of SSR180711 in mg/kg p.o. *Post hoc* analyses following a two-way ANOVA: $^{*}P<0.05$, $^{**}P<0.01$, *vs* control (Veh + MK-801)-treated rats. $N=12$ rats per group.

(Dunnett's *post hoc* tests: all F's >2.30, all P's <0.05), following a significant treatment effect ($F(3196)=11.15$, $P<0.0001$) and learning sessions effect ($F(3588)=16.43$, $P<0.0001$) from the two-way ANOVA).

SSR180711 Prevented the Impairment of Memory Induced by an Acute Low Dose of PCP in the Object Recognition Task, in Rats Sensitized to PCP

In rats treated subchronically with vehicle, an acute challenge with 1 mg/kg i.p. of PCP on day 11 did not affect the ratio of the time spent preferentially investigating the novel *vs* familiar object by almost two-fold (Table 1). In rats subchronically injected with PCP and challenged with vehicle on the test day, the ratio was similarly not affected. However, following an acute challenge with PCP, these subchronic PCP-treated rats did not spend more time

Table 1 Sub-Chronic Treatment with PCP (10 mg/kg i.p. o.d. during Days 1–5) Induces a Sensitization to the Disrupting Effect of an Acute Challenge with PCP (1 mg/kg i.p., Day 11) in the Object Recognition Task in Rats

	Chronic vehicle acute vehicle	Chronic vehicle acute PCP	Chronic PCP acute vehicle	Chronic PCP acute PCP
Familiar object (*F*)	7.4 ± 0.8	8.2 ± 1.0	8.2 ± 0.8	14.6 ± 1.7
Novel object (*N*)	13.5 ± 1.3**	16.6 ± 1.2**	17.5 ± 1.3**	11.9 ± 1.8
Ratio *N*/*F*	1.82	2.02	2.13	0.81^{++}

Each number is the average (±SEM).
**$P<0.01$, *vs* novel/familiar object group, Winer *post hoc* tests following significant two-way ANOVA.
$^{++}P<0.01$, *vs* the Chronic vehicle/Acute vehicle group, Dunn's *post hoc* test following significant Kruskal–Wallis test. $N=8$–10 per group.

investigating the novel than the familiar object. All this was confirmed by statistics (Winer analysis comparing the time spent investigating the novel *vs* the familiar object, at each treatment condition, following significant object and treatment × object interaction effects, two-way ANOVA: $F(1,35)=48.58$, $P<0.0001$ and $F(3,35)=11.48$, $P<0.0001$, respectively). These data suggest that subchronic treatment with PCP produced a sensitization to the effects of an acute challenge with PCP (Table 1).

In a separate experiment, administration of SSR180711 (1 mg/kg p.o.) reversed the effects of an acute challenge with 1 mg/kg i.p. PCP in rats treated repeatedly with PCP (Figure 9). This was confirmed by the Winer analysis comparing the time spent investigating the novel *vs* the familiar object, at each treatment following significant object and treatment × object interaction effects, two-way ANOVA: $F(1,31)=14.07$, $P<0.001$ and $F(2,31)=8.66$, $P=0.001$, respectively). In other words, SSR180711 reversed the visual memory deficits induced by PCP sensitization, which is evoked by an acute challenge with a low dose of PCP in animals sensitized by a repeated treatment with a high dose of PCP.

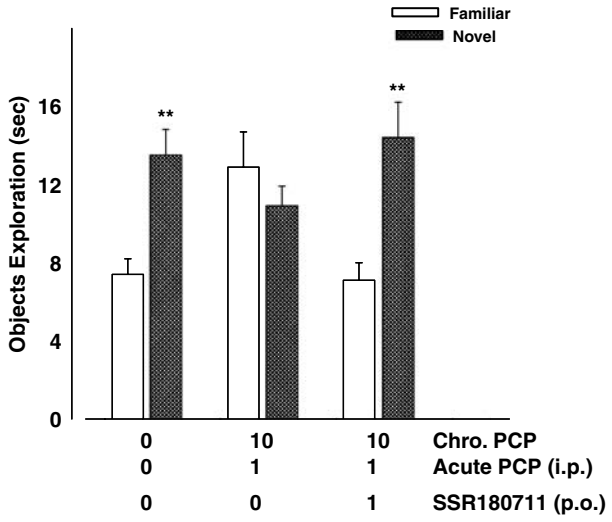

Figure 9 Reversal by SSR180711 of a PCP-induced deficit of episodic memory in an object recognition task in rats sensitized to PCP. Refer to legend of Figure 4 for details. *Post hoc* analyses following a two-way ANOVA: **$P<0.01$, novel *vs* familiar object at the considered treatment condition. $N=10$–12 rats per group.

SSR180711 Antagonized the Impairment of Novelty Discrimination in Adult Rats Treated with PCP at the Neonatal Stage

Under control conditions (ie acute injection of vehicle), adult rats pretreated with vehicle at the neonatal stage spent approximately four-fold more time investigating the novel than the familiar juvenile (NDI = 4.39 ± 1.10, Figure 10). By contrast, adult rats neonatally pretreated with PCP presented an NDI roughly half that of vehicle-neonatal rats. This indicates that PCP-neonate adult rats spent less time exploring the novel juvenile, which can be interpreted as an impairment of selective attention (see Terranova *et al*, 2005, for in-depth discussion). Treatment with SSR180711 dose-dependently normalized this impairment: This was supported by *post hoc* statistical analysis (see legend of Figure 10 for details) following a two-way ANOVA with a significant neonatal pretreatment effect and acute treatment effect ($F(1,8)=13.09$, $P<0.01$ and $F(4,32)=4.41$, $P<0.05$, respectively). It is noteworthy that there is also a significant difference between PCP-treated control animals and subjects treated with SSR180711. Here, one-way ANOVA showed a global treatment effect for the PCP neonates group ($P=0.035$), and *post hoc* analysis indicates a significant difference at the highest dose of SSR180711 (1 mg/kg). Note that in the vehicle-neonatal control group, SSR180711 had no effect by itself (compare the five white bars in Figure 10).

MLA Abolished the Reversing Effect of SSR180711 on the Impairment of Novelty Discrimination in Adult Rats Treated with PCP at the Neonatal Stage

MLA, at 3 mg/kg s.c., antagonized the effects of SSR180711 (Figure 11). By itself, MLA had no effect. This was confirmed by the statistical analysis following a two-way ANOVA with a significant neonatal pretreatment effect, acute treatment effect, and neonatal × acute treatments interaction ($F(1,8)=31.64$, $P<0.01$, $F(3,24)=4.05$, $P<0.05$ and $F(3,24)=4.68$, $P<0.05$, respectively).

SSR180711 Partially Reversed PCP-Induced Deficit of Sequential Memory in a Linear Maze Task in Rats

Control rats displayed a low number of errors across the 5 days of learning (Figure 12a). PCP at 1.5 mg/kg i.p. induced a marked deficit of learning, as shown by a greatly increased number of errors, particularly during the first three sessions. SSR180711, at 1 and 3 mg/kg i.p., partially

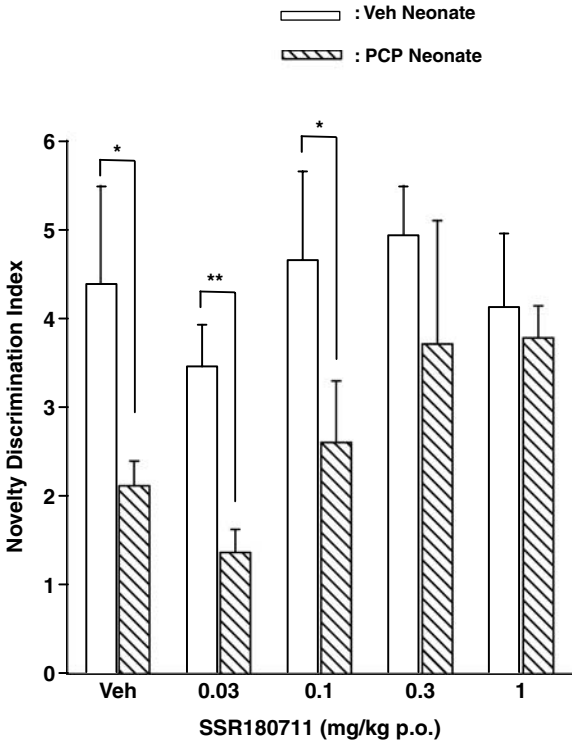

Figure 10 Antagonism by SSR180711 of the impairment of novelty discrimination in adult rats treated with PCP administration at the neonatal stage. Each bar represents the average (+SEM) novelty discrimination index (ratio of the time spent investigating the novel juvenile divided by the time spent investigating the familiar juvenile, in seconds). *Post hoc* analyses following a two-way ANOVA: $^{*}P<0.05$, $^{**}P<0.01$, neonatal PCP-treated compared to neonatal vehicle-treated rats, at the considered dose of SSR180711. $N=5$ rats per group.

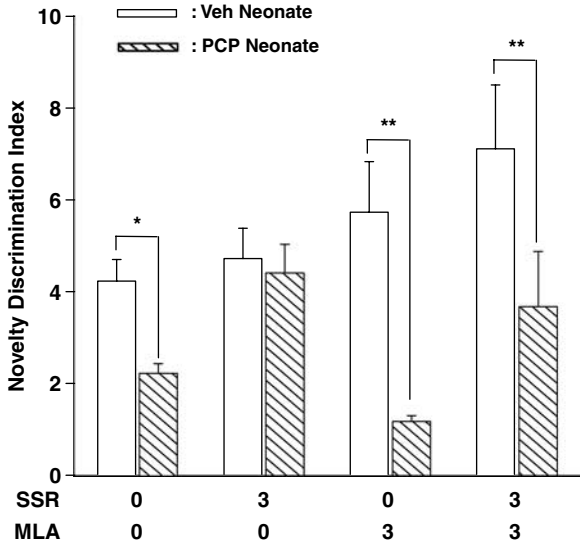

Figure 11 Reversal by the α7 n-AChR antagonist MLA of the antagonism by SSR180711 of the impairment of novelty discrimination in adult rats treated with PCP administration at the neonatal stage. Refer to legend of Figure 8 for details. *Post hoc* analyses following a two-way ANOVA: $^{*}P<0.05$, $^{**}P<0.01$, neonatal PCP-treated compared to neonatal vehicle-treated rats, at the considered dose of SSR180711. $N=5$ rats per group.

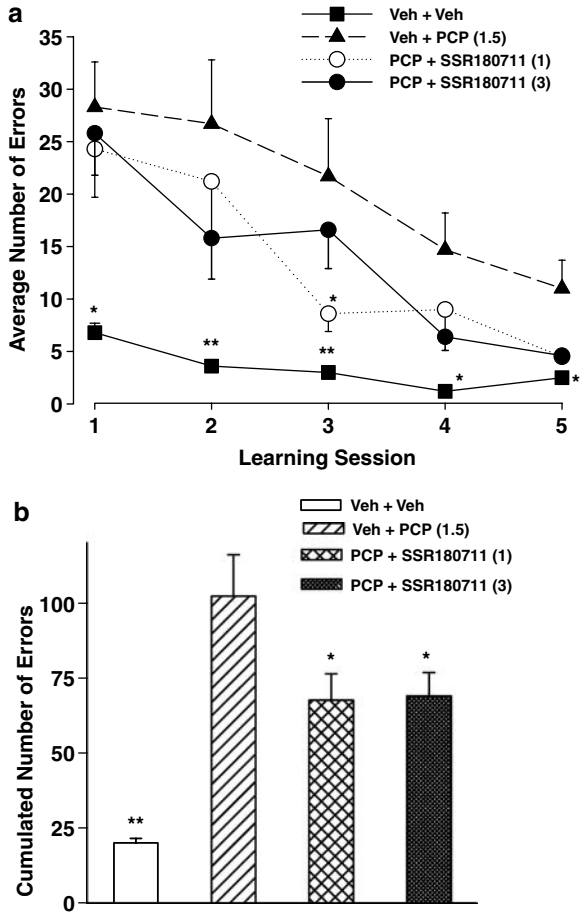

Figure 12 Attenuation by SSR180711 of a phencycline-induced deficit of sequential memory in a linear maze task in rats. Each symbol or bar represents the average (±SEM) number of errors as a function of the daily learning session (a) or cumulated across all five sessions (b). Numbers in parentheses in the graph legend indicate the dose in mg/kg (p.o. for SSR180711, i.p. for PCP). $N=20$ rats per group. Panel a: $^{*}P<0.05$, $^{**}P<0.01$, *vs* the vehicle/phencyclidine (Veh + PCP)-treated group at the considered learning session, *post hoc* test following significant two-way ANOVA. Panel b: $^{*}P<0.05$, $^{**}P<0.01$, *vs* the vehicle/phencyclidine-treated group, *post hoc* tests following significant one-way ANOVA.

diminished the number of errors induced by PCP, across all five learning sessions (see Figure 12a for details of statistical analysis, following a significant treatment and learning sessions effects, two-way ANOVA: $F(3,76)=12.88$, $P<0.0001$ and $F(4,76)=33.51$, $P<0.0001$, respectively). When the number of errors was cumulated across all five learning sessions (Figure 12b), both doses of SSR180711 were found to significantly lower the errors rate with respect to the PCP-injected group (all t's >2.36, all P's <0.05, Dunnett's *post hoc* test, following significant one-way ANOVA: $F(3,76)=12.88$, $P<0.0001$).

SSR180711 Enhanced the Spontaneous Firing Rate of Retrosplenial Cortex Neurons of Rats

With respect to basal conditions, the low dose of SSR180711 (1 mg/kg i.v., Figure 13) had a marginal effect on neuronal firing, with a small (−19%) but nonetheless significant

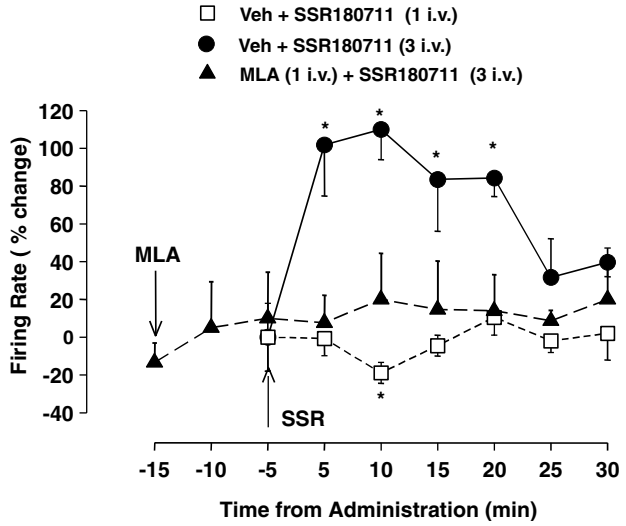

Figure 13 Potentiation by SSR180711 of the spontaneous firing rate of retrosplenial cortex neurons. Each symbol represents the mean (±SEM) percentage change in firing rate (with respect to basal levels recorded 5 min before administration of SSR180711). Numbers in parentheses in the graph legend indicate the dose in mg/kg. *$P<0.05$ *vs* basal level, *post hoc* tests following a one-way ANOVA, for the considered treatment. $N=3$–6 per treatment.

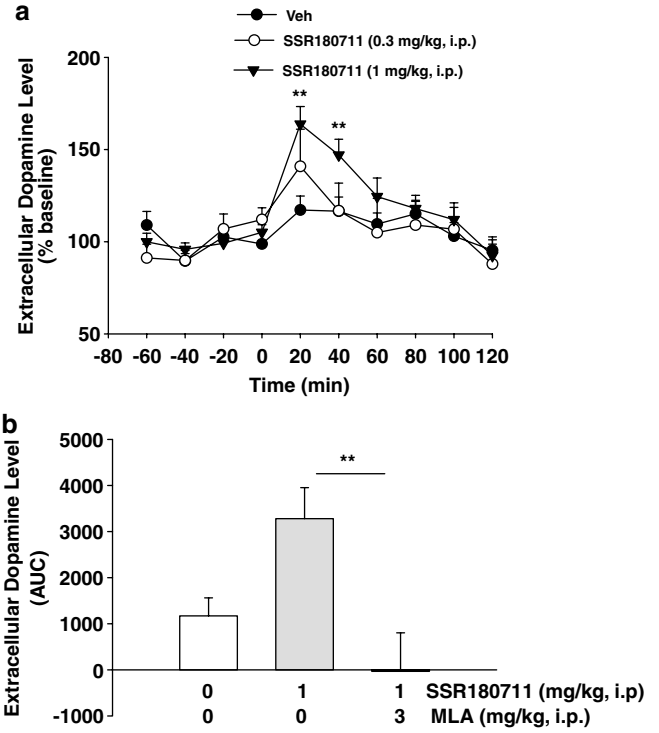

Figure 14 Increase by SSR180711 of extracellular dopamine levels in the prefrontal cortex of freely moving rats. (a) Time-course effect. Changes in extracellular levels of dopamine are expressed as a percentage of the mean value of the four basal samples before administration of SSR180711 or vehicle (administered at 0 time). Each symbol represents the mean±SEM. **$P<0.01$ *vs* the vehicle (Veh) value at the corresponding time of sampling, *post hoc* tests following a two-way ANOVA. $N=$ 7–9 rats per group. (b) Reversal by the α7 n-AChR antagonist MLA. Each bar represents the mean + SEM of the area under the curve (AUC) for the 120 min period following injection of vehicle or SSR180711. **$P<0.01$, *post hoc* tests following a one-way ANOVA. $N=7$–9 rats per group.

decrease at a single time point (10 min: Duncan's *post hoc* test following significant one-way ANOVA: $F(4,29)=3.77$, $P<0.05$). At 3 mg/kg, the compound doubled the firing rate, with significant effects observed from 5 to 20 min post-administration (Duncan's *post hoc* test, following significant ANOVA: $F(4,19)=3.44$, $P<0.05$). MLA (1 mg/kg i.v.), administered 15 min before SSR180711 (3 mg/kg), while having no effect on its own (first two filled triangles), fully reversed the increase produced by SSR180711. This was confirmed by a nonsignificant one-way ANOVA ($F(6,20)=0.50$, $P>0.05$).

SSR180711 Increased Extracellular Dopamine Levels in the Prefrontal Cortex of Rats

The average basal extracellular dopamine level in the prefrontal cortex was estimated to be 7.6±1.0 fmol by fraction ($N=36$): SSR180711 significantly ($F(6,84)=9.81$, $P<0.01$) increased extracellular dopamine levels, with a maximal effect (164±10% of basal value) observed at 20 min for the dose of 1 mg/kg, i.p.: Figure 14a).

MLA (3 mg/kg, i.p.), which had no effect by itself (data not shown), completely prevented the increase in extracellular dopamine levels induced by 1 mg/kg SSR180711 (Figure 14b: Dunnett's *post hoc* test following a significant one-way ANOVA: $F(1,13)=9.63$, $P<0.01$).

SSR180711 was Active in Models Predictive of an Antidepressant Activity

SSR180711 dose-dependently and significantly ($F(3,24)=12.25$, $P<0.001$) decreased immobility time in the forced-swimming test at all three doses administered (1–10 mg/kg, p.o.), and reduced ($H(3)=17.06$, $P<0.001$) ultrasonic distress calls emitted by rat pups separated from their mother and littermates, with an MED of 3 mg/kg (Table 2).

Table 2 SSR180711 is Active in Tests Predictive of an Antidepressant/Anxiolytic Activity

Test	Doses (mg/kg)	Time spent in immobility (s)	Number of vocalizations
Swimming test in rats (p.o. route)	0	214.7±11.4	—
	1	170.9±15.4*	—
	3	150.4±12.9**	—
	10	114.9±5.9**	—
Ultrasonic distress calls in rat pups (s.c. route)	0	—	100.9±20.1
	1	—	53.0±11.0
	3	—	$26.6\pm2.7^{++}$
	10	—	$19.4\pm7.2^{++}$

Each number is the average (±SEM).
*$P<0.05$, **$P<0.01$, *vs* the vehicle (0) group, *post hoc* tests following significant one-way ANOVA.
$^{++}P<0.01$, *vs* the vehicle (0) group, Dunn's *post hoc* test following significant Kruskal–Wallis test. $N=7$–8 rats per group.

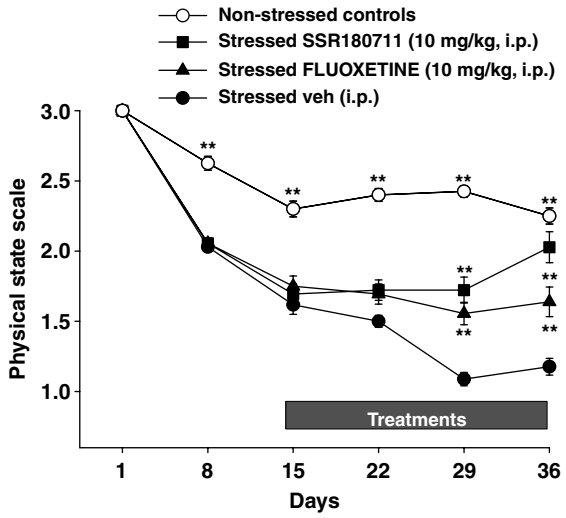

Figure 15 Reversal by SSR180711 and fluoxetine of the physical degradation induced by the application of CMS in mice. Each line represents the average physical state scale score (±SEM). *Post hoc* analyses following significant Kruskal–Wallis test: $^{**}P<0.01$, *vs* stressed vehicle group. $N = 17$–20 mice per group.

In the CMS procedure, SSR180711 and fluoxetine (administered from day 14 to day 36, 10 mg/kg i.p. once a day for each compound) significantly attenuated stress-induced degradation of the physical state (coat appearance) of mice, at day 29 ($H(3) = 53.31$, $P<0.0001$) and day 36 of treatment ($H(3) = 40.35$, $P<0.0001$). At the end of the stress period, the stressed/SSR180711 group reached the level of nonstressed controls, whereas the score of the stressed/vehicle group was about half that of the nonstressed controls (Figure 15).

DISCUSSION

The present data indicate that the novel and selective α7 n-AChR partial agonist, SSR180711, possesses cognitive-enhancing properties on its own, and is able to reverse deleterious effects consecutive to pharmacologically induced hypoglutamatergic states in several memory tests.

Profile of SSR180711 in Tests Predictive of Therapeutic Activity against Cognitive Impairment Associated with Schizophrenia

The cognitive deficits observed in schizophrenic patients are more and more considered to form the core symptom of the pathology, representing an obstacle to reinsertion and proper societal functioning of patients (Freedman, 2003; Green *et al*, 2004). These deficits include attentional disturbances, memory impairment (more particularly working, episodic, and sequential memory), and information processing deficits. Multiple lines of evidence suggest that a dysfunction in glutamatergic neurotransmission, via NMDA receptors, might be involved in the pathophysiology of schizophrenia (Javitt and Zukin, 1991; Olney and Farber, 1995; Coyle, 1996; Krystal *et al*, 1999; Hashimoto *et al*, 2004). Noncompetitive NMDA receptor antagonists such as PCP are known to induce schizophrenia-like symptoms, including cognitive deficits, in human volunteers (Allen and Young, 1978; Javitt and Zukin, 1991; Lahti *et al*, 1995; Krystal *et al*, 1999; for a critical review, see Goff and Coyle, 2001). There is also substantial evidence from animal studies that NMDA receptor antagonists such as MK-801, PCP, and ketamine impair learning and memory performances when administered both before learning and during the consolidation phase (Morris *et al*, 1986; Monaghan *et al*, 1989; Bliss and Collingridge, 1993; Kesner and Dakis, 1993).

Our results demonstrate that SSR180711 was able to reverse spontaneous or NMDA receptor antagonist-induced deficits of several types of memory known to be affected in schizophrenia. When given acutely, SSR180711 improved memory performances in several variants of the object recognition task, using either a long-term episodic memory procedure in nontreated animals or a short-term episodic memory protocol using animals impaired either by an acute dose of MK-801, or by an acute low dose of PCP in rats sensitized by repeated treatment with high doses of PCP. The latter property is particularly interesting, in the light of the observation that schizophrenic patients exhibit more pronounced negative symptoms than normal volunteers following an acute challenge with ketamine (Lahti *et al*, 2001), indicating that the former are sensitized to the effects of this PCP analog. Moreover, SSR180711 reversed MK-801-induced spatial learning deficit in the Morris water test in rats using a working memory protocol. It is important to note that SSR180711 reversed the deleterious effects of MK-801 in the object recognition task when the psychotomimetic was administered in the immediate post-acquisition interval, thus affecting consolidation and/or recall processes. It can be speculated that the effects of SSR180711 are mediated via α7 n-AChRs that indirectly mediate glutamate release in the hippocampus and the amygdala, two structures well known to be important for information processing in the post-training period (Rossato *et al*, 2004). It is important to note that the positive effects of SSR180711 in the object recognition test in mice using a long-term forgetting delay are mediated by the α7 n-AchR, as they were abolished by the genetic deletion of this receptor subtype.

Sequential memory, as assessed for example by the recall of a list of letters, is affected in schizophrenic patients (Elvevag *et al*, 2001). The linear maze task was originally developed as a model of long-term memory, with the recall session taking place days if not weeks after acquisition (Sara *et al*, 1980). Here, the protocol was modified in order to reproduce more accurately the characteristics of sequential memory. In the linear maze, PCP-treated animals demonstrated a marked deficit in their ability to remember a sequence of left–right turns. Such a deficit could reflect an inability to remember sequences or lists, but may also include elements of disorientation or perseverative behavior, features of cognitive impairment induced by PCP. In this task, SSR180711 reduced the effects of acute PCP on sequential memory performances. Furthermore, the observation of PCP-treated animals revealed a typical pattern of behavior when entering a blind alley: they almost systematically returned to the start box, and reiterated this strategy over and over (often more than six times in a row during the first learning session. This perseverative-like pattern of behavior is reminiscent of the well-described phenomenon

of perseveration presented by schizophrenic patients (Crider, 1997). SSR180711, by normalizing performance, contributed to reduce this behavioral perseveration, and it is hoped that this antiperseverative profile will also translate into the clinic.

Based on the neurodevelopmental hypothesis of schizophrenia (Weinberger, 1986; Lieberman *et al*, 1997), several animal models that rely on the expression of behavioral deficits in adulthood after neonatal brain lesions have been developed. For example, treatment with PCP at the neonatal stage has been shown to retard the acquisition of a delayed spatial alternation task, to produce a spontaneous deficit in prepulse inhibition, and to potentiate the hyperlocomotor effects of an acute challenge of PCP. Here, we have used an approach that combines a paradigm that probes selective attention capacities at the adult stage (for an in-depth discussion, see Terranova *et al*, 2005) with a treatment (high doses of PCP at the neonatal stage: Wang *et al*, 2001, 2003) that has a major negative impact on this selective attention. The fact that both juveniles move freely and quickly as well as play with each other induces perpetual changes in spatial location of the two stimuli. This renders more difficult a preferential interaction with the relevant stimulus (novel juvenile) when the attentional system of the adult rat is prevented from working under optimal conditions by pharmacological treatment. Results with SSR180711 show that this compound reversed attentional disturbances in this situation, an effect that would appear to be mediated by α7 nAChRs, as MLA fully antagonized this effect.

To sum up, considering the well-described deficiency of working, sequential and episodic memory in schizophrenic patients, the efficacy of SSR180711 against memory deficits driven by an hypoglutamatergic state in rats indicate that the compound could potentially alleviate a cluster of symptoms considered to constitute the core of the pathology. A putative mechanism for the beneficial effect of SSR180711 against hypoglutamatergy-driven memory/cognitive deficits would be an activation of presynaptic α7 n-AChRs present on glutamatergic neurons, which would result in increasing levels of glutamate as suggested by the SSR180711-induced increase in the amplitudes of evoked EPSCs in mouse hippocampal slices, which was no longer observed in α7 knockout mice (Biton *et al*, submitted). Furthermore, considering the marked attentional disturbances that afflict schizophrenic patients (Brebion *et al*, 2000), the positive activity of SSR180711 on this parameter adds further weight to the therapeutic potential of the compound in this population.

Profile of SSR180711 in Electrophysiological and Neurochemical Tests Related to Cognitive Deficits in Schizophrenia

SSR180711 enhanced basal extracellular levels of DA in the prefrontal cortex (PFC), and increased spontaneous neuronal activity in the retrosplenial cortex (RSC). Both limbic structures have been implicated in the etiopathology of schizophrenia: hypofunctioning of the former has been linked to the emergence of negative symptoms and cognitive deficits (Goldman-Rakic and Selemon, 1997), whereas dysfunction of the latter has been postulated to participate to memory impairment, in particular of the verbal type (Tendolkar *et al*, 2004). An hypodopaminergic tone has been linked to reduced activity of the PFC (Goldman-Rakic and Selemon, 1997), and the ability of current antipsychotics to augment prefrontal DA tone is considered to be a neurochemical marker indicative of their capacity to alleviate negative and cognitive symptoms in patients (Kapur and Remington, 1996). Furthermore, suboptimal activity of the DA system in the PFC has been suspected to lead to hyperfunctioning of the subcortical dopaminergic system, leading to the genesis of positive symptoms of schizophrenia (Grace, 1991). By virtue of its ability to potentiate DA transmission in the PFC and neuronal activity in the RSC, SSR180711 is anticipated to be effective in combating negative/cognitive deficits, social dysfunctioning seen in schizophrenic patients, as well as to possibly have an indirect beneficial effect on positive symptoms (via a diminution of subcortical DA tone).

Alterations of the cholinergic neurotransmission have been suspected to occur in schizophrenia (Bymaster *et al*, 1999; Hyde and Crook, 2001). The atypical nature of clozapine, and in particular its ability to ameliorate—albeit to a marginal extent—cognitive deficits in schizophrenic patients (Meltzer and McGurk, 1999) has been suggested by some authors to arise from an indirect enhancement of central cholinergic transmission. This hypothesis stems from the observation that a metabolite of clozapine (*N*-desmethylclozapine) is a fairly potent partial agonist at the M1 variety of muscarinic-acetylcholine receptors (Sur *et al*, 2003). SSR180711 was, in addition, found to increase the extracellular levels of acetylcholine in the hippocampus and prefrontal cortex (Biton *et al*, submitted). This twin-mechanistic activity, pro-cholinergic and pro-dopaminergic (see above) is anticipated to further reinforce the efficacy of the compound against cognitive deterioration associated with schizophrenia.

SSR180711 has Efficacy in Tests Predictive of Activity Against Depressive Disorders

SSR180711, at a dose range that overlapped with that shown to be efficacious in cognitive tests, was active in several models of depression, including the rat pup mother separation-induced ultrasonic vocalization paradigm, the forced-swim test in rats, and the CMS procedure in mice. It is thus hoped that SSR180711 will show additional beneficial effects on depressive states, a comorbid situation that has a fairly high prevalence in schizophrenic patients (Barnes *et al*, 1989).

Additional Properties and Safety Profile of SSR180711

As discussed above, α7 n-AChRs show a propensity to desensitize following activation by full agonists. Based on the findings that SSR180711 possesses a partial agonist profile *in vitro* (intrinsic activity: 39 or 51% that of acetylcholine, in GH4C1 cells or xenopus oocytes transiently transfected with human α7 n-AChRs, respectively; Biton *et al*, submitted), we anticipated that *in vivo*, there would be no tolerance (ie tachyphylaxia) to the effects of SSR180711 upon repeated treatment. Results from the subchronic administration experiment in the object recognition test

clearly show that there is no such tolerance, suggesting that SSR180711 does not produce desensitization of α7 n-AChRs *in vivo*, in a test predictive of a sought-after (procognitive) clinical activity. In addition, SSR180711 has no functional activity at the nicotinic receptor subtypes related to adverse side effects of nicotine, such as the central $\alpha3\beta4$ and $\alpha4\beta4$ (possibly mediating the addictive, convulsive, and emetic activities) and peripheral $\alpha3\beta2$, $\alpha3\beta4$, and $\alpha1\beta1\gamma\delta$ (thought to be responsible for the gastro-intestinal, vaso-constrictive, and respiratory side-effects). In particular, it was verified that SSR180711 does not induce convulsions in mice up to 100 mg/kg p.o. This is in line with the demonstration that nicotine induced convulsions with similar potency in wild-type and α7 n-AChRs knock-out (homozygous and heterozygous) mice, suggesting that the α7 subtype is not implicated in the convulsive activity of nicotine (Franceschini *et al*, 2002; but see Damaj *et al*, 1999).

Conclusion

SSR180711, a selective α7 n-AChR partial agonist, displayed pro-mnesic effects under baseline conditions in several models of memory (working and spatial types), and reversed deleterious effects induced by hypoglutamatergic (PCP and MK-801-induced) states in models of working, spatial, and sequential memory. It was also active in paradigms postulated to model some aspects of schizophrenia (selective attention deficit following neonatal treatment with PCP, episodic memory impairment produced by PCP in PCP-sensitized rats) and in electrophysiological and neurochemical markers putatively relevant to the pathology (eg spontaneous neuronal activity, cortical DA level). Implication of α7 n-AChRs in the beneficial effects of SSR180711 was confirmed by the observation that, in all tests in which antagonist/agonist interaction was studied, the selective α 7 n-AChR antagonist MLA reversed these effects. This preclinical profile positions SSR180711 as a promising drug candidate for the treatment of cognitive/negative symptoms of schizophrenic patients. Lastly, the antidepressant-like properties of SSR180711 are of added interest, in view of the high prevalence of depressive symptoms in schizophrenic patients.

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