Antidepressant effect of ketamine: Regulation of MMP9 in corticosterone mouse model

Master in Molecular Biology and Biomedicine

Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC)

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Abstract

Over the past years, development of antidepressants targeting the monoamine system has been the approach to treat major depressive disorder (MDD). However, approximately 10-30% of patients do not respond adequately to conventional treatments, while others become treatment resistant. Thus, ongoing research on novel fast-acting antidepressants is a key area to understand the complex neurobiology of this disorder. Recent studies have indicated that ketamine, a methyl-D-aspartate receptor (NMDA) antagonist, is a promising fast-acting drug with sustained antidepressant effects. Moreover, human brain imaging and post-mortem studies of patients with depression have reported structural changes in the prefrontal cortex and hippocampus and increased plasma matrix metalloproteinases (MMPs) levels. Antidepressants are known to induce remodeling of these regions and this process occurs in the extracellular matrix (ECM) regulated by MMPs. Previous studies in the mouse corticosterone model of depression show increased matrix metallopeptidase-9 (MMP-9) protein levels in prefrontal cortex and hippocampus. Thus, we hypothesize that acute ketamine treatment may downregulate MMP-9 expression and function, as well as its endogenous substrates in the corticosterone model, in parallel to its antidepressant-like effect. In addition, experiments in primary hippocampal cell cultures would also contribute to unravel its effect. Current research suggests that a better understanding on the mechanisms that underlie the antidepressant effect of ketamine on the glutamatergic system and other signaling cascades, is essential to develop more efficient treatments. These results could help to find a novel brain biomarker that may be linked to the development of mood disorders and/or the antidepressant effect.

1. Background

1.1 Major Depressive Disorder

Depression is a common mood disorder worldwide. According to a systematic analysis for The Global Burden of Diseases, Injuries, and Risk Factors Study 2017, depressive disorders affect more than 264 million people worldwide, major depressive disorder accounting for more than 163 million of the cases (James et al., 2018).

This psychiatric condition is characterized by having constant and long-lasting feelings of sadness, hopefulness, loss of interest, and alterations in normal body functions such as sleep and appetite, but also in cognitive or psychomotor activities (Fava, 2000).

According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), one of the symptoms to diagnose depression should be depressed mood or anhedonia and requires five or more of other symptoms to be present for 2 weeks, which can include appetite, weight, or sleep changes, fatigue, concentration difficulties, feeling of worthless or suicidal thoughts (American Psychiatric Association, 2013).

Accurate diagnosis of depression is a major challenge since depressive symptoms are not exclusive of this condition, and the right clinical understanding of the neurobiology of depression, as well as identifying depression severity and response to previous medication is crucial to achieving a successful antidepressant treatment for the patient.

According to the World Health Organization, in 2020 depression is a leading cause of disability worldwide and it has been estimated that its prevalence over a lifetime is around 17% for people who suffer a depressive episode. There are effective treatments for depression that are based on a combination of antidepressant medications and psychotherapy or cognitive behavioral therapy (Wang et al., 2007), however, antidepressants are effective in 30%-40% of the cases, and patients who are not correctly diagnosed with depression and remain untreated might eventually generate serious medical complications, including diabetes, cerebrovascular disease, heart attack, and dementia (Eaton et al., 2012).

1.2 Neurobiology of depression

Advances in the neurobiology of neuropsychiatric disorders are based on the research of the neurochemical imbalance in the brain monoamines like serotonin, noradrenaline, and dopamine. Given

that early studies and clinical trials of antidepressants indicate the ability of some medications to relieve common depressive symptoms by increasing serotonin and noradrenaline levels, research has traditionally focused on the monoamine approach (Cowen, 2015).

Depression has been also associated with alterations in limbic brain areas, responsible for emotional regulation and memory (Figure 1). The main areas of the limbic brain implicated in depression are the amygdala, hippocampus, and thalamus, where functional al structural changes have been reported in patients with depression. Moreover, depression is associated with reduced neurotransmission of serotonin or 5-hydroxytryptamine (5-HT) (Jans et., al 2007), and altered 5-HT receptors like 5-HT$_{1A}$, which negatively regulates 5-HT neurotransmission when 5-HT is released. 5-HT$_{1A}$ is located in presynaptic and postsynaptic neurons, and is involved in the regulation of mood and emotion (Blier and El Mansari, 2013). Postmortem studies in patients with depression have reported an increase in the 5-HT receptors (Boldrini et al., 2008), consistent with a PET imaging study with depressed patients that observed an increase in the presynaptic 5-HT$_{1A}$ receptor levels (Hesselgrave and Parsey, 2013). Other studies reviwed by Savitz and Drevets, report both an upregulation or a downregulation of the 5-HT$_{2A}$ receptor in patients with MDD (Savitz and Drevets, 2013). These results suggest that depression is correlated with changes in some 5-HT receptor subtypes and reduced 5-HT neurotransmission, altering the limbic system.

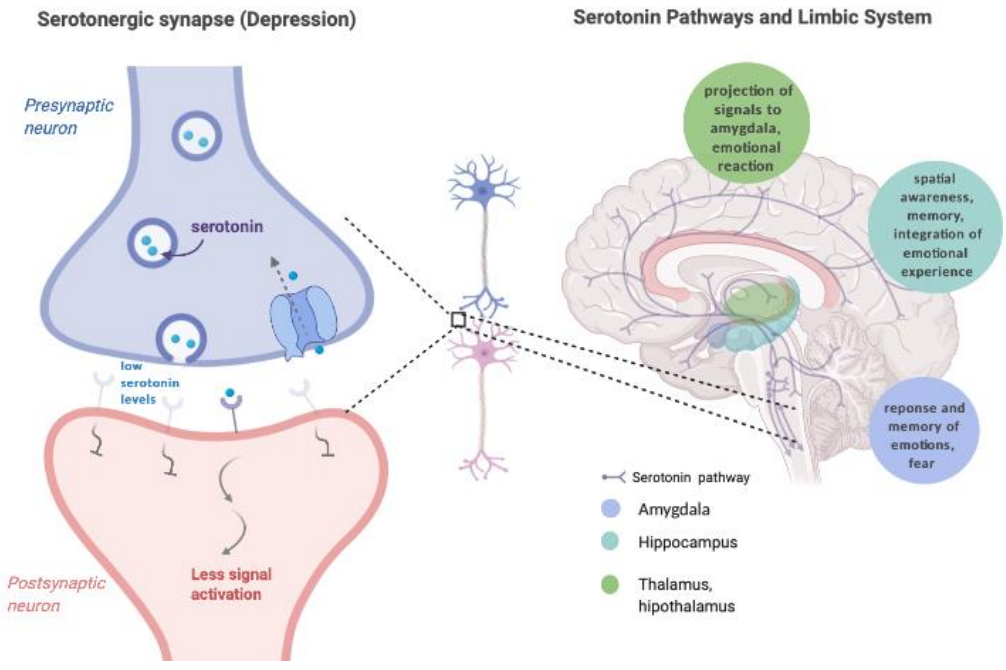

Figure 1. Serotonin and the limbic system in depression. An imbalance in serotonin neurotransmission affects serotonin pathways, and has been associated with the regulation of mood, emotion and behavior. Reduced serotonin receptor activation in depression causes a dysregulation in different limbic structures: hippocampus, amygdala, and thalamus. *Figure source: personal collection.*

1.2.1 Monoamine hypothesis

This hypothesis is based on the fact that depression is the consequence of a dysfunctional neurotransmission caused by a deficit of 5-hydroxytryptamine (5-HT or serotonin) (Figure 2). 5-HT is different from other biological amines since its synthesis rate depends on the concentration of the precursor tryptophan. Initially, when neurobiological researchers observed the relationship between low 5-HT and depression, tryptophan was suggested as a potential molecule to treat depression. However, as not only the synthesis of 5-HT is altered in depression, depression research began to develop drugs that interact with 5-HT receptors and the 5-HT and norepinephrine (NE) signaling pathways (Cosci, 2019).

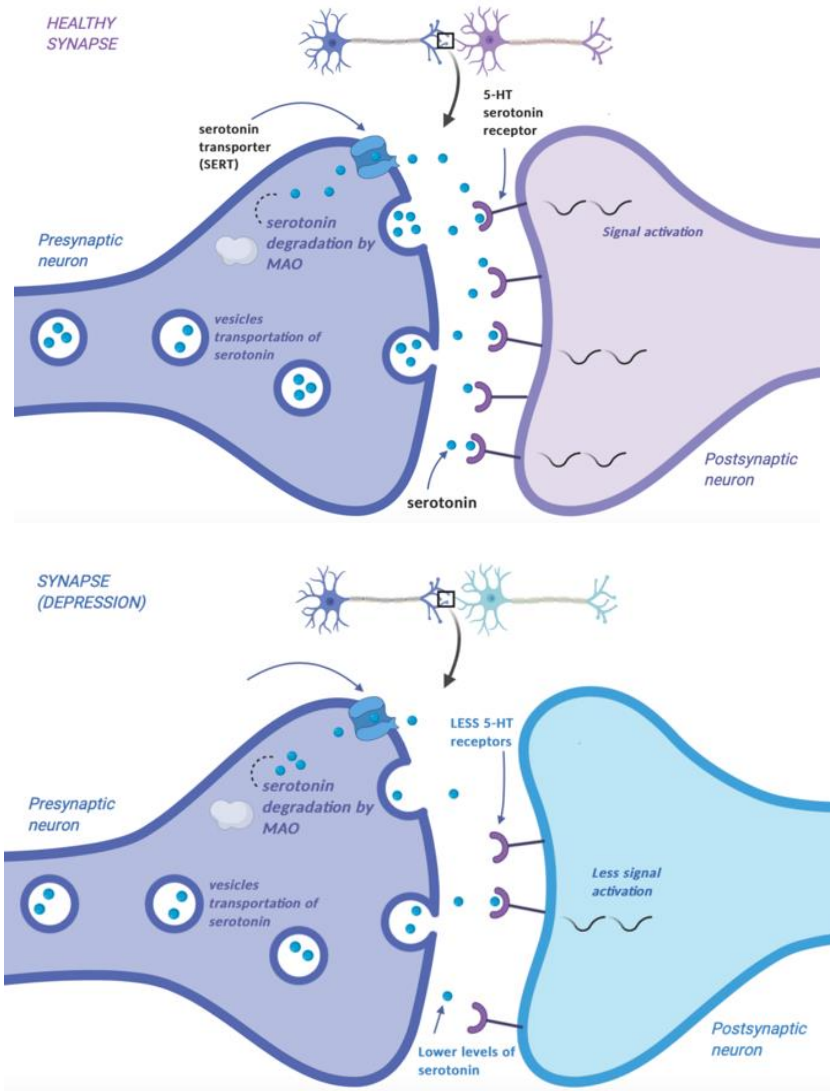

Figure 2. Serotonin role in depression. Comparison of a healthy synapse (upper figure) and a synapse of a depressed person (lower figure). In normal synapses, serotonin is transported into vesicles in order to be released into the synaptic cleft, then serotonin binds to its receptors in the postsynaptic or presynaptic neurons, where initiate different signalling cascades, and finally receptors are cleared and transporters reuptake serotonin (SERT)

and later it is broken down by monoamine oxidase (MAO). In depression, there are lower levels available of serotonin and less 5-HT receptors are activated. Antidepressants based on this monoamine hypothesis inhibit the MAO enzyme degradation of serotonin or block the SERT to inhibit reuptake in order to restore the monoamine deficiencies to normal levels. *Figure source: personal collection.*

The monoamine deficiency approach focuses on restoring the normal concentrations of monoamines and is the target of most of the antidepressant drugs. Antidepressants that have an effect on monoaminergic transmission include monoamine oxidase inhibitors (MAOIs), which increase the levels of both 5-HT and NE through the inhibition of enzymes that degrade them, tricyclic antidepressant compounds (TCAs), selective serotonin reuptake inhibitors (SSRIs) and NE reuptake inhibitors (SNRIs), through the inhibition of 5-HT or NE transporters (White et al., 2008). The majority of antidepressants prescribed since the 1990s belong to this SSRI family, however, more recent drugs act on both the norepinephrine and the serotonin transporters, while other antidepressants act as partial agonists/antagonist of serotonin receptors (Massart et al., 2012).

Overall, the monoamine hypothesis is only a part of the complex neurobiology of depression, and new studies keep changing the variables and approaches to develop antidepressant drugs. In fact, a review by Andrews et al., even suggested that depression is correlated with increased levels of 5-HT based on the melancholia phenotype in patients with depression, as well as animal models that show elevations of 5-HT in different brain areas, contrary to the previously mentioned 5-HT deficiency hypothesis (Andrews et al., 2015).

1.2.2 Neurotrophic hypothesis

This hypothesis establishes that low levels of brain-derived neurotrophic factor (BDNF) cause depressive symptoms (Castren et al., 2007). BDNF is a neurotrophin that plays a role in neuroplasticity and survival of adult neurons and glia, and when BDNF levels are low, neuroprotection is compromised. Other important neurotrophins are the nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), and they bind specifically to tyrosine kinase receptors (Trk). Precursors of these proteins called pro-neurotrophins such as pro-BDNF can bind to other receptors and mediate neuronal death and induce long term depression in the synapse, however, the mature form of BDNF binds selectively to TrkB receptors, promoting neuronal survival (Neto, 2011).

Studies indicate a relation of depression with low levels of BDNF in serum and conversely, treatment with antidepressants has been observed to increase these levels (Shimizu et al., 2003). As mentioned before, BDNF participates in neuroprotection, but also in neurogenesis, which is the generation and maturation of neurons and other progenitors from neural stem cells (Cavallucci, 2016). This process

occurs in parts of the brain as the subgranular zone (SGZ), of the dentate gyrus (DG) of the hippocampus. Downregulation of BDNF in hippocampus and prefrontal cortex has been observed in patients with major depression, and conversely, chronic treatment with antidepressants has been observed to cause BDNF upregulation (Autry and Monteggia, 2012). Studies reviewed by Duman et al., indicate that *in vitro* and *in vivo* experiments with fast-acting antidepressants like ketamine activate AMPA receptors and increase BDNF release, causing activation of mTORC and increased synapsis (Duman et al., 2019).

Overall, the neurotrophic hypothesis suggests that reduced neurotrophin expression in the hippocampus is associated with depression, causing neuronal atrophy and loss of hippocampal volume (MacQueen and Frodl, 2011). BDNF and other neurotrophins have been used as biomarkers for major depressive disorders (Jiang and Salton, 2013) and antidepressant treatment has been observed to increase BDNF levels at the hippocampal level, having a positive effect on adult neurogenesis (Neto, 2011).

1.2.3 Glutamatergic hypothesis

Glutamate is the primary excitatory neurotransmitter in the brain, on the contrary GABA is the primary inhibitor and GABAergic neurons can convert glutamate to GABA. Different ionotropic receptors, as NMDA, AMPA or kainate receptors, and metabotropic receptors (mGluRs) are targets of glutamate. Regulation of these receptors is important to control the concentration of glutamate to avoid excitotoxicity via glutamate transporters (Stahl, 2013).

This hypothesis suggests that there is an imbalance of glutamate and GABA, excitatory and inhibitory neurotransmitter systems. In *postmortem* brain tissue of patients who suffered major depression, glutamate levels are altered, as well as the mRNA and expression of glutamate receptors and their levels are restored after treatment with antidepressants (Deschwanden et al., 2011). This suggests that in depressive disorders, there is a dysregulation of the glutamatergic signaling and reuptake systems. Additionally, glutamate accumulation in areas of the brain involved in cognitive and behavioral regulation has been observed to cause changes in the structure of the brain, such as loss of hippocampal and prefrontal cortex volume, and synaptic activity (Musazzi et al., 2012).

Some antidepressant drugs based on this hypothesis, act as glutamate receptor antagonist (Figure 3). This is the case of ketamine, a noncompetitive glutamate receptor antagonist that shows faster action, but its clinical use for the treatment of depression remains limited due to its narrow therapeutic window (Musazzi et al., 2012). In this sense, low doses of ketamine have been observed to induce the activation of AMPA receptors, causing a signaling cascade through Ca^{2+} channels. This leads to BDNF release

and the subsequent stimulation of TrkB receptors, activating mTOR1 signaling pathway, which participates in the expression of proteins required for synaptic plasticity (Figure 3) (Fogaça and Duman, 2019).

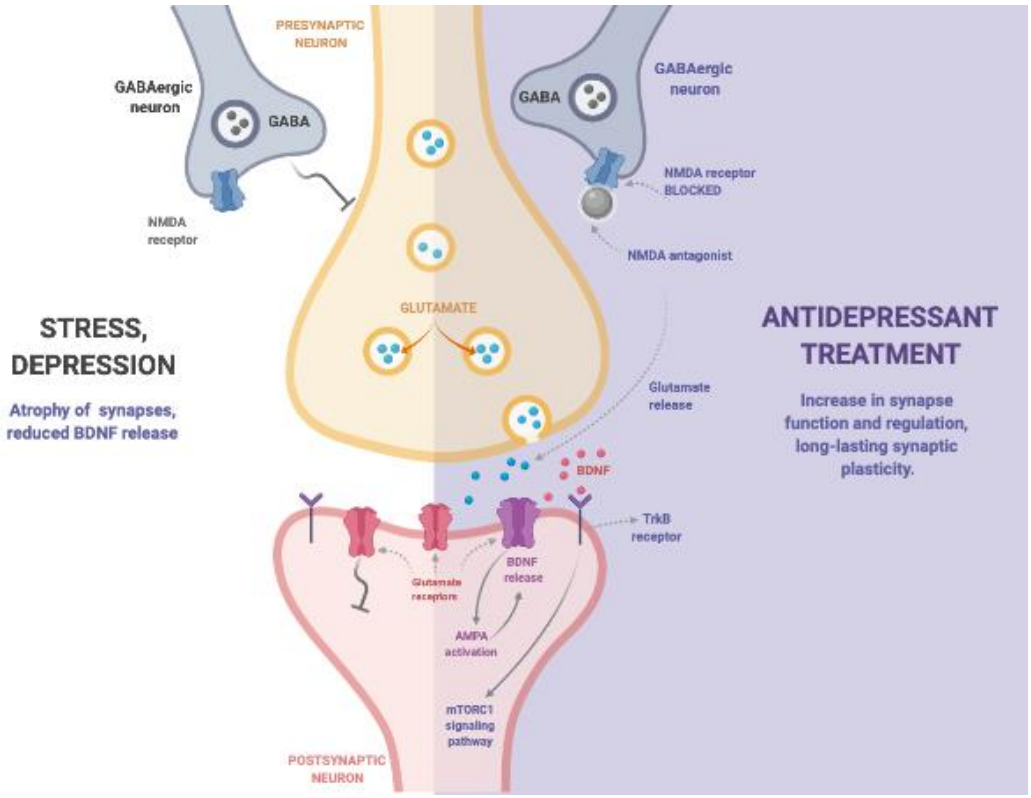

Figure 3. Glutamate and GABA dysfunction in depression. Depression has been associated with a dysregulation of glutamatergic signaling and reuptake systems. Prolonged stress and depression cause a decrease of both GABA and glutamate levels. Antidepressant treatment with NMDA glutamate receptor antagonists block GABAergic neuron activity, disinhibiting glutamatergic pyramidal neurons, increasing the levels of glutamate, and activating AMPA glutamate receptors on postsynaptic neurons, resulting in the depolarization and induction of BDNF release. BDNF binds to its receptor TrkB and drives the signaling pathway of mTORC1, which participates in synapse regulation via synthesis of synaptic proteins and inducing long-lasting synaptic plasticity. *Figure source: personal collection.*

1.2.4 Neuroinflammatory hypothesis

Clinical studies have indicated a relationship between immunocompromised patients with depression and how certain stress factors affect their symptoms and treatment. Cytokines are a group of proteins that participate in the immune response and affect the communication between cells. They act as chemical messengers in two systems associated with depression, the hypothalamic-pituitary-adrenal (HPA) axis, and the catecholamine/sympathetic nervous system. Internal or external stress factors cause alterations in these systems, which include changes in the expression and regulation of neurotransmitters (Sang, 2016). The neuroinflammatory hypothesis suggests that depression is a

consequence of low-grade inflammation triggered by cytokines in response to stress factors (Berk et al., 2013). Given that early studies and clinical trials of antidepressants indicate the ability of some medications to relieve common depressive symptoms by increasing serotonin and noradrenaline levels, research has traditionally focused on the monoamine approach (Cowen, 2015).

Several studies in animals have reported that the administration of cytokines induces depressive behavior, while other studies of patients with depression identified increased expression of proinflammatory cytokines like IL-6, tumor necrosis factor (TNF) and IL-1β (Farooq et al., 2016). Moreover, a correlation between an increase of IL-6 in the CSF and mood impairment has been demonstrated in healthy patients (Engler et al., 2017).

1.2.5 Stress, cortisol and HPA hypothesis

Cortisol is a steroid hormone that also participates in the immune response, specifically in the body's response to stress, and it is produced when the HPA-axis is activated. If cortisol levels are low, the number of proinflammatory cytokines rises and decreases when cortisol are high (Miller et al., 2017). This regulation is mediated by neurotransmitters like dopamine, acetylcholine and serotonin, and according to this hypothesis, imbalances of these neurotransmitters and cytokines affect depressive symptoms.

A 14-year period study with adult patients with depression showed that elevated cortisol levels and HPA-axis hyperactivity are associated with persistent depressive symptomps (Iob et al., 2019). This is consistent with a previous meta-analysis where approximately 80% of patients with major depression exhibit some kind of HPA-hyperactivation. In addition, the degree of hyperactivity was higher in patients with melancholic and pyschotic types of depression (Stetler and Miller, 2011).

Corticosterone (CORT) is the rodent equivalent hormone of cortisol, and it is released by adrenal glands in response to stress factors. Increased levels of CORT have effects on the HPA axis and cause the release of glucocorticoids that target different systems, also producing the negative feedback on the HPA axis. This HPA axis can be dysregulated in depressed animals exposed to stress, presenting a hyperactive HPA axis (Xie et al., 2018). CORT has also been associated with the modulation of hippocampal neuronal plasticity. Studies have demonstrated that repeated administration of CORT and exposure to stress factors induce depressive behavior via the mediation of the HPA axis (Shen et al., 2007). Overall, the expression of depressive symptoms is a result of a dysregulation of the HPA-axis and cortisol levels that affect neurotransmitter signaling.

1.3 Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are the main group of enzymes that participate in the extracellular matrix (ECM) degradation process. The ECM is a complex network that surrounds cells in tissues, serving as a physical barrier, providing structure, and maintaining tissue specificity. It is composed of collagen, glycosaminoglycans, proteoglycans, and glycoproteins. Collagen degradation in the ECM is a process required for tissue remodeling, reparation, and development. MMPs are the main enzymes participating in collagen degradation in the ECM. They are a type of enzymes called endopeptidases, which become active under zinc and calcium rich conditions. There are 24 known MMPs, 23 in humans, and are related to biological process like embryogenesis, tissue regeneration and reparation, development of bone tissue, and formation of blood vessels (Cui et al., 2017). The activity of MMPs is dependent of the expression of the MMP genes, MMP peptide secreted by cells, pro-forms of MMP and inhibition factors. MMPs activity can be inhibited by tissue inhibitors of metalloproteinases (TIMPs), controlling the activation of the pro-forms of MMPs (pro-MMPs). Additionally, TIMP activation or inhibition can be influenced by inflammatory cytokines and other growth factors in response to stress factors (Jabłońska-Trypuć et al., 2016).

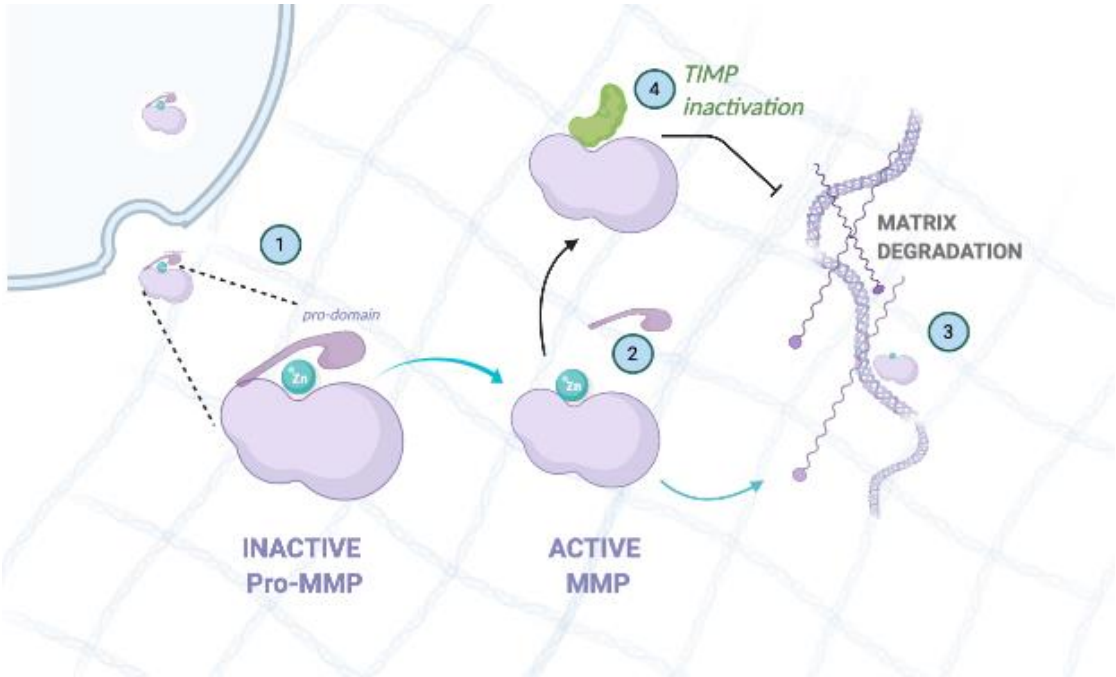

Figure 4. Activation and inhibition of matrix metalloproteinases (MMPs). (1) MMPs are initially synthetized as an inactive pro-form (pro-MMP). (2) MMP activation requires the separation of the pro-domain from the catalytic site; this can be achieved by the partial or complete removal of the pro-domain, and is a process mediated by other MMPs, proteases, binding of reactive oxygen species (ROS), and other reagents like SH or mercury compounds. (3) MMP activation results in ECM degradation. (4) Tissue inhibitors of metalloproteinases (TIMPs) are endogenous proteins that specifically conduct the inhibition of MMPs and maintain balance in the ECM. *Figure source: personal collection*

In general, MMPs are synthesized as inactive pro-forms called zymogens, that require activation through a cofactor zinc (Zn) in the catalytic site and the proteolytical activation by separating the pro-domain from the catalytic site (Figure 4). There is evidence that under Zn limited conditions, the activity of MMP is reduced (Nosrati et al., 2019).

1.3.1 MMP9

Matrix metalloproteinase-9 (MMP-9), or gelatinase B, is a 92 kDa protein and type IV collagenase that participates in the degradation of gelatin, which is a polypeptide and denatured product of collagen (Mohamadi and Hamidi, 2017), and type IV collagen, present in the ECM. Other recognized substrates of MMP-9 include growth factors, receptors and adhesion molecules, some of which are derived from neuronal activity, like BDNF. MMP-9 regulation is essential under normal conditions in the brain (Vafadari et al., 2015).

MMP-9 levels are higher in early development and its expression decreases with age. However, in adulthood, MMP-9 is expressed in different brain areas associated to the synapsis, where it is first secreted as an inactive pro-MMP9 form, then activated by proteolytic cleavage of domains by other MMPs, proteases or binding of reactive oxygen species (ROS), and finally inhibited by TIMP-1 (Reinhard et al., 2015).

Under normal conditions, the expression of MMP-9 in the brain is low and it is mainly found in the hippocampus and cortex. MMP-9 expression can be increased when there is neuronal activity and its mRNA has been localized near synapse areas (Beroun, 2019).

MMP-9 has been widely studied in cancer, lung pathologies, and neurological disorders. MMP-9 is involved in the immune response and inflammation processes through activation of cytokines, and increased MMP-9 activity has been associated with neuronal injury and development of depressive symptoms, since depression is related to a systemic inflammation (Bobinska et al., 2016). Moreover, recent studies indicated that MMP-9 is involved in synaptic plasticity and long-term potentiation (LTP) of synaptic transmission in hippocampal regions, which are reduced in depression (Alaiyed, 2019).

Another study analyzed plasma of patients with major depressive disorder and found significantly decreased levels MMP-9 (Bobinska et al., 2016). Moreover, a study in cancer patients has established a relationship of high MMP-9 levels with the severity of depressive symptoms (Lutgendorf et al., 2008).

Furthermore, animal models of depression have shown that chronic stress caused an increase in MMP-9 levels in the hippocampus and cleavage of nectin-3, an adhesion molecule that participates in hippocampal plasticity (Vafadari, 2015). Another study examined the expression and activity of MMP-9 in the adult rat hippocampus. They described an increase MMP-9 expression after chronic but not acute ECS treatment, and its activity after both acute and chronic treatment. However, classical antidepressants as fluoxetine, tranylcypromine and desipramine did not modify the neither the expression nor the activity of these proteins (Benekareddy et al., 2008). Moreover, a recent study with corticosterone treated male mice, indicated that MMP-9 levels are increased in samples of the prefrontal cortex after chronic administration of venlafaxine compared to vehicle groups (Alaiyed et al., 2020).

1.3.2 Nectin 3

Nectin cell adhesion molecule 3, or nectin-3, is part of the nectin family of immunoglobulins and participates in adhesive junctions in the hippocampus. Findings show decreased levels of nectin-3 in adult mice exposed to chronic stress, and knockout mice for nectin-3 presented disrupted memory processes and neuronal plasticity and maturation, causing damage to the structure of neurons and contributing to memory loss (Wang, 2017).

It has been described that the cleavage of nectin-3 is mediated by the glutamate receptor NMDA and MMP-9 activity (Van der Kooij et al., 2014). As mentioned before, stress can increase glutamate levels in the hippocampus and prefrontal cortex (PFC) and studies with cultured hippocampal cells determined that glutamate treatment enhanced the cleavage of nectin-3, resulting in high levels of a nectin-3 proteolytic fragment and that this process is NMDA-dependent. To address that nectin-3 cleavage is induced by both NMDA and MMP-9 activation, cultures were also treated separately with NMDA receptor antagonists and an MMP-9 inhibitor, and no proteolytic fragments where registered. Experiments with animal models of chronic stress with altered social behaviour, show decreased levels of nectin-3 and altered social behaviour, and that nectin-3 overexpression prevents this unpaired behaviour (Van der Kooij et al., 2014). These findings indicate that MMP-9 and NMDA play an important role in the stress-induced response in the hippocampus and in behavioural alterations, where nectin-3 cleavage is linked to deficits in cognition, memory, and social interaction in animal models.

1.4 Current therapies: fast-acting antidepressant drugs

Current treatment approaches include a combination of pharmacotherapy and psychotherapy. Commonly the most used antidepressants include selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), based on restoring the neurotransmitter balance. Similary, tricyclic antidepressants (TCAs) inhibit the reuptake of serotonin and norepinephrine, while

monoamine oxidase inhibitors (MAOIs) inhibit the monoamine oxidase enzyme, responsible for breaksown of serotonin, dopamine and norepinephrine (Sheffler and Abdijadid, 2020). However, approximately 30% of depressive patients do not respond to common antidepressants and continue to experience depressive symptoms, facing treatment-resistant depression (TRD) (DiBernardo, 2018).

Current treatments for depression fail to achieve a short-term improvement of the symptoms. Therefore, improving antidepressant efficacy still remains a challenge in neuropharmacology. In fact, alternative antidepressants are required with different mechanisms of action to achieve a fast-acting effect and long-term remission. Ketamine has been evaluated as a novel antidepressant for its fast-acting mechanism of action, involving the glutamatergic system (Yang et al., 2015).

Ketamine is a non-competitive antagonist that targets NMDA glutamate receptors on GABAergic neurons (Rosenbaum et al., 2020). Ketamine is currently FDA approved for anaesthesia; however, it is not FDA approved for the treatment of depression due to its high rate of side effects, which include euphoria, hallucinations, and confusion (Bush, 2013). Ketamine consists of a mixture of R-ketamine and S-ketamine. In 2019, the S-isomer of ketamine, esketamine, was approved for TRD as a nasal spray (FDA, 2019), that is administered in combination with an oral antidepressant.

In addition to the blockade of NMDA receptors, ketamine´s mechanism of action includes the indirect activation of AMPA receptors in postsynaptic neurons that mediate rapid synapse transduction that leads to synaptic plasticity (Maeng, 2008). In addition, studies carried out in BDNF knockout mice using TrkB inhibitors (Yang et al., 2015) have shown that the fast-acting antidepressant effects of ketamine are dependent on the BDNF-TrkB cascade.

1.5 Corticosterone model of depression

As mentioned before, depression has been associated with stress and the HPA axis response, where elevated levels of cortisol have been reported. Based on this connection, animal models of depression have been developed to study the effect of potential antidepressant treatments. Moreover, in several studies with adult mice, dysregulation of the HPA axis followed by exogenous CORT administration, caused increased immobility time in the forced swimming test (FST) and reduced sucrose preference, reflecting anhedonia, which is described as the inability to experience pleasure (Xie et al., 2018). Interestingly, there is evidence which supports that antidepressant treatment can reverse the HPA axis hyperactivity and restore glucocorticoid levels in animal models of depression (Juruena, 2014).

2. Hypothesis and objectives

Research hypothesis: The antidepressant effect of the acute administration of ketamine in the corticosterone mouse model will be parallel to a reduced activity and expression of MMP-9 in mouse hippocampal and cortical tissue.

Objectives:

- Study the effect of acute administration of ketamine in the corticosterone mouse model.
- Evaluate MMP-9 activity by gelatin zymography and in situ zymography.
- Evaluate MMP-9 and nectin-3 expression by Western Blot.
- Evaluate MMP-9 expression in mouse cortex and hippocampal tissue by in situ hybridization.

3. Experimental design

3.1 Animals and experimental groups

The proposed study will be conducted using C57BL/6J mice (2-3 months) old housed (n=4-5 animals per cage) in the animal house facility of the Universidad de Cantabria in a temperature-controlled environment with 12 h light/dark cycle, with food and water *ad libitum*. All experiments were carried out with the approval of the Animal Care Committee of the Universidad de Cantabria and were performed following the Spanish legislation and the European Communities Council Directive on "Protection of Animals Used in Experimental and Other Scientific Purposes".

A set of animals is proposed to be used. The set of C57BL/6J mice (2-3 months) will be chronically administered with corticosterone (5-10 mg/kg/day) in the drinking water or vehicle (drinking water) for 28 days and tested using novelty suppressed feeding, followed by acute ketamine administration (10 mg/kg, i.p.) and a battery of anxiety and depression related test according to an experimental time schedule (Figure 5); mice will be then sacrificed and their brains will be used for the in vitro studies.

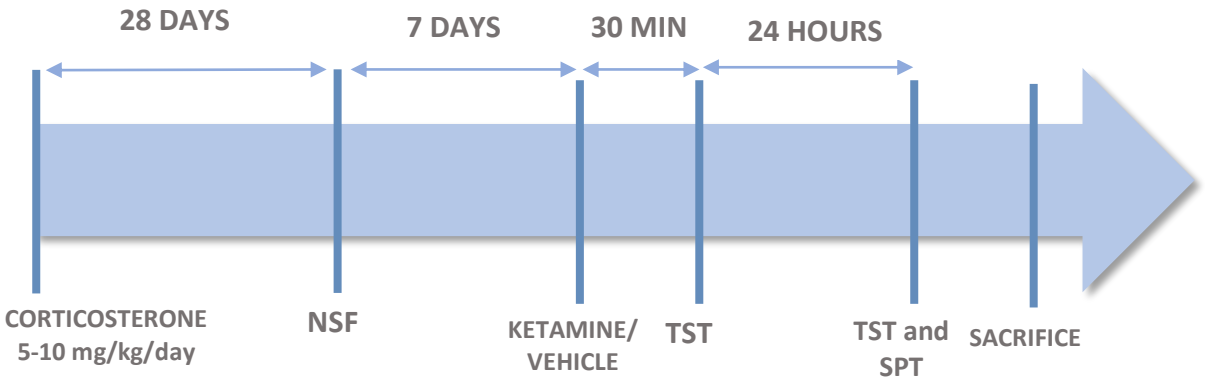

Figure 5. Proposed experimental time schedule. NSF: Novelty Suppressed Feeding; TST: Tail Suspension Test; SPT: Sucrose Preference Test.

3.2 Behavioural testing in mice

Anxiety and depression behavioural tests will be performed during the light phase, as described in detail (Linge et al., 2016). C57BL/6J mice will be placed in the experimental room 1 h before the start of each experiment to acclimatize.

The novelty suppressed feeding test (NSF) measures anxiety behavior by analyzing the latency to approach and eat in a new environment. This test will be performed as previously described (Linge et al., 2013). After 24 h of food deprivation (water ad libitum), mice are removed from their home cage and placed in a corner of an open arena (50cmx 50cm x 30cm) with a food pellet in the center. The latency (in seconds) to approach and eat the pellet, and the average speed is evaluated with a computerized system (Any-maze Video-Tracking software, Stoelting Co., USA).

The tail suspension test (TST) is based on the short-term suspension of animals by their tails leading to an immobile posture and the measurement of behavioral despair. This test will be performed by individually suspending the mouse by its tail in an inescapable position. The mouse movements will be recorded for 6 min and the immobility time will be evaluated by a trained observer.

The sucrose preference test (SPT) is a reward-based test that analyzes the sucrose intake and is used as an indicator of anhedonia. The test will be performed as previously described (Linge et al., 2016). After single housing, mice are trained to drink a sucrose solution for 48 h by placing a bottle of tap water and another with a sucrose solution (1% w/v). The volume of both water and the sucrose solution is evaluated in a period lf 24 h. The ml of sucrose consumed are measured to calculated the percentage of sucrose versus total intake.

3.1.3 Gelatin zymography

Gelatin zymography will be performed to detect gelatinase activity in biological samples using SDS-PAGE with gelatin. Mice will be sacrificed and their brains removed and dissected to obtain the prefrontal cortex and hippocampus, then samples will be frozen immediately on dry ice and then stored at -80°C. The protocol for protein extraction is adapted from Szklarczyl et al. (2002).

Tissue samples are weighted and homogenized 1:20 (w/v) in SAMPLE BUFFER (10 mM $CaCl_2$ and 0.25% Triton X-100 in water) (20 µl of buffer/1 mg of tissue), and then centrifuged at 6000xg for 30 min at 4°C. Supernatant 1 is discarded and the pellet 1 is resuspended in 100-200 ul of PELLET BUFFER 1 (50 mM Tris pH 7.4, 0.1 M $CaCl_2$), incubated 15 min at 60°C, and centrifuged at 10,000xg for 30 min at 4°C. Pellet 2 is discarded and supernatant 2 (Triton X-100-insoluble fraction) is kept in ice. Protein quantification is performed by Lowry assay using BSA for the standard curve. For SDS-PAGE, preparation of 150 µl of samples + Laemmli buffer + 4 µl of PELLET BUFFER 2 (PELLET BUFFER 1 + 10% TRITON X-114) (about 75 µg of protein/well) and positive control 2% fetal bovine serum (FBS) in extraction buffer (2% Triton X-114, 10mM Tris HCl, 150 mM NaCl pH 7,4, 1 µM protease inhibitors aprotinin and PMSF) + Laemmli buffer. Marker and samples are loaded in a 8,5% SDS-PAGE gel containing 0,1% gelatin and run for 100V for 15 min, then 160V for 50 min in 1X RUNNING BUFFER (10 X: 0,25M TRIS BASE, 1,91M Glycine, 0,03M SDS , H2O 1 L) at 4°C. After running, the gel is incubated with 100 mL of 1X WASHING BUFFER (100 ml H_2O, 2,5% TRITON X-100) at room temperature with agitation for 15 minutes twice, and then in 100 ml of 1X DEVELOPING BUFFER (50 mM Tris-HCl, pH 7.4; 200 mM NaCl; 6,7 mM $CaCl_2$; 1 µM $ZnCl_2$; 0.2% Brij35) for 30 min at room temperature with agitation. The gel is incubated with fresh 1X DEVELOPING BUFFER at 37°C for 48 h. After incubation, DEVELOPING BUFFER is removed and the gel was washed 3 times with 100 ml of water and left at room temperature with agitation for 5 minutes. The gel is scanned before staining with SNAPSCAN 1236 AGFA and AGFA FotoLook software. The gel is stained with STAINING SOLUTION (0,5% Coomassie Blue R-250, 5% methanol, 10% acetic acid) for 1 h at room temperature with agitation, and later distained with DESTAINING SOLUTION (10% methanol, 5% acetic acid) until areas of proteolytic activity were visible. Finally, DESTAINING SOLUTION is removed and the gel is scanned with SNAPSCAN 1236 AGFA and AGFA FotoLook software.

3.4 *In situ* zymography

In situ zymography and the immunohistochemistry are performed following the protocol of George and Johnson, 2010 and Amantea et al, 2008. Brain slides of 14 µm thickness are obtained with a cryostat

and stored at -80°C. The brain slides are kept at room temperature for 1 h and then they are re-hydrated in PBS 1X for 5 minutes. The slides are then incubated with a solution containing DQTM Gelatin fluorescein-conjugated (20 µg/ml DQTM Gelatin), 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) (Molecular Probes, Inc., Eugene, OR, USA) in a dark and humid chamber, at 37°C for 18 hours. Negative control is prepared by adding 50 mM EDTA to the gelatin solution. After incubation, the slides are washed with PBS 1X 3 times, 5 min with agitation. Finally, slides are fixed with 4% paraformaldehyde in PBS for 5 min and washed again 3 times for 5 min each with PBS.

For immunohistochemistry, slides are blocked with PBS-TS (PBS containing 5% normal donkey serum; 0,2 % Triton X-100) for 30 min at room temperature, followed by the incubation with primary mouse anti-NeuN antibody (1:100) (Millipore) in PBS-T (PBS, 0,2% Triton X-100) overnight at 4°C in a dark and humid chamber. Next, slides are washed with PBS 1X 5 times for 5 min each with agitation. This is followed by incubation with secondary antibody Alexa 568 anti-mouse (1:200) in PBS-TS for 2 hours at room temperature and washed with PBS 1X. Slides are then incubated with DAPI (1:1000) in PBS 1X for 5 min, then washed 3 times and let at room temperature until dry. All the slides are coated with Gerbatol and covered with a coverslip. The fluorescent signal is detected using a Zeiss Axio Imager M1 fluorescence microscope, 12 bits B&W camera (AxioCam MRm). The images are analyzed using the software ImageJ (NIH, USA).

3.5 Western Blot

Protein extraction for western blot is performed following the protocol by Van der Kooij et al, 2014. After mice are sacrificed by cervical dislocation, their brains are removed and dissected to obtain prefrontal cortex and hippocampus; tissue samples are stored at -80°C. Samples are 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). The sample is filtered with a 40 µm filter, previously wet with a 1 ml of homogenization buffer, and then centrifuged at 1000xg for 10 min at 4℃. The pellet is resuspended in 100 µl of 1% SDS buffer (SDS in homogenization buffer and inhibitors) and boiled for 1 min. Lowry assay is carried out for protein quantification and then samples are prepared for SDS-PAGE with a loading buffer containing β-mercaptoethanol, boiled at 100℃ for 5 min and kept in ice for 3 min. The aliquots are centrifuged at 956xg for 5 min at 4℃ and the supernatant is stored at -20℃ until used.

About 35 µg of protein per sample are loaded per duplicate on an 8,5% SDS-PAGE gel. The gel runs at 100 V for 15 min and then at 160 V for 50 min. Transference to a nitrocellulose membrane (GE Healthcare Europe GmbH, Munich, Germany) is performed using cold transfer buffer (2.5 mM Tris,

19.2 mM glycine, pH 8.3; 20% methanol) at 100 V for 90 min at 4ºC. Following the transference, the membrane is blocked with 5% (w/v) nonfat dry milk in TBST for 1 hour. The membrane is incubated overnight at 4℃ with rabbit anti-MMP9 (RayBiotech, RayBiotech Life, Georgia, GA, USA) (1:3000) or rabbit anti-nectin-3 (MBL, MBL International, Woburn, MA, USA) (1:10000) primary antibodies in blocking solution. After incubation, the membrane is washed with TBST and then incubated with fluorophore-conjugated IRDye 800CW Donkey anti-Rabbit secondary antibody (LI-COR Biosciences, Lincoln, NE, USA) (1:15000 in milk 5%) for 1 hour at room temperature. After washing with TBST, the signal is visualized using an Odyssey CLx Imaging System (LI-COR Bioscience, Lincoln, USA). The densitometric values are normalized using mouse anti-tubulin (1:20000) as housekeeping. The images are analyzed with the use of Image Studio™ Lite software (LICOR Bioscience, Lincoln, USA).

3.7 *In situ* hybridization

Mice will be sacrificed, and their brains will be removed and stored el -80°C. Brain coronal slides of 14 µm thickness are obtained with a cryostat and stored at -80°C for in situ hybridization. Brain sections are pretreated using 4% paraformaldehyde in PBS for 5 minutes and then rinsed twice with a solution of PBS. Section are then acetylated with a solution of 0.25% acetic anhydride in 0.1 M triethanolamine buffer for 10 min. After this, sections are dehydrated with a graded ethanol wash (70%, 80%, 95% and 100%), and then immersed in chloroform for 10 min, and rehydrated with 100% and 95% ethanol. Finally, sections are air-dried and stored at -20°C prior to use. This protocol is adapted from Castro (Castro et al., 2003a), using the following oligonucleotides complementary to MMP9 mRNAs:

- MMP9-1antisense : 5'-TCTCCGTGCTCCGCGACACCAAACTGGAT-3'
- MMP9-2 antisense: 5'-GAGAGAAATAGTTACATAATACCTTCCAGGG-3'
- MMP9-3 antisense: 5'-TTAGAGCCACGACCATACAGATACTGGATG-3'

These oligonucleotides are 3'end-labelled with [^{35}S]dATP using terminal deoxy-nucleotide transferase (TdT). To label the oligonucleotides, 3 pmoles of the specific oligonucleotide are mixed with 1.6 pmoles of [^{35}S]dATP with an specific activity of 1250 Ci/mmol, 15 U of TdT, 2.5 µl TDT buffer and DEPC water. The mixture is incubated for 1 hour at 37°C, and the reaction is stopped with 87.5 µl of DEPC water. The probe is purified using Sephadex chromatography micro column illustra ProbeQuant G-50 (GE Healthcare life science, UK). After the purification, the labelled probe (250000 c.p.m./slide) is mixed with hybridization buffer (50% deionized formamide, 4x standard saline citrate (SSC), 10 mM sodium phosphate pH = 7.0, 1 mM sodium pyrophosphate, 10% dextran sulphate, 5x Denhardt's solution, 200 µg/ml salmon sperm DNA, 100 µg/ml poly A, 0.12 mg/ml heparin and 20 mM dithiothreitol) and then homogenized. 200 µl of the mixture is scattered for every slide, and then coated

with cover glasses (Menzel-Gläser, Germany). Slides are incubated at 42°C for 16 hours in a humidified chamber, and then washed to remove excess probe and reduce unspecific binding. Slides are washed at 50°C in 2x SSC buffer with 1M DTT twice for 30 minutes followed by three washes of 5 minutes at room temperature with 1x SSC, 0.1x SSC, and ethanol 80% consecutively. Finally, slides are washed in ethanol 96% for 1 minute at room temperature. Sections are air- dried and exposed to film BioMax MR together with ^{14}C microscales at -20°C for 2-4 weeks depending on the abundance of mRNA in the tissue. The control of specificity is done with the probe without labelling (at a concentration 1000 times higher). The films are scanned to generate a digital image to further analysis.

3.8 Possible difficulties

- Gelatin zymography: poor extraction of MMP9 protein in tissue samples leads to undetectable bands in gel. The quantity of MMP-9 mRNA is not enough.

- Selected oligos for *in situ* hybridization are not sensitive enough.

3.9 Schedule of activities

- Weeks 1-3: experimental design and probing protocols and material oligonucleotide probes (gel zymography, in situ zymography, in situ hybridization)

- Weeks 1-4: development of corticosterone model in C57BL/6J mice

- Week 5: NSF

- Week 6: acute ketamine administration/vehicle, TST and SPT, and sacrifice.

- Week 7-11: tissue sectioning, in situ hybridization

- Weeks 9-10: gelatin zymography and western blot

- Weeks 11-12: in situ zymography

- Week 13: data analysis

4. Expected outcomes

4.1 General outcomes

- It is expected to be an antidepressant-like effect observed in the animal model of corticosterone after acute administration of ketamine.

- It is expected that MMP-9 activitiy will be lower in the corticosterone mice after acute administration of ketamine, compared to the vehicle corticosterone group.

Groups:

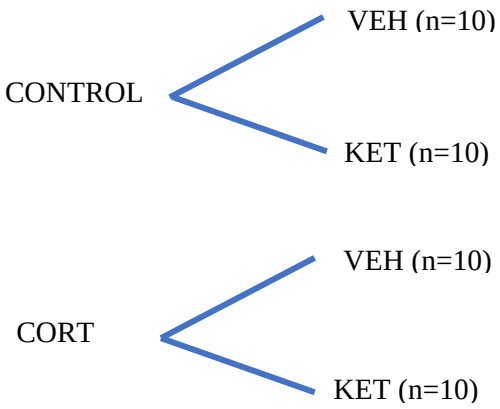

Figure 6. Experimental group design: CONTROL+ VEHICLE (n=10), CONTROL+KETAMINE (n=10), CORT+VEHICLE (n=10), CORT+KETAMINE(n=10)

4.2 Behavioral tests outcomes

4.2.1 Novelty spressed feeding (NFS) test

Results from previous behavioral experiments with corticosterone treated mice indicated an increased latency time of feeding compared to the vehicle animals in the NSF test (Breviario, 2019). This is the expected NSF test outcome for the control and CORT group before the vehicle/ketamine treatment.

4.2.2 Tail suspension test (TST)

Results from previous behavioral experiments with corticosterone treated mice indicated the CORT group exhibits a higher immobility time compared to the vehicle group (Breviario, 2019). This is the expected TST outcome for the vehicle and CORT group (Figure 7A). Following acute ketamine administration in the CORT+KET group we expect a reduction in the immobility time after 30 min (Figure 8B). 24 hours after ketamine administration a similar reduction in inmobility time will be observed in the CORT+KETgroup (Figure 7C). A reduction in the immobility time is spected in the CONTROL+KET at least 30 min after ketamine administration.

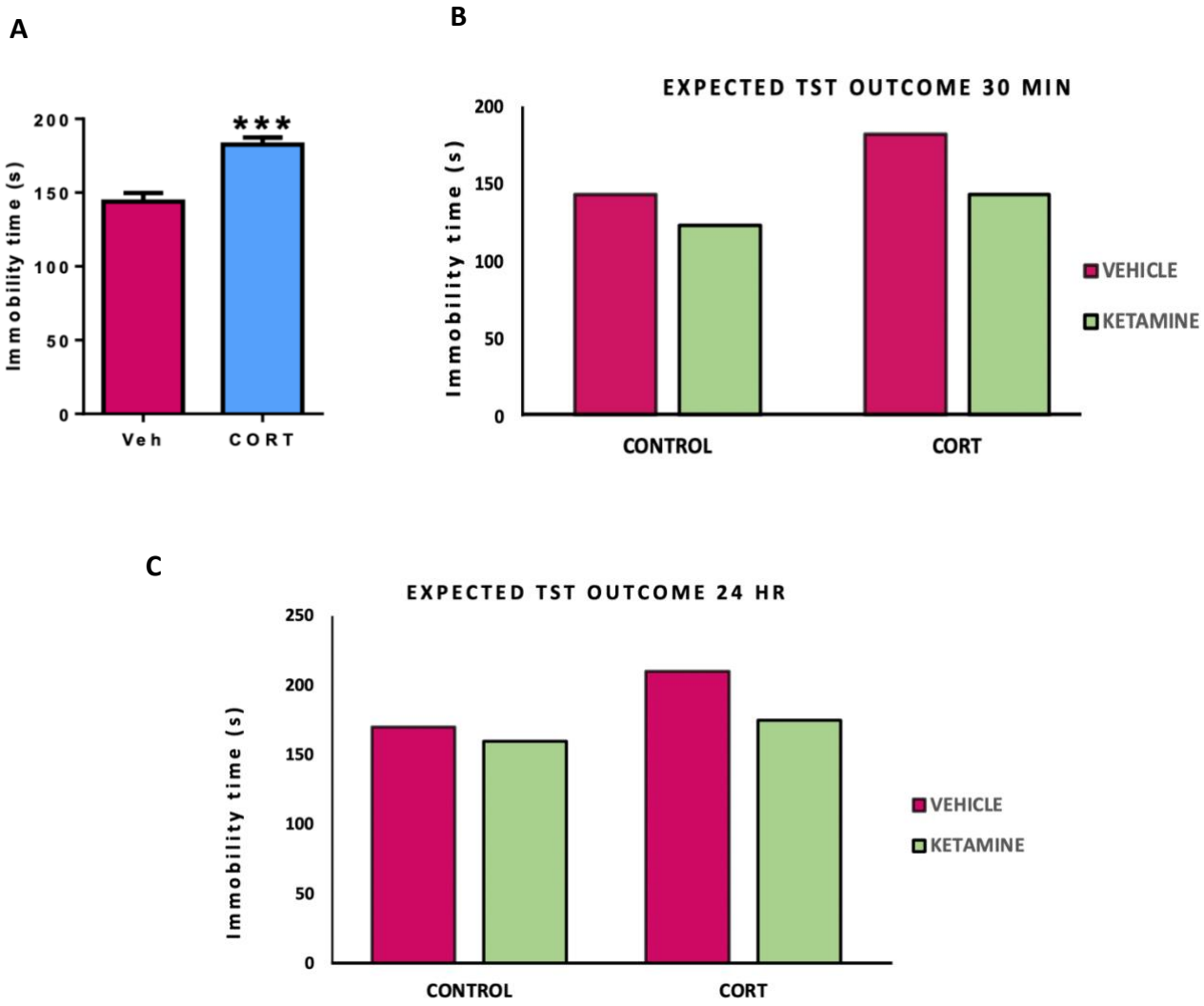

Figure 7. Expected tail suspension test (SPT) outcome. A) TST results from previous experiments with the immobility time of corticosterone treated mice in a 6 min test (Breviario, 2019). B) Expected TST outcome using CONTROL and CORT groups 30 min after vehicle and ketamine administration. C) Expected TST outcome using CONTROL and CORT groups 24 h after vehicle and acute ketamine administration.

4.2.3 Sucrose preference test (SPT)

Results from previous behavioral experiments with corticosterone treated mice indicated a significant reduction in the sucrose preference compared to vehicle animals (Breviario, 2019) (Figure 8A). After 24 h of acute ketamine administration it is expected that ketamine reverses the decrease % of sucrose preference observed in CORT group reaching similar levels as CONTROL+VEH group (Figure 8B).

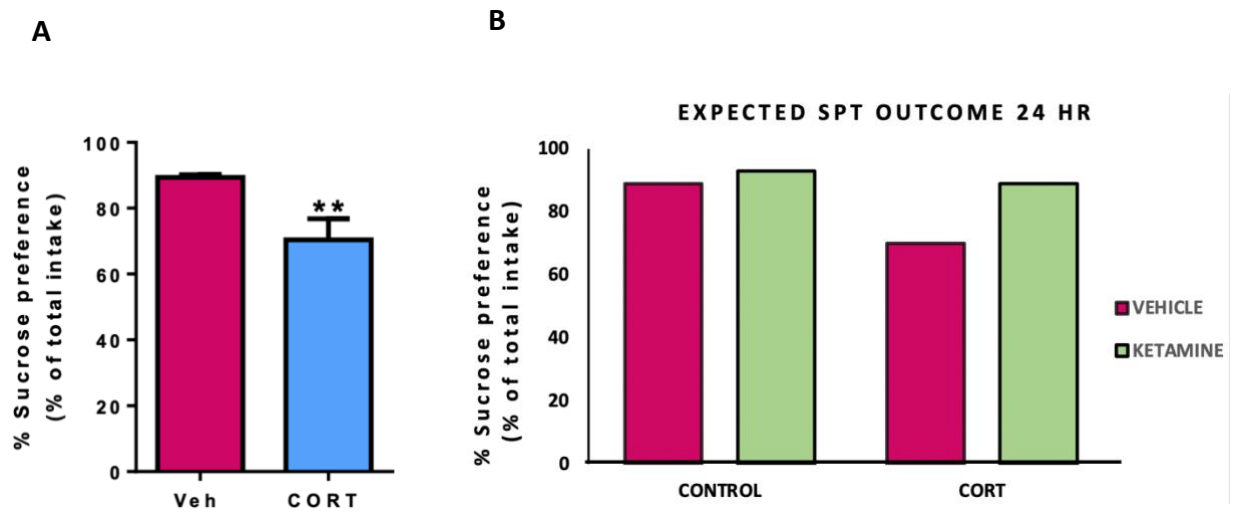

Figure 8. Expected Sucrose preference test (SPT) outcome. A) SPT results from previous experiments with corticosterone treated mice (Breviario, 2019). B) Expected SPT outcome using CONTROL and CORT groups after 24 h of vehicle and acute ketamine administration.

4.2.4 Gelatin Zymography

Results from previous gelatin zymography using cortex and hippocampal samples of corticosterone treated mice indicated a significant increase in the MMP-9 activity in the cortex compared to vehicle (Figures 9A and B) and a tendency to increased activity in the hippocampus compared to vehicle (Figures 9C and D) (Breviario, 2019). Following acute ketamine administration in the CORT+KET group we expect that ketamine, after 24 hours administration, reverts the increased MMP9 activity induced by the corticosterone model in the cortex and hippocampus, reaching similar levels as CONTROL+VEH (Figures 9E and F). A small reduction in MMP-9 activity compared to CONTROL+VEH group could be expected in the control group following ketamine administration (Figures 9E and F).

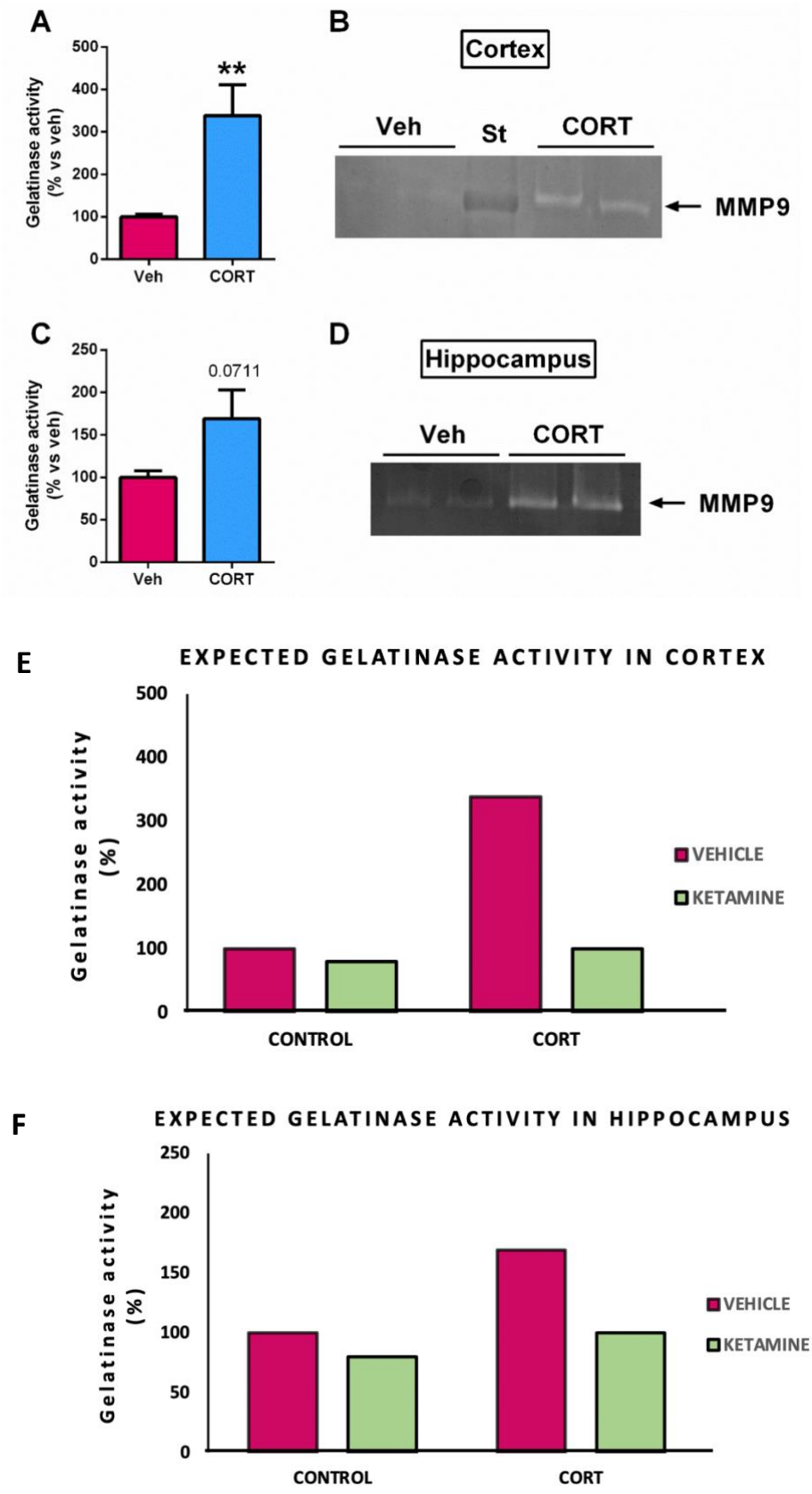

Figure 9. Expected Gelatin Zymography outcome. (A-D) Results from previous experiments in cortex and hippocampus samples of corticosterone treated mice vs vehicle mice (Breviario, 2019). E-F) Expected gelatin zymopgraphy results in cortex (E) and hippocampus (F) using CONTROL and CORT group after vehicle and ketamine administration.

4.2.5 *In situ* Zymography

Results from previous *in situ* zymography studying gelatinolytic activity of MMP9 in the CA1, CA3, and in the dentate gyrus (DG) of the hippocampus, indicated a significant higher activity in mice treated with corticosterone compared to vehicle group (Breviario, 2019) (Figure 10A-I). Following acute ketamine administration in the CORT+KET group we expect that ketamine reverts the increase in the gelatinolytic activity of MMP9 in the DG, CA1 and CA3 regions of the hippocampus, reaching similar levels as CONTROL+VEH. Also, a small reduction of MMP-9 activity in the CONTROL+KET group compared to CONTROL+VEH group is expected (Figure 10J-L). Similar results are expected in the prefrontal cortex.

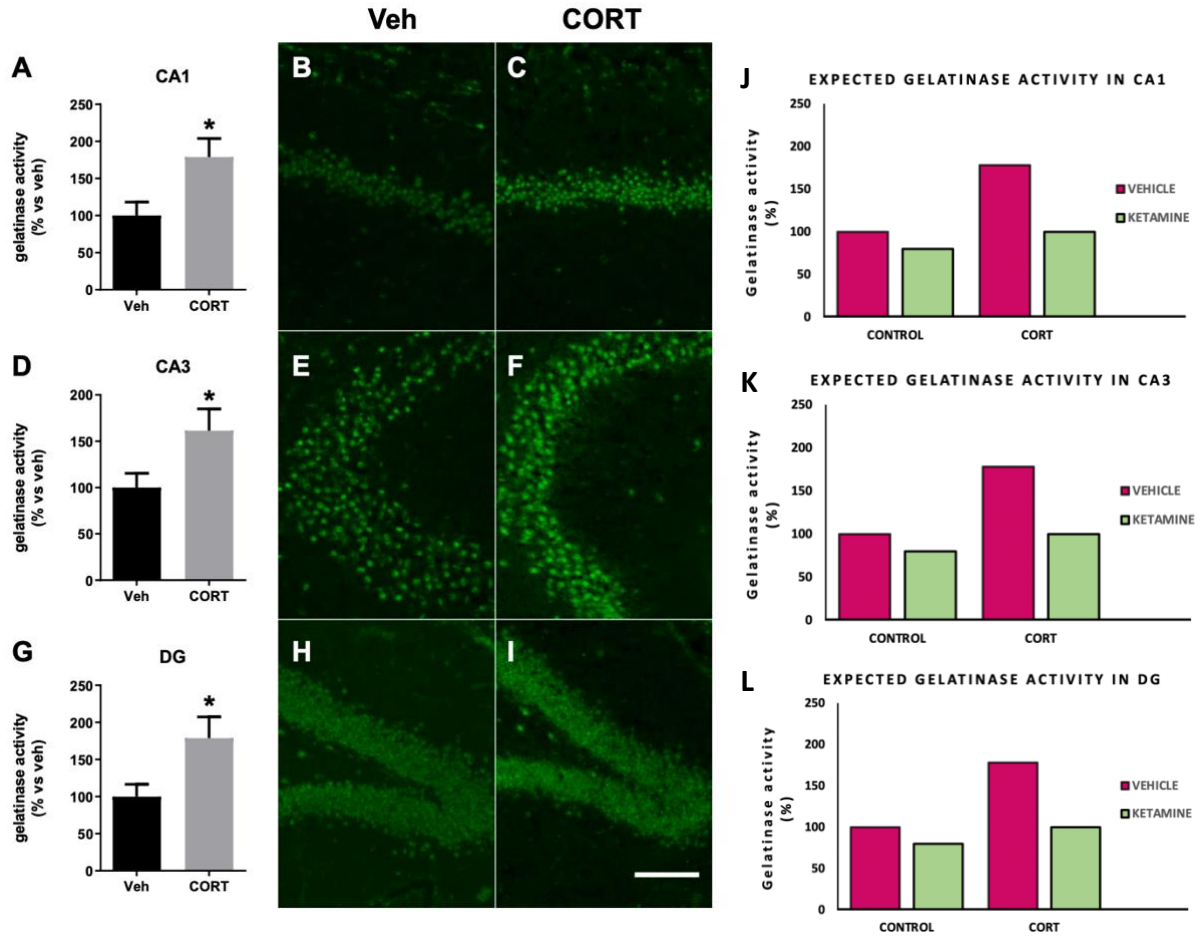

Figure 10. Results form previous *in situ* Zymography. A) CA1 field; D) CA3 field and G) DG field of the hippocampus. Images of the *in situ* zymography in the CA1 field in the vehicle (B) and corticosterone-treated mice (C); in the CA3 field in the vehicle (E) and corticosterone-treated mice (F); and in the dentate gyrus in the vehicle (H) and corticosterone-treated mice (I) (Breviario, 2019). J-L) Expected results of gelatinase activity in CA1(J), CA3(K) and DG(L) using CONTROL and CORT group after vehicle and ketamine administration.

4.2.5 Expression of MMP9

Results from previous western blot of cortex and hippocampal samples of corticosterone treated mice indicated a significant increase in the expression of MMP9 in the cortex compared to the vehicle group, and a similar increase of expression in the hippocampus (Breviario, 2019) (Figure 11A-D). Following acute ketamine administration in the CORT+KET group it is expected that ketamine reverts the increase in the expression of MMP9 in the both areas, reaching similar levels as CONTROL+VEH group (Figure 11E-F). There is expected a small reduction of MMP-9 activity in cortex and hippocampus in the CONTROL+KET group compared to CONTROL+VEH group.

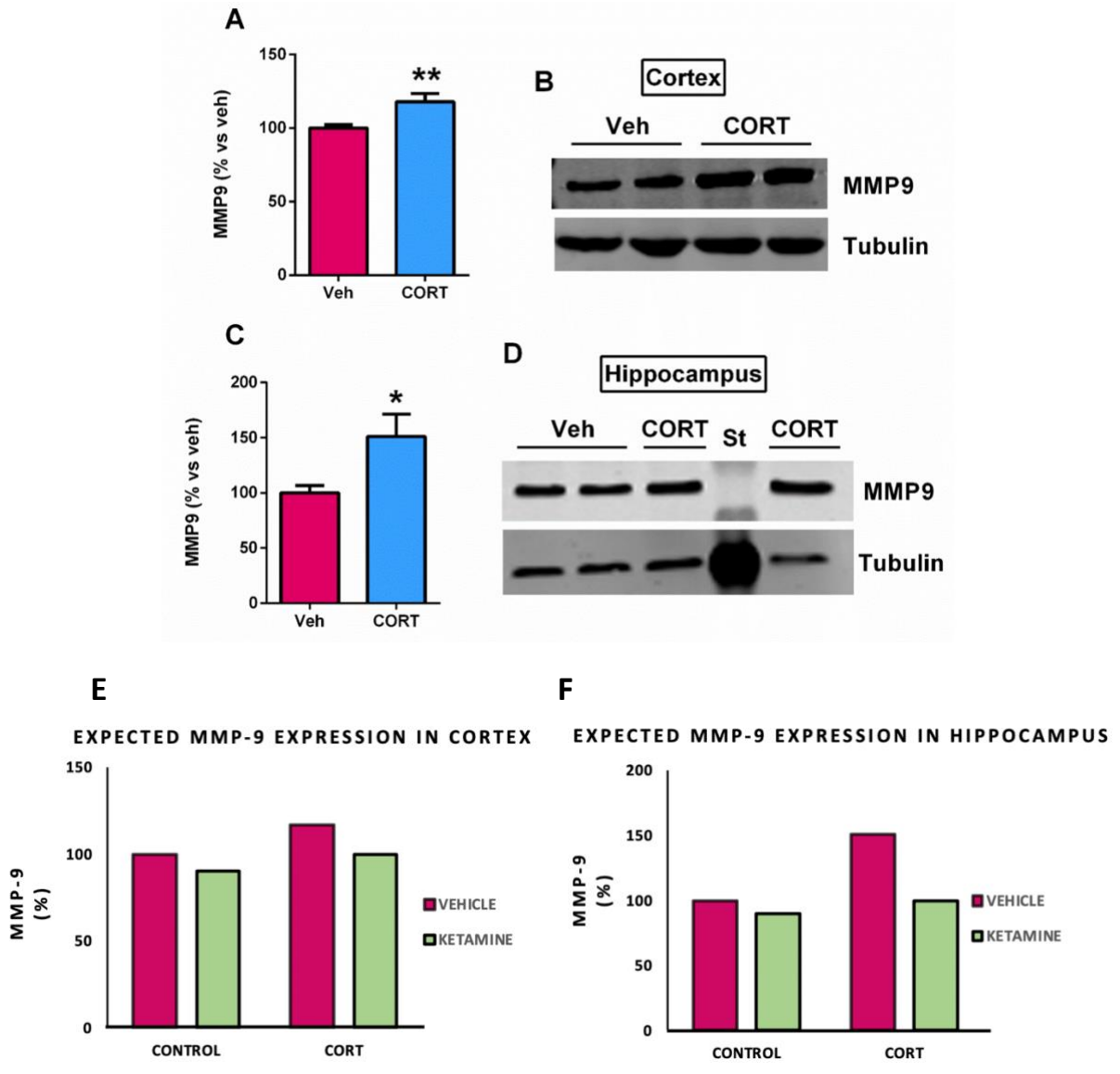

Figure 11. Expected MMP9 expression outcome. A-D) Expression results from previous experiments with corticosterone treated mice (Breviario, 2019). E-F) Expected expression outcome of MMP-9 using CONTROL and CORT groups in cortex and hippocampus after vehicle and ketamine administration.

4.2.6 *In situ* hybridization

Results from previous experiments in adult rats using *in situ* hybridization show a representative autoradiogram of MMP-9 mRNA in the hippocampus (Benekareddy et al., 2008) (Figure 12). Following acute ketamine administration in the CORT+KET group we expect similar results as the ones previously described for protein expression (see section 4.2.5). Acute ketamine administration is expected to revert the increased MMP-9 mRNA expression induced by the corticosterone model in the cortex and hippocampus, reaching similar levels as CONTROL+VEH group. There is expected a small reduction in MMP-9 mRNA expression in the CONTROL+KET group compared to CONTROL+VEH group (Figure 13)

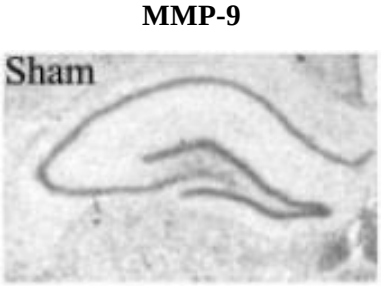

Figure 12. Representative autoradiograms of MMP-9 mRNA in the rat hippocampus. (Benekareddy et al., 2008)

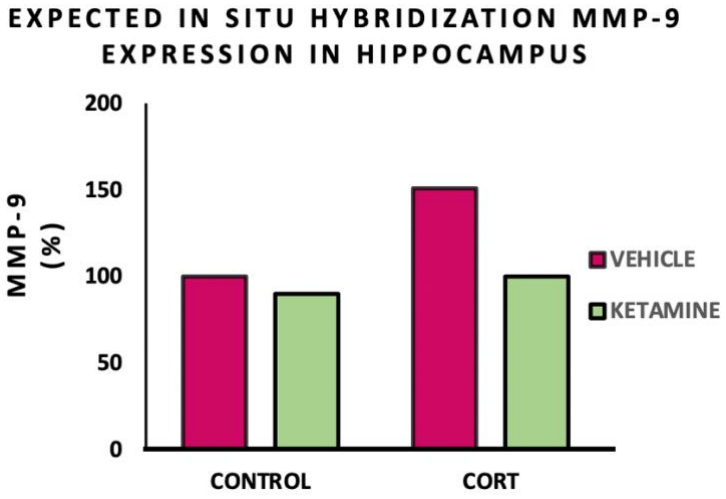

Figure 13. Expected in situ hybridization MMP9 expression outcome

5. Discussion

This experimental proposal suggests that acute ketamine administration may alter behavior in mice under a corticosterone model of depression, and that one of the underlying mechanisms of this fast-acting antidepressant effect is affecting metalloproteinases activity in the brain. Previous experiments in corticosterone-treated mice have shown an increase in the MMP-9 activity in cortex and in

hippocampus (Van der Kooij et al., 2014). Specifically, the corticosterone model show a significant higher MMP-9 activity in CA1, CA3 and in the dentate gyrus of the hippocampus, and layers 2/3 and 5 of the medial prefrontal cortex (Breviario, 2019). It is expected that the acute ketamine administration induce an antidepressant-like effect, as observed by the reduction in the immobility time in the TST and the reversion of the anhedonic state observed in this animal model of depression. It is also expected that the increased expression of MMP-9 protein and mRNA, as well as the increased gelatinolytic activity of MMP9 observed in the CORT group is reverted in both cortex and hippocampus.

The corticosterone model of depression used in this study induces a depressive-like behavior characterized by behavioural despair and an anhedonic state (Gourley and Taylor, 2009). This model is in accordance with other animal models as the chronic stress model (reviewed in Willner, 2016), acute LPS administration (O'Connor et al., 2009), or genetic models as the BDNF Val66Met66 knock-in mice (Duman and Adhajanian, 2012), 5-HT_4 knockout mice (Amigó et al., 2016), and the GLAST+ β-catenin knockout mice (Vidal et al., 2018; Garro-Martínez et al., 2020). Regarding the behavioural effects of ketamine, we propose that the acute administration of this drug may induce an antidepressant-like effect observed as a decrease in the immobility time in the TST in our corticosterone model. Several studies carried out in animal models of depression, including the chronic stress (Krzystyniak et al., 2019), and the unpredictable chronic stress (UCS) (Fitzgerald et al., 2019) showed that ketamine (3-10 mg/kg) induces an antidepressant-like effect evatuated in the sucrose preference test and the FST, respectively, although no data are published to date using the TST. Moreover, a prophylactic administration of ketamine has been described to promote resilience and an faster recovery to chronic stress models (Krzystyniak et al., 2019). All these data are in agreement with the fast-acting antidepressant effect of ketamine observed in patients with treatment-resistant depression (Zarate et al., 2006; Abdallah et al., 2015; Han et al., 2016; Corriger and Pickering, 2019).

In this study we also propose an antidepressant-like effect of ketamine in non stressed animals. Regarding the acute effect of ketamine in behavioural tests is contradictory depending on the animal, strain, dose, and test used to evaluate this effect. To the best of our knowledge, only one study performed in other mouse strain evaluating the antidepressant-like effect of ketamine in the tail suspension test showed no changes after ketamine administration (Khakpai et al., 2019). In the forced swimming test (FST) an antidepressant-like effect has been described in naïve rats both after acute systemic and bilateral intracerebral administration of ketamine (López-Gil et al., 2019). Moreover, in this test it has been described opposing effects of ketamine as a decrease in the immobility time (Pham et al., 2017), or an increase in the immobility time in non-stressed mice (Fitzgerald et al., 2019). In this sense, a recent publication reports that ketamine can induce depressive symptomps in healthy controls (Nugent et al., 2019). Further preclinical and clinical studies need to be done to clarify these findings.

MMP-9 has been associated with depression and described as a potential biomarker for this condition (Domenici et al., 2010). In previous experiments in the corticosterone model, there are increased MMP-9 expression and activity levels (Breviario, 2019). Several studies report an increase in MMP-9 plasma levels in patients with depression (Domenici et al., 2010; Rybakowski et al., 2013; Bobinska et al., 2016). Moreover, MMP-9 serum levels present a negative correlation with the severity of depression (Yoshida et al., 2012). These alterations are reversed following fast-acting antidepressant strategies as the electroconvulsive therapy (Shibasaki et al., 2018). However, preclinical studies in näive animals only reported an increase in MMP-9 in hippocampus after chronic but not acute electroconvulsive treatment (Benekareddy, 2008). All this data point to a possible downregulation of MMP-9 in brain samples following ketamine administration.

To date there is no clear explanation of the molecular mechanism underlying either the increased MMP-9 brain or plasma levels in animal models of depression and human samples, or the downregulation promoted by antidepressant treatments. Here we propose some putative explanations taking into account the possible interaction of the MMPs and different molecular markers commonly associated to depression. In this sense, there are several studies reporting decreased BDNF levels in animal models of depression (Duman and Monteggia, 2006; Castren et al., 2007) and clinical studies (Chen et al., 2001; Shimizu et al., 2003; Autry and Monteggia, 2012). Is is worth to mention, that MMP-9 is involved in BDNF maturation. proBDNF is an immature form of BDNF that is synthesized as a precursor, which is then cleaved by MMPs such as MMP-9 and converted into its mature form (Hashimoto, 2010; Mizoguchi et al., 2011). The increased MMP-9 expression and/or activity observed in the corticosterone model could be a compensatory mechanism underlying the low brain BDNF level in this model.

We propose that ketamine administration could revert the previously reported increase in MMP-9 expression levels and activity in the cortiscosterone model, as well as the nectin-3 fragment in hippocampal and prefrontal cortex tissue samples (Breviario, 2019). Studies in animal models of depression, most of them associated to chronic stress exposure, reported a decrease in BDNF expression in the hippocampus and prefrontal cortex (Smith, Makino, Kvetnansky, & Post, 1995; Duman & Monteggia, 2006; Krishnan & Nestler, 2008, 2010). Chronic treatments with classical antidepressant drugs normalize this alteration in BDNF levels (reviewed in Duman et al., 2019), including the fast-acting antidepressant drug ketamine (Li et al., 2010; Duman and Aghajanian, 2012). In fact, ketamine antidepressant effect is BDNF-dependent (Lepack et al., 2015). The increase in BDNF in models of depression after ketamine administration could underlie the normalization of MMP-9 levels expected in our experiments.

The increased levels of MMP-9 activity and the cleaved form of nectin-3 in hippocampus and cortex in our corticosterone model of depression have been also reported in studies with mice exposed to chronic stress (Van der Kooij et al., 2014). These authors described that the increased cleveage of nectin-3 was dependent of the NMDA receptor function, as this effect could be counteracted by the incubation with the NMDA receptor antagonist MK-801 (Van der Kooij et al., 2014). As mentioned above, we expect a similar result using ketamine, reverting the MMP-9 and increased nectin-3 cleveage, as ketamine is an indirect NMDA receptor antagonist. On contrast, chronic venlafaxine in mice did not modify MMP-9 levels in the corticosterone model (Alaiyed et al., 2020), although an increase was reported in näive animals (Alaiyed et al., 2019). These authors correlate the increased MMP-9 levels following venlafaxime treatment with the formation of dendritic spines (Alaiyed et al., 2020).

Moreover, animal models in other neural pathologies have been associated the high MMP-9 levels with aberrant dendritic spines (Bilousova et al., 2009), and has been associated with the formation of new dentritic spines and dentritic branches to create synapses (Wang et al., 2008). The treatment with minocycline reverted the MMP-9 levels, promoting dendritic spine maturation (Dziembowska et al., 2013), although some authors report an stimulation of dendritic formation parallel to the increased MMP-9 promoted by chronic venlafaxine treatment (Alaiyed et al., 2020). Taking into account the decreased dendritic spine density in both chronic stress animal models of depression, we speculate that the changes in dendritic spines could be associated to the increased MMP-9 levels, and they are reverted by ketamine, as already reported (Li et al., 2010; Krzystyniak et al., 2019).

6. Conclusion

The studies mentioned before suggest that the antidepressant properties of ketamine may show discrepancies based on the model, dose, behavioral tests and depressive state. It is important to consider that both animal and human studies show that ketamine has different behavioral effects in depressed and healthy controls, indicating an important implication of the brain's depressive profile in response to ketamine. Different approaches have been used to study the interactions between the extracellular matrix, MMPs, and neurotransmission in depression. The present research suggests that ketamine is involved in the regulation of MMP-9 levels in depression, since ketamine's effects may be interacting with the NMDA receptors. Further research is needed to clarify the relevance and interaction between MMPs and putative fast-acting antidepressant drugs.

7. Acknowledgements

Throughout the writing of this TFM, I have received a great deal of support and assistance.

I would first like to thank my director, María Fuencisla Pilar Cuellar, whose expertise was invaluable in formulating this research structure, perspectives, and methodology, and to my co-director, Maria Elena Castro Fernández, whose support and insightful feedback, made it possible for me to successfully complete this work. I would also like to thank my master's director, Gabriel, for all his support since the beginning of this master and his dedication to all the students, as well as his counsel that helped resolve any doubts and complete the dissertation process.

I would like to acknowledge my colleagues from my internship at IBBTEC for their wonderful collaboration, and the University of Cantabria for the opportunity to work in a professional research environment. Finally, I would like to thank my friends for their support, discussions, and insights that lead me to look for new opportunities in the field of biomedicine.

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