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RESEARCH PAPER

Stress-induced anhedonia is associated with an increase in Alzheimer's disease-related markers

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BACKGROUND AND PURPOSE
Stress is believed to be associated with the development of neuropsychiatric disorders, including Alzheimer's disease (AD). We have studied mechanisms implicated in vulnerability to stress and the relationship with changes in AD-related markers.

EXPERIMENTAL APPROACH
Anhedonia induced by a chronic mild stress (CMS) procedure, applied for 6 weeks, was used to select rats vulnerable or resistant to stress. Sucrose intake, the Porsolt forced swimming test and cognitive deficits in the novel object recognition test (NORT) were used to characterize vulnerable and resilient rats. The antidepressant venlafaxine (20 $mg \cdot kg^{-1}$ p.o.) or saline was administered daily during the last 2 weeks of CMS. Biochemical markers affected by stress, PKB, ERK and synaptophysin, and those associated with AD, amyloid β-protein (Aβ), β-secretase (BACE1) and τ phosphorylation, were measured in the hippocampus.

KEY RESULTS
After CMS, 40% of rats were resistant to the development of anhedonia (CMS-resistant to stress), whereas the remaining were responsive [CMS-anhedonic (CMSA)]. Only CMSA rats displayed significant increases in immobility time in the forced swimming test and cognitive deficits in the NORT, and significant decreases in synaptophysin, phosphorylated PKB and phosphorylated ERK1/2 expression in the hippocampus. Increased levels of Aβ40, BACE1 and τ phosphorylation were also found only in CMSA rats. All these effects in CMSA rats were reverted by treatment with venlafaxine.

CONCLUSIONS AND IMPLICATIONS
Vulnerability to stress might constitute a risk factor for the development of AD, and pharmacological treatment with venlafaxine may represent a therapeutic strategy for the treatment of stress-related disorders, including AD.

Abbreviations
Aβ, amyloid β-protein; AD, Alzheimer's disease; BACE1, β-secretase; CMS, chronic mild stress; CMSA, CMS-anhedonic; CMSR, CMS-resistant to stress; HPA, hypothalamic-pituitary-adrenal; NORT, novel object recognition test

Introduction

Stressful experiences are believed to be closely associated with the development of psychological alterations and neuropsychiatric disorders. There are also important memory disturbances in stress-related psychiatric disorders (Bremner and Narayan, 1998; Bremner *et al.*, 2003). Even more, major stressors experienced throughout the lifespan have also been hypothesized to contribute to variability in the ageing process (McEwen, 2002). Clinical data suggest that a stressful lifestyle can be a risk factor for Alzheimer's disease (AD) (Wilson *et al.*, 2005) and stress-related psychiatric disorders (i.e. major depression) have been identified as a risk for developing AD (Ownby *et al*., 2006). There is a dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis in AD and cognitive status was negatively associated with glucocorticoid levels (Elgh *et al*., 2006; Popp *et al*., 2009; Gil-Bea *et al*., 2010). Csernansky *et al*. (2006) also showed that initial, higher serum glucocorticoids in the predementia clinical stage of AD predicts a more rapid cognitive decline. This view has gained support from studies in transgenic mouse models of AD in which stress or glucocorticoids exacerbated AD-like neuropathology (Green *et al*., 2006; Jeong *et al*., 2006).

It is well established that individuals are not equally resilient to stress and, therefore, some suffer greater neuropsychiatric pathophysiology than others (Charney, 2004; Southwick *et al*., 2005). Hence, it is possible that individuals vulnerable to stress could be at a higher risk of developing AD. In which case, understanding the factors that influence stress resistance may help to develop better pharmacotherapies to treat stress-related disorders, including AD. In fact, in a recent paper by Wilson *et al*. (2011), it has been demonstrated in a longitudinal clinical-pathological cohort study that higher levels of anxiety and vulnerability to stress are associated with increased risk of AD and a more rapid decline in global cognition. At present, the neural substrates and molecular mechanisms that mediate resistance to the deleterious effects of stress are not completely understood. It has been shown that resistance to the development of stress-induced behavioural alterations could be associated with alterations in brain-derived neurotrophic factor (BDNF) (Krishnan *et al*., 2007; Taliaz *et al*., 2011), apoptotic pathways (Bergström *et al*., 2008; Shishkina *et al*., 2010), AMPA receptors (Schmidt *et al*., 2010) or the potassium channel TREK1 (Heurteaux *et al*., 2006). Importantly, critical individual differences in resilience to both the behavioural and the neurochemical effects of stress have been reported (Feder *et al*., 2009).

Insights into the biology of variations in susceptibility can be gained by understanding models of individual differences in response to stress (Rutter, 2006). We used the validated chronic mild stress (CMS) paradigm to induce anhedonia, a core symptom of major depression, in rats. Almost 40% of animals exposed to CMS are resistant to the development of anhedonia, whereas the remaining are susceptible, CMS-resistant and CMS-vulnerable, respectively (Strekalova *et al*., 2004; Bergström *et al*., 2008). As resistance to anhedonia in the CMS can be generalized to other behavioural measures [behavioural despair in the Porsolt forced swimming test and cognitive deficits in the novel object recognition test (NORT)] we performed a characterization of vulnerable and resilient rats. We investigated the biochemical mechanisms underlying vulnerability to stress-induced psychopathology and their relationship to AD markers. In addition, we studied the possible effects of pharmacological intervention in stress-vulnerable rats with the antidepressant venlafaxine. Venlafaxine is a 5-HT and noradrenaline re-uptake inhibitor, which, in epidemiological analyses, has demonstrated better short-term efficacy than other antidepressants, such as fluoxetine. Altogether, the results from the present study support the notion that vulnerability to stress might constitute a risk factor for the development of AD, and that pharmacological treatment with venlafaxine may represent a therapeutic strategy for the treatment of stress-related disorders, including AD.

Methods

Animals

Male Wistar rats (Charles River Laboratories, Barcelona, Spain), weighing 180–200 g, were housed in a temperature- (21 ± 1°C) and humidity- (55 ± 5%) controlled room on a 12 h light/dark cycle with food and water freely available, except during the scheduled CMS sessions. All animal care and experimental procedures were performed in strict compliance with the recommendations by the European Union (DOCE L 358/1/18/2/1986) for the care and use of laboratory animals. Behavioural experiments were conducted between 9 h and 13 h. Animals were randomly assigned to the control and stressed groups. Animals were housed in groups except during the scheduled CMS sessions (sucrose intake test), in which rats were individually housed.

CMS paradigm and experimental design

The CMS procedure was applied for 6 weeks. Unpredictable mild stressors (two to three in any 24 h period) were randomly applied (Elizalde *et al*., 2008; Andreasen *et al*., 2011). The experimental timeline of the CMS procedure was as follows: Monday: soiled bedding (6 h), white noise (4 h); Tuesday: paired housing (2 h), 45° cage tilt (8 h); Wednesday: paired housing (2 h), overnight illumination; Thursday: stroboscopic illumination (8 h), removal of nesting material (12 h); Friday: stroboscopic illumination (in dark cycle) (8 h), soiled cage bedding (6 h); Saturday: mice odour (8 h), confinement (1 h); Sunday: stroboscopic illumination (in dark cycle; 8 h). Control animals were left undisturbed in the home cages with the exception of general handling (i.e. regular cage cleaning and measuring body weight). Food and water were freely available to all animals during the CMS procedure. Sucrose intake (see later discussion) was monitored weekly. Venlafaxine (Almirall SA, Barcelona, Spain, 20 mg·kg^{-1} p.o.) or saline was administered daily during the last 2 weeks of CMS. The dose of venlafaxine was chosen according to previous studies (Reneric and Lucki, 1998; Czubak *et al*., 2009).

The time course of the experiments performed is shown in Figure 1. On the last week of CMS, the NORT (days 1 and 2) and Porsolt forced swimming test (days 3 and 4) were performed. Venlafaxine was administered after the behavioural testing (20 h before the following behavioural session).

Behavioural tests

Sucrose intake test. Anhedonic-like behaviour was evaluated by weekly monitoring of sucrose intake. Rats were trained to drink a sucrose solution for 1 week. After this preliminary phase, once a week, rats were given a 15 h exposure to two standard drinking bottles, one containing 2% sucrose and the other tap water. The position of the two bottles (right/left) was varied randomly from trial to trial. Body weight measurements were taken weekly in both stressed and non-stressed groups, and the relative sucrose intake was calculated as absolute intake (g) per rat body weight. In the CMS group, rats

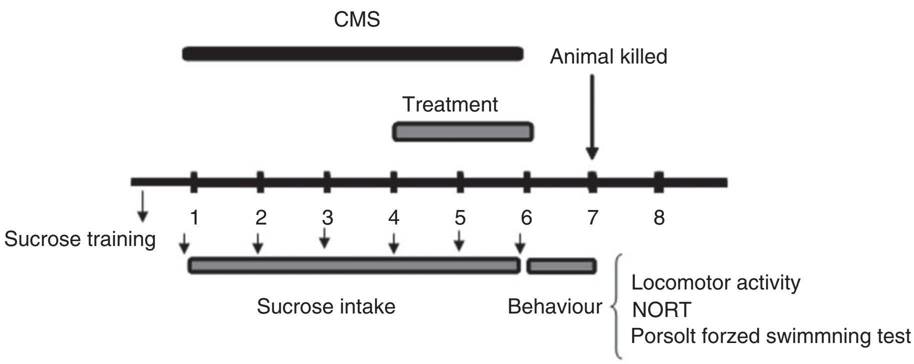

Figure 1

Experimental time course. The chronic mild stress procedure was applied for 6 weeks. Venlafaxine or saline was administered daily for the last 2 weeks of CMS. Anhedonic-like behaviour was evaluated by weekly monitoring of sucrose intake.

were considered to be resistant to stress [CMS-resistant to stress (CMSR)] when sucrose intake was similar to controls for three or more weeks during the CMS paradigm.

Forced swimming test. As described by Porsolt *et al*. (1977), two swimming sessions were conducted: an initial 15 min pretest followed 24 h later by a 5 min test. Rats were placed individually in a vertical plexiglass cylinder (height: 45 cm, diameter: 19 cm) filled with 28–30 cm of 26°C water. Immobility was considered as rats floating passively, making only small movements to keep its nose above the surface.

Object recognition

The object recognition test was adapted from Ennaceur and Delacour (1988). The open field consisted of a square open field (65 cm × 65 cm × 45 cm) made of black wood. On the previous day to the experiment, animals were familiarized with the field for 30 min. During the first trial of the experiment, two objects similar in shape, size, colour, texture, etc., equidistant from the sides (10 cm) were placed within the chamber. The animal was placed in the centre of the open field and allowed to freely explore for 5 min. It was considered that the animal was exploring the object when the head of the rat was orientated towards the object with its nose within 2 cm of the object. One hour later, a second trial took place, in which one object was replaced by a different one, and exploration was scored for 5 min. In order to eliminate olfactory stimuli, chamber and objects were cleaned after testing each animal. To avoid preference for one of the objects, the order of the objects was balanced between testing animals. Results are expressed as percentage of time spent with the novel object with respect to the total exploration time (discrimination index).

Tissue and blood collection

Fasting rats were killed between 8 h 00min and 10 h00min. Adrenal glands were weighed. Brains were removed and dissected on ice to obtain the hippocampus, or frozen immediately and stored at –80°C, according to random assignment. Trunk blood was placed in EDTA tubes, centrifuged at 12 500× *g* (15 min, 4°C), and plasma was frozen until corticosterone levels were determined.

Corticosterone measurement

Plasma corticosterone (30 µL) was determined using a commercially available enzyme immunoassay kit (Coat-A-Count Rat Corticosterone, Siemens, Los Angeles, CA, USA). All assays were performed in duplicate. Limit of detection was 5.7 ng·mL^{-1} and intra- and interassay coefficients of variation were less than 10% for all comparisons. Corticosterone concentration values were expressed as ng ml^{-1}.

Western blotting

Assays were performed as described in Table 1. Immunopositive bands were visualized using an enhanced chemiluminescense Western blotting detection reagent (ECL, Amersham, Buckinghamsire, England). The OD of reactive bands visible on X-ray film was determined densitometrically. β-actin was used as internal control. Results are expressed as percentage of OD values of non-stressed saline (control) rats.

Amyloid β-protein (Aβ) levels

Aβ40 and Aβ42 levels were determined using a commercially available high-sensitive ELISA kits (Wako Pure Chemical Industries, Tokyo, Japan) following manufacturer instructions.

Data analysis and statistics

Data were analysed by SPSS for Windows, release 11.0 (SPSS Inc., Chicago, IL, USA). Normality was checked by Shapiro–Wilks's-test ($P > 0.05$). Behavioural and biochemical data were analysed by two-way ANOVA (resistance to stress × treatment), followed by Student's *t*-test adjusted by Bonferroni correction. *Post hoc* comparisons were conducted if appropriate, using Tukey's protected least significance test.

Results

Segregation of rats into susceptible (anhedonic) and unsusceptible (resistant) populations

Sucrose intake was used instead of sucrose preference as criteria for anhedonia, among other reasons, to account for the

Table 1

Conditions used in Western blotting experiments

Protein	SDS–polyacrylamide gel	Primary antibody (dilution)
synpatophysin	13%	Anti-synpatophysin (1:2000)[a]
pPKB	13%	Anti-pPKB Ser473 (1:1000)[b]
Total PKB	13%	Anti-PKB (1:1000)[b]
pGSK3β	13%	Anti-pGSK3 Ser 9 (1:1000)[b]
Total GSK3β	13%	Anti-pGSK3 (1:1000)[b]
pERK1/2	13%	Anti-p44/42 Thr202/Tyr204 (1:2000)[b]
Total ERK1/2	13%	Anti-ERK 1/2 (1:2000)[b]
pτ	13%	Anti-pτ Ser202/Thr205 AT8 (1:1000)[c]
Total τ	13%	Anti-τ T46 (1:3000)[d]
GR	8%	Anti-GR (1:2000)[e]
BACE1	13%	Anti-*BACE1* (1:1000)[a]

GR, glucocorticoid receptor. Homogenization buffer: 50 mmol·L^{-1} Tris–HCl, pH 8; 150 mmol·L^{-1} NaCl, 2 mmol·L^{-1} EDTA, 2 mmol·L^{-1} EGTA, 0.5 mmol·L^{-1} PMSF, 1 mmol·L^{-1} sodium vanadate and 10 mg·mL^{-1} leupeptin, 1% Nonidet P-40, 1:100 of phosphatases inhibitors cocktail set II (Calbiochem, Darmstadt, Germany). Source of antibodies: [a]Abcam Inc., Cambridge, MA, USA; [b]Cell Signaling Technology, Beverly, MA, USA; [c]Pierce, Rockford, IL, USA; [d]Sigma-Aldrich, St. Louis, MO, USA; [e]Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA.

decrease in body weight induced by CMS. The criteria for anhedonia in each rat was taken at 4 weeks of CMS application, as both a decrease of sucrose intake below 65% in the 4th week (oe-way ANOVA, $F_{2,39} = 16.056$; $P < 0.001$) and significant lower sucrose intake compared with non-stress rats in 3 weeks (one-way ANOVA repeated measures, $F_{2,37} = 9.223$; $P < 0.01$). This criterion was based on the fact that animals with a sucrose preference <65% in other stress models had shown features of anhedonia and depression, such as an increased threshold of intracranial self-stimulation and sleep disturbances (Willner, 1997). Rats that matched this definition (approximately 60% of rats) were assigned to the anhedonic group [CMS-anhedonic (CMSA)]. The rest of the stressed animals were considered to be non-anhedonic or resistant to stress (CMSR). Rats were assigned to CMSA or CMSR groups before further testing (Figure 2A).

Anhedonic behaviour was reversed after 2 weeks of venlafaxine treatment (6th week of the whole CMS procedure), and CMSA saline was the only group that displayed a lower sucrose intake (Figure 2B, two-way ANOVA, interaction resistance to stress × treatment, $F_{2,39} = 3.502$; $P < 0.05$).

To examine whether resistance to CMS defined by anhedonia generalizes to other stress-related markers, a further phenotypic characterization of CMSA and CMSR rats was performed. Only CMSA rats displayed a significant increase in the immobility time in the Porsolt forced swimming test, consistent with increased depression-like behaviour. This effect was reversed by venlafaxine (Figure 3A, two-way ANOVA, interaction resistance to stress × treatment, $F_{2,39} = 6.004$; $P < 0.01$). Levels of the stress hormone corticosterone, as well as adrenal gland weight, were increased only in the CMSA group, and these increases were counteracted by venlafaxine (Figure 3B and C, two-way ANOVA, interaction resistance to stress × treatment, $F_{2,39} = 3.045$; $P < 0.05$ and $F_{2,39} = 24.610$; $P < 0.001$ respectively). Glucocorticoid receptor (GR) expression was significantly lower in CMSA rats, and this effect was also reversed by venlafaxine (Figure 3D, two-way ANOVA, interaction resistance to stress × treatment, $F_{2,29} = 5.091$; $P < 0.05$).

Vulnerability to anhedonia is associated with deficits in plasticity markers: effects of venlafaxine

Three different markers, already known to be affected by stress, were selected: synaptophysin, widely used to estimate synaptic density (Valtorta *et al.*, 2004); PKB, as part of one of the most critical pathways in regulating cell survival; and ERK1/2, which play essential roles in neuronal survival and synaptic plasticity related to learning and memory formation (Eckel-Mahan *et al.*, 2008).

Significant decreases, around 30%, in synaptophysin levels were found in the hippocampus of CMSA rats (Figure 4A). Decreases in phosphorylated PKB (pPKB) normalized to total PKB (Figure 4B) and phosphorylated ERK1/2 normalized to total ERK1/2 (Figure 4C) were also found. Unsusceptible (CMSR) rats did not show any of these changes. Treatment with venlafaxine reversed all these effects on markers of synaptic plasticity (two-way ANOVA, interaction resistance to stress × treatment, $F_{2,23} = 4.810$; $P < 0.05$; $F_{2,31} = 3.550$; $P < 0.05$; $F_{2,24} = 3.265$; $P < 0.05$ for synaptophysin, PKB and ERK1/2, respectively).

Cognitive performance in anhedonic and resistant rats: effects of venlafaxine

Only CMSA rats displayed cognitive deficits in the NORT, as shown by a significantly decreased discrimination index (Figure 5). Statistical analysis indicates a significant interaction between resistance to stress and venlafaxine treatment on the measure of discrimination between new and familiar

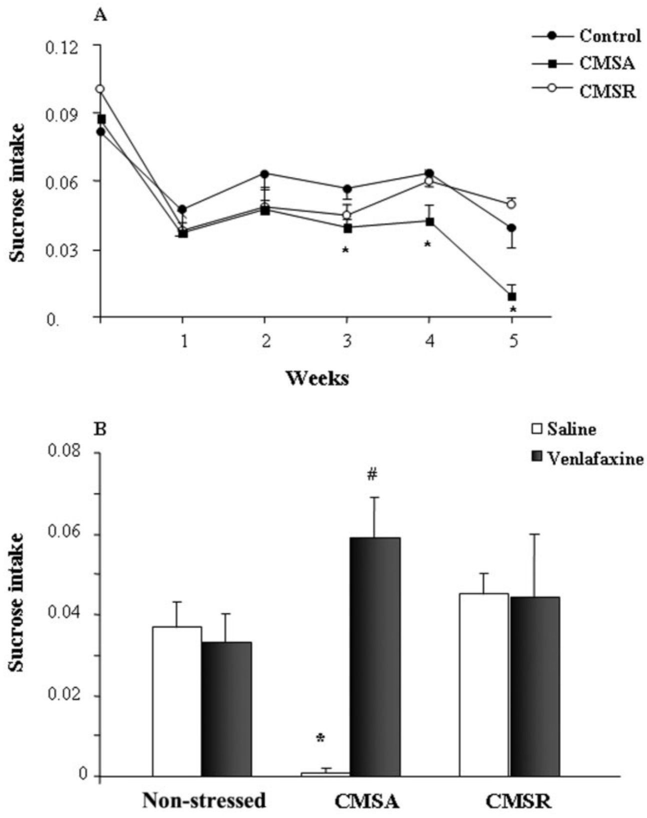

Figure 2

Sucrose preference (anhedonic behaviour). In (A) chronic stress leads to a decreased sucrose intake in a subgroup of rats. According to the criterion for anhedonia (see text), the group of stressed rats was split into anhedonic (CMSA) and resistant (CMSR) subgroups. *P < 0.05 versus non-stressed group, Tukey's protected least significance test. In (B), venlafaxine treatment was able to reverse anhedonic behaviour in CMSA rats. Two-way ANOVA (resistance to stress × treatment), *P < 0.01 versus non-stressed saline group, #P < 0.05 versus CMSA saline, Student's *t*-test. The number of animals per group was n = 10 (non-stressed) or n = 4–6 (CMS).

objects (two-way ANOVA, interaction resistance to stress × treatment, $F_{2,39} = 3.176$; $P < 0.05$). Further analysis (Student's *t*-test) revealed that CMSA animals treated with venlafaxine did not show any memory impairment (Figure 5).

Differences in locomotor activity among groups does not seem to be implicated in these cognitive effects, as there was no difference in the total amount of time spent exploring two identical objects among the groups in the NORT, and the discrimination index was around 50% in all cases. In addition, total distance travelled was similar among all experimental groups (two-way ANOVA, interaction resistance to stress × treatment, $F_{2,39} = 2.710$; $P > 0.05$).

Anhedonic rats display increased levels of AD markers that are reverted by venlafaxine treatment

The principal constituent of amyloid plaques observed in AD, is the amyloid β-protein (Aβ), with Aβ40 and Aβ42 being the predominant Aβ species. CMSA rats had significantly higher hippocampal levels of Aβ40, but not Aβ42 (Figure 6A). Aβ is cleaved from amyloid precursor protein by β-secretase (BACE1). Significant increases in BACE1 levels were also found in CMSA, and not in CMSR rats (Figure 6B). These alterations in AD-related markers were normalized by venlafaxine (two-way ANOVA, interaction resistance to stress × treatment, $F_{2,31} = 3.501$; $P < 0.05$ and $F_{2,34} = 6.056$; $P < 0.01$ for Aβ40 and BACE1, respectively).

The second main feature of AD is τ hyperphosphorylation, as it leads to the formation of neurofibrillary tangles. Levels of pτ normalized to total τ (Figure 6C) were significantly increased in the hippocampus of CMSA rats, and venlafaxine again was able to counteract this increase ($F_{2,25} = 4.971$; $P < 0.05$).

Discussion

The goal of the present study was to identify molecular mechanisms underlying vulnerability to stress-induced psychopathology and a purported relationship of vulnerability to stress with changes in the expression of AD-related markers. We found that stress-induced anhedonia was associated with behavioural features that were not seen in stressed rats without hedonic deficits. Predisposition for stress-induced anhedonia was associated with decreases in the expression of synaptic markers, cognitive deficits and alterations in markers of AD pathology. Interestingly, pharmacological treatment with the antidepressant venlafaxine was able to counteract all these effects. We suggest that venlafaxine, by modulating stress responses, may constitute a new therapeutical approach for the treatment of AD.

Definition of susceptibility to stress by anhedonia (sucrose intake test)

In the present chronic stress protocol, a decrease of preference for sucrose occurred in a subgroup of stress-exposed rats that we categorized as anhedonic. However, another subgroup of stress-exposed rats did not exhibit a decrease in sucrose preference. Since these animals were exposed to the same extent of stress as those rats that developed anhedonia, they were therefore defined as non-anhedonic or resilient to stress. Several caveats have been found when defining hedonic status by sucrose test (Nestler *et al*., 2003). Therefore, we have followed the modified protocol by Strekalova *et al*. (2004), in which a decrease in sucrose preference below 65% was also associated with a reduction in physiological correlates of a depressive syndrome in rodents, such as sexual activity and alterations in circadian rhythms. It is also worth mentioning that anhedonia in response to CMS seems to be a persistent behaviour as, in a previous work from our group, it was found that anhedonic behaviour persisted in stressed animals, even 1 month after the cessation of the chronic stress procedure (Elizalde *et al*., 2008).

The behavioural analysis of both groups revealed that anhedonia in stressed rats is accompanied by other features of depressive-like behaviour, such as increased immobility time in the Porsolt forced swimming test (as previously shown in mice by Strekalova *et al*., 2004), alterations in the HPA axis activity or changes in synaptic plasticity. A point of caution

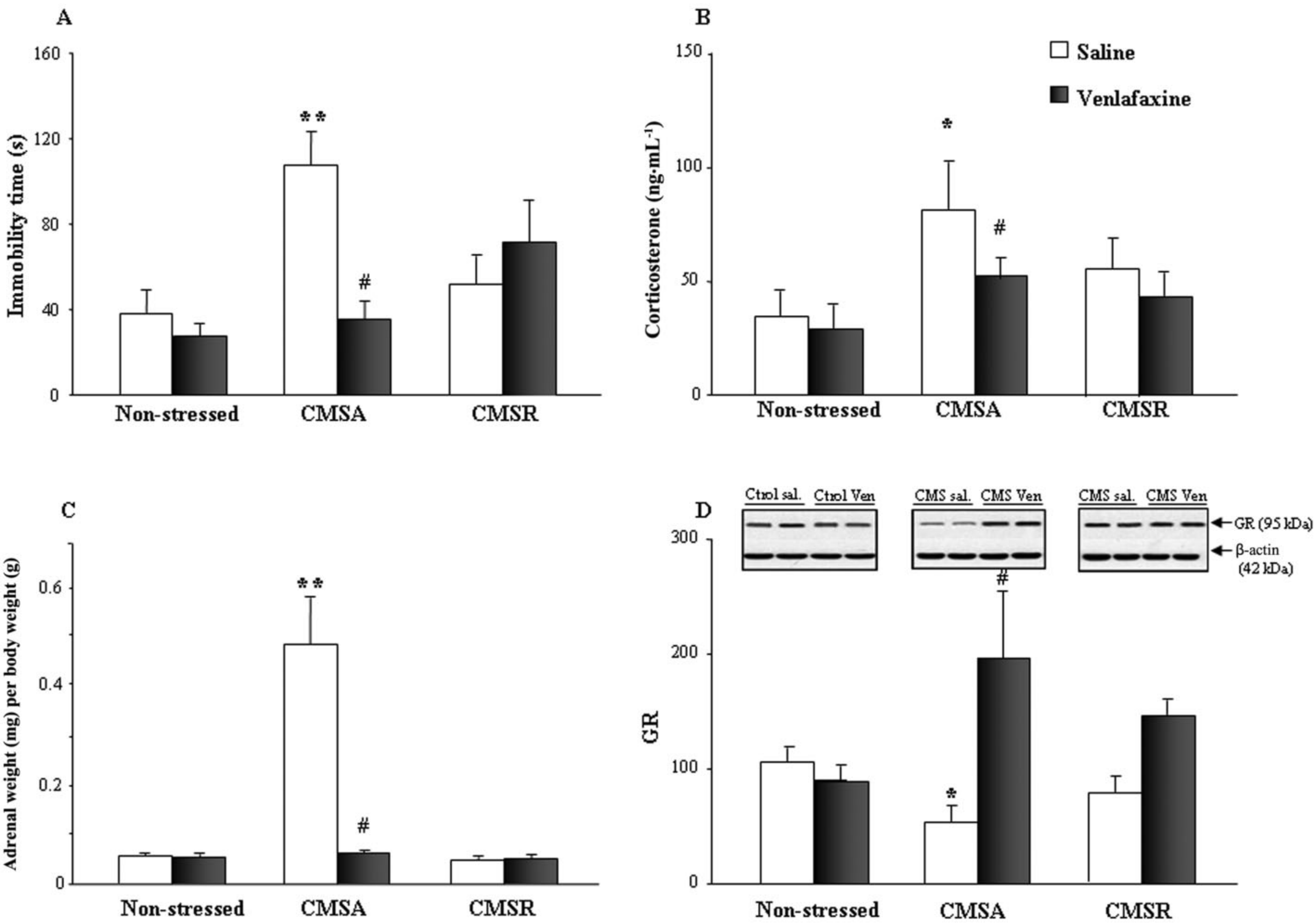

Figure 3

Vulnerability to stress-induced anhedonia generalizes to other stress-related markers. Treatment with venlafaxine was able to counteract the depressive-like behaviour in the Porsolt swimming test and normalize HPA axis hyperactivity. (A) Porsolt forced swimming test. (B) Corticosterone levels in plasma. (C) Adrenal gland weight relative to body weight. (D) GR expression in the hippocampus. Data are shown as % OD of non-stressed saline-treated rats. Upper panel, representative picture of Western blot. CMSA: anhedonic group, rats vulnerable to stress. CMSR: rats resilient to stress. Two-way ANOVA (resistance to stress × treatment), $^*P < 0.05$ or $^{**}P < 0.01$ versus non-stressed saline group, $^{\#}P < 0.05$ versus CMSA saline, Student's *t*-test. The number of animals per group was $n = 10$ (non-stressed) or $n = 4$–6 (CMS). Ctrol, control; sal., saline; Ven, venlafaxine.

should be considered at this point, as due to the segregation of animals into susceptible/resistant to stress, the number of animals in each group might be considerer low. Therefore, the statistical outcomes of the behavioural studies should be considered with caution. It has been shown that alterations in the behavioural phenotype associated with stress are related to the increased HPA axis responsiveness to stressors, similar to humans in which a significant percentage of depressed patients has been shown to hypersecrete cortisol (Pariante and Lightman, 2008). HPA axis hyperactivity is probably due to a reduced GR-mediated negative feedback, which, in turn, increases production and secretion of glucocorticoids (de Kloet *et al.*, 2006; McGowan *et al.*, 2009; Numakawa *et al.*, 2009), thereby contributing to a pathological allostatic overload. Therefore, it is tempting to speculate that changes found in rats susceptible to stress are associated with the specific HPA hyperactivity found in CMSA rats, which in turn would lead to deleterious effects.

Stress-induced anhedonia was associated with changes in markers of plasticity and survival in the neuron that were not seen in stressed rats without hedonic deficit. The hippocampus is critically involved in long-term memory formation (Morris *et al.*, 2003; Poldrack and Packard, 2003) and is also a primary CNS target of stress hormones (de Kloet *et al.*, 1999; McEwen, 1999). But plasticity of hippocampal circuitry, essential for its function in learning and memory, may increase its vulnerability to various insults including stress (McEwen, 1999). In fact, changes in the expression of plasticity markers could represent one of the main mechanisms that account for behavioural/cognitive disturbances observed in stress-related neuropsychiatric disorders. Essentially, 'brains sensitive to stress' may be unable to produce appropriate adaptive neuronal responses (e.g. such as changes in synaptic connections or dendritic branching required to deal with stressors), hence rendering individuals vulnerable to emotional and cognitive disturbances. The molecular mechanisms underlying resis-

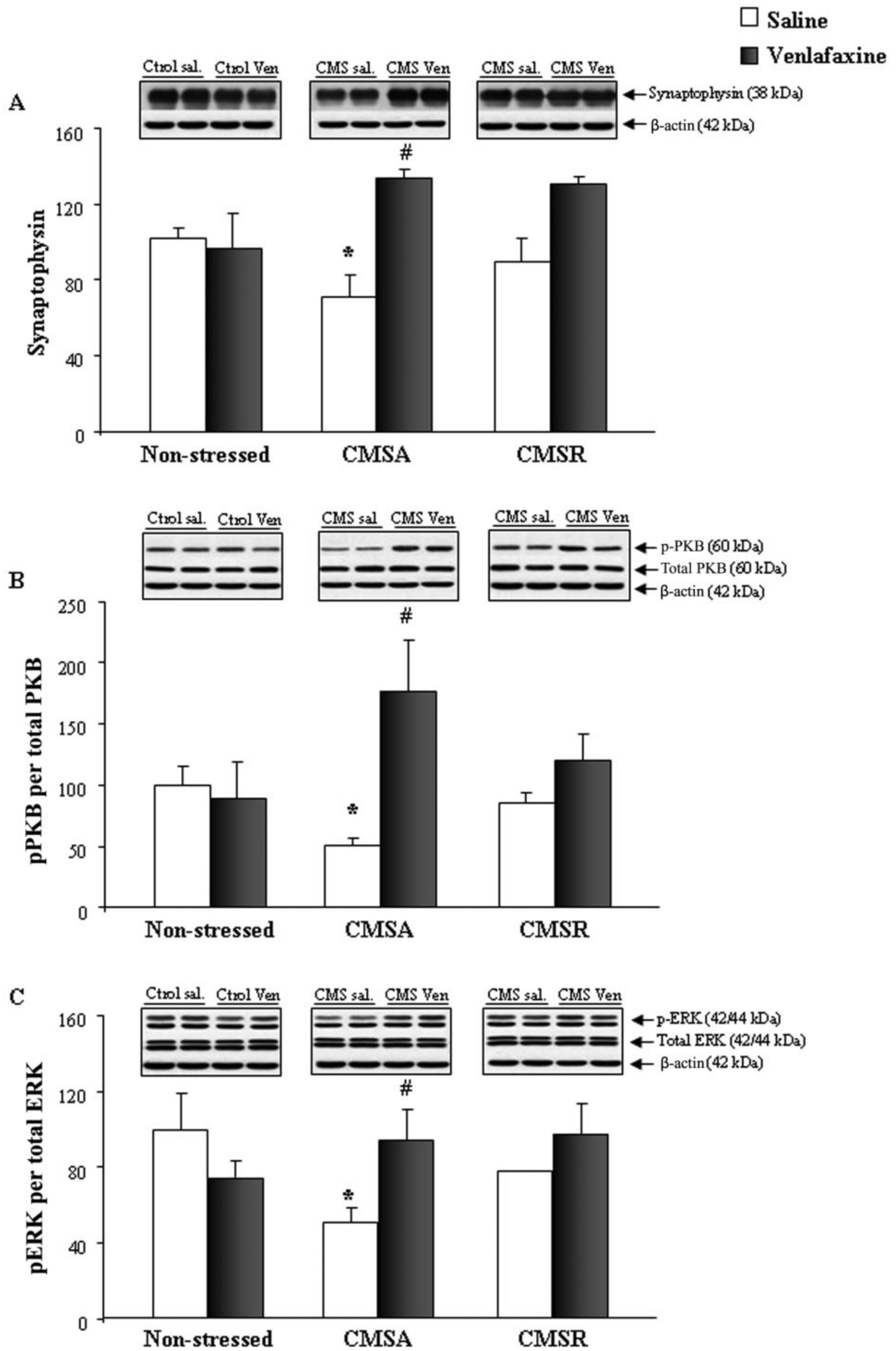

Figure 4

Decreases in plasticity markers are present only in rats vulnerable to stress (CMSA), and are reverted by venlafaxine treatment. (A) Synaptophysin levels, expressed as % OD of non-stressed saline rats. (B) Activation of PKB, expressed as ratio pPKB per total PKB levels. Data are shown as % OD of non-stressed saline rats. (C) Activation of ERK1/2, expressed as ratio pERK per total ERK. Data are shown as % OD of non-stressed saline rats. In all cases, representative picture of Western blot is shown. CMSA: anhedonic group, rats vulnerable to stress. CMSR: rats resilient to stress. Two-way ANOVA (resistance to stress × treatment), $*P < 0.05$ versus non-stressed saline group, $^{\#}P < 0.05$ versus CMSA saline, Student's *t*-test. The number of animals per group was $n = 10$ (non-stressed) or $n = 4$–6 (CMS). Ctrol, control; sal., saline; Ven, venlafaxine.

tance to stress are poorly understood (see Introduction). In a recent paper *in situ* hybridization studies showed an up-regulation of BDNF mRNA in the CMS resilient group and a down-regulation of VEGF mRNA in the CMS-sensitive group (Bergström *et al.*, 2008). Since the rat strain used in the present experiment is the Wistar strain, which is generally considered as an outbred strain, genetic influence may be one of the factors influencing resistance to stress.

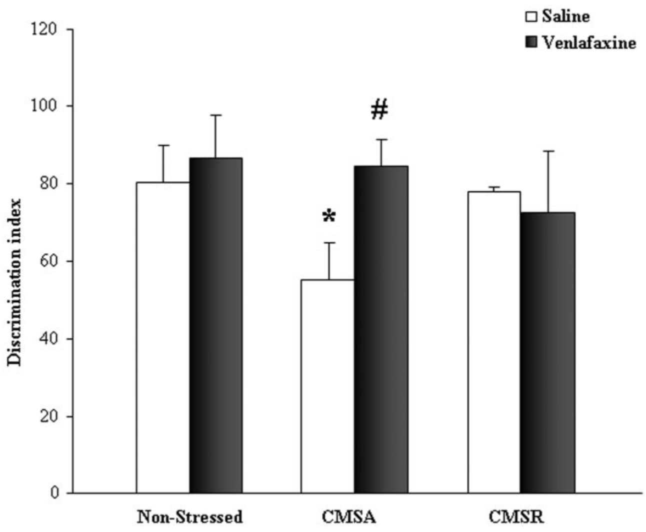

Figure 5

Cognitive deficits were observed in the NORT only in rats vulnerable to stress (CMSA). The decreased discrimination index in the CMSA group was reverted by venlafaxine treatment. CMSR: rats resilient to stress. Two-way ANOVA (resistance to stress × treatment), $*P < 0.05$ versus non-stressed saline group, $^{\#}P < 0.01$ versus CMSA saline, Student's *t*-test. The number of animals per group was $n = 10$ (non-stressed) or $n = 4–6$ (CMS).

Susceptibility to stress as a risk factor for AD

It has been suggested that elevated glucocorticoids and stress may contribute to the development or maintenance of AD. In fact, hyperactivity of the HPA axis is a well-described feature in AD (Elgh *et al.*, 2006; Popp *et al.*, 2009; Gil-Bea *et al.*, 2010). Related to this purported association between stress and AD, cognitive deficits shown in different models of chronic stress (Aisa *et al.*, 2007; Garcia-Garcia *et al.*, 2009) have been suggested to be associated with increased levels of glucocorticoids (Aisa *et al.*, 2007). Supporting this hypothesis, in the present work, only CMSA rats showed cognitive deficits. It is noteworthy that Henningsen *et al.* (2009) reported cognitive deficits in contextual fear conditioning in anhedonic CMS rats, although working memory was altered in all rats subjected to CMS, irrespective of the hedonic status.

It has been suggested that this hypercortisolaemia, leading to hippocampal atrophy and further HPA axis disinhibition (i.e. 'the glucocorticoid cascade hypothesis'), would initiate a chain of events, ultimately culminating in the development of lesions typical of AD (Dhikav and Anand, 2007). This view gained support from studies in transgenic mouse models of AD in which stress or glucocorticoids exacerbated AD-like neuropathology (Green *et al.*, 2006; Jeong *et al.*, 2006). It has been shown experimentally that increased corticosterone levels provoke misprocessing of amyloid precursor peptide in the rat hippocampus, resulting in increased levels of Aβ (Catania *et al.*, 2009; Solas *et al.*, 2010). As a glucocorticoid response element in the promoter region of the *BACE1* gene has been demonstrated (Sambamurti *et al.*, 2004), it is likely that glucocorticoids mediate the regulatory actions of stress on *BACE1* expression by directly increasing transcription of this gene. Accordingly, increased *BACE1* expression in CMSA rats could lead to increased Aβ production. Increased Aβ levels could be also facilitating synaptic disruption in rats sensitive to stress, as it has been shown that treatment with Aβ disrupts the activation of PKB and ERK (Townsend *et al.*, 2007). Different Aβ fragments (40 or 42 amino acids) have been identified, indicating that multiple cleavage sites exist. Aβ40 is the most abundant form, while Aβ42 is the least soluble and most prone to form extracellular deposits. Aβ40 is a critical, built-in mechanism against Aβ42 aggregation. Our data suggested that the increased Aβ40 levels might be protective by perhaps sequestering the more toxic Aβ42 and facilitating its clearance (Kumar-Singh *et al.*, 2006).

Together with synaptic loss, the best correlate to cognitive dysfunction in AD is τ hyperphosphorylation (Gómez-Isla *et al.*, 1997). Aberrant τ phosphorylation may result from dysregulation of different kinase pathways, namely GSK3β, which is a downstream effector in the PKB pathway. The PKB/GSK3β pathway promotes neuronal survival by directly inactivating the pro-apoptotic machinery. Interestingly, it is this same route that is required for the induction of long-term potentiation and depression, basic processes underlying learning and memory (van der Heide *et al.*, 2006). Therefore, hyperphosphorylation of τ may result from dysregulation of the PKB–GSK pathway and would contribute to the detrimental effect of stress on AD in susceptible individuals. τ might be phosphorylated in multiple sites. Among them, GSK3β is responsible for phosphorylating Ser202 of τ protein (Mandelkow *et al.*, 1992), and therefore, following our hypothesis of the involvement of the PKB-GSK pathway, we checked and observed an increase on this phosphorylation at 202/204. Previous reports have also shown that animals subjected to CMS showed increased expression of τ phosphorylated at Ser 202 (Cuadrado-Tejedor *et al.*, 2011).

On the other hand, previous works showing that stress can induce hippocampal τ phosphorylation in rodents have suggested a specific involvement of the CRF signalling system in stress-associated τ hyperphosphorylation (Rissman *et al.*, 2007; Rissman, 2009; Zhang *et al.*, 2011).

Venlafaxine, by modulating the HPA axis, reverses the deleterious effects of stress

In experimental models of chronic stress, different molecular mechanisms in the hippocampus have been implicated in the antidepressant effects of venlafaxine, such as significant increases in BDNF levels (Czubak *et al.*, 2009; Larsen *et al.*, 2010), up-regulated expression of B-cell lymphoma extra large (Bcl-xl) and down-regulated expression of B-cell lymphoma-2-associated X protein (Bax) (Wang *et al.*, 2011). Among other mechanisms, the effects of antidepressant treatment on the HPA axis and feedback inhibition by glucocorticoids may be important in understanding the mechanism by which antidepressants exert their clinical activity. In this sense, it has been suggested that antidepressants, such as venlafaxine, could exert their clinical activity through a modulation of the HPA axis.

Following our hypothesis that changes found in rats susceptible to stress are associated with the specific HPA hyperactivity found in CMSA rats, which in turn would lead to deleterious effects, it is suggested that venlafaxine, by reversing alterations in the HPA axis, was able to counteract the effects of stress on behaviour, synaptic markers or even AD markers. It is speculated then that pharmacological treatment

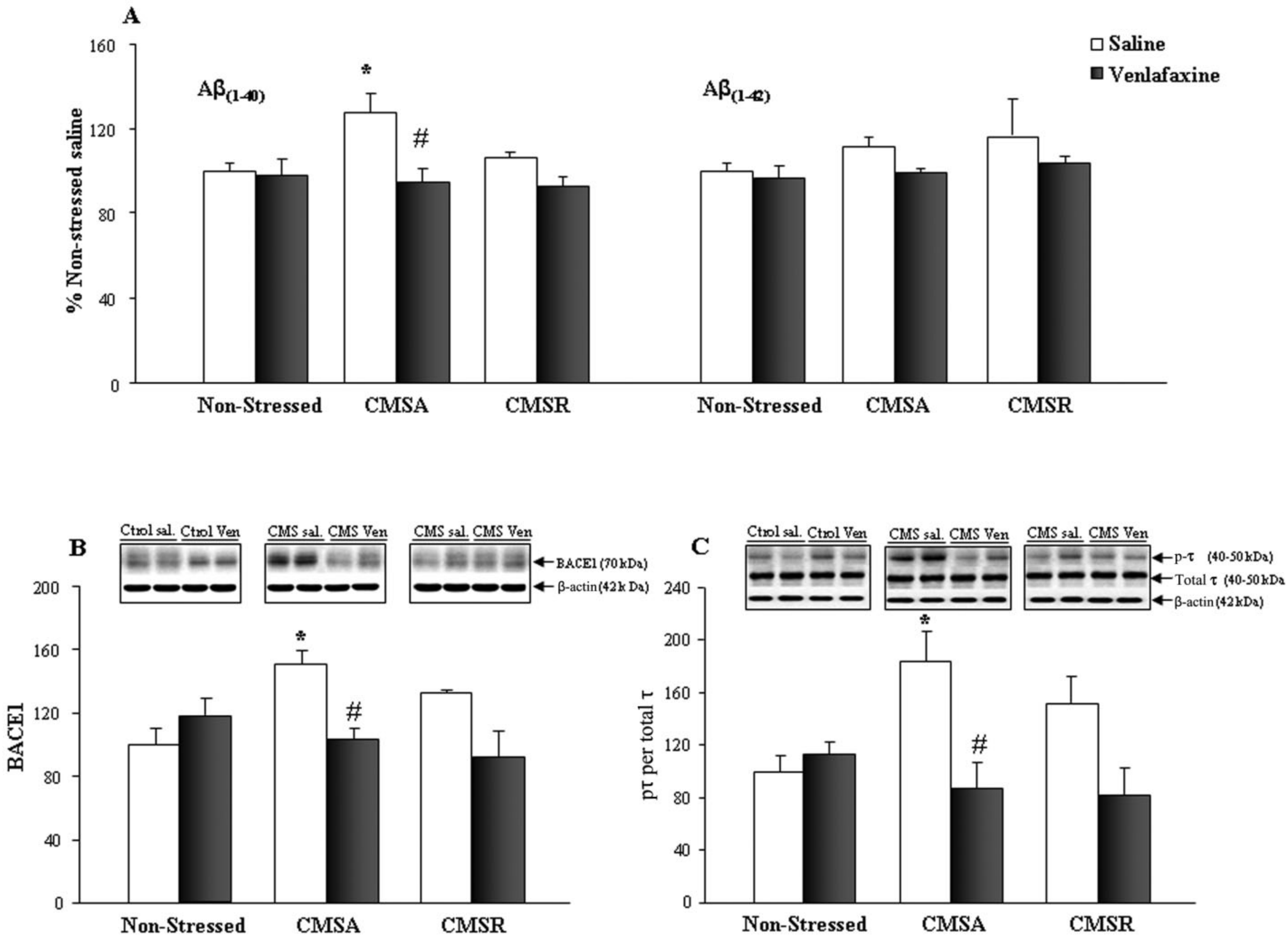

Figure 6

Effects of vulnerability to stress on the expression of AD related markers in the hippocampus. (A) Aβ40 and Aβ42 levels. (B) *BACE1* expression. Data are shown as % OD of non-stressed saline-treated rats. Upper panel, representative picture of Western blot. (C) τ hyprephosphorylation at Ser202/Thr205, expressed as ratio pτ per total τ. Data are shown as % OD of non-stressed saline-treated rats. Upper panel, representative picture of Western blot. CMSA: anhedonic group, rats vulnerable to stress. CMSR: rats resilient to stress. Two-way ANOVA (resistance to stress × treatment), $^{*}P < 0.05$ versus non-stressed saline group, $^{\#}P < 0.05$ versus CMSA saline, Student's *t*-test. The number of animals per group was $n = 10$ (non-stressed) or $n = 4–6$ (CMS).

with venlafaxine, an already clinically used antidepressant treatment, might be considered a new approach for the treatment of both cognitive and behavioural symptoms and neuropathological markers of AD. Whether this new therapeutic outcome could be extended to other antidepressants or is a specific feature of venlafaxine cannot be predicted. The modulation of the HPA axis by different antidepressants has been observed. However, clinical efficacy and even latency to reach effect is different among antidepressants, and venlafaxine has been found to be more effective than fluoxetine in clinical studies (Bauer *et al*., 2009; Cipriani *et al*., 2009).

Conclusions

It is proposed here that anhedonic behaviour after chronic stress may serve as a model for studying the more general phenomenon of resistance/sensitivity to stress in humans. In addition, our results suggest that individuals sensitive to stress are more prone to the appearance of memory impairment, τ hyperphosphorylation and synaptic/plastic alterations, which could contribute to the development of AD. The biological basis for all of these alterations seems to be related to HPA axis dysfunctions and the associated increase in glucocorticoids. Therefore, treatments aimed at normalizing the HPA axis could have purported therapeutical interest for the treatment of AD and other stress-related disorders.

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Conflicts of interest

None.

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