

6

Main findings and general discussion

MAIN FINDINGS

The general aim of this thesis was to investigate neuroreceptor imaging of mood disorder related systems and to study these in a multimodality approach, using positron emission tomography (PET) and structural and functional magnetic resonance imaging (MRI and fMRI, respectively), in combination with assessment of hypothalamic pituitary adrenal (HPA) axis functioning. In this concluding chapter, main findings are summarized and discussed, starting with [^{11}C]flumazenil studies and followed by clinical effects of sleep deprivation. Methodological aspects, clinical implications and forthcoming lines of research are addressed.

[^{11}C]FLUMAZENIL GABA_A-BENZODIAZEPINE RECEPTOR BINDING IN MAJOR DEPRESSIVE DISORDER VERSUS HEALTHY CONTROLS

Gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the brain. In major depressive disorder (MDD), GABA has repeatedly been found to be decreased in plasma, cerebrospinal fluid and cortical tissue. As the synaptic effects of GABA are mediated mainly through the GABA_A receptor, altered GABA_A receptor binding was hypothesized in MDD. Therefore, using the PET tracer [^{11}C]flumazenil ([^{11}C]FMZ), postsynaptic GABA_A central benzodiazepine receptor status was assessed in MDD and compared with that in healthy controls.

In order to find the optimal kinetic model for analysing this clinical [^{11}C]FMZ study, reference tissue models were validated against the most accurate tracer kinetic model at a region of interest (ROI) level (**Chapter 2**). Using a Hill-type metabolite corrected arterial input function, the single-tissue (1TC) compartmental model was preferred over the two-tissue (2TC) compartmental model across all structures and subjects investigated, based on a lower rejection rate and a stronger performance in structures with minor levels of GABA_A benzodiazepine receptors, such as white matter and pons, and intermediate levels such as putamen and thalamus.

The simplified reference tissue model (SRTM) with pons or white matter as reference tissue was superior to the full reference tissue model (FRTM). Ultimately, pons was preferred as reference tissue, as no fits were rejected. In contrast, when white matter was used as reference tissue, fits of structures with intermediate receptor density had

to be rejected. On the basis of an excellent correlation between SRTM derived binding potential (BP_{ND}) and 1TC volume of distribution (V_T), it was concluded that SRTM with pons as reference tissue, rather than a plasma input model, could be used for the analysis of clinical [^{11}C]FMZ studies.

In order to find the optimal voxel based parametric method for evaluating statistically significant group differences between MDD and healthy controls, accuracy and precision of a wide range of parametric methods for quantifying [^{11}C]FMZ studies were investigated, by comparing with nonlinear full compartmental analysis (**Chapter 3**). As changes in specific [^{11}C]FMZ binding between MDD and healthy controls were much smaller than global intersubject variability, proportional scaling was applied to the parametric images to account for these non-pathology related differences in global binding. With the exception of standardized uptake value (SUV) images, providing large under- and overestimations of [^{11}C]FMZ activity, various parametric methods showed comparable *quantitative* performance to 1TC V_T and SRTM BP_{ND} methods.

Using statistical parametric mapping (SPM), virtually all parametric methods showed decreased [^{11}C]FMZ binding in the bilateral parahippocampal gyrus (PHG) in MDD. The basis function method (BFM) and receptor parametric mapping (RPM1-2) methods showed a reduced rate of tissue tracer delivery (K_1 and R_1) in this area (**Chapter 3, 4**). For the PHG, SUV images provided the best *qualitative* contrast between groups, especially when taken over a late time interval. However, given their poor performance in quantitative comparisons, and hence in evaluating effects of therapy, for use in clinical studies, Logan V_T , MRTM2 and both RPM methods are preferred.

When comparing MDD with healthy controls, using manually placed regions of interest (ROI), no significant differences in [^{11}C]FMZ V_T or BP_{ND} values were demonstrated. Using voxel based Logan V_T as the preferred parametric method, decreased [^{11}C]FMZ binding was additionally shown in the right temporal gyrus (**Chapter 4**). For V_T based values, decreased [^{11}C]FMZ binding indicates a reduced ratio of the concentration of [^{11}C]FMZ in the tissue under study, versus the concentration of [^{11}C]FMZ in plasma. A decrease in [^{11}C]FMZ BP_{ND} implicates reduced affinity and/or density of the GABA_A benzodiazepine receptor. This may be due to occupation of the GABA_A benzodiazepine receptor site by an endogenous ligand, or an absolute reduction in GABA_A receptor numbers due to atrophy. V_T also contains a nondisplaceable component and changes

in V_T should be interpreted with more care. When levels of nonspecific binding are low, however, V_T is a good substitute for BP_{ND} , especially if no reference tissue is available.

Montgomery Åsberg Depression Rating Scale (MADRS) depression severity scores were inversely related to [^{11}C]FMZ V_T binding in the right posterior temporal gyrus, bordering the parahippocampal gyrus, and the ventrolateral prefrontal cortex, areas involved in the pathophysiology of anxiety and depression. State anxiety scores significantly differentiated MDD from controls and were, at the participants' group level, strongly inversely correlated with [^{11}C]FMZ binding in the temporal and parahippocampal region. Therefore, low or decreased [^{11}C]FMZ binding is probably partly correlated with increased anxiousness as part of the depressive syndrome (**Chapter 4**).

In MDD, basal adrenocorticotrophic hormone (ACTH) levels after dexamethasone suppression were significantly higher than in controls (**Chapter 4**). Despite the absence of differentiating plasma GABA levels, increased HPA axis activity was associated with low [^{11}C]FMZ V_T binding in the bilateral insular area, extending into the superior temporal lobe and giving support to the hypothesis of an inhibiting GABAergic system, providing a partial structural basis for the excitatory regulations of the HPA axis.

In the depressed patients, MADRS and anxiety scores improved significantly after treatment with citalopram, a serotonergic antidepressant drug, in line with an attenuated corticosteroid output. Decreased [^{11}C]FMZ binding was shown in the right dorsolateral prefrontal cortex and temporal gyrus, both involved in MDD related pathophysiology (**Chapter 4**). Although disputable, this may imply a *relative* and not an *absolute* decrease in [^{11}C]FMZ binding, i.e. a lower increase than elsewhere in the cortex due to citalopram induced increased GABA availability (1,2).

SLEEP DEPRIVATION AS A THERAPEUTIC INTERVENTION FOR MOOD DISORDER

In MDD, sleep disturbances are frequently occurring, whereas sleep deprivation as a modulator of the circadian rhythm is an effective non-pharmacological treatment option, improving symptoms in a large proportion of patients. Sleep deprivation may be used to target multiple mood disorder related systems. These include cognitive and affective cortical and subcortical networks, and underlying neurobiological stress related systems such as the HPA axis and dopamine system, but have not been studied in their mutual relationship.

In healthy adults, total sleep deprivation (TSD) induced decreased perceived energy levels, concentration and speed of thought, but no significant alteration of mood (**Chapter 5**). In the fMRI semantic affective classification task, reduced response speed and accuracy was shown for both neutral and positive targets, despite increased regional prefrontal and limbic activity, suggesting failing top-down control. Processing of solely affective stimuli was accompanied by increased activation in the paralimbic region, suggesting augmented affective perturbation.

TSD resulted in a significantly lower saliva cortisol awakening response (CAR) and cortisol output over the day. Using the postsynaptic PET dopamine D2/D3 receptor ligand [^{11}C]raclopride, decreased parametric RPM2 derived BP_{ND} binding was observed in left caudate, putamen and thalamus, signifying either increased dopamine release, occupying the D2/D3 receptor, and/or decreased D2/D3 receptor affinity or numbers, though this could not be differentiated on the basis of the present study design. Therefore, in TSD, activation of the dopaminergic system may compensate for a blunted cortisol response, whereas D2 receptor deactivation, by e.g. internalization of receptors, may result in HPA axis attenuation through the D2 mediated corticotrophin releasing hormone (CRH) receptor. Sleep deprivation requires involvement of adaptive neurobiological systems, providing a comprehensive mechanism for the therapeutic effects of TSD in mood disorders.

GENERAL DISCUSSION

METHODOLOGICAL ISSUES [¹¹C]FLUMAZENIL STUDY

In the first part of this thesis, different methods are described to analyse [¹¹C]flumazenil studies, with the main reason to validate reference tissue models. Use of a reference tissue strongly simplifies the implementation of [¹¹C]FMZ studies, as it is patient friendly by obviating the need for arterial cannulation, and investigator friendly in precluding the need for measurement of metabolites, thereby reducing the risk of erroneous results.

Reference tissue

In the present study, pons was preferred as a reference tissue for analysing [¹¹C]FMZ binding to the GABA_A benzodiazepine receptors, which are mainly concentrated in cortical grey matter. In theory, however, pons is considered to be white matter, which may not have an equivalent level of nonspecific binding as grey matter. This would be a violation of the assumption underlying reference tissue models, namely identical levels of nonspecific binding in reference tissue and region of interest. As V_T of white matter was even slightly higher than that of pons (containing white and minor amounts of grey matter in the pontine nuclei), white matter probably contained an even higher level of nonspecific binding (Chapter 2).

Depressive illness is associated with widespread regions of decreased white matter integrity, as documented by diffusion tensor imaging, including regions in the superior longitudinal fasciculus, which was included in the 'white matter' ROI (3). Therefore, in theory, potential bias in V_T or BP_{ND} might be different for MDD patients versus healthy controls. However, given the absence of significant differences between MDD and healthy controls for all ROIs (similar average values and ranges), 'centrum semiovale' white matter is either not seriously affected by pathology in the MDD group, or alterations in the grey matter are offset by analogous alterations in white matter in this study.

As pons is not devoid of benzodiazepine receptors, V_T pons contains both a specific and a nonspecific binding signal. In theory, this may be taken into account in using an extended reference tissue model (4), requiring an independent assessment of

the level of specific binding in the pons. Given the good correlation between V_T and BP_{ND} across subjects, the [^{11}C]FMZ nonspecific binding signal does not seem to affect results significantly (Chapter 2).

The ROI for the pons consisted of a small, elliptical structure, which could be sensitive to movement. [^{11}C]FMZ scans were checked retrospectively for movement. We found no systematic differences between pons ROI volumes in patients and controls, using independent samples *t*-testing, as this would affect all reference tissue based methods in the study. Although in PET any small structure is subject to partial volume effects and contamination or spill-over effects of nearby structures, these effects would presumably affect image data in patients and controls similarly, and do not explain differences in BP_{ND} between groups.

Alternative options for kinetic analysis

In order to prevent manual selection of the reference tissue, automatic approaches to extract reference tissue kinetics are appealing, as long as they are sensitive and robust. Recently, within our PET group, supervised cluster analysis (SVCA) algorithms have been used to improve quantification of dynamic PET studies (5). In SVCA, PET voxels are segmented, based on differences in time-activity curves (TACs), involving a linear sum of predefined kinetic classes, associated with grey matter with and without specific binding, white matter, blood, bone, and soft tissue regions. The algorithm selects reference tissue voxels primarily from grey matter tissue without specific binding (6). Though for [^{11}C]FMZ it is difficult to find grey matter without specific binding, a corresponding SVCA method may be developed.

Ultimately, arterial sampling remains the gold standard for obtaining the arterial input function. In order to obviate this procedure, Mourik et al. (7) characterized the [^{11}C]FMZ extracted image derived arterial input function (IDIF) of dynamic PET studies by using the four hottest pixels per plane, over the carotid arteries, together with reconstruction based partial volume correction. Area under the curve (AUC), Logan V_T , and Basis Function Method (BFM) K_1 and V_T were similar to values obtained with conventional blood sampler input function (BSIF), offering an alternative non-invasive method for analysing [^{11}C]FMZ studies.

CLINICAL ISSUES [¹¹C]FLUMAZENIL STUDY

GABA and [¹¹C]flumazenil binding to the GABA_A benzodiazepine receptor in major depressive disorder

In MDD, GABA has repeatedly been found to be decreased, although we did not confirm this in plasma in the present study. This may be due to sample size, or MDD patient characteristics like gender (slightly more females) and moderate severity of depression in this outpatient sample.

Generally, in neurobiology, shortage of a neurotransmitter induces an upregulation of the postsynaptic receptor, such as the 5-HT_{1A} receptor in the serotonin model of depression (8). As GABA is decreased in MDD, and mainly binds to the GABA_A receptor, in theory, upregulation of GABA_A receptor affinity or number may have been expected in MDD, with a parallel increase in BP_{ND} and/or V_T of [¹¹C]FMZ. However, in other neuropsychiatric conditions such as panic disorder (9) or epilepsy (10), GABA was also found to be decreased, but so was [¹¹C]FMZ binding, suggesting alternative adaptive or explanatory neuroreceptor mechanisms. As mentioned earlier, this could be due to occupation of the benzodiazepine receptor by an endogenous ligand, or to altered receptor affinity or an absolute decrease in GABA_A receptor numbers. The cortical GABA_A receptor is sensitive to damage, and hence [¹¹C]FMZ binding also represents a reliable marker of neuronal integrity (11).

The most abundant composition of the GABA_A receptor in the human brain is two α-, two β- and one γ-subunit. Therefore, theoretically, an upregulation of the GABA binding site at the α/β subunit interface could induce a downregulation of GABA_A benzodiazepine binding at the α/γ2 subunit. Support for this theory was found in a recent overview by Houser et al. (12), who in animal epilepsy models identified opposing expression of different GABA_A receptor subunits in response to increased excitability, with uncertain effects on global receptor binding affinity and functionality. Therefore, in MDD, low GABA may well result in altered GABA binding due to GABA_A α/β subunit upregulation, with ambiguous outcome on flumazenil binding to the α/γ2 subunit interface. Recently, Frankle et al. (2) showed dose dependency for increases in extracellular endogenous GABA release and [¹¹C]FMZ binding in healthy controls, though this has to be repeated in disease models.

Reduced [¹¹C]flumazenil binding in the bilateral parahippocampal gyrus

In MDD and healthy controls, no significant differences in [¹¹C]FMZ V_T and BP_{ND} values were found for any of the manually placed elliptical ROIs, including the hippocampal ROI. Absence of such a relationship could be due to limited power caused by the small sample size, volumes or heterogeneity of the ROIs, though this finding was in line with Kugaya et al. who used [¹²³I]iomazenil SPECT, with a much lower sensitivity compared with [¹¹C]FMZ (13,14).

In a subsequent volume of interest (VOI) approach, using the standardized segmentation toolbox of Svarer et al. (15), tissue VOI were projected onto parametric images. Various parametric methods showed only marginal group differences between MDD and healthy controls. In contrast, at the voxel level virtually all methods showed statistically significant decreased binding in the bilateral parahippocampal gyrus in MDD. The localization maxima of these regions were, however, too peripheral to be included in Svarer's hippocampal or entorhinal VOI segments (personal communication). As regional differences are computed from the average signal intensities, actual differences within the volume may have been diluted, though it can not be excluded that our findings are due to a type I error (16,17).

The parahippocampal gyrus is reciprocally connected to parts of the hippocampal formation (18), involved in the medial and dorsal prefrontal network (19). It plays a role in a range of cognitive functions, including episodic memory, planning for the future, and spatial navigation (20). Pathological changes in the parahippocampal gyrus occur during the early stages of neuropsychiatric disorders, including Alzheimer, schizophrenia and epilepsy, suggesting a high sensitivity to neurotoxic actions (11).

Ultimately, decreased [¹¹C]FMZ binding in this area could be due to reduced GABA_A (benzodiazepine) receptor density, attributable to atrophy, with a concomitant decrease in tracer flow to the region of interest, as was shown in the parametric K_1 and R_1 [¹¹C]FMZ images (Chapter 3). Additional evidence for atrophy was found in a posthoc voxel based morphometry (VBM) DARTEL (21) analysis, using SPM8 and 5 mm smoothing, finding decreased grey matter in the parahippocampal gyrus and right prefrontal gyrus in our MDD group (data not published). Therefore, our results of decreased [¹¹C]FMZ binding in the parahippocampal gyrus are probably explained by diminished GABA_A benzodiazepine receptor numbers and local atrophy, which may have even further increased partial volume effects. Atrophy in this area is in line with

Abe et al. (22) and Drevets and Price (23). As both depression and anxiety scores were related to this area, increased glucocorticoid activity may either precede, provoke, maintain, or be a consequence of impaired local GABA_Aergic inhibition.

Intriguing additional evidence for parahippocampal involvement in MDD stems from a study by Montag et al. (24), finding brain derived neurotrophic factor (BDNF) Val66Met polymorphism to be associated with smaller parahippocampal volumes. Increases in BDNF signalling are linked to neurotrophic support and sprouting after treatment with antidepressants (25), whereas glucocorticoids are known to inhibit effects of BDNF in the hippocampus, leading to neuronal loss (26). As BDNF has also been shown to affect the phenotype of inhibitory GABAergic interneurons (27), a mediating role is suggested in the interaction of GABA and glucocorticoids in the pathophysiology of MDD.

Alternative options for altered [¹¹C]flumazenil binding

Recently, it was shown that flumazenil is a weak P-glycoprotein (P-gp) substrate in rodents (28). P-gp is able to transport drugs against a concentration gradient across the blood-brain barrier (BBB) back into plasma, thereby reducing the bioavailability in the brain. Therefore, in theory, increased P-gp activity could result in decreased cerebral [¹¹C]FMZ uptake. However, in human, for flumazenil this was not confirmed in *in vitro* studies (29). Due to the pathophysiological dysfunction of the BBB and blood-cerebrospinal fluid barrier in MDD, flumazenil entrance may have been altered, compared with healthy controls, though this should not influence [¹¹C]FMZ receptor binding, as both BP_{ND} and V_T are independent of flow.

Enhanced P-gp activity in prefrontal and temporal regions has been reported in MDD patients on antidepressants (30). Citalopram, which was used as an antidepressant drug treatment in the study, may be a substrate for P-gp (31). Therefore, regional differences in V_T may reflect localized regions of altered P-gp function, thereby offering an alternative explanation for the voxel based reduced Logan V_T binding in the right lateral temporal gyrus and dorsolateral prefrontal gyrus.

HPA axis

In MDD patients, visiting the outpatient department, basal adrenocorticotropic hormone (ACTH) levels after dexamethasone suppression were significantly increased, compared with controls, in line with a recent meta-analysis (32). This

was not confirmed in cortisol in blood or urine, thought to be mainly due to large between-subject variability. Additional exogenous corticotrophin releasing hormone (CRH) showed a trend-wise difference in ACTH_{AUC} output between MDD and healthy controls, again accompanied by large variability. At the high end, overactivity of the HPA axis was hypothesized, not responding to shut-down signals, though not directly linked to decreased [¹¹C]FMZ binding in the parahippocampal gyrus. Still, in an MDD group of dexamethasone-CRH non suppressors, Aihara et al. (33), found glucose hypermetabolism in the parahippocampal gyrus, using [¹⁸F]-fluoro-2-deoxy-D-glucose (FDG) PET, signifying increased local activity, normalizing after treatment. This suggests HPA axis hyperactivity to be related to increased metabolic demands, which may ultimately result in atrophy in neurotoxicity sensitive areas.

Order of interaction for GABA, stress and the HPA axis

In this study, HPA axis hyperactivity was thought to be due to reduced GABAergic tone on the paraventricular nucleus (PVN), finding its origin in hypothalamic and adjacent forebrain regions, and projecting to stress-integrative CRH neurons (34). [¹¹C]FMZ binding in the PVN could not be quantified due to small size and associated partial volume effects.

GABAergic deficits might actually be secondary to stress induced HPA axis hyperactivity, modulating the levels of several proteins regulating glutamate and GABAergic transmission in the hippocampus, resulting in volume reductions. Blocking hippocampal neurogenesis in turn is sufficient to increase HPA axis activity, which may sustain or amplify hippocampal neuropathology (35-37). Chronic stress may downregulate expression and function of GABA_A receptors in the frontal cortex, partly due to loss of cell bodies (38). In mice, early life stress is known to result in altered GABA_A receptor subunit composition, though GABA_A-ergic receptor dysfunction by itself may induce anxiety and depressive like behaviour accompanied with cognitive deficits and elevated baseline corticosterone concentrations (39).

Ultimately, stress induced output of steroid hormones may alter synaptic transmission of GABA by altering receptor expression. Under basal conditions, stress derived neurosteroids such as tetrahydrodeoxycorticosterone (THDOC), exert a negative feedback onto the HPA axis by binding to extrasynaptic GABA_A receptors on CRH neurons. Recently, Sarkar et al. showed that, following acute restraint stress, GABA

is no longer inhibitory, but excitatory in CRH neurons, increasing corticosterone levels (40).

Major depressive disorder versus anxiety disorder

The MDD group in this study experienced a high anxiety level, as expressed by (state) anxiety scores (Chapter 4). Symptoms of anxiety are highly prevalent among depressed patients, but not all anxiety symptoms represent an actual comorbid anxiety disorder (41). As genetic risk factors for lifetime MDD and general anxiety disorder are strongly correlated (42), shared pathways may be expected. Neuroanatomically, both anxiety disorders and MDD show dysfunction of limbic regions, possibly sharing failing prefrontal top-down control (43).

In the [¹¹C]FMZ literature, different anxiety disorders have shown to involve GABA_A benzodiazepine receptor binding deficits in variable brain regions, related to severity and subtypes of anxiety disorders. Given the excellent therapeutic efficacy of classical benzodiazepines as anxiolytic drugs, it is not surprising to find alterations at the GABA_A receptor in anxiety disorders. Recent studies suggest that benzodiazepine binding involves a global conformational rearrangement of the GABA_A receptor, thereby affecting receptor gating, instead of affecting GABA binding directly (44). In MDD, meta-analyses of clinical data have concluded that the antidepressant activity of benzodiazepines is limited to alprazolam with classical benzodiazepines being ineffective beyond their established role as anxiolytics. Intriguingly, alprazolam was effective for all symptoms of depression, except for depressed mood (45). Therefore, it seems likely that additional neurotransmitter systems are involved in the experience of depressed mood.

Drugs that induce enhancement of GABA transmission act as anxiolytics, as shown by pregabalin, a GABA agonist and antiepileptic drug, which also is registered for general anxiety disorder. Agents acting at GABA_A or GABA_B receptors have been found to exhibit antidepressant-like activity in animal screening procedures, though this excludes the human apprehension of the depressed state. The use of non-sedating, anxiolytic selective $\alpha 2/\alpha 3$ GABA_A receptor agonists, such as TPA023, as novel antidepressants are currently under study (46,47).

The results in the present [¹¹C]FMZ study in MDD patients may, in part, reflect their anxiety state. According to a retrospective analysis by Nikolaus et al., general anxiety

disorder showed a significant reduction in temperocortical GABA_A receptors (48). Deficits were shown in a comparable area (Chapter 4). In panic disorder, Cameron et al. found reduced [¹¹C]FMZ benzodiazepine receptor distribution volume (V_T) in the insula, mainly accounted for by comorbid depressive disorder. Although none of the participants was suffering from panic disorder, state anxiety scores (STAI) and ACTH_{AUC} showed a strong inverse relationship with decreased [¹¹C]FMZ binding in the insula.

In contrast to MDD, initial investigations of CRH dysregulation in anxiety disorders have been mostly negative, with notably general anxiety and panic disorder patients exhibiting no difference from controls (49). The majority of studies have shown normal or slightly increased output of ACTH and cortisol in the dexamethasone suppression and DEX-CRH tests (46,50), though lower than in MDD. Given the presence of high 'state anxiety', as measured in the STAI and HAM-A rating scales, in the absence of a clinical anxiety disorder, our MDD group showed a mixed profile, with large between-subject variability. Anxiety disorders and MDD may be part of a clinical mood disorder spectrum, with HPA axis regulation in anxiety disorders primarily being governed by brain structures involved in hyperarousal, perception of external and internal stimuli and cognitive judgement and in MDD by disturbances of feedback mechanisms at the higher hypothalamic level (51).

Surely, this study only provides information about the [¹¹C]FMZ benzodiazepine GABA_A binding site in MDD, not on GABA, or the GABA_A receptor in full. In this study, decreased [¹¹C]FMZ binding in the temporal areas was found to be related to HPA axis hyperactivity and increased anxiousness, as part of the depressive syndrome. Using [¹¹C]FMZ as a marker of neuronal integrity, decreased [¹¹C]FMZ binding in the parahippocampal gyri may reflect atrophy of GABA_A benzodiazepine receptor carrying neurons, possibly due to increased glucocorticoid levels.

CLINICAL EFFECTS OF SLEEP DEPRIVATION IN HEALTHY ADULTS

Affective changes

Sleep deprivation is an attractive and effective treatment for depressive disorder and is therefore an appealing approach to study distinct neurobiological systems involved in the pathophysiology of mood disorders. In the present sample of healthy adults, TSD did not result in a clear impairment of affect, as opposed to decreased self perceived

energy and concentration, which points towards a buffer function to cope with the emotional effects of TSD (Chapter 5). Most medical residents and shift workers are familiar with a feeling of giddiness after night duty and in healthy controls euphoric reactions and increases in impulsiveness and drive are noted occasionally, but hardly commented upon in the literature (52).

In response to TSD, decreased sensitivity was shown to detect positive valences in a semantic affective classification task, adapted for use in fMRI. Processing of solely affective stimuli increased paralimbic activation, mainly driven by words with a negative valence, suggesting increased emotional reactivity, coupled to an impaired top-down modulatory regulation of emotional responses. This perturbation towards emotional stimuli is in line with findings by Zohar et al. (53). In medical residents, he found sleep disturbance amplifying the negative emotive effects of next days' disruptive events, while reducing the positive effect of motivational goal-enhancing events.

In clinical practice, comparative ambiguity towards positive stimuli is seen in patients with major depressive disorder (MDD), processing sad information selectively and more intense (54), with diminished capacity to sustain and process positive emotions (55). However, in MDD, total sleep deprivation does elevate mood (56,57), whereas in bipolar disorder, sleep reduction or deprivation may even trigger a manic or hypomanic episode in about 10% of patients (58). As presynaptic dopamine transporter blockers like methylphenidate or dopamine releasers like amphetamines are supposed to increase wakefulness (59), partly through the D2 receptor (60), TSD is suggested to involve the D2 dopaminergic system. The role of dopamine itself in improving mood remains inconclusive; according to Volkow et al. (61), no preclinical evidence exists for dopamine increases in striatum following TSD. In healthy adults, Liggins et al. (62) recently showed that the dopamine augments L-DOPA does not affect positive mood, in contrast to inducing hypomania in patients with bipolar disorder, suggesting a sensitized dopaminergic system in mood disorder.

Cognitive changes

After 25 hours of wakefulness, increased dorsomedial prefrontal metabolic activity was shown, probably related to monitoring of emotional arousal (63) and underscoring the effort to maintain cognitive top-down control. These results are in apparent contrast with reduced prefrontal metabolic activity in TSD of progressively longer duration (24-72 h), with corresponding decrements in performance on tasks requiring complex

cognitive executive processing (64). Increased activation in the proximity of the medial prefrontal gyrus, combined with a reduced response speed on neutral words and positive targets with neutral distracters is in line with Drummond et al. (65). He found in a psychomotor vigilance 'go/no-go' task slow responses to be associated with greater activation of the medial prefrontal regions implicated in the 'default mode network' (DMN). The DMN is normally active when the brain is not involved in active goal-directed behaviour. Sleep loss may result in inappropriate activation of the default systems or lead to failure to effectively re-allocate resources to task relevant brain regions when needed. Taking these results into account, we expect to have captured a time frame in which adaptive cognitive brain stress systems were activated and subjects were struggling to keep control and awake.

Although some disinhibition due to increased impulsiveness was hypothesized, this was not encountered. The fMRI affective classification task may need further adaptation to become a potent 'go/no-go' design, gathering information on response inhibition as the underlying cognitive process (64,65) though processing emotional valent words may be invariably arousing. In response to TSD, in solving moral dilemmas, Tempesta et al. only found decreased reaction time for impersonal problems, involving low emotional engagement (66).

Finally, speed of thought was reported to be reduced. According to Harrison and Horne, TSD significantly impairs the ability to think laterally, innovatively and flexibly (67). These findings are akin to cognitive symptoms in MDD. Whereas reduced sleep is a neurobiological core feature in the majority of patients with (hypo)mania, the clinical presentation involves increased associative thinking, along with cognitive and affective disinhibition, suggesting a differential effect of lack of sleep on cognitive processing in various mood disorders.

Neurophysiological and endocrine changes

From an evolutionary perspective, staying awake has served to guard against outside threats, requiring the ability to mobilize stress related systems and to exert motivational control over the waking state.

HPA axis activity, one of the key endocrine response mechanism to a stressful situation, was significantly blunted, as shown by the saliva cortisol awakening response (CAR) and cortisol output over the day. As the CAR is influenced by the suprachiasmatic

nucleus, and fine-tuned by neuronal input to the adrenal cortex by the sympathetic nervous system (68), the circadian rhythm may be deregulated by sleep deprivation. For the remainder of the day, robust attenuation of the HPA mediated response was thought to be a consequence of the absence of the initial physiological awakening response. In line with the present study, Vzontgas et al. found lowered, though not significantly, 24 hour plasma cortisol levels in blood (69). Both results are in contrast with findings of high glucocorticoid levels in acute sleep loss, interpreted as reflecting the stress effort to stay awake (70).

In response to TSD, in the striatum significantly decreased [^{11}C]raclopride BP_{ND} binding was shown, in line with Volkow et al. (61,71). Using a methylphenidate dopamine transporter (DAT) blocking design, Volkow et al. showed decreased [^{11}C]raclopride BP_{ND} binding not to be due to an increase in dopamine release, but to decreased D2/D3 receptor affinity. This was hypothesized to be in accordance with D2 receptor internalization, to readjust excitability (61), possibly in response to a temporal increase in dopamine availability to promote wakefulness. Along this reasoning, decreased alertness would be in line with reduced D2 receptor availability, involved in wakefulness (60).

Evidently, it is appealing to repeat this design in a group of MDD patients. According to Yoder et al. even slight differences in cognitive states between groups may have an effect on BP_{ND} , mediated by changes in endogenous dopamine concentration (72). In MDD, sleep deprivation is hypothesized to work as a motivational psychostimulant by first increasing dopamine, and subsequently reducing dopamine sensitivity in the limbic system, normalizing increased transmission (73). Additional information on cortical dopamine receptor transmission may be acquired using extrastriatal PET D2 receptor ligands such as [^{11}C]FLB 497 (74) and [^{18}F]fallypride (75). Despite the availability of D1 tracers like [^{11}C]SCH 23390 and [^{11}C]NNC-112 for imaging of prefrontal cortical dopaminergic functioning, their use is currently hampered by lack of specificity (76,77).

As both D1 and D2 receptors contribute to the dopaminergic regulation of the HPA axis functioning, partly by acting as a modulator of CRH activation (78,79), decreased affinity of the D2 receptor is congruent with the downscaling of the CRH mediated HPA response in the present study. Translated to clinical practice, blunting of the HPA axis response may possibly explain some of the beneficial effects of sleep deprivation in MDD, as depressed patients tend to be hypervigilant and overaroused, though this hypothesis needs to be proven in future studies.

FUTURE PERSPECTIVES

GABA

Mood disorders are all related to inhibition, with either too much *behavioural* inhibition, as in depressive disorder, or too little, as in (hypo)mania in bipolar disorder. However, at the molecular level, *deficits* in GABAergic inhibition are implicated in the pathophysiology of depressive disorder (80).

Relatively new is the discovery of GABA's role in mediating rest–stimulus interactions. According to Price and Drevets, fMRI resting state hyperactivity is evident in MDD, with a substantial role for the limbic system (81), GABA and glutamate (82–84). Transcranial magnetic stimulation (TMS) allows for measurements of resting-state activity in terms of neural inhibition (85). By potentiating GABAergic tone, cortical inhibition is supposed to reduce excessive cortical excitability, forming an intriguing intervention. With respect to neuropharmacology, GABA will remain of interest due to the finding of new GABA_A and GABA_B drugs, and by recognizing the strengths of receptor genetics in altering the conformation and distribution of GABA_A receptors. In MDD, altered expression of GABA_A receptor subunit transcripts have been found, suggesting GABAergic gene expression to be subject to epigenetic control (86). In a genome wide association analysis (GWAS) study of bipolar disorder, polymorphisms within the gene encoding the GABA_A receptor β 1 subunit were found (87), especially in individuals with schizo-affective and psychotic features, although not replicated in a larger study (88). GABA receptor genomics may yield an individualized therapeutic tool.

Circadian rhythm and sleep deprivation

Diagnostic and therapeutic interventions aimed at restoring the circadian rhythm in mood disorders are both highly applicable and desirable. Apart from providing additional therapeutic means with low economic costs, they provide an understanding of mood disorder related mechanisms, and may hold predictive power for the course of the illness.

In the new VUmc Neuro Imaging Center, a combined PET-MRI is available. Since PET is unrivalled for quantification of specific molecular targets, whereas MRI adds the anatomical localisation and functional neuronal activity, this provides great opportunities for further imaging of mood disorder related systems.

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