The two sides of the coin of psychosocial stress: evaluation by positron emission tomography
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CHAPTER 1
GENERAL INTRODUCTION
1. Introduction

“Men ought to know that from the brain and from the brain only arise our pleasures, joys, laughter, and just as well our sorrows, pains, grieves and tears.” - Hippocrates

Transient sadness is a natural part of life. However, for some individuals, sad mood is present in a more intense and persistent manner. Many centuries after the Hippocratic view that emotions are derived from our brain only, this paradigm was broken and studies demonstrated that several systems and organs, including the brain, interact and communicate in the realm of emotions and mood disorders. Depression is one of the leading causes of disability worldwide, predicted to cause the biggest economic burden to society by 2030 (1). Despite major research efforts, the complete pathophysiology of depression remains unknown and it is plausible that multiple subtypes exist with different pathophysiological mechanisms (2).

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM), depression consists of several symptoms related to low mood. The DSM-V revision (3) recognizes sad mood and loss of pleasure (anhedonia) as core symptoms of depression. Next to the core symptoms, somatic correlates of a negative mood state are included as symptoms. Somatic symptoms comprise aberrant levels of energy and sleep, as well as mood, weight and food intake fluctuations. Cognitive correlates of a negative mood are also recognized and include subjective impairments of concentration and decision-making, as well as thought patterns of guilt, worthlessness and suicidal ideation.

A number of large population studies generated statistics regarding the prevalence, onset and history of the disease. In the United States of America (USA), the National Comorbidity Survey gave lifetime prevalence estimates of 15-20% and one-month prevalence estimates of 5% for adults (4). In the aged population, depression is the most commonly diagnosed psychiatric condition (5). This is very alarming considering that roughly 20% of the worldwide population will be over 65 years old by the year of 2030 (6). The median age at the onset of major depressive disorder (MDD) is around 20-25 years old (7). Estimates of occurrence range from 50-90%, with increasing odds of recurrence and chronicity with each new episode (8). These findings substantiate that MDD has a very large economic impact to society, mainly due to healthcare costs and loss of productivity (1). Not only the high incidence, disability, mortality (suicide) and the economic burden associated with the disease, but also the high rates of inadequate
treatment of the disorder are a serious concern. Main antidepressant therapies used in the clinical practice modulate monoaminergic neurotransmitter function. This includes the antidepressant classes of selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), tricyclic/tetracyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs) and atypical antidepressants (9). It is estimated that 30-50% of the patients do not respond to treatment with antidepressants (10) due to either lack of efficacy or intolerable side effects (11). Moreover, psychotherapeutic interventions have reported disappointing results (12). The complex and heterogeneous nature of MDD may contribute to these modest results and suggests that other neuromolecular pathways than an imbalance in monoamines targeted by conventional antidepressants are involved. Treatment that works for one specific individual might not work for another, resulting in an attenuated treatment effect. Therefore, unaccounted heterogeneity in symptoms of depression may arrest our knowledge about the aetiology and effective treatments for MDD.

Figure 1: Psychosocial and physical stressors combined with a possible pre-existing predisposition to major depressive disorder (MDD) might induce a first depressive episode. Biochemical processes such as (neuro)inflammation, HPA axis dysfunction, imbalance in neurotransmitter systems, disturbances in neurotrophic factors and astrocyte excitotoxicity interact and may induce cellular damage and apoptosis and inhibit neuronal growth and survival. All these factors might influence treatment response to conventional treatment with antidepressants, increasing the vulnerability of the depressed individuals to further depressive episodes. Moreover, each episode can contribute to cognitive decline and alterations in brain structures. (Adapted from Moylan et al, 2013 (14)).
1.1 Depression as a multifactorial mental disorder

Besides genetic predisposition (35-40% heritable) (13) and dynamic environmental influences, numerous neurobiological mechanisms have been proposed to contribute to the pathogenesis of MDD. Possible pathways include neurotransmitter systems, neurotrophins (14), astrocyte excitotoxicity (2), (neuro)inflammation and HPA axis dysfunction (15) (Figure 1).

1.1.1 Neurotransmitter dysfunction (serotonergic, dopaminergic and noradrenergic systems)

Serotonin (5-HT) is the neurotransmitter most extensively associated with mood disorders such as MDD. 5-HT is produced from tryptophan, an essential amino acid catabolized by indoleamine-2,3-deoxygenase (IDO). Pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6 and tumour necrosis factor-α (TNF-α) present during an inflammatory state can activate IDO, leading to 5-HT depletion and synthesis of tryptophan catabolites, including kynurenine (KYN) and quinolinic acid (QUIN) in the plasma and in the brain (16). Many antidepressants aim to counteract this effect by increasing synaptic 5-HT levels, for example by inhibiting the neuronal reuptake of the neurotransmitter.

Noradrenergic alterations have been strongly associated with MDD and many antidepressants elevate the synaptic availability of norepinephrine by inhibition of reuptake and/or blockade of presynaptic α-2 adrenoceptors (17). Indeed, post-mortem and functional imaging studies revealed altered density and sensitivity of α-2 adrenoceptors (which modulate noradrenaline release) in the prefrontal cortex of depressed suicidal victims (17; 18).

Diminished levels of dopamine (DA) are also related to MDD. The suggested physiological alterations underlying reduced DA signalling could result from diminished DA release from the presynaptic neurons or impaired signal transduction, either due to changes in receptor number or function and/or altered intracellular signal processing (19). DA is a neurotransmitter involved in motivation, which many MDD patients lack. Interestingly, the increase in 5-HT levels following SSRIs administration might reduce DA neuronal activity. Thus, alterations in dopamine function may partially underpin the resistance towards antidepressants (19). Also, a decreased turnover of homovanillic acid which is the primary metabolite of dopamine has been found in individuals with MDD (20), a finding consistent with depressogenic effects of dopamine depletion in MDD patients (21).
1.1.2 Neurotrophins

Neurotrophins are key mediators of normal neurogenesis and numerous findings support a role of neurotrophins and neurogenesis in MDD. MDD is associated with reduced levels of neurotrophins such as the brain-derived neurotrophic factor (BDNF) (22) and vascular endothelial growth factor (23). Administration of these factors have demonstrated antidepressant effects in animal models and traditional antidepressants were shown to normalize neurotrophin levels in responding patients (24; 25).

1.1.3 Astrocyte excitotoxicity

Astrocytes play a role in synaptic transmission and information processing (26). They express glutamate receptors and therefore, respond to glutamate that spills over from synapses. In turn, this spill over triggers the release of glutamate by glial cells that can modulate neuronal activity, synaptic transmission, plasticity, and also induce excitotoxicity and contribute to neuronal damage and/or dysfunction (27). Glutamate is a major excitatory neurotransmitter in the human brain and its reuptake is critical for regulating concentrations in the synaptic cleft and maintaining normal synaptic activity. Impairment in glutamate transport may thus result in excessive or dysregulated glutamate receptor signalling (28). Under physiological conditions, astrocytes prevent excitotoxicity by maintaining extracellular glutamate levels in the micromolar range via high-affinity glutamate transporters: excitatory amino acid transporter (EAAT) 1 and 2. However, this balance may be disrupted by oxidative stress or (neuro)inflammation leading to necrosis and/or apoptosis through excessive stimulation of glutamate receptors (28).

Furthermore, glutamate transmission via N-methyl-D-aspartate receptors (NMDAR) is crucial for neuronal survival and synaptic plasticity (29). The extrasynaptic NMDAR has been an increasing focus of attention regarding the deleterious effects of glutamate. Extrasynaptic neurotransmission inhibits extracellular signal-regulated kinases. Moreover, extrasynaptic NMDAR activation enhances nitric-oxide production, which is mainly responsible for synaptic damage (29; 30). Ketamine, a NMDAR antagonist has been implicated as a rapid antidepressant, possibly exerting its mechanism of action through the inhibition of extrasynaptic NMDAR 2C and 2D subunits (29).
1.1.4 (Neuro)inflammation and HPA axis dysfunction

Over the last two decades psychiatric research has provided support for the hypothesis that inflammatory processes and brain-immune interactions are involved in the pathogenesis of MDD and may contribute to the serotonergic and noradrenergic dysfunction (31). Inflammation, infection, cell damage or stress might trigger glial cells, in particular microglia cells, to release pro-inflammatory cytokines that may affect the hypothalamic-pituitary-adrenal (HPA) axis and serotonergic and noradrenergic signalling, ultimately leading to MDD and neurodegeneration (15; 32).

The immune and neuroendocrine systems function together in order to restore and maintain physiological homeostasis during inflammation or other harmful stimuli which induce cytokine production. Increased cytokine production may contribute to the development of depression directly via activation of the HPA axis or indirectly through cytokine-induced glucocorticoid (GC) receptor resistance (33). The release of TNF-α and IL-6 increases the production of corticotrophin-releasing hormone, adrenocorticotropic hormone and cortisol by acting directly on hypothalamic and pituitary cells (5). Cytokines might also increase GC receptor resistance through several signalling pathways, including activation of the p38 mitogen-activated protein kinase (MAPK) and by stimulating changes in the expression of GC receptors (33; 34). HPA hyperactivity has been associated with the pathophysiology underlying suicidal behaviour, excessive activity of the noradrenergic system and dysfunction of the serotonergic system (35; 36).

As previously mentioned, pro-inflammatory cytokines increase the activity of IDO and reduce the production of 5-HT through the kynurenine pathway (16; 37; 38), producing KYN and QUIN (39; 40). QUIN exerts agonistic effects on NMDAR leading to excitotoxicity, inhibits glutamate uptake and may cause degeneration of nerve cells and hippocampal cell death. Furthermore, pro-inflammatory cytokines influence neurotransmitter function through disruption of tetrahydrobiopterin (BH4). BH4 is an essential co-factor for the enzymes phenylalanine hydroxylase, tryptophan hydroxylase and tyrosine hydroxylase, which are rate-limiting enzymes for the synthesis of 5-HT, DA and norepinephrine, respectively (41). BH4 is also a co-factor for the enzyme nitric oxide synthase (NOS) that is responsible for the conversion of arginine to nitric oxide (NO) (42). Pro-inflammatory cytokines stimulate the production of NO, increasing the utilization of BH4 and thus decreasing neurotransmitter synthesis (41).

Since the present thesis will mainly focus on the (neuro)inflammatory hypothesis of depression, this topic will be further discussed in Chapter 2.
1.2 Stress as a risk factor for MDD development

Stress has been shown to be a major risk factor for developing depression and further sensitization to stress may occur as the disorder progresses (43–45). Further investigation of biological pathways related to stress in the depressed population might help to understand the stress-related etiology of depression (46). The HPA axis is functionally linked to the immune system as GCs (e.g. cortisol) regulate inflammatory responses and increased inflammation is a consequence of stress system activation (47). Exposure to early life stress, for example, is a distal risk factor that is considered a predictor of MDD with an onset in adolescence or early adulthood. Major life events such as loss of a loved one, job loss and divorce often precede depressive episodes later in life (48). This concerns up to 80% of the episodes observed in the general population (49). Virtually anyone will experience major negative life events during life, yet only 20-25% of the population develop depression afterwards (50).

1.3 Vulnerability and resilience to depression

Prolonged stress induces neuroimmune and neuroendocrine responses, and individual differences in these responses likely shape behavioural vulnerability and resilience. In some individuals, overactive unresolved stress responses may increase stress vulnerability and ultimately the development of mood disorders, such as MDD. However, most individuals mount adaptive coping mechanisms (i.e. response in reaction to a stressor) that promotes resilience when facing stress (51). These coping strategies involve reactive or passive strategies. A passive coping involves low aggressiveness, impulsivity and flexibility, with a general tendency to passively accept or introvertly shy away from similar stressful situations. In turn, a reactive coping style is generally characterized by a high level of aggression, impulsivity and other bold/extrovert actions, indicating active attempts to counteract a stressful stimulus (52). These different coping styles have also been associated with distinct patterns of the neuroendocrine (re)activity patterns (53).

The HPA axis activation in response to stress increases circulating GCs by promoting their synthesis and release from the adrenal cortex, resulting in widespread physiological, hormonal and neurobiological effects. This circuit may be altered in the chronically stressed brain (51). GCs binds to steroid receptors expressed ubiquitously throughout the brain, altering gene expression and affecting synaptic plasticity, structural remodelling, and ultimately behavioural responses to stress and adaptive coping mechanisms of resilience (54). Moreover, GCs may produce a persistent sensitization of
microglia – maintaining a pro-inflammatory state despite resolution of the inflammatory state - that primes neuroimmune responses to subsequent events (51; 55).

1.4 Animal models of stress-induced depressive-like behaviour

Even though considerable progress has been made in non-invasive human studies of brain structure and function, such studies are still limited in their ability to investigate a causal role in the physiology and molecular biology of the depressed brain (56; 57). This has resulted in a demand for animal models of depression for hypothesis testing and to further understand underlying mechanisms in MDD. However, the choice of which biological correlates to study is not easy, since problems with animal models of human psychiatric disorders include: 1) the difference between the human and animal nervous system; 2) the difficulty in determining analogous behaviours among species (Table 1); and 3) the need of extrapolation of results from animals to humans (58). Such problems most likely reflect a significant difference in aetiology and complexity of depressive behaviour.

Table 1: Comparison of core symptoms of depression in humans with the possible analogous parameters assessable in rodents (59).

<table>
<thead>
<tr>
<th>Core symptoms in humans</th>
<th>Analogous parameters in rodents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of enjoyment</td>
<td>Anhedonia</td>
</tr>
<tr>
<td>Loss of motivation</td>
<td>Passive coping strategies; low locomotor activity</td>
</tr>
<tr>
<td>Sleep disturbances</td>
<td>Altered sleep/activity patterns</td>
</tr>
<tr>
<td>Anxiety</td>
<td>Anxiety-related behaviour</td>
</tr>
<tr>
<td>Hypercortisolism</td>
<td>Hyperactivity of the stress system</td>
</tr>
</tbody>
</table>

Ideally, an animal model should fulfil at least three major criteria: i.e. having face validity, construct validity, and predictive validity, supplemented by a criterion for aetiological validity (60; 61). To summarize these criteria: animal models must resemble the human condition in several aspects, including 1) similarity between the behavioural phenotype and the clinical-symptom profile (face validity); 2) amelioration or attenuation by clinical effective antidepressant treatments and, conversely, absence of changes by clinically ineffective treatment of the human disorder (predictive validity); 3) triggering by events that are known to be important for eliciting the human disorder (aetiological validity); and 4) similar neurobiological underpinnings (construct validity) (61).

An important aspect to consider when selecting an animal model for depression, is that depression is a symptomatic heterogeneous disease. Thus, individual animal models would be expected to simulate endophenotypes or a subset of symptoms, which
are likely defined by the conditions applied. It is essential to use realistic induction conditions (aetiological validity) and to ensure the legitimacy of the underlying pathology (61). In that sense, many depressive-like behaviour animal models have been developed and applied, either based on natural or artificial animal behaviours (58).

In general, stress models with a good aetiological, face and constructive validity have been described (61). From a biological point of view, the social environment should be considered as a source of stress and the processes of fighting for control and losing control are of central importance to the psychosocial situation of the individual. In humans, loss of rank, social status, and/or control are examples of a more general class of loss events, which are increasingly recognized as the specific type of “life events” that are associated with depression (61). Moreover, the animal models should have heuristic value since they investigate the environmental challenges that an animal may meet in its everyday life (i.e. “natural model”). In social settings, this might mean loss of control by social defeat. As described by Koolhaas et al., social defeat is a very special kind of stressor and distinguishes itself from other stress paradigms with respect to the magnitude and the quality of the stress response (62). Moreover, a very interesting feature of this model is that, besides allowing the investigation of physiological, neurobiological and behavioural alterations caused by stress in defeated animals, we can also study these alterations in the winning (i.e. dominant) rat, in particular the brain effects of repetitive victorious confrontations.

Besides the social defeat model, other stress-induced animal models of depression (not used in the present thesis) have been developed. An interesting animal model of stress is the chronic mild stress (CMS) model. It focuses on a core symptom of depression, named anhedonia (in humans) or anhedonia-like behaviour (in animals). The CMS paradigm involves the exposure of animals to a series of mild stressors in an unpredictable manner (isolation or crowded housing, food or water deprivation, disruption of the dark-light cycle, tilting of home cages, dampened bedding, etc.) over a period of several weeks or even months (61). The learned helplessness model is also frequently used, since feelings of helplessness are core symptoms of MDD. It displays good face, construct and predictive validity, however lacking the aetiological validity. The classic design is composed of three groups, one that receives unpredictable, uncontrollable and unavoidable electrical shocks, one that receives controllable electrical shocks and the control group which is not exposed to stress (for a recent review, see (63)). Early-life stress models are based on the observation that negative life events during critical periods
of development may increase the vulnerability for psychiatric conditions later in life, mainly MDD. Maternal separation, for example, is an experimental procedure that is widely used in this context. Previous studies demonstrated that a single or repeated separation of pups from their mother leads to acute or long-term effects on physiology and behaviour. Schmidt et al. recently published a comprehensive review on early-life stress animal models in rodents, questioning the validity of early-life stress paradigms, such as maternal separation, as robust models of depression (59).

2. Nuclear medicine

Nuclear medicine comprises a range of imaging techniques that provide detailed information about a wide range of biological processes at the molecular and cellular level. As opposed to medical imaging techniques, such as computed tomography (CT), X-ray and magnetic resonance imaging (MRI), which provide anatomical images (64), nuclear medicine allows for the in vivo visualization and analysis of the underlying pathology and tissue function (65). In the attempt to complete our knowledge of the pathophysiological mechanisms underlying MDD, nuclear medicine could be a unique tool to be applied in such investigation.

In order to diagnose or characterize disease states with this molecular imaging technology, it is necessary to intravenously administer tracer amounts of a radiolabelled compound (radiotracer, radiopharmaceutical or simply “tracer”) with high specificity and affinity for the target of interest. After administration, the radiotracer is distributed throughout the body (65). The distribution is mainly determined by the characteristics of the compound, such as affinity for the desired target (binding potential of a drug) (66), and also by the physiology of the tissue under investigation. The distribution of the radiotracer can be measured with a dedicated camera. A great advantage of nuclear medicine is the availability of radiotracers to image molecular targets involved in specific physiological and pathological processes. Two different nuclear medicine modalities are available: single photon emission computed tomography (SPECT) and positron emission tomography (PET). This thesis focuses on the use of PET for brain imaging.

2.1 Positron emission tomography (PET)

PET is an imaging technology that measures the distribution and concentration of tracers, labelled with positron (i.e. $\beta^+$ particle) emitting radioisotopes (e.g. $^{18}$F, $^{11}$C, $^{15}$O and $^{13}$N). The emitted positron collides with an electron from the surrounding matter in a process
called annihilation, which results in the generation of two gamma (γ) rays of equal energy (511 keV) and traveling in opposite direction (180°). The PET camera detects the two opposing γ rays through coincidence detection, generating a line of response on which the original decay had occurred (Figure 2).

![Positron emission and positron-electron annihilation](image)

**Figure 2**: Positrons are emitted by radioisotopes rich in protons. Once emitted, the positron travels a short distance before annihilating with an electron from the surrounding matter. When annihilating, the mass of positron and electron is converted into two gamma rays with an energy of 511 keV each, traveling at an angle of 180°. The gamma rays are detected by coincidence detectors in a PET scanner system (for humans or small animals).

Through the combination of measurement of many coincidences, the system can reconstruct the 3D distribution of the radiotracer as function of time. After the collected data is subjected to physical corrections for dead-time, attenuation, randoms and scatter, the radioactivity concentration in the region of interest can be accurately measured, and the biological processes under investigation can be analysed in a quantitative manner. When combined with anatomical information from another imaging technique, the functional information of PET can be accurately localized and related to specific structures.

### 2.1.1 PET data quantification methods

Data obtained from PET studies can be evaluated in several different ways. In clinical practice, visual inspection of PET images is the main method for image interpretation (67). Usually, this is performed when a static image is obtained after a certain period of radiotracer uptake in the tissue, considering the observed PET signal in the tissue corresponds to the underlying process of interest. However, a more quantitative approach might be required in cases when the static PET signal does not properly correspond to the
state of interest or when disease progression, treatment response or subtle physiological states are evaluated.

A method to obtain semi-quantitative PET data, frequently used in clinical practice and research, is calculation of the Standardized Uptake Value (SUV). Its main advantage is the simplicity of application, since it requires only the tissue radioactive concentration at a carefully pre-defined time. The radioactivity concentration in tissue is subsequently corrected for the injected dose and some anthropometric characteristic of the subject (generally the body weight or the body surface area), according to Equation 1.

\[
SUV = \frac{\text{Measured Activity Concentration} \ [kBq/mL]}{\text{Injected dose} \ [MBq]/\alpha}
\]

Where \(\alpha = \begin{cases} 
\text{Body Weight (kg)} \\
\text{Body surface area (m}^2) \\
\ldots
\end{cases}
\]

**Equation 1:** Equation to calculate the SUV from a PET measurement. The measured activity concentration is derived from the PET data, and the injected dose is the amount of radioactivity administered to the patient.

The calculation of the SUV does not require invasive procedures such as arterial blood sampling, which improves patient comfort and enables longitudinal preclinical studies. However, its validity is affected by a number of technical and physiological factors. In fact, the SUV is dependent on, for example, the clearance of the tracer from circulation, metabolism and changes in perfusion and blood flow (67). Due to the aforementioned reasons, SUV is often referred to as a semi-quantitative metric.

In research or when the main purpose of a PET study is to obtain values of parameters that characterize a physiological, biochemical or pharmacokinetic process, a fully quantitative approach is required. In other words, a radiotracer kinetic model, describing the radiotracer under study, is required to translate the measurements of radioactivity into quantitative values of the biological parameter of interest (68). In order to perform the analysis, compartment models are generally used (69). These compartments are not necessarily distinct anatomical compartments, but a convenient way to describe different kinetic “states” of the radiotracer (68). This is especially useful for the analysis of PET data, since the total radioactivity concentration measured from each image voxel is a sum of radiotracer concentrations in different tissues (e.g. brain...
parenchyma, vasculature) and physiological states. In this context, different compartments can describe the different specific states in which the radiotracer can be found, such as unbound in plasma, unbound in brain tissue, metabolized, or bound to a specific receptor (70). Moreover, since compartmental modelling provides the most exhaustive description of radiotracer kinetics, it is usually considered the gold standard for PET data analysis (71). The parameters of compartmental models describe changes in radioactive concentrations by first-order differential equations. Exchanges between compartments are described by mass/balance equations, defined by tracer concentrations and kinetic rates constants ($K_1$, $k_2$, $k_3$, etc). Macro-parameters of interest such as metabolic and enzymatic rates, receptor concentrations and others can be obtained from these micro-parameters.

In order to perform the kinetic modelling of the data from the PET study, two datasets are necessary: 1) the time-activity (TAC) curve of the tissue of interest (provided by the PET scanner); 2) the radiotracer concentration in the circulating plasma as function of time (input function), measured in the arterial blood collected at different time points. The radioactivity concentration in blood is the sum of the radioactivity in plasma and the radioactivity associated with the red blood cells. The TAC in tissue corresponds to the radioactivity concentration measured in a specific volume of interest which is the sum of the radioactive concentration in the extra- and intra-cellular compartments, as well as the concentration in the blood pool. In some cases, not all radiotracer in the plasma or tissue is in its original form (e.g. if it is metabolized). Furthermore, not all the radiotracer in the tissue may be involved in the specific process of interest for the study (72). A correct approach to the PET data quantification should account for all these contributions. Correction for radioactive metabolites in plasma is usually performed by assessment of the percentage of intact tracer in the plasma samples.

Different kind of radiotracers are available for brain PET imaging: those that only enter and exit the brain without binding to a target; those that bind to a receptor or a transporter; and those that are metabolized by enzymatic action. Data from such radiotracers need to be analysed with a kinetic model that best suits their kinetic properties.

For radiotracers that only enter and exit the brain without binding, the 1-tissue compartmental model (1TCM) can be used. In this case, there are two compartments, the plasma compartment and the tissue compartment. This model calculates the rate constants $K_1$ and $k_2$, which describe the rate of the radiotracer from plasma to tissue, and the rate of
the radiotracer from tissue to plasma, respectively. $K_1/k_2$ (73) describes the ratio of the radiotracer in the tissue to the arterial plasma concentration at equilibrium and is defined as the distribution volume ($V_T$).

The 2-tissue compartmental model (2TCM) assumes that the radiotracer goes from arterial plasma to the central non-displaceable compartment, where part of the radiotracer molecules binds to the tissue non-specifically and reaches the equilibrium rapidly and some molecules retain in the tissue fluid as free ligand (Figure 3). The rest of the ligand is transferred from the non-displaceable compartment to the specific compartment, in which it is specifically bound to the target molecule (e.g. a specific receptor). In this case, four parameters, $K_1$, $k_2$, $k_3$ and $k_4$, are calculated. $K_1$ and $k_2$ indicate the rate of the radiotracer from plasma to the non-displaceable compartment, and the rate of the radiotracer from the non-displaceable compartment to plasma, respectively. $k_3$ reflects the rate of the radiotracer from the non-displaceable compartment to the specific compartment (e.g. binding to a receptor) and $k_4$ describes the rate of the radiotracer from the specific compartment to the non-displaceable compartment (e.g. release from a receptor). The $V_T$ can be obtained using the equation $K_1/k_2*(1+k_3/k_4)$. Another important macro-parameter derived from the 2TCM is the non-displaceable binding potential $BP_{ND}$, which is the ratio of $k_3$ and $k_4$. $BP_{ND}$ indicates how well the radiotracer binds specifically to the target.

In case radiotracer molecules are trapped in the specific compartment, the $k_4$ is equal to or approaches zero. $2'\cdot[18F]$fluoro-2'-deoxyglucose ($^{18F}$FDG), a glucose analogue, is a typical example of an irreversibly bound tracer. The most important pharmacokinetic parameter that can be obtained from an irreversible 2TCM fit is the metabolic rate $Ki$, calculated as $Ki = K_1*k_3/(k_2+k_3)$ (74).

**Figure 3:** Representation of the 2-tissue compartment model (2TCM). The model assumes that the radiotracer goes from arterial plasma ($C_P$) to non-displaceable compartment ($C_{ND}$), where part of the radiotracer molecules binds to the tissue non-specifically (NS) and reach the equilibrium rapidly and some molecules retain in the tissue fluid as free ligand (F). From the non-displaceable compartment, the tracer can go to the specific compartment ($C_S$), in which the radiotracer is specifically bound to the target molecule. $K_1$, $k_2$, $k_3$ and $k_4$ represent the exchange rates between compartments.
A disadvantage of performing the full kinetic modelling of the radiotracer in study is that it often requires long image acquisition protocols for accurate pharmacokinetic modelling. Also, data analysis is laborious and time-consuming. Furthermore, obtaining the input function is an invasive method and uncomfortable for patients. In preclinical studies, due to the required amount of arterial blood extracted for constructing an input function, it is often a terminal procedure which precludes longitudinal designs. For that reason, alternatives for the measurement of an arterial input function, including image-derived input functions (75), population-based input functions (76) and the use of reference regions (77), have been developed.

\[ \begin{align*}
C_p & \quad \text{Target region} \\
C_{ND} & \quad \text{Nondisplaceable (F+NS)} \\
C_S & \quad \text{Specific bound (S)} \\
\end{align*} \]

**Figure 4:** Schematic representation of a reference tissue compartmental model. The method is based on the use of a region devoid of specific binding (reference region, \( C_{ND} \)), from which it is possible to infer the receptor binding in the region of interest (target region, \( C_S \)). The target and reference region exchange radiotracer with the plasma at a similar rate.

In PET, the use of reference regions is the most popular approach and it is based on the use of a region non-existent specific binding, from which it is possible to infer the receptor binding in the remaining regions of the brain. The method assumes that the non-displaceable distribution volume is the same for both target and reference region and that the \( K_1 \) and \( k_2 \) in the reference region are equal to those in the target region. Under these assumptions, reference-based models relate the radiotracer kinetics in target region to those in the reference region (Figure 4). The use of this approach is, however, limited. Many receptors are not restricted to particular anatomical regions and therefore no reference region devoid of these receptors can be defined.

### 3. Thesis aim and outline

As already discussed, depression is multifactorial disease, with high incidences in the general population and high associated disability, mortality and economic burden to
society. Until the present moment, the pathophysiology of depression has not been fully elucidated. As depression hampers the quality of life of nearly half of the patients due to ineffective treatment, it is of utmost importance to elucidate pathophysiological mechanisms underpinning the disorder. Psychosocial and physical stressors capable of inducing (neuro)inflammation seem to be a possible causal role for MDD, in particular in the subgroup of treatment-resistant depressive patients.

In the past, brain alterations related to the disorder could only be obtained post mortem, sadly many times in depressed suicide victims. In this context, PET is an attractive tool for non-invasive in vivo brain imaging that allows to investigate possible alterations in the brain of living depressed patients. With this in mind, this thesis aimed to address the neuroinflammatory hypothesis of depression, using psychosocial stress as a predisposing factor for mood disorders such as depression and aggression, evaluated through PET imaging.

In chapter 2, we review the current knowledge on the (neuro)inflammatory hypothesis of depression. Based on literature findings of preclinical and clinical studies of depressive patients with an elevated inflammatory profile and unresponsiveness to conventional antidepressant therapy, we discuss the usage of non-steroidal anti-inflammatory agents for MDD, as well as the anti-inflammatory properties of some antidepressants.

Since stress seems to be a strong predictor of depression, we used a psychosocial stress rodent model with high ethological validity, namely repeated social defeat (RSD). In chapter 3, we aimed to evaluate if RSD was capable of inducing depressive-like behaviour and if these behavioural changes were associated with glial activation and alterations in brain metabolism using $^{11}$C-PK11195 PET and $^{18}$F-FDG PET, respectively. Furthermore, the persistence of the evaluated parameters was evaluated up to 6 months after the exposure to RSD.

In the pursuit of a more suitable and sensitive radiotracer for detection of glial activation, in chapter 4 the TSPO ligand $^{11}$C-PBR28 was validated and compared to $^{11}$C-PK11195 in the neuroinflammatory rodent model of herpes encephalitis (HSE). A full pharmacokinetic modelling was applied, as well as voxel and volume of interest (VOI) analysis for comparison of both radiotracers.
Major stressful life events at young age or adolescence seems to play a crucial role in predisposing individuals for psychiatric disorders, such as MDD, at any point in their life. In chapter 5, we tested how a previous exposure to RSD in adolescence affects the neuroendocrine, (neuro)inflammatory, behavioural and brain metabolic response to a second RSD exposure in aged rats of 14 months. The previously validated $^{11}$C-PBR28 radiotracer was used to evaluate glial activation and $^{18}$F-FDG PET was used for assessment of brain metabolism alterations.

In the RSD paradigm, a resident (dominant) male rat is used to attack and defeat intruder (submissive) rats. Whereas the submissive rats develop depressive-like behaviour, in the resident rats an escalation in the levels of aggressiveness upon exposure to repeated victorious confrontations was observed. In chapter 6, we therefore investigated if the dopaminergic D2 receptors, largely associated with the reward system, are altered in the striatal area of the brain and whether changes in the availability of these receptors are related to the behavioural alterations.

References


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