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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

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ABSTRACT

The selective antagonist at the CRF_1 receptor, SSR125543, has been shown to produce anxiolytic-like effects in a number of animal models. The aim of the present study was to verify whether these effects are mediated by an action on the hypothalamic pituitary adrenal (HPA) axis. SSR125543 effects were evaluated in a mouse model of post-traumatic stress disorder. Animals received two unavoidable electric foot-shocks (1.5 mA/2 s). Two weeks later they were placed in the shock context and fecal and plasma corticosterone levels were measured by enzyme-immunoassay. Their cognitive performances were evaluated using the object recognition task following administration of SSR125543 at 3, 10 and 30 mg/kg or paroxetine at 20 mg/kg (i.p.), used as positive control. To assess the involvement of the HPA axis in the drug effects, a separate group of animals was subjected to the same procedure and drug regimen, but was treated with dexamethasone to blunt the HPA axis. Stressed mice had higher levels of corticosterone following re-exposure to the context and displayed impaired cognitive performance as compared to control animals. Corticosterone levels were normalized in stressed mice by SSR125543 and the cognitive deficit was significantly attenuated by SSR125543 and paroxetine, whether the HPA axis was blunted or not. These findings confirm that SSR125543 is able to attenuate the deleterious effects of stressful exposure. Importantly, the observation that these effects were still present in dexamethasone-treated mice indicates that this action does not necessarily involve pituitary-adrenal axis blockade, thereby suggesting that extra-pituitary CRF_1 receptors may play a role in these effects.

1. Introduction

Corticotropin-releasing factor (CRF) is a 41 amino acid neuropeptide which has been identified as the main physiological regulator of stress. CRF is synthesized in neurons of the paraventricular hypothalamic nucleus and released into the pituitary portal blood where it triggers the secretion of adrenocorticotropin (ACTH) from the anterior lobe. Subsequently, corticosterone (in rodents) or cortisol (in human) is secreted from the adrenal cortex into blood and exerts a negative feedback on the hypothalamic pituitary adrenal (HPA) axis (Sapolsky and McEwen, 1985; De Kloet, 2000). Besides its function as a major physiological regulator of the HPA system, CRF plays a neuromodulatory role. CRF-containing neurons and receptors are found in brain areas involved in stress responses, including the amygdala, the lateral septum, the locus coeruleus and the brainstem raphe nuclei (Keegan et al., 1994; Van Pett et al., 2000; Holmes et al., 2003). Studies in animals have shown that central administration of CRF or CRF fragments to rodents as well as CRF overexpression in transgenic mice resulted in increased anxiety- and depression-like behaviors (Stenzel-Poore et al., 1994).

Two CRF receptors have been identified, namely CRF_1 and CRF_2. The peptide acts predominantly through the CRF_1 subtype to modulate anxiety- and depression-related responses, an observation which resulted in extensive validation of the CRF_1 receptor as potential drug target and the discovery of several selective non-peptide CRF_1 receptor antagonists (Holsboer and Ising, 2008). Among the most investigated CRF_1 receptor antagonists is SSR125543, which has been reported to produce anxiolytic- and antidepressant-like effects in a variety of rodent models. For example, the compound reduced anxiety-like behaviors in models involving inescapable stress, such as conflict procedures, following social defeat or predator exposure (Griebel et al., 2002). In addition, SSR125543 produced antidepressant-like effects in several procedures in rodents (Griebel et al., 2002), including the forced swimming test (Overstreet and Griebel, 2004) and the chronic mild stress (Alonso et al., 2003;

Abbreviations: ACTH, adrenocorticotropin; CM, corticosterone metabolite; CRF, corticotropin releasing factor; DEX, dexamethasone; EIA, enzyme-immunoassay; HPA, hypothalamic pituitary adrenal; ORT, object recognition task; PTSD, post-traumatic stress disorder.

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Surget et al., 2008, 2009). A recent study also demonstrated that SSR125543 prevented cognitive impairment induced by predator exposure in mice (Urani et al., 2011).

Nevertheless, the question whether the effects of the compound on emotionality involve an action on the HPA axis, on extra-pituitary CRF_1 receptors or on both, still remains unclear. Multiple lines of evidence suggest that a dysregulation of the HPA axis plays an important role in the pathogenesis of mood and anxiety disorders. Both clinical and preclinical studies support the view that CRF may be hypersecreted from the hypothalamus in anxiety disorders and report a blunted ACTH response to CRF challenge in depressed patients (Gold et al., 1986; Holsboer, 1986; Belzung and Billette de Villemeur, 2010). Elevated levels of CRF in cerebrospinal fluid and plasma have been found in patients suffering from post-traumatic disorder (PTSD) (Baker et al., 1999; Bremner et al., 1997; de Kloet et al., 2008). One plausible mechanism is down-regulation of pituitary CRF receptors, presumably secondary to increased hypothalamic CRF release. Preclinical studies also suggest that CRF effects are rather mediated by CRF receptors present in the brain. Indeed, intracerebroventricular administration of CRF was shown to produce physiological and behavioral alterations similar to those observed in laboratory animal in response to stress; however these effects were not observed after systemic administration of CRF and were not blocked by hypophysectomy, vagotomy, adrenalectomy or pretreatment with dexamethasone (Griebel, 1999). In addition, Muller et al. (2003), studying conditional CRF_1 receptor knockout mouse line, showed that limbic CRF_1 receptor modulates anxiety-related behavior and that this effect is independent of HPA system function. Moreover, Lu et al. (2008) reported that the administration of a CRF_1 receptor antagonist was able to revert abnormal active stress-coping behavior and to blunt the hypersensitive HPA responses induced by stress on conditional mouse model of CRF brain overexpression, suggesting that extra-hypothalamic CRF_1 receptors are implicate in these responses.

In this context, the aim of the present study was to verify whether the anxiolytic-like effects of the CRF_1 receptor antagonist SSR125543 are mediated by a direct action on CRF_1 receptors located within components of the HPA axis. We used a recently established mouse model of PTSD (Philbert et al., 2011), which is based on the exposure of mice to electric foot-shocks, followed two weeks later by the assessment of their cognitive performance in the object recognition task (ORT). The rationale of investigating the effects of stress on cognitive function originates from the observation that PTSD patients display alterations in cognitive processes, such as an impairment in non trauma-related episodic memory performance (*for review see* Brewin et al., 2007). The ORT assesses short-term visual episodic memory (Dodart et al., 1997; Ennaceur and Delacour, 1988). It is based on the natural tendency of rodents to explore a novel object more than a familiar one and it has the advantage of not involving goal-oriented behaviors (e.g., reward, escape). Our previous study has demonstrated that foot-shock stress produced a significant impairment in recall performance in this test two weeks later (Philbert et al., 2010). The effects of SSR125543 were tested in intact animals and in mice treated by the synthetic glucocorticoid agonist, dexamethasone, in order to blunt the HPA axis. In a parallel experiment using a similar procedure, corticosterone levels were measured following re-exposure to the shock apparatus, two weeks after the application of stress, and the ability of SSR125543 to normalize stress-induced changes in corticosterone levels was assessed. The prototypical 5-HT reuptake inhibitor (SSRI), paroxetine, was used as a positive control throughout experiments.

2. Material and methods

2.1. Animals

Swiss male mice (Janvier, Le Genest St-Isle, France) weighing 20 to 22 g at the start of the experiment were used. They were housed individually in plastic cages (30 cm × 18 cm × 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.

2.2. 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.

2.3. Drug administration

Paroxetine (Sigma-Aldrich, CAS 110429-35-1), dexamethasone (Sigma-Aldrich, CAS 50-02-2), and SSR125543, synthesized by Sanofi Medicinal Chemistry, were suspended in saline with methylcellulose (0.6%) and Tween 80 (0.1%). Paroxetine and SSR125543 were administered via intraperitoneal (i.p.) route and dexamethasone (DEX) was given subcutaneously (s.c.). Concentrations were adjusted to administer a final volume of 10 ml/kg of body weight. Control animals received vehicle administration, i.e. saline with methylcellulose (0.6%) and Tween 80 (0.1%).

2.4. Assessment of corticosterone levels

2.4.1. Collection of feces, extraction procedure and analysis of fecal corticosterone metabolites

The collected fecal samples were analyzed for immunoreactive corticosterone metabolites (CM) using a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay (EIA). Before EIA analysis, the fecal samples were dried and homogenized, and aliquots of 0.05 g were extracted with 1 ml of 80% methanol. Details of the extraction procedure and the assay performance have been described by Touma et al. (2003, 2004).

Data were analyzed using a two-way ANOVA with repeated measures with *stress* and *delay* as variables followed by a post-hoc complementary analysis on stress effect for each level of factor *delay* (Winer analysis). A Student's *t*-test was performed to analyze the effect of the delay from context re-exposure on CM levels in stressed and control mice.

2.4.2. Blood collection and corticosterone level analysis

Blood was sampled between 10:00 and 11:30 a.m. by cardiac puncture under isoflurane anesthesia (3.5%) and collected into Microvette® 500 LH-Gel tubes. Plasma was separated from whole blood by centrifugation (5 min, 10,000 *g*) and stored in low-binding Eppendorf tubes at −20 °C until analyzed. On the day of the assay, samples were diluted in buffer (1/40). Corticosterone levels were then analyzed in diluted plasma by a corticosterone EIA kit (Enzo® Life Sciences, *Catalog No. ADI-900-097*) according to the manufacturer's instructions using Victor™2 counter plate reader for photometry (Wallac, 1420 Multilabel Counter). The sensitivity threshold of the assay was 27 pg/ml. Statistical analyses were performed using two-way ANOVAs using *stress* and *treatment* as variables.

2.5. Object recognition task (ORT)

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 and a plastic

piece of construction game. During the first session, Swiss mice were allowed to become familiar with the experimental environment for 7 min and the 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 15 s of object exploration (acquisition session). 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 was recorded. Combinations of orders of presentation and locations of objects were balanced to reduce potential biases owing to spatial or object preferences. Under a short-term inter-trial procedure, animals spent more time exploring the new object compared to the familiar one, reflecting a remembering 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) (Fig. 1).

The following parameters were analyzed: (a) time to reach 15 s of exploration of the 2 identical objects in the acquisition session; (b) time of exploration of each object during retrieval session; (c) total time of exploration (sum of both object exploration times) and (d) the ratio of the time exploring the new object of the total time of exploration. For exploration time, data were analyzed using a two-way ANOVA with repeated measures with *object* as a fixed factor to analyze the ability of animals to discriminate between the familiar and the novel objects. Effect of *object* factor was then analyzed by Winer analysis for each level of *group* factor. A Student's *t*-test vs 0.5 (chance level value) was performed to analyze the ratios. For the time to reach 15 s of exploration during acquisition and the total time of exploration during retrieval session, a one-way ANOVA was performed to analyze the differences between groups, followed by a Dunnett's post-hoc analysis.

2.6. Experimental procedures

Experiment 1 examined the effect of re-exposure to the shock context on CM levels. Thirteen days after foot-shock application, mice were placed again at 9 a.m. (+/−10 min) for 1 min into the shock apparatus, before being returned to their homecage. Four, six, eight and ten hours later, cage sawdust was changed. Feces samples were collected either 6 to 8 (3 to 5 p.m.) or 8 to 10 (5 to 7 p.m.) h after the context re-exposure for CM analysis.

Experiment 2 tested whether acute SSR125543 treatment reduces corticosterone level increase induced by re-exposure to the shock context. Mice received one administration of SSR125543 at 10 mg/kg (i.p) or vehicle at day 13 following acute inescapable stress exposure. One hour later, they were re-exposed to the shock apparatus for 1 min. Blood was sampled 15 min after re-exposure to the shock context for corticosterone analysis.

Experiment 3 evaluated the cognitive performance of stressed mice using the ORT following acute administration of SSR125543 (3, 10 or 30 mg/kg, i.p.) or paroxetine (20 mg/kg, i.p.). The test was performed at days 14 and 15 following the stress procedure. Treatments were administered 1 h before session 2 (acquisition) of the ORT. In parallel, a control experiment was performed in non-stressed mice to determine potential effects of SSR125543 (30 mg/kg) or paroxetine (20 mg/kg) on cognitive performance per se.

Experiment 4 investigated whether the effects of SSR125543 on stress-induced alterations of cognitive performance in the ORT are mediated by an action on the HPA axis. Animals were subjected to the procedure described in experiment 3, but they were treated with DEX (500 μg/kg, s.c.) to blunt the HPA axis 90 min before treatment administration (i.e. paroxetine at 20 mg/kg, SSR125543 at 10 mg/kg or vehicle, i.p.).

Experiment 5 assessed the ability of the DEX dose tested to blunt the HPA axis. Blood was collected 90 min after DEX (500 μg/kg, s.c.) or vehicle (methylcellulose 0.6%, NaCl 0.9%) administration to assess corticosterone baseline level in stressed and non-stressed mice.

3. Results

3.1. Experiment 1: effects of acute inescapable stress exposure on fecal CM levels in mice

Since the time-course of CM excretion has been shown to be around 8 to 10 h in mice (Touma et al., 2003), feces were collected either 6 to 8 or 8 to 10 h after re-exposure to the shock context for CM analysis. CM levels were not significantly different between samples collected from control and stressed mice 6 to 8 h after re-exposure to the shock context (Fig. 2A: $F_{(1;54)}=3.192$, $p=0.0777$). CM levels were significantly increased in samples collected between 8 and 10 h post-context re-exposure when compared to samples collected between 6 and 8 h (Fig. 2A: $F_{(1;54)}=13.193$, $p=0.0006$ and $F_{(1;54)}=41.135$, $p<0.0001$ for control and stressed groups, respectively). Further analysis showed that the CM increase was higher in stressed than in control mice (Fig. 2B: $t=-2.831$, $p=0.0065$) which results in higher CM levels in stressed mice compared to controls when samples were performed between 8 and 10 h post-context re-exposure (Fig. 2A: $F_{(1;54)}=3.192$, $p=0.0004$).

3.2. Experiment 2: effects of SSR125543 on the increase in corticosterone levels following stress exposure

In line with the previous experiment measuring CM level in feces, plasma levels of corticosterone were significantly higher in vehicle-treated stressed mice compared to non-stressed controls following re-exposure to the stress context (Fig. 3: $F_{(1;45)}=4.186$, $p=0.0466$). SSR125543 given at 10 mg/kg (i.p.) 1 h before context re-exposure, prevented the increase in plasma corticosterone in stressed mice (Fig. 3: $F_{(1;45)}=10.784$, $p=0.0020$). In non-stressed mice, SSR125543 had no significant effect on baseline corticosterone levels (Fig. 3: $F_{(1;45)}=2.293$, $p=0.1370$, vehicle- vs SSR125543-treated non-stressed mice).

3.3. Experiment 3: effects of SSR125543 on long-term cognitive deficit induced by acute inescapable stress in untreated mice

Under control conditions (no stress, vehicle treatment), mice spent more time exploring the new object than the familiar one (Fig. 4A: $F_{(1;85)}=30.387$, $p<0.0001$) with a discrimination ratio significantly different from the chance value 0.5 (Fig. 4B: $t=5.212$, $p=0.0002$). Conversely, mice previously exposed to electrical foot-shocks two weeks before the ORT spent the same amount of time exploring the new and the familiar objects (Fig. 4A: $F_{(1;85)}=0.383$, $p=0.5376$), indicating that stressed mice did not discriminate between the two objects.

The administration of SSR125543 1 h before the learning session did not affect the discrimination ratio in non-stressed animals during recall (Fig. 4A: $F_{(1;85)}=6.660$, $p=0.0116$; Fig. 4B: $t=2.980$, $p=0.0125$). However, it prevented significantly the effects of stress exposure at all three doses tested (i.e. 3, 10 and 30 mg/kg, i.p.) as it increased the amount of time spent investigating the novel object compared to the familiar one (Fig. 4A: $F_{(1;85)}=4.929$, $p=0.0291$; $F_{(1;85)}=7.138$, $p=0.0090$; $F_{(1;85)}=6.376$, $p=0.0134$ for 3, 10 and 30 mg/kg, respectively). Expressed as a ratio, the relative time of exploration of the novel object was different from the chance value 0.5 for SSR125543-treated stressed groups (Fig. 4B: $t=3.439$, $p=0.0055$; $t=4.166$, $p=0.0016$; $t=2.689$, $p=0.0227$, for 3, 10 and 30 mg/kg, respectively).

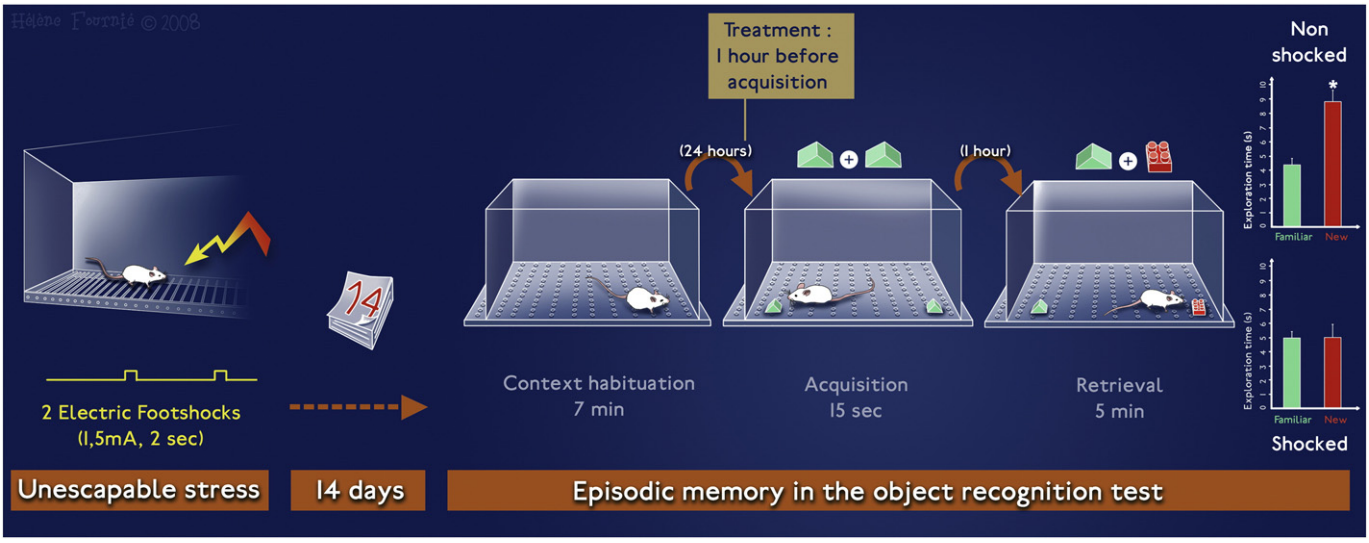

Fig. 1. Experimental design.

Paroxetine treatment (20 mg/kg, i.p.) produced a significant effect in non-stressed mice, as they were unable to discriminate between the two objects during recall (Fig. 4A: $F_{(1;85)}=3.191$, $p=0.0776$), they spent less time exploring objects (Table 1: $F_{(7;85)}=2.50$, $p=0.0016$) and the ratio was not different from chance value (Fig. 4B: $t=2.165$, $p=0.0532$). However, when administered to stressed mice, paroxetine prevented the stressed-induced deficit in object discrimination (Fig. 4A: $F_{(1;85)}=8.955$, $p=0.0036$; Fig. 4B: $t=3.339$, $p=0.0087$).

In stressed mice, neither drug treatment modified significantly time to reach 15 s of object exploration during acquisition and total exploration during retrieval (Table 1), suggesting that the effects of SSR125543 and paroxetine have not been contaminated by motor effects.

3.4. Experiments 4 and 5: effects of SSR125543 on long-term cognitive deficit induced by acute inescapable stress in DEX-treated mice

DEX-treated mice exhibited a significant decrease in plasma corticosterone levels compared to vehicle-treated mice (Fig. 5: $F_{(1;43)}=7.613$, $p=0.0085$). Winer analysis indicated that corticosterone levels were significantly decreased in DEX-treated stressed mice compared to vehicle-treated stressed animals (Fig. 5: $F_{(1;43)}=7.325$, $p=0.0097$), whereas DEX-treated non-stressed mice exhibited only a non-significant tendency to a decrease in corticosterone levels compared to vehicle-treated control mice (Fig. 5: $F_{(1;43)}=1.634$, $p=0.2080$).

DEX treatment did not affect performance of mice in the ORT as non-stressed mice were able to discriminate between both objects during the recall session, (Fig. 6A: $F_{(1;53)}=23.907$, $p<0.0001$; Fig. 6B: $t=5.111$, $p=0.0006$) and as DEX-treated stressed mice displayed similar impaired episodic memory to stressed controls (Fig. 6A: $F_{(1;53)}=1.800$, $p=0.1854$) with a discrimination ratio not different from the chance value 0.5 (Fig. 6B: $t=1.846$, $p=0.0979$). The administration of SSR125543 (10 mg/kg, i.p.) was devoid of effect in DEX-treated non-stressed mice, animals being able to discriminate between the two objects (Fig. 6A: $F_{(1;53)}=13.069$, $p=0.0007$). As was the case in the previous experiment, paroxetine (20 mg/kg, i.p.), here administered to DEX-treated non-stressed mice produced an impairment in episodic memory. When tested in DEX-treated stressed mice, both compounds prevented the occurrence of cognitive deficit as shown by the amount of time investigating the novel object compared to the familiar one (Fig. 6A: $F_{(1;53)}=4.538$, $p=0.0378$; $F_{(1;53)}=6.908$, $p=0.0112$ for paroxetine- and or SSR125543-treated groups, respectively). Expressed as a ratio, the relative time of exploration of the novel object was different from the chance value 0.5 (Fig. 6A: $t=3.522$, $p=0.0065$; $t=5.677$, $p=0.0003$ for paroxetine- and SSR125543-treated groups, respectively). The time to reach 15 s of object exploration during acquisition and total exploration during retrieval was not significantly affected by the drug treatments (Table 2).

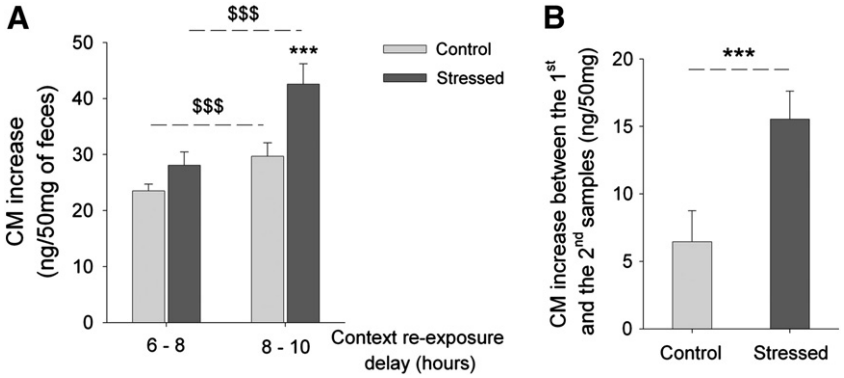

Fig. 2. Effect of re-exposure to the shock context on CM levels in control and stressed mice. (A) Data represent mean concentrations of fecal CM expressed in ng/50 mg feces (±s.e.m.). $^{\S\S\S}p<0.001$ first (6–8 h) vs second (8–10 h) fecal samples. (B) Bars represent mean (±s.e.m.) fecal CM concentration increase between the first and the second fecal samples in control and stressed mice. $^{***}p<0.001$ control vs stressed mice. ($n=27$–29).

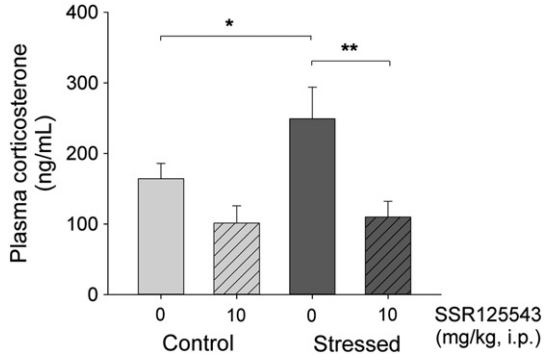

Fig. 3. Effect of re-exposure to the shock context on plasma corticosterone levels in acute SSR125543-treated mice. Bars represent means (±s.e.m.) of plasma corticosterone concentration. *p<0.05, ***p<0.001 between line-connected groups. (n=12–13).

Table 1
Activity measures after drug administration during the acquisition session (time to reach 15 s exploration) and during the retrieval session (total time exploration).

	Treatment	n	Time to reach 15 s of object exploration (s)	Sum of object exploration during retrieval (s)
Control mice	Vehicle	13	146.1 ± 14.4	21.9 ± 2.22
	SSR125543 (30 mg/kg)	12	142.1 ± 14.05	18.3 ± 1.98
	Paroxetine (20 mg/kg)	12	249.8 ± 33.23	12.2 ± 1.36**
Stressed mice	Vehicle	11	150.2 ± 17.43	15.4 ± 1.36
	SSR125543 (3 mg/kg)	12	191 ± 31.06	16 ± 2.32
	SSR125543 (10 mg/kg)	12	172 ± 15.59	16 ± 1.63
	SSR125543 (30 mg/kg)	11	186.3 ± 22.37	14.5 ± 1.3
	Paroxetine (20 mg/kg)	10	206.8 ± 20.9	15.6 ± 1.93

** p<0.01 vs vehicle-treated unstressed group.

4. Discussion

The main objective of this study was to verify whether the anxiolytic-like effects of the CRF_1 receptor antagonist, SSR125543, in a mouse model of traumatic stress exposure involve an action on the HPA axis. Results demonstrated that the blunting of the HPA axis did not alter the action of the drug on the long-lasting cognitive deficit induced by stress, thereby suggesting that extra-pituitary CRF_1 receptors may have been involved in these effects.

The assessment of corticosterone levels showed that when re-exposed to the traumatic context thirteen days after the stress (i.e. two electric foot-shocks of 1.5 mA), stressed mice exhibited greater corticosterone levels compared to controls. This observation was supported by both investigations of corticosterone in blood samples and CM levels in feces. It is important to note that baseline levels of corticosterone did not differ between control and stressed mice and that CM levels were not significantly different between both groups when collected between 6 and 8 h following context exposure. This later observation fits well with the findings of Touma et al. (2003) who reported that the peak concentration of radioactivity was observed only about 10 h after [^{3}H]-corticosterone injection in mice. Previous studies have reported long-lasting HPA axis disturbance following acute stress exposure by measuring corticosteroids in response to the same (Marti et al., 2001; Munoz-Abellan et al., 2011) or to novel stressors (Belda et al., 2008). Increased corticosterone levels upon re-exposure to the shock context have been reported by Hagewoud et al. (2011) in rats 24 h after the stress. In the present study, the corticosterone level increase following re-exposure to the shock context was still present when measured two weeks after the initial stress. These results parallel clinical observations which report increased cortisol levels during confrontation with trauma reminders (Gola et al., 2012; Elzinga et al., 2003).

The behavioral findings showed that the application of electric foot-shocks led to an impairment in episodic memory two weeks later. Although cognitive deficits in rodents following stress exposure have been reported many times, most of these studies used either chronic stress (Yun et al., 2010; Elizalde et al., 2008; Wang et al., 2011a), early-life stress (Oitzl et al., 2000; Aisa et al., 2007; Rice et al., 2008; Wang et al., 2011b) or have observed the deficit immediately following an acute stress exposure (Urani et al., 2011; Sandi et al., 2005; Diamond et al., 1999, 2006; Morrow et al., 2000). To the best of our knowledge, only one study has reported cognitive impairment that persisted over time following a one time predator exposure (El Hage et al., 2006a). The current findings of long-term memory deficit following traumatic stress exposure may be reminiscent of some aspects of the cognitive impairment observed in humans suffering from PTSD. Indeed, a large number of clinical studies have reported alterations in learning and memory in patients with PTSD (Vasterling et al., 2002; Brewin et al., 2007; El Hage et al., 2006b).

The drug experiments showed that the SSRI, paroxetine, given 1 h before the learning session, attenuated the cognitive deficit induced by stress. SSRIs are widely used for the treatment of PTSD, including its cognitive symptoms (Bremner and Vermetten, 2004; Meltzer-Brody et al., 2000; Stein et al., 2009). The current data are in line with those reported previously by El Hage et al. (2004) and Urani et al. (2011) showing that acute SSRI treatment was able to prevent episodic memory deficit in the object recognition test two days following unavoidable cat exposure and in a modified version of the ORT, respectively. In contrast, acute paroxetine administration impaired

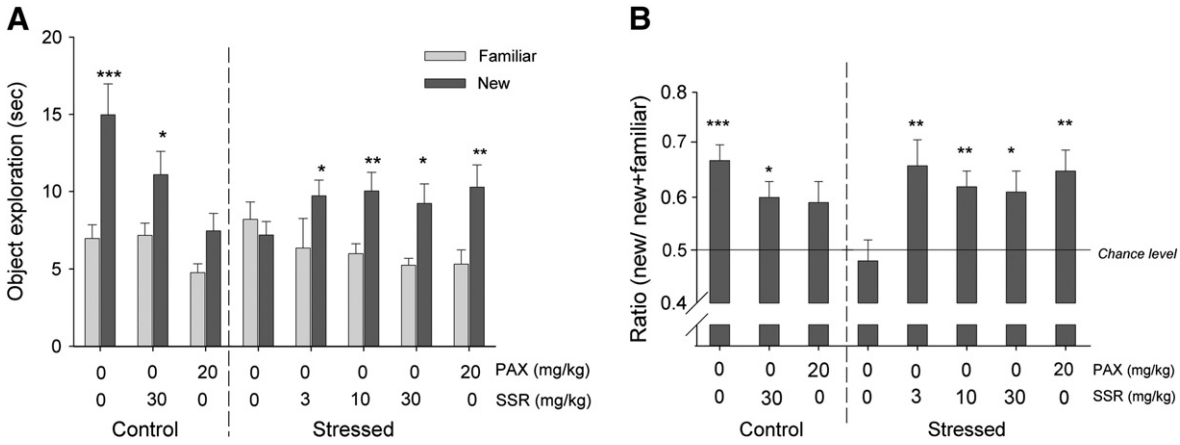

Fig. 4. Effects of SSR125543 (SSR) and paroxetine (PAX) treatment on short-term episodic memory impairment induced by an acute inescapable stress in mice. (A) Bars represent mean (±s.e.m.) of time spent exploring new and familiar objects. *p<0.05, **p<0.01, ***p<0.001 new vs familiar object. (B) Bars represent mean (±s.e.m.) of novelty index, i.e. the ratio of the time spent exploring the new object on the sum of time exploring the two objects. *p<0.05, **p<0.01 and ***p<0.001 vs 0.5. (n=10–13).

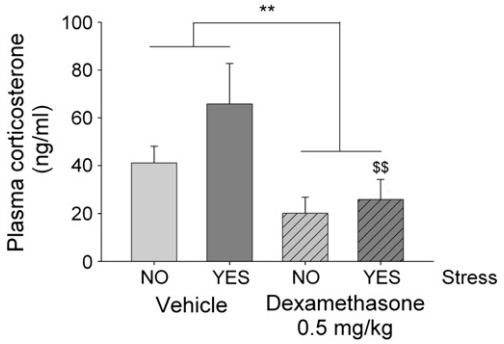

Fig. 5. Effects of DEX on corticosterone levels two weeks after inescapable stress in mice. Bars represent means (±s.e.m.) of plasma corticosterone concentration. **p<0.01 between vehicle- and DEX-treated groups. $$p<0.01 between vehicle- and DEX-treated stressed groups (n=12–13).

Table 2
Activity measures after drug administration during the acquisition session (time to reach 15 s exploration) and during the retrieval session (total time exploration).

	Treatment	n	Time to reach 15 s of object exploration (s)	Sum of object exploration during retrieval (s)
Control mice	DEX	10	154.7 ± 13.5	21.3 ± 1.64
	DEX + paroxetine	9	235 ± 42.65	14.7 ± 2.21
	DEX + SSR125543	10	157.4 ± 16.86	21.4 ± 2.74
Stressed mice	DEX	10	197.4 ± 38	15.8 ± 2.61
	DEX + paroxetine	10	253.9 ± 37.80	14.7 ± 2.41
	DEX + SSR125543	10	167.3 ± 19.95	17.5 ± 2.42

memory performance in unstressed mice. This result was previously reported in the ORT by Naudon et al. (2007) and may be due to anticholinergic side effects (Fujishiro et al., 2002). The CRF_1 receptor antagonist, SSR125543, also reversed the cognitive deficit induced by acute stress in the absence of effect on cognition per se. This finding is consistent with a recent study which reported that SSR125543 prevented cognitive impairment induced by predator stress exposure (Urani et al., 2011). Moreover, another study has explored recently the potential effect of a CRF_1 receptor antagonist on cognitive processes following cat exposure stress (Adamec et al., 2010). They showed that CRF_1 receptor blockade can interfere with the acquisition and consolidation of stressor effects on startle and returns risk assessment to baseline levels in stressed mice. These findings demonstrate the role of CRF_1 receptors in initiation and post-trauma consolidation of predator stress effect on anxiety-like behavior. These studies along with our data support the idea that CRF_1 antagonists can attenuate the deleterious effects of traumatic stress exposure and, as such, may be useful for the treatment of PTSD.

In the experiments where corticosterone levels were assessed, the administration of SSR125543 1 h before re-exposure to the stress context significantly reduced stress-induced corticosterone release, a finding which confirms a previous study showing that the drug decreased restraint-stress induced ACTH secretion (Gully et al., 2002). It is therefore tempting to suggest that the beneficial effect of SSR125543 on cognition in stressed mice may involve an action of the drug on the HPA axis. Preventing CRF, ACTH and glucocorticoids to exert their action has been suggested to be a possible strategy for short circuiting the deleterious effect of stress (Holsboer and Ising, 2008). To investigate this possibility, we have tested the effects of SSR125543 in HPA-blunted mice subjected to the same experimental stress procedure. DEX, a potent glucocorticoid, was used to cause suppression of the HPA axis. Note that this treatment did not affect memory performance in both control and stressed mice. However, the corticosterone suppression following DEX administration was more important in stressed than in control mice. This latter observation is in line with reported clinical data from PTSD patients, which demonstrated significantly more salivary cortisol suppression in these patients compared to healthy controls in response to DEX administration (De Kloet et al., 2007). Our drug experiment showed that the cognitive-normalizing effects of SSR125543 following stress were still present in DEX-treated mice. This result suggests that extra-pituitary CRF_1 receptors may have been involved in the action of SSR125543. This hypothesis is consistent with the results of Ivy et al. (2010) who reported that central or peripheral administration of a CRF_1 receptor blocker improved memory performance of early-life stressed rats in the ORT and prevented dendritic atrophy in the hippocampus.

These results also raise the question whether the action of stress on memory retrieval involves to some extent the HPA axis. Our data indicate that the blockade of the HPA axis with DEX in stressed mice did not modify memory performance. Zhou et al. (1996) showed that DEX treatment produced a significant decrease in CRF_1 receptor mRNA levels in the anterior pituitary, but not in extra-pituitary brain regions in rats. Similarly, Britton et al. (1986a) and Britton et al. (1986b) showed that DEX treatment, while suppressing the activity of the HPA axis, did not modify the behavioral effects of centrally-administered CRF. More recently, studies on conditional knockout mice in which CRF_1 receptor function was inactivated only in anterior forebrain and limbic structures allowed Muller et al. (2003) to

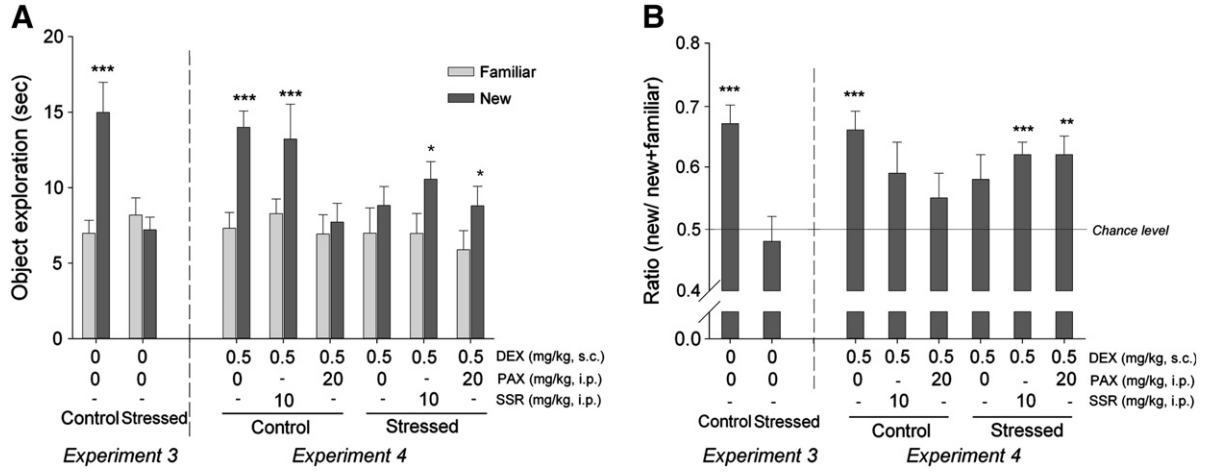

Fig. 6. Effects of SSR125543 on short-term episodic memory impairment induced by an acute inescapable stress in DEX-treated mice. (A) Bars represent mean (±s.e.m.) of time spent exploring new and familiar objects. *p<0.05 and ***p<0.001 new vs familiar object. (B) Bars represent mean (±s.e.m.) of novelty index, i.e. the ratio of the time spent exploring the new object on the sum of time exploring the two objects. **p<0.01 and ***p<0.001 vs 0.5. (n=9–10).

genetically differentiate CRF/CRF_1 receptor neuronal pathways modulating behavior from those regulating neuroendocrine (HPA system) function. They concluded that limbic CRF_1 receptors modulate anxiety-related behavior and that this effect is independent of HPA-system activity. In addition, Dedic et al. (2011) generated two conditional CRF-overexpressing mice lines and reported that while mice ubiquitously overexpressing CRF exhibited increased anxiety-related behavior, overexpression of CRF in the pituitary did not produce alterations in emotional behavior. These results suggest that HPA disturbances are not sufficient to duplicate the behavioral consequence of stress but rather that central CRF hyperdrive on its own or in a combination with elevated glucocorticoids is responsible for the increase in anxiety-related behavior. Regarding stress-induced cognitive alterations, Wang et al. (2011a) reported that chronically stressed mice with forebrain CRF_1 deficiency exhibit normal dendritic morphology of CA3 neurons and mild impairment in spatial memory while in stressed wild-type mice, spatial memory was disrupted and the complexity of apical dendrite of CA3 neurons was reduced. These findings underscore that forebrain CRF/CRF_1 signaling plays a critical role in the modulation of memory function and brain structural adaptation under stress.

In conclusion, these experiments showed further that CRF_1 receptor blockade may reverse the deleterious effects of stress. More precisely, these findings suggest that CRF_1 receptor antagonists may be useful for the treatment of PTSD. Finally, our data demonstrate that the effects of SSR125543 on cognitive impairment following traumatic stress exposure does not involve an action on the HPA axis, but may be mediated by central CRF_1 receptors. Further studies are warranted to determine which brain structures may play a role in these effects.

Disclosure/conflict of interest

The authors of this paper have no conflict of interest to declare.

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References

Adamec R, Fougere D, Risbrough V. CRF receptor blockade prevents initiation and consolidation of stress effects on affect in the predator stress model of PTSD. Int J Neuropsychopharmacol 2010;13:747–57.
Aisa B, Tordera R, Lasheras B, Del Rio J, Ramirez MJ. Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. Psychoneuroendocrinology 2007;32:256–66.
Alonso R, Griebel G, Pavone G, Stemmelin J, Le Fur G, Soubrie P. Blockade of CRF_1 or V1b receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. Mol Psychiatry 2003;9:278–86.
Baker DG, West SA, Nicholson WE, Ekhator NN, Kasckow JW, Hill KK, et al. Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. Am J Psychiatry 1999;156:585–8.
Belda X, Fuentes S, Nadal R, Armario A. A single exposure to immobilization causes long-lasting pituitary-adrenal and behavioral sensitization to mild stressors. Horm Behav 2008;54:654–61.
Belzung C, Billette de Villemeur E. The design of new antidepressants: can formal models help? A first attempt using a model of the hippocampal control over the HPA-axis based on a review from the literature. Behav Pharmacol 2010;21:677–89.
Bremner JD, Vermetten E. Neuroanatomical changes associated with pharmacotherapy in posttraumatic stress disorder. Ann N Y Acad Sci 2004;1032:154–7.
Bremner JD, Licinio J, Darnell A, Krystal JH, Owens MJ, Southwick SM, et al. Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. Am J Psychiatry 1997;154:624–9.
Brewin CR, Kleiner JS, Vasterling JJ, Field AP. Memory for emotionally neutral information in posttraumatic stress disorder: a meta-analytic investigation. J Abnorm Psychol 2007;116:448–63.
Britton DR, Varela M, Garcia A, Rosenthal M. Dexamethasone suppresses pituitary-adrenal but not behavioral effects of centrally administered CRF. Life Sci 1986a;38:211–6.
Britton KT, Lee G, Dana R, Risch SC, Koob GF. Activating and 'anxiogenic' effects of corticotropin releasing factor are not inhibited by blockade of the pituitary-adrenal system with dexamethasone. Life Sci 1986b;39:1281–6.
De Kloet ER. Stress in the brain. Eur J Pharmacol 2000;405:187–98.
De Kloet CS, Vermetten E, Heijnen CJ, Geuze E, Lentjes EG, Westenberg HG. Enhanced cortisol suppression in response to dexamethasone administration in traumatized veterans with and without posttraumatic stress disorder. Psychoneuroendocrinology 2007;32:215–26.
De Kloet CS, Vermetten E, Geuze E, Lentjes EG, Heijnen CJ, Stalla GK, et al. Elevated plasma corticotrophin-releasing hormone levels in veterans with posttraumatic stress disorder. Prog Brain Res 2008;167:287–91.
Dedic N, Touma C, Romanowski CP, Schieven M, Kuhne C, Ableitner M, et al. Assessing behavioural effects of chronic HPA axis activation using conditional CRH-overexpressing mice. Cell Mol Neurobiol 2011. http://dx.doi.org/10.1007/s10571-011-9784-0.
Diamond DM, Park CR, Heman KL, Rose GM. Exposing rats to a predator impairs spatial working memory in the radial arm water maze. Hippocampus 1999;9:542–52.
Diamond DM, Campbell AM, Park CR, Woodson JC, Conrad CD, Bachstetter AD, et al. Influence of predator stress on the consolidation versus retrieval of long-term spatial memory and hippocampal spinogenesis. Hippocampus 2006;16:571–6.
Dodart JC, Mathis C, Ungerer A. Scopolamine-induced deficits in a two-trial object recognition task in mice. Neuroreport 1997;8:1173–8.
El Hage W, Peronny S, Griebel G, Belzung C. Impaired memory following predatory stress in mice is improved by fluoxetine. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:123–8.
El Hage W, Griebel G, Belzung C. Long-term impaired memory following predatory stress in mice. Physiol Behav 2006a;87:45–50.
El Hage W, Gaillard P, Isingrini M, Belzung C. Trauma-related deficits in working memory. Cogn Neuropsychiatry 2006b;11:33–46.
Elizalde N, Gil-Bea FJ, Ramirez MJ, Aisa B, Lasheras B, Del Rio J, et al. Long-lasting behavioral effects and recognition memory deficit induced by chronic mild stress in mice: effect of antidepressant treatment. Psychopharmacology (Berl) 2008;199:1-14.
Elzinga BM, Schmahl CG, Vermetten E, Van Dyck R, Bremner JD. Higher cortisol levels following exposure to traumatic reminders in abuse-related PTSD. Neuropsychopharmacology 2003;28:1656–65.
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.
Fujishiro J, Imanishi T, Onozawa K, Tsushima M. Comparison of the anticholinergic effects of the serotonergic antidepressants, paroxetine, fluvoxamine and clomipramine. Eur J Pharmacol 2002;454:183–8.
Gola H, Engler H, Schauer M, Adenauer H, Riether C, Kolassa S, et al. Victims of rape show increased cortisol responses to trauma reminders: a study in individuals with war- and torture-related PTSD. Psychoneuroendocrinology 2012;37:213–20.
Gold PW, Calabrese JR, Kling MA, Avgerinos P, Khan I, Gallucci WT, et al. Abnormal ACTH and cortisol responses to ovine corticotropin releasing factor in patients with primary affective disorder. Prog Neuropsychopharmacol Biol Psychiatry 1986;10:57–65.
Griebel G. Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders? Pharmacol Ther 1999;82:1-61.
Griebel G, Simiand J, Steinberg R, Jung M, Gully D, Roger P, et al. SSR125543A: a potent and selective corticotrophin-releasing factor1 receptor antagonist. II. Characterization in rodent models of stress-related disorders. J Pharmacol Exp Ther 2002;301:333–45.
Gully D, Geslin M, Serva L, Fontaine E, Roger P, Lair C, et al. 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4- methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A): a potent and selective corticotrophin-releasing factor(1) receptor antagonist. I. Biochemical and pharmacological characterization. J Pharmacol Exp Ther 2002;301:322–32.
Hagewoud R, Bultsma LJ, Barf RP, Koolhaas JM, Meerlo P. Sleep deprivation impairs contextual fear conditioning and attenuates subsequent behavioural, endocrine and neuronal responses. J Sleep Res 2011;20:259–66.
Holmes A, Heilig M, Rupniak NM, Steckler T, Griebel G. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol Sci 2003;24:580–8.
Holsboer F. Corticotropin-releasing hormone—a new tool to investigate hypothalamic–pituitary–adrenocortical physiology in psychiatric patients. Psychopharmacol Bull 1986;22:907–12.
Holsboer F, Ising M. Central CRH system in depression and anxiety—evidence from clinical studies with CRH1 receptor antagonists. Eur J Pharmacol 2008;583:350–7.
Ivy AS, Rex CS, Chen Y, Dube C, Maras PM, Grigoriadis DE, et al. Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. J Neurosci 2010;30:13005–15.
Keegan CE, Herman JP, Karolyi IJ, O'Shea KS, Camper SA, Seasholtz AF. Differential expression of corticotropin-releasing hormone in developing mouse embryos and adult brain. Endocrinology 1994;134:2547–55.
Lu A, Steiner MA, Whittle N, Vogl AM, Walser SM, Ableitner M, et al. Conditional mouse mutants highlight mechanisms of corticotropin-releasing hormone effects on stress-coping behavior. Mol Psychiatry 2008;13:1028–42.
Marti O, Garcia A, Valles A, Harbuz MS, Armario A. Evidence that a single exposure to aversive stimuli triggers long-lasting effects in the hypothalamus–pituitary–adrenal axis that consolidate with time. Eur J Neurosci 2001;13:129–36.
Meltzer-Brody S, Connor KM, Churchill E, Davidson JR. Symptom-specific effects of fluoxetine in post-traumatic stress disorder. Int Clin Psychopharmacol 2000;15:227–31.
Morrow BA, Roth RH, Elsworth JD. TMT, a predator odor, elevates mesoprefrontal dopamine metabolic activity and disrupts short-term working memory in the rat. Brain Res Bull 2000;52:519–23.

Muller MB, Zimmermann S, Sillaber I, Hagemeyer TP, Deussing JM, Timpl P, et al. Limbic corticotropin-releasing hormone receptor 1 mediates anxiety-related behavior and hormonal adaptation to stress. Nat Neurosci 2003;6:1100–7.

Munoz-Abellan C, Rabasa C, Daviu N, Nadal R, Armario A. Behavioral and endocrine consequences of simultaneous exposure to two different stressors in rats: interaction or independence? PLoS One 2011;6:e21426.

Naudon L, Hotte M, Jay TM. Effects of acute and chronic antidepressant treatments on memory performance: a comparison between paroxetine and imipramine. Psychopharmacology (Berl) 2007;191:353–64.

Oitzl MS, Workel JO, Fluttert M, Frosch F, De Kloet ER. Maternal deprivation affects behaviour from youth to senescence: amplification of individual differences in spatial learning and memory in senescent Brown Norway rats. Eur J Neurosci 2000;12: 3771–80.

Overstreet DH, Griebel G. Antidepressant-like effects of CRF_1 receptor antagonist SSR125543 in an animal model of depression. Eur J Pharmacol 2004;497:49–53.

Philbert J, Pichat P, Biton B, Griebel G. P.2.b.009 Paroxetine and D-cycloserine attenuate deficits in cognitive processes following acute stress: an animal model of PTSD European. Neuropsychopharmacology 2010;20(Suppl. 3):S358.

Philbert J, Pichat P, Beeské S, Decobert M, Belzung C, Griebel G. Acute inescapable stress exposure induces long-term sleep disturbances and avoidance behavior: a mouse model of post-traumatic stress disorder (PTSD). Behav Brain Res 2011;221: 149–54.

Rice CJ, Sandman CA, Lenjavi MR, Baram TZ. A novel mouse model for acute and long-lasting consequences of early life stress. Endocrinology 2008;149:4892–900.

Sandi C, Woodson JC, Haynes VF, Park CR, Touyarot K, Lopez-Fernandez MA, et al. Acute stress-induced impairment of spatial memory is associated with decreased expression of neural cell adhesion molecule in the hippocampus and prefrontal cortex. Biol Psychiatry 2005;57:856–64.

Sapolsky RM, McEwen BS. Down-regulation of neural corticosterone receptors by corticosterone and dexamethasone. Brain Res 1985;339:161–5.

Stein DJ, Ipser J, McAnda N. Pharmacotherapy of posttraumatic stress disorder: a review of meta-analyses and treatment guidelines. CNS Spectr 2009;14:25–31.

Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, Vale WW. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. J Neurosci 1994;14:2579–84.

Surget A, Saxe M, Leman S, Ibarguen-Vargas Y, Chalon S, Griebel G, et al. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biol Psychiatry 2008;64:293–301.

Surget A, Wang Y, Leman S, Ibarguen-Vargas Y, Edgar N, Griebel G, et al. Corticolimbic transcriptome changes are state-dependent and region-specific in a rodent model of depression and of antidepressant reversal. Neuropsychopharmacology 2009;34: 1363–80.

Touma C, Sachser N, Mostl E, Palme R. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol 2003;130:267–78.

Touma C, Palme R, Sachser N. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. Horm Behav 2004;45: 10–22.

Urani A, Philbert J, Cohen C, Griebel G. 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 2011;98:425–31.

Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, et al. Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. J Comp Neurol 2000;428:191–212.

Vasterling JJ, Duke LM, Brailey K, Constans JI, Allain Jr AN, Sutker PB. Attention, learning, and memory performances and intellectual resources in Vietnam veterans: PTSD and no disorder comparisons. Neuropsychology 2002;16:5-14.

Wang XD, Chen Y, Wolf M, Wagner KV, Liebl C, Scharf SH, et al. Forebrain CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendritic remodeling. Neurobiol Dis 2011a;42:300–10.

Wang XD, Rammes G, Kraev I, Wolf M, Liebl C, Scharf SH, et al. Forebrain CRF_1 modulates early-life stress-programmed cognitive deficits. J Neurosci 2011b;31: 13625–34.

Yun J, Koike H, Ibi D, Toth E, Mizoguchi H, Nitta A, et al. Chronic restraint stress impairs neurogenesis and hippocampus-dependent fear memory in mice: possible involvement of a brain-specific transcription factor Npas4. J Neurochem 2010;114: 1840–51.

Zhou Y, Spangler R, LaForge KS, Maggos CE, Ho A, Kreek MJ. Modulation of CRF-R1 mRNA in rat anterior pituitary by dexamethasone: correlation with POMC mRNA. Peptides 1996;17:435–41.