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ARTICLE

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Behavioural and neurochemical changes induced by stress-related conditions are counteracted by the neurokinin-2 receptor antagonist saredutant

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Abstract

These experiments were undertaken to assess the mechanisms underlying the antidepressant-like effects of the neurokinin-2 (NK_2) receptor antagonist saredutant (SR48968) in rats tested in the forced swim test (FST), by analysing hippocampal brain-derived neurotrophic factor (BDNF) and plasma corticosterone [as index of hypothalamic-pituitary-adrenal (HPA) axis activity]. Male Wistar rats received three intraperitoneal injections over 24 h of vehicle, saredutant (5 mg/kg), citalopram (15 mg/kg), clomipramine (50 mg/kg). Rats were subjected to restraint stress (4 h) 24 h prior to the FST procedure. This stress procedure increased immobility and decreased swimming behaviour in the FST; furthermore, it lowered hippocampal BDNF protein expression and increased plasma corticosterone levels. Saredutant and clomipramine or citalopram, used here as positive controls, reduced the immobility time in the FST both under basal conditions and after stress exposure. This effect was not attributable to changes in locomotion, because locomotor activity was unchanged when assessed in the open field test. Pretreatment with para-cholorophenylalanine (150 mg/kg, 72 h and 48 h prior to FST) abolished the effect of citalopram and saredutant on immobility time. At neurochemical level, saredutant attenuated activation of HPA axis in stressed animals more than clomipramine or citalopram. The behavioural effects of saredutant support the hypothesis that NK_2 receptor activity is involved in stress-related disorders. These effects of saredutant may be related to normalization of the HPA axis. Moreover, saredutant increases BDNF expression in the hippocampus, confirming the role of NK_2 receptor blockade in BDNF activation following stressor application.

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Key words: Brain-derived neurotrophic factor, corticosterone, forced swim test, NK_2 receptor antagonist, restraint stress.

Introduction

Saredutant (SR48968) is a selective, non-peptide, neurokinin-2 (NK_2) receptor antagonist that exhibits antidepressant/anxiolytic activity in rodents, lacking side-effects such as sedation, disinhibition or memory impairment (Griebel *et al.* 2001; Louis *et al.* 2008; Micale *et al.* 2008*b*; Overstreet *et al.* 2010; Rogacki *et al.* 2011; Salomé *et al.* 2006). Little is known of the mechanism(s) underlying saredutant antidepressant/anxiolytic activity. While an effect on monoaminergic (i.e. 5-HT, norepinephrine) and γ-aminobutyric acid (GABA)ergic neurotransmission has been proposed (Ribeiro & de Lima, 2002; Steinberg *et al.* 2001; Walsh *et al.* 1995), an influence of NK_2 receptor antagonism on hypothalamic-pituitary-adrenal (HPA) axis is also conceivable, since saredutant reduces corticotropin-releasing factor, a neuropeptide involved in stress-coping behaviour (Risbrough & Stein, 2006; Steinberg *et al.* 2001).

The biological basis of stress-related disorders like depression as well as the precise mechanisms of

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antidepressant efficacy of drug treatment remain largely unknown; increasing evidence, however, links stress to depression and antidepressant action and suggests that the stressors act by inducing a disruption in cellular mechanisms governing neuronal plasticity and disturbances in the HPA axis (Kunugi *et al.* 2010; Schmidt & Duman, 2007). Because stressful life events are major predisposing risk factors for developing depression, preclinical studies should take into account both the effects of pre-training stressors, such as drug injection procedure, inescapable foot-shocks, restraint and forced swim on the behavioural and neuroendocrine responses of animals, and the influence of these stressful stimuli on anxiolytic and/or antidepressant drug action (Briones-Aranda *et al.* 2002; Consoli *et al.* 2005; de Kloet *et al.* 2005; Drago *et al.* 2001; McEwen, 2005; Micale *et al.* 2008*a*; Tamburella *et al.* 2010; Teixeira & de Lima, 2003).

Antidepressant drugs counteract stress-induced atrophy of hippocampus and exert neurotrophic activity, by increasing the expression of factors as cyclic adenosine monophosphate-response element binding protein (CREB) and brain-derived neurotrophic factor (BDNF), and also affect HPA axis hyperactivity (Duman *et al.* 1999; Kunugi *et al.* 2010; Luo *et al.* 2004; Malberg *et al.* 2000; Nibuya *et al.* 1996; Xu *et al.* 2003). Hence, the long-term beneficial effects of antidepressant treatment could partially be related to their delayed effect on synaptic plasticity and neuroendocrine function.

Based on the above premise, this study was undertaken to assess the mechanisms underlying the effect of the tachykinin NK_2 receptor antagonist saredutant in the forced swim test (FST), an experimental model widely used for preclinical screening of new antidepressant drugs (Porsolt *et al.* 1978). For this purpose, we evaluated the changes of BDNF protein levels in the hippocampus, a brain region involved in emotional processes, and the plasma corticosterone levels as index of neuroendocrine response to stressful situations (Herman *et al.* 2005). The influence of stress-related behaviour was assessed by applying an acute (4 h) restraint stress prior to the FST, as previously described (Tamburella *et al.* 2010). Furthermore, the open field test (OFT) was carried out in order to ensure that coping behaviours in the FST were not secondary to non-specific effects of treatment or stressor on locomotor activity (Cryan *et al.* 2005). We also investigated whether the neurochemical effects of acute restraint stress could be counteracted by treatment with the NK_2 antagonist, saredutant. The tricyclic antidepressant (TCA) clomipramine and the selective serotonin reuptake inhibitor (SSRI) citalopram were used as comparators, under the same experimental conditions.

Materials and method

Animals

Wistar male rats (Harlan, Italy) weighing 220–240 g (aged 8–10 wk) were used throughout all experiments. For at least 1 wk prior to the experiment, the animals were housed four per cage at a temperature of 22 ± 1 °C and under a 12-h light/dark cycle (lights on 08:00 hours), with food and tap water available *ad libitum*.

After being randomly assigned to a given treatment group, the animals were tested only once in the behavioural experiments and then killed. The experiments were performed in a laboratory maintained at a temperature of 22 ± 1 °C, between 10:00 and 17:00 hours, according to the behavioural procedures of the test used. All the experiments were carried out according to the European Community Council 86/609/EEC and efforts were made to minimize animal suffering and to reduce the number of animals used. The rationale, design and methods of this study had been approved by the Ethical Committee for Animal Research, University of Catania.

Behavioural tests

Forced swim test

The procedure was based on the behavioural test described by Porsolt *et al.* (1978). A single experiment consisted of a pre-test and a swim test. Naive rats were individually placed inside vertical cylinders (height: 40 cm, diameter: 30 cm) containing 25 cm water at 23–25 °C for 15 min. Following the pre-test, animals were removed and allowed to dry in a heated enclosure before returning to their home cages. After 24 h, the swim test was performed: the rats were individually placed again in the cylinder for 5 min and the total duration of immobility and escape behaviour was measured. Swim tests were videotaped and subsequently assessed for the following behaviours: immobility (the animal remains floating passively in the water without struggling and shows the minimal movements necessary to keep its head above water); swimming (active movements, more than those necessary to keep the head of the rat above the water and mainly propelling the rat around the cylinder); climbing (very vigorous, active movements with the animal's forepaws breaking the water surface, usually against the wall of the water container). Total time

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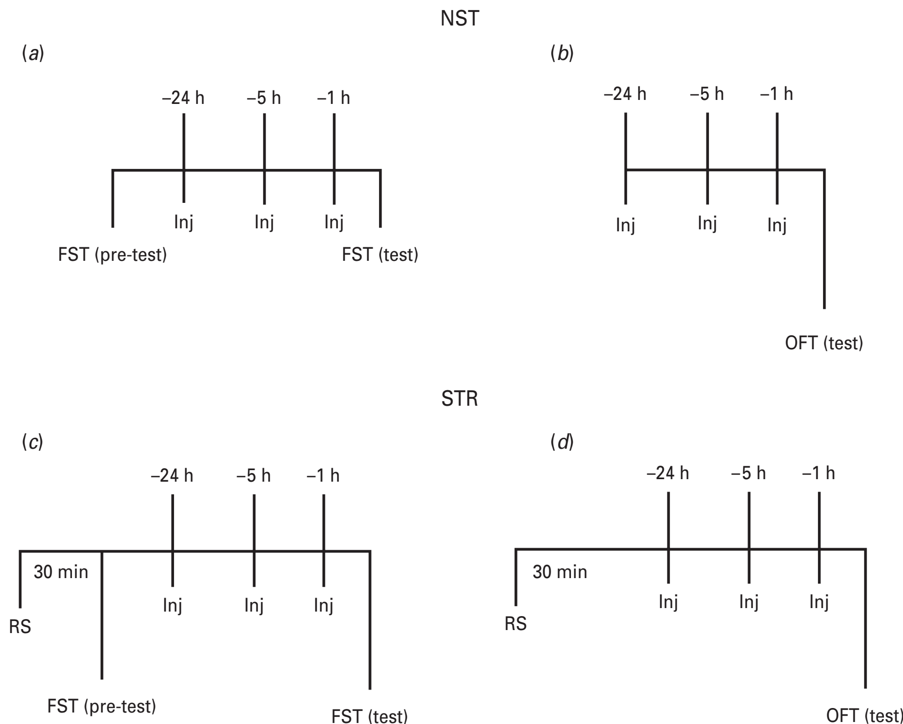

Fig. 1. Representation of experimental design. NST, Non-stressed group; Inj, injection; FST, forced swim test; OFT, open field test; STR, stressed group; RS, restraint stress.

spent engaged in each activity was recorded and analysed by two 'blind' observers.

Open field test

Activity in the OFT was evaluated in order to ensure that the decreased immobility or the increased active behaviours in the FST were not secondary to a non-specific change in motor activity produced by the treatments (Cryan *et al.* 2005). The experiment was performed in a soundproof and moderately illuminated (~50 lx) cubic observation chamber (2×2×2 m), using a white wooden open field (100×100 cm, walls 40 cm high). At the beginning of the test, animals were gently placed in the centre of the arena and allowed to explore. The exploratory activity in the open field, i.e. the number of squares crossed with all paws (crossing) was counted in a 5 min session, recorded on a tape using a video camera (Hitachi Videocam; Hitachi Ltd., Japan) and then scored by video tracking software (Ugo Basile, Italy).

Drugs and experimental design

All compounds were administered i.p. in a volume of 1 ml/kg body weight. Clomipramine hydrochloride and citalopram hydrobromide were freshly solubilized in distilled water. Saredutant {N-[(2S)-4-(4-acetamido-4-phenylpiperidin-1-yl)- 2-(3,4-dichlorophenyl)butyl]-N-methylbenzamide} was solubilized in physiological saline, containing 0.1% Tween 80. The doses were: 50 mg/kg clomipramine; 15 mg/kg citalopram; 5 mg/kg saredutant. Two groups of control animals were injected i.p. with clomipramine and citalopram vehicle or with saredutant vehicle, respectively. As similar results were obtained from these two control groups, vehicle data were pooled. In some experiments, endogenous serotonin was depleted by administering para-cholorophenylalanine (PCPA, 150 mg/kg) to rats 72 and 48 h prior to the swim test, according to Page *et al.* 1999). All chemical substances were purchased from Sigma (USA), except for saredutant, which was donated by Sanofi-Aventis (Italy).

Two different experiments were carried out (Fig. 1). In the first experiment, different groups of rats ($n=5$–6) received i.p. injections of drugs 24, 5 and 1 h prior to the FST (A) or the OFT (B) without prior stress application (non-stressed groups are indicated as NST). In the second experiment, different groups of rats ($n=5$–6) were first exposed to an acute restraint stress procedure (stressed groups are indicated as STR; Tamburella *et al.* 2010) and then subjected to the

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FST (C) or OFT (D) procedure. In this respect, the animals were placed into individual plastic rodent restrainers for 4 h, until 30 min prior to the pre-test of the FST procedure. The treatment started right after the pre-test. Clomipramine and citalopram were selected as positive controls as they are well-known antidepressant agents active in the FST. The doses of saredutant, clomipramine and citalopram were selected based on previous experiments (Consoli *et al.* 2007; Micale *et al.* 2006, 2008*b*; Tamburella *et al.* 2009, 2010).

Immediately after the 5 min test, all animals were killed by decapitation, the brains removed and whole hippocampus dissected, frozen on dry ice and stored at −80 °C until analysis. Trunk blood was collected in EDTA-tubes and centrifuged at 4 °C for 15 min at 1500 *g*; plasma was stored at −20 °C until analysis.

Biochemical methods

Western blotting analysis of BDNF protein expression

Western blot analysis was used to measure protein levels of BDNF, whose expression is related to stress-related alteration of hippocampal plasticity. Rat hippocampi were lysed in a buffer containing 40 mM Tris (pH 7.5), 1% Triton, 0.2% sodium dodecyl sulphate (SDS), 0.2% deoxicholate and 1.6% NaCl. Protease inhibitor cocktail (P8340, Sigma) containing 4-(2-aminoethyl) benzenesulfonyl fluoride, pepstatin A, bestatin, leupeptin, E-64 and aprotinin was added to inhibit proteolysis in the samples. Tissues were sonicated for around 30 s at medium power at 4 °C and lysates were centrifuged for 30 min at 10 000 *g*. The supernatant was used for SDS-polyacrylamide gel electrophoresis (PAGE) and the pellet discarded. Protein concentration was determined with the Bradford assay (Bradford, 1976). Equal amounts of hippocampal protein were diluted with SDS buffer, loaded onto 15% acrylamide gels (60 μg per lane) and run in SDS-PAGE. Proteins were electroblotted to nitrocellulose membrane (Bio-Rad, USA) and stained with Ponceau S Red (Sigma) to confirm the efficiency of transfer. Gel retention was assessed by staining with Coomassie Blue (Pierce, USA). Non-specific binding was blocked for 1 h at 4 °C with 3% non-fat dry milk in Tween-Tris-buffered saline (TTBS). Membranes were incubated overnight at 4 °C with antibody to BDNF [rabbit polyclonal immunoglobulin G (IgG), 1:750, Santa Cruz Biotechnology (USA)], prepared in 3% non-fat dry milk solution in TTBS. Actin was probed to verify equal loading of protein (mouse monoclonal IgG, 1:5000, Sigma). Appropriate horseradish-conjugated secondary antibodies, anti-rabbit (goat IgG; Santa Cruz Biotechnology) or anti-mouse (goat IgG, Sigma) were applied at 1:10 000. Negative controls were carried out by omitting primary antibody. Visualization was obtained by using a chemiluminescence system (ECL kit; Amersham Pharmacia Biotec, USA). Protein bands on films were digitally quantified with the use of an acquisition and analysis program (Scion Image; Scion Corporation, USA).

Corticosterone enzyme immunoassay

Enzyme immunoassay of corticosterone was carried out in plasma samples using a commercially available kit (Cayman Chemicals, USA), according to manufacturer's instructions; inter- and intra-assay coefficients of variance were $<10\%$; lower limit of detection was approximately 30 pg/ml.

Statistical analysis

Data were analysed using one- or two-way analysis of variance (ANOVA). The *post-hoc* Student–Newman–Keuls test was used for multiple comparisons. A p value of $\leqslant 0.05$ was considered as indicative of a significant difference.

Results

The influence of drug treatment, alone or combined to acute restraint stress, on behavioural performance in the FST is shown in Fig. 2. Two-way ANOVA (factor 1: stress; factor 2: drug treatment) revealed for the immobility time a significant effect of stress ($F_{1,42}=22.044$; $p<0.001$) and drug treatment ($F_{4,42}=34.751$; $p<0.001$) as well as a significant stress × drug treatment interaction ($F_{4,42}=4.515$; $p<0.01$). For the swimming time, there was a significant effect of treatment ($F_{4,42}=34.445$; $p<0.001$) and a significant stress × drug treatment interaction ($F_{4,42}=2.763$; $p<0.05$) but not an effect of stress ($F_{1,42}=1.785$; $p=$n.s.). For the climbing time, there was a significant effect of treatment ($F_{4,42}=10.970$; $p<0.001$), but not of stress ($F_{1,42}=0.839$; $p=$n.s.) and there was no stress × treatment interaction ($F_{4,42}=0.262$; $p=$n.s.). Saredutant (5 mg/kg) produced a decrease of immobility time in the FST paradigm in the NST rats, which was similar in amplitude to that elicited by clomipramine (50 mg/kg) and citalopram (15 mg/kg; $p<0.001$ for all drug treatments *vs.* control, Fig. 2). Application of acute restraint stress, the day before the FST, modified the behavioural performance by increasing the immobility time and decreasing swimming behaviour ($p<0.001$, Fig. 2). Saredutant, clomipramine and citalopram significantly counteracted the behavioural effect of the stress

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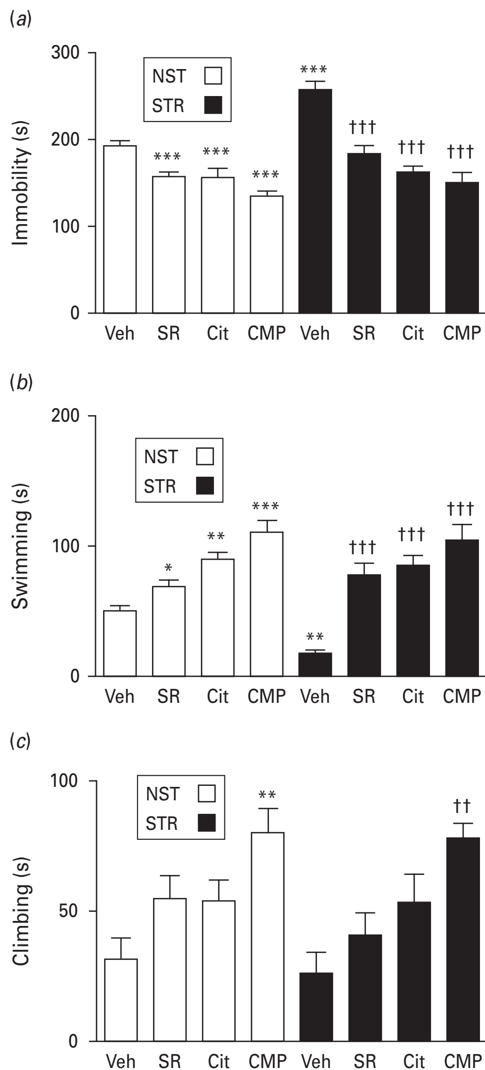

Fig. 2. Behavioural performance of rats in the forced swim test under the basal condition (NST) or after application of acute restraint stress (STR). (*a*) Immobility time; (*b*) swimming time; (*c*) climbing time. Saredutant (SR; 5 mg/kg), citalopram (Cit; 15 mg/kg), clomipramine (CMP; 50 mg/kg) or vehicle (Veh) were administered i.p. 24, 5 and 1 h prior to behavioural testing. Columns represent mean values ± S.E.M. of time measurements ($n = 5$–6). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ *vs.* Veh-NST; †† $p < 0.01$, ††† $p < 0.001$ *vs.* Veh-STR. Two-way analysis of variance and Student–Newman–Keuls *post-hoc* test.

procedure ($p < 0.001$, Fig. 2), by decreasing the immobility time and increasing swimming behaviour ($p < 0.001$, any drug treatment *vs.* vehicle-stressed animals, Fig. 2). As mentioned above, the stress procedure did not change climbing behaviour, while there was a tendency of drug treatments to increase climbing, which reached significance only for clomipramine ($p < 0.01$).

In order to assess the involvement of 5-HT system on the behavioural effect of saredutant in the FST, we repeated the experiment in animals pretreated with PCPA, a selective and irreversible inhibitor of tryptophan hydroxylase, which depletes 5-HT content in rat brain (Jéquier *et al.* 1967). As shown in Fig. 3, PCPA-pretreatment abolished the effect of both citalopram and saredutant on immobility time and strongly reduced their effect on swimming.

The influence of drug treatment, alone or combined to acute restraint stress, on behavioural performance in the OFT is shown in Fig. 4. Two-way ANOVA (factor 1: stress; factor 2: treatment) did not show an effect of stress ($F_{1,42} = 0.486$; $p =$ n.s.) or drug treatment ($F_{4,42} = 1.349$; $p =$ n.s.). Furthermore, there was no stress × drug treatment interaction ($F_{4,42} = 2.171$; $p =$ n.s.) in motor ability as assessed in the OFT.

The effects of pharmacological treatment, alone or combined to acute restraint stress, on the hippocampal BDNF expression was assessed by Western blot in hippocampal lysates (Fig. 5). Two-way ANOVA (factor 1: stress; factor 2: drug treatment) revealed a significant effect of stress ($F_{1,40} = 80.846$; $p < 0.001$) and drug treatment ($F_{4,40} = 13.144$; $p < 0.001$) as well as a significant stress × drug treatment interaction ($F_{4,40} = 27.028$; $p < 0.001$) on BDNF in hippocampi of rats tested in the OFT. Application of acute restraint stress lowered the hippocampal expression of BDNF ($p < 0.01$). Saredutant, clomipramine and citalopram did not change BDNF expression in NST, but increased BDNF by about two-fold in the STR group ($p < 0.001$, Fig. 5*a*). In rats tested in the FST, two-way ANOVA (factor 1: stress; factor 2: treatment) showed a significant effect of stress ($F_{1,40} = 5.053$; $p < 0.05$), drug treatment ($F_{4,40} = 19.035$; $p < 0.001$) as well as a significant stress × drug treatment interaction ($F_{4,40} = 4.930$; $p < 0.01$) for BDNF protein expression. Again, acute restraint stress diminished BDNF levels ($p < 0.05$), an effect reversed by pharmacological treatments ($p < 0.001$). Interestingly, saredutant, but not clomipramine or citalopram, was also able to increase BDNF expression in the NST group ($p < 0.01$, Fig. 5*b*).

To assess the effect of drug treatments and/or acute restraint stress on the HPA axis, corticosterone levels were measured in plasma (Fig. 6). Two-way ANOVA (factor 1: stress; factor 2: drug treatment) showed a significant effect of stress ($F_{1,40} = 34.959$; $p < 0.001$) and drug treatment ($F_{4,40} = 14.517$; $p < 0.001$), as well as a significant stress × drug treatment interaction ($F_{4,40} = 9.916$; $p < 0.001$) for plasma corticosterone levels in rats tested in the OFT. Application of acute restraint

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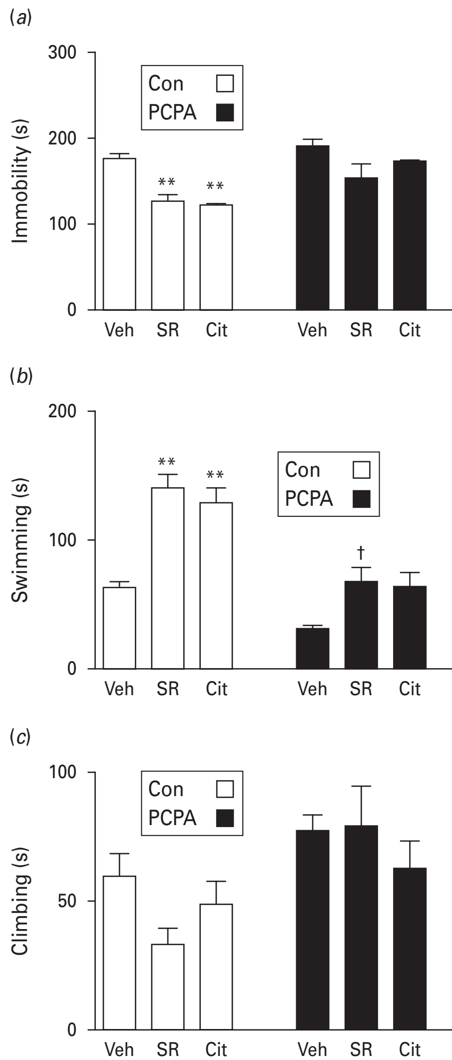

Fig. 3. Behavioural performance of rats in the forced swim test under the basal condition (Con) and after pretreatment with para-cholorophenylalanine (PCPA, 150 mg/kg, 72 h and 48 h prior to behavioural testing). (*a*) Immobility time; (*b*) swimming time; (*c*) climbing time. Saredutant (SR; 5 mg/kg), citalopram (Cit; 15 mg/kg) or vehicle (Veh) were administered i.p. 24, 5 and 1 h prior to behavioural testing. Columns represent mean values ± S.E.M. of time measurements ($n=5$–6). ** $p<0.01$ *vs.* Veh-Con; † $p<0.05$ *vs.* Veh-PCPA. One-way analysis of variance and Student–Newman–Keuls *post-hoc* test.

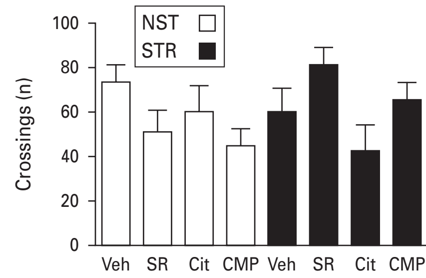

Fig. 4. Locomotor activity of rats in the open field test under the basal condition (NST) or after application of acute restraint stress (STR). Saredutant (SR; 5 mg/kg), citalopram (Cit; 15 mg/kg), clomipramine (CMP; 50 mg/kg) or vehicle (Veh) were administered i.p. 24, 5 and 1 h prior to behavioural testing. Columns represent mean values ± S.E.M. of crossing area numbers ($n=5$–6).

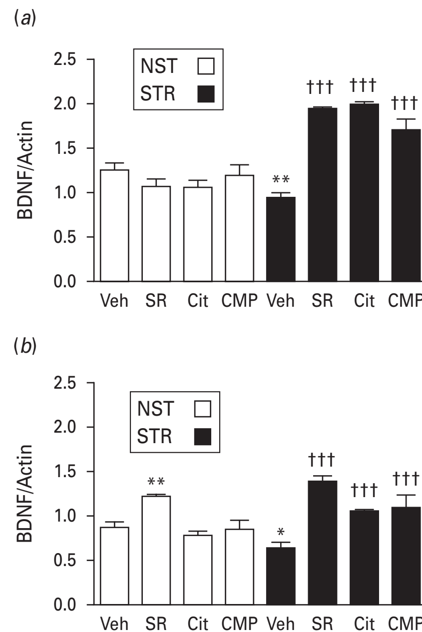

Fig. 5. Hippocampal expression of brain-derived neurotrophic factor (BDNF) in rats subjected to the open field test (*a*) or the forced swim test (*b*), under basal conditions (NST) and after application of acute restraint stress (STR). Saredutant (SR; 5 mg/kg), citalopram (Cit; 15 mg/kg), clomipramine (CMP; 50 mg/kg) or vehicle (Veh) were administered i.p. 24, 5 and 1 h prior to behavioural testing. Columns represent mean values ± S.E.M. of BDNF/actin ratios of protein band densities ($n=5$). All groups were represented in each gel and band densities were normalized as percentage of change from respective control (Veh-NST or Veh-STR). * $p<0.05$, ** $p<0.01$, *vs.* Veh-NST; ††† $p<0.001$, *vs.* Veh-STR. Two-way analysis of variance and Student–Newman–Keuls *post-hoc* test.

stress increased corticosterone as compared to the NST group ($p<0.01$). Saredutant and clomipramine, but not citalopram, decreased corticosterone ($p<0.001$), reversing the neuroendocrine stress responses (Fig. 6*a*). No difference was found in the corticosterone plasma levels among the NST groups (Fig. 6*a*). In rats tested in the FST, two-way ANOVA

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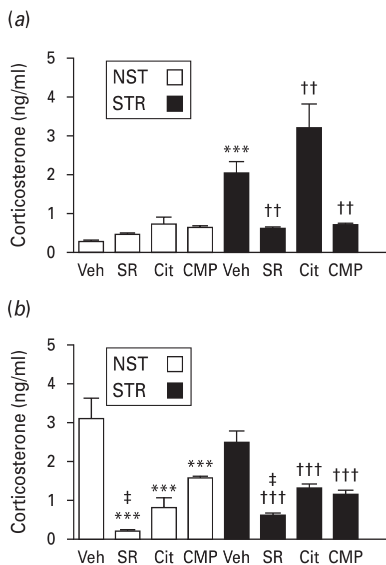

Fig. 6. Corticosterone levels in rats after the open field test (*a*) or the forced swim test (*b*), under basal conditions (NST) or after application of acute restraint stress (STR). Saredutant (SR; 5 mg/kg), citalopram (Cit; 15 mg/kg), clomipramine (CMP; 50 mg/kg) or vehicle (Veh) were administered i.p. 24, 5 and 1 h prior to behavioural testing. Columns represent mean values ± S.E.M. of plasma corticosterone ($n = 5$–6). *** $p < 0.001$, *vs.* Veh-NST; †† $p < 0.01$, ††† $p < 0.001$ *vs.* Veh-STR; ‡ $p < 0.05$ *vs.* Cit and CMP. Two-way analysis of variance and Student–Newman–Keuls *post-hoc* test.

(factor 1: stress; factor 2: treatment) showed a significant effect of drug treatment ($F_{4,40} = 34.810$; $p < 0.001$), but not of stress ($F_{1,40} = 0.470$; $p =$ n.s.); furthermore, there was no stress × drug treatment interaction ($F_{4,40} = 1.902$; $p =$ n.s.). A subsequent one-way ANOVA, carried out separately for each group (NST or STR), confirmed a significant effect of drug treatment (NST: $F_{4,20} = 16.355$; $p < 0.001$; STR: $F_{4,20} = 25.471$; $p < 0.001$); all drugs decreased the corticosterone levels as compared to vehicle ($p < 0.001$), saredutant treatment resulting in being more effective than citalopram or clomipramine ($p < 0.05$, Fig. 6*b*).

The drug treatments as well as the stress procedure did not induce any change in the body weight of the animals (data not shown).

Discussion

In the present study, we confirm that the NK_2 non-peptide antagonist saredutant, given systemically, is effective, both under basal conditions and after stress application, in reducing immobility time of rats in a FST, an experimental model widely used to assess antidepressant-like activity (Porsolt *et al.* 1978). Moreover, for the first time, we show that a short-term treatment with saredudant (three injections over 24 h) increases hippocampal BDNF protein expression and reduces plasma corticosterone levels in stressed animals. NK_2 receptor antagonism has been associated with antidepressant/anxiolytic-like activity in experimental models (Borelli *et al.* 2010; Griebel *et al.* 2001; Louis *et al.* 2008; Micale *et al.* 2008*b*; Overstreet *et al.* 2010; Salomé *et al.* 2006; Steinberg *et al.* 2001), including FST. Here we found that a relatively low dose of saredutant (5 mg/kg) reproduces the effect on immobility and swimming time induced in FST by commonly used doses of the TCA clomipramine (50 mg/kg) or the SSRI citalopram (15 mg/kg). The precise mechanisms underlying the effects of NK_2 receptor antagonists on FST are unknown. It is generally assumed that antidepressants increase swimming behaviour predominantly by enhancing serotoninergic neurotransmission (Cryan *et al.* 2005). To test the hypothesis that the effect of saredutant was related to a facilitator effect on the serotoninergic system, we attempted to blunt 5-HT transmission by pretreating the animals with PCPA. The paradigm of PCPA-treatment we used here has been validated in rats, where it depletes 5-HT content in brain tissue by about 90% and abolishes the effect of SSRI on immobility time in FST, whereas it does not change the effect of desipramine (Page *et al.* 1999). As expected, we found that citalopram was unable to reduce immobility time in PCPA-treated rats. Saredudant also appeared to be unable to reduce immobility time in PCPA-treated rats, indicating that 5-HT transmission was involved in its action. An involvement of 5-HT transmission on the behavioural effects of saredutant is also consistent with the observation that direct infusion of NK_2 antagonists into the dorsal raphe nucleus, a major source of 5-HT innervation of the forebrain, elicits disinhibitory effects on emotional behaviour of rodents (Sandford *et al.* 2000; Stratton *et al.* 1993). Other effects of NK_2 blockade, including inhibition of catecholamine release, have been reported in stressed animals (Steinberg *et al.* 2001). The neurotransmitter systems involved in the antidepressant-like effects of saredutant are therefore likely to be not limited to 5-HT. A specific concern in antidepressant research with the FST is that compounds endowed with psychomotor stimulating activity could reduce immobility even if devoid of antidepressant action (Jacobson & Cryan, 2007). Therefore, false positive for an antidepressant-like profile may result from altered locomotor activity. In this respect, it should be pointed out that none of the drugs tested in the present study affected rat motor

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behaviour, as assessed in the OFT, although all drugs produced a reduction in FST immobility. Thus, these results indicate that the decreased immobility time of treated animals in the FST is not due to an effect on locomotor activity.

As human stressful life events are associated with an increased risk of depression, behavioural models of depression utilize several stress paradigms to induce depressive-like symptoms in laboratory animals (McEwen, 2005; Schmidt & Duman, 2007). Increasing evidence suggests that exposure to stress paradigms leads to cell loss in the hippocampus, a brain area implicated in the pathophysiology of mood disorders, and affects the expression of neurotrophic/growth factors. Antidepressant drug treatments counteract these changes, supporting the 'neurotrophic' hypothesis as an explanation for antidepressant drug action (McLaughlin *et al.* 2007; Warner-Schmidt & Duman, 2006). Interestingly, saredutant has been shown to counteract the hippocampal decrease in newly generated neurons induced by a chronic mild stress paradigm in mice, even more effectively than fluoxetine (Stemmelin *et al.* 2008). This neurogenic action of saredutant is reinforced by *in vitro* data, showing that it stimulates differentiation of rat neural stem cells into neurons, without affecting astrogenesis (Stemmelin *et al.* 2008).

Among potential targets of antidepressant drug treatments, neurotrophins, such as BDNF, seem relevant because they promote the growth and development of immature neurons and enhance the survival and the function of adult neurons. Antidepressant drugs have been proposed to exert their actions partially via BDNF, whose expression is in turn regulated by CREB (Steinberg *et al.* 2001; Tardito *et al.* 2006). BDNF regulates synaptic transmission and activity-dependent plasticity (Bramham & Messaoudi, 2005; Gottmann *et al.* 2009), promotes neurogenesis (Scharfman *et al.* 2005) and exerts an antidepressant activity in a time-dependent manner, as it has been shown that a single bilateral infusion of BDNF into the dentate gyrus of the hippocampus produces an antidepressant-like effect in both the learned helplessness paradigm and FST, within 3 d after infusion (Shirayama *et al.* 2002). Importantly, pre-training stressors that affect behavioural response induce neuronal plasticity changes (Briones-Aranda *et al.* 2002; Chaki *et al.* 2004; Consoli *et al.* 2005; Drago *et al.* 2001; Micale *et al.* 2008*b*; Pittenger & Duman, 2008; Tamburella *et al.* 2010; Teixeira & de Lima, 2003).

In the present study, rats exposed to acute restraint stress exhibited an impaired behavioural performance in the FST (i.e. increased immobility and decreased swimming time), which was reversed by drug treatments. However, drug treatments also affected the behavioural performance in the NST group, suggesting that their effect on FST response is, at least in part, independent of previously applied stressors. On the other hand, at molecular level, the stress procedure led to decreases in BDNF protein expression, counteracted by drug treatments. However, the drugs failed to induce any change in the BDNF levels of NST groups tested in the OFT, indicating that exposure to stressors as restraint and forced swim is a key factor for decreasing BDNF and that the effect of drug treatments on BDNF is not exerted in the absence of stressors. Steinberg *et al.* (2001) reported that repeated administration of saredutant (once per day for 21 d; 1 mg/kg i.p. in rats) increases the expression of CREB mRNA in hippocampus (dentate gyrus and CA1) whereas an acute administration does not change CREB expression. Because BDNF is a CREB-regulated gene (Tardito *et al.* 2006), it is not expected to be changed by acute saredutant treatment. Consistent with this view, we did not observe changes in BDNF expression in control (non-stressed) animals, following acute saredutant. In stressed animals, however, we observed a reduction of BDNF that was partially reversed by drug treatment. We did not study CREB expression in detail here; however, we previously reported that acutely applied stressors reduce CREB expression in the hippocampus (Tamburella *et al.* 2010).

Worthy of note, in rats not subjected to restraint stress and challenged in the FTS, saredutant, but not clomipramine or citalopram, increased hippocampal BDNF expression, whereas all three drugs increased BDNF in rats exposed to both restraint and FST. Because FST represents a stressful paradigm *per se*, it appears that blockade of NK_2 receptors could be effective on BDNF following milder stress conditions, not sufficient for detecting the effect of TCA or SSRI. The new important finding here is therefore that saredutant reverses the effects of acute stressors on BDNF expression. The impact of this observation on neuronal plasticity, however, remains to be elucidated.

Neuronal plasticity related to BDNF expression might contribute to the long-term effect of antidepressant drugs. However, because our present experimental paradigm was short term (three drug injections in 24 h), it is unlikely that changes in BDNF expression that we found with saredutant, citalopram and clomipramine could have already significantly modified synaptogenesis and/or neurogenesis to affect behavioural response in the FST.

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A potential, fast-acting, candidate related to behavioural effects observed in this short-term experimental paradigm is the HPA axis. For a long time, the major role of the HPA axis in stress-related conditions has been recognized, both as a marker of stress response and as a mediator of additional downstream pathophysiological changes. Disruption of the glucocorticoid receptor function in the forebrain of mice induces alterations in despair-like behaviour and HPA axis function, reminiscent of major depressive disorder (Kolber & Muglia, 2009). Furthermore, corticosteroid × serotonin interactions in the neurobiological mechanisms of stress-related disorders have been reported (Lanfumey *et al.* 2008). Here we found that physical restraint led to increased plasma corticosterone in vehicle-treated animals as compared to NST animals tested in the OFT. By contrast, no effect of physical restraint was detected in animals subsequently subjected to the FST, suggesting that the FST represents a stressor sufficient to enhance corticosterone release, consistent with other reports (Pintér *et al.* 2011; Rittenhouse *et al.* 2002). All drug treatments were able to counteract corticosterone increase following stressor procedures, except citalopram, which induced a further increase of corticosterone in the OFT-STR group. This latter observation is in line with other studies that have reported an increase in adrenocorticotropic hormone and corticosterone in rats following acute citalopram treatment (Hesketh *et al.* 2005; Jensen *et al.* 1999). The reduction of plasma corticosterone by saredutant was even stronger than that induced by clomipramine and citalopram, indicating that NK_2 blockade attenuates activation of the HPA axis in stress-related conditions, as also described by other studies (Culman *et al.* 2010; Steinberg *et al.* 2001). We therefore hypothesize that the effect of NK_2 receptor blockade in stress-related conditions, as observed in the present, short-term, experimental paradigm, could be related to a reduction of the activity of the HPA axis.

In conclusion, we confirm the antidepressant-like behavioural effect of saredutant in an animal model and suggest that normalization of HPA axis activity altered by stressors, as revealed by changes in plasma corticosterone levels, could be related to this effect. Moreover, because saredutant was able to counteract the decrease of BDNF expression in the hippocampus under stress-related conditions, NK_2 blockade seems able to affect the stress-induced activation of the hippocampal neurotrophic pathway and this may have a pathophysiological significance in longer-lasting experimental paradigms, allowing synaptogenesis and neurogenesis to take place.

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Statement of Interest

None.

References

Borelli KG, Defensor EB, Blanchard DC, Blanchard RJ, *et al.* (2010). Effects of intra-hippocampal injections of the NK2 receptor antagonist saredutant on the elevated plus maze, and the mouse defense test battery. *Neuroscience Letters* **485**, 241–245.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.

Bramham CR, Messaoudi E (2005). BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Progress in Neurobiology* **76**, 99–125.

Briones-Aranda A, Lopez-Rubalcava C, Picaro O (2002). Influence of forced swimming-induced stress on the anxiolytic-like effect of 5HT(1A) agents in mice. *Psychopharmacology* **162**, 147–155.

Chaki S, Nakazato A, Kennis L, Nakamura M, *et al.* (2004). Anxiolytic- and antidepressant-like profile of a new CRF1 receptor antagonist, R278995/CRA0450. *European Journal of Pharmacology* **485**, 145–158.

Consoli D, Fedotova J, Micale V, Sapronov NS, *et al.* (2005). Stressors affect the response of male and female rats to clomipramine in a model of behavioral despair (forced swim test). *European Journal of Pharmacology* **520**, 100–107.

Consoli D, Leggio GM, Mazzola C, Micale V, *et al.* (2007). Behavioral effects of the beta3 adrenoceptor agonist SR58611A: is it the putative prototype of a new class of antidepressant/anxiolytic drugs? *European Journal of Pharmacology* **573**, 139–147.

Cryan JF, Page ME, Lucki I (2005). Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology* **182**, 335–344.

Culman J, Das G, Ohlendorf C, Haass M, *et al.* (2010). Blockade of tachykinin NK1/NK2 receptors in the brain attenuates the activation of corticotrophin-releasing hormone neurones in the hypothalamic paraventricular nucleus and the sympathoadrenal and pituitary-adrenal responses to formalin-induced pain in the rat. *Journal of Neuroendocrinology* **22**, 467–476.

de Kloet ER, Joëls M, Holsboer F (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience* **6**, 463–475.

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Drago F, Nicolosi A, Micale V, Lo Menzo G (2001). Placebo affects the performance of rats in models of depression: is it a good control for behavioural experiments? *European Neuropsychopharmacology* **11**, 209–213.

Duman RS, Malberg J, Thome J (1999). Neural plasticity to stress and antidepressant treatment. *Biological Psychiatry* **46**, 1181–1191.

Griebel G, Perrault G, Soubrié P (2001). Effects of SR48968, a selective non-peptide NK2 receptor antagonist on emotional processes in rodents. *Psychopharmacology* **158**, 241–251.

Gottmann K, Mittmann T, Lessmann V (2009). BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses. *Experimental Brain Research* **199**, 203–234.

Herman JP, Ostrander MM, Mueller NK, Figueiredo H (2005). Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **29**, 1201–1213.

Hesketh S, Jessop DS, Hogg S, Harbuz MS (2005). Differential actions of acute and chronic citalopram on the rodent hypothalamic-pituitary-adrenal axis response to acute restraint stress. *Journal of Endocrinology* **185**, 373–382.

Jacobson LH, Cryan JF (2007). Feeling strained? Influence of genetic background on depression-related behavior in mice: a review. *Behavior Genetics* **37**, 171–213.

Jensen JB, Jessop DS, Harbuz MS, Mørk A, *et al.* (1999). Acute and long-term treatments with the selective serotonin reuptake inhibitor citalopram modulate the HPA axis activity at different levels in male rats. *Journal of Neuroendocrinology* **11**, 465–471.

Jéquier E, Lovenberg W, Sjoerdsma A (1967). Tryptophan hydroxylase inhibition: the mechanism by which p-chlorophenylalanine depletes rat brain serotonin. *Molecular Pharmacology* **3**, 274–278.

Kolber BJ, Muglia LJ (2009). Defining brain region-specific glucocorticoid action during stress by conditional gene disruption in mice. *Brain Research* **1293**, 85–90.

Kunugi H, Hori H, Adachi N, Numakawa T (2010). Interface between hypothalamic-pituitary-adrenal axis and brain-derived neurotrophic factor in depression. *Psychiatry and Clinical Neurosciences* **64**, 447–459.

Lanfumey L, Mongeau R, Cohen-Salmon C, Hamon M (2008). Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. *Neuroscience and Biobehavioral Reviews* **32**, 1174–1184.

Louis C, Stemmelin J, Boulay D, Bergis O, *et al.* (2008). Additional evidence for anxiolytic- and antidepressant-like activities of saredutant (SR48968), an antagonist at the neurokinin-2 receptor in various rodent-models. *Pharmacology, Biochemistry and Behavior* **89**, 36–45.

Luo C, Xu H, Li XM (2004). Post-stress changes in BDNF and Bcl-2 immunoreactivities in hippocampal neurons: effect of chronic administration of olanzapine. *Brain Research* **1025**, 194–202.

McEwen BS (2005). Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. *Metabolism* **54**, 20–33.

McLaughlin KJ, Gonez JL, Baran SE, Conrad CD (2007). The effects of chronic stress on hippocampal morphology and function: an evaluation of chronic restraint paradigms. *Brain Research* **1161**, 56–64.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000). Chronic antidepressant treatment increases neurogenesis in adult hippocampus. *Journal of Neuroscience* **20**, 9104–9110.

Micale V, Arezzi A, Rampello L, Drago F (2006). Melatonin affects the immobility time of rats in the forced swim test: the role of serotonin neurotransmission. *European Neuropsychopharmacology* **16**, 538–545.

Micale V, Scapagnini G, Colombrita C, Mazzola C (2008*a*). Behavioral effects of dietary cholesterol in rats tested in experimental models of mild stress and cognition tasks. *European Neuropsychopharmacology* **18**, 462–471.

Micale V, Tamburella A, Leggio GM, Mazzola C, *et al.* (2008*b*). Behavioral effects of saredutant, a tachykinin NK2 receptor antagonist, in experimental models of mood disorders under basal and stress-related conditions. *Pharmacology, Biochemistry and Behavior* **90**, 463–469.

Nibuya M, Nestler EJ, Duman RS (1996). Chronic antidepressant administration increases the expression of cAMP element binding protein (CREB) in rat hippocampus. *Journal of Neuroscience* **16**, 2365–2372.

Overstreet DH, Naimoli VM, Griebel G (2010). Saredutant, an NK2 receptor antagonist, has both antidepressant-like effects and synergizes with desipramine in an animal model of depression. *Pharmacology, Biochemistry and Behavior* **96**, 206–210.

Page ME, Detke MJ, Dalvi A, Kirby LG, *et al.* (1999). Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology* **147**, 162–167.

Pintér O, Domokos A, Mergl Z, Mikics E, *et al.* (2011). Do stress hormones connect environmental effects with behavior in the forced swim test? *Endocrine Journal* **58**, 395–407.

Pittenger C, Duman RS (2008). Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* **33**, 88–109.

Porsolt RD, Bertin A, Jalfre M (1978). 'Behavioural despair' in rats and mice: strain differences and the effects of imipramine. *European Journal of Pharmacology* **51**, 291–294.

Ribeiro RL, De Lima TC (2002). Participation of GABAA receptors in the modulation of experimental anxiety by tachykinin agonists and antagonists in mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **26**, 861–869.

Risbrough VB, Stein MB (2006). Role of corticotropin releasing factor in anxiety disorders: a translational research perspective. *Hormones and Behavior* **50**, 550–561.

Rittenhouse PA, López-Rubalcava C, Stanwood GD, Lucki I (2002). Amplified behavioral and endocrine responses to forced swim stress in the Wistar–Kyoto rat. *Psychoneuroendocrinology* **27**, 303–318.

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Rogacki N, Lopez-Grancha M, Naimoli V, Potestio L, *et al.* (2011). The neurokinin NK2 antagonist, saredutant, ameliorates stress-induced conditions without impairing cognition. *Pharmacology, Biochemistry and Behaviour* **98**, 405–411.

Salomé N, Stemmelin J, Cohen C, Griebel G (2006). Selective blockade of NK2 or NK3 receptors produces anxiolytic- and antidepressant-like effects in gerbils. *Pharmacology, Biochemistry and Behavior* **83**, 533–539.

Sandford JJ, Argyropoulos SV, Nutt DJ (2000). The psychobiology of anxiolytic drugs. Part 1: basic neurobiology. *Pharmacology and Therapeutics* **88**, 197–212.

Scharfman H, Goodman J, Macleod A, Phani S, *et al.* (2005). Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Experimental Neurology* **192**, 348–356.

Schmidt HD, Duman RS (2007). The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. *Behavioural Pharmacology* **18**, 391–418.

Shirayama Y, Chen AC-H, Nakagawa S, Russell DS, *et al.* (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *Journal of Neuroscience* **22**, 3251–3261.

Steinberg R, Alonso R, Griebel G, Bert L, *et al.* (2001). Selective blockade of neurokinin-2 receptors produces antidepressant-like effects associated with reduced corticotropin-releasing factor function. *Journal of Pharmacology and Experimental Therapeutics* **299**, 449–458.

Stemmelin J, Canolle B, Roudières V, Labie C, *et al.* (2008). Saredutant (SR48968), a NK2 receptor antagonist, blocks chronic stress-induced decrease in neurogenesis in Balb/c mice. *European Neuropsychopharmacology* **18**(S4), S356.

Stratton SC, Beresford IJ, Harvey FJ, Turpin MP (1993). Anxiolytic activity of tachykinin NK2 receptor antagonists in the mouse light-dark box. *European Journal of Pharmacology* **250**, 11–12.

Tamburella A, Micale V, Leggio GM, Drago F (2010). The beta3 adrenoceptor agonist, amibegron (SR58611A) counteracts stress-induced behavioral and neurochemical changes. *European Neuropsychopharmacology* **20**, 704–713.

Tamburella A, Micale V, Navarria A, Drago F (2009). Antidepressant properties of the 5-HT4 receptor partial agonist, SL65.0155: behavioral and neurochemical studies in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **33**, 1205–1210.

Tardito D, Perez J, Tiraboschi E, Musazzi L, *et al.* (2006). Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic, mechanisms in the action of antidepressants: a critical overview. *Pharmacological Reviews* **58**, 115–134.

Teixeira RM, De Lima TCM (2003). Involvement of tachykinin NK1 receptor in the behavioral and immunological responses to swimming stress in mice. *Neuropeptides* **37**, 307–315.

Walsh DM, Stratton SC, Harvey FJ, Beresford IJ, *et al.* (1995). The anxiolytic-like activity of GR159897, a non-peptide NK2 receptor antagonist, in rodent and primate models of anxiety. *Psychopharmacology* **121**, 186–191.

Warner-Schmidt JL, Duman RS (2006). Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus* **16**, 239–249.

Xu H, Richardson JS, Li XM (2003). Dose-related effects of chronic antidepressants on neuroprotective proteins BDNF, Bcl-2 and Cu/Zn-SOD in rat hippocampus. *Neuropsychopharmacology* **28**, 53–62.

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