Psychopharmacology (2013) 228:97–107
DOI 10.1007/s00213-013-3020-1

ORIGINAL INVESTIGATION

The CRF_1 receptor antagonist SSR125543 prevents stress-induced cognitive deficit associated with hippocampal dysfunction: Comparison with paroxetine and D-cycloserine

J. Philbert · C. Belzung · G. Griebel

Received: 14 November 2012 / Accepted: 31 January 2013 / Published online: 14 February 2013

Abstract
Rationale The selective CRF_1 (corticotropin releasing factor type 1) receptor antagonist SSR125543 has been previously shown to attenuate the long-term cognitive deficit produced by traumatic stress exposure. Memory disturbances described in post-traumatic stress disorder (PTSD) patients are believed to be associated with changes in neuronal activity, in particular at the level of the hippocampus.
Objectives The present study aims at investigating whether the effects of SSR125543 (10 mg/kg/day for 2 weeks) on cognitive impairment induced by traumatic stress exposure are associated with changes in hippocampal excitability. Effects of SSR125543 were compared to those of the 5-HT reuptake inhibitor, paroxetine (10 mg/kg/day), and the partial *N*-methyl-D-aspartate (NMDA) receptor agonist, D-cycloserine (10 mg/kg/day), two compounds which have demonstrated clinical efficacy against PTSD.
Methods Mice received two unavoidable electric footshocks. Then, 1 or 16 days after stress, they were tested for their memory performance using the object recognition test. Neuronal excitability was recorded during the third week post-stress in the CA1 area of the hippocampus. Drugs were administered from day 1 post-stress to the day preceding the electrophysiological study.
Results Application of electric shocks produced cognitive impairment 16, but not 1 day after stress, an effect which was associated with a decrease in hippocampal neuronal excitability. Both stress-induced effects were prevented by repeated administration of SSR125543, paroxetine and D-cycloserine.
Conclusions These findings confirm that the CRF_1 receptor antagonist SSR125543 is able to attenuate the behavioral effects of traumatic stress exposure and indicate that these effects are associated with a normalization of hippocampal neuronal excitability impaired by stress.

Keywords Paroxetine · D-Cycloserine · CRF_1 receptor antagonist · Memory · Input–output curve · Electrophysiology · Excitability · Hippocampus · PTSD

J. Philbert · G. Griebel (✉)
Sanofi, Exploratory Unit, 1, Avenue Pierre Brossolette,
91385 Chilly-Mazarin, France
e-mail: guy.griebel@sanofi.com

C. Belzung
INSERM U-930, Université François Rabelais Tours,
UFR Sciences et Techniques, Parc Grandmont,
Tours, France

Introduction

There are numerous clinical reports on decreased performance in learning and memory in post-traumatic stress disorder (PTSD) patients (Brewin et al. 2007). It has been hypothesized that these cognitive impairments may relate to functional and morphological changes in brain structures implicated in memory function, such as the prefrontal cortex (Arnsten 2009), the amygdala (Roozendaal et al. 2009) and the hippocampus. This latter structure has received particular attention and there are several clinical studies that have reported hippocampal alterations in PTSD patients, such as a volume reduction (Woon et al. 2010), hippocampal metabolic deficits characterized by reduced ratio of *N*-acetylaspartate relative to creatine in hippocampus (Schuff et al. 2008) and functional impairments (Acheson et al. 2012).

In animal studies, stress-induced cognitive impairments have been reported to be associated with damage to the hippocampus, such as reduced dendritic arborisation in the CA3 region (Sousa et al. 2000), dendritic atrophy of CA1

pyramidal cells (Ivy et al. 2010) and a structural remodelling of excitatory axo-spinous synaptic connectivity in CA1 area (Donohue et al. 2006). Interestingly, treatment of PTSD patients with selective serotonin reuptake inhibitors (SSRIs), such as paroxetine resulted in an improvement in memory function and an increase in hippocampal volume (Bremner and Vermetten 2004; Nemeroff and Owens 2004; Vermetten et al. 2003).

SSRIs represent the mainstay of treatment of PTSD, but several new treatment avenues for this condition are being investigated. One of them is targeting the *N*-methyl-D-aspartate receptor (NMDAR), well known to be involved in hippocampal synaptic plasticity and memory function (Li and Tsien 2009). Clinical studies examining the effects of D-cycloserine, a partial agonist of the NMDAR, have reported that this compound both promoted the extinction of fear and protected against reinstatement of fear in patients suffering from PTSD (Litz et al. 2012), and facilitated fear extinction in other anxiety disorders such as acrophobia (Ressler et al. 2004) or social anxiety disorder (Hofmann et al. 2006). Moreover, de Kleine et al. (2012) reported recently a greater symptom reduction in severe PTSD patients receiving D-cycloserine compared to those from the placebo group during exposure therapy. These clinical findings are supported by experiments in rodents, which showed that D-cycloserine promoted fear extinction (Ledgerwood et al. 2004, 2005; Yamamoto et al. 2008). These latter authors demonstrated that repeated administration of D-cycloserine reduced the enhanced levels of mRNAs of NMDARs in the hippocampus of rats exposed to a single prolonged stress, suggesting that NMDAR-mediated signal transduction in the hippocampus may be involved in fear extinction.

Blockade of the corticotropin releasing factor type 1 (CRF_1) receptor is another new concept which is being explored for the development of alternative treatments for PTSD (Holsboer and Ising 2008). The CRF_1 receptor is a key player in the response to stress and it was notably observed that PTSD patients exhibit increased cerebrospinal fluids levels of the endogenous peptide, CRF (Baker et al. 1999; Bremner et al. 1997). The CRF_1 receptor has been validated as a potential drug target for antidepressants and anxiolytics in numerous studies in animals (Adamec et al. 2010; Griebel et al. 2002; Overstreet and Griebel 2004; Philbert et al. 2012; Surget et al. 2009; Urani et al. 2011). Although clinical studies with CRF_1 receptor antagonists in depression have been disappointing so far, their potential for treating PTSD remains to be determined (Griebel and Holsboer 2012).

The objective of this study was to test the hypothesis that protracted CRF_1 receptor-targeted blockade using the selective antagonist, SSR125543, would prevent the development of a PTSD-like phenotype in a mouse model of PTSD and to determine if these effects are associated with functional changes in the hippocampus. SSR125543 has been reported to produce anxiolytic- and antidepressant-like effects in a variety of rodent models. We used a recently established mouse model of PTSD (Griebel et al. 2002; Philbert et al. 2011, 2012) based on the exposure of mice to electric foot-shocks followed by the assessment of (1) their cognitive performance using the object recognition task, 2 weeks later, and (2) extracellular recordings of population spike amplitude in the hippocampal CA1 area, representing the summation of action potentials, i.e., synaptic transmission of a population of neurons within this network. SSR125543 was administered repeatedly and its effects were compared to those of paroxetine and D-cycloserine, two clinically active compounds in PTSD patients.

Materials and methods

Animals

Swiss male mice (Janvier, Le Genest St-Isle, France) weighing 20–22 g at the start of the experiment were used. They were housed individually in plastic cages (30×18×18 cm) with free access to food and water ad libitum. They were maintained at a constant temperature of 21±1 °C, humidity at 50±10 % and under a 12:12 light/dark cycle (light on at 7:00 A.M.). Experiments were conducted in accordance with the "Guide and Care and Use of Laboratory Animals" (National Institute of Health) and were approved by the internal Animal Ethics Committee.

Shock application

Animals were placed into the shock chamber for a 190-s habituation period following which two electric foot-shocks (1.5 mA; for 2 s, 6 s apart) were delivered through the metal grid floor. Animals remained in the shock chamber for another 60-s period before they were returned to their home cage. Control animals were exposed to the same procedure, but without receiving any foot-shock.

Drug administration

Paroxetine (Sigma-Aldrich; CAS 110429-35-1), D-cycloserine (Sigma-Aldrich; CAS 68-41-7), and SSR125543, synthesized by Sanofi Medicinal Chemistry, were suspended in saline with methylcellulose (0.6 %) and Tween 80 (0.1 %). Drug treatments began 5 h after stress. Mice received one intraperitoneal (i.p.) administration per day of 10 mg/kg. The dose and administration route were chosen on the basis of previous work with the compounds using mice as subjects (Griebel et al. 2002; Poleszak et al. 2008; Rorick-Kehn et al. 2005; Urani et al. 2011). A protracted treatment regimen was used because

it is closer to the clinical situation where drugs are generally given on a chronic basis, following the traumatic event (see, e.g., the ongoing clinical trials involving the CRF_1 receptor antagonist, GSK561679, in PTSD patients; http://clinicaltrials.gov/ct2/show/NCT01018992). During the object recognition task, performed 2 weeks after stress exposure, treatments were administered 1 h before the first session and 1 h before the second one. Treatments lasted until the day before mice were sacrificed for the electrophysiological study. Concentrations were adjusted to administer a final volume of 10 ml/kg of body weight.

Object recognition task

The test took place in a square open-field, which consisted of a uniformly lit (20±2 lx) plexiglass enclosure (52×52× 40 high cm). The objects to be discriminated were a metal triangle (3.3 cm high, 5.5 cm wide) and a plastic piece of construction game (3 cm high and 3 cm wide). During the first session, Swiss mice were allowed to become familiar with the experimental environment for 8 min, time spent in activity was measured. Twenty-four hours later, mice were again placed in the enclosure in the presence of two identical objects until they reached 10 s of object exploration (acquisition session) with a limit of 5 min. Exploration of an object was defined as pointing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. After a 60-min interval, mice were placed again in the enclosure with a previously presented familiar object and a new one for 5 min (retrieval session). Time spent exploring the familiar and the new objects were recorded. Under a short-term inter-trial procedure, animals spent more time exploring the new object compared to the familiar one, reflecting a memory of the familiar one. Animals displaying impaired recall performance spent the same amount of time exploring both objects, indicating a forgetting of the familiar object (short-term visual memory deficit). For more details on the procedure, see Pichat et al. (2007).

The following parameters were analysed: (a) activity during context habituation session; (b) time to reach 10 s of objects exploration in the acquisition session; (c) time of exploration of each object during retrieval session and (d) ratio of the time exploring the new object over the total time of exploration. For exploration time, data were analysed using a two-way ANOVA with repeated measures with *object* as a fixed factor to analyse the ability of animals to discriminate between the familiar and the novel object. Effect of *object* factor was then analysed by Winer analysis for each level of *group* factor. Student's *t*-test vs. 0.5 (chance level value) was performed to analyse the ratios.

Two separate groups of mice were use to investigate short- and long-term effects of stress exposure on memory, respectively. In the first group (short-term), habituation session was performed 5 h after the shock procedure, and acquisition and recall sessions took place on the following day. In the second group (long-term) memory performance were assessed on the 14th and the 15th days following shock application. This latter procedure was used to investigate the effects of drug treatments and is depicted in Fig. 1.

Synaptic transmission

Between days 20 and 24 after shock application, mice were anesthetised with isoflurane (4 %) before being decapitated and their brains were quickly removed. Transverse hippocampal slices (400 μm thick) were cut using a Leica vibroslicer VT1000S in artificial cerebrospinal fluid solution containing 126 mM NaCl, 3.5 mM KCl, 1 mM $MgCl_2$, 1.15 mM KH_2PO_4, 1 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose, at approximately 3 °C, aerated with 95 % O_2, 5 % CO_2 to a final pH of 7.35. Then, brain sections were immediately trimmed, while still immersed in ice-cold artificial cerebrospinal fluid solution, so that they contained only the hippocampal structure. Hippocampal slices were transferred with a wide-bore Pasteur pipette to a holding chamber containing the oxygenated artificial cerebrospinal fluid solution at room temperature. One hour later, a slice was placed into the recording chamber and superfused with 34 °C oxygenated artificial cerebrospinal fluid solution (2–4 ml/min^{-1}).

The Schaffer collaterals were stimulated once per 30 s by a stimulating electrode made from Teflon-coated stainless steel wire (75 μm diameter; A&M Systems, Carlsborg, WA, USA). Extracellular evoked population spikes were measured with a recording electrode made from borosilicate capillary-filled glass. It was placed into the CA1 cell bodies by an electronic micromanipulator MPC200 (Digitimer Ltd.). Signals were amplified (DAM80; World Precision Instruments) and analysed offline using Notocord-hem software. Population spike amplitude was defined as the perpendicular distance from the negative peak to a tangent drawn between the first and second positive peaks. Once a stable population spike was obtained, the stimulus intensity was increased from 2 to a maximum of 1,400 μA in gradual increments in order to determine input–output curves.

The relationship between the applied intensity and the resultant population spike amplitude, expressed as a percentage of the maximal response, is a four-parameter sigmoid: $Y=b+/(1+\exp[-s*(\log(x)-\log(I_{50}))])$, where t denotes the top of the curve, b is the bottom of the curve, s is the slope, and I_{50} is the stimulation intensity required to obtain half-maximal excitability. Experimental unit considered (x) was the hippocampus slice. A model was adjusted for each condition on all available data. Next, some parameters of the model were shared and statistical tests were then conducted to determine if the fit was significantly degraded by sharing

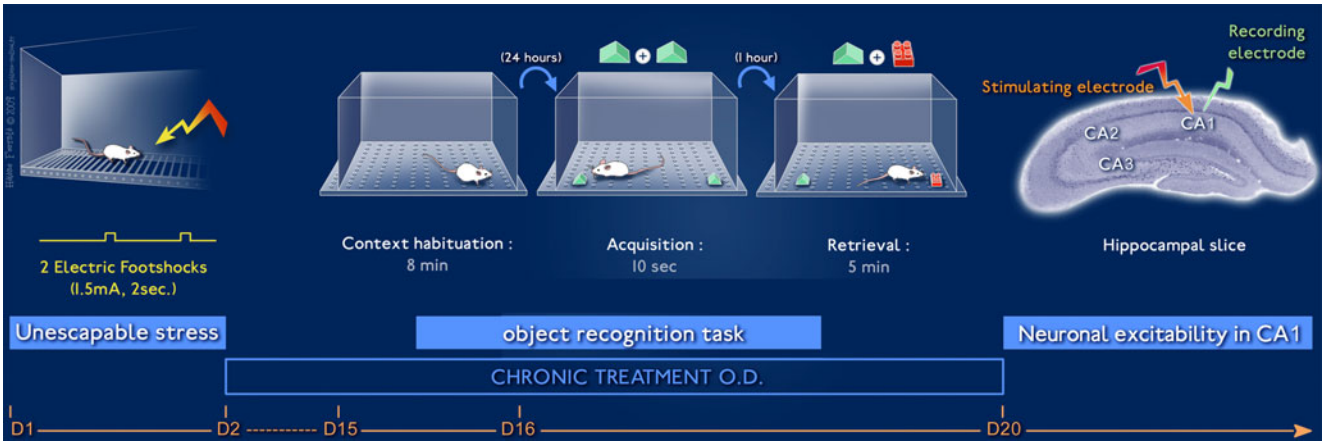

Fig. 1 Experimental design used to investigate the long-term effects of foot-shock stress

parameters. I_{50} rates were estimated for each slice of each animal and comparisons between groups were performed using one-way ANOVA.

Results

Object recognition task

Analysis of time spent exploring the objects during acquisition revealed no significant difference across treatment, stress manipulation and object (Table 1). During the retrieval session (session 3), control mice spent more time exploring the new object than the familiar one (Fig. 2a: $F_{(1,\ 22)}$=16.195; p<0.001), with a discrimination ratio significantly different from the chance value 0.5 (Fig. 2b: t=4.412, p<0.001). Mice exposed to electrical foot-shocks on the day preceding the test also spent more time exploring the novel object than the known one (Fig. 2a: $F_{(1,\ 22)}$=9.002; p<0.01) with a ratio significantly different from the chance value 0.5 (Fig. 2b: t=3.936, p<0.01). These results indicate that short-term memory performance was not altered in mice on the day following exposure to electrical foot-shocks.

Table 1 Time spent exploring the objects during the acquisition session in the object recognition test

Condition	Time object 1 (s)	Time object 2 (s)
Paroxetine		
Non-stressed control	5.55±0.41	4.44±0.41
Stressed vehicle	5.33±0.29	4.66±0.29
Stressed+10 mg/kg/day	5.31±0.31	4.50±0.29
D-Cycloserine		
Non-stressed control	5.92±0.39	4.08±0.39
Stressed vehicle	5.08±0.41	4.92±0.41
Stressed+10 mg/kg/day	5.38±0.56	4.62±0.56
SSR125543		
Non-stressed control	5.11±0.26	4.88±0.26
Stressed vehicle	4.51±0.37	5.48±0.37
Stressed+10 mg/kg/day	5.00±0.41	4.99±0.39

Data represent means ± SEM

The long-term effects of exposure to electrical foot-shocks on memory performance are presented in Fig. 3. Under control (vehicle) conditions, mice spent more time exploring the new object than the known one (Fig. 3a$_1$: $F_{(1,44)}$=14.749, p<0.001; Fig. 3b$_1$: $F_{(1,43)}$=13.925, p<0.001; Fig. 3c$_1$: $F_{(1,95)}$=18.589, p<0.001), with a discrimination ratio significantly different from the chance value 0.5 (Fig. 3a$_2$: t=2.752, p<0.05; Fig. 3b$_2$: t=2.996, p<0.01; Fig. 3c$_2$: t=5.810, p<0.001). Conversely, mice previously exposed to electrical foot-shocks 2 weeks before, spent the same amount of time exploring the new and the known object (Fig. 3a$_1$: $F_{(1,44)}$=0.009, p=0.924; Fig. 3b$_1$: $F_{(1,43)}$=0.016, p=0.901; Fig. 3c$_1$: $F_{(1,95)}$=1.805, p=0.1823). This indicates that stressed mice lost their ability to discriminate between the two objects, which is indicative of a forgetting of the familiar object.

Chronic treatment by paroxetine, D-cycloserine and by SSR125543 for 2 weeks significantly increased in stressed mice the amount of time preferentially spent investigating the novel object compared to the known one (Fig. 3a$_1$: $F_{(1,\ 44)}$=18.772, p<0.001; Fig. 3b$_1$: $F_{(1,43)}$=7.860, p<0.01; Fig. 3c$_1$: $F_{(1,95)}$=20.671, p<0.001). In the same way, discrimination ratios were significantly different from the chance value 0.5 for stressed mice treated by paroxetine (Fig. 3a$_2$: t=5.280, p<0.001), D-cycloserine (Fig. 3b$_2$: t=4.154, p<0.001) and by SSR125543 (Fig. 3c$_2$: t=5.556, p<0.001). Neither locomotor activity recorded during the context habituation session, nor total time spent in exploring both objects during the acquisition and the recall sessions was significantly modified by treatments (data not shown). Thus, effects of these compounds did not result from non-specific biases such as sedation and/or motor effects. These

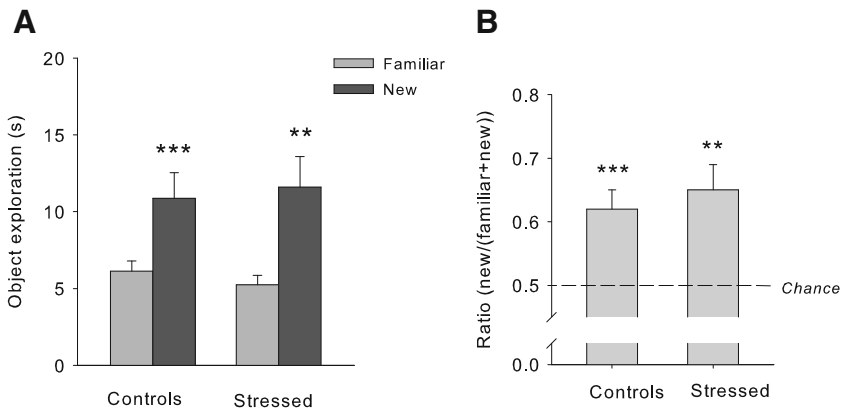

Fig. 2 Short-term memory was not impaired on the day following acute inescapable stress exposure in mice. **a** Bars represent means (±SEM) of time spent exploring new or familiar objects. Two-way ANOVA with repeated measures on factor "object". $n=12$ mice per group. $^{**}p<0.01$, $^{***}p<0.001$, new vs. familiar object. **b** Bars represent means (±SEM) of novelty ratio, Student's test vs. 0.5. $^{**}p<0.01$, $^{***}p<0.001$

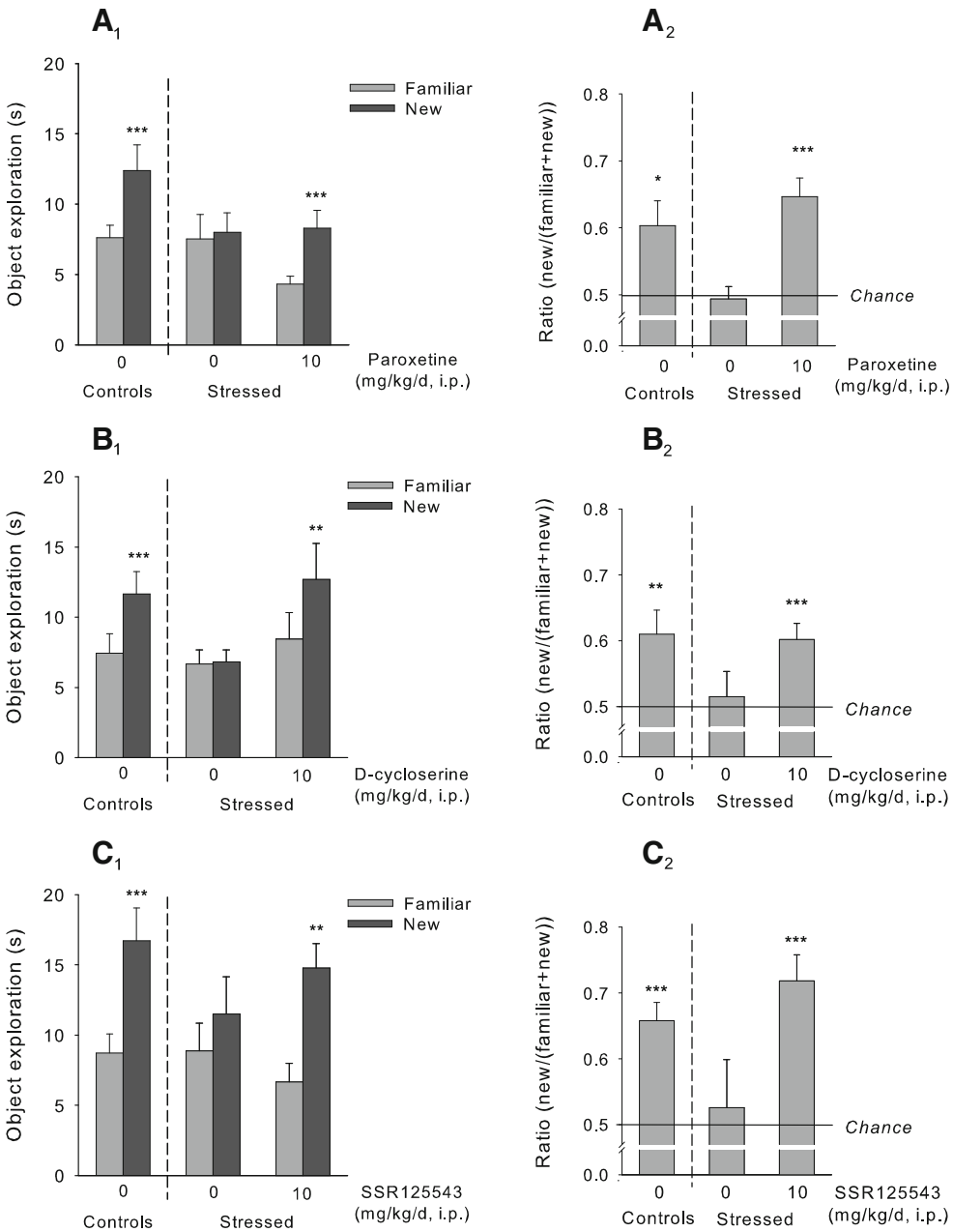

Fig. 3 Effect of chronic paroxetine (**a**), D-cycloserine (**b**), and SSR125543 (**c**) treatments on short-term memory impairment induced by acute inescapable stress in mice. ($\mathbf{a_1}$, $\mathbf{b_1}$, $\mathbf{c_1}$ Bars represent means (±SEM) of time spent exploring new or familiar objects. Two-way ANOVA with repeated measures on factor "object". $n=15$–16 mice per group. $^{**}p<0.01$, $^{***}p<0.001$, new vs. familiar object. $\mathbf{a_2}$, $\mathbf{b_2}$, $\mathbf{c_2}$ Bars represent means (±SEM) of novelty ratio, Student's test vs. 0.5. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

findings demonstrate that chronic treatments by paroxetine, by D-cycloserine and by the SSR125543 prevent the memory deficit induced by acute inescapable stress exposure.

Synaptic transmission

As shown in Figs. 4a, 5a and 6a, I_{50} of stressed mice are significantly different from control animals (Fig. 4a: $F_{(9,2E3)}$=1.187, p<0.001; Fig. 5a: $F_{(4,753)}$=2.042, p<0.001; Fig. 6a: $F_{(4,411)}$=0.824, p<0.001). In addition, I_{50} were significantly higher in stressed mice compared to controls (Fig. 4b: $F_{(3,56)}$=6.81, p<0.001; Fig. 5b: $F_{(3,52)}$=6.69, p<0.001; Fig. 6b: $F_{(3,37)}$=7,37, p<0.001). These findings demonstrate that exposure to two unavoidable electric foot-shocks significantly reduced neuronal excitability in CA1 hippocampal area.

Chronic treatments with paroxetine, D-cycloserine and SSR125543 at 10 mg/kg/day (i.p.) during 3 weeks significantly reversed stressed-induced decrease of hippocampal neuronal excitability. The I_{50} parameter cannot be shared between untreated stressed and stressed mice treated by paroxetine (Fig. 4a: $F_{(9,2E3)}$=1.187, p<0.001), by D-cycloserine (Fig. 5a: $F_{(2,720)}$=0.335, p<0.001), or by SSR125543 (Fig. 6a: $F_{(2,565)}$=0.312, p<0.001). I_{50} from stressed mice receiving paroxetine were significantly lower than I_{50} from stressed mice receiving vehicle (Fig. 4b: $F_{(3,56)}$=6.81, p< 0.001). Similar results were obtained in stressed mice treated by D-cycloserine (Fig. 5b: $F_{(3,52)}$=6.69, p<0.001) and by SSR125543 (Fig. 6b: $F_{(3,37)}$=7,37, p<0.001).

D-Cycloserine and SSR125543 have no effect on their own on hippocampal neuronal excitability. The four parameters can be shared (Fig. 5a: $F_{(4,753)}$=2.04, p=0.087; Fig. 6a: $F_{(4,411)}$=0.824, p=0.51) and I_{50} from D-cycloserine- and SSR125543-treated non-stressed mice were not significantly different from controls (Fig. 5b: $F_{(3,52)}$=6.69, p= 0.824; Fig. 6b: $F_{(3,37)}$=7.37, p=0.867). On the contrary, 3-week paroxetine treatment (10 mg/kg/day, i.p.) produced a significant decrease in hippocampal neuronal excitability in non-stressed mice, I_{50} cannot be shared between groups (Fig. 4a: $F_{(3,1E3)}$=1.36, p=0.254) and was significantly increased when compared to controls (Fig. 4b: $F_{(3,56)}$=6.81, p<0.001).

Discussion

The present study demonstrated that chronic treatment with the CRF_1 receptor antagonist, SSR125543 prevented the decrease of hippocampal neuronal excitability associated with a cognitive deficit in a mouse model of PTSD. These effects were shared with the SSRI paroxetine and the NMDAR partial agonist, D-cycloserine.

Exposing mice to two unavoidable electric foot-shocks led to an impairment of their memory performance and to a decrease in hippocampal CA1 neuronal excitability as observed 2 and 3 weeks later, respectively. The finding that there was no cognitive impairment 24 h following stress exposure, is consistent with our previous data showing that trauma-related symptoms, such as sleep disturbances and generalized avoidance behavior, appeared after a delay of at least 7 days (Philbert et al. 2011). It is important to note that we did not assess hippocampal neuronal excitability 24 h after shock exposure, so that we cannot exclude the possibility that changes in this response may have occurred shortly after stress. However, taken as a whole, these findings are compatible with the hypothesis that the current procedure may be a valid animal model of PTSD requiring that behavioral alterations are expected to persist for a long time or become more intensive with the passage of time (Siegmund and Wotjak 2006).

Long-lasting effects following an acute stressful event on hippocampal synaptic transmission have received little

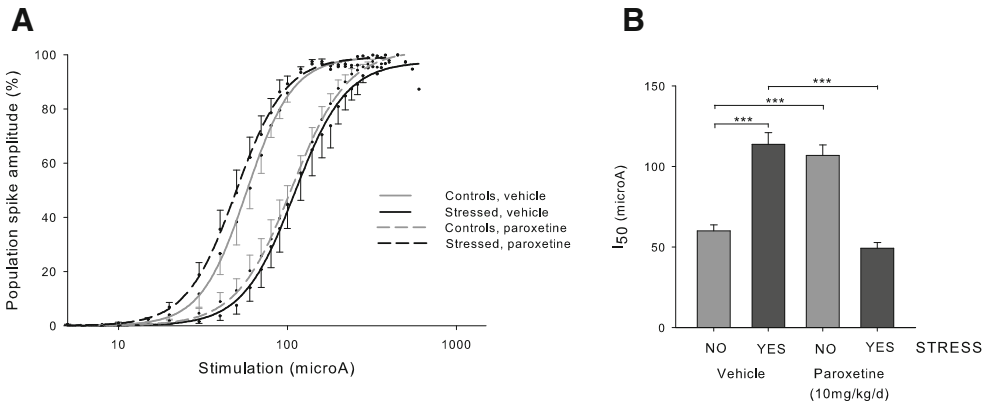

Fig. 4 Effect of paroxetine chronic treatment on neuronal excitability in CA1 hippocampal field 3 weeks after an inescapable stress in mice. Input–output curves were obtained from CA1 following Schaffer collateral stimulation in hippocampal slices. n=11–14 slices per group. **a** Curves represent fitted sigmoïdal functions (±SEM) of population spike mean amplitude. **b** Bars represent means (±SEM) of stimulation intensity required to obtain half-maximal excitability (I_{50}). One-way ANOVA, ***p<0.001

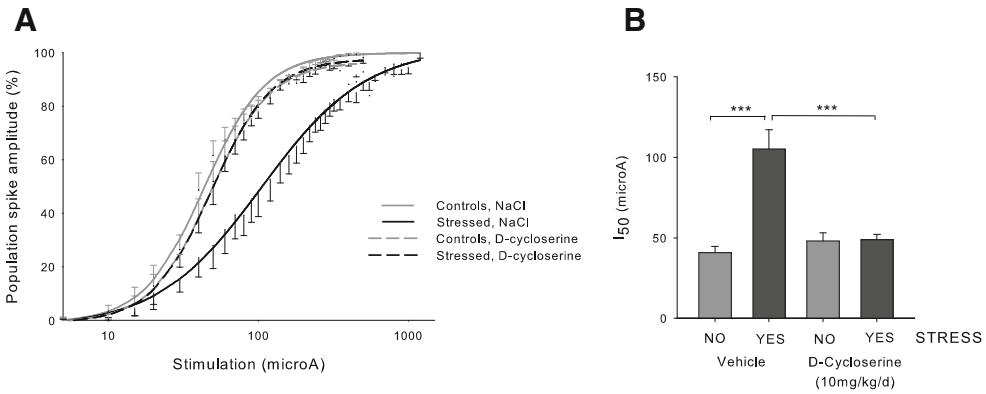

Fig. 5 Effect of D-cycloserine chronic treatment on neuronal excitability in CA1 hippocampal field 3 weeks after an inescapable stress in mice. Input–output curves were obtained from CA1 following Schaffer collateral stimulation in hippocampal slices. n=11–16 slices per group. **a** Curves represent fitted sigmoïdal functions (±SEM) of population spike mean amplitude. **b** Bars represent means (±SEM) of stimulation intensity required to obtain half-maximal excitability (I_{50}). One-way ANOVA, ***p<0.001

attention in studies in animals. Sousa et al. (2000) reported alterations in the lengths of terminal dentritic segments of pyramidal cells in rat CA1 hippocampus 3 weeks after exposure to unpredictable chronic stress and demonstrated that these effects persisted 1 month later. Donohue et al. (2006) reported a remodelling of CA1 excitatory synaptic circuitry 2 days after a chronic restraint stress of 3 weeks. Other studies have reported that PTSD-like symptoms, such as enhanced acoustic startle or impaired spatial memory, observed in rats 7 days after exposure to single prolonged stress were associated with deficit of hippocampal long-term potentiation in the CA1 region, reflecting an alteration of synaptic plasticity (Kohda et al. 2007; Li et al. 2010). However, none of these studies described long-term residual effects of acute stress on hippocampal synaptic activity. A reduction of the population spike amplitude threshold in the CA1 area following stress exposure was previously described in rats by Kerr et al. (1991), but this decrease was induced by a 6-month exposure to moderate stress using the shuttle escape task paradigm. Here, we demonstrated for the first time that one brief inescapable exposure to two electric foot-shocks induced long-lasting decrease of population spike amplitude in hippocampal CA1 area, indicating a diminished synaptic transmission within this network.

The precise mechanism by which stressful exposure decreases hippocampal synaptic transmission is unclear. Data from the literature suggest that the increase in glucocorticoïd release induced by stress affects the hippocampus. This hypothesis is notably supported by studies showing that hippocampal pyramidal cells display high expression of glucocorticoïd receptors, and are therefore susceptible to the effects of stress (De Kloet et al. 1999; McEwen 2000). In addition, exposure of rat hippocampus slices to a single high dose of corticosterone, the primary glucocorticoïd hormone in rodents, reduced the amplitude of the population spike in the CA1 pyramidal cell layer by stimulation of Schaffer collaterals (Rey et al. 1987; Vidal et al. 1986). Moreover, Zahorodna et al. (2006) demonstrated

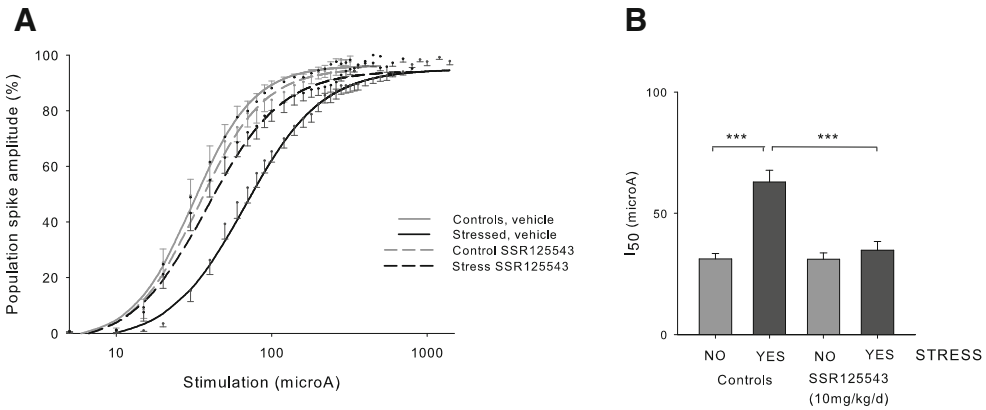

Fig. 6 Effect of SSR125543 chronic treatment on neuronal excitability in CA1 hippocampal field 3 weeks after an inescapable stress in mice. Input/output curves were obtained from CA1 following Schaffer collateral stimulation in hippocampal slices. n=9 – 12 slices per group. **a** Curves represent fitted sigmoïdal functions (±SEM) of population spike mean amplitude. **b** Bars represent means (±SEM) of stimulation intensity required to obtain half-maximal excitability (I_{50}). One-way ANOVA, ***p<0.001

in rats that 21-day, but not 7-day, treatment with corticosterone (10 mg/kg/day) resulted in a decrease of population spike amplitude by stimuli of small and intermediate intensity without change in maximum amplitude of responses in comparison with control hipoccampal slices. Other constituents that parallel glucocorticoïd release from the adrenal gland, such as CRF, could have been involved in stress-induced alteration of synaptic transmission in CA1 hippocampal field (Ivy et al. 2010). CRF-containing neurons and receptors are found in brain areas involved in the stress response, including the hippocampus (Holmes et al. 2003; Van et al. 2000). Rebaudo et al. (2001) reported that bath-applied CRF reduced population spike amplitude in CA1 hippocampal region at the concentration of 1 nM in slices prepared from rats. In addition, there is ample evidence that stressful exposure or glucocorticoïd release can increase glutamatergic tone in hippocampus, an effect which has been associated with excitotoxicity (Sapolsky 2000). Youssef et al. (2006) reported that 15 μM NMDA applied for 10 min caused abolition of the population spike potential in rat hippocampal slices and was followed by only partial (33 %) recovery, suggesting that NMDA impaired neuronal function that could eventually lead to cell death and decreased neuronal density in the field CA1. Thus, stress-induced hippocampal dysfunctions could also result from excitotoxic insults such as high glutamate release.

The idea that glutamate/NMDA and CRF neurotransmissions may have been involved in the current effects of stress is supported by the drug experiments which showed that both memory deficit and alteration of hippocampal neuronal excitability following stress were prevented by the NMDAR partial agonist, D-cycloserine, and the CRF_1 receptor antagonist, SSR125543. As mentioned above, studies that investigated the effects of D-cycloserine in animal models of PTSD demonstrated that repeated administration of the drug normalized impaired fear extinction and stress-induced increase of NMDAR mRNA levels in the hippocampus of rats exposed to single prolonged stress (Ledgerwood et al. 2004, 2005; Yamamoto et al. 2008). In addition, Rouaud and Billard (2003) showed that D-cycloserine facilitated NMDAR-mediated signal transduction and synaptic plasticity in the CA1 field of rat hippocampal slices. These data, together with our results, suggest that chronic D-cycloserine treatment normalises stress-impaired synaptic transmission in the CA1 field, via NMDAR activation leading to increased synaptic plasticity, which resulted in a restoration of memory performance.

The effect observed after a chronic treatment with the CRF_1 receptor antagonist, SSR125543, on the cognitive deficit induced by acute stress is consistent with previous results showing that acute administration of this drug attenuated memory deficit in mice following exposure to rats (Urani et al. 2011) or to electric foot-shocks 2 weeks post-stress (Philbert et al. 2012). This latter study demonstrated that the effects of SSR125543 effects are unrelated to an action at the level of the HPA axis, but may certainly involve extra-hypothalamic CRF_1 receptors. This hypothesis is strengthened by the current findings showing that chronic treatment with SSR125543 normalized hippocampal neuronal excitability in stressed mice, thereby suggesting that hippocampal CRF_1 receptors may have been involved in the cognitive effects of the compound. It is additionally supported by the observations that the blockade of CRF_1 receptor by a specific CRF_1 receptor antagonist reduced hippocampal spine loss in CA3 area of mice exposed to restraint stress (Chen et al. 2008). More recently, central or peripheral administration of a CRF_1 receptor antagonist to early-life stressed rats, was shown to improve memory performance in the object recognition task and prevented dentritic atrophy and long-term potentiation attenuation in CA1 Schaffer collateral synapses (Ivy et al. 2010). It is likely that similar mechanisms may have been involved in the current effects of SSR125543 on stress-induced changes of population spike amplitude in the CA1 area. It is worth mentioning that other brain structures, such as the basolateral amygdala, have been shown to be involved in the beneficial effects of CRF_1 receptor antagonists on fear reactions following stressful exposure (Hubbard et al. 2007). However, a role of hypothalamic CRF_1 receptors in these effects cannot be totally ruled out because exposure of corticosterone affects hippocampal neural activity (De Kloet et al. 2008) and a CRF_1 receptor antagonist may improve this effect by normalizing HPA axis dysfunction. Moreover, in this study SSR125543 was given chronically, starting immediately after stress. This treatment design could involve a different mechanism of action as that observed when the drug is given at the time of testing (Philbert et al. 2012). It is possible, that the blockade of the HPA axis immediately after stress may have inhibited the consolidation of enduring effects of stress as observed in previous studies (e.g., Adamec et al. 2007) indicating that the chronic regimen was not necessary.

The experiment with the SSRI paroxetine demonstrated that repeated treatment with this drug prevented both the cognitive deficit and the decrease in population spike amplitude in CA1 area induced by stress. The mechanisms underlying these effects may involve the neurotrophic and neuroprotective actions of the drug. Chronic administration of SSRIs has been shown to induce a robust increase in pyramidal cell dentritic spine synapse density in the CA1 hippocampal field in ovariectomised female rats (Hajszan et al. 2005). Sairanen et al. (2007) demonstrated in rats that chronic antidepressant treatment increases the expression of GAP-43 in the CA1 region of the hippocampus, a protein which plays an important role in synaptogenesis, axonal path finding as well as regulation of cytoskeletal organisation

(Benowitz and Routtenberg 1997). These studies suggest that paroxetine treatment, in the current study, may increase synaptic connectivity in the CA1 area of the hippocampus of stressed mice. This hypothesis is consistent with a recent study showing that repeated paroxetine treatment, starting immediately after stress, reversed structural damages and apoptosis in hippocampus in a mouse model of PTSD (Wang et al. 2012). It is important to note that paroxetine impaired neuronal excitability in non-stressed animals. This observation may be consistent with that of Tokarski et al. (1996) who reported that chronic treatment (14 days, twice daily, 10 mg/kg, p.o.) with paroxetine decreased the amplitude of population spike hippocampal slices from non-stressed rats. Moreover, since it is widely acknowledged that altered hippocampal excitability may be associated with cognitive impairment (McEwen and Sapolsky 1995), it is worth mentioning that in a previous study using the object recognition task in non-stressed mice, paroxetine, given acutely, altered memory performance (Philbert et al. 2012), effects also reported by others (Naudon et al. 2007), and which have been suggested to be related to the anticholinergic side effects of paroxetine (Fujishiro et al. 2002). Although paroxetine was not tested in the object recognition test in non-stressed animals in this study, it can be hypothesized that similar mechanisms may have been involved in its effects on hippocampal excitability in non-stressed mice.

In conclusion, the results of the present study demonstrate that protracted CRF_1 receptor blockade is able to prevent the deleterious effects of unavoidable stress exposure on memory performance and hippocampal excitability at the level of the CA1 area, suggesting that CRF_1 receptor antagonists may be useful for PTSD symptoms prophylaxis. Since there is evidence that CRF_1 receptor antagonists given acutely after stress have some efficacy to block both acute and enduring effects of stressors in other animal models of PTSD, it would be interesting to determine in future experiments whether the current effects can be reproduced following a single administration of SSR125543 given immediately after stress.

Acknowledgments We thank Drs. Bruno Biton, Christophe Lanneau and Philippe Pichat for their advice, and Pauline Cervello, and Nicolas Redon for technical help with the electrophysiological studies.

Conflict of interest This study was sponsored by Sanofi. Julie Philbert and Guy Griebel are employees of Sanofi. Catherine Belzung has no financial interests to disclose.

References

Acheson DT, Gresack JE, Risbrough VB (2012) Hippocampal dysfunction effects on context memory: possible etiology for posttraumatic stress disorder. Neuropharmacology 62:674–685

Adamec R, Fougere D, Risbrough V (2010) CRF receptor blockade prevents initiation and consolidation of stress effects on affect in the predator stress model of PTSD. Int J Neuropsychopharmacol 13:747–757

Adamec R, Muir C, Grimes M, Pearcey K (2007) Involvement of noradrenergic and corticoid receptors in the consolidation of the lasting anxiogenic effects of predator stress. Behav Brain Res 179:192–207

Arnsten AF (2009) Stress signalling pathways that impair prefrontal cortex structure and function. Nat Rev Neurosci 10:410–422

Baker DG, West SA, Nicholson WE, Ekhator NN, Kasckow JW, Hill KK, Bruce AB, Orth DN, Geracioti TD Jr (1999) Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. Am J Psychiatry 156:585–588

Benowitz LI, Routtenberg A (1997) GAP-43: an intrinsic determinant of neuronal development and plasticity. Trends Neurosci 20:84–91

Bremner JD, Licinio J, Darnell A, Krystal JH, Owens MJ, Southwick SM, Nemeroff CB, Charney DS (1997) Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. Am J Psychiatry 154:624–629

Bremner JD, Vermetten E (2004) Neuroanatomical changes associated with pharmacotherapy in posttraumatic stress disorder. Ann N Y Acad Sci 1032:154–157

Brewin CR, Kleiner JS, Vasterling JJ, Field AP (2007) Memory for emotionally neutral information in posttraumatic stress disorder: a meta-analytic investigation. J Abnorm Psychol 116:448–463

Chen Y, Dube CM, Rice CJ, Baram TZ (2008) Rapid loss of dendritic spines after stress involves derangement of spine dynamics by corticotropin-releasing hormone. J Neurosci 28:2903–2911

de Kleine RA, Hendriks GJ, Kusters WJ, Broekman TG, van Minnen A (2012) A Randomized placebo-controlled trial of D-cycloserine to enhance exposure therapy for posttraumatic stress disorder. Biol Psychiatry 71:962–968

De Kloet ER, Karst H, Joels M (2008) Corticosteroid hormones in the central stress response: quick-and-slow. Front Neuroendocrinol 29:268–272

De Kloet ER, Oitzl MS, Joels M (1999) Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci 22:422–426

Donohue HS, Gabbott PL, Davies HA, Rodriguez JJ, Cordero MI, Sandi C, Medvedev NI, Popov VI, Colyer FM, Peddie CJ, Stewart MG (2006) Chronic restraint stress induces changes in synapse morphology in stratum lacunosum-moleculare CA1 rat hippocampus: a stereological and three-dimensional ultrastructural study. Neuroscience 140:597–606

Fujishiro J, Imanishi T, Onozawa K, Tsushima M (2002) Comparison of the anticholinergic effects of the serotonergic antidepressants, paroxetine, fluvoxamine and clomipramine. Eur J Pharmacol 454:183–188

Griebel G, Holsboer F (2012) Neuropeptide receptor ligands as drugs for psychiatric diseases: the end of the beginning? Nat Rev Drug Discov 11:462–478

Griebel G, Simiand J, Steinberg R, Jung M, Gully D, Roger P, Geslin M, Scatton B, Maffrand JP, Soubrié P (2002) 4-(2-Chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluoro-4-me thylphenyl)ethyl]5-methyl-*N*-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor$_1$ receptor antagonist: II. Characterization in rodent models of stress-related disorders. J Pharmacol Exp Ther 301:333–345

Hajszan T, Maclusky NJ, Leranth C (2005) Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. Eur J Neurosci 21:1299–1303

Hofmann SG, Meuret AE, Smits JA, Simon NM, Pollack MH, Eisenmenger K, Shiekh M, Otto MW (2006) Augmentation of

exposure therapy with D-cycloserine for social anxiety disorder. Arch Gen Psychiatry 63:298–304
Holmes A, Heilig M, Rupniak NM, Steckler T, Griebel G (2003) Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol Sci 24:580–588
Holsboer F, Ising M (2008) Central CRH system in depression and anxiety—evidence from clinical studies with CRH1 receptor antagonists. Eur J Pharmacol 583:350–357
Hubbard DT, Nakashima BR, Lee I, Takahashi LK (2007) Activation of basolateral amygdala corticotropin-releasing factor 1 receptors modulates the consolidation of contextual fear. Neuroscience 150:818–828
Ivy AS, Rex CS, Chen Y, Dube C, Maras PM, Grigoriadis DE, Gall CM, Lynch G, Baram TZ (2010) Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. J Neurosci 30:13005–13015
Kerr DS, Campbell LW, Applegate MD, Brodish A, Landfield PW (1991) Chronic stress-induced acceleration of electrophysiologic and morphometric biomarkers of hippocampal aging. J Neurosci 11:1316–1324
Kohda K, Harada K, Kato K, Hoshino A, Motohashi J, Yamaji T, Morinobu S, Matsuoka N, Kato N (2007) Glucocorticoid receptor activation is involved in producing abnormal phenotypes of single-prolonged stress rats: a putative post-traumatic stress disorder model. Neuroscience 148:22–33
Ledgerwood L, Richardson R, Cranney J (2004) D-Cycloserine and the facilitation of extinction of conditioned fear: consequences for reinstatement. Behav Neurosci 118:505–513
Ledgerwood L, Richardson R, Cranney J (2005) D-Cycloserine facilitates extinction of learned fear: effects on reacquisition and generalized extinction. Biol Psychiatry 57:841–847
Li F, Tsien JZ (2009) Memory and the NMDA receptors. N Engl J Med 361:302–303
Li X, Han F, Liu D, Shi Y (2010) Changes of Bax, Bcl-2 and apoptosis in hippocampus in the rat model of post-traumatic stress disorder. Neurol Res 32:579–586
Litz BT, Salters-Pedneault K, Steenkamp MM, Hermos JA, Bryant RA, Otto MW, Hofmann SG (2012) A randomized placebo-controlled trial of D-cycloserine and exposure therapy for post-traumatic stress disorder. J Psychiatr Res 46:1184–1190
McEwen BS (2000) Effects of adverse experiences for brain structure and function. Biol Psychiatry 48:721–731
McEwen BS, Sapolsky RM (1995) Stress and cognitive function. Curr Opin Neurobiol 5:205–216
Naudon L, Hotte M, Jay TM (2007) Effects of acute and chronic antidepressant treatments on memory performance: a comparison between paroxetine and imipramine. Psychopharmacology (Berl) 191:353–364
Nemeroff CB, Owens MJ (2004) Pharmacologic differences among the SSRIs: focus on monoamine transporters and the HPA axis. CNS Spectr 9:23–31
Overstreet DH, Griebel G (2004) Antidepressant-like effects of CRF1 receptor antagonist SSR125543 in an animal model of depression. Eur J Pharmacol 497:49–53
Philbert J, Pichat P, Beeske S, Decobert M, Belzung C, Griebel G (2011) Acute inescapable stress exposure induces long-term sleep disturbances and avoidance behavior: a mouse model of post-traumatic stress disorder (PTSD). Behav Brain Res 221:149–154
Philbert J, Pichat P, Palme R, Belzung C, Griebel G (2012) The CRF_1 receptor antagonist SSR125543 attenuates long-term cognitive deficit induced by acute inescapable stress in mice, independently from the hypothalamic pituitary adrenal axis. Pharmacol Biochem Behav 102:415–422
Pichat P, Bergis OE, Terranova JP, Urani A, Duarte C, Santucci V, Gueudet C, Voltz C, Steinberg R, Stemmelin J, Oury-Donat F, Avenet P, Griebel G, Scatton B (2007) SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (II) efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia. Neuropsychopharmacology 32:17–34
Poleszak E, Wlaz P, Wrobel A, Fidecka S, Nowak G (2008) NMDA/glutamate mechanism of magnesium-induced anxiolytic-like behavior in mice. Pharmacol Rep 60:655–663
Rebaudo R, Melani R, Balestrino M, Izvarina N (2001) Electrophysiological effects of sustained delivery of CRF and its receptor agonists in hippocampal slices. Brain Res 922:112–117
Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, Hodges L, Davis M (2004) Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. Arch Gen Psychiatry 61:1136–1144
Rey M, Carlier E, Soumireu-Mourat B (1987) Effects of corticosterone on hippocampal slice electrophysiology in normal and adrenalectomized BALB/c mice. Neuroendocrinology 46:424–429
Roozendaal B, McEwen BS, Chattarji S (2009) Stress, memory and the amygdala. Nat Rev Neurosci 10:423–433
Rorick-Kehn LM, Hart JC, McKinzie DL (2005) Pharmacological characterization of stress-induced hyperthermia in DBA/2 mice using metabotropic and ionotropic glutamate receptor ligands. Psychopharmacology 1–15
Rouaud E, Billard JM (2003) D-Cycloserine facilitates synaptic plasticity but impairs glutamatergic neurotransmission in rat hippocampal slices. Br J Pharmacol 140:1051–1056
Sairanen M, O'Leary OF, Knuuttila JE, Castren E (2007) Chronic antidepressant treatment selectively increases expression of plasticity-related proteins in the hippocampus and medial prefrontal cortex of the rat. Neuroscience 144:368–374
Sapolsky RM (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57:925–935
Schuff N, Neylan TC, Fox-Bosetti S, Lenoci M, Samuelson KW, Studholme C, Kornak J, Marmar CR, Weiner MW (2008) Abnormal *N*-acetylaspartate in hippocampus and anterior cingulate in posttraumatic stress disorder. Psychiatry Res 162:147–157
Siegmund A, Wotjak CT (2006) Toward an animal model of posttraumatic stress disorder. Ann N Y Acad Sci 1071:324–334
Sousa N, Lukoyanov NV, Madeira MD, Almeida OF, Paula-Barbosa MM (2000) Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. Neuroscience 97:253–266
Surget A, Wang Y, Leman S, Ibarguen-Vargas Y, Edgar N, Griebel G, Belzung C, Sibille E (2009) Corticolimbic transcriptome changes are state-dependent and region-specific in a rodent model of depression and of antidepressant reversal. Neuropsychopharmacology 34:1363–1380
Tokarski K, Czyrak A, Mackowiak M, Wedzony K, Bijak M (1996) Prolonged treatment with antidepressants increases the 5-HT1A-mediated inhibition of hippocampal neurons without changing the 5-HT1A receptor binding. Acta Physiol Hung 84:343–344
Urani A, Philbert J, Cohen C, Griebel G (2011) The corticotropin-releasing factor 1 receptor antagonist, SSR125543, and the vasopressin 1b receptor antagonist, SSR149415, prevent stress-induced cognitive impairment in mice. Pharmacol Biochem Behav 98:425–431
Van PK, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE (2000) Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. J Comp Neurol 428:191–212
Vermetten E, Vythilingam M, Southwick SM, Charney DS, Bremner JD (2003) Long-term treatment with paroxetine increases verbal

declarative memory and hippocampal volume in posttraumatic stress disorder. Biol Psychiatry 54:693–702

Vidal C, Jordan W, Zieglgansberger W (1986) Corticosterone reduces the excitability of hippocampal pyramidal cells in vitro. Brain Res 383:54–59

Wang H, Zuo D, He B, Qiao F, Zhao M, Wu Y (2012) Conditioned fear stress combined with single-prolonged stress: a new PTSD mouse model. Neurosci Res 73:142–152

Woon FL, Sood S, Hedges DW (2010) Hippocampal volume deficits associated with exposure to psychological trauma and posttraumatic stress disorder in adults: a meta-analysis. Prog Neuropsychopharmacol Biol Psychiatry 34:1181–1188

Yamamoto S, Morinobu S, Fuchikami M, Kurata A, Kozuru T, Yamawaki S (2008) Effects of single prolonged stress and D-cycloserine on contextual fear extinction and hippocampal NMDA receptor expression in a rat model of PTSD. Neuropsychopharmacology 33:2108–2116

Youssef FF, Addae JI, Stone TW (2006) NMDA-induced preconditioning attenuates synaptic plasticity in the rat hippocampus. Brain Res 1073–1074:183–189

Zahorodna A, Tokarski K, Hess G (2006) Imipramine treatment ameliorates corticosterone-induced alterations in the effects of 5-HT1A and 5-HT4 receptor activation in the CA1 area of rat hippocampus. Eur Neuropsychopharmacol 16:383–390