Summary
Major Depressive Disorder (MDD) is considered by the World Health Organization one of the most burdensome diseases in the world, with a lifetime prevalence of approximately 16%. By 2020, it is estimated to be the second leading cause of disability, secondary only to ischemic heart diseases. Not only the economic impact is problematic, but also the high associated mortality rates (suicide). Of special concern are the 30-50% of the patients who do not respond to treatment with conventional antidepressants, impacting the quality of life and increasing the vulnerability to further depressive episodes. The high rates of ineffective treatment point out to unknown mechanisms that play a role in the development and progression of this debilitating psychiatric disease, warranting further research in order to improve the patient’s quality of life, with the ultimate goal of achieving remission.

For almost three decades, the neuroinflammatory hypothesis of depression has been explored and evidence has been found indicating that inflammatory processes and brain-immune interactions are involved in the pathogenesis of MDD. In chapter 2, we summarized the most recent data regarding the (neuro)inflammatory hypothesis of MDD (the main focus of this thesis). Moreover, we reviewed preclinical and clinical data available regarding anti-inflammatory treatments for MDD, in the form of monotherapy or augmentative strategies to conventional antidepressants. Furthermore, we discussed the anti-inflammatory properties of some antidepressants. Even though the results obtained so far are promising, the lack of proper study design makes it difficult to draw firm conclusions and to establish a solid foundation for future clinical guideline modifications.

Previous research has provided data that stress (either in physical or psychosocial form) is a major risk factor for the development of depression. Almost 25% of the patients exposed to highly stressful situations might develop MDD. In response to stressful situations, the hypothalamic-pituitary-adrenal (HPA) system releases glucocorticoids (i.e. cortisol in humans and corticosterone in animals) to regulate inflammatory responses as a consequence of stress system activation. However, prolonged stressful situations might induce neuroimmune, neuroendocrine and behavioural alterations, leading to MDD. In the proof-of-concept study designed in chapter 3, we investigated how repeated exposure to psychosocial stressful conditions in the form of the repeated social defeat (RSD) was able to induce neuroinflammation and alterations in brain metabolism (brain activity) in adolescent defeated rats. One of our main goals was to evaluate if those alterations could be visualized and quantified through positron emission tomography (PET), since this
technique allows in vivo visualization of tissue function and investigation of possible mechanisms underlying disease. For investigation of neuroinflammation, we used the $^{11}$C-PK11195 PET tracer. $^{11}$C-PK11195 has been widely used for imaging and quantification of translocator protein (TSPO) overexpression in the brain’s immune cells – mainly microglia and to a lesser extent, astrocytes (glial cells). Evaluation of brain activity was performed through the glucose analogue, $^{2}$-$[^{18}$F]$fluoro$-^{2}$-deoxyglucose ($^{18}$F-FDG). In addition, we investigated behavioural and physiological biomarkers in response to RSD, shortly after RSD (1 month) and 3 and 6 months afterwards. In summary, defeated rats showed transient depressive- and anxiety-like behaviour, increased corticosterone and brain pro-inflammatory cytokine IL-1β levels, as well as glial activation and brain hypometabolism in the first month after RSD. During the 3- and 6-month follow-up, no between-group differences in any investigated parameter were found. PET imaging demonstrated to be a useful tool for the detection of RSD-induced brain alterations, which included transient glial activation and reduced brain glucose metabolism in rats. These imaging findings were associated with stress-induced behavioural changes and provide support for the hypothesis that neuroinflammation could be a contributing factor in the development of depression.

Even though $^{11}$C-PK11195 is still widely used for TSPO PET imaging, second generation tracers have already been developed and proved to have superior imaging properties than $^{11}$C-PK11195, such as improved signal-to-noise ratio and higher affinity for TSPO. $^{11}$C-PBR28 is a second-generation tracer for TSPO imaging, which in the past has already been used in the clinics and without a full pharmacokinetic analysis in animal models of neuroinflammation. For that reason, in chapter 4, we evaluated $^{11}$C-PBR28 as a tool for detection and quantification of neuroinflammation in the animal model of herpes encephalitis (HSE) and compared the results with those obtained with $^{11}$C-PK11195 in the same animal model. Image-derived analysis such as volume-of-interest and voxel-based analysis demonstrated that $^{11}$C-PBR28 is capable of detecting more brain regions affected by HSE than $^{11}$C-PK11195, and the results were corroborated by the pharmacokinetic analysis (considered the gold standard of quantitative PET analysis). These results suggest that further preclinical studies would benefit from using $^{11}$C-PBR28 as TSPO tracer instead of $^{11}$C-PK11195, specially for mild-to-moderate animal models of neuroinflammation.

Early-life trauma and adversities in developmental stages of life are predisposing factors for developing psychiatric conditions, including MDD, at any point in life. For
that reason, we sought to investigate how a recurrence of RSD affects the neurobiological and behavioural profile of aged rats in chapter 5. Rats used in chapter 3 were allowed to age during 12 months under monitored conditions. At 14-months old, stress-naïve (SN; controls at adolescence) and stress-sensitized rats (SS; RSD-exposed rats at adolescence) were subjected to a 5-day RSD protocol, with neuroinflammation (i.e. glial activation) and brain activity being evaluated with the previously validated tracer $^{11}$C-PBR28 and $^{18}$F-FDG. Moreover, behavioural outputs, corticosterone and anti- and pro-inflammatory cytokine levels were measured at the end of the protocol. SN aged rats demonstrated a similar response as adolescent rats exposed to RSD – i.e. increased glial activation, decreased brain activity, elevated corticosterone levels and increased levels of both anti- and pro-inflammatory brain cytokines. Behaviourally, SN rats demonstrated anxiety-like behaviour. On the other hand, SS rats differed already at baseline measurements from SN rats. SS rats demonstrated increased $^{11}$C-PBR28 uptake at baseline in several brain regions (indicative of glial activation), suggesting that a prior exposure to stressful conditions exacerbates glial activation during ageing. Interestingly, after the recurrence of RSD, SS rats demonstrated a decrease of $^{11}$C-PBR28 uptake overtime, blunted corticosterone response followed by decreased levels of IL-1β and IL-10, as compared to SN rats. Behaviourally, SS rats showed both anxiety- and depressive-like behaviour. The neurobiological, endocrine and behavioural discrepancies observed between groups in this study cannot be explained with the current design. We hypothesize that SS rats might develop an adaptive and thus protective mechanism to cope with stress and decrease further brain damage; or these results points to a maladaptive response, demonstrating inability of SS rats to cope with stress overload. Nonetheless, other mechanisms might be involved in the alterations provoked by early-life adversities, such epigenetics, and further research is warranted to investigate the present results.

Repeated social defeat, also termed the resident-intruder paradigm, is a well-known psychosocial stress animal model capable of inducing depressive-like behaviour in defeated rats. In order to defeat a rat, a trained aggressive dominant rat is required. Thus, RSD animal model allows the investigation of both defeated and aggressive rats used in the paradigm. As the resident rats were subjected to repeated winning confrontations (in chapter 3 and 5), a unique opportunity was presented to investigate how these exposures could induce neurobiological alterations. Since we observed a reduced time to attack the intruder (i.e. attack latency; AL) as the residents won more confrontations, in chapter 6, we hypothesized that repetitive winning could have
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