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ARTICLE

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The antidepressant agomelatine blocks the adverse effects of stress on memory and enables spatial learning to rapidly increase neural cell adhesion molecule (NCAM) expression in the hippocampus of rats

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Abstract

Agomelatine, a novel antidepressant with established clinical efficacy, acts as a melatonin receptor agonist and 5-HT_{2C} receptor antagonist. As stress is a significant risk factor in the development of depression, we sought to determine if chronic agomelatine treatment would block the stress-induced impairment of memory in rats trained in the radial-arm water maze (RAWM), a hippocampus-dependent spatial memory task. Moreover, since neural cell adhesion molecule (NCAM) is known to be critically involved in memory consolidation and synaptic plasticity, we evaluated the effects of agomelatine on NCAM, and polysialylated NCAM (PSA-NCAM) expression in rats given spatial memory training with or without predator stress. Adult male rats were pre-treated with agomelatine (10 mg/kg i.p., daily for 22 d), followed by a single day of RAWM training and memory testing. Rats were given 12 training trials and then they were placed either in their home cages (no stress) or near a cat (predator stress). Thirty minutes later the rats were given a memory test trial followed immediately by brain extraction. We found that: (1) agomelatine blocked the predator stress-induced impairment of spatial memory; (2) agomelatine-treated stressed, as well as non-stressed, rats exhibited a rapid training-induced increase in the expression of synaptic NCAM in the ventral hippocampus; and (3) agomelatine treatment blocked the water-maze training-induced decrease in PSA-NCAM levels in both stressed and non-stressed animals. This work provides novel observations which indicate that agomelatine blocks the adverse effects of stress on hippocampus-dependent memory and activates molecular mechanisms of memory storage in response to a learning experience.

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Key words: Antidepressant, cell adhesion molecules, hippocampus, memory, stress.

Introduction

Agomelatine is a novel antidepressant which acts as an agonist of melatonergic MT_1 and MT_2 receptors (Ying et al., 1996; Yous et al., 1992) and as an antagonist of 5-HT_{2C} receptors (Chagraoui et al., 2003; Millan et al., 2003). Clinical trials have shown that agomelatine has a powerful antidepressant efficacy (Kennedy and Emsley, 2006; Loo et al., 2002; Olie and Kasper, 2007) with fewer side-effects than are found with other commonly prescribed antidepressants (Kennedy and Emsley, 2006; Montgomery, 2006) Agomelatine is an effective treatment for depression because it resynchronizes disrupted circadian rhythms (Armstrong et al., 1993; Martinet et al., 1996; Quera

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Salva et al., 2007; Redman et al., 1995; Van Reeth et al., 1997) which are disturbed in depression. Indeed, researchers have long speculated that the disorganization of internal circadian rhythms plays a critical role in the development of major depression (Armitage, 2007; Goodwin et al., 1982; Hallonquist et al., 1986; Healy and Waterhouse, 1995; McClung, 2007).

In preclinical studies on rodents, agomelatine has been shown to have both antidepressant (Barden et al., 2005; Bourin et al., 2004) and anxiolytic (Millan et al., 2005; Papp et al., 2006) properties. Chronic administration of agomelatine improves avoidance learning deficits induced in the learned helplessness model of depression (Bertaina-Anglade et al., 2006) and produces antidepressant-like effects in the chronic mild stress model of depression (Papp et al., 2003). Stress is a known risk factor in the development of many neuropsychiatric disorders, including depression (Bremner and Vermetten, 2001; Heim and Nemeroff, 1999; Mazure, 1995; Tsuang, 2000). Moreover, as stress-induced memory impairments are commonly reported in stress-related psychopathologies (de Quervain et al., 2000; Kirschbaum et al., 1996), there is therapeutic relevance to the use of antidepressant treatments to prevent stress-induced cognitive deficits. For example, there is considerable work showing that the adverse effects of acute and chronic stress on diverse hippocampal functions can be blocked by treatment of rats with antidepressants that have serotonergic (Hitoshi et al., 2007), as well as non-serotonergic (Campbell et al., 2008; Diamond et al., 2004; McEwen and Olie, 2005; Vouimba et al., 2006), modes of action.

At a more reductionistic level of analysis, extensive research has revealed evidence of structural changes in the brain and impairments of neuroplasticity in response to stress and depression. Clinical studies have described decreases in hippocampal volume in patients with stress-related major depression (Bremner et al., 2000; MacQueen et al., 2003; Sheline et al., 2003; Stockmeier et al., 2004), and chronic stress in rats alters synaptic morphology in the CA1 (Donohue et al., 2006) and CA3 (Stewart et al., 2005) regions of the hippocampus. Changes in molecular forms of plasticity in stress and depression have been described, as well. For example, the three main isoforms of the neural cell adhesion molecule (NCAM), consisting of NCAM-180, -140 and -120, abundantly expressed proteins which are critically involved in memory consolidation (Foley et al., 2000; Venero et al., 2006), and whose expression is markedly reduced in the hippocampus following chronic stress exposure (Sandi et al., 2001). In related work, we reported that NCAM is a central regulator of the pro-plasticity effects of learning and the anti-cognitive effects of stress. Correspondingly, hippocampal NCAM expression is decreased in response to stress (Touyarot and Sandi, 2002) and is up-regulated in response to learning (Venero et al., 2006). We have also shown that a reduction in hippocampal and prefrontal cortex NCAM expression correlates with predator stress-induced memory impairments in rats (Sandi et al., 2005). As prior work has demonstrated that chronic stress can reduce NCAM mRNA levels and that this effect can be reversed with antidepressant treatment (Alfonso et al., 2006), we evaluated here, whether NCAM expression would be a molecular correlate associated with agomelatine-induced changes in stress-memory interactions. Importantly, previous studies would support the role of NCAM as a downstream target of agomelatine treatment. For example, 7 d treatment with melatonin has been shown to enhance hippocampal NCAM-180 (Baydas et al., 2002) and learning and memory (Baydas et al., 2005).

The polysialylated form of NCAM (PSA-NCAM) is also a powerful mediator of synaptic plasticity. Learning in a number of hippocampal-dependent memory tasks increases the expression of PSA-NCAM (Lopez-Fernandez et al., 2007; Murphy et al., 1996; Venero et al., 2006). Moreover, chronic treatment of rats with the antidepressant imipramine increases hippocampal PSA-NCAM expression (Sairanen et al., 2007). Similarly, chronic treatment with fluoxetine increases PSA-NCAM levels in CA3 stratum lucidum of the hippocampus (Varea et al., 2007b), and also PSA-NCAM expression in the prefrontal cortex (Varea et al., 2007a). Cognition-enhancing therapies also increase hippocampal expression of PSA-NCAM (Murphy et al., 2006). Therefore, we addressed here the effects of agomelatine in the modulation of this plasticity marker in the dorsal, as well as the ventral, hippocampus.

Overall, the goal of the present study was to investigate whether chronic agomelatine treatment can protect hippocampus-dependent memory from being impaired by acute predator stress. Rats were trained in the radial-arm water maze (RAWM), a hippocampus-dependent spatial memory task (Campbell et al., 2008; Diamond et al., 2006; Park et al., 2006; Sandi et al., 2005; Woodson et al., 2003; Zoladz et al., 2006), to learn the location of a hidden escape platform in a water maze. Once the rats learned the platform's location they were exposed to a cat, a powerful fear-provoking stressor that impairs hippocampus-dependent spatial memory (Alfonso et al., 2006; Campbell et al., 2008; Diamond et al., 1999, 2004, 2006;

Park et al., 2006; Woodson et al., 2003) and blocks hippocampal synaptic plasticity (long-term potentiation; LTP) (Meshes et al., 1999; Vouimba et al., 2006). Studies have shown that stress-induced impairments of memory and LTP can be reversed by antidepressants, including fluoxetine and tianeptine (Rocher et al., 2004; Shakesby et al., 2002; Vouimba et al., 2006). Therefore, in the present study we have combined the study of antidepressant treatment with an examination of the molecular mechanisms of stress-memory interactions in rats. Specifically, we have tested the hypothesis that agomelatine will improve memory performance under stress conditions via a mechanism that involves training-induced alterations in hippocampal NCAM and PSA-NCAM expression. Our work focused on analysis of the dorsal and ventral divisions of the hippocampus, based on their differential involvement in emotional and cognitive components of memory processing (Bannerman et al., 2004).

Material and Methods

Animals

Sprague–Dawley rats (Harlan, Indianapolis, IN, USA), weighing $\sim$300 g at the time of testing, were housed on a 12-h light–dark schedule (lights on 07:00 hours) in Plexiglas cages (two per cage) with food and water provided ad libitum. Colony room temperature and humidity were maintained, respectively, at 20 ± 1 °C. All rats were given 1 wk to acclimate to the housing room environment before any experimental manipulations took place. The Institutional Animal Care and Use Committee at the University of South Florida approved all procedures.

Pharmacological agents and treatment regimen

Rats were treated daily with intraperitoneal (i.p.) injections of agomelatine (Servier Pharmaceuticals, Orleans, France), at a dose of 10 mg/kg, or vehicle (1 ml/kg; a 1% solution of hydroxyethylcellulose). All injections occurred at 17:00 hours (2 h before lights off) and continued daily for 22 d.

On the final day of injections (day 22), a subgroup of vehicle-treated (baseline vehicle, $n = 5$) and agomelatine-treated (baseline agomelatine, $n = 5$) animals were sacrificed without behavioural testing to examine the effects of agomelatine on basal NCAM and corticosterone levels. The remaining rats underwent training in the RAWM. All behavioural testing, tissue harvesting and blood sampling took place 2–5 h following the final injection, between 19:00 and 22:00 hours.

RAWM

The RAWM task has been described previously (Campbell et al., 2008; Diamond et al., 2006; Park et al., 2006; Sandi et al., 2005; Woodson et al., 2003; Zoladz et al., 2006). Briefly, the RAWM consists of a black, galvanized round tank (168 cm diameter, 56 cm height, 43 cm depth) filled with clear water (22 °C). Six V-shaped stainless steel inserts (54 cm height, 56 cm length) were positioned in the tank to produce six swim arms radiating from an open central area. A black, plastic platform (12 cm diameter) was located 1 cm below the surface of the water at the end of one arm (referred to as the 'goal arm'). At the start of each trial, rats were released in one arm (referred to as the 'start arm') facing the centre of the maze. If a rat did not locate the hidden platform within 60 s, it was guided to the platform by the experimenter. Once a rat found or was guided to the platform, it was left there undisturbed for 15 s. An arm entry was operationally defined as the rat passing at least halfway down the arm. For each trial, the experimenter recorded the number of arm entry errors and latency for the rat to find the platform. An arm entry error consisted of the rat entering an arm that did not contain the hidden platform or, in rare circumstances, entering the goal arm and not climbing on the platform. The goal arm was different across rats within a day to eliminate a scent build-up in the vicinity of the hidden platform. The start arms varied pseudo-randomly across trials so that a different start arm was used on sequential trials.

Rats were given 12 acquisition trials (T1–T12) to locate the hidden platform. Immediately after trial 12 they were either exposed to a cat (see 'Predator stress' below) or placed in their home cages for 30 min. Following this 30-min period, all rats were given a single memory test trial to assess their memory for the platform location. This resulted in four RAWM-trained groups: vehicle-no stress, vehicle-stress, agomelatine-no stress and agomelatine-stress.

Predator stress

Rats in the stress groups were placed in a small clear Plexiglas box ($25 \times 10 \times 15$ cm) with numerous small (1 cm diameter) holes. Then the rats were transported, in the box, to the cat housing room, where they were placed in a large cage ($61 \times 53 \times 51$ cm) with an adult gonadally intact female cat for 30 min. The Plexiglas box prevented any physical contact between the rats

and cat, but enabled the rats to be exposed to all non-tactile sensory stimuli generated by the cat. Canned cat food was smeared on the top of the Plexiglas box to direct cat activity toward the rats.

Tissue preparation

Immediately following the 30-min memory test, rats were rapidly decapitated, their brains were removed and a sample of trunk blood was collected. The brains were kept on a cold plate and each hippocampus was dissected out and divided into dorsal and ventral sections. The tissue was stored at −80 °C until analysed.

Crude synaptosomal pellets were obtained according to a modified protocol from Lynch and Voss (1991). In brief, tissue was homogenized in 10 vol ice-cold sucrose (0.32 M) and Hepes (4 mM) containing a cocktail of protease inhibitors (Complete TM, Boehringer Mannheim, Lewes, UK) with 16 strokes and centrifuged at 1000 *g* for 5 min. The supernatant was centrifuged at 15 000 *g* for 15 min, and the pellet re-suspended in PBS, containing protease inhibitors. Protein concentration were estimated by the method of Lowry et al. (1951).

Quantitative immunoblotting of NCAM

Two major NCAM isoforms, NCAM-140 and NCAM-180, were measured in crude synaptosomal preparations by Western blotting. Synaptosomal samples from each rat were incubated overnight at room temperature with EndoN (AbCys, Paris, France; final dilution 1:120) to selectively cleave the PSA moiety of NCAM. The reaction was stopped by boiling samples at 100 °C for 5 min in 70 mM Tris–HCl (pH 6.8), 33 mM NaCl, 1 mM EDTA, 2% (w/v) sodium dodecyl sulphate, 0.01% (w/v) Bromophenol Blue, 10% glycerol and 3% (v/v) dithiothreitol. Then 15 μg of each sample was separated on 7.5% (w/v) SDS–PAGE and transferred to nitrocellulose membrane (Biotran BA85, Schleicher & Schuell). After saturation of non-specific sites with 5% (w/v) skimmed milk in 10 mM Tris–HCl (pH 7.4), containing 150 mM NaCl, 0.05% (v/v) Tween-20 (TBST), blots were incubated for 2 h at room temperature with polyclonal rabbit anti-NCAM (1:5000) (Millipore, Zug, Switzerland), then washed with TBST, incubated for 1 h with secondary antibody (Molecular Probes, Basel, Switzerland), and finally developed using the SuperSignal West Dura Substrate (Pierce, Rockford, IL, USA). Bands were detected using the ChemiDoc XRS system (Bio-Rad, Hercules, CA, USA) and quantified using Quantity One® software (Bio-Rad). The sum of the intensities of the pixels within a volume boundary multiplied by pixel area was determined for each band. Expression of NCAM in each group was expressed as a percent change from basal level (combination of basal vehicle and agomelatine-treated samples).

PSA-NCAM ELISA

PSA-NCAM was quantified by ELISA, according to a previously described protocol (Merino et al., 2000). In brief, a flat-bottomed 96-well microplate was allowed to absorb a coating solution [0.1 M Na_2CO_3/0.1 M $NaHCO_3$ (pH 9.4)] for 2 h at room temperature. The solution was removed, and 50 μl of pellet samples was added at a concentration of 10 μg/ml to each well. The plates were incubated overnight at 4 °C and then washed three times with 1 M PBS containing 0.05% Tween-20 (pH 7.4). Additional binding sites were blocked with BSA (3%) for 2 h at room temperature. The wells were rinsed three times and incubated with 50 μl aliquots of a monoclonal antibody against PSA-NCAM (AbCys) overnight at 4 °C. Then, the wells were washed, and 50 μl aliquots of peroxidase-conjugated second antibody, an IgM anti-mouse peroxidase conjugate (Sigma-Aldrich, Buchs, Switzerland) at a 1:1000 dilution, was added for a 2-h incubation period. Afterwards, 50 μl of citrate buffer [50 mM Na_2HPO_4, 25 mM citric acid (pH 4.5)] containing 1 mg/ml *o*-phenylene diamine and 0.06% H_2O_2, added just before use, was placed in each well, and allowed to react for 10 min at room temperature. The reaction was terminated by the addition of 50 μl of 10 M H_2SO_4 to each well. The optical density was determined by measuring absorbency at 492 nm with a Microplate Reader (GE-Bioscience, Otelfingen, Switzerland).

Corticosterone assay

The blood collected at time of sacrifice was centrifuged (3000 rpm for 5 min at 4 °C), and the serum was extracted and stored at −80 °C until it was assayed for corticosterone by an ELISA immunoassay kit (Assay Design, Ann Arbor, MI, USA).

Statistics

Arm entry errors made in the RAWM during the acquisition phase (T1–T12) and on the 30-min memory test (T13) were analysed separately using SPSS software (SPSS Inc., Chicago, IL, USA). The acquisition phase was analysed with a mixed-model ANOVA, with group serving as the between-subjects factor and trial serving as the within-subjects factor. Performance on the 30-min memory test trial was analysed with a

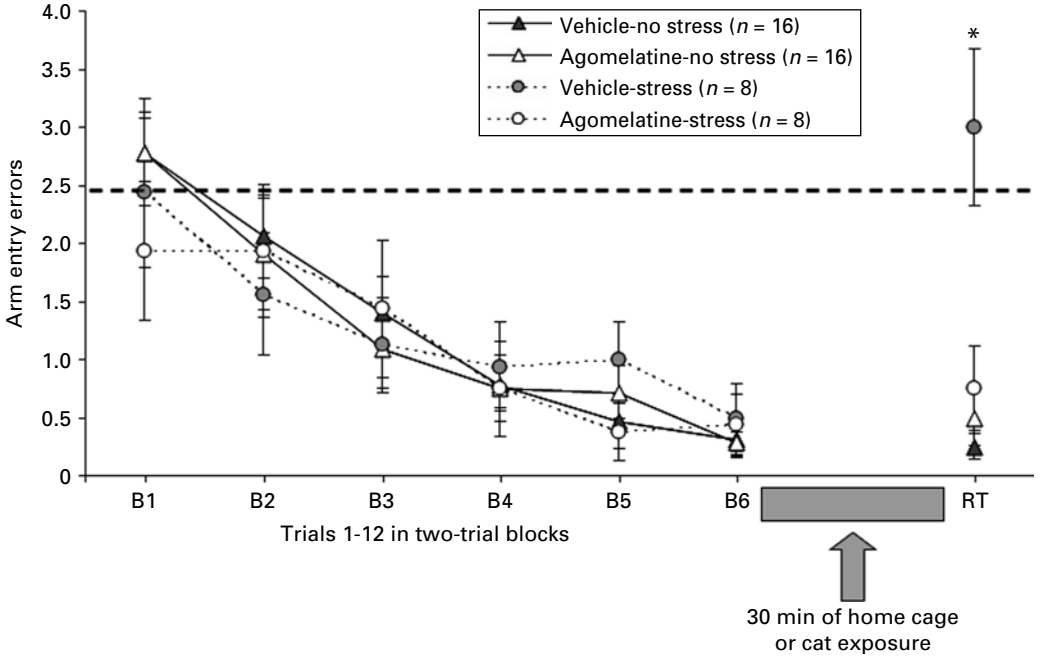

Figure 1. Chronic agomelatine treatment prevented the predator stress-induced impairment of spatial memory in the radial-arm water maze. During acquisition, the groups learned the location of the hidden platform at equivalent rates. Predator stress during the 30-min delay period between learning and retention (indicated by the shaded grey bar) impaired memory retrieval in the vehicle-treated, but not agomelatine-treated, group. The data are presented as mean number of arm entry errors (±S.E.M.) made during acquisition (two-trial blocks) and on the retention trial (RT). The dashed line at 2.5 errors indicates chance level of performance (Diamond et al., 1999). * $p<0.001$ compared to all other groups (Bonferroni post-hoc tests).

one-way ANOVA, with group serving as the between-subjects factor.

The levels of NCAM and PSA-NCAM in all water maze-trained groups were expressed as a percent of baseline. NCAM data were analysed with one-way ANOVAs on NCAM-180 and NCAM-140 expression, with group serving as the between-subjects factor in each analysis. PSA-NCAM and serum corticosterone data were analysed with one-way ANOVAs, with group serving as the between-subjects factor. Alpha was set at 0.05 for all analyses, and Bonferroni-corrected post-hoc tests were used when necessary. In all figures, the data are presented as means ± S.E.M.

Results

RAWM

Analysis of the acquisition phase revealed a significant main effect of trial [$F(7,294)=17.37$, $p<0.0001$], indicating that the groups made fewer errors, i.e. learned the location of the hidden platform, as the trials progressed (Figure 1). There was no significant effect of group [$F(1,44)=0.19$, $p>0.90$], and the trial × group interaction was not significant [$F(20,294)=0.79$, $p>0.1$]. Analysis of performance on the 30-min memory test trial revealed a significant main effect of group [$F(3,44)=13.00$, $p<0.0001$]. Bonferroni post-hoc tests indicated that the vehicle-treated group exposed to predator stress during the 30-min delay period made significantly more errors than all other groups ($p<0.001$). In contrast, the agomelatine-treated group exposed to predator stress (0.75 ± 0.36 errors) was not significantly different from the vehicle-treated (0.25 ± 0.11 errors) and agomelatine-treated (0.50 ± 0.24 errors) no-stress groups, indicating chronic agomelatine treatment blocked the amnesic effects of predator stress on hippocampus-dependent spatial memory (Figure 1).

NCAM-180 and NCAM-140

Chronic treatment with agomelatine in the absence of water-maze training had no significant effect on ventral hippocampus synaptic NCAM-180 expression [vehicle: 100.2 ± 12.92 vs. agomelatine: 99.77 ± 8.16; $t(8)=0.03$, $p>0.97$] and NCAM-140 [vehicle: 110.8 ± 10.74 vs. agomelatine: 89.16 ± 5.29; $t(8)=1.81$,

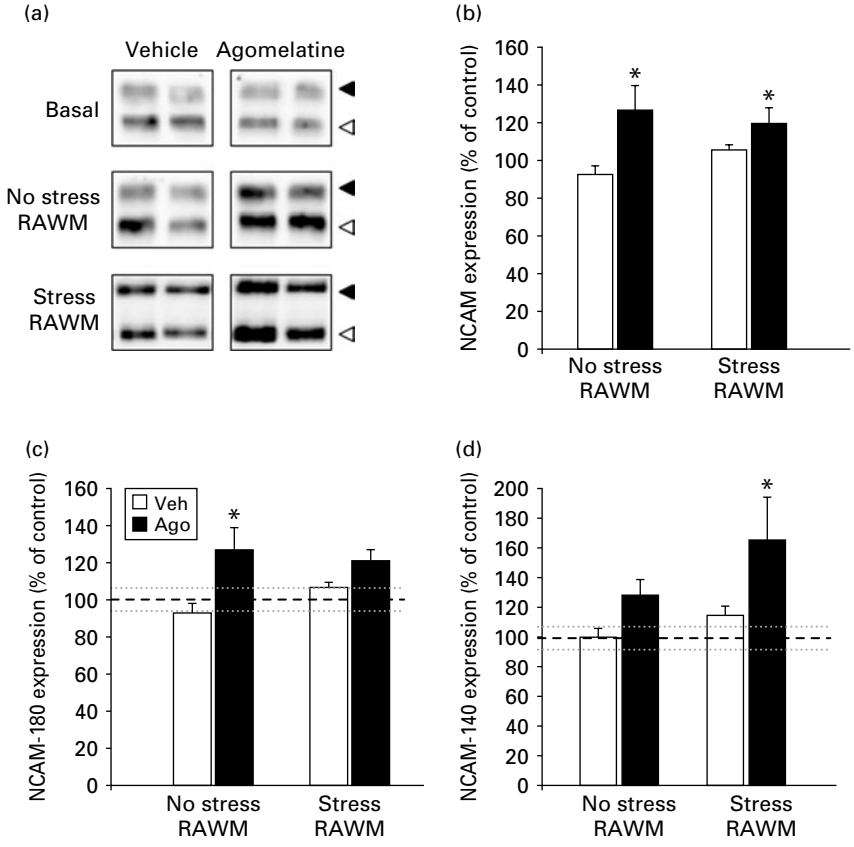

Figure 2. Neural cell adhesion molecule (NCAM)-180 and NCAM-140 expression in the ventral hippocampus was examined in vehicle- and agomelatine-treated animals under basal and post-water maze training conditions, with and without predator stress. (a) Representative immunoblots from ventral hippocampal crude synaptosomal extracts. (b) Agomelatine-treated animals, independent of stress exposure, had enhanced expression of overall NCAM following water maze training. (c) NCAM-180 expression was significantly increased in the agomelatine-treated no-stress group, relative to the vehicle-treated no-stress group. (d) NCAM-140 expression was significantly increased in the agomelatine-treated stress group, relative to the vehicle-treated stress group. In all panels, NCAM expression (quantitative densitometric analysis) is displayed as a percent change from basal expression (i.e. basal vehicle and basal agomelatine combined). Values shown are the mean ± S.E.M. The sample sizes were: basal vehicle ($n=5$), basal agomelatine ($n=5$), no stress-vehicle ($n=13$), no stress-agomelatine ($n=5$), stress-vehicle ($n=8$) and stress-agomelatine ($n=7$). * $p<0.05$ compared to the respective vehicle-treated group (Bonferroni post-hoc tests).

$p>0.10$]. Therefore, to increase the power of the statistical analysis, data from these two groups were combined into one group, which is referred to as 'basal level NCAM'. All total NCAM expression in the four water-maze-trained groups was expressed as a percent of basal level. A one-way ANOVA for total NCAM revealed a significant main effect of group [$F(4,38)=6.05$, $p<0.001$]. Bonferroni post-hoc tests indicated total NCAM expression in the ventral hippocampus was significantly elevated in the agomelatine-treated no-stress (127.84 ± 12.71) and agomelatine-treated stress (143.07 ± 7.44) groups, compared to the vehicle-treated no-stress (95.94 ± 4.83%) and vehicle-treated stress (109.64 ± 3.62%) groups (p values <0.05) (Figure 2).

A one-way ANOVA performed on the NCAM-180 data indicated a significant main effect of group [$F(4,38)=4.442$, $p<0.01$]. Bonferroni post-hoc test demonstrated that NCAM-180 was significantly elevated in the agomelatine-treated no-stress group (127.24 ± 11.36) compared to the vehicle-treated no-tress group (92.69 ± 4.83) ($p<0.05$). Vehicle-treated stress NCAM-180 (105.70 ± 3.63) was not significantly different from that of the agomelatine-treated stress group (120.36 ± 6.89), again agomelatine tended to enhance NCAM-180 expression following RAWM

training in combination with predator stress. There was no significant difference in NCAM-180 expression in vehicle-treated vs. vehicle-treated stress animals, or in either of these groups compared to basal NCAM expression.

A one-way ANOVA performed on the NCAM-140 data indicated a significant main effect of group [$F(4, 38) = 5.38$, $p < 0.01$]. Bonferroni post-hoc tests revealed that NCAM-140 expression was significantly elevated in the agomelatine-treated stress group (165.24 ± 26.16) compared to the vehicle-treated stress group (113.59 ± 6.03) ($p < 0.05$). Vehicle-treated expression (99.20 ± 6.48) was not significantly different from that of the agomelatine-treated group (128.45 ± 9.08). There was no significant difference in NCAM-140 expression in vehicle-treated vs. vehicle-treated stress animals, or in either of these groups compared to basal NCAM expression.

In contrast to the ventral hippocampus, we found no significant change in NCAM expression in the dorsal hippocampus under any condition (all statistical comparisons, $p > 0.1$; data not shown).

PSA-NCAM

Polysialylation of the NCAM molecule has been shown to be induced by learning and memory (Lopez-Fernandez et al., 2007; Sandi et al., 2003), as well as by administration of fluoxetine (Varea et al., 2007a,b). Therefore, we assessed the effects of agomelatine treatment and spatial learning and memory on PSA-NCAM expression in the ventral hippocampus. Chronic treatment with agomelatine had no effect on basal expression of PSA-NCAM in the ventral hippocampus [vehicle: 106.74 ± 6.54 vs. agomelatine: 93.26 ± 4.38; $t(8) = 1.71$, $p = 0.12$]. Therefore, the vehicle baseline and agomelatine baseline groups were combined, and PSA-NCAM expression in the remaining four groups was expressed as a percent of basal level. A one-way ANOVA on PSA-NCAM expression revealed a main effect of group [$F(4, 37) = 3.95$, $p < 0.01$]. Bonferroni post-hoc tests indicated that RAWM training (vehicle-no stress: $85.60 \pm 2.55\%$) induced a significant reduction of PSA-NCAM expression in the ventral hippocampus, relative to baseline ($100.00 \pm 4.57\%$) ($p < 0.05$) (see Figure 3). A reduction in PSA-NCAM expression was also observed following water-maze training combined with predator stress (vehicle-stress: $78.69 \pm 7.6\%$). Chronic agomelatine treatment ameliorated the training-induced reduction of PSA-NCAM expression in the no-stress group ($95.16 \pm 4.16\%$) as well as the stress group (87.75 ± 2.11) ($p < 0.05$).

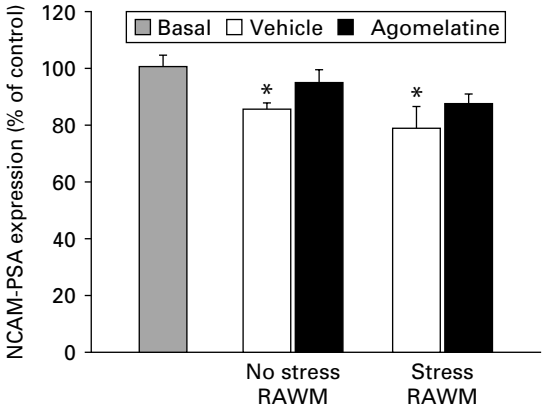

Figure 3. Polysialylated neural cell adhesion molecule (PSA-NCAM) expression in the ventral hippocampus was examined in vehicle- and agomelatine-treated animals under basal and post-radial-arm water maze (RAWM) ($\pm$stress) conditions. The vehicle-treated groups trained in the water maze, exhibited a significant reduction of PSA-NCAM expression, independent of stress exposure compared to basal conditions. Chronic agomelatine treatment blocked this training-induced reduction of PSA-NCAM expression in non-stressed and stressed conditions. The sample sizes were: basal ($n = 10$), no stress-vehicle ($n = 10$), no stress-agomelatine ($n = 5$), stress-vehicle ($n = 8$) and stress-agomelatine ($n = 7$). * $p < 0.05$ compared to the basal group (Bonferroni post-hoc tests).

As with NCAM expression, we found no significant changes in any measure of PSA-NCAM levels in the dorsal hippocampus (all p values > 0.1) (data not shown).

Serum corticosterone levels

We examined the effect of chronic agomelatine treatment on basal and post-training serum corticosterone levels. Chronic treatment with agomelatine had no effect on basal corticosterone relative to the vehicle-treated group [197.2 ± 29.57 ng/ml vs. 183.6 ± 13.37 ng/ml; $t(12) = 0.42$, $p > 0.68$]. Therefore, to increase the power of the statistical analysis we combined the data from these two groups to form a single baseline measure. A one-way ANOVA on serum corticosterone levels revealed a main effect of group [$F(4, 47) = 7.43$, $p < 0.001$]. Bonferroni post-hoc tests indicated that both of the trained stress groups (i.e. vehicle-stress and agomelatine-stress) had significantly greater serum corticosterone levels than baseline ($p < 0.05$) (see Figure 4).

Discussion

There were three primary findings in the present study. First, and most important, chronic agomelatine

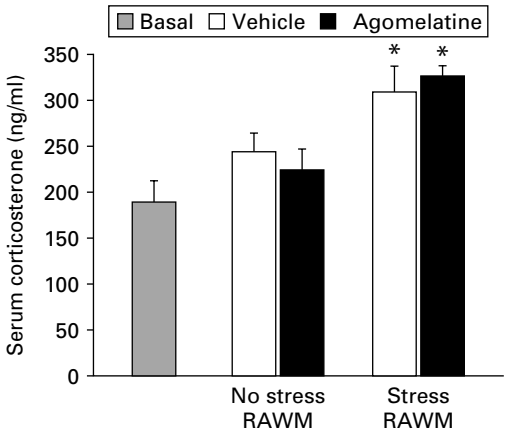

Figure 4. The vehicle- and agomelatine-treated groups exposed to a cat for 30 min displayed significant elevations in serum corticosterone levels, relative to baseline. The sample sizes were: basal ($n=10$), no stress-vehicle ($n=15$), no stress-agomelatine ($n=7$), stress-vehicle ($n=8$) and stress-agomelatine ($n=8$). * $p<0.05$ compared to the basal group (Bonferroni post-hoc tests).

treatment prevented the predator stress-induced impairment of spatial memory. This memory-protective effect of agomelatine was accomplished independently of any general effects of the treatment on hypothalamus–pituitary–adrenal axis, as we did not observe an effect of agomelatine on the stress-induced increase in serum corticosterone levels. This result is in agreement with previous data where no effect of agomelatine was observed on corticosterone after immobilization stress in transgenic mice with low glucocorticoid receptor function (Barden et al., 2005).

Second, rats treated with agomelatine exhibited a rapid (within 30 min) learning-induced increase in total NCAM in the ventral hippocampus, as analysed in the synaptosomal fraction. Indeed, rats trained in the RAWM exhibited a significant increase in NCAM only if they had been chronically treated with agomelatine. This increase in NCAM expression occurred in stressed as well as non-stressed rats, indicating that it was specifically induced by agomelatine interacting with learning and memory consolidation processes. Previous studies focusing only on the 24-h post-water-maze training time-point showed that synaptically localized NCAM levels are elevated in the hippocampus (Venero et al., 2006). Here, we show that chronic agomelatine treatment primed the hippocampus of rats in such a way that training animals in the water maze led to what appears to be an 'accelerated' expression of NCAM levels in the rat hippocampus within 30 min for both the stress and no-stress groups. It should be noted that melatonin, which also has antidepressive properties, has also previously been shown to enhance NCAM-180 and NCAM-140 expression (Baydas et al., 2002, 2005).

Third, agomelatine prevented the reduction in PSA-NCAM expression occurring in the synaptosomal fraction of the ventral hippocampus 30 min after water-maze training, an effect which was found in both stressed and non-stressed vehicle-treated trained animals (i.e. the effect occurred independently of the predator stress manipulation and was found only in trained rats). A rapid reduction in PSA-NCAM was previously shown within 15 min of NMDA receptor-dependent stimulation of synapses in the adult dorsal vagal complex (Bouzioukh et al., 2001). PSA-NCAM can inhibit glutamate-induced activation of the NR2B receptors (Hammond et al., 2006), while activation of NMDA receptors increases NCAM-180 in hippocampal slices (Hoffman et al., 2001). NMDA receptor activation is critically involved in the early phase of spatial learning (Bannerman et al., 1995; Morris et al., 1986; Morris, 1989) thus indicating an NMDA-dependent reduction in PSA-NCAM that may pre-empt a later modulation in the NCAM expression and function during memory consolidation.

Although PSA-NCAM in the dentate gyrus has been linked to neurogenesis (Bonfanti, 2006), there are many examples in which regulation of hippocampal PSA-NCAM expression has been dissociated from neurogenesis (Foley et al., 2008; Lopez-Fernandez et al., 2007; Pham et al., 2003). The fact that in our study the reduction in PSA-NCAM occurred rapidly, after a single training session, supports the view that neurogenesis is not implicated in the effect, since our experimental protocol lasted for <1 h and PSA-NCAM typically starts in newly generated cells a few days after proliferation takes place.

One difference between the present and previous findings from our group is that we previously reported that predator exposure produced a rapid (30 min) reduction in NCAM levels in the dorsal hippocampus of water-maze-trained rats (Sandi et al., 2005), which was not found in the present study. There are two primary methodological differences between the studies which may provide insight into how NCAM levels change in the dorsal vs. ventral hippocampus under different learning and stress conditions. In our previous work, rats were given two training sessions which were separated by 1 wk. In that work, the first session gave the rats the opportunity to learn the task and to acclimate to the water-maze-training conditions. It was only after the rats were given the second session, 1 wk later, that they were exposed to

the cat, which then led to their memory impairment for that day's platform location, as well as the rapid reduction of NCAM levels in the dorsal hippocampus. In the present study, maze-naive rats were given only a single water-maze training session, followed either by control (home cage) or predator stress conditions, which was terminated by the 30-min memory test. Therefore, one important difference between the two studies was that the rapid NCAM reduction found in our first study was in rats that had already had experience with spatial learning in the water maze, and in the present study the rats were naive, and were therefore presumably more stressed by the training procedures.

It is also important to note that in our previous study the rats did not receive any pre-training injections (Sandi et al., 2005), but in the present study all animals had been given daily injections of either vehicle or agomelatine for three consecutive weeks, a procedure that can be considered a mild chronic stress protocol. Based on work demonstrating that chronic stress procedures can result in marked reductions in NCAM expression (Sandi, 2004), it is possible that the repeated exposure to daily injections might have caused, by itself, alterations in NCAM regulation that might underlie the lack of changes in NCAM expression in the dorsal hippocampus following predator stress found in the present study.

One final perspective on the findings of our two NCAM/predator stress studies is that they may reveal differences in functional characteristics between the dorsal and ventral hippocampus. Studies have shown that spatial learning can differentially depend on the dorsal, but not ventral, hippocampus (Moser et al., 1993), however, other evidence indicates that the ventral hippocampus also contributes to spatial learning. Ferbinteanu et al. (2003) found that lesions of either the ventral or dorsal hippocampus disrupted one-trial matching-to-position water-maze learning. Interestingly, Ferbinteanu et al. reported this ventral hippocampus role in spatial memory when rats were trained in relatively cold water (21 °C), while Moser and colleagues (Moser et al., 1993, 1995) found little contribution of the ventral hippocampus to spatial learning when animals were trained with warmer water (25 °C). Similarly, we previously reported that altering the water temperature in the water maze effects the production of corticosterone and the rate of learning (Akirav et al., 2004; Sandi et al., 1997). Specifically, rats trained in cold water (19 °C) learned more rapidly and displayed a greater corticosterone response than rats trained in warm water (25 °C). The ventral hippocampus is believed to play a preferential role in brain processes associated with anxiety-related behaviours (Bannerman et al., 2004). In situations of heightened anxiety or stress, such as those associated with a rat's first exposure to the water maze, the relative contributions of the ventral and dorsal hippocampus to spatial memory processing appear to be equally distributed. In contrast, when the animals have had prior experience with water-maze training, as occurred in our first NCAM/water-maze study, dorsal hippocampus functioning may predominate because of a reduced anxiety component to water-maze testing. Although speculative, the findings of our two NCAM/water-maze studies suggest that conditions of heightened anxiety, produced by daily injections and the rats' first exposure to the water maze, generated a greater involvement of the ventral hippocampus in spatial learning and memory processing than when the rats were trained under lower anxiety conditions.

In this study, no difference in PSA-NCAM expression in basal conditions was observed after chronic agomelatine treatment. This result is in contrast to previously reported findings where PSA-NCAM expression decreased after chronic agomelatine treatment (Banasr et al., 2006). Factors such as genetic background (Wistar vs. Sprague–Dawley rats) and/or differences in experimental conditions (time of sampling after drug treatment: 16 h after last drug treatment in Basnar et al. vs. 30 min in present study) could explain the differences in findings across studies. Moreover, the study by Banasr et al. examined PSA-NCAM expression by immunohistochemistry in an area restricted to the granule cell layer of the ventral part of the dentate gyrus, whereas we examined overexpression of PSA-NCAM in the synaptosomal fraction of the ventral hippocampus. In contrast, our results are in agreement with those obtained by Morley-Fletcher et al. (unpublished observations) who have demonstrated that chronic agomelatine does not affect PSA-NCAM expression in the hippocampus in control Sprague–Dawley rats, the same as used in the present study.

Modulations of NCAM and PSA-NCAM are routinely observed as a result of the induction of synaptic plasticity, but under normal circumstances, these molecular changes require 24 h to develop (Venero et al., 2006). In the present work we found that following chronic agomelatine treatment, learning-induced changes in NCAM occurred rapidly, detectable 30 min after training. Taken together, these results suggest that agomelatine may have beneficial effects on cognition in people with depression. Moreover, at a mechanistic level, the learning-induced enhancement

of NCAM expression observed in agomelatine-injected rats might be interpreted as a potential compensatory mechanism to overcome the deleterious effects induced by other (as yet unknown) mechanisms mediating the stress-induced impaired retrieval. Subsequent work will therefore address the possibility that chronic treatment with agomelatine may block the impairing effects of predator stress on long-term (24 h) spatial memory (Diamond et al., 2006; Park et al., 2008), as well as memory-related changes in hippocampal dendritic spine density (Diamond et al., 2006).

Overall, these findings suggest that agomelatine potentiates learning-associated synaptic plasticity by facilitating a rapid training-induced induction of NCAM in the ventral hippocampus. The precise cellular/subregional location of the learning and stress-related changes described in our study can not be determined from this work. Previous work with other antidepressants identified neuropil in the CA3 stratum lucidum (Varea et al., 2007b) and the subgranular zone of the dentate gyrus (Sairanen et al., 2007) as particularly responsive to chronic fluoxetine and imipramine treatments, respectively, in terms of changes in PSA-NCAM immunostaining.

In summary, this work provides novel observations which indicate that agomelatine blocks the adverse effects of acute stress on relatively short-term (30 min) hippocampus-dependent memory and rapidly activates molecular mechanisms of memory storage in response to a learning experience. Collectively, these findings show striking correlations between the memory-protective effects of agomelatine and the expression levels of NCAM and its post-translational modification, PSA-NCAM. They suggest that agomelatine enables learning to rapidly induce forms of molecular plasticity which are necessary components of memory formation.

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Statement of Interest

None.

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