



Characterization of SSR103800, a selective inhibitor of the glycine transporter-1 in models predictive of therapeutic activity in schizophrenia[☆]

Denis Boulay^{a,*}, Philippe Pichat^a, Gihad Dargazanli^a, Genevieve Estenne-Bouhtou^a, Jean Paul Terranova^b, Nancy Rogacki^c, Jeanne Stemmelin^a, Annick Coste^a, Christophe Lanneau^a, Christophe Desvignes^b, Caroline Cohen^a, Richard Alonso^b, Xavier Vigé^a, Bruno Biton^a, Régis Steinberg^b, Mireille Sevrin^a, Florence Oury-Donat^b, Pascal George^a, Olivier Bergis^a, Guy Griebel^a, Patrick Avenet^a, Bernard Scatton^a

^a Sanofi-Aventis, CNS Department, 31 avenue Paul Vaillant Couturier, Bagneux, France

^b Sanofi-Aventis, Montpellier, France

^c Sanofi-Aventis, Bridgewater, NJ, USA

ARTICLE INFO

Article history:

Received 24 September 2007

Received in revised form 13 June 2008

Accepted 18 June 2008

Available online 24 June 2008

Keywords:

Atypical antipsychotic

Antidepressant

Glycine

GlyT1 inhibitor

Schizophrenia

SSR103800

ABSTRACT

On native human, rat and mouse glycine transporter-1 (GlyT1), SSR103800 behaves as a selective inhibitor with IC₅₀ values of 1.9, 5.3 and 6.8 nM, respectively. It reversibly blocked glycine uptake in mouse brain cortical homogenates, increased extracellular levels of glycine in the rat prefrontal cortex, and potentiated NMDA-mediated excitatory postsynaptic currents in rat hippocampal slices. SSR103800 (30 mg/kg, p.o.) decreased MK-801- and PCP-induced locomotor hyperactivity in rodents. SSR103800 (1 and 10 mg/kg, p.o.) attenuated social recognition deficit in adult rats induced by neonatal injections of PCP (10 mg/kg, s.c., on post-natal day 7, 9 and 11). SSR103800 (3 mg/kg, p.o.) counteracted the deficit in short-term visual episodic-like memory induced by a low challenge dose of PCP (1 mg/kg, i.p.), in PCP-sensitized rats (10 mg/kg, i.p.). SSR103800 (30 mg/kg, i.p.) increased the prepulse inhibition of the startle reflex in DBA/1J mice. SSR103800 decreased defensive- and despair-related behaviors in the tonic immobility test in gerbils (10 and 30 mg/kg, p.o.) and in the forced-swimming procedure in rats (1 and 3 mg/kg, p.o.), respectively. These findings suggest that SSR103800 may have a therapeutic potential in the management of the core symptoms of schizophrenia and comorbid depression states.

© 2008 Elsevier Inc. All rights reserved.

1. Introduction

A widely accepted theory suggests that a dopaminergic (DA) hyperfunction is the primary cause of schizophrenia symptoms. However, the observation that antagonists of the glutamate N-methyl-D-aspartate (NMDA) receptor produced schizophrenic-like symptoms in humans (Javitt and Zukin, 1991) and precipitated psychotic episodes in otherwise stabilized schizophrenia patients (Lahti et al., 2001) has prompted speculation about an alternate hypothesis to the DA one, which relies on the idea of a hypoglutamatergic state, involving the NMDA receptor. This hypothesis is further substantiated by several findings showing for example a decreased concentration of glutamate in the cerebrospinal fluid (CSF) of schizophrenic patients (Kim et al., 1980). Endogenous kynurenic acid concentration, an antagonist of the glycine B site on the NMDA

receptor in the human brain, is increased in the CSF (Erhardt et al., 2001) as well as in the postmortem prefrontal cortex of patients with schizophrenia (Schwarcz et al., 2001). Another, though more indirect argument for this hypo-NMDA hypothesis, is based on the observation that compounds such as D-cycloserine, glycine, alanine and D-serine, which putatively increase NMDA receptor sensitivity to glutamate, provide additional benefit when associated with antipsychotics on negative symptomatology and cognitive dysfunction (Tsai et al., 2006; Evins et al., 2002). Due to the pivotal role of the glutamate system in psychomotor and cognitive processes (Carlsson, 1995; Riedel et al., 2003), a compound that would enhance this system, by increasing glycine levels at the vicinity of the NMDA receptor, may alleviate the negative and cognitive symptoms of schizophrenia (Depoortere et al., 2005).

Glycine elevation can be achieved by inhibition of the glycine transporter 1 (GlyT1), which is responsible for glycine removal from the synaptic cleft. Levels of synaptic glycine are tightly controlled by two types of specific transporters (Glycine Transporters, GlyT1 and GlyT2). The GlyT1 is classically considered to be localized on glial cells – but recently described on neurons as well (Cubelos et al., 2005). The GlyT1 is closely associated with the NMDA receptor, whereas GlyT2 is colocalized

[☆] Note: All experiments were carried out in accordance with the “Guide for the Care and Use of Laboratory Animals” adopted and promulgated by the NIH.

* Corresponding author. Sanofi-Aventis, CNS Department, 31 avenue Paul Vaillant Couturier, 92220 Bagneux, France. Tel.: +33 1 45 36 24 90; fax: +33 1 45 36 20 70.

E-mail address: denis.boulay@sanofi-aventis.com (D. Boulay).

with inhibitory strychnine-sensitive glycine receptors (see: Gomeza et al., 2003 for review). GlyT1 inhibitors are expected to increase glutamatergic transmission, and as such represent a promising therapeutic approach for schizophrenia (Vandenberg and Aubrey 2001; Depoortere et al., 2005). In line with this hypothesis, there is increasing evidence that GlyT1 inhibitors, such as ORG24461, CP-802,079, sarcosine, NFPS, SSR504734 are active in models of schizophrenia, which address the positive, negative and cognitive symptoms of the disease (Harsing et al., 2003; Kinney et al., 2003; Le Pen et al., 2003; Martina et al., 2004; Depoortere et al., 2005). We have recently synthesized a novel selective and reversible GlyT1 inhibitor, SSR103800.

We presently report on the *in vitro* binding profile, as well as the biochemical, electrophysiological and behavioral data, which demonstrate that elevation of central glycine levels induced by SSR103800, functionally impacts central glutamate transmission and prevents various effects produced by a hypo-glutamatergic state. The therapeutic potential of SSR103800 was evaluated in various animal models addressing the different symptoms of schizophrenia, namely the positive/agitation symptoms (MK-801 and PCP induced motor activity in rodents), the cognitive deficits (social attention selective deficit in adult rats treated with PCP at a neonatal stage and visual episodic-like memory deficit in chronic PCP sensitized rats), and the sensory gating impairment (prepulse inhibition of the startle response). In regard to the reported co-morbid depression symptoms in schizophrenia (Subotnik et al., 1997), additional experiments assessing the antidepressant-like potential of SSR103800 were performed.

2. Materials and methods

2.1. Animals

Animals were supplied by Iffa-Credo (Les Oncins, France) or Charles-River (St Aubin-les-Elbeuf, France), unless specified otherwise. Animals were kept in temperature- and humidity-controlled rooms (22 °C, 50%) with lights on from 7 am to 7 pm (except when indicated otherwise), with water and food available *ad libitum*. All experiments were performed in accordance with the "Guide and Care and Use of Laboratory Animals" (National Institutes of Health) and were approved by the Institutional Animal Care & Use Committee of Sanofi-Aventis.

2.2. Drugs

SSR103800 the (S,S) enantiomer, SSR103713 the (R,R) enantiomer of SSR103800, fluoxetine and phencyclidine were synthesized by the CNS Medicinal Chemistry Department of Sanofi-Aventis. Glycine and MK-801 were obtained from Sigma Aldrich (Saint Quentin Fallavier, France) or Bio-rad Laboratories (LifeScience Group Marnes-la-Coquette, France). Olanzapine was purchased from Toronto Research (North York, Ontario). Drugs were diluted in dimethyl sulfoxide (DMSO) for *in vitro* studies and, in saline alone or with a few drops of Tween 80 for intra-peritoneal injection, or in distilled water with methylcellulose (0.6%) for oral administration, unless specified otherwise. Doses refer to the weight of the free base, except when indicated otherwise. All drug solutions were prepared fresh daily and administered *i.p.* or *p.o.* (10 or 20 ml/kg in mice or gerbils, 2 or 5 ml/kg in rats), *s.c.* (0.1 ml in rat pups) or *i.v.* (1 ml/kg in rats).

2.3. Effects of SSR103800 and its (R,R) enantiomer SSR103713 on *in vitro* [¹⁴C]glycine uptake

Human neuroblastoma (SK-N-MC) and rat astrocytoma (C6) cell lines, obtained from the American Type Culture Collection (Rockville, MD, USA), were maintained at 37 °C, in humidified air with 5% CO₂ in monolayer culture in growth medium (MEM for SK-N-MC cells and

HAM-F12 for C6 cells) containing 10% fetal-calf serum. In SK-N-MC and C6 cells, the presence of GlyT1 was assessed as reported in Depoortere et al. (2005). Forty eight hours before [¹⁴C]glycine uptake experiments, cells were plated at a density of 20,000 to 30,000 per well in 96-well culture dishes previously coated with fibronectin for SK-N-MC cells or poly-D-lysine for C6 cells. For native mice GlyT1, brain cortices were dissected out and homogenized (10 ml/g) in HEPES-buffer (HB) containing (in mM): NaCl (147), KCl (5), MgCl₂ (2), CaCl₂ (2), HEPES (10), D-glucose (10) and L-alanine (5); pH 7.4. Cells or homogenates were incubated at 37 °C in 200 µl of HEPES-buffer with test compounds. Uptake was initiated by adding 10 µM [¹⁴C]glycine (112.4 mCi/mmol; NEN Life Science Products, Paris, France). Non-specific uptake was determined with 10 mM unlabeled glycine. After 10 min, incubations were stopped by filtration or washing with HB. Radioactivity was measured by liquid or solid scintillation in a Wallac MicroBeta counter.

2.4. Effects of SSR103800 and SSR103713 on *ex vivo* [¹⁴C]glycine uptake

Male OF1 mice (20–25 g) were sacrificed 60 min after *p.o.* administration. Cortical tissues were rapidly dissected and homogenized on ice using a polytron apparatus (1 g tissue for 10 volumes of cold HB). The assay performed on fresh homogenates was started by adding 10 µM [¹⁴C]glycine to 20 µl of tissue in HB (final protein concentration: 0.8–0.9 mg/ml). Non-specific uptake was determined with 10 mM unlabeled glycine. The uptake was performed for 10 min at 25 °C, and stopped by aspiration onto MultiScreen glass fiber filter plate using a MultiScreen vacuum manifold. The filter was washed twice with ice-cold HB, dried and soaked with Meltilex (Meltilex, Wallac, Perkin Elmer, Life Sciences, Courtaboeuf, France). Radioactivity was measured by solid scintillation in a Wallac MicroBeta counter.

In the results, the concentration or the dose of compound inhibiting 50% (IC₅₀ or ID₅₀, respectively) of specific [¹⁴C]glycine uptake are calculated from percentage of inhibition of [¹⁴C]glycine uptake of test group versus control group. IC₅₀ and ID₅₀ values are obtained using the 4-parameter logistic model according to Ratkowsky and Reedy (1986). The adjustment was obtained by non linear regression using the Levenberg Marquardt algorithm.

The IC₅₀ were calculated using SAS system SUN4 via Everstat 5.0 software by the arithmetic mean and its respective SEM values obtained from 2 to 6 independent dose–response studies. The ID₅₀ mean value was calculated by the arithmetic mean and its respective SEM value obtained from 3 independent dose–response studies.

A time-course study was performed at 5, 15 min and 1, 4, 7, 16 and 24 h after oral treatment with 10 mg/kg of SSR103800, using the protocol described above. Results are expressed as the percentage of [¹⁴C]glycine uptake versus the control (vehicle-treated) group. For the time-course experiment, data were analyzed with Kruskal–Wallis test, followed by multiple two-sided comparisons test versus time treatment of control group with the Bonferroni–Holm correction in case of significance of the global analysis.

2.5. Effect of SSR103800 on extracellular levels of glycine measured in the prefrontal cortex of freely moving rats

Male Sprague–Dawley rats (320–350 g) were housed two per cage. Two days before the dialysis assay, they were anesthetized with chloral hydrate (400 mg/kg, *i.p.*, 10 ml/kg of body weight) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Anesthesia was maintained throughout surgery as necessary with supplementary doses of chloral hydrate. Body temperature was monitored by a rectal probe and adjusted (37±1 °C) by a homeothermic blanket. The skull and the dura were opened to allow the implantation of a guide cannula in the medial prefrontal cortex (PFC). The coordinates were 2.5 mm anterior to bregma, 0.6 mm lateral to the midline and 1.3 mm below the dural surface (Paxinos and Watson,

1998). A dental cement cap held the cannula in place, and three screws anchored the cap to the skull. The rats were individually housed post-surgery and allowed two days of recovery before the start of the experiment. On the day of the experiment, animals were placed in a microdialysis bowl, the cannula cap was removed, and a 3 mm microdialysis probe (CMA12, Carnegie Medicine AB, Stockholm, Sweden) was inserted into the guide cannula. The probe was perfused at a constant flow rate of 1 μ l/min using a microinjection pump (CMA100; Carnegie Medicine AB) with a gassed Ringer's solution containing (in mM): NaCl (145), KCl (2.7), CaCl_2 (1.2), MgCl_2 (1), Na_2HPO_4 (2.3), NaH_2PO_4 (0.45); pH 7.4. Microdialysis sampling started 120 min after probe placement into the PFC. The outlet of the probe was connected to an on-line derivatization system allowing direct analysis of dialysate samples collected every 15 min. Glycine levels were measured in 15 μ l dialysate samples using capillary electrophoresis (CE) with laser-induced fluorescence detection. Before analysis, the samples were derivatized using naphthalene-2,3-dicarboxaldehyde and sodium cyanide, as previously described (Bert et al., 1996). CE experiments were performed on a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Villepinte, France) coupled to an external Zetlif fluorescence detector (Picometrics S.A., Toulouse, France). The excitation was performed by an Omnichrome (Melles Griot Laser Products) helium-cadmium laser at a wavelength of 442 nm with a 30 mW excitation power. The emission intensity was measured at a wavelength of 490 nm. Separations were carried out with a fused-silica capillary (Polymicro Technology, Phoenix, AZ, USA) of 50 μ m i.d. and 375 μ m o.d. having a total length of 55 cm and an effective length of 38.9 cm with an applied voltage of 25 kV (i.e., 65 μ A current). Borate buffer (75 mM) containing β -Cyclodextrin (1 mM), pH 10.5, was used for CE running. At the end of the experiments, an injection of sky blue solution was performed through the probe and animals were sacrificed with an overdose of pentobarbital. The brain was removed, frozen, and 50 μ m thick sections were cut with a cryostat to verify correct placement of the microdialysis probe. Glycine levels in fractional samples were converted to a percentage of the mean value of the 90 min baseline measurements before treatment. Time-course effect of SSR103800 on glycine levels was analyzed by two-way ANOVA with treatment as a between-subjects factor and time of sampling as a within-subjects factor, followed by Dunnett's post-hoc tests. Dose-effects of SSR103800 were evaluated by comparing the area under the curve during the first 180 min after p.o. injection of the drug or vehicle. Statistical analysis was carried out by a one-way ANOVA followed by Dunnett's post-hoc tests.

2.6. Effect of SSR103800 on evoked NMDA-mediated excitatory postsynaptic currents (eEPSC's) in rat hippocampal slices

Sprague-Dawley rats (17 to 22 days-old) were sacrificed and their brains removed and sectioned (coronal 0.3 mm thick slices) with a Campden 752M microslicer in an artificial cerebrospinal fluid (aCSF) solution containing (in mM): NaCl (126), KCl (3), MgCl_2 (1), KH_2PO_4 (1), CaCl_2 (1), NaHCO_3 (25) and glucose (11), pH 7.35, at approximately 0 °C and aerated with 95% O_2 , 5% CO_2 . After at least 1 h in aCSF at room temperature, slices were transferred to the recording chamber and superfused continuously with aCSF at 30–32 °C. Patch-clamp whole-cell recordings of layer CA1 pyramidal cells were obtained with borosilicate glass electrodes (resistance: 4–7 M Ω), filled with a 290 mosmol solution containing (in mM): K MeSO₄ (or MgSO_4) (130), EGTA (10), ATP (2), GTP (0.5) and QX314 (5); pH 7.2. Electrical recordings were made from CA1 pyramidal cell somata under visual control with a $\times 10$ water-immersion lens on an upright microscope (BX50WI; Olympus Optical, Tokyo, Japan). A concentric bipolar stimulation electrode (platinum/iridium, 12.5 μ m i.d., Frederick Haer and Co., ME, USA) connected to a constant current isolated stimulator (DS3, Digitimer Ltd, UK) was placed near the pyramidal layer of CA1 to generate eEPSC's. The bath solution was supplemented

with 20 μ M bicuculline methiodide, 1 μ M CGP-52432, 1 μ M strychnine and 1 μ M NBQX (antagonists for GABA_A, GABA_B, inhibitory strychnine-sensitive glycine and AMPA/kainate receptors, respectively) in order to isolate the NMDA receptor-mediated component of eEPSC's. The concentration of external magnesium was maintained at 1 mM, to minimize polysynaptic transmission due to high activity of NMDA transmission. In all experiments, 10 μ M of glycine was added to the aCSF medium, experimental conditions that are similar to those previously reported in Depoortere et al. (2005). This extracellular "basal" glycine concentration did not affect the amplitude of eEPSC's but was added to avoid glycine depletion of the permanently superfused slices. Parameters of stimulation (square pulses, duration: 10–100 μ s and amplitude: 2–10 mA) were adjusted to obtain half maximal amplitude of monophasic eEPSC's and elicited at a fixed frequency of 0.2 Hz. Data are expressed as the percentage of the eEPSC's amplitude obtained under control conditions. Statistical significance of drug-induced effects was assessed using a one-way analysis of variance with repeated measures on treatment (pre-application or drug concentrations) followed by Dunnett's test to compare NMDA-mediated EPSC maximum amplitude obtained under drug concentration to control period's one. In order to study the effects of SSR103713 at 1 μ M on EPSC amplitude, a paired Student *t* test is performed between the pre-application period and under drug application.

2.7. Effects of SSR103800 on locomotor hyperactivity induced by MK-801 in mice

Male Swiss mice (18–21 g) were individually isolated in boxes and pretreated p.o. with SSR103800 or vehicle, immediately followed by vehicle or MK-801 (0.2 mg/kg, i.p.). Thirty minutes after the second injection, they were placed in actimeter devices (20 cm diameter, 9.5 cm high, Apelex, France) equipped with 2 perpendicular light beams 1.5 cm above the floor. Locomotor activity (number of interrupted light beams) was recorded for a period of 30 min after placing the mouse into the actimeter. The statistical analyses were performed using SAS® V8.2 software (SAS Institute Inc., Cary, NC, USA). The variable analyzed (i.e. the number of beam breaks (variable NBB)) was not normally distributed according to Poisson's Law and required a square root transformation. For each model, descriptive statistics of NBB and square root of NBB (*n*, mean, Std, SEM) were calculated. First, for evaluating MK-801 effects and its reversal by SSR103800, a two-way analysis of variance was used with all groups of mice, which have received one dose of SSR103800 (20 or 30 mg/kg) or vehicle and either injection of MK-801 (0.2 mg/kg) or saline (six groups). A Winer test was used for evaluating the MK-801 effect at each dose of SSR103800 (20 and 30 mg/kg). Secondly, a Dunnett's test was used to evaluate the effect on its own of the highest doses of SSR103800 (20, 30 mg/kg). Thirdly, one-way analysis of variance for evaluating SSR103800 dose effect was used with all groups of mice which have received MK-801 (0.2 mg/kg). Dunnett's test was performed for comparing vehicle versus each dose of SSR103800 (Vehicle, 3, 10, 15, 20, 30 mg/kg).

2.8. Effects of SSR103800 on locomotor hyperactivity induced by acute PCP injection in rats

Male juveniles OFA rats (220–270 g) were pretreated p.o. with either SSR103800 (1, 3, 10 mg/kg, p.o.) or vehicle. Sixty minutes later, they were intraperitoneally administered with either vehicle or PCP (4 mg/kg) and immediately placed in actimeters (dimensions: 40 cm \times 40 cm square and 23 cm high, in house apparatus developed by the Electronic department of Sanofi-Aventis (Bagneux, France) equipped with 12 perpendicular blind infra-light beams (one beam every 3 cm (half-step precision = 1.5 cm), 2.3 cm above the floor). Over a period of 30 min, acquisition and analysis software recorded the

number of beam breaks. The statistical analyses were performed using SAS® V8.2 software (SAS Institute Inc., Cary, NC, USA). We used a non-parametrical test (Kruskal–Wallis analysis), since values (number of beam breaks) were not distributed normally. Data were analyzed using the non-parametric Kruskal–Wallis tests and multiple comparisons versus the vehicle/saline or the vehicle/PCP4 groups.

2.9. Effect of SSR103800 on the deficit in the object recognition task in rats sensitized to PCP (short-term episodic-like memory procedure)

The object recognition task, which has been claimed to model certain aspects of episodic-like memory was similar to that described by Ennaceur and Delacour (1988) in young rats. The apparatus consisted in a uniformly lit (100 lux) wooden enclosure (65 L×45 l×45 H 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. Experiment consisted of three sessions: during the first session (context habituation), the subjects ($n=8-10$ animals per group) were allowed 2 min to become acquainted to the apparatus. Time spent in locomotor activity was manually recorded. The animals were placed again in the enclosure 24 h thereafter for the second session (acquisition), during which they were exposed to a pair of identical objects (A or B) (either 7 L×3 l×8 H cm metal triangles or 9 L×3 l×7 H 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, within a 5 min-limit. 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 5 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. During the third session (recall), the objects A and B were placed in the open-field and rats were exposed to the familiar (i.e. presented during the acquisition session) and to the novel (i.e. 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. Combinations of the nature and location of the familiar object were balanced to reduce potential biases due to spatial or objects preferences. This third session took place 90 min after the second session. At this inter-session interval, there is high discrimination between the two objects that is the rat spends more time exploring the novel than the familiar object, suggestive of an intact short term episodic-like memory. This interval was used to evaluate a possible impairment of performance following acute PCP injection in naïve or in PCP-chronically treated rats and its possible reversal by acute administration of SSR103800.

For the PCP-chronic treatment, rats were administered daily with PCP (10 mg/kg, i.p.) or its vehicle (distilled water + Tween 80) for five consecutive days. Rats ($n=8-10$ animals per group) were then left unused for 6 days before starting the experiment. On the 11th day, rats were first exposed to the context habituation session and 24 h later to the acquisition session. They were injected with a challenge dose of PCP (1 mg/kg, i.p.) or vehicle (distilled water + Tween 80) and with vehicle or SSR103800 (3 mg/kg, p.o.) immediately after the end of the acquisition session. They were thereafter subjected to the recall session 90 min later.

Data (time spent 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 versus the novel object for each treatment. A recognition index was also defined as the ratio $[N/(N+F)]$, which corresponds to the novelty preference for the

different treated animals. Data for this index were analyzed with a one-way ANOVA with the treatment as the between factor followed by Dunnett's test for each treatment. These analyses and all subsequent ones were performed using the SAS software (SAS Institute Inc., Cary, NC, USA).

2.10. Effect of SSR103800 on the impairment of novelty discrimination in adult rats treated with phencyclidine at the neonatal stage

Female Wistar Han rats with 10 male pups on postnatal day 3 (PN 3) were obtained. 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 vehicle. 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 were housed 5 per cage until two weeks before the beginning of behavioral experiments, when they were housed individually. Behavioral experiments were performed once they reached the adult stage (between PN 60 and PN 108). Juvenile male Wistar rats (three-week old, 45–50 g on arrival) were housed ten per cage until the 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 7.00 pm to 7.00 am). 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. SSR103800 or vehicle was administered p.o. to the adult rat 60 min before exposure to the first juvenile. Each adult rat was submitted to 4 treatments: vehicle and 3 different doses of SSR103800. Data are expressed as the mean of a novelty discrimination index (NDI) that 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 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 for repeated measures, 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 Dunnett's tests.

2.11. Effect of SSR103800 on the endogenous deficit of prepulse inhibition of the startle reflex in DBA/1J mice

Male DBA/1J mice (20–22 g) were obtained from Jackson Laboratories (Bar Harbor, ME). They were housed four mice per cage and maintained on a 12:12 light:dark cycle with lights on at 6.00 am. Mice were tested in eight startle boxes (SR-LAB, San Diego Instruments, San Diego, CA). The startle reflex was detected via a piezoelectric transducer situated below the startle platform and recorded via a computer (SR-LAB software). Mice were injected i.p. with vehicle, olanzapine (3 mg/kg, solubilized with one drop of glacial acetic acid, pH 5.0) or SSR103800 (3, 10, 30 mg/kg) 30 min prior to test trial initiation. They were placed into a restraint cylinder (3.7×12.8 cm) fixed on top of the startle platform. The test session began with a background white noise of 70 dB with a 10 min duration. Next, five consecutive trials of a 40-ms 120 dB startle pulse were administered, but excluded from the data analysis. Fifty-six test trials of eight differing types were administered. Each prepulse followed by

a pulse trial began with a 50 ms null period, followed by a 20 ms prepulse white noise stimulus of 74, 78 or 86 dB, with a 100 ms delay preceding the startle. During the null period, there was no presentation of acoustic stimulus with the exception of the background noise. Ten trials of each of the three prepulse intensities plus startle occurred, as well as ten trials of a startle pulse alone. Four trials each of the three prepulses alone were given, as well as four trials without stimulation to record baseline. Sixty-five milliseconds after the onset of startle or prepulse (in prepulse alone trials), behavioral responses were measured every ms. The variable inter-trial interval was averaged at 15 s. An additional 5 startle pulses ended the test session, but were not included in the analysis.

Prepulse inhibition was calculated for each mouse from averaged startle amplitudes for each of the three prepulse intensities across all test trials. Prepulse inhibition (expressed as percentage) was calculated as follows:

$$\frac{(\text{amplitude}(\text{pulse}) - \text{amplitude}(\text{prepulse}/\text{pulse})) \times 100}{\text{amplitude}(\text{pulse})}$$

Prepulse inhibition percentages were analyzed by two-way ANOVA for repeated measures (between subjects factor: drug dose; within subjects factor: intensity of prepulse stimuli), followed by Dunnett's post hoc analysis. In addition, basal startle amplitudes were analyzed across treatment groups using a Kruskal–Wallis multiple comparison test.

2.12. Effect of SSR103800 in the forced-swimming test in rats

The procedure was a modification of the technique described by Porsolt et al. (1978). Wistar rats (260–300 g) were placed in individual glass cylinders (40 cm in height and 17 cm in diameter) containing water (water depth was 30 cm; 23 ± 1 °C). Two swimming sessions were conducted (an initial 15-min pretest followed 24 h later by a 6-min test). The total duration of immobility was scored continuously for a 5-min period manually by an experimenter unaware of the drug treatment. Vehicle or fluoxetine (10 mg/kg) or a dose of SSR103800 (0.3, 1, 3 mg/kg) were administered p.o. twice (15 min after the first session on day 1, and 60 min before session 2 on day 2). This administration schedule is optimal for revealing drug effects. Data were analyzed by one-way ANOVA followed by a Dunnett's test.

2.13. Effects of SSR103800 on tonic immobility in gerbils

The test is based on that described by Simiand et al. (2003) and has been claimed to be sensitive to antidepressant drugs (Salomé et al., 2006). To induce tonic immobility, animals (6–9 per group) were held on a flat surface and were firmly pinched for 15 s at the scruff of the neck using the thumb and the index finger. They were then placed on parallel bars (4 mm in diameter, 28 cm long, spaced 5 cm apart and having a 3 cm difference in height). The front paws were placed gently on the upper bar (situated at 43 cm above the base) and the hind paws on the lower bar. The duration of tonic immobility was measured in five successive trials with a 30-second intertrial interval. Each trial ended when an animal started to move or after 90 s of immobility. Data were analyzed using the non-parametric Kruskal–Wallis multiple comparison test versus vehicle group. Experiments were performed 60 min after p.o. administration of SSR103800 (1, 3, 10, 30 mg/kg) or vehicle.

3. Results

3.1. SSR103800 blocks the *in vitro* [¹⁴C]glycine uptake

SSR103800 inhibited [¹⁴C]glycine uptake in human SK-N-MC cells, rat C6 cells and in mice brain homogenates with IC₅₀ values of 2.0 ± 0.4 nM,

5.3 ± 2.2 nM and 6.8 ± 1.1 nM respectively (Table 1). The (R,R) inactive enantiomer SSR103713 was approximately 25 fold less potent in blocking the uptake of [¹⁴C]glycine in human and in rat GlyT1. SSR103800 had no effect on other classical transporter systems including murine proline, glutamate and GABA transporters, and on human GlyT2 transporter. SSR103800 was inactive (inhibition lower than 50% at 10 μM) against 100 targets, including glycine, glutamate, dopamine, serotonin, adrenaline, noradrenaline, histamine and muscarinic receptors, enzymes such as MAO and uptake systems such as dopamine, noradrenaline transporters (assays performed by Cerep, Celle l'Evescault, France, data available upon request).

3.2. SSR103800 blocks *ex vivo* [¹⁴C]glycine uptake in mouse cortical homogenate

Oral administration of SSR103800 produced an inhibition of *ex vivo* specific [¹⁴C]glycine uptake in the mouse cerebral cortex. ID₅₀ value was 3.0 mg/kg p.o. for SSR103800. The (R,R) enantiomer SSR103713 was not active up to 10 mg/kg, p.o. A time-course study, performed at 10 mg/kg p.o., showed that SSR103800 induced a rapid (within 15 min) and significant decrease of specific [¹⁴C]glycine uptake, that was maintained between 60–80% of inhibition from 1 to 4 h after administration (Fig. 1b). The inhibition of specific [¹⁴C]glycine uptake was reversible, and values recorded at 16 and 24 h returned to the control levels.

3.3. SSR103800 increases extracellular levels of glycine in the prefrontal cortex of freely moving rats

The mean basal extracellular glycine level in the PFC was estimated to be 3.3 ± 0.6 μM ($n=20$). Administration of SSR103800 (3, 10, 30 mg/kg, p.o.) produced a rapid and sustained increase in PFC extracellular levels of glycine in freely moving rats [$F(3,16)=18.58$, $p<0.0001$ and $F(33,176)=9.26$, $p<0.0001$ for the treatment and treatment × time interaction factor, respectively; Fig. 2 on the left]. The maximal increase was observed for the dose of 30 mg/kg p.o. (at 105 min, $+161.2 \pm 26.2\%$ of control, $n=5$) and lasted 3 h (at 180 min, $+133.2 \pm 19.2\%$). Areas under the curve during the 180 min after drug injection confirm the effect of SSR103800 [$F(3, 16)=13.06$, $p<0.0001$; Fig. 2 on the right]. Additionally, SSR103800, at the same dose-range, did not increase levels of other amino acids such as aspartate, glutamate, taurine, alanine and D-serine (data not shown).

3.4. SSR103800 augments evoked NMDA-mediated excitatory postsynaptic currents (eEPSCs) in rat hippocampal slices

The experimental conditions that were chosen allowed to only record the NMDA receptor component of pyramidal neuron eEPSCs (see Materials and methods) after electrical stimulation within the CA1 area. This was confirmed by the strong voltage-dependence (due to the magnesium block) of the eEPSCs and the full block obtained by bath application of 100 μM 5,7-DCK (or 50 μM L-AP5, data not shown), two antagonists acting at the glycine recognition site and glutamate binding site of the NMDA receptor, respectively. SSR103800 increased the amplitude of eEPSCs (Fig. 3), with a maximal enhancement of $328 \pm 59\%$

Table 1

In vitro affinity of SSR103800 and SSR103713 for native human (h) rat (r) GlyT1 and mice (m) GlyT1

Transporter IC ₅₀ (nM)	SSR103800	SSR103713
hGlyT1 (SK-N-MC cells)	2.0 ± 0.4	51 ± 23
rGlyT1 (C6 cells)	5.3 ± 2.2	123 ± 47
mGlyT1	6.8 ± 1.1	84 ± 7

IC₅₀ values are the mean ± SEM for 2–6 independent determinations.

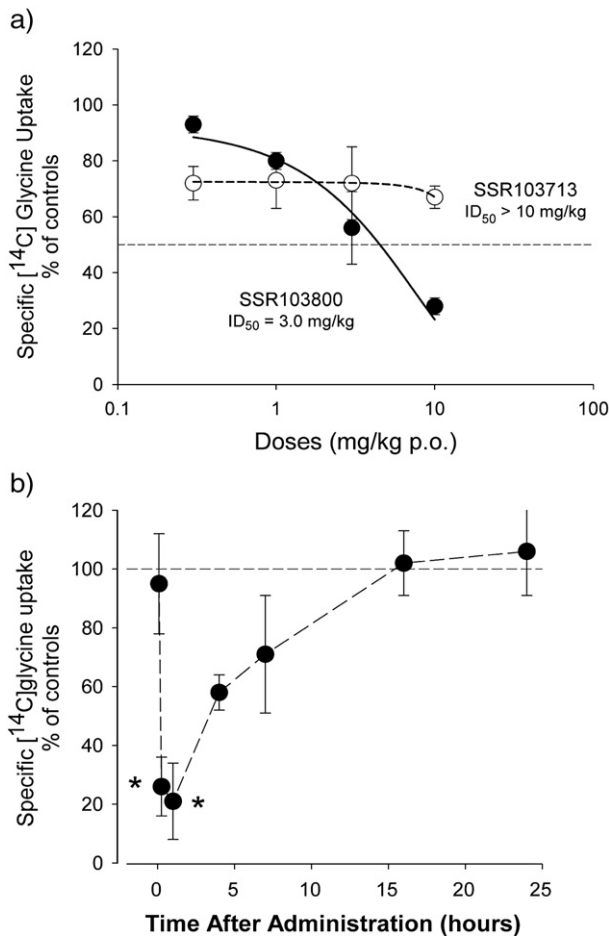


Fig. 1. a) Blockade by SSR103800 of the *ex vivo* uptake of [¹⁴C]glycine in mouse cortical homogenate. Each point represents the mean±SEM of percentages of specific [¹⁴C]glycine uptake expressed as a percentage of the controls (vehicle-treated) group values. Injection times were 60 min (p.o.) before sacrifice. The SSR103713 (open circle symbols) is the (R,R) inactive enantiomer of SSR103800 (black circle symbols). ID₅₀ expresses the dose of compound inhibiting 50% (ID₅₀) of specific [¹⁴C]glycine uptake. b) SSR103800 (10 mg/kg, p.o.) produced a rapid, long lasting but reversible blockade of *ex vivo* uptake of [¹⁴C]glycine in mouse cortical homogenate. Each point represents the mean±standard deviation of percentages of specific [¹⁴C]glycine uptake expressed as a percentage of the control group values (3 to 6 mice by experimental group). ** *p*<0.01, Kruskal–Wallis Post hoc test versus time treatment of control group.

(*n*=7) at 1 μM as compared to the control amplitude. Statistical analysis showed a significant increase in maximum peak amplitude for 0.5 μM and 1 μM of SSR103800 (*p*=0.0007 and *p*<0.0001 Dunnett's test for 0.5 μM and 1 μM respectively). In contrast, the less active enantiomer SSR103713 (Fig. 3) had no significant effect at 1 μM (91±8% of the initial amplitude *n*=4), a concentration at which SSR103800 produced a more than two-fold increase of eEPSCs. The increase in eEPSCs amplitude observed with SSR103800 was reversible within 8 min upon removal of the compound.

3.5. SSR103800 reverses the locomotor hyperactivity induced by MK-801 in mice

Statistical analyses of the number of interrupted light beams (motor activity) using a global two-way Anova with SSR103800/saline and their respective SSR103800/MK-801 groups, revealed significant effects of the MK-801 and SSR103800 treatments [respectively *F*(1,74)=31.7, *p*<0.01) and (*F*(2,74)=71.86, *p*<0.01); but also significant interaction [*F*(2,74)=33.79, *p*<0.01], which demonstrate that SSR103800 modulates MK-801-induced motor response.

Indeed, MK-801 (0.2 mg/kg i.p.) significantly increased (approximately three-fold) the number of interrupted light beams, illustrated by the two foremost left symbols of Fig. 4 (from 484±52 to 1577±101) [*F*(1,74)=105.32, *p*<0.01 symbols **, with respect to vehicle group].

A significant hypo-locomotor effect on its own has been observed only at the highest dose of SSR103800 (30 mg/kg) [*F*(2,37)=4.54; *p*<0.01, symbols **, with respect to vehicle group in Fig. 4].

For evaluating the minimal effective dose of SSR103800 reducing MK801-induced motor hyperactivity, an additional one-way analysis of variance was used across all groups of mice which received MK-801 (0.2 mg/kg, i.p.). A significant SSR103800 effect was described [*F*(5,74)=39.64; *p*<0.01] which suggests that SSR103800 antagonized MK-801-induced hyperactivity. A Dunnett's test compared to the Vehicle/MK801 group confirmed that the minimal effective dose of SSR103800 to decrease the MK-801-induced hypermotility is 15 mg/kg, p.o. (MED) [*p*<0.05, symbols * on Fig. 4]. The dose of 20 mg/kg, p.o. also decreased significantly MK-801-induced hypermotility [*p*<0.01, symbols ** on Fig. 4]. The dose of 30 mg/kg completely reversed MK-801-induced hyperactivity [*p*<0.01, symbols ** on Fig. 4].

3.6. SSR103800 decreases the locomotor hyperactivity in PCP treated rats

PCP at the dose of 4 mg/kg, i.p. markedly increased locomotor activity in rats (+200% as compared to Vehicle/Saline group) as reflected by the significant increase of the number of beam breaks [DF=5, Chi2=33.26, statistical value for comparison between vehicle/PCP4 and vehicle/saline groups=3.23, *p*<0.01] (Fig. 5). SSR103800 decreased the spontaneous motor activity on its own at the dose of 30 mg/kg, but this effect just failed to reach statistical significance [Statistical value=1.82, *p*=0.07]. SSR103800 significantly decreased locomotor hyperactivity in PCP treated rats [DF=3, Chi2=14.64] at the three tested doses: 10, 20 and 30 mg/kg, p.o. [statistical values for each comparison: 2.05, *p*<0.05; 1.7, <0.05; 3.81, *p*<0.01, respectively].

3.7. SSR103800 prevents the impairment of short term episodic-like memory induced by an acute low dose of PCP in the object recognition task, in rats sensitized to PCP

Neither the acute low challenge dose of PCP (1 mg/kg, i.p.) alone nor the chronic treatment with the high dose of PCP (10 mg/kg, i.p.) alone, affected the short-term recall performance in the object recognition task, as shown by an identical discrimination ratio of these both groups compared to vehicle/vehicle control animals. In contrast, rats with chronic PCP and treated with the acute challenge dose were unable to show a significant discrimination between the novel and familiar object as shown by a significantly lower discrimination index and a lack of significant difference in exploration time of the novel and familiar object, compared to the three other groups. Statistical analysis supported this effect: Winer analysis comparing the time spent investigating the novel versus 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 results (Table 2) suggest that a PCP-sensitization has developed in rats several days after the ending of a sub-chronic treatment with a high dose of PCP and impair their short-term episodic-like memory abilities in the object recognition task.

In a separate experiment, administration of SSR103800 (3 mg/kg, p.o.) abolished the deleterious effect of the acute challenge with PCP (1 mg/kg, i.p.) in rats sub-chronically treated with PCP (Fig. 6). This was confirmed by a Winer analysis comparing the time spent investigating the novel versus the familiar object, at each treatment following two-way ANOVA with significant object, treatment and treatment×the object interaction effects: [*F*(1,23)=15.0 *p*<0.001, *F*(2,23)=3.67, *p*<0.05, and *F*(2,23)=18.4, *p*<0.0001, respectively] and by the Dunnett's analysis comparing the novelty index (inserted Fig. 6) between

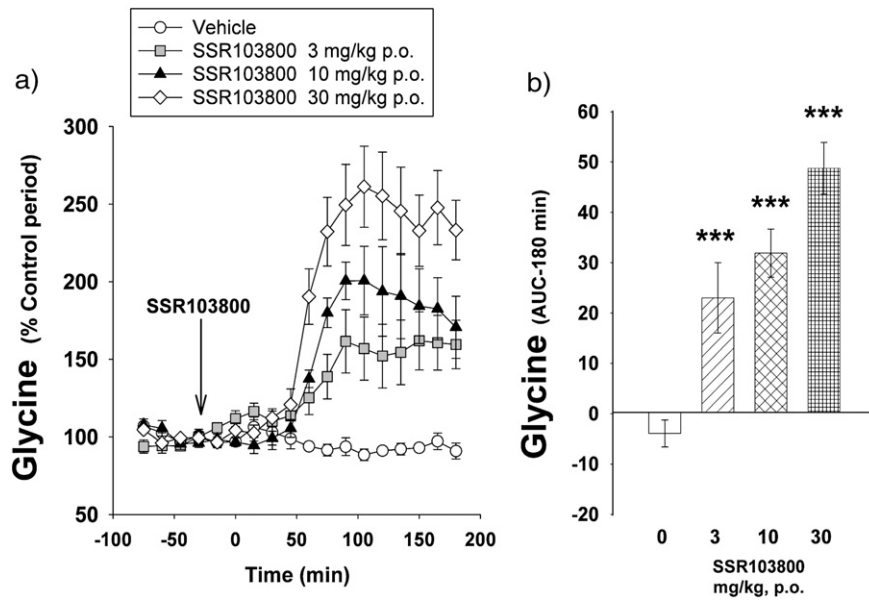


Fig. 2. Increase by SSR10380 of extracellular levels of glycine in the prefrontal cortex of freely moving rats. a) Time-course of the effect of SSR10380. Changes in glycine levels are expressed as a percentage of the mean value of the six basal samples collected before the start of the treatment (indicated by the vertical arrow). Each symbol represents the mean±SEM. n=8–9 rats per group. b) Dose–effect relationship of SSR10380. Each bar represents the mean±SEM of the area under the curve (AUC) for the 180 min period following injection of SSR10380 or vehicle. *** p<0.001 compared with the vehicle group (Dunnett’s post-hoc tests following a one-way ANOVA). n=8–9 rats per group.

different treated groups (ANOVA: $F(2,23)=17.1$ $p<0.0001$). 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, were significantly modified by the treatment with SSR10380 (data not shown). In other words, SSR10380 completely blocked the memory deficit induced by a PCP-sensitization in rats and did not result from non-specific biases such as sedation and/or motor effects.

3.8. SSR10380 antagonizes the impairment of novelty discrimination in adult rats treated with phencyclidine at the neonatal stage

Under control conditions (i.e. acute administration of vehicle), adult rats pretreated with saline at the neonatal stage (farthest left white bar, Fig. 7) spent approximately three and a half more time investigating the novel rather than the familiar juvenile ($NDI=3.53 \pm 0.44$). In contrast, adult rats neonatally pretreated with PCP presented

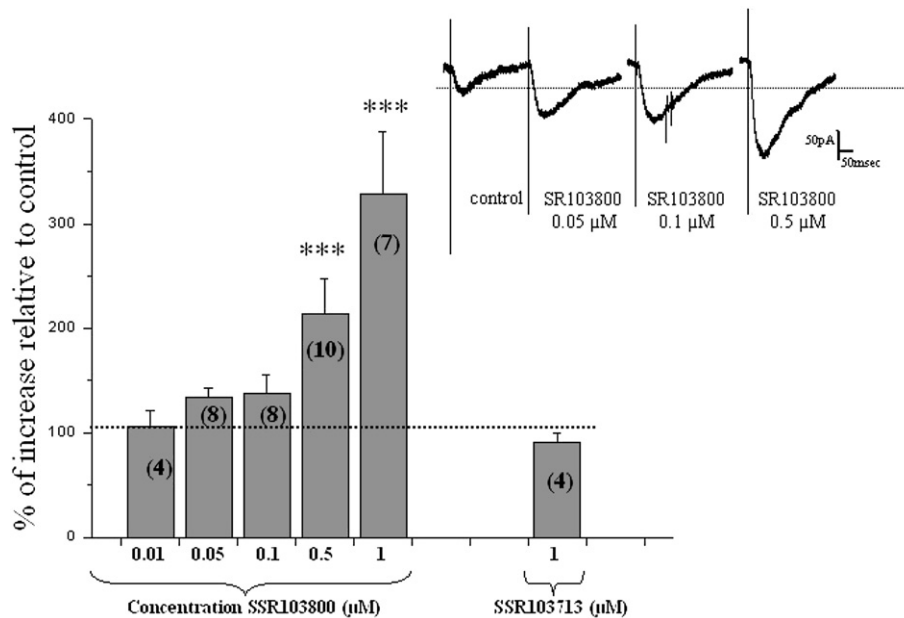


Fig. 3. Potentiation by SSR10380 of evoked NMDA-mediated excitatory post-synaptic currents (eEPSC’s) in rat hippocampal slices. Each symbol represents the mean±SEM ratio change of maximal amplitudes of eEPSC’s obtained against a background concentration of glycine (10 mM). Experiments were performed at $V_h=-20$ mV, to maximize NMDAR-mediated currents. *** p<0.001 with respect to control condition (10 μM glycine alone), Dunnett’s tests. Numbers of cells per group are given in brackets. Inset: single traces of eEPSC’s showing potentiating effects of SSR10380. The SSR103713 is the (R,R) inactive enantiomer of SSR10380.

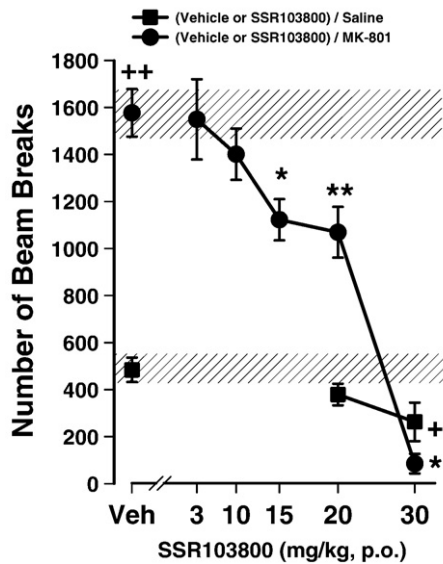


Fig. 4. Antagonism by SSR103800 of MK-801-induced locomotor hyperactivity in mice. Each symbol represents the mean (with SEM) number of light beam interruptions recorded for 30 min, 30 min following an i.p. injection of MK-801 (0.2 mg/kg, i.p.) or saline. $^{**}p < 0.01$ compared to the vehicle (Veh)/saline group (square symbols). $^*p < 0.05$; $^{**}p < 0.01$, compared to the vehicle (Veh)/MK-801 group (circle symbols). $n = 8$ –20 mice per group.

a NDI (1.42 ± 0.17) roughly half that of neonatal saline-treated rats (compare the farthest left pair of bars, Fig. 7). This indicates that neonatal PCP-treated rats spent less time exploring the novel juvenile, which can be interpreted as an impairment of social recognition. Treatment with SSR103800 normalized this deficit ($p < 0.05$). The significant difference of novelty discrimination observed between the two groups (saline-neonates and PCP-neonates) under acute treatment of vehicle was abolished when rats were treated with the three doses of SSR103800 (0.1 to 10 mg/kg) (the three foremost right pairs of

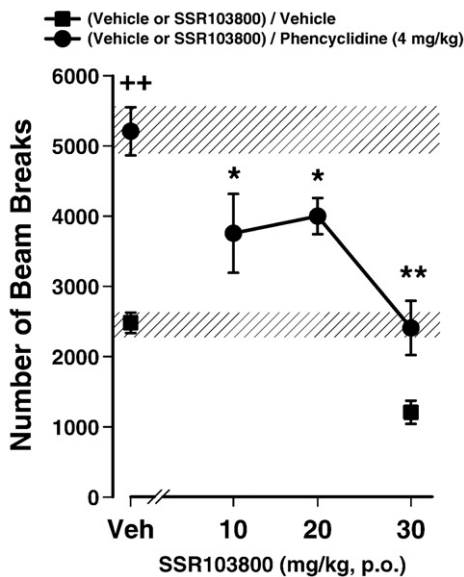


Fig. 5. Antagonism by SSR103800 of the PCP-induced locomotor hyper-activity in rats. Each symbol/bar represents the mean (with SEM) number of light beam interruptions recorded for 30 min, 30 min following an i.p. injection of either vehicle or PCP (4 mg/kg, i.p.). Non parametric Kruskal–Wallis tests, $^{**}p < 0.01$ (versus the vehicle/saline group) and $^*p < 0.05$; $^{**}p < 0.01$ (versus the vehicle/PCP4 group). $n = 8$ rats per group.

Table 2

PCP-induced deficit of episodic-like memory in an object recognition task in rats sensitized to PCP

Time spent in exploration (s)	Chronic vehicle	Chronic vehicle	Chronic PCP	Chronic PCP
	Acute vehicle	Acute PCP	Acute vehicle	Acute PCP
Familiar object (F)	7.4 ± 0.8	8.2 ± 1.0	8.2 ± 0.8	14.6 ± 1.7
Novel object (N)	$13.5 \pm 1.3^{**}$	$16.6 \pm 1.2^{**}$	$17.5 \pm 1.3^{**}$	11.9 ± 1.8
Ratio N/F	2.05 ± 0.27	2.53 ± 0.51	2.26 ± 0.22	$0.86 \pm 0.12^{**}$

Each value represents the mean (+SEM) of the time spent in exploration for the familiar and the novel object, and the exploration ratio for the novel and the familiar object. $^{**}p < 0.01$ for significant difference between time spent in investigation for the novel vs familiar objects and for significant difference on Ratio N/F for novelty index in groups compared to the chronic vehicle / acute vehicle group.

bars, Fig. 7). This was supported by post-hoc statistical analysis following a two-way ANOVA with a significant neonatal pre-treatment effect and acute treatment effect [$(1,8) = 9.379$, $p < 0.05$ and $F(3,24) = 4.145$, $p < 0.05$, respectively]. Note that in the neonatal saline-treated group, SSR103800 had no effect by itself (compare the five white bars in Fig. 7). SSR103800 dose-dependently normalized at the adult stage, social recognition deficits induced by a neonate injection of PCP in rats.

3.9. SSR103800 increases the prepulse inhibition of the startle reflex in DBA/1J mice

DBA/1J mice were demonstrated in our laboratory to have PPI baseline level, in a range between [$3.41 \pm 4.31\%$ and $48.59 \pm 4.89\%$] depending of the applied acoustic prepulse intensity, in the vehicle condition (see Fig. 8).

A two-way repeated measures ANOVA revealed a significant effect of treatment on endogenous PPI in DBA/1J mice [$F(4,45) = 7.16$, $p < 0.001$]. SSR103800 at 30 mg/kg, i.p. significantly enhanced PPI at the 78 dB ($p < 0.05$, Dunnett's *t* test) and 86 dB ($p < 0.001$) intensity

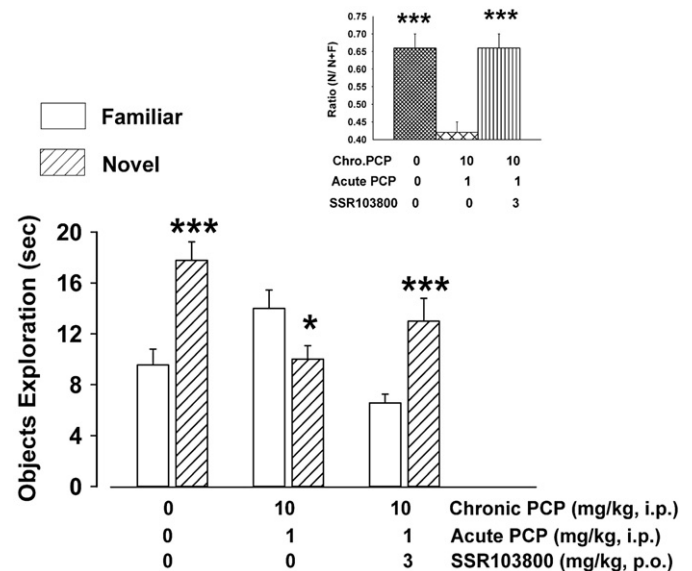


Fig. 6. Reversal by SSR103800 of a PCP-induced deficit of episodic-like memory in an object recognition task in rats sensitized to PCP. Each bar represents the mean (+SEM) time spent exploring a novel or a familiar object. The interval between the acquisition and the recall session was 1.5 h. Post-hoc analyses following a two-way ANOVA: $^{***}p < 0.001$, novel versus familiar object at the considered treatment condition. $n = 8$ –10 rats per group. In insert, each bar represents the mean (+SEM) novelty index calculated from time spent exploring a novel or a familiar object in PCP-sensitized control animals and SSR103800 treated rats. Post-hoc analyses following a one-way ANOVA: $^{***}p < 0.001$ versus PCP-sensitized control group.

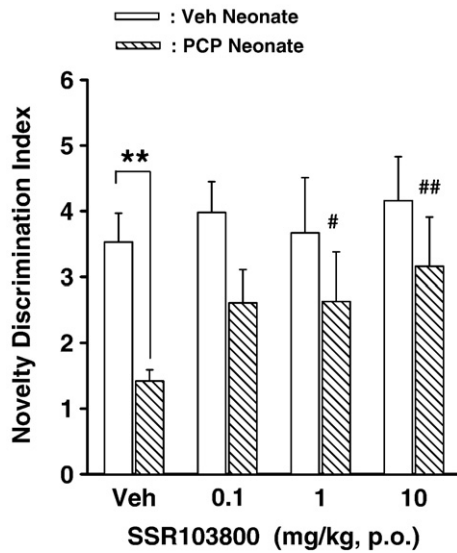


Fig. 7. Reversal by SSR103800 of the social recognition deficit in adult rats which have been treated with PCP at the neonatal stage. Each bar represents the mean (+SEM) novelty discrimination index (ratio of the time spent investigating the novel juvenile divided by the time spent investigating the familiar juvenile, in sec). Post-hoc analyses following a two-way ANOVA: * $p < 0.05$, neonatal PCP-treated compared to neonatal vehicle-treated rats, at each acute treatment condition. ## $p < 0.01$, compared to vehicle (Veh)-treated rats for the cohort of neonatal PCP-treated rats. $n = 5$ rats per group.

levels. This potentiated effect of PPI was similar to that demonstrated by the antipsychotic olanzapine at 3 mg/kg [$p < 0.001$ for 78 dB and $p < 0.01$ for 86 dB]. A Kruskal–Wallis multiple comparison test [DF=4, $\chi^2 = 20.80$] revealed that the basal startle amplitude (in the absence of any prepulse) was not significantly altered by SSR103800 at any dose. In contrast, olanzapine elicited a suppression of the startle

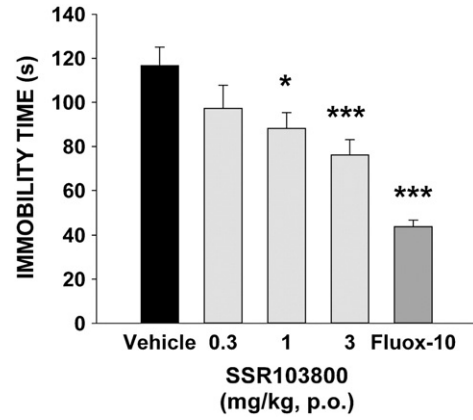


Fig. 9. Effect of SSR103800 in the forced-swimming test in rats. Effects of SSR103800 and fluoxetine in the forced swim test in Wistar rats. Data represent mean (+SEM), * $p < 0.05$, *** $p < 0.001$ (Dunnett’s test versus control group, $n = 10$ –12 rats per group).

amplitude compared to vehicle-treated mice (see inserted figure) (statistical value 3.41, $p < 0.01$).

3.10. SSR103800 decreases immobility time in the forced-swimming test in rats

The duration of immobility measured in the forced swimming test in rats was significantly reduced by SSR103800 [$F(4,50) = 13.6$, $p < 0.001$, Fig. 9] at 1 mg/kg, p.o. ($p < 0.05$) and 3 mg/kg, p.o. ($p < 0.001$). A decrease of immobility is also observed with fluoxetine at 10 mg/kg, p.o. ($p < 0.001$).

3.11. SSR103800 blocks tonic immobility in gerbils

The duration of tonic immobility measured after a neck/back pinch in gerbils was significantly decreased by SSR103800 [Kruskal–Wallis

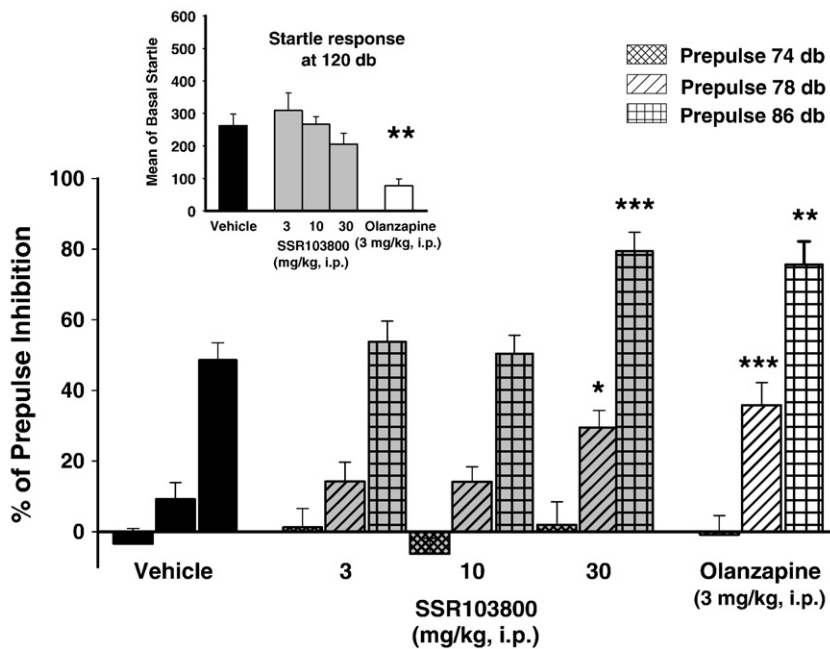


Fig. 8. Reversal by SSR103800 of the endogenous deficit in prepulse inhibition of the startle reflex and effects on basal startle amplitude in DBA/1J mice. Each bar represents the mean (+SEM) of the prepulse inhibition of the startle reflex induced by a pulse of 120 db (expressed in percentage of the basal startle reflex in lack of prepulse stimulus), as a function of the three prepulse (PP) intensities (in dB). Post-hoc Dunnett’s t test analyses following a two-way ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus vehicle at the corresponding prepulse intensity. $n = 10$ mice per group. In the inserted figure: Each bar represents the mean (+SEM) of the startle reflex measured in arbitrary units and induced by a pulse of 120 dB (measured in arbitrary units) in the absence of prepulse signal. Kruskal–Wallis multiple comparison test, ** $p < 0.01$ versus vehicle. $n = 10$ mice per group.

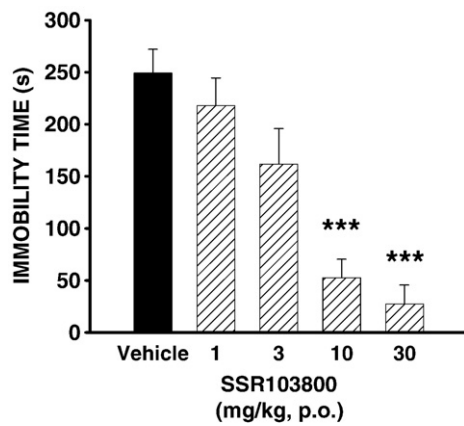


Fig. 10. Blockade by SSR103800 of Tonic immobility in gerbils. Effects of SSR103800 on tonic immobility in Mongolian gerbils. Data represent mean (+SEM), *** $p < 0.001$ (Kruskal–Wallis multiple comparison test versus control group, $n = 7–9$ gerbils per group).

DF=4, $\chi^2 = 26.3$, $p < 0.001$, Fig. 10] at 10 and 30 mg/kg, p.o ($p < 0.001$ for each dose versus controls).

4. Discussion

This paper describes the neurochemical, electrophysiological and psychopharmacological profiles of a novel selective and reversible GlyT1 inhibitor, SSR103800.

SSR103800 blocked the uptake of [14 C]glycine in the native human SK-N-MC cell line expressing the GlyT1a isoform, in the rat C6 cell line that expresses the glycine transporter GlyT1, and in murine cortical tissues expressing native GlyT1. This effect was stereo-selective since the (R,R) enantiomer, SSR103713, was much less potent. The IC_{50} values of glycine uptake blockade by SSR103800 (human=2.0 nM, rat=5.3 nM, and mouse=6.8 nM) indicate a potency better than that reported for ALX5407 (220 nM for hGlyT1, (Herdon et al., 2001); 26 nM for hGlyT1, (Smith et al., 2004); 10 nM for rGlyT1, (Kinney et al., 2003) and for ORG 24598 (120 nM for hGlyT1, (Brown et al., 2001), and significantly more potent than sarcosine or glycyldodecylamide, two earlier GlyT1 inhibitors (IC_{50} 's greater than 10 μ M, (Javitt and Frusciante 1997). SSR103800 displayed a low *in vitro* activity for human GLYT-2 (694 nM) and mice proline (460 nM) transporters, and had no activity for the glutamate, GABA or the dopamine transporter. Unlike current antipsychotics, it had no affinity for dopamine receptors, and for other receptors that have been associated with various side-effects, such as weight gain (5-HT $_2c$, histaminergic H $_1$), hypotension (alpha adrenergic), dry mouth/constipation/cognitive defects (muscarinic), sedation (histaminergic), for example.

Ex-vivo, SSR103800 blocked in a reversible manner the uptake of glycine in mouse brain. Reversibility of such an effect at GlyT1 is of importance as non-reversible blockade might contribute to an abnormal sustained activation of NMDA transmission with a higher risk of seizure. In addition, SSR103800 increased basal extra-cellular levels of glycine measured by microdialysis in the prefrontal cortex of rats as reported by several authors with other glycine transporter inhibitors (Depoortere et al., 2005). Such an increase of the prefrontal glycinergic tone is anticipated to result in a potentiating of the NMDA/ glutamatergic transmission (see below) and may ultimately correct a deficiency in this neurotransmission. The increase in central levels of glycine induced by SSR103800 had a functional impact on central glutamatergic neurotransmission in the hippocampal area as shown *in vitro* by the finding that SSR103800 potentiated NMDA-mediated eEPSC's in rat hippocampal slices. This later effect may be of particular interest in light of the reported hippocampal dysfunction in schizophrenia, which may play a role in the cognitive impairment observed

in schizophrenic patients (Sweatt 2004). It can be anticipated that SSR103800 may be useful to treat the cognitive symptoms of this condition.

To support this idea are the findings from the object recognition task in which SSR103800 was found to block PCP-induced episodic-like memory deficit in rats sensitized to PCP. This experimental procedure is believed to relate to certain aspects of the cognitive deficits in schizophrenia such as impairment in visual episodic-like memory (see for eg, Danion et al., 2007). Moreover, the pharmacological sensitization phenomenon consecutive to the chronic administration of PCP has been shown to induce schizophrenic-like symptoms in humans and aberrant behaviours in animals with a relative isomorphism with schizophrenia (Jentsch and Roth 1999). The observation that schizophrenic patients exhibit more pronounced cognitive and negative symptoms than normal volunteers following an acute challenge with ketamine (Lahti et al., 2001), indicates that the former are sensitised to the effects of this PCP-like compound.

The effects of SSR103800 on cognitive processes were assessed further in a model involving repeated neonatal injections of PCP in rats, which were subsequently tested in adulthood in a task based on social recognition. The neonatal PCP model is based on the neurodevelopmental hypothesis of schizophrenia (Weinberger, 1986; Deutsch et al., 1998). In the early development of the central nervous system, stimulation of NMDA receptor may be critical for neuronal cell survival and differentiation, as well as the establishment of neural networks. In a social context, adult rats spent more time exploring a novel than a familiar juvenile rat. This novelty discrimination capacity was disrupted in adult rats neonatally treated with PCP, an effect prevented by SSR103800. It can therefore be hypothesized that the drug may be useful to treat the symptoms related to the inability to differentiate relevant from irrelevant information, which are considered to underlie many cognitive deficits of schizophrenia (Terranova et al., 2005). Furthermore, the recent demonstration that the direct glycine agonist D-serine reverses the deleterious effects produced by a similar neonatal PCP treatment in a spatial memory task in rats (Andersen and Pouzet 2004), further supports the idea that a pro-glycinergic strategy might have a positive impact on multiple facets of cognitive deficiency in schizophrenia.

Together, the effects of SSR103800 on episodic-like memory and social recognition suggest that the compound may have a therapeutic potential in the treatment of the cognitive deficits in schizophrenic patients.

Prepulse inhibition of the startle reflex (PPI), a measure of sensorimotor gating used to identify antipsychotics, is reduced in schizophrenic patients, in rodents treated with dopamine agonists or glutamate/NMDA antagonists, or spontaneously in several rodent strains (Yee et al., 2004; Willott et al., 2003). For instance, we observed that DBA/1J display a level of PPI (sensorimotor gating performance) which can be improved by several antipsychotic drugs. SSR103800, when given at the dose of 30 mg/kg, increased PPI. This effect was comparable to that observed with olanzapine, but unlike this latter drug, SSR103800 did not suppress basal startle amplitude, which is often indicative of a non-specific (i.e. sedative) action in this test. These effects of SSR103800 in the PPI model are in line with several recent studies, which reported PPI enhancing effects of several GlyT1 inhibitors such as SSR504734, ORG 24598 or ALX5407 in rodent strains with spontaneous or low levels of PPI following MK-801 treatment or ventral hippocampal lesion (Depoortere et al., 2005; Le Pen et al., 2003; Lipina et al., 2005). PPI which results from a "pre-attention" process is an experimental method to measure the sensorial input filtering ability of individuals, possibly impaired in auditory hallucinations and psychosis. Such abnormalities in PPI have been claimed to be predictive of or lead to cognitive deficits but also to positive symptoms (Meincke et al., 2004a,b). However, the effects of SSR103800 on sensory gating abilities observed in DBA/2 mice in the PPI test need to be confirmed in pharmacological-based models of PPI,

using a psychotic-like drug challenge. The effects of SSR103800 on PPI therefore suggest that the drug has a strong potential in alleviating sensorial gating deficit in schizophrenic patients with beneficial therapeutic impact on both the cognitive and positive symptoms.

In models which are claimed to address the positive aspects of schizophrenia, (Jackson et al., 1994), SSR103800 antagonized MK-801-induced hyperactivity in mice and decreased motor activity in PCP treated rats. Harsing et al. (2003) reported similar effects of NFPS and/or ORG 24461 for PCP-induced hyperactivity in mice, suggesting that selective GlyT1 inhibitors may be useful to treat some of the aspects of the positive symptoms of schizophrenia.

Since schizophrenia is often associated with comorbid symptoms, such as depressed mood, along with experimental evidence that GlyT1 inhibitors have antidepressant-like properties in rodents, we evaluated the effects of SSR103800 in two models of depression. In the forced-swimming test, probably the most common popular behavioural assay to detect antidepressant-like activity (for a review, see (Cryan et al., 2005)), SSR103800 reduced despair-like behaviour, as did the reference antidepressant fluoxetine under similar experimental conditions. The potential of SSR103800 on depressive-like behaviours was confirmed by its ability to decrease time spent by gerbils in tonic immobility, an adaptive reaction to threat in this species. This temporary motor inhibition was described to be highly sensitive to acute administration of SSRI-s or tricyclic antidepressants (Simiand et al., 2003). It was described that major depression is accompanied with decreased levels of glycine (Altamura et al., 1995) suggesting that GlyT1 inhibitors may be useful in the treatment of this condition.

In conclusion, the present findings demonstrated that SSR103800 is a potent, selective and orally active GlyT1 inhibitor, exhibiting activity in animal models of schizophrenia and depression. Its mechanism of action targets what is considered as being one of the primary causes of schizophrenia, namely a hypo-glutamatergic state. As such, it is expected to be efficacious not only against positive, but also negative symptoms and cognitive deficits, as well as co-morbid depression.

Acknowledgements

We wish to thank M. Bensaid for the characterization of the subtype of GlyT1 in Sk-N-MC cells. We express our gratitude to the following persons for their expert technical assistance: Martine Lacave, Marie-Thérèse Lucas, Sophie Ho-Van, Bernard Kleinberg, Patricia Senneville.

References

- Altamura C, Maes M, Dai J, Meltzer HY. Plasma concentrations of excitatory amino acids, serine, glycine, taurine and histidine in major depression. *Eur Neuropsychopharmacol* 1995;5:71–5 Suppl.
- Andersen JD, Pouzet B. Spatial memory deficits induced by perinatal treatment of rats with PCP and reversal effect of d-serine. *Neuropsychopharmacology* 2004;29:1080–90.
- Bert L, Robert F, Denoroy L, Stoppini L, Renaud BJ. Enhanced temporal resolution for the microdialysis monitoring of catecholamines and excitatory amino acids using capillary electrophoresis with laser-induced fluorescence detection. Analytical developments and in vitro validations. *Chromatogr A* 1996;755: 99–111.
- Brown A, Carlyle I, Clark J, Hamilton W, Gibson S, McGarry G, et al. Discovery and SAR of org 24598 – a selective glycine uptake inhibitor. *Bioorg Med Chem Lett* 2001;11:2007–9.
- Carlsson A. Neurocircuitries and neurotransmitter interactions in schizophrenia. *Int Clin Psychopharmacol* 1995;10(Suppl 3):21–8.
- Cryan JF, Page ME, Lucki I. Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology (Berl)* 2005;182:335–44.
- Cubelos B, Gimenez C, Zafra F. Localization of the GLYT1 glycine transporter at glutamatergic synapses in the rat brain. *Cereb Cortex* 2005;15:448–59.
- Danion JM, Huron C, Vidailhet P, Berna F. Functional mechanisms of episodic memory impairment in schizophrenia. *Can J Psychiatry* 2007;52:693–701.
- Depoortere R, Dargazanli G, Estenne-Bouhtou G, Coste A, Lanneau C, Desvignes C, et al. Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. *Neuropsychopharmacology* 2005;30:1963–85.

- Deutsch SI, Mastropaolo J, Rosse RB. Neurodevelopmental consequences of early exposure to phencyclidine and related drugs. *Clin Neuropharmacol* 1998;21:320–32.
- Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* 1988;31:47–59.
- Erhardt S, Blennow K, Nordin C, Skogh E, Lindstrom LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neurosci Lett* 2001;313:96–8.
- Evins AE, Amico E, Posever TA, Tokar R, Goff DC. d-Cycloserine added to risperidone in patients with primary negative symptoms of schizophrenia. *Schizophr Res* 2002;56:19–23.
- Gomez J, Ohno K, Betz H. Glycine transporter isoforms in the mammalian central nervous system: structures, functions and therapeutic promises. *Curr Opin Drug Discov Dev* 2003;6:675–82.
- Harsing Jr LG, Gacsalyi I, Szabo G, Schmidt E, Sziray N, Sebban C, et al. The glycine transporter-1 inhibitors NFPS and Org 24461: a pharmacological study. *Pharmacol Biochem Behav* 2003;74:811–25.
- Herdon HJ, Godfrey FM, Brown AM, Coulton S, Evans JR, Cairns WJ. Pharmacological assessment of the role of the glycine transporter GlyT-1 in mediating high-affinity glycine uptake by rat cerebral cortex and cerebellum synaptosomes. *Neuropharmacology* 2001;41:88–96.
- Jackson DM, Johansson C, Lindgren LM, Bengtsson A. Dopamine receptor antagonists block amphetamine and phencyclidine-induced motor stimulation in rats. *Pharmacol Biochem Behav* 1994;48:465–71.
- Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* 1991;148:1301–8.
- Javitt DC, Frusciante M. Glycyldodecylamide, a phencyclidine behavioral antagonist, blocks cortical glycine uptake: implications for schizophrenia and substance abuse. *Psychopharmacology (Berl)* 1997;129:96–8.
- Jentsch JD, Roth RH. The neuropharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* 1999;20:201–25.
- Kim JS, Kornhuber HH, Schmid-Burgk W, Holzmüller B. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. *Neurosci Lett* 1980;20:379–82.
- Kinney GG, Sur C, Burno M, Mallorga PJ, Williams JB, Figueroa DJ, et al. The glycine transporter type 1 inhibitor N-[3-(4-fluorophenyl)-3-(4-phenylphenoxy)propyl] sarcosine potentiates NMDA receptor-mediated responses in vivo and produces an antipsychotic profile in rodent behavior. *J Neurosci* 2003;23:7586–91.
- Lahti AC, Weiler MA, Tamara Michaelidis BA, Parwani A, Tamminga CA. Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology* 2001;25:455–67.
- Le Pen G, Kew J, Alberati D, Borroni E, Heitz MP, Moreau JL. Prepulse inhibition deficits of the startle reflex in neonatal ventral hippocampal-lesioned rats: reversal by glycine and a glycine transporter inhibitor. *Biol Psychiatry* 2003;54:1162–70.
- Lipina T, Labrie V, Weiner I, Roder J. Modulators of the glycine site on NMDA receptors, D-serine and ALX 5407, display similar beneficial effects to clozapine in mouse models of schizophrenia. *Psychopharmacology (Berl)* 2005;179:54–67.
- Martina M, Gorfinkel Y, Halman S, Lowe JA, Periyalwar P, Schmidt CJ, et al. Glycine transporter type 1 blockade changes NMDA receptor-mediated responses and LTP in hippocampal CA1 pyramidal cells by altering extracellular glycine levels. *J Physiol* 2004;557:489–500.
- Meincke U, Light GA, Geyer MA, Braff DL, Gouzoulis-Mayfrank E. Sensitization and habituation of the acoustic startle reflex in patients with schizophrenia. *Psychiatry Res* 2004a;126:51–61.
- Meincke U, Mörrth D, Voss T, Thelen B, Geyer MA, Gouzoulis-Mayfrank E. Prepulse inhibition of the acoustically evoked startle reflex in patients with an acute schizophrenic psychosis – a longitudinal study. *Eur Arch Psychiatry Clin Neurosci* 2004b;254:415–21.
- Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Sydney: Academic press; 1998. Ref Type: Magazine Article.
- Porsolt RD, Anton G, Blavet N, Jalife M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur J Pharmacol* 1978;47:379–91.
- Ratkowsky DA, Reedy TJ. Choosing near-linear parameters in the four-parameter logistic model for radioligand and related assays. *Biometrics* 1986;42:575–82.
- Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. *Behav Brain Res* 2003;140:1–47.
- Salomé N, Stemmelin J, Cohen C, Griebel G. Selective blockade of NK2 or NK3 receptors produces anxiolytic- and antidepressant-like effects in gerbils. *Pharmacol Biochem Behav* 2006;83:533–9.
- Schwarz C, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenic acid content in schizophrenia. *Biol Psychiatry* 2001;50:521–30.
- Simiand J, Guitard J, Griebel G, Soubrie P. Tonic immobility in gerbils: a new model for detecting antidepressant-like effects. *Behav Pharmacol* 2003;14(S40) Ref Type: Magazine Article.
- Smith G, Ruhland T, Mikkelsen G, Andersen K, Christoffersen CT, Alifrangis LH, et al. The synthesis and SAR of 2-arylsulfanyl-phenyl piperazinyl acetic acids as glyT-1 inhibitors. *Bioorg Med Chem Lett* 2004;14:4027–30.
- Subotnik KL, Nuechterlein KH, Asaroun RF, Fogelson DL, Goldstein MJ, Talovic SA. Depressive symptoms in the early course of schizophrenia: relationship to familial psychiatric illness. *Am J Psychiatry* 1997;154:1551–6.
- Sweatt JD. Hippocampal function in cognition. *Psychopharmacology (Berl)* 2004;174:99–110.
- Terranova JP, Chabot C, Barnouin MC, Perrault G, Depoortere R, Griebel G, et al. SSR181507, a dopamine D(2) receptor antagonist and 5-HT(1A) receptor agonist, alleviates disturbances of novelty discrimination in a social context in rats, a

- putative model of selective attention deficit. *Psychopharmacology (Berl)* 2005;181:134–44.
- Tsai GE, Yang P, Chang YC, Chong MY. D-alanine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* 2006;59:230–4.
- Vandenberg RJ, Aubrey KR. Glycine transport inhibitors as potential antipsychotic drugs. *Expert Opin Ther Targets* 2001;5:507–18.
- Weinberger DR. The pathogenesis of schizophrenia: a neurodevelopmental theory. Nasrallah HAW *The Neurology of Schizophrenia*; 1986. p. 397–406. Ref Type: Abstract.
- Willott JF, Tanner L, O'Steen J, Johnson KR, Bogue MA, Gagnon L. Acoustic startle and prepulse inhibition in 40 inbred strains of mice. *Behav Neurosci* 2003;117:716–27.
- Yee BK, Chang DL, Feldon J. The effects of dizocilpine and phencyclidine on prepulse inhibition of the acoustic startle reflex and on prepulse-elicited reactivity in C57BL6 mice. *Neuropsychopharmacology* 2004;29:1865–77.