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Introduction and outline of thesis

INTRODUCTION

MOOD DISORDERS

Mood disorders are common and recurrent psychiatric disorders, affecting the lives of many individuals worldwide (1-3). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) classification system (4), 'mood disorder' is a term for a group of diagnoses, in which disturbance of mood is the primary symptom. Mood may be abnormally low, as in a depressive episode in 'major depressive disorder (MDD)', or be alternated with an abnormally elevated mood, known as (hypo)mania, within the course of 'bipolar disorder'. In this thesis, emphasis will be on MDD. Although numerous studies have sought to identify the brain mechanisms associated with mood disorders, their exact pathogenesis is still unclear. Neuroreceptor imaging provides a unique tool to depict mood disorder related systems in the brain *in vivo*.

Major depressive disorder

MDD or unipolar depression is a severe and prevalent psychiatric illness, with a lifetime prevalence ranging from 6 to 12%. In The Netherlands, 19% of the population under 65 years has experienced at least one depressive episode (5). MDD is clinically diagnosed according to the criteria in the DSM-IV for a 'major depressive episode', and characterized by at least five of the symptoms listed beneath (Table 1) (4). Depressed patients may show additional symptoms of anxiety or psychosis. Severity of an episode is judged as mild, moderate or severe, based on the degree of impairment in daily occupational and social functioning. The course may either be limited to a singular episode, or follow a recurrent pattern.

NEUROBIOLOGY OF MOOD DISORDER RELATED SYSTEMS

A wide range of pathophysiological mechanisms, including cognitive, motor, autonomic, endocrine and circadian abnormalities are implicated in the clinical phenomenology of mood disorders (6). In MDD, brain systems that are involved regulate mood and anxiety, but also reward processing, attention, motivation, stress responses, social cognition, and neurovegetative functions such as sleep-wake abnormalities, appetite, energy and libido (7).

Models of depression

Contemporary neurobiological models of depression posit dysregulation of limbic and cortical regions involved in emotional regulation and experience. These include subregions of the dorsal prefrontal cortical network, regulating emotion, and a ventral network involved in emotional experience, including hippocampus, amygdala, ventral anterior cingulate cortex, orbitofrontal cortex and basal ganglia. Within the basal ganglia, the ventral striatum is implicated in reward and motivation, whereas the dorsal striatum is involved in motor and cognitive control (8).

Alterations of monoaminergic neurotransmitter systems have provided the rationale for currently available pharmacological treatments in MDD, by upregulating levels of serotonin and norepinephrine throughout the network of limbic, striatal and prefrontal cortices. Recently, attention has been directed towards dopamine (DA) (9). Monoamine reuptake inhibitors and other modulators of monoaminergic function may reduce symptoms in about 50% of depressed patients and result in remission in 30-40% of patients (10). Therefore, the monoaminergic theory alone cannot explain MDD. Other relevant mechanisms likely include dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis together with the major inhibitory and excitatory neurotransmitters gamma-aminobutyric acid (GABA) and glutamate.

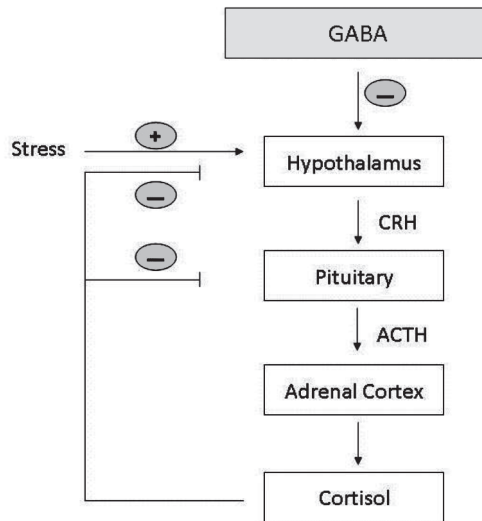
Table 1: DSM-IV criteria of Major Depressive Episode

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- A. Five (or more) of the following symptoms, including at least one of the first two, which have been present during the same 2-week period and represent a change from previous functioning.
- (1) Depressed mood most of the day, nearly every day, as indicated by either subjective report or observation made by others.
 - (2) Loss of interest or pleasure (anhedonia).
 - (3) Significant weight loss when not dieting, or weight gain.
 - (4) Insomnia or hypersomnia.
 - (5) Psychomotor agitation or retardation.
 - (6) Fatigue or loss of energy.
 - (7) Feelings of worthlessness or excessive or inappropriate guilt.
 - (8) Diminished ability to think or concentrate, or indecisiveness.
 - (9) Recurrent thoughts of death.
- B. The symptoms do not meet criteria for a Mixed Episode.
- C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
- D. The symptoms are not due to the direct physiological effects of a substance or a general medical condition.
- E. The symptoms are not better accounted for by bereavement.
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HPA axis

Considerable research has focused upon a central role for a dysregulated HPA axis in MDD. The HPA axis is a major neuroendocrine system in the body, controlling reactions to stress and regulating many body processes. In response to stressful stimuli, corticotrophin releasing hormone (CRH) is released from the paraventricular nucleus (PVN) of the hypothalamus, which in turn acts on the pituitary gland, stimulating the release of adrenocorticotrophic hormone (ACTH) into the circulation. Glucocorticoids are secreted from the adrenal gland under ACTH control and mediate negative feedback to the pituitary, hypothalamus, and higher-order brain centres, ensuring equilibrium of the system (11,12) (Figure 1).

Figure 1



HPA axis and influence of GABA

Secretion of cortisol, the main glucocorticoid in humans, follows a circadian cycle, with peak levels at awakening and a declining pattern thereafter (13). The cortisol awakening response (CAR) is the cortisol secretory activity in the first 45-60 minutes after awakening (14,15), which happens under the influence of the hypothalamic suprachiasmatic nucleus (SCN) (16).

The most consistent finding in MDD is impaired regulation of the HPA-axis during an acute episode, possibly due to impaired corticosteroid receptor functioning, and its normalization after successful treatment. Stress induced hypercortisolemia may lead to central downregulation of glucocorticoid receptors, impairing cortisol's negative feedback and enhancing levels of CRH and ACTH (17). Consequently, CRH producing neurons are found to be hyperactive in idiopathic depression (18-21). Functional alterations in the HPA system are challenged by the combined dexamethasone/CRH test, which is a sensitive measure for the negative feedback function of the HPA system. In MDD, evening dexamethasone administration, followed by next day's stimulation with CRH, results in inadequate cortisol and ACTH suppression (22).

An increase in the level of circulating glucocorticoids may exert neurotoxic effects on cerebral structures. In animal studies, volumetric changes in the limbic and prefrontal cortex have been associated with an abnormal glucocorticoid response, as these structures are rich in mineralocorticoid (MR) and glucocorticoid (GR) receptors and are sensitive to neurotoxic actions (6,23). In human studies, in the presence of elevated cortisol and/or recurrent depression (24), magnetic resonance imaging (MRI) studies have shown a reduction in the volume of the hippocampus (25).

GABA

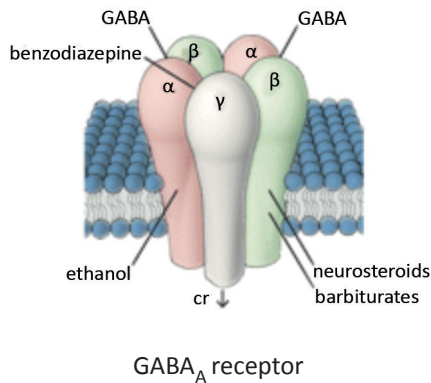
In depressive disorder, GABA levels in blood, cerebrospinal blood, and occipital cortex are lower than in controls (26-28). GABA is the major inhibitory neurotransmitter within the brain, with concentrations at least 1000 times greater than that of monoamines. One third of all synapses are GABAergic, located at cortical interneurons and cerebellar Purkinje cells (29). At nerve endings, GABA is synthesized by α -decarboxylation of glutamic acid, under control of two isoforms of L-glutamate decarboxylase (GAD), GAD-65 and GAD-67 (30). In MDD, post-mortem studies have reported reduced levels of GAD-67 proteins (31). GABA regulates neuronal excitability through inhibitory feedback loops. GABA is known to be one of the strongest inhibitors of the HPA axis and the noradrenergic system (32), and projects upon the CRH nuclei in the hypothalamic PVN, providing part of the structural basis for inhibitory regulation of the HPA-axis (33). A decrease in GABAergic inhibition probably leads to excitation.

GABA_A receptor

The synaptic effects of GABA are mainly mediated through the GABA_A receptor (Figure 2), and to a lesser extent through GABA_B receptors. GABA-binding to the

GABA_A receptor channel results in a net influx of negative chloride (Cl⁻) ions, thereby hyperpolarizing the membrane and reducing neuronal firing (34). The postsynaptic GABA_A receptor is made up out of five subunits, expressed by multiple genes, and it contains binding sites for various ligands and drugs, including benzodiazepines (35). In mammals, at least 19 different subunits have been identified (α 1-6; β 1-3; γ 1-3, δ , ϵ , θ , π , ρ 1-3). Differential expression of GABA_A receptor subunits has been observed amongst brain regions and cell types (36-38). The most abundant composition of the GABA_A receptor in the human brain is the two α -, two β - and one γ -subunit (39).

Figure 2



The GABA binding site is located at the interface of the α/β subunits. Flumazenil, a benzodiazepine that is used as a ligand in positron emission tomography (PET) research, binds at the α/γ_2 subunit interface (38). [¹¹C]Flumazenil (FMZ) is a selective, reversibly bound, high affinity neutral antagonist of the benzodiazepine site, showing rapid uptake and a high specific to nonspecific binding ratio (40).

There is substantial evidence for involvement of the GABA_A complex in human anxiety states, and it is well established that benzodiazepine medication reduces anxiety symptoms (41,42). Anxiety and major depressive disorder have considerable overlap and co-occurrence (43) and symptoms may respond to the same pharmacological treatment, suggesting the possibility of common neurobiological dysfunctions.

Using [^{11}C]flumazenil, global reductions in GABA_A benzodiazepine receptor distribution volume have been shown in the right orbitofrontal cortex and insula in patients with panic disorder (44), further amplified in comorbid depression (45). In general, in anxiety disorders, GABA_A receptor deficits have been shown in the temporal lobe (46) and in posttraumatic stress disorder in the prefrontal cortex (47). Collectively, these data suggest that different anxiety disorders involve GABA_A receptor deficits primarily in prefrontal and temporal brain regions. No PET studies have looked into GABA_A benzodiazepine receptor binding in major depressive disorder. Only Kugaya et al. found no differences in the density of GABA_A [^{123}I]iomazenil benzodiazepine binding between MDD patients and controls, using single photon emission computed tomography (SPECT), which has lower sensitivity than PET for detecting differences in binding (48).

Dopamine

Dopamine has not only been associated with psychosis and substance related disorders, but also with affective disorders (49). Dopamine originates in the ventral tegmental area (VTA) and substantia nigra, and is formed out of L-phenylalanine, which is then converted to L-tyrosine and L-DOPA. Dopamine acts on both pre- and postsynaptic receptors.

A number of psychological and physical functions are impaired during depressive episodes, pointing towards direct or indirect involvement of the dopamine system in the pathophysiology of MDD (9). These include (1) anhedonia, which is the inability to feel pleasure or be stimulated to activity, likely due to impaired motivation and emotional responsiveness (mesocortical dopaminergic pathway), (2) altered sensitivity to reward (mesolimbic pathway), and (3) psychomotor slowing (nigro-striatal pathway).

Dopamine receptor

The two major dopamine receptors, D1 and D2, are expressed in the cerebral cortex. D1, D2, D3 and D5 are expressed in the striatum. Genetically, the D3 receptor is closely related to the D2 receptor. For PET research, the benzamide raclopride, labelled with carbon-11, is used as a reversible dopamine D2/D3 postsynaptic receptor antagonist ligand (50). D2/3 receptor ligands compete with dopamine for receptor binding, thus a decrease in radiotracer binding potential (BP_{ND}) is interpreted as an increase in dopamine release, a decrease in D2/3 receptor density, or a decrease in affinity of the receptor (51).

THERAPEUTIC INTERVENTIONS IN MOOD DISORDER

For treatment of mood disorders, numerous pharmacological treatments and interventions have received empirical support. Sleep deprivation as a modulator of the circadian rhythm is an effective non-pharmacological therapeutic intervention for MDD, thereby improving symptoms in 40-60% of patients (52). In bipolar patients, sleep reduction or deprivation may trigger mania or hypomania (53). Sleep deprivation also has an effect on healthy subjects, leading to tiredness, impairment in mood, and neurocognitive slowing, with varying effects on autonomic, biochemical, hormonal and immunologic functioning (54).

Sleep deprivation essentially leads to disruption of the sleep-wake cycle, which is regulated by a homeostatic mechanism, the so-called 'process S', a substance expressing itself in Slow Wave Sleep (SWS) activity in the electro-encephalogram (55) and by a circadian mechanism, arising from the endogenous pacemaker in the hypothalamic suprachiasmatic nucleus (56). Sleep deprivation causes a rebound in SWS and resets process S (57). The SCN contains a dense serotonergic plexus (58) and is sensitive to the input of light, but also responsive to non-photic cues as sleep deprivation, arousal, locomotor activity, social cues, feeding and temperature (59).

Sleep deprivation may be used to target multiple mood disorder related systems, including the HPA-axis and the dopamine system. Dopamine release is supposed to modulate wakefulness, partly through its D2 receptor (60-63) acting as a stimulator of CRH (60,64) and ultimately releasing cortisol from the adrenal cortex.

NEURO-IMAGING: BASIC CONCEPTS

In order to image the brain *in vivo*, a number of methods may be applied. MRI is best suited to depict anatomy, whereas neuronal activity may be registered using functional MRI (fMRI). Neuroreceptor systems and transporters for the major neurotransmitters may be visualized using corresponding radioligands and PET (65).

Magnetic resonance imaging

MRI uses a strong, permanent static magnetic field to align nuclei in the brain. With the aid of a second magnetic gradient field, nuclei are brought to a higher energy level. Upon removal of the gradient field, nuclei slowly return to their original states. The emitted energy is measured with a coil.

fMRI uses the blood-oxygen-level-dependent (BOLD) contrast to map neuronal activity in the brain, by imaging changes in local magnetization resulting from oxygen-rich as opposed to oxygen-poor haemoglobin in blood. Changes in blood flow and oxygenation are closely linked to neuronal activity. The resulting brain activation is graphically presented by colour-coded statistical Z- or T-maps, indicating the strength of activation across the brain or the specific region studied (66).

Neuroreceptor imaging using PET

PET is a dynamic imaging technique, producing images of the distribution of radiolabelled compounds as a function of time.

Radioligands

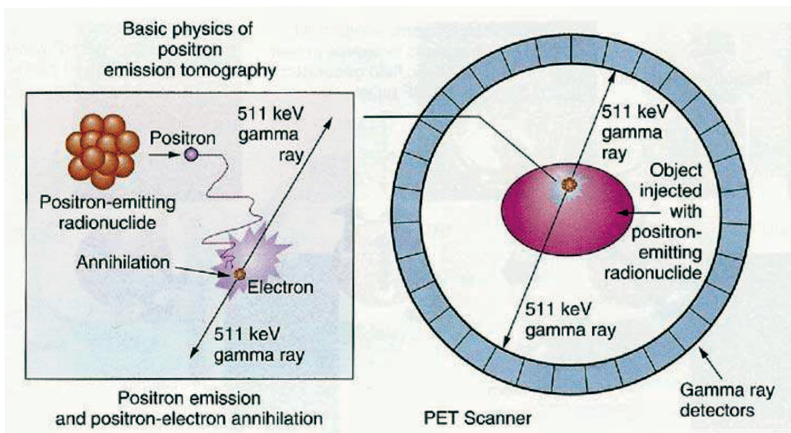
PET is based on the decay characteristics of positron emitters. PET ligands are synthesized by a cyclotron, an accelerator of subatomic particles. In general, the half-life of a positron emitter is short. For example the half-life of carbon-11 (^{11}C) is 20.3 minutes, which means that virtually no radioactivity is left after two hours. PET ligands can be radiolabelled with very high specific activity, allowing for PET measurements after injecting an extremely low amount of the compound (so called tracer dose), thereby minimizing any pharmacological effect (65). Examples of common PET radioligands are ^{11}C flumazenil and ^{11}C raclopride.

PET physics

At the start of a PET scan, a radioactive tracer such as ^{11}C flumazenil or ^{11}C raclopride is injected intravenously. Through the blood stream, the tracer is carried to reach the specific target area(s) in the brain. Meanwhile, the carbon-11 radionuclide emits positrons. A positron travels at most a few mm in tissue before colliding with an electron. The combination of positron and electron is very unstable, and decays almost immediately by emitting two gamma rays (photons) (Figure 3), with a fixed energy of 511 keV, in opposite directions. If both gamma rays are detected simultaneously by two opposing detectors of the PET scanner, this forms a valid coincidence event, defining

the line of response along which the original annihilation took place. A PET scanner consists of many of those detectors, usually arranged in a ring surrounding the patient (Figure 3). As the total path length of both annihilation photons together is known, accurate correction for tissue attenuation can be made using a separate transmission scan. A PET scan consists of the acquisition of large numbers of these coincidence events. Raw PET data must be reconstructed into three-dimensional image volumes using either analytic filtered back projection or iterative reconstruction algorithms.

Figure 3



Basic physics of PET

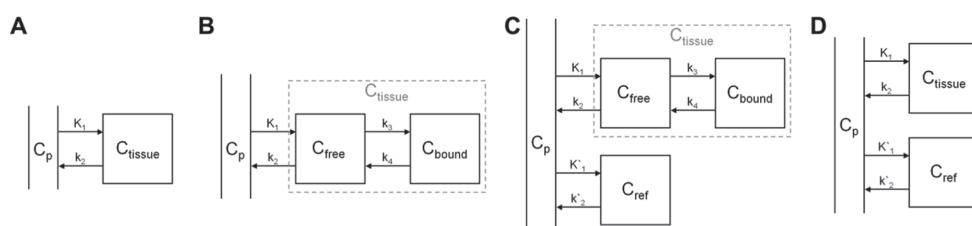
Kinetic modelling

The resulting *in vivo* brain signal is due to specific tissue binding to the neuroreceptor (C_{bound}), nonspecific binding to other intracerebral sites (e.g. protein binding) and free ligand in the brain, not bound to the receptor (C_{free}), and intravascular activity (Figure 4).

All of these concentrations vary in time. Cerebral uptake and clearance of radioactive tracers may be measured using dynamic scanning, which implies acquisition of multiple frames or scans over time. Kinetic methods can then be used to extract quantitative information about the receptors under study, by estimating rate constants (K_1 and k_{2-4}) that govern transfer of tracer between arterial, nondisplaceable (i.e. free and

nonspecific) brain, and receptor compartments (68). In order to derive these kinetic parameters, an appropriate compartmental model is needed. A compartment is a physiological or biochemical 'space' in which the concentration of the radioactive tracer is assumed to be homogeneous. As the kinetics of the nondisplaceable compartment are very fast, free and nonspecific compartments are generally combined into a single nondisplaceable compartment. For quantification purposes, the metabolite corrected arterial plasma input curve (C_p) is used, indicating delivery of the tracer to the brain.

Figure 4: Various compartmental models to quantify PET data (67)*



- A One-tissue (1T) compartmental model (1TC)
 B Reversible two-tissue (2T) compartmental model (2TC)
 C Full reference tissue model (FRTM)
 D Simplified reference tissue model (SRTM)
 C_p Concentration in arterial plasma, or plasma input curve ($\text{kBq}\cdot\text{mL}^{-1}$)
 C_{tissue} Concentration in tissue compartment, consisting of C_{free} and C_{bound} ($\text{kBq}\cdot\text{mL}^{-1}$)
 C_{ref} Concentration in reference tissue
 K_1 Rate constant for transfer between arterial plasma and tissue compartments ($\text{mL}\cdot\text{cm}^{-3}\cdot\text{min}^{-1}$)
 k_2 - k_4 Rate constant for transfer between compartments (min^{-1})
 * reproduced with consent J Mourik

If the exchange rate is fast between free ligand (C_{free}) and specific binding to the receptor (C_{bound}), a simplified one-tissue compartmental model (1TC) may be used (Figure 4A), instead of a two-tissue compartment model (2TC) (Figure 4B). The resulting parameter is the volume of distribution of the radioligand (V_T), containing free, nonspecific and specific binding, which is the ratio of the concentration of the radioligand in tissue to that in plasma at equilibrium. A reversible one-tissue compartmental model with a metabolite corrected plasma input function is the established method for analysing [^{11}C]flumazenil studies (69-71).

If a region within the brain is devoid of receptors it can be used as an indirect (reference tissue) input function, provided it is not affected by the pathology under study (72,73). For [^{11}C]flumazenil studies, reference tissue approaches using pons and white matter have been reported (74-76), but these studies used small sample sizes and were not fully validated against the gold standard of an arterial input function.

Just like the 2TC model can be simplified to a 1TC model when the rate of exchange between free and specific compartments is fast enough, the full reference tissue model (FRTM) (Figure 4C) can be reduced to a simplified reference tissue model (SRTM) (Figure 4D), thereby creating one simplified physiological (target) compartment (C_{tissue}), containing free, specific and nonspecific binding. Essential for the use of reference tissue models (i.e. for both FRTM and SRTM), is the assumption that the levels of free and nonspecific binding (together called the nondisplaceable compartment) are the same in target and reference tissues (73,77). The outcome of this reference tissue approach is nondisplaceable binding potential (BP_{ND}). This is the free fraction within the nondisplaceable compartment \times the ratio of B_{avail} (maximum concentration of available receptor sites) to apparent K_d (dissociation constant for the receptor), which is directly related to the density of receptors and the binding affinity of the receptor for the radioligand (68). The free fraction is assumed to be constant and, if the affinity is also relatively constant, then differences in BP_{ND} reflect differences in receptor density or receptor occupancy by a drug or an endogeneous ligand. SRTM with the cerebellum as reference tissue is the established method for analysing [^{11}C]raclopride studies (73). V_T is related to the specifically bound signal, represented by BP_{ND} , but contains an additional nonspecific binding component.

For the 1TC and 2TC plasma input compartmental models, and the reference tissue models SRTM and FRTM, in general nonlinear regression (NLR), applied to time-activity curves derived from predefined 'region of interest' (ROI), is used to estimate the various kinetics parameters. Analysis at the voxel level requires simplifications of the models (e.g. linearisations or basis function approaches), but then results in parametric images, where the intensity of each voxel is proportional to the value of the parameter of interest, such as V_T or BP_{ND} .

OUTLINE OF THIS THESIS

Mood disorders are characterized by heterogeneity in psychobiological dimensions and involve both cortical and subcortical brain systems. The general aim of this thesis is to study mood disorder related brain systems, in two clinical neuroreceptor imaging studies, using a multimodality approach.

In **Chapter 1**, the concept of major depressive disorder (MDD) as a subtype of mood disorder is explained, together with its related neuropathophysiology of the HPA-axis, and both GABAergic and dopaminergic systems. Sleep deprivation as a mechanism to target mood related systems, and the neuro-imaging methods fMRI and PET are introduced.

At the symptom level, MDD and anxiety respond to the same pharmacological treatment, supporting the possibility of common neurobiological dysfunctions. In anxiety disorders, GABAergic drugs are anxiolytic, and in PET studies, GABA_A receptor binding has been found to be decreased. In MDD, GABA levels have been found to be decreased. As the synaptic effects of GABA are mainly mediated through the GABA_A receptor, it was hypothesized that GABA_A receptor binding is altered in MDD compared with healthy controls, and that this normalizes after pharmacological treatment. Therefore, the first clinical aim was to assess cerebral GABA_A receptor status in MDD, before and after treatment, compared with healthy controls using [¹¹C]flumazenil and PET.

In MDD, persistent hyperactivity of the HPA axis is a common finding. GABA is known to be one of the strongest inhibitors of the HPA axis and projects directly upon the CRH nuclei in the hypothalamic paraventricular nucleus (PVN). As decreased GABA levels are thought to be related to a deficient inhibiting GABAergic system, this mechanism provides a partial structural basis for hyperactivity of the HPA-axis in MDD. Therefore, the second clinical aim was to determine whether HPA-axis hyperactivity in MDD is associated with decreased GABA_A binding, again using [¹¹C]flumazenil as a tracer.

In order to analyse clinical [¹¹C]flumazenil studies, the optimal kinetic model should be used. As mentioned above, a single tissue compartmental model with arterial plasma input is the established method. Arterial cannulation, however, is invasive and measurement of plasma metabolites is laborious. Accordingly, use of reference tissue

models is appealing, but insufficiently validated for [^{11}C]flumazenil. Therefore, the first methodological aim was to assess whether a reference tissue model is an option for analysing clinical [^{11}C]flumazenil studies, when compared with the most accurate tracer kinetic model. To determine the optimal model, data were analysed using both single and reversible two-tissue plasma input compartmental models, together with both full and simplified reference tissue models. For the latter models, both pons and white matter were investigated as reference tissues (**Chapter 2**).

In order to identify disorder related changes in MDD versus healthy controls, various methods of analysis may be used. Drawing regions of interest (ROI) suggests a predefined idea about the localization of pathology. However, subtle disease specific changes may be better identified in a voxel by voxel representation, which are only limited by the spatial resolution of the PET scanner rather than by the size of the volume of interest. To this end, accuracy and precision of a wide range of parametric methods for quantifying [^{11}C]flumazenil studies were investigated and compared with full compartmental analysis. The aim of this study was to find the optimal parametric model for quantitative and qualitative comparison between groups (**Chapter 3**).

Successively, in **Chapter 4**, clinical results of the [^{11}C]flumazenil study are described. In MDD and healthy controls, GABA levels and baseline [^{11}C]flumazenil GABA_A benzodiazepine receptor binding were investigated, in relation to HPA axis activity. In a MDD subgroup effects of treatment with citalopram were assessed.

In the second clinical study in this thesis, neurophysiological effects of total sleep deprivation were investigated in the healthy brain (**Chapter 5**). Sleeping problems frequently co-occur with mood disorders, and sleep deprivation is an effective non-pharmacological therapeutic intervention for MDD, targeting e.g. the dopaminergic D2 receptor system. It was hypothesized that staying awake would (a) induce affective and cognitive alterations and (b) mobilize stress related systems, such as the HPA-axis and the dopaminergic system. The aim was to study their mutual relationship, using an emotional fMRI task, cortisol saliva sampling, and assessment of the dopamine D2/D3 receptor status using [^{11}C]raclopride PET.

In **Chapter 6**, the main results of these studies are summarized and discussed with focus on clinical implications and future research perspectives.

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