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Brain matrix metalloproteinase-9 activity is altered in the corticosterone mouse model of depression

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ABSTRACT

Major depressive disorder is a highly prevalent psychiatric condition. Metalloproteinase 9 (MMP-9), a gelatinase involved in synaptic plasticity, learning and memory processes, is elevated in both chronic stress animal models and human peripheral blood samples of depressed patients. In this study we have evaluated the MMP-9 activity and protein expression in brain areas relevant to depression using the chronic corticosterone mouse model of depression. These mice show a depressive- and anxious-like behaviour. The MMP-9 activity and protein levels are significantly elevated in both the hippocampus and the cortex, and nectin-3 levels are lower in these brain areas in this model. In particular, these mice display an increased gelatinase activity in the CA1 and CA3 subfields of the hippocampus and in the internal layer of the prefrontal cortex. Moreover, the immobility time in the tail suspension test presents a positive correlation with the cortical MMP-9 activity, and a negative correlation with nectin-3 levels.

In conclusion, the chronic corticosterone model of depression leads to an increase in the protein expression and activity of MMP-9 and a reduction of its substrate nectin-3 in relevant areas implicated in this disease. The MMP-9 activity correlates with behavioural despair in this model of depression. All these findings support the role of MMP-9 in the pathophysiology of depression, and as a putative target to develop novel antidepressant drugs.

1. Introduction

Major depressive disorder (MDD) is a widespread psychiatric condition affecting >250 million people worldwide, and the incidence of this disease has increased by 14% in the last 10 years (COVID-19 Mental Disorders Collaborators, 2021). It is also associated with an increased risk of premature death, including a high rate of suicide, and a high economic and social cost.

The etiopathogenesis of MDD is not fully known and different neurobiological hypotheses have been proposed to date. Initially, a monoamine hypothesis was postulated, which proposes the existence of a dysfunctional brain monoaminergic system in depressed patients (Schildkraut, 1965). Then, a neurotrophic hypothesis was proposed, that associates depression with a decrease in neurotrophic factors and synaptic plasticity in limbic areas, and a reduction in cell proliferation in the hippocampus (Duman et al., 1997; Duman and Monteggia, 2006). In the last years, several studies demonstrated the correlation between inflammation and neuropsychiatric diseases, including depression (Bower et al., 2002; Meyers et al., 2005), which resulted in the development of the neuroinflammatory hypothesis of depression. Moreover, the increase in inflammatory markers is associated with a decrease in synaptic plasticity markers, such as the brain derived neurotrophic factor (BDNF) (Barrientos et al., 2003; Wu et al., 2007; Ben Menachem-Zidon et al., 2008; Koo and Duman, 2008), and with a reduction in hippocampal proliferation (Zonis et al., 2015).

Metalloproteinases (MMPs) are a group of enzymes sharing a

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conserved zinc-binding motif in their catalytic active site. They are responsible for the cleavage of the extracellular matrix proteins, cell surface receptors and cell adhesion molecules, such as nectins. Interestingly, in the last few years, they have gained importance since they act not only as regulators of extracellular signalling networks but also of intracellular ones (Xie et al., 2017). Among them, the MMP-9 is a gelatinase expressed in both the peripheral and central nervous systems (CNS), playing a pivotal role in the regulation of synaptic plasticity. MMP-9 is translated locally, and it is released from glutamatergic excitatory synapses in response to neuronal activity mediated by NMDA receptors (Vafadari et al., 2016). In the CNS, extrasynaptic MMP-9 is implicated in processes such as the growth and maturation of dendritic spines, and the accumulation and immobilization of AMPA receptors, which make the excitatory synapses more efficient in modulating the AMPA/NMDA receptors ratio (Vafadari et al., 2016). In this sense, overexpression of MMP-9 in rats promotes a higher proportion of silent synapses, lower AMPA/NMDA receptor ratio, and impaired long-term potentiation (LTP) (Nagy et al., 2006). On the contrary, the administration of MMP-9 inhibitors restores these changes (Vafadari et al., 2016). These MMPs also participate in the modulation of inflammatory processes, as well as in neurogenesis, axonal growth and regeneration, and the formation of myelin (Reinhard et al., 2015). All these biological functions justify the involvement of MMP-9 in the etiopathogenesis of certain neurodegenerative disorders such as epilepsy (Michaluk and Kaczmarek, 2007), and multiple sclerosis among others (Milward et al., 2008).

The involvement of these MMPs has been extended to the field of neuropsychiatric diseases, such as depression. Few human studies performed in peripheral blood samples report an increase in the metalloproteinases MMP-2, MMP-7, and MMP-9 (Domenici et al., 2010; Rybakowski et al., 2013; Bobińska et al., 2016), and a reduction in the tissue inhibitors of metalloproteinases (TIMPs) (Bobińska et al., 2016) in major depressive patients. The MMP-9 serum levels showed a positive correlation with the severity of depression (Yoshida et al., 2012; Shibasaki et al., 2016). In addition, electroconvulsive therapy reduces serum levels of MMP-9 in depressed patients in responders, but not in the group of patients that relapse (Shibasaki et al., 2018). However, these results must be taken with caution, since findings in peripheral samples do not always correlate with those observed in the CNS. In fact, only one study using *post-mortem* brain samples confirmed the existence of higher hippocampal MMP-9 enzymatic activity in MDD patients who committed suicide (Bijata et al., 2022). High MMP-9 activity is also associated with other diseases such as epilepsy (Konopka et al., 2013) and ovarian cancer (Lutgendorf et al., 2008) that can present comorbidity with major depression. All these findings highlight MMP-9 as a possible diagnostic marker of depression (Jönsson et al., 2014), that correlates with treatment response and disease progression.

Regarding the brain MMP levels in animal models of depression, there are only two studies reporting an increased MMP-9 activity in a chronic stress model (van der Kooij et al., 2014), and chronic unpredictable stress model (CUS) in mice (Bijata et al., 2022). Moreover, there is also one article reporting the modulation of MMP-9 expression and activity after the electroconvulsive therapy in the hippocampus *versus* the lack of effect of chronic administration of three clinically used antidepressants in naïve rats (Benekareddy et al., 2008).

A deeper understanding of the role of MMP-9 in the neurobiology of depression would help to detect novel therapeutic targets in order to obtain faster and more efficacious antidepressant drugs. Therefore, in this study, we have evaluated the protein expression and activity of the metalloproteinase MMP-9 in hippocampus and cortex using the corticosterone mouse model of depression (CORT), an experimental paradigm endowed with construct, face and predictive validity (Gourley and Taylor, 2009). The hippocampus and cortex are the main areas implicated in the regulation of mood and cognition. Several studies indicate that these areas show decreased neuronal synapses (Duman and Aghajanian, 2012), and a reduction in volume and neural plasticity in patients diagnosed with MDD and chronic stress in the hippocampus and prefrontal cortex (Belleau et al., 2019), and other cortical areas as the temporal lobe (Papmeyer et al., 2015).

2. Methods

2.1. Animals

C57BL/6J male mice, 2–3 months old, were group-housed (4–5 mice per cage) with a 12 h light-dark cycle, and with food and water *ad libitum*. All procedures were carried out with the previous approval of the Animal Care Committee of the University of Cantabria and according to the Spanish legislation (RD 53/2013) and the European Communities Council Directive on "Protection of Animals Used in Experimental and Other Scientific Purposes" (2010/63/UE).

2.2. Corticosterone model

C57BL/6J mice were randomized in two groups and treated chronically with corticosterone hemisuccinate (4-Pregnen-11β, 21-diol-3, 20-dione 21-hemisuccunate) in the drinking water for 4 weeks (45 mg/l, equivalent to a dose of 6–10 mg/kg/day per animal). The corticosterone solution was placed in opaque bottles to avoid degradation and it was changed every 4 days (Gourley and Taylor, 2009; Amigo et al., 2021) and the vehicle group received water. Then, mice were behaviourally assessed in anxiety- and depression-related paradigms (Fig. 1). Following behavioural evaluation, animals were randomized in two sets that were used for: a) gel zymography and western blot experiments; and b) *in situ* zymography experiments.

2.3. Behavioural tests

Mice were subjected to a battery of behavioural tests performed during the light phase and were transported to the experimental room 1 h before each experiment to let them acclimatize. 24 h after the sucrose preference test, the animals were sacrificed.

2.3.1. Open field test (OF)

Mice were placed in one of the corners of the arena (50 × 50 × 30 cm) illuminated with 350 lx, and video-tracked by a computerized system (Any-maze Video-Tracking software, Stoelting Co., USA) for 5 min. The parameters analysed were the total ambulatory distance and the time spent in the centre of the box *versus* total time.

2.3.2. Tail suspension test (TST)

Mice were suspended by the tail using adhesive tape placed 1 cm from the tip of the tail, and at a distance of 20–25 cm from the floor (as previously described in Garro-Martínez et al., 2021). The animals' behaviour was recorded during a 5 min session. The time spent immobile was scored by an observer blind to the experimental group.

2.3.3. Novelty suppressed feeding test (NSF)

Mice were food-deprived for 24 h before performing the test. Animals were placed in one corner of an open field arena illuminated in the centre (40 lx), with the floor covered with clean wood chip bedding, and a food pellet placed in the centre. The latency time to eat the pellet was recorded for a maximal time of 10 min, using a video tracked software

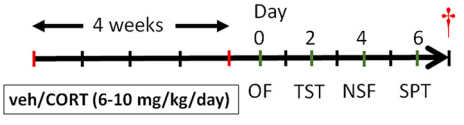


Fig. 1. Experimental time schedule: veh: vehicle; CORT; corticosterone; OF: Open Field; TST: Tail Suspension Test; NSF: Novelty Suppressed Feeding; SPT: Sucrose Preference Test. †: sacrifice.

(Any-maze Video-Tracking software, Stoelting Co., USA). After the test, mice were transferred to their home cages and a post-test of 5 min was done to check the amount of food consumed. The animals that showed no food intake in their home cages were excluded from the data analysis.

2.3.4. Sucrose preference test (SPT)

Mice were single-housed and habituated for 48 h to sucrose consumption putting two bottles in their cage, one containing sucrose in water (1% *w/v*) and one containing tap water. During the habituation period, the position of the bottles was exchanged every 12 h to avoid possible place preferences. The day after, the sucrose preference was evaluated as the percentage of sucrose solution intake *vs* total intake (sucrose+water) during a 24 h period.

2.4. Gel zymography

Mice were sacrificed by cervical dislocation and the brains were rapidly removed and dissected on an ice-cold platform to obtain cortex and hippocampus, and kept at −80 °C until used for western blot or gel zymography experiments.

2.4.1. Zymography's protein extraction

Protein extraction was performed following the protocol of Szklarczyk (Szklarczyk et al., 2002). Briefly, the tissues were homogenized (1:20 w/v) in sample buffer (10 mM $CaCl_2$; 0.25% Triton X-100 in water) and then centrifuged at 6000 x*g* for 30 min at 4 °C. The supernatant was removed, and the pellet was resuspended in 100 μl of pellet buffer (50 mM Tris pH 7.4; 0.1 M $CaCl_2$), heated for 15 min at 60 °C, and then centrifuged at 10000 x*g* for 30 min at 4 °C. The pellet was discarded, and 4 μl of pellet buffer, containing 10% triton X114 was added to the supernatant in order to increase the resolution of the digested band.

2.4.2. Gel zymography

The protein was quantified by the Lowry method (Lowry et al., 1951) and the samples were prepared using Laemmly buffer without β-mercaptoethanol. 75 μg of protein were loaded per duplicate on an 8.5% SDS-PAGE gel containing 0.1% gelatine. As a positive control, 10% fetal bovine serum (FBS) was prepared in extraction buffer [2% Triton X114; 10 mM Tris- HCl, pH 7.4; 150 mM NaCl; 1 μM protease inhibitors aprotinin and phenylmethylsulfonyl fluoride (PMSF)]. The gel was run at 100 V for 15 min, then 160 V for 50 min in running buffer [25 mM Tris-HCl, pH 7.4; 20 mM glycine; 0.1% sodium dodecyl sulfate, (SDS)] at 4 °C. After the electrophoresis, the gel was washed twice in washing buffer (2.5% Triton X100) at room temperature with gentle agitation for 15 min. Then the washing buffer was discarded, and the gel was incubated for 30 min at room temperature in incubation buffer (50 mM Tris-HCl, pH 7.4; 200 mM NaCl; 6.7 mM $CaCl_2$; 1 μM $ZnCl_2$; 0.2% Brij35). Later, the gel was incubated with fresh incubation buffer at 37 °C for 48 h. Following this incubation, the gel was washed 3 times with water for 5 min each at room temperature. Before staining, the gel was scanned to record the exact position of the protein standard bands. The gel was stained with Coomassie Blue R-250 for 1 h at room temperature and then was destained (10% methanol; 5% acetic acid) at room temperature until bands of proteolytic activity were clearly visible. Finally, the gel was scanned using a SnapScan 1236 AGFA scanner and the AGFA FotoLook software. The bands were quantified with the software ImageJ (NIH, USA).

2.5. In situ zymography

Brain slices (14 μm thick) were obtained with a cryostat and were kept at −80 °C until used. The *in situ* zymography was performed following previously described protocols (George and Johnson, 2010). The brain slices were dried for 1 h at room temperature and then were rehydrated in phosphate buffered saline (PBS) for 5 min. The slides were incubated with 20 μg/ml DQ™ Gelatin fluorescein-conjugated (Molecular Probes, Inc., Eugene, OR, USA), in MMP activity buffer (100 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM $CaCl_2$, 20 μM $ZnCl_2$, 0.2 mM sodium azide, and 0.05% Brij35, in MilliQ water), in a dark and humid chamber, at 37 °C for 18 h. As a negative control, 50 mM EDTA was added to the gelatine solution. After overnight incubation, the slides were washed with PBS 1× three times for 5 min each, with gentle agitation. Then the slides were fixed with 4% paraformaldehyde in PBS for 5 min and washed 3 × 5 min with PBS. The slides were mounted using Vectashield® (Vector Laboratories, USA). The fluorescent signal was detected using a Zeiss Axio Imager M1 fluorescence microscope, 12 bits B&W camera (AxioCam MRm). The images were analysed using the software ImageJ (NIH, USA).

2.6. Western blot

2.6.1. Synaptoneurosomal protein extraction

The protein extraction was performed following the protocol of Van der Kooij (Van der Kooij et al., 2014). Briefly, the tissues were homogenized (1:15 *w/v*) in homogenization buffer (10 mM HEPES pH 7.4; 1 mM EDTA; 2 mM EGTA; 0.5 mM DTT; 0.1 mM PMSF), containing a protease inhibitor cocktail (Sigma). The homogenate was filtered with a 40 μm filter and centrifuged at 1000 x*g* for 10 min at 4 °C. The pellet was resuspended in 100 μl of 1% SDS buffer (SDS in homogenization buffer plus inhibitors) and boiled for 1 min. The protein concentration was quantified using the Lowry method. The samples were prepared with a loading buffer containing β-mercaptoethanol, boiled at 100 °C for 5 min, and put on ice for 3 min. Then the aliquots were centrifuged at 956 x*g* for 5 min at 4 °C and the supernatant was stored at −20 °C until used.

2.6.2. Western blot

35 μg of protein per sample were loaded per duplicate on an 8,5% SDS-PAGE gel. The electrophoresis was run at 100 V for 15 min and then at 160 V for 50 min, and then transferred to a nitrocellulose membrane (GE Healthcare Europe GmbH, Munich, Germany). The membrane was blocked with 5% (*w/v*) nonfat dry milk in TBST for 1 h. Afterward, the membrane was incubated overnight at 4 °C with rabbit anti-MMP9 (1:3000) (RayBiotech, RayBiotech Life, Georgia, GA, USA) or rabbit anti-nectin-3 (1:10000) (MBL, MBL International, Woburn, MA, USA) primary antibodies in blocking solution. The day after, the membrane was washed with TBST and then incubated in fluorophore-conjugated IRDye 800CW Donkey anti-rabbit secondary antibody (LI-COR Biosciences, Lincoln, NE, USA) (1:15000 in milk 5%) for 1 h at room temperature. After washing, the specific signal was visualized using an Odyssey CLx Imaging System (LI-COR Bioscience, Lincoln, USA). The densitometric values were normalized using mouse anti-tubulin (1:20000) as housekeeping. The images were analysed with the use of Image StudioTM Lite software (LICOR Bioscience, Lincoln, USA).

2.7. Data analysis

Values are expressed as mean ± standard deviation (SD). Data normality was checked using Kolmogorov-Smirnov test for normality. When normality was confirmed, data were analysed using a two-tailed Student's *t*-test, unpaired data. For the correlation studies, the Pearson's correlation coefficient (r) was used. The statistical analyses were performed using the GraphPad Prism 6 version 8.4.3 for Windows (GraphPad Software, Inc., La Jolla, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Depressive- and anxious-like behaviour in the corticosterone model

The corticosterone-treated mice exhibited a higher immobility time compared to the control group in the TST (174.7 ± 19.6 s in the

corticosterone *vs* 148.6 ± 21.0 s in vehicle treated animals, $p < 0.05$; Fig. 2A) and showed a significant decrease in the sucrose preference compared to their control group (74.4 ± 12.5% in corticosterone *vs* 89.1 ± 2.6% in the vehicle treated animals, $p < 0.01$; Fig. 2B).

Regarding the anxious-like behaviour, the corticosterone-treated mice presented an increased latency time of feeding compared to the control group in the NSF (441.9 ± 111.2 s in corticosterone *vs* 334.2 ± 83.7 s in vehicle treated animals, $p < 0.05$; Fig. 2C). No differences were observed in the food eaten in their home-cage (Fig. 2D), in the freezing time and in the ambulation, as evidenced by the mean speed of the animals during the test (Fig. S1). Mice treated with corticosterone also presented a reduction in the time spent in the centre of the OF compared to the control group (4.4 ± 1.9 s in the corticosterone *vs* 8.3 ± 3.1 s in the vehicle treated animals, $p < 0.01$; Fig. 2E). The total distance travelled in the corticosterone treated mice was also lower compared to the vehicle treated mice (14.1 ± 2.1 m in corticosterone *vs* 17.1 ± 2.1 m in vehicle treated animals, $p < 0.01$; Fig. 2F).

No correlation was observed between the immobility time in the TST, and the distance travelled in the OF (Fig. S2).

3.2. MMP-9 and nectin-3 protein expression in cortex and hippocampus

The mice chronically treated with corticosterone showed a significant increase in MMP9 protein levels compared to the control group in

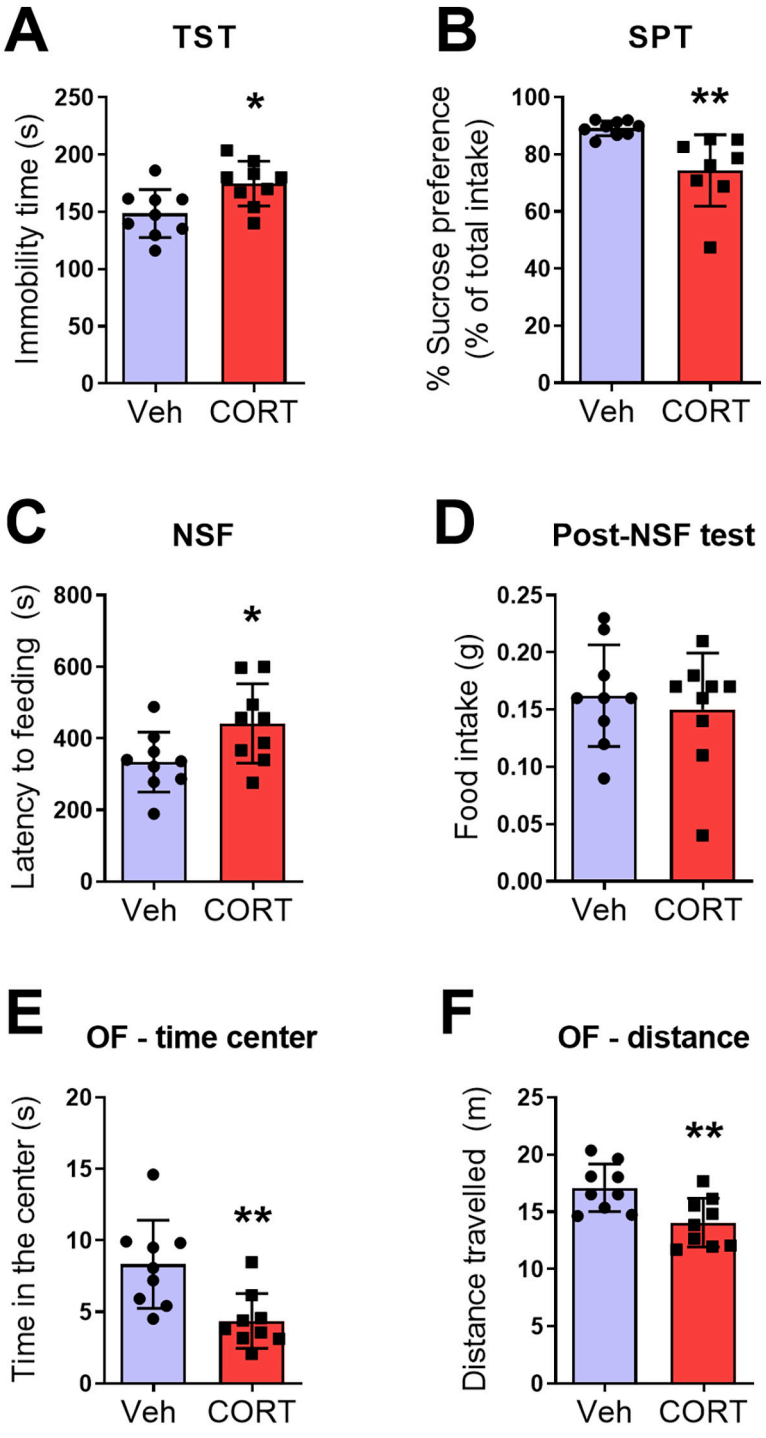


Fig. 2. Depressive- and anxious-like behaviour in CORT. Immobility time in the tail suspension test (A); percentage of the sucrose consumed *versus* total intake in the sucrose preference test (B); latency of feeding (C) and food eaten in the post-NSF test (D) of the novelty suppressed feeding test; time spent in the centre (E) and distance travelled (F) in the open field test. Data are expressed as mean ± SD. Two-tailed Student's t-test, unpaired data. *$p < 0.05$, **$p < 0.01$. $n = 9$ animals per group. Veh: vehicle group; CORT: corticosterone model; TST: tail suspension test; SPT: sucrose preference test: NSF: novelty suppressed feeding test; OF: open field test.

the cortex (117.8 ± 14.7% in corticosterone *vs* 100.0 ± 2.4% in the vehicle treated animals, $p < 0.01$) (Fig. 3A), and in the hippocampus (151.1 ± 61.0% in the corticosterone *vs* 100.0 ± 6.7% in the vehicle treated animals, $p < 0.05$) (Fig. 3B).

A significant reduction of nectin-3 protein level, one of the main substrates of MMP-9, was observed in the cortex of corticosterone treated mice compared to the control group (78.5 ± 13.9% in the corticosterone *vs* 100.0 ± 7.0% in the vehicle treated animals, $p < 0.05$) (Fig. 3C), and in the hippocampus (67.3 ± 23.7% in the corticosterone *vs* 100.0 ± 9.0% in the vehicle treated animals, $p < 0.05$) (Fig. 3D).

3.3. Metalloproteinase activity in cortex and hippocampus

Gel zymography was used to evaluate the gelatinase activity in CORT. Mice treated chronically with corticosterone displayed a significant increase in MMP-9 gelatinase activity compared to their control group in the cortex (338.3 ± 219.5% in the corticosterone *vs* vehicle treated animals, $p < 0.01$) (Fig. 4A). In the hippocampus, we also observed a tendency to increased gelatinase activity in the corticosterone model (169.0 ± 89.9% in the corticosterone *vs* vehicle treated animals, $p = 0.07$) (Fig. 4B).

MMP-2 activity was also analysed in the cortex (101.5 ± 25.5% in corticosterone *vs* 100.0 ± 36.5% in the vehicle treated animals, *ns*), and the hippocampus (159.0 ± 161.3% in corticosterone *vs* 100.0 ± 31.3% in the vehicle treated animals, *ns*), not observing statistical differences between the experimental groups. Moreover, the ratio MMP-9/MMP-2 in cortex (1.564 ± 0.681 in corticosterone *vs* 1.936 ± 1.106 in vehicle treated animals, *ns*) and hippocampus (2.754 ± 1.638 in corticosterone *vs* 2.724 ± 1.231 in vehicle treated animals, *ns*), did not present statistical differences.

An *in situ* zymography was also performed to study the regional localization of the gelatinolytic activity. Mice chronically treated with corticosterone presented a higher gelatinolytic activity compared to the control group in different regions of the hippocampus: CA1 field (178.8 ± 66.9% in the corticosterone *vs* 100.0 ± 48.2% in the vehicle treated animals, $p < 0.05$; Fig. 5A, B and C); CA3 field (179.5 ± 70.9% in the corticosterone *vs* 100.0 ± 41.0% in the vehicle treated animals, $p < 0.05$; Fig. 5D, E and F). However, no changes were observed in the dentate gyrus (Fig. 4G, H, and I).

In the cortex, the corticosterone treated animals also showed a higher gelatinolytic activity compared to their control group in layer 5 (152.1 ± 38.2% in the corticosterone *vs* 100.0 ± 45.2% in the vehicle treated animals, $p < 0.05$). The outer layers 2/3 presented a tendency to increased activity (139.4 ± 36.8% in the corticosterone *vs* 100.0 ± 37.3% in the vehicle treated animals, $p = 0.07$; Fig. 5J, K, L).

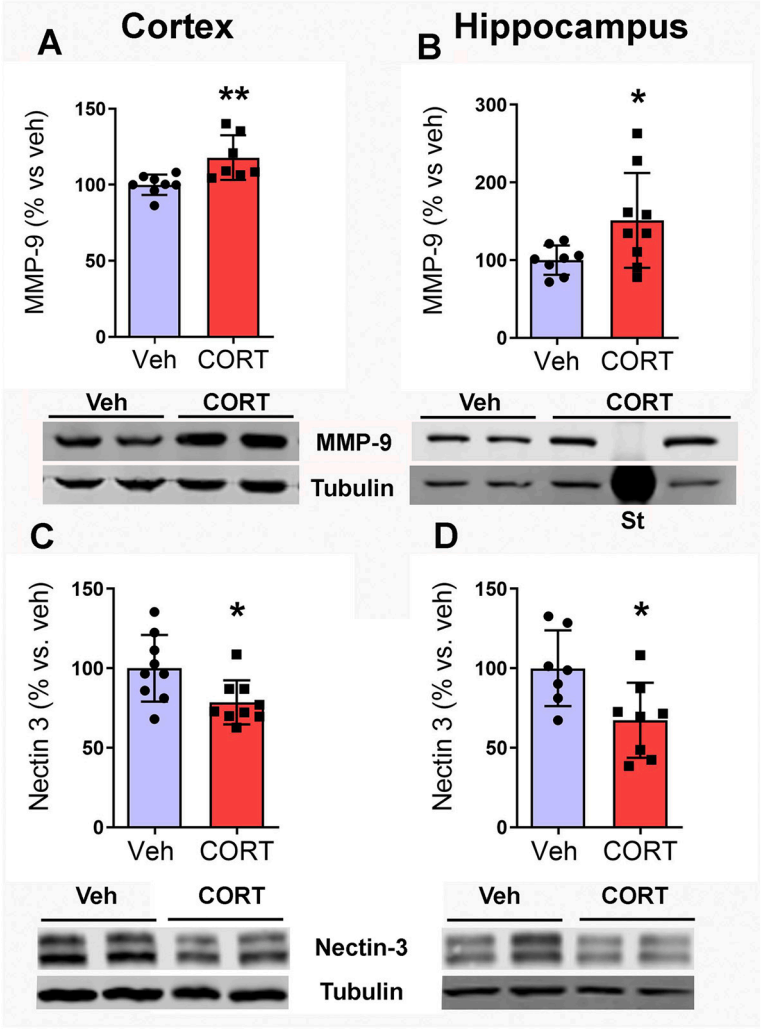


Fig. 3. MMP-9 and nectin-3 protein expression levels in the cortex (A and C, respectively) and the hippocampus (B and D, respectively) of corticosterone-treated mice. Representative images of MMP-9 and nectin-3 western blots are shown below the corresponding graphs. Data are expressed as mean ± SD. Two-tailed Student's *t*-test, unpaired data. * $p < 0.05$; ** $p < 0.01$. $n = 7$–9 animals per group. Veh: vehicle group; CORT: corticosterone model; St: protein standard.

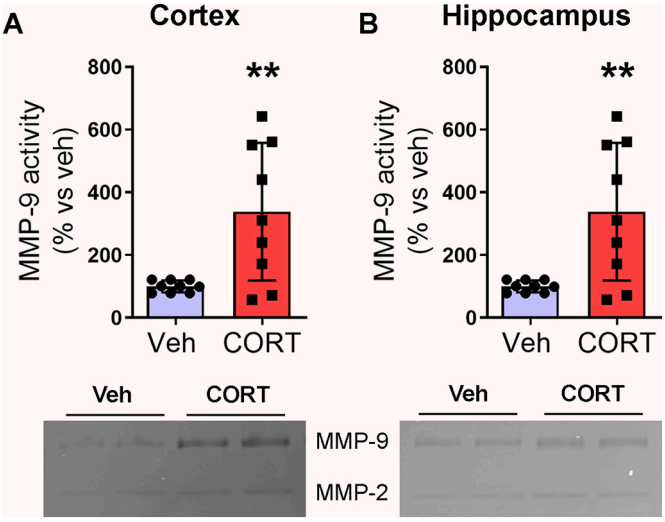


Fig. 4. MMP-9 gelatinase activity in CORT and vehicle mice in the cortex (A) and the hippocampus (B). Representative images of the gel zymography showing MMP-9 and MMP-2 bands are shown. Data are expressed as mean ± SD. Two-tailed Student's t-test, unpaired data. $^{**}p < 0.01$. n = 7–9 animals per group. Veh: vehicle group; CORT: corticosterone model; St: protein standard.

3.4. Correlation of MMP-9 protein expression/activity and behavioural despair in the TST

A correlation analysis was performed between different behavioural and molecular parameters in the cortex (Fig. 6). The immobility time in the TST positively correlated with the MMP-9 activity (Fig. 6B) and negatively correlated with nectin-3 protein levels (Fig. 6C). However, no correlation was found between the molecular markers and anhedonia (sucrose preference test) (Fig. 6D–F), or anxiety evaluated as the central time in the open field test (Fig. 6G–I) and the latency to feeding in the novelty suppressed feeding test (data not shown).

The analysis performed between the behavioural and molecular data in the hippocampus did not show any correlation (Fig. S3).

4. Discussion

This is the first study reporting an increase in the MMP-9 activity and protein expression in the chronic corticosterone animal model of depression (CORT), in brain areas relevant to the neurobiology of this disease. Moreover, we have found an association between the MMP-9 activity levels and the behavioural despair in this animal model.

It is well established that CORT is endowed with construct, face and predictive validity (Gourley and Taylor, 2009) as it resembles the impairment in the HPA axis and shows some of the manifestations of depression/anxiety disorders (Johnson et al., 2006). This model also presents neurochemical and molecular alterations similar to those reported in depressed patients (Murray et al., 2008; David et al., 2009).

In our study, the animals treated with chronic corticosterone presented anhedonia, shown as a reduction in sucrose preference (Belovicova et al., 2017), and depressive-like behaviour evidenced by increased behavioural despair in the TST (Porsolt et al., 2001; Belovicova et al., 2017). The lower ambulation found in the open field test, would difficult the interpretation of the TST. However, the lack of correlation between the immobility time and the distance travelled could be interpreted as indicative of the depressive-like behaviour, and not associated to the lower mobility in animal models of depression (Florensa-Zanuy et al., 2021). Moreover, this reduced locomotor activity is shown in the open field test, and not in the NSF. One of the factors that could contribute to this difference is the light intensity used in the arena (350 lx in the OF and 40 lx in the NSF), which may result in a higher freezing time in the open field test, as previously reported in association to conditional fear (Warthen et al., 2011).

This model also manifested conflict-based anxiety in the NSF test (Belovicova et al., 2017), and innate-anxiety in the OF test (Amigo et al., 2021), according with previous studies. These behavioural outcomes are in line with the results reported by other authors in CORT (Murray et al., 2008; David et al., 2009), and several models of chronic stress (reviewed in Willner, 2016). By contrast, Demuyser et al. (2016) reported that the CORT model does not present anxious-like manifestations, a discrepancy that may be due to differences in the methodological procedures used in their study, especially the housing conditions (individually housed animals) and the higher doses of corticosterone used during shorter periods of treatment (20 mg/kg/day, s.c, 21 days).

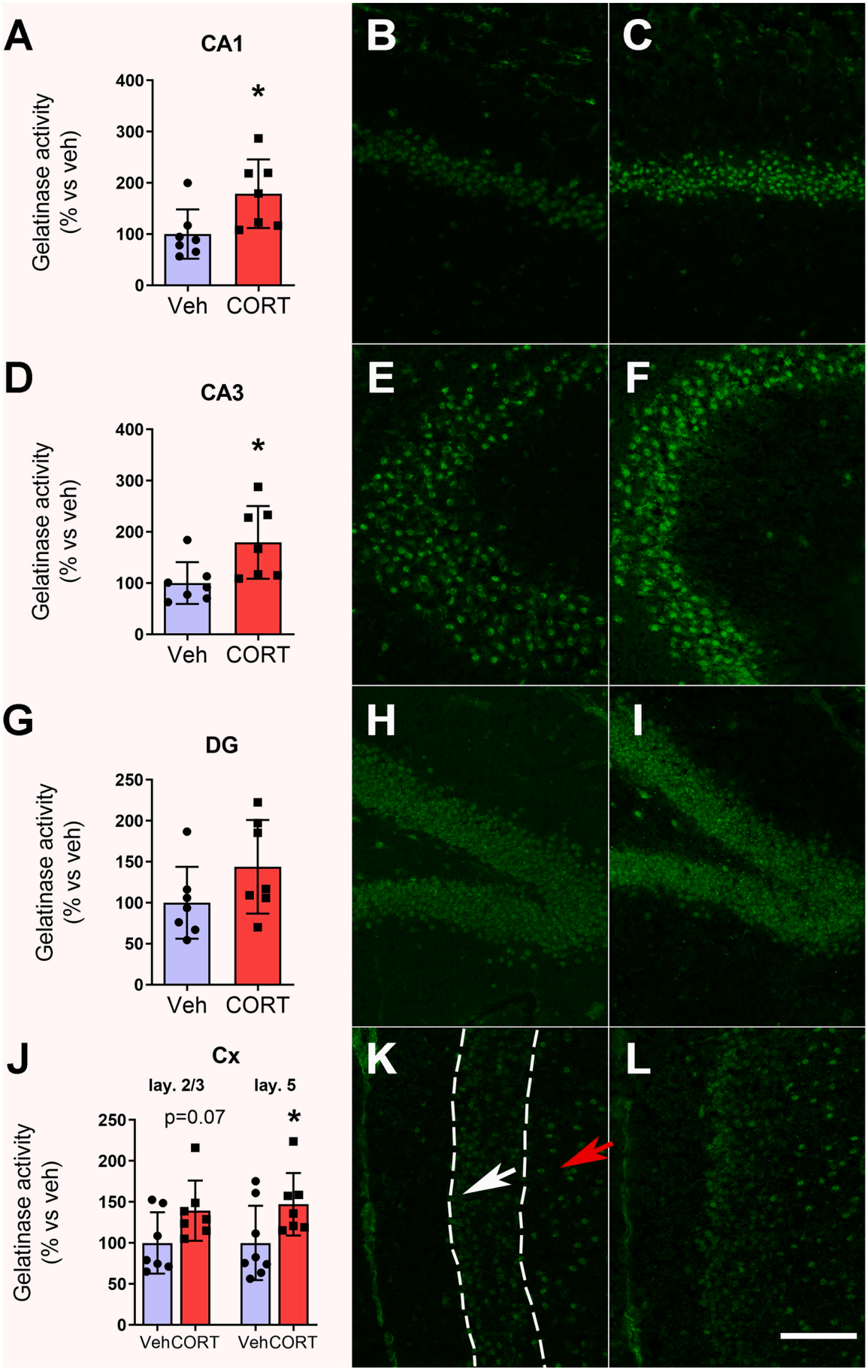
This is the first study reporting an increase in the gelatinolytic activity and MMP-9 protein expression in CORT in cortex. It is worth to note that this increase in cortical MMP-9 activity has been also reported in a mouse model of post-traumatic stress disorder (Chevalier et al., 2021). Moreover, the increase in MMP-9 activity was also observed in the CA1 and CA3 subregions of the hippocampus, with no changes in the dentate gyrus, confirming previous findings in other animal models of depression the chronic unpredictable stress model (Bijata et al., 2022). In contrast, other studies using a different model and species —the chronic restraint stress in rat—, only reported the MMP-9 increase in the CA1 subregion (van der Kooij et al., 2014). An MMP-9 increase in the CA1 and the dentate gyrus was also reported after 24 h of acute restraint stress (Aguayo et al., 2018). The CA1 subregion of the hippocampus appears to be associated to the chronic stress-mediated increase of MMP-9 expression by specifically activating the 5-HT_7 receptors (Bijata et al., 2022). The discrepancies found in MMP-9 activity in the different hippocampal areas may be linked to a differential activation of glutamatergic receptors in animal models of stress, since the CA3 area experiences an enhancement of NMDA-receptor dependent transmission (Kole et al., 2002), and the dentate gyrus an increase in AMPA-receptor dependent synaptic transmission (Karst and Joëls, 2003). Interestingly, our results showed a significant correlation between the immobility time in the TST and the MMP-9 activity or the nectin-3 protein expression in the cortex, but not in the hippocampus, indicating that a higher MMP-9 activity in cortical areas could lead to an increased behavioural despair.

This increased MMP-9 protein and activity observed in CORT is in line with the excitotoxicity linked to chronic stress (Popoli et al., 2011). In this sense, the enhanced glutamatergic activity mediated by NMDA activation has been associated with higher MMP-9 activity in primary cell cultures (van der Kooij et al., 2014).

Moreover, the increased MMP-9 activity observed in the CORT model is also evidenced by the reduction in nectin-3 levels —one of the substrates of this metalloproteinase— in the cortex and the hippocampus. In fact, some authors have reported a similar nectin-3 reduction in animal models associated to stress, in which this reduction was associated with alterations in social behaviour and cognition (Wang et al., 2011; van der Kooij et al., 2014). However, we did not find a significant correlation between MMP-9 activity and nectin-3 protein levels (*data not shown*), as MMP-9 degrades not only nectin-3, but also other molecules as neuroligin-1 (Peixoto et al., 2012), and β-dystroglycan (Michaluk and Kaczmarek, 2007). In addition, there are no clear evidence whether MMP-9 is the only enzyme able to degrade nectin-3 (van der Kooij et al., 2014).

The overactivation of MMP-9 has been associated to the generation of immature spines and silent synapses, lower AMPA/NMDA receptors ratio, and impaired LTP, leading to depression and other neuropathologies (Magnowska et al., 2016). These neuronal changes are in line with the neuroplasticity theory of depression that links high glucocorticoid levels, associated to chronic stress, to the atrophy of mature neurons, the shortening and the decreased density of dendritic spines in the hippocampus, leading to impaired neuronal plasticity (Boku et al., 2018).

The results obtained herein and in other animal models resemble those observed in human studies. Initial studies in peripheral blood



(caption on next page)

Fig. 5. Gelatinase activity in hippocampal regions: A–C) CA1 field; D–F) CA3 field and G–I) DG field of the hippocampus, and prefrontal cortex: layers 2/3 and layer 5 (J–L). Representative images of *in situ* zymography in the CA1 field in the vehicle (B) and corticosterone-treated mice (C); in the CA3 field in the vehicle (E) and corticosterone-treated mice (F); in the dentate gyrus in the vehicle (H) and corticosterone-treated mice (I); in the prefrontal cortex in the vehicle (K) and corticosterone-treated mice (L). In K, red arrow shows layers 2–3 and white arrow shows layer 5. Data are expressed as mean ± SD. Two-tailed Student's *t*-test, unpaired. * $p < 0.05$. Veh: vehicle group; CORT: corticosterone model. Scale bar 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

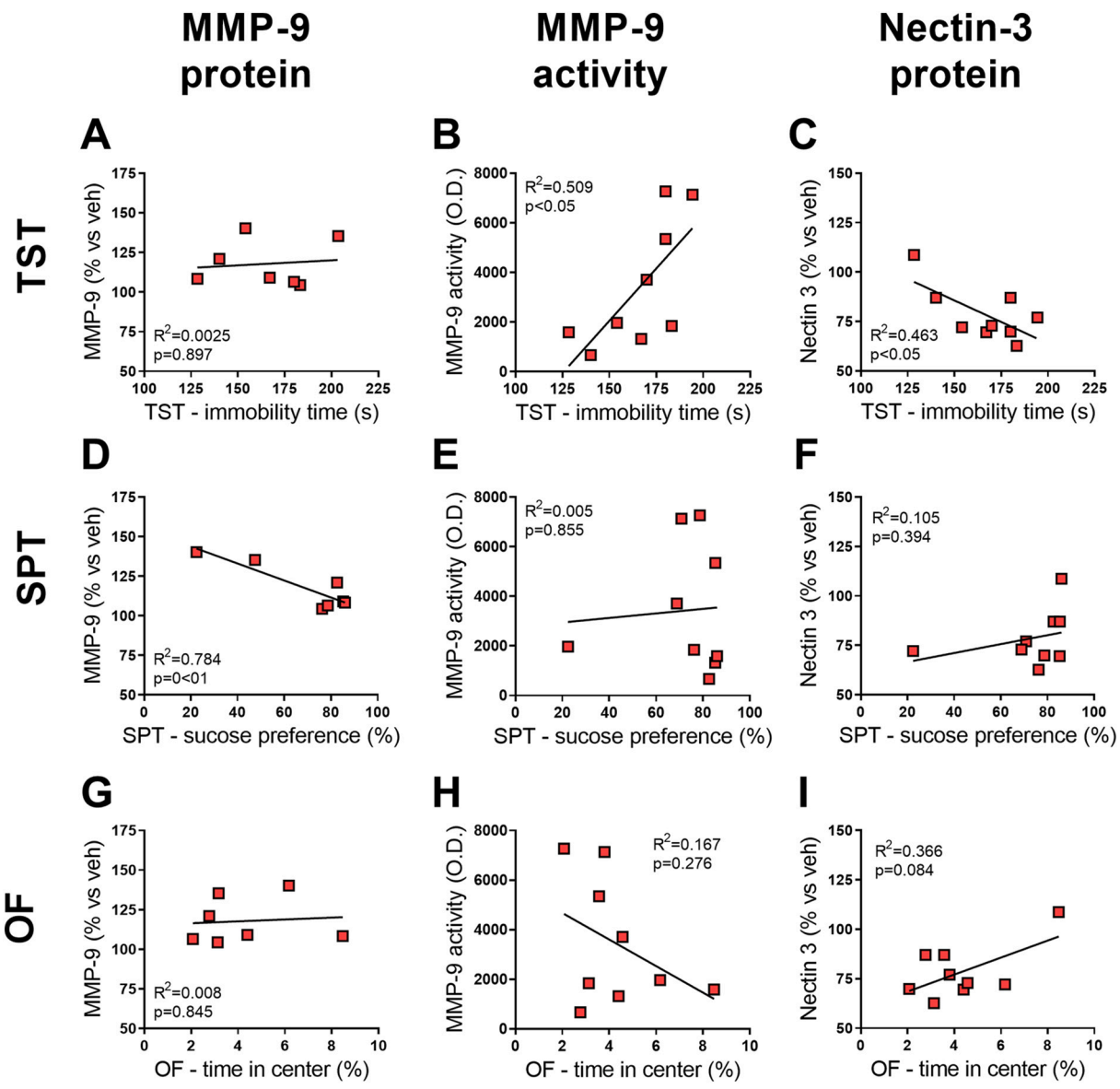


Fig. 6. Correlation in the corticosterone group between the behavioural outcomes (TST, tail suspension test; SPT, sucrose preference test; and OF, open field test) and: MMP-9 protein levels (A, D and G), MMP-9 activity (B, E and H), and nectin-3 protein levels (C, F and I) in the cortex. $n = 7$–9 animals per group. r: Pearson's correlation coefficient.

indicated higher MMP-9 levels in depressed patients compared to healthy subjects (Domenici et al., 2010; Rybakowski et al., 2013; Bobińska et al., 2016; Che et al., 2019), which were associated with the severity of the depressive symptoms (Yoshida et al., 2012; Shibasaki et al., 2016). Therefore, the correlation activity-behaviour reported here supports the importance of the MMP-9 activity level in the severity of depressive symptoms, as previously reported (Shibasaki et al., 2016). In this line, our data, together with the reduction in the level of peripheral MMP-9 after electroconvulsive therapy (Shibasaki et al., 2018), support the putative role of MMP-9 levels as a marker of the MDD severity or the responsiveness to the antidepressant-treatment. However, in our study, this correlation is only observed between MMP-9 activity and behavioural despair, and not with anhedonia or anxiety. In fact, some authors propose an association of MMP9 expression only with specific manifestations (Chevalier et al., 2021), which may account for the different correlations observed here for behavioural despair and anhedonia. These differences might be also associated to the brain area studied. In this sense, anhedonia is more associated to other brain areas such as the ventral tegmental area, nucleus accumbens (NAc), ventral striatum (VTA-NAc pathways) (Dunlop and Nemeroff, 2007; reviewed in Höflich et al., 2019), or central amygdala (Puścian et al., 2021), and anxiety to areas as the amygdala and the bed nucleus of the stria terminals

(Moreira et al., 2007; Duvarci et al., 2009; reviewed in Adhikari, 2014). In fact, a reduction of MMP-9 activity in the central amygdala induced by the chronic administration of the antidepressant drug fluoxetine to non-stressed mice, is associated to the appearance of anhedonia (Puścian et al., 2021).

One of the limitations of our study is the lack of data in areas more associated to other depressive manifestations as anhedonia and anxiety, as indicated above. Another limitation of the study is the impossibility to discriminate between MMP-9 and proMMP-9 in the gel zymography. This fact has been widely described by different authors, assuming that the enzymatic activity obtained in this technique could be associated to both enzyme forms (Cauwe and Opdenakker, 2010; Bijata et al., 2022). Other limitation in our study is the lack of specificity of the *in situ* zymography, as this technique only allows the evaluation of the gelatinase activity, which is mainly due to both MMP-9 and MMP-2 enzymes.

5. Conclusion

The chronic corticosterone model exhibits an increase in the MMP-9 protein and activity and a reduction of its substrate nectin-3 in relevant areas implicated in depression as the cortex and the hippocampus. The MMP-9 activity level correlates with behavioural despair in this model of depression. Our data support the role of MMP-9 in the pathophysiology of depression and in the severity of this illness, and act as a putative target to develop more efficacious antidepressant drugs.

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Ethical statement

We state that this work is original, and has not been submitted elsewhere for publication, completely or in part.

Authors statement

SB: performing experiments, analysing and interpreting data, writing the original draft; JS performing experiments, analysing and interpreting data, writing the original draft; EF-Z: methodology and data analysis; EG-M: methodology; AD: conceptualization and writing review; EC: conceptualization and writing review; AP: funding acquisition, writing review; and FP-C: conceptualization, funding acquisition, project administration, writing original draft.

Declaration of Competing Interest

None of the authors report potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2022.110624.

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