Dose-escalation in the picture: pharmacological and imaging studies in depression

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Citation for published version (APA):

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SUMMARY, CONCLUSIONS AND GENERAL DISCUSSION
Summary

In this chapter, a summary of the conclusions of our studies and the discussion of these findings will be presented. Possible limitations and strengths of the thesis will be discussed and directions for future research will be presented.

Summary of this thesis and conclusions

This thesis addressed several questions:

1. **Is a short, easy to use clinician rated questionnaires as effective and precise as the routine Hamilton depression rating scale (HDRS)?**
   In chapter 3, we reanalyzed the treatment-outcomes of two antidepressant-psychotherapy trials. In line with previous reports, we found that the effect-sizes of two 6-item sub-scales of the HDRS (17-items) – the Maier6 and Bech7 sub-scales – were comparable to the original HDRS in the measurement of depression severity, and the sensitivity to measure changes. Furthermore, this comparability was stable across the full range of response to treatment, across both pharmacotherapy and psychotherapy, and for patients with different baseline severities of their depression. With an item response theory approach, we calculated a conversion table linking HDRS-scores and Maier and Bech scores, and determined cut-off points for remission for these subscales compared with conventional HDRS definitions. With these subscales clinicians can measure depression severity and clinical response more efficient than with the original HDRS.

2. **What is the evidence for dose-escalation as a strategy for non-response to a first SSRI?**
   In chapter 4, we presented a systematic review of the evidence for the dose response relationship of selective serotonin reuptake inhibitors (SSRIs) in major depressive disorder (MDD). In our literature-search, we identified 8 dose-escalation studies that increased dosages after at least 3 weeks of a standard dosage. Furthermore, 3 systematic reviews were published by then, which included three or four of the eight identified dose-escalation studies. Only one of the dose-escalation studies approached stringent methodological criteria. We found no evidence for increased efficacy of dose-escalation within the first 4 weeks of treatment. Dose-escalation after 6 weeks appeared less effective than continuing the same dose. We found some, but limited evidence for efficacy of dose-escalation after 8 weeks, particularly in partial responders. This effect was seen within 4 weeks after dose-escalation. Irrespective of efficacy, dose-escalation unequivocally increased side-effects, but effects on drop-out rates due to side effects were less straightforward. We therefore concluded that the available evidence for dose-escalation neither unequivocally confirmed its efficacy, nor deemed it ineffective. This conclusion was the starting point for the DELPHI-study, described in the chapters 2 and 7 to 10.

3. **What is the evidence for switching antidepressants as a strategy for non-response to a first SSRI?**
   In chapter 5, we presented a systematic review of the evidence for switching after failure of a first SSRI in MDD. In addition to our literature-search we included four studies released after these searches, of which three studies from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial. We identified eight randomized controlled trials (RCTs) and 23 open switch studies. Studies were of variable methodological quality and carried out in heterogeneous populations. The STAR*D results largely increased the amount, and quality of the available evidence, but did not show differential class effects to guide switching. Three randomized switch-studies investigating the switch from SSRI to another SSRI or SNRI (venlafaxine) were eligible for a meta-analysis. This meta-analysis showed that in the most favorable analysis, venlafaxine was slightly more effective as a second antidepressant,
compared with other SSRIs (number needed to treat (NNT) = 10 (95% confidence interval (95% CI) 6.3-33.3). We therefore concluded that after the failure of a first SSRI switching is open to all antidepressant classes (except irreversible MAO-inhibitors), without clear recommendations other than those that apply for the selection of initial treatment.

4 Does the depletion of monoamine (5-HT and NA/DA) systems lower mood in humans, and is this lowering of mood different across different populations?
In chapter 6, we presented a systematic review of monoamine depletion studies reporting mood effects of depletion. As an extension of previous systematic reviews of monoamine depletion studies, we pooled the results of 53 small-sized depletion studies with an adapted pooling technique (modified from conventional meta-analyses of RCTs to handle the statistically paired cross-over designs, and including an adjustment for small sample bias). Pooling is important because it quantifies the balance of positive versus negative studies.

We pooled 45 acute tryptophan depletion (ATD) studies and 8 acute phenylalanine/tyrosine depletion (APTD) studies, acutely lowering serotonergic and dopaminergic/norepinephrinergic neurotransmission, respectively. Serotonin or dopamine/norepinephrine depletion did not decrease mood in healthy controls, but slightly lowered mood in healthy controls with a family history of MDD. In drug-free patients with MDD in remission, a moderate mood decrease was found for ATD, without an effect of APTD. ATD induced relapse in patients with MDD in remission who used serotonergic antidepressants. MDD patients did not experience mood deterioration after ATD while depressed.

We concluded that simple, direct correlations of serotonin or dopamine/norepinephrine levels in the brain and mood do not exist. Because the serotonin or dopamine/norepinephrine depletion induced by ATD or APTD most clearly decreases mood in vulnerable individuals (patients who are in remission from MDD and healthy controls with a positive family history for MDD), we concluded that the monoamine systems are important systems in the vulnerability to become depressed. Moreover, the changes in brain metabolism in remitted patients who relapse after ATD or AMPT suggest that the serotonergic and norepinephrinergic systems give input to a final common pathway which needs further research to be clarified.

5 Do MDD-patients and healthy controls differ in the number of central serotonin transporters, and is the amount of available SERTs correlated with depression severity?
In chapter 7, we measured the availabilities of the central serotonin transporters (SERTs) in baseline SPECT scans of the DELPHI-SPECT participants, and compared these with SERT availabilities in age- and sex-matched healthy controls. Because of earlier reports, we investigated potential covariates as smoking behaviour, age, season of scanning and possible interactions with gender in multivariate models. We found interaction effects of smoking and diagnosis with gender for diencephalon SERT availability. For midbrain SERT availability, an interaction of gender with diagnosis was found, while season of scanning was a covariate. These interaction effects were expressed as lower SERT availability in midbrain and diencephalon in depressed males, and higher SERT availability in diencephalon in depressed (non-smoking) females compared with healthy controls. These findings point to complex effects of gender, smoking and season of scanning on the serotonergic system in healthy controls and patients with MDD, which must be considered in future studies comparing SERT availabilities in MDD patients versus healthy controls.

6 Does a common genetic polymorphism of the promoter region of the serotonin transporter gene (SLC6A4) modify the association between the SERT occupancy by paroxetine and the clinical response?
In chapter 8, we studied the relationship between clinical response after 6 weeks of paroxetine treatment and SERT occupancy (which is the change in SERT availability after 6 weeks of treatment relative to the pre-treatment SERT availability). Because clinical response to SSRIs is likely associated with polymorphisms of the serotonin transporter gene promoter region (5-HTTLPR), we also aimed to investigate whether this relation of
response and SERT occupancy was modified by the 5-HTTLPR genotype. We obtained study-entry and week 6 SPECT scans for 44 patients treated with paroxetine 20 mg/day, of which 42 scans were analyzable (10 responders and 32 non-responders).

We found that for all patients, SERT occupancy was not associated with clinical response (expressed as the proportional decrease in HDRS relative to study entry). However, when we grouped patients by 5-HTTLPR polymorphism, we found that in patients with the (favorable) L/A/LA variant a significant relation between SERT occupancy and clinical response (absolute and proportional change in HDRS) existed. A previous study found a better amygdala-cingulate coupling in subjects with an L-allele relative to the unfavorable S/S variant, probably reflecting differences in the development of the serotonergic system induced by 5-HTTLPR. As an explanation of our findings, we therefore hypothesized that patients with the 5-HTTLPR L/A/LA genotype have a more flexible serotonergic system, which is more easily influenced by serotonergic antidepressants.

7 Is dose-escalation of paroxetine an effective clinical strategy for non-response in MDD?

In chapter 9, we studied the strategy of dose-escalation which was found equivocally efficacious in the systematic review, as described in chapter 4. In this study, we addressed the methodological flaws found in previous dose-escalation trials, but moreover, we also measured whether paroxetine dose-escalation increased SERT occupancy more than placebo dose-escalation. After inclusion of 49 SPECT patients (with successful follow-up of 31 patients participating in the randomized placebo-controlled dose-escalation SPECT study), we performed an interim analysis including all randomized patients (n=57), and tested differences between placebo and true dose-escalation with pre-defined cut-off values for futility and superiority. We found no clinical benefits of true dose-escalation compared with placebo, and so the trial was stopped because of futility.

Most important, our data also showed that true dose-escalation of paroxetine did not increase SERT occupancy more than placebo dose-escalation, probably because a plateau was reached at standard doses already. We concluded, that in line with previous, though more equivocal evidence, dose-escalation is not beneficial in MDD. Therefore, two clinical options remain for the treatment of MDD-patients who do not respond to standard doses: either continuation of treatment until 10 weeks while waiting for a potential delayed response, or a switch to a different and potentially more effective treatment strategy (e.g. another SSRI or SNRI or TCA, or psychotherapy, or a combination).

8 Does treatment with paroxetine normalize amygdala hyperactivation in MDD?

In chapter 10, we describe the (negative) faces task of the DELPHI-fMRI sub-study. We studied the effects of paroxetine treatment on the hyperactivation of the amygdala (and other brain areas) during a depressive episode. We specifically investigated: 1) whether amygdala-activation by (negative) facial expressions differed from healthy controls, 2) whether amygdala activation changed after 6 and 12 weeks of treatment, 3) whether the amygdala activation merely changed by paroxetine treatment or in relation with clinical response, and 4) whether dose-escalation of paroxetine in week 6 non-responders affected activations, compared with placebo-dose-escalation. Apart from the amygdala, we also investigated these questions for other brain areas. We performed fMRI scans in 22 MDD patients and 21 age- and sex-matched healthy controls (controls were scanned once).

Compared with healthy controls, we found increased activations in bilateral (extended) amygdala and left insula in MDD-patients. In contrast with previous studies, we found an increase in right amygdala activation after 6 weeks of treatment, which was attributed to the high number (12/20) of treatment non-responders. This increased amygdala-activation was reduced thereafter until week 12. When we analyzed the differences in week 6 and 12 treatment responders versus week 6 and 12 non-responders, bilateral amygdala activation was reduced in responders versus non-responders. Amygdala activations in week 6 and 12 were associated with HDRS-scores. Furthermore, non-responders had higher activations in
right orbitofrontal cortex (OFC) and insula, while treatment responders showed higher activation in right dorsolateral prefrontal cortex (DLPFC) and left nucleus accumbens. These differences were non-existent at study-entry.

Although randomized groups became small, changes over time in the true dose-escalation group showed decreased activations in ventral and dorsal regions, while activations of the right hippocampus and left subgenual cingulate increased, when compared with changes observed after placebo dose-escalation. We concluded that in line with previous treatment studies paroxetine treatment over 12 weeks reduced activations in the amygdala and other (ventral) emotion-generating structures, and increased cortical function (in the dorsal regulatory structures; see chapter 1, pages 18 and 21), especially in treatment responders. These findings may point to increased fronto-limbic control as a mechanism of paroxetine drug-response effects, which was supported by findings of other groups.

9 **What are the changes in hemostasis and blood platelet parameters when patients are treated with paroxetine, and are these changes modified by dose-escalation or a genetic polymorphism of the promoter region of the serotonin transporter gene?**

In chapter 11, we present the results of a study investigating an infrequent, but dangerous adverse effect of SSRIs: increased bleeding tendency. In this study, we measured platelet parameters and coagulation, while we applied a secondary (non-randomized) dose-escalation in paroxetine treatment non-responders. We additionally investigated whether 5-HTTLPR polymorphisms modified these effects on platelet parameters. We found that a standard dose of paroxetine (20 mg/day) already significantly decreased platelet serotonin levels and platelet β-thromboglobulin (β-TG), without further decreases after dose-escalation. Moreover, we found that 5-HTTLPR polymorphisms modified these effects: compared with L_A/L_A-carriers, bleeding time significantly increased in <2L_A-allele carriers, and platelet serotonin decrease was larger in patients without L_A-alleles. Furthermore, the platelet function analyzer closure time significantly increased in patients without L_A-alleles. Although the observed bleeding tendency presumably is not clinically relevant in patients without trauma or surgery (unless a preexisting platelet abnormality is present), these findings are applicable to patients with a previous severe bleeding episode. For them genotyping may be considered; when an S'/S' genotype is found, switching to another, non-serotonergic antidepressant might be advisable.

**Clinical relevance**

Antidepressants have comparable efficacy, however only 50% of the MDD-patients respond to the first antidepressant trial given, while fewer achieve full remission of symptoms. When the miracle doesn't happen, there are 5 strategies for non-response: prolongation of the initial trial, dose-escalation, switching to another drug, augmentation with another drug or a combination of antidepressants. This thesis addresses switching strategies and dose-escalation.

**Switching**

Despite a small benefit of a switch to venlafaxine compared with a second SSRI (in the most favorable analysis only), we concluded that switching-options after a first SSRI are open to all antidepressant classes, without clear recommendations other than those that apply for the selection of initial treatment. One more RCT comparing venlafaxine and citalopram, and three open studies, one with venlafaxine (with randomization to different doses), one with a venlafaxine-mirtazapine combination strategy, and one with duloxetine appeared thereafter.

In addition, another meta-analysis was published, comparing a non-SSRI switch (venlafaxine (3 studies) or mirtazapine (1 study) or bupropion (1 study)) versus a second SSRI. This meta-analysis included 2 different studies (abstracts from symposia) compared with our meta-analysis, and excluded one study with questionable methodology, like we did in our most
favorable analysis. Their conclusion was that the NNT for remission was 22 (confidence interval not given), modestly in favor of a non-SSRI switch, which is far below the standard for clinical relevance (NNT ≤ 10) as suggested by the United Kingdom’s National Institute of Clinical Excellence. When they considered response as an outcome, they found no significant benefit, comparable to our results. In the final manuscript of the previous abstract, Lenox-Smith and Jiang reported increased efficacy of venlafaxine over citalopram (flexible doses) in a subgroup of patients with severe MDD (HDRS21 > 31), who participated in a multicenter RCT of SSRI non-responders. However, this difference was only significant for the continuous scores of the HDRS21 (difference approximately 4.7 points; SD not given), and not for the remission rates. Therefore, we think that only at the level of public health, the difference between switching-strategies might be interesting, but not at the level of individual patients.

**Dose-escalation**

In our systematic review of dose-escalation it was concluded that the available evidence neither unequivocally confirmed its efficacy, nor deemed it ineffective. This was in agreement with another review, performed by an independent group, and published just before ours. The results of the dose-escalation RCT in this thesis first of all addressed the methodological shortcomings of previous studies, and was stopped for futility after an interim analysis. Even though the numbers of patients randomized to placebo or true dose-escalation were modest (n= 27 and n= 30, respectively), the changes in HDRS-scores and subscales were far from significantly different, while the level of this significance was far above the a prioricut-off for futility (which was undisclosed at the time of analysis). This finding aligns with the dose-escalation study by Licht et al. Unfortunately, the premature termination of the trial precluded planned secondary analyses to distinguish subgroups who still might benefit form dose-escalation (e.g. partial responders; defined as 25-50% reduction of HDRS). In our sample the subgroup of partial responders consisted of 24 patients (from 60 randomized; 40%), and did not show any indication of beneficial treatment-effects for true dose-escalation. Nevertheless this observation cannot replace true subgroup analyses in larger samples.

Our sub-study of the changes in SERT occupancy during the randomized, placebo-controlled dose-escalation phase further corroborates our clinical findings. We therefore conclude that our systematic review, our dose-escalation study and our imaging study provide substantial evidence for clinicians, that dose-escalation is not an effective strategy for depressed patients who do not respond to 6 weeks of a first SSRI at a standard dose.

In a recent meta-analysis of 9 placebo-controlled, fixed-dose, dose-finding studies of SSRIs, Papakostas et al. found a modest but statistically significant difference of 4% higher response rates in patients who started with higher than standard doses of citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline (p= 0.04). This 4% difference in response rates corresponds with a NNT of 25, which is clinically not impressive, nor relevant. In their meta-analysis, Papakostas et al. could not investigate whether this difference was attributable to a single SSRI. However, when inspecting their data, all paroxetine trials did not show benefits of higher paroxetine doses compared with standard 20 mg/day doses, with relative risks of 1.00 (95% CI 0.67-1.50), 1.02 (95% CI 0.69-1.52) and 0.94 (95% CI 0.65-1.35). In contrast, the rate of discontinuation due to adverse events was also higher in the patients treated in the above standard doses group (16.5% compared with 9.8%). Therefore, to our opinion, it remains also questionable whether higher doses of SSRIs at the initiation of treatment indeed are advantageous to MDD-patients. Instead, they could give patients more adverse effects and preclude prolonged exposure to tolerable standard doses. Finally, but most important for this thesis, these findings presumably hardly apply to paroxetine.

**Other findings of clinical relevance**

Besides the switching and dose-escalation strategies, two other relevant points from this thesis emerge. First, we showed that the Maier and Bech subscales of the HDRS17 are equivalent to the full HDRS17. Others even suggested that these subscales might measure the core-symptoms and
severity of MDD more specifically, and their use might even be more efficient, because of the reduction of noise by less responsive items. We therefore propose that the Maier or Bech-subscales should be used in the clinical assessment of (treatment of) MDD patients, instead of, or besides self-rating scales like the Inventory for depressive symptoms self-rated (IDS-SR) or Beck depression inventory (BDI). This will be of great importance in patients who cannot reliably fill out self-rated questionnaires, like in MDD with psychotic features or MDD with severe cognitive dysfunction. Of course, providing a shorter, easy to use clinician rated scale will not translate in direct implementation of one of these subscales, but may at least reduce the time burden of rating scales for physicians.

Second, our findings regarding the increased bleeding tendency in patients merit attention. Increased bleeding tendency, especially in combination with non-steroidal anti-inflammatory drugs (NSAIDs) was observed before. This even led to the recommendation to use TCAs when concomitant NSAIDs should be used in the guideline for Dutch general practitioners. However, the serotonergic TCA clomipramine, presumably has comparable effects. Because of the relatively low incidence of dangerous gastro-intestinal bleeding complications in general (4.3/1000 SSRI treatment years; 14.5/1000 SSRI+NSAID/aspirin treatment years; relative to 1.2/1000/year in untreated humans), the increased bleeding tendency by SSRIs is of no clinical relevance in patients without trauma, surgery or a previous severe bleeding episode. However, in patients with a previous severe bleeding episode, or in patients who need elective surgery, and maybe also in patients regularly using NSAIDs, genotyping of the 5-HTTLPR may be considered. When an S'/S' genotype is found, switching to another, non-serotonergic antidepressant might be advisable. For which of the aforementioned subgroups this genotyping will be cost-effective, remains to be investigated.

Neurobiology of the treatment of major depressive disorder

With our studies we also aimed to investigate the neurobiology of paroxetine treatment effects in MDD in vivo. The enhancement of serotonergic neurotransmission by paroxetine is undoubted, caused by a reduced serotonin uptake after SERT occupancy as the primary mechanism. Therefore, the most important question which we addressed was whether dose-escalation of paroxetine would increase SERT occupancy compared with placebo.

SERT occupancy after short or prolonged treatment with SSRIs was investigated before even at variable and higher doses. However, without a pre-assessment of SERT availability before treatment and without randomization to a placebo-controlled dose-escalation, one can never exclude the possibility that non-responders after 6 weeks of a standard dose consist of a selected sample who had lower SERT occupancy. This hypothetically lower SERT occupancy could then be considered explanatory for non-response, and might increase by subsequent dose-escalation compared with placebo dose-escalation.

Our SPECT measurements in the subgroup of the DELPHI study showed no overall relation between SERT occupancy and clinical response in the open phase of treatment with paroxetine over 6 weeks. Furthermore, we found no evidence for the postulated 80% SERT occupancy as a requisite for clinical response. In addition, the second, randomized, placebo-controlled dose-escalation phase of DELPHI showed no differences in changes in SERT occupancy between placebo and true dose-escalation for 6 more weeks. With this latter finding, we are the first group that studied sequential dose-escalation in non-responders, controlling for placebo-effects and measurement-bias by repeated SPECT scans. Interestingly, we did find an association between SERT occupancy and clinical response in the subgroup of patients with the (tri-allelic) L_A/L_A polymorphism of the 5-HTTLPR. Because the tri-allelic L_A:L_C ratio is approximately 5:1, our finding aligns with data from previous treatment studies which found increased response rates in subjects with the bi-allelic L/L 5-HTTLPR polymorphism.
However, the mechanism why increased SERT occupancy is associated with increased clinical response in L/A/LA carrying patients, cannot be explained by higher SERT occupancies in L/A/LA patients.\textsuperscript{45} Probably, the explanation must be sought in differences in the function of the serotonergic system (and its development) mediated by 5-HTTLPR polymorphisms. From previous lines of research, it may be concluded that the S-allele predisposes to a more reactive arousal system, and that the L-allele accounts for more flexibility and/or the possibility to react and adapt better to external changes imposed on the subject.\textsuperscript{98} Furthermore an elegant fMRI-study showed that both the anatomy and functional connections between limbic, subcortical and cortical regions are mediated by 5-HTTLPR polymorphisms, with S/S carriers showing relative uncoupling of the amygdala-pregenual cingulate connectivity.\textsuperscript{48}

The biochemical consequences of chronic administration of SSRIs are thought to include an increase in extracellular levels of serotonin followed by neuroadaptive alterations in serotonin receptors and postsynaptic intracellular signalling pathways (see figure 1.2, page 20), as well as time-dependent effects on neurogenesis.\textsuperscript{99,100} Not surprisingly, animal studies demonstrated that in mice knocked out for the murine Slc6a4 SERT transporter (Slc6a4\textsuperscript{-/-}), fluoxetine and fluvoxamine are ineffective on behavioural and other measures, while mice with at least one copy of the allele (Slc6a4\textsuperscript{S+L} or Slc6a4\textsuperscript{S+S}) showed the typical antidepressant effects.\textsuperscript{99} This is pivotal evidence for the working mechanism of SSRIs, for which SERTs are apparently required to be present. The Slc6a4\textsuperscript{S+L} mice have ~50% less expression and transport function and can be viewed as a model for humans with the S/S genotype.\textsuperscript{101} Therefore, the findings of almost identical effects of SSRIs in Slc6a4\textsuperscript{S+L} or Slc6a4\textsuperscript{S+S} mice\textsuperscript{102} do not support the findings of differential effects for 5-HTTLPR polymorphisms from meta-analyses of human studies.\textsuperscript{46,47} However, the 5-HTTLPR polymorphism is only present in humans and higher order primates (e.g. macaques). To our knowledge, no studies in primates investigated the mediating effects of 5-HTTLPR polymorphisms on the relation between SERT occupancy and behavioural effects. Furthermore, we are unaware of studies investigating dose-response relationships of SSRIs in animals. Finally, although a robust increase in anxiety-like behaviour was found in Slc6a4\textsuperscript{S-/-} mice compared with other genotypes, so far no studies were performed to investigate the differences in brain development and functional connections for mice with a Slc6a4\textsuperscript{S-/-}, Slc6a4\textsuperscript{S+L} or Slc6a4\textsuperscript{S+S} genotype.\textsuperscript{103}

Serotonin has an important role in the regulation of neuronal information processing by constraining (instead of inhibiting) the reactivity of the brain to internal and external stressors.\textsuperscript{104} In general, serotonin appears 1) to prevent an overshoot of other dynamic systems (e.g. constraining the release of dopamine after rewarding stimuli), and 2) to control the sensitivity of the system to perturbation by new elements entering the system (e.g. constraining a natural propensity to switch to alternate behaviours; this can be provoked by serotonin depletion). This constraint can be experimentally challenged by acute tryptophan depletion (ATD), which results in increased perception of aversive stimuli, increased food intake, increased sexual, aggressive, depression-like and anxiety-like behaviours, but also decreased sensitivity to cues of punishment, and less flexibility to change behaviour in rats.\textsuperscript{104}

Besides the cognitive effects,\textsuperscript{98} our meta-analysis quantified the mild mood lowering effects by ATD in humans, which were especially seen in healthy controls with depressed relatives, and patients who had remitted from a previous MDD-episode.\textsuperscript{32} Because mood-effects can be considered as more complex behaviour, these may be more difficult to provoke in relatively short-term ATD studies compared with longer-lasting studies in animals. Nevertheless, the large body of research on the serotonergic system has now changed the categorical model – decreased serotonin is specific for depression – into a model in which alterations in the serotonergic neurotransmission can be seen as a biological risk-factor, which interacts with innate and/or external factors. This so-called serotonergic vulnerability is now seen as neither a sufficient, nor necessary factor to develop and maintain MDD, but in combination with other factors increases the risk for MDD.\textsuperscript{98}
Our fMRI-study, corroborates the hypothesis that paroxetine treatment interferes with the (maladaptive) functioning of various interconnected regions in the limbic-subcortical-cortical network. Like others, we found increased ventral* activations (e.g. in the amygdala and insula), and decreased dorsal* activations (anterior cingulate, DMPFC, DLPFC) in MDD-patients relative to healthy controls. We furthermore found that the ventral hyperactivation and dorsal hypoactivation was normalized in treatment responders, but not in non-responders. Recently, Chen et al. demonstrated increased functional coupling of the amygdala with the frontal and (pregenual) cingulate cortex (and striatum and thalamus) after 8 weeks of sertraline treatment. Although we did not investigate this coupling per se, we found that the amygdala responses in our patients were correlated with pregenual cingulate and frontal regions.

Furthermore, in responders we found increased activation in the nucleus accumbens, pointing to the involvement of the limbic-cortico-striato-pallido-thalamic network, which indirectly suggests the involvement of changes in dopaminergic neurotransmission after paroxetine treatment. Two coinciding explanations can be given for this increased activation of the nucleus accumbens. First, in rodents, chronic treatment with antidepressants (among others SSRIs) potentiated dopamine transmission, due to an increased sensitivity of postsynaptic dopamine receptors and possibly also due to a decreased sensitivity of presynaptic (inhibitive) dopamine autoreceptors, preferentially in the limbic system. Second, again in rodents, decreases in serotonin are associated with a release of normally inhibited behaviour, including impaired suppression of behaviour inducing punishment. Activation of the nucleus accumbens is associated with anticipation of reward, while the nucleus accumbens is deactivated by loss or punishment. Therefore, it might be postulated that the increase in serotonergic neurotransmission by SSRIs, reduces a lack of constraint on dopaminergic neurotransmission in the nucleus accumbens, with subsequently less punishment perceived. This would then result in less deactivation of the nucleus accumbens. The fact that this was demonstrated in treatment responders relative to non-responders might suggest that in non-responders this enhanced dopaminergic constraint might not be achieved.

As interesting, but contradictive finding, we did not find any indication that dose-escalation increased SERT occupancy, but at the other hand, we found indications that a true dose-escalation decreased hyperactivations in the ventral compartment (OFC, insula, VMPFC, ventrolateral thalamus), and increased activation in the dorsal compartment (hippocampus, DLPFC, and parietal cortex). Also we counter intuitively found an increased activation in the subgenual cingulate after dose-escalation. As explained, we could unfortunately not disentangle response- and drug-effects. If the decreases in the ventral, and the increases in the dorsal compartment could have been attributed to responders to dose-escalation, while the increased activation in the subgenual cingulate was associated with non-responders (despite dose-escalation), this would have made our findings less surprising.

**Limitations**

Limitations of the studies in this thesis were discussed in each chapter. However, some must be recapitulated in this general discussion.

At the moment of publication of this thesis, the systematic reviews presented in the chapters 4, 5, and 6 might have become outdated, since the literature searches were performed until October 2006 or February 2005. Although we did not repeat the searches until July 2008, for the dose-escalation and switching reviews, we commented on additional studies that we encountered thereafter in this general discussion. We did not review additional monoamine depletion studies, as this should have resulted in new meta-analyses.

* For explanation of the ventral and dorsal compartments of the limbic-subcortical-cortical network, see chapter 1, page 18.
Furthermore, in contrast with other authors, we did not perform a meta-analysis for the dose-escalation studies, and only for the venlafaxine versus second SSRI switch studies. At this point, we considered the dose-escalation studies with various moments of randomization after the initiation of treatment too heterogeneous to pool. For switching, we decided not to pool (by then unpublished) data on various dual acting antidepressants versus second SSRIs.

In the SPECT-studies, we used $[^{23}I] \beta$-CIT for SPECT imaging, which is a non-selective radioligand, and also binds to the dopamine transporter (DAT; e.g. substantia nigra) and norepinephrine transporter (NET; e.g. locus coeruleus). Nowadays, selective SERT ligands like $[^{11}C]$DASB for PET or $[^{23}I]$ADAM for SPECT are available. However, previous imaging study in primates, showed that $[^{23}I] \beta$-CIT uptake in midbrain and diencephalon predominantly reflects SERT, as these structures are rich of SERT relative to DAT and NET. Although this non-selectivity might have slightly concealed changes in SERT occupancies due to additional DAT- or NET-binding, we think our findings in diencephalon and midbrain mainly reflect SERT occupancy. Furthermore, if one would consider non-selectivity as relevant, the use of SPECT-scans at randomization as a reference for changes in occupancy will further reduce the magnitude of this non-selectivity bias, because non-selectivity will be measured systematically between T0 and T1. Despite this rationale, in future studies, a selective ligand for SERT ($[^{11}C]$DASB or $[^{23}I]$ADAM) should be considered. Furthermore, it would be a challenge to replicate our study using one of these selective ligands.

Although the region-of-interest (ROI) approach to determine non-displaceable binding potential (BP$_{ND}$) and SERT occupancies is a valid and widely accepted method, we did not co-register individual SPECT images with individual structural MRI scans. This might have reduced signal-to-noise variance in our measurements. However, because we applied a randomized, double-blind dose-escalation, and analyzed the SPECT scans while blinded for the moment in treatment (T0 or T1) and intervention, we think that this ROI approach only affected measurement-bias, and did not introduce differential bias between the intervention groups.

Scientific studies are powered to investigate major questions, but often additional questions will be explored. In the case-control study of SERT availability of MDD-patients versus controls, only small subgroups remained for effect-modifying variables, which might have increased the chance of detecting spurious results. We therefore presented these differences as exploratory analyses only. For the SERT occupancy-response relation by genotype interaction, we also compared small groups, but nevertheless found significant results, which we consider valid. Nevertheless, replication in a larger sample would strengthen these results. Because of the interim-analysis, we stopped our trial for futility. Thereafter, our modest sample size of the randomized dose-escalation for example precluded proper subgroup analyses. Our fMRI-study, which was relatively large for imaging studies found robust effects. However, the comparison of the dose-escalation versus placebo dose-escalation in our fMRI study was hindered by a modest number of patients in the true dose-escalation group. This precluded the investigation of the 3-way interaction intervention by time by response, which could have properly answered which effects were drug-induced. Therefore, as indicated, these results must be considered with some restraint. Finally, in the hemostasis study, we studied 19 patients, but did so in a within-subject design, which has more power to detect changes. Again the number of subjects receiving a dose-escalation was modest, which might have precluded changes in other platelet parameters with smaller effect-sizes.

A general limitation is that our neurobiological investigations did not investigate adaptive neuronal effects caused by paroxetine. We either investigated the pre-synaptic effects of dose-escalation on SERT occupancy, or the changes in the final common pathway: the changes in activations of the limbic-subcortical-cortical network. Many adaptive pre- and post-synaptic effects of SSRIs have been documented, mostly in animal studies, but also in humans. We did not investigate any of these specific secondary effects of SSRIs. The radiation burden for scientific research did not allow additional radioligand scans of other receptors (e.g. 5-HT$_{2A}$ or 5-HT$_{1A}$) or other monoamines (e.g. endogenous dopamine release). Furthermore, additional challenges (e.g. gepirone to assess 5-HT$_{1A}$ receptor sensitivity) were considered too burdensome in these patients. We did not perform animal studies to investigate dose-escalation,
for example in microdialysis experiments or studies investigating SERT occupancy, SERT down-regulation, or other secondary effects of serotonergic neurotransmission on receptors and/or other pathways.

Nevertheless these limitations, one should bear in mind that our, and previous clinical studies did not support dose-escalation as a rational strategy for SSRI non-responders in MDD. Therefore, the merits of additional dose-escalation studies would be to provide fundamental insights in working mechanisms.

Finally, this thesis might also make clinicians wonder why initial, high doses of SSRI have been found effective in (some) fixed-dose, dose-finding studies in anxiety disorders, and especially in obsessive compulsive disorder (OCD). Of course, anxiety disorders are beyond the scope of this thesis, but this question remains intriguing and might warrant an approach as we did for MDD.

**Strengths**

First of all, a major strength of this thesis is its clinical starting point. All psychiatrists recognize the problem of non-response to a first SSRI, and most of them increase the dose as a first strategy, followed by switching. As such, this thesis covers an important clinical topic, which could be very relevant for future treatment guidelines.

Our systematic reviews were conducted in accordance with the stringent guidelines of the Cochrane Collaboration. We performed ‘sensitive’ literature searches in several databases, decreasing the chance of missing valuable studies. All studies were critically appraised and abstracted. We only performed meta-analyses when clinical heterogeneity allowed pooling, a methodological requirement which is often violated by referring to a random effects approach. In the obvious heterogenous populations investigated in the monoamine depletion review, we achieved better homogeneity by stratification. In this latter meta-analyses, we acknowledged the statistical problems of pooling crossover studies (having dependent measurements) and adapted the standard statistical approach to solve this problem.

The empirical studies in this thesis were designed carefully and the methodology of the dose-escalation trial was based on a thorough review of the previous dose-escalation studies. We therefore randomized patients after sufficient time to expect not many further delayed clinical responses. Also, we gradually increased doses which obviously prevented early drop-out due to intolerable adverse effects. Most importantly, our SERT occupancy SPECT protocol was unique, and provided the rationale why dose-escalation was ineffective.

With the fMRI-study, which could be started after a second grant was obtained, we looked beyond SERT occupancy to investigate the effects of paroxetine treatment on emotion-regulation. Although the dose-escalation results of this study are not unambiguous, this study is only the sixth fMRI-study investigating the treatment-effects of SSRIs. The fact that we scanned patients three times, and that many patients initially were treatment non-responders allowed us to investigate the distinction in brain activations between drug-effects and clinical response, which is an important addition to the existing literature.

Finally, we also looked at peripheral effects of SSRIs, far beyond the brain, but also considering the inhibition of SERTs by paroxetine in association with hemostasis. Increased bleeding tendency is an infrequent but potentially dangerous adverse effect. We showed that even standard doses of paroxetine might affect hemostasis, and suggested subgroups who are eligible for secondary prevention by genetic screening of the 5-HTTLPR.
Future research

Future studies with data from DELPHI

Several lines of research on data from the DELPHI-study still need to be worked out. We collected salivary samples at study-entry, To and T1 (see chapter 2, page 36). We will investigate the changes in cortisol by treatment over time, and might be able to associate these changes with clinical response or other clinical or imaging (e.g. SERT) variables. We therefore will collaborate with the group of Kirschbaum and Rohleder. In addition, we collected blood-samples at study-entry, To and T1, to determine ex-vivo SERT and norepinephrine inhibition by paroxetine. Finally, we determined omega-3 and omega-6 polyunsaturated fatty acids again at study-entry, To and T1, which will be analyzed in relation with clinical response to paroxetine, and additionally in relation with SERT availability and occupancy.

Furthermore, we are currently working on a sub-study which investigates the association of midbrain and amygdala SERT occupancy with the changes in amygdala-activation after (negative) facial expressions in the patients who underwent both SPECT and fMRI scans.

Future clinical studies

The findings presented in this thesis may be the starting point of new research.
- For better evidence-based recommendations about when to choose between switching, augmentation, combination, or psychotherapeutic strategies as a next step, comparisons of these strategies relative to each other are necessary. This will require future algorithm-based switch studies, including psychotherapy and augmentation or combination strategies to improve our knowledge to guide treatment for SSRI non-responders. In these studies, one arm should be to continue treatment (e.g. for another 6-8 weeks, depending on the timing of switching) as a ‘placebo’ control. Of course the somehow disappointing results from STAR*D should be reconsidered when such a major endeavor is set up again.
- As a smaller enterprise, the relative benefits of switching after 6 weeks versus a continuation of the same treatment for another 6 weeks would provide important guidance to clinicians. Quitkin et al. showed that MDD-patients treated with fluoxetine 20 mg/day who were non-responders at week 6, showed remission rates at week 12 between 31%-41%, indicative of a substantial delayed remission. However studies directly comparing such prolonged treatment versus switching have not yet been performed in MDD.
- The controversial evidence advocating initial, high doses of SSRI in panic disorder, posttraumatic stress disorder, OCD and other anxiety disorders necessitates a well-performed meta-analysis of fixed-dose, dose-finding studies in anxiety disorders, stratified by disorder and drug. In addition, dose-escalation studies in these disorders would be interesting, especially when combined with the investigation of neurobiological mechanisms by neuroimaging. For example, the effects of a randomized, placebo-controlled dose-escalation on dopaminergic D2-like receptors, or dopamine transporters in OCD would be interesting.

Future neurobiological studies

In order to develop more successful treatment approaches, we need to understand better which effects of treatment are required for recovery, and which biomarkers are associated with treatment response. Therefore, more fundamental, neurobiological studies should aim at several gaps in the evidence.
- In order to elucidate SSRI working-mechanisms, the increased coupling between amygdala and frontal and cingulate cortices must be investigated with other SSRIs. Secondly, it should be investigated whether 5-HTTLPR polymorphisms mediate this coupling sec or the increase after SSRIs, or both. If the magnitude of coupling would be mediated by 5-HTTLPR polymorphisms, this would also corroborate our findings for the association between SERT occupancy with clinical response in L_A/L_A carriers.
In order to study secondary effects of SSRI treatment, neuroimaging approaches to quantify the effects on 5-HT$_{1A}$ receptors during treatment and after dose-escalation are warranted in patients. Although a desensitization of 5-HT$_{1A}$ was demonstrated for chronic SSRI treatment in animals, the question remains whether this desensitization is also associated with 5-HT$_{1A}$ downregulation and/or whether dose-response effects occur. This study should ideally be performed in combination with gepirone-challenges at different moments during treatment to assess 5-HT$_{1A}$ receptor sensitivity.

In order to investigate secondary effects of serotonin, e.g. on endogenous dopamine release in the nucleus accumbens and other brain regions, microdialysis experiments in animals, chronically treated with SSRIs, will further investigate dose-escalation and time dependent changes in SSRI treatment.

The hypothesis of the involvement of dopamine in mood and MDD must be investigated. Serotonergic antidepressants indirectly influence the dopaminergic system. Responders to amitriptyline showed decreased [$^{123}$I]IBZM SPECT binding to striatal dopamine D$_2$ receptors, relative to pretreatment. This decrease correlated with a decrease in HDRS-score. This might represent an increased tonic dopamine release after amitriptyline, but may also be attributed to improved psychomotor activity in responders. Nevertheless, a failure to achieve alterations in the dopaminergic system by antidepressants, might explain non-response or residual symptoms (sleep disturbances, diminished pleasure, loss of interest, fatigue, and decreased motivation), which are often observed in clinical practice. Therefore, investigation of the dopaminergic effects of SSRIs, in combination with or versus behavioural approaches (re-activation) in combined fMRI and SPECT/PET studies in (retarded) MDD-patients are necessary.

The effects of SSRIs on neurogenesis also merit further research. After prolonged antidepressant administration in rodents, increased hippocampal neurogenesis was found. The second-messenger effects of SSRIs upregulate cAMP responsive element binding protein (CREB) in the neuron’s nucleus, which consequently regulates CREB-directed gene transcription. To date, it is unclear which specific genes transcription are affected by CREB. Therefore the exact role of second messenger systems (and CREB in particular) in response to SSRIs remain to be elucidated. One of the genes that is (positively) influenced by CREB is the brain derived neurotrophic factor (BDNF) gene. BDNF is a plethoric factor, which regulates neuronal survival, migration, phenotypic differentiation, axonal and dendritic growth and synapse formation. As such BDNF has a role in cognitive functions (e.g. in memory and hippocampal plasticity). BDNF and serotonin are closely interlinked: BDNF promotes production of serotonin, upregulates serotonin uptake/release, and modifies firing rates of neurons in the raphe nuclei, while serotonin upregulates BDNF. This stimulating feedback mechanism may be involved in the selection of useful synapses, which could be hypothesized to be a requisite for clinical response. Few studies investigated the long-term changes in BDNF measured in venous blood of MDD patients treated with antidepressants. Although different effects for different drugs were seen, generally an increase in BDNF after 6 months was found, significantly associated with a decrease in HDRS. Nevertheless, the role of BDNF as biomarker for antidepressant response is equivocal. Furthermore, due to dilution, peripheral measures may not be adequate, so sampling from an internal jugular vein might be better. Therefore, this line of research might need development of the methods to measure BDNF (and CREB) more locally in the brain of humans.
Summary and general discussion

Conclusion

This thesis addresses the pharmacological treatment of Major Depressive Disorder (MDD), and focuses on what to do when patients do not respond to a standard dose of an antidepressant. In two systematic reviews, we summarized the evidence for various switch strategies and dose-escalation, and concluded that for both strategies insufficient evidence existed to unambiguously guide clinicians. We thereafter performed a randomized controlled trial in which we studied dose-escalation in depressed patients who had not responded to 6 weeks of paroxetine (20 mg/day), and combined clinical measurements with SPECT and fMRI neuroimaging in subgroups of the participants (DELPHI-study). With the DELPHI-study, we show that dose-escalation of paroxetine has no clinical benefit over placebo dose-escalation, and furthermore quantify the absence of a pharmacological effect on the target of paroxetine: the SERT.

Another systematic review and four studies in this thesis, investigate the role of monoamines and SERT in the etiology of MDD and the neurobiological effects of pharmacological treatment with paroxetine. From these studies we conclude that MDD is not necessarily caused by a malfunction of the serotonergic system *per se*. MDD may better be considered as a disturbance of the emotional and cognitive functioning in the limbic-subcortical-cortical network. The increase of serotonergic neurotransmission by paroxetine (and likely other antidepressants), appears to improve the constraint of neuronal information processing, with less emotional activation and more cognitive control in treatment responders relative to non-responders. The findings of this thesis can be the starting point for further investigation of biomarkers for treatment response, in order to develop better understanding of MDD and a more effective treatment approaches.

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Chapter 12


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