Dose-escalation in the picture: pharmacological and imaging studies in depression
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CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS
General introduction

The present thesis concerns the pharmacological treatment of Major Depressive Disorder (MDD). It especially focuses on what to do when patients do not respond to a standard dose of an antidepressant.

In this thesis, the results of studies that were performed since 2001, while working at the Program for Mood Disorders of the department of Psychiatry of the Academic Medical Center will be reported. These studies first aimed at systematically reviewing the existing literature about treatment strategies for non-response to the first antidepressant (included in Part II). These reviews identified an important gap between equivocal evidence for dose-escalation and the firm recommendation of this strategy in clinical guidelines. This was the starting point for the experimental studies to further investigate the clinical efficacy and the mechanism behind dose-escalation, described in Part IV. Meanwhile, the findings, and further reading of conflicting results of others raised questions about the pathophysiological origin of MDD. Therefore, the generally known serotonin hypothesis for MDD, will be addressed in two additional studies (Part III). Part V of the thesis comprises the general discussion and will address directions for future research.

In this introduction chapter, the reader will be introduced to some backgrounds of MDD, its pharmacological treatment, the monoamine hypothesis about the etiology of MDD, and briefly on the mechanisms of action of antidepressant drugs. After a summary, the separate studies of the thesis will be introduced. In chapter 2, the methods of the Dose-Escalation Legitimate? Pharmacology and Imaging studies in depression (DELPHI-study) will be described. However, let’s start with some history.

MELANCHOLY, the subject of our present discourse, is either in disposition, or habit. In disposition is that transitory melancholy which comes and goes upon every small occasion of sorrow, need, sickness, trouble, fear, grief, passion or perturbation of the mind, any manner of care, discontent or thought, which causeth anguish, dullness, heaviness and vexation of spirit, any wayes opposite to pleasure, mirth, joy, delight, causing forwardness in us, or a dislike. In which equivocal and improper sense, we call him melancholy, that is dull, sad, sower, lumpish, ill disposed, solitary, any way mov’d or displeased. And from these melancholy dispositions, no man living is free. […] Melancholy, in this sense, is the character of mortality. […] It falleth out oftentimes that these dispositions become habits, and many affects con temptuous make a disease. […] for that which is but a flea-biting to one, causeth unsufferable torment to another; and which one by his singular moderation and well composed carriage can happily overcome, a second is no whit able to sustain; but upon every small occasion of mis-conceived abuse, injury, grief, disgrace, loss, cross, rumour, etc. (if solitary, or idle) yields so far to passion, that his complexion is altered, his digestion is hindered, his sleep gone, his spirits obscured, and his heart heavy, his hypocondries mis-affected; wind, crudity, on a sudden overtake him, and he himself overcome with melancholy. […] If any discontent seize upon a patient, in an instant, all other perturbations will set upon him; and then, like a lame dog or broken-winged goose, he droops, and pines away, and is brought at last to that ill habit or malady of melancholy it self. […] But all these melancholy fits, […] displeasing, violent and tyrannizing over those whom they seize on for the time—yet these fits, I say, or men affected, are but improperly so called, because they continue not, but come and go, as by some objects they are moved. This melancholy, of which we are to treat, is an habit, morbus sonticus, or chronicus, a chronic or continue disease, a settled humour, not errant, but fixed; and as it was long increasing, so, now being (pleasant or painful) grown to an habit, it will hardly be removed. […] 

INVETERATE melancholy, howsoever it may seem to be a continuant, inexorable disease, hard to be cured, accompanying them to their graves most part, yet many times it may be helped, even that which is most violent, or at least it may be mitigated and much eased. Nil desperandum. It may be hard to cure, but not impossible for him that is, most grievously affected, if he be but willing to be helped.”
Burton’s work *The anatomy of melancholy* (first published in 1621) was a best-seller. It describes many aspects of the disease that we currently recognize as depression: the sometimes difficult distinction of the illness melancholy (depression) from ‘normal’ sadness, pain or sorrow after misery in life, the relation between stressful life-events and the development of the illness, the interaction of life-events and coping styles to develop the illness or remain well, the clinical symptoms, the enormous impact of the illness on a depressed person’s life, social dysfunction and the tendency of recurrence and chronicity.

Nowadays, MDD is one of the most prevalent and disabling illnesses in psychiatry, after ischemic heart disease the second most common cause of disability worldwide and expected to be the world’s second cause of disability by 2030, behind HIV/AIDS. MDD is diagnosed by the occurrence of a cluster of different symptoms affecting mood, pleasure, attention, activities, vital somatic functions (eating, sleep), and (ruminative) thoughts over oneself, guilt or suicide over a prolonged period of time. For clinical and scientific uniformity, MDD is currently defined by criteria specified in the Diagnostic and Statistical Manual (4th edition; DSM-IV), which is a non-theoretical classification-system.

**Epidemiology of MDD**

The 12-month prevalence of MDD is estimated to be 5.8% in the Netherlands, and 5.3% in the United States. Prevalence rates are two times higher in women and higher in widowed, divorced, unemployed or disabled people, people with somatic disease and first degree relatives of patients with MDD. In the general Dutch population, the median duration of MDD is 3 months, with 63% of new MDD episodes recovering within 6 months (regardless whether treatment is offered or the natural course of the disease is awaited), however, this prognosis is less favourable for people who seek help. Estimations of the lifetime prevalence are 15.4% in the Netherlands, >16% in Europe, and 13.2%-16.2% in the USA, with the same 2:1 female-male distribution. Most epidemiological studies indicate that nowadays 51-69% of depressed patients seek or receive treatment, which has increased over the last decade relative to the nineties of the previous century. However, only 50% of patients who are treated for MDD meet diagnostic criteria of MDD or meet indication criteria for treatment, and it is estimated that only 21.7% of MDD-patients in the USA receive adequate treatment. Therefore, depending on one’s view, MDD is frequently over-treated, under-treated or mis-treated.

MDD is a recurrent and potentially chronic disease. After the first episode, 50-80% of patients have a recurrence within 10 years. Of incident episodes of MDD 15-20% will become a chronic depression (defined as a duration of ≥2 years), although higher percentages (46%) have been reported. MDD is associated with high direct treatment costs as well as indirect costs of loss of productivity and quality of life. In the Netherlands, treatment costs of MDD (€250 million) in 1994 were estimated to comprise 1% of the total healthcare budget, and 13.6% of the total mental health budget. In 2003, MDD treatment costs in the Netherlands had increased to €660 million, still consuming 1.1% of the total healthcare budget. In 1999 10% of newly assigned disability-payments (WAO) in the Netherlands was due to MDD.

**Treatment of MDD**

In order to treat major depressive disorder effectively, many national clinical guidelines were developed. Besides various forms of psychotherapy, pharmacotherapy is often applied as monotherapy or in combination with psychotherapy. Antidepressants are effective drugs for MDD when compared to placebo, although effect sizes are moderate, and some critics claim that effects are exaggerated by pharmaceutical companies. Antidepressants are prescribed for MDD, but also for other psychiatric disorders, and are of considerable financial interest. In 2006, approximately 780,000 inhabitants (4.7%) of the Netherlands used

* Melancholy is derived from the Greek Μελαγχολια (melancholia), which refers to the black choler (bile) that was seen as the material cause of the illness those days.
antidepressants, mostly Selective Serotonin Reuptake Inhibitors (SSRIs). Annual prescriptions of antidepressants increased over the last decade by ~6% every year. The costs of prescribed antidepressants were €156 million in 2006, again, most costs represent the prescription of SSRIs.

A range of antidepressants have been developed, all aiming at the stimulation of serotonergic and/or noradrenergic neurotransmission. Four classes of antidepressants exist: SSRIs, Tricyclic antidepressants (TCAs), Monoamine-oxidase inhibitors (MAOIs) and a miscellaneous group. TCAs and SSRIs, but also ‘dual action’ antidepressants block the transporters at the pre-synaptic nerve ending, which causes inhibition of the reuptake of either serotonin (5-HT), noradrenaline (NA = norepinephrine), or both. The classic MAOIs irreversibly inhibit the enzymes mono-amine oxidase A and B, which degrades serotonin, noradrenaline and dopamine. The miscellaneous group harbours ‘dual action’ antidepressants (e.g. venlafaxine or duloxetine), and antidepressants with pre- and post-synaptic targets different from serotonin or noradrenaline transporters.

By nature of their largely similar, but also slightly distinct pharmacological affinities for transporters and receptors, antidepressants have (almost) comparable efficacy, but are different in their adverse effects. Because SSRIs are tolerated better than TCAs, SSRIs have become the antidepressants of first choice in most countries. Within their class, different SSRIs also have comparable efficacy, but different side effect profiles.

Achieving remission is the aim of treatment, because persistence of (residual) symptoms increases the risk of a future relapse or recurrence and is associated with ongoing psychosocial dysfunction. If remission is achieved, patients are advised to continue their antidepressants (at least 6 months) in order to prevent relapse, although guidelines’ recommendations are inconsistent. Nevertheless, only 50% of the patients respond (defined as a 50% decrease in depression severity) to the initial antidepressant, and remission rates (defined as a depression severity score below a certain cut-off) are lower (28-35%). Therefore, clinicians who treat MDD are often confronted with non-response, for which evidence-based approaches are necessary.

Insufficient response to antidepressants: A challenge for the clinician.

The clinician faces several dilemmas when a patient shows insufficient response to an antidepressant. First, how does one ascertain clinical improvement? A variety of measurement scales is available to measure depression severity and improvement of therapy. Psychopathology rating scales with clear cut-off scores provide a more direct representation of the effects of treatment than quality of life scales or a functional outcome. Thus, the use of a symptom-rating scale is highly recommended to assess treatment effects, either clinician-rated (e.g. the Hamilton Depression Rating Scale (HDRS), Montgomery Åsberg Depression Rating Scale (MADRS) or Inventory for Depressive Symptoms (IDS-C)) or self-rated (Inventory for Depressive Symptoms (IDS-SR), Quick-IDS (QIDS-SR) or Beck Depression Inventory (BDI)). Despite criticism on internal validity, selection and rating of items, and sensitivity to change, most scales correlate with each other. Frank et al. proposed tentative definitions for response (>50% decrease of pretreatment rating scale scores) and remission (e.g. HDRS ≤7, MADRS ≤7, IDS-SR ≤13, BDI ≤9). Therefore, measurements should be routinely performed before the start of treatment and at consecutive critical decision points. However, scales are not routinely implemented in daily practice. Apparently, the current challenge of implementation is to persuade clinicians to measure MDD severity repeatedly with appropriate scales.

The next dilemma is when to measure the effects of the antidepressants. Although a recent meta-analysis indicated that the effects of antidepressants on depressive symptoms can be observed within the first weeks of treatment, patients may need 6-10 weeks to achieve substantial improvement. Therefore, the initiated treatment should not be changed too early, neither too late. Changing a regimen early may forestall a potentially late effect of the current treatment, but may also offer an increased chance of response. Contrary, unchanged treatment may increase despair due to the failure of response, but may prevent the (possible premature)
start of a more aggressive treatment with increased side effects. Over the last decades, the time
to declare that an antidepressant trial failed, increased from 3 weeks\textsuperscript{71} to 6-8 weeks\textsuperscript{70,72}
Interestingly, recommendations on this lag-time were never derived from studies primarily
designed to quantify the timing of the determination of response. Current guidelines for
depression differentially recommend a minimal duration of antidepressant therapy between 4
and 6 weeks.\textsuperscript{13,21,23,25,26,28,29,67,73} At these time points (‘critical decision points’)\textsuperscript{67} a next treatment-
strategy must be considered.

A final dilemma is to adequately re-evaluate the diagnosis, without the (counter-) transference
of helplessness. Critical decision points are important for the reassessment of somatic or
psychiatric co-morbidity, re-evaluation of persistent psychosocial problems and treatment
adherence. However, when none of these issues apply, the patient may be accused of ‘a failure to
cooperate’, or blamed for the non-response by ‘having a personality disorder’ or ‘not being
motivated for treatment’. Although these reproaches occasionally may be justified, they could
also indicate the patient’s and/or clinician’s helplessness, which is often transferred from a
depressed person to the persons surrounding him or her. Most depressed patients want to
improve, but are indeed disabled in their coping-styles, their motivation and their problem solving
capacities. As such, routinely administered behavioural interventions (registration, planning and
gradual reactivation)\textsuperscript{74} are valuable in the pharmacological treatment of MDD, also deferring
increased helplessness.

**Insufficient response to antidepressants: What to do when the miracle doesn’t
happen?**

Five pharmacological strategies for insufficient response to an antidepressant can be distin-
guished: 1. prolongation, 2. dose-escalation, 3. switching, 4. augmentation and 5. combina-
tion, all regarding the antidepressant initially given. Of course, a sixth strategy could be the addition
of psychotherapy to the monotherapy of pharmacotherapy\textsuperscript{75,76} but as this is not a topic of this
thesis, only the first 5 strategies are described.

1. **Prolongation of the trial for another 2-4 weeks.** This strategy is applicable only in case of doubt
   of the nature of the non-response (e.g. amendable psychosocial stressors, non-adherence),
   but is not favourable given the relative importance assigned to critical decision points.

2. **Dose-escalation.** With this strategy the dose of the prescribed antidepressant is increased to
   the maximal tolerable dose. Dose-escalation assumes a dose-response relationship, which is
equivocal for SSRIs. The advantage of dose-escalation is that it is easy and quick to apply.

3. **Switching of the antidepressant.** With this strategy the prescribed antidepressant is
   discontinued (eventually after tapering and a wash-out period) and the antidepressant is
   switched to another antidepressant either belonging to the same class, or to a different class
   compared to the initial antidepressant. The obvious advantage of switching is that it facilitates
   a new pharmacological approach, possibly targeting different neurotransmitter systems. Its
   disadvantage is that it requires some time, and that one may unwontedly discard achieved
   improvement when the chosen antidepressant appears to be a less effective one for this
   patient.

4. **Augmentation of the prescribed antidepressant.** With this strategy a drug is added that has no
   strong antidepressant effects by itself, but is known to increase the effects of antidepressants
   (e.g. lithium). The advantage of augmentation is that the effect of the antidepressant initially
   prescribed is maintained. Furthermore augmentation might result in faster responses than
   switching (as tapering is not required, nor a wash-out). Disadvantage is the risk of poly-
   pharmacy and the related interactions.

5. **Combination of antidepressants.** With this strategy another antidepressant drug, mostly with
different pharmacological properties compared to the antidepressant already prescribed, is
added to the initial antidepressant. This strategy has the same advantages and disadvantages
as augmentation strategies, but the risks of severe interactions are higher.
Neurotransmission, the serotonergic system and neuronal networks

The brain passes information through electrical signals in neurons. Synapses form the functional contacts between neurons and mainly communicate through neurotransmitter secretion. Neurotransmitters are stored in synaptic vesicles. The vesicles are released into the synaptic cleft in response to presynaptic depolarisation. After the release of neurotransmitters in the synapse, they are either metabolized or transported back into the terminal to be used again.\(^\text{77}\)

Neurotransmitters bind to specific receptor proteins on the membrane of the pre- and postsynaptic neurons. Postsynaptically, receptors either induce a change in postsynaptic ion channels (ionotropic receptors), causing a depolarisation, or they activate G-protein mediated complexes (metabotropic receptors), which activate one or more metabolic steps (‘second messengers’). Activation of metabotropic receptors is generally responsible for forming enzymes that regulate gene expression, neurotransmitter synthesis, receptors and neuroplasticity. Which of the mechanisms is used depends on the particular postsynaptic receptor type.\(^\text{78}\) Three major ‘monoamine’ neurotransmitters are associated with psychiatric disorders: serotonin, noradrenaline and dopamine, of which the latter is traditionally related to psychotic disorders. SSRIs target the serotonergic system and increase serotonergic neurotransmission. From here, the serotonergic system and the neuronal networks that are believed to be involved in MDD will be described.\(^\text{79}\)

Serotonin
The serotonin neurons of the brain start in the raphe nuclei in the midbrain and project to the neocortex, basal ganglia, temporolimbic zones, hypothalamus, cerebellum and the brain stem (Figure 1.1).\(^\text{80,81}\) Serotonin is involved in several functions: sleep and wakefulness, appetite, nausea, migraine, headaches and regulation of mood.\(^\text{77}\) The serotonin receptors comprise of 5-HT\(_{1A}, 1B, 1C, 1D, 1E, 1F, 5-HT\(_{2A}, 2B, 2C, 5-HT\(_{4}, 5-HT\(_{5}, 5-HT\(_{6}\) and 5-HT\(_{7}\) (metabotropic) and the 5HT\(_{3}\) (ionotropic) receptors.\(^\text{78}\) Serotonin plays an important role in brain development via regulation of neurite outgrowth, synaptogenesis and cell survival.\(^\text{82,83}\) Serotonin that is released into the synaptic cleft is either taken up back into the presynaptic nerve ending by the serotonin transporter (SERT), or degraded by MAO-A.\(^\text{77,78}\)

Figure 1.1. Serotonergic pathways through the human brain.

![Serotonergic system](image)

Serotonergic system. From the raphe nuclei in the midbrain, neurons project to the neocortex, basal ganglia, temporolimbic zones, hypothalamus, cerebellum and the brain stem.

The serotonin (and noradrenaline) deficiency hypothesis
In the late fifties of the previous century, TCAs appeared to be effective in treating MDD by increasing serotonergic and noradrenergic neurotransmission. This discovery led to the monoamine hypothesis: MDD might etiologically be explained by a deficiency in monoamine neurotransmitters: serotonin or noradrenaline. Since then, the working mechanism of AD is believed to be by (1) increased neurotransmission by increased synaptic levels of serotonin, noradrenaline and/or (2) specific agonistic effects on serotonin or noradrenaline (sub-)receptors.
Depletion of the available serotonin and noradrenaline is used as a model to test the involvement of monoaminergic systems in MDD. Serotonin depletion can be achieved by rapidly lowering the essential amino-acid tryptophan which cannot be synthesized by the body and must be ingested to enable formation of serotonin. To achieve depletion, a tryptophan free amino-acid mixture is administered (acute tryptophan depletion).84 Depletion of noradrenaline and dopamine occurs simultaneously, and uses the same concept (acute depletion of the essential amino-acids phenylalanine and tyrosine).85 As an alternative to induce a state of depletion, enzyme-blocking agents decrease the production of the monoamines. Para-chlorophenylalanine blocks serotonin synthesis,86 and Alpha-methyl-para-tyrosine blocks noradrenaline and dopamine synthesis.87

Since 1975 an increasing number of depletion studies have been conducted, with different effects in different study-populations. In general in healthy controls no clear mood-effects were found, unless they had relatives with MDD. In remitted MDD patients who used antidepressants (or shortly after tapering) approximately 50% of the patients experienced a relapse after depletion. However this occurred only after depletion of the monoamine that their antidepressant targeted. In depressed patients no consistent deterioration of the mood effects were found.88-93

Thus, the monoamine-deficiency theory, in its purest form, states that depression can be cured by the increase of serotonergic and/or noradrenergic neurotransmission. However, the reverse train of thought, that depression is bio-etiologically caused by a deficiency of monoamines (e.g. serotonin and/or noradrenaline) has attractive face-validity, but probably is an untenable, superficial simplification.75-94 Therefore, the current, less pertinent view is that the monoamine hypothesis only partially explains MDD and the response to AD.95-98

The limbic-cortical dysregulation hypothesis
In a more multidimensional, systems-level model, MDD can be viewed as a disorder affecting discrete but functionally integrated pathways; neural networks, which can be identified by neuroimaging techniques.† In such a network, dysfunction in one or more of the elements (e.g. after cognitive or somatic stress), will initially be tried to be influenced (or compensated) by other, remaining parts of the network, that try to maintain homeostatic emotional control. Therefore, results from neuroimaging studies investigating differences between healthy controls and MDD patients, must be considered as the identification of regions to be either etiologically abnormal or regions involved in (mal-)adaptive compensatory processes.99

Because MDD is an affective disorder, the neurobiology of emotion processes is likely involved. For the processing of emotions two systems are important: a ventral system (consisting of amygdala, insula, ventral striatum, ventral anterior cingulate gyrus, and ventral prefrontal cortex) and a dorsal system (consisting of hippocampus, dorsal anterior cingulate gyrus, and dorsal prefrontal cortex). The ventral system serves to identify the emotional significance of a stimulus, the production of mood states, and automatic regulation of emotional responses, while the dorsal system serves to effortfully regulate mood states and subsequent behaviour.100

Initial lesion-deficit studies, early Positron Emission Tomography (PET) studies (measuring regional resting state glucose metabolism or blood flow) and later functional Magnetic Resonance Imaging (fMRI)-studies identified several brain regions to be affected by MDD: the limbic structures (amygdala, hippocampus, hypothalamus and brainstem), the subcortical (basal ganglia and thalamus) and the cortical (dorsolateral prefrontal, ventrolateral prefrontal and orbitofrontal cortex (DLPFC, VLPFC, OFC respectively) structures. In these, the most consistent finding is a hypoactivity of the (dorsal) frontal lobe, while often a hyperactivity in the (ventral) VLPFC and OFC is found.99,101 Additionally, an increased activity of the (rostral, subgenual) anterior cingulated gyrus99,101 and the amygdala, anterior insula, and ventral striatum was found, although less consistently.101 Furthermore, fMRI studies point to a increased sensitivity of the amygdala for

† Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) use a variety of radioligands with various half-lives to quantify different targets (transporters, receptors, or blood-flow or metabolism). Functional Magnetic Resonance Imaging (fMRI) is a technique to image brain activity (hemodynamic response) related to a specific task or stimulus.
emotional stimuli, and a bias to interpret these stimuli in a negative context. Interestingly, induction of sad mood in healthy volunteers also produces increased blood flow in the insula, subgenual anterior cingulate gyrus and decreased blood flow in the dorsomedial prefrontal cortex (DMPFC).

The above abnormalities were combined in the limbic-cortical dysregulation model, which includes a dorsal neocortical hypofunction, which results in ventral (para)limbic hyperactivity, with a reciprocity in this inverse relationship.

**Which effects in the brain cause antidepressants to be antidepressants?**

The various treatment forms available to relieve MDD and their moderate efficacy poses the question: How do antidepressants bring about their therapeutic effects? For this question, three levels of action must be distinguished: 1. direct neurotransmission effects, 2. second messenger effects, and 3. change in neuronal networks. For levels 1. and 2. see Figure 1.2. At all three levels, there appears to be a time dependent differentiation of the effects that occur, e.g. not all effects are the same in the consecutive weeks after the initiation of treatment. This may explain why it sometimes takes several weeks before the therapeutic effects become apparent. Because most of the recent research was done with SSRIs and SNRIs, the effects of these drugs are described hereafter, unless indicated differently.

**Direct effects of antidepressants on neurotransmission**

When an SSRI is ingested, the blockade of the target transporter (i.e. the serotonin transporter (SERT)) occurs within several minutes. Therefore, the antidepressant effects cannot only be based on increased neurotransmission by decreased serotonin reuptake, as these effects take much longer than this direct pharmacological effect. Further research revealed that after two days of treatment, the firing-rate of SERT-containing neurons in rats decreased, but that this firing-rate was restored within 2 weeks of continued treatment. This was attributed to somatodendritic 5-HT1A autoreceptors, which normally have a negative feedback on the neuron’s firing rate. These 5-HT1A autoreceptors appeared to desensitize. Microdialysis-experiments in rats showed that the restored firing-rate after 14 days was responsible for a 6-fold increase in intrasynaptic serotonin level, while after the acute blockade of the SERT this increase of the serotonin level was only small and transient. Furthermore, in humans, serotonin autoreceptors (5-HT1B/1D) (and also noradrenergic α2 autoreceptors) in the synapse normally inhibit serotonin release by feedback-mechanisms as well. Prolonged treatment with SSRIs again desensitize these receptors, resulting in increased serotonin release in the synaptic cleft. As such, desensitisation of HT1A autoreceptors in the raphe nuclei in the midbrain may have effects on serotonergic neurotransmission in critical brain areas where these serotonergic neurons project to. Finally, in rats, after prolonged administration of SSRIs the SERT itself is downregulated by 80-90%. This is probably caused by trafficking and internalization of the SERTs instead of altered SERT-gene-regulation, because mRNA expression in the cells studied was unaltered by the treatment.

Other antidepressants have rather different effects. TCAs (except clomipramine) do not change the pre-synaptic serotonin-containing neurons, but appear to sensitize the postsynaptic 5-HT receptors for serotonin. Along with serotonin, the responsiveness for noradrenaline was also found to be enhanced, likely due to enhanced α-adrenoceptor-mediated transmission. MAOIs also increase serotonergic transmission by desensitisation of 5-HT1A autoreceptors, but do not desensitize other 5-HT autoreceptors, and desensitize noradrenergic α2 autoreceptors, which indirectly enhances serotonergic transmission.
Figure 1.2. Transporters, receptors and second messenger systems involved in the effects of antidepressants.

In the presynaptic neuron, serotonin is synthesized from tryptophan by tryptophan hydroxylase and stored in vesicles. Likewise, norepinephrine is synthesized from tyrosine by tyrosine hydroxylase. These vesicles merge with the cell membrane when the neuron is depolarized, thereby releasing their contents into the synaptic cleft. After release, serotonin and norepinephrine are transported back into the presynaptic neuron by serotonin and norepinephrine transporters. Furthermore, serotonin and norepinephrine are catabolized by the monoamine-oxidase A (MAO-A) enzyme. In the synaptic cleft, serotonin and norepinephrine affect both the pre- and post-synaptic neuron. The pre-synaptic 5-HT₁A and 5-HT₁B auto-receptors decrease serotonin release by inhibitory feedback; the α₂-adrenergic receptor does the same for the release of norepinephrine.

Post-synaptically, serotonin and norepinephrine bind to G-protein-coupled monoamine receptors (MARS): the cyclicAMP (cAMP)-coupled receptor, which activates protein kinase A (PKA), and the Phosphatidylinositol (PI)-coupled receptor, which activates phospholipase C (PLC) which thereafter form inositol triphosphate (IP₃) and diacylglycerol (DAG). IP₃ and DAG activate protein kinase C (PKC). Both PKA and PKC finally activate cAMP responsive element binding (CREB) protein, which stimulates DNA transcription. For example, this might result in the production of brain derived neurotrophic factor (BDNF).
Second message effects of antidepressants

The secondary effects of antidepressants can be divided in post-synaptic effects on the sensitivity and availability of receptors, and the effects caused by the activation of post-synaptic receptors. Effects on post-synaptic receptors include a decrease of 5-HT1A and 5-HT2A receptor density, and 5-HT1D and 5-HT2C/2B responsivity. Because these effects are either investigated in animals or by using indirect measures (e.g. growth-hormone release), it is important that Meyer et al. only demonstrated 10% in-vivo 5-HT2A receptor downregulation in MDD patients between 20-30 years, but not in older patients. Interestingly, in rodents, the activation of post-synaptic 5-HT1A receptors in the hippocampus stimulates neurogenesis, which appears to be required for therapeutic effects of antidepressants.

After the activation of the G-protein metabotropic receptors (either by serotonin or noradrenaline), second messenger systems mediate signals to the neuron’s nucleus, where cAMP responsive element binding protein (CREB) regulates CREB-directed gene transcription. Most evidence indicates that CREB is upregulated by chronic antidepressant use, but not for all antidepressants, and results of studies appear to be biased by the cell type investigated, the brain region where these cells originate from and the timing after the first antidepressant exposure. One of the genes that is (positively) influenced by CREB is the brain derived neurotrophic factor (BDNF) gene. BDNF is a plethoric growth factor, which regulates neuronal survival, migration, differentiation, axonal and dendritic growth and synapse formation. The genomic structure of BDNF is complex, which facilitates differential activation by diverse and variable stimuli, which can be different in different brain regions and even in different parts of the cell.

Changes in neuronal networks

At the neuro-anatomical level, the novel neuroimaging techniques have ‘opened’ the brain to study the in-vivo effects of psychiatric treatment. In MDD, early PET studies found time dependent changes during the treatment with fluoxetine. After 1 week, glucose metabolism increased in the hippocampus and brainstem, and decreased in posterior cingulate gyrus, striatum and thalamus compared to the pre-treatment scan. After 6 weeks, patients who responded to the treatment had a decrease in metabolism in the subgenual cingulate gyrus, the hippocampus, the pallidum and insula and an increase in the anterior and posterior cingulate gyrus, prefrontal and parietal cortex. However, the non-responders showed a persistent, unchanged pattern of change as seen in week 1. The changes in prefrontal cortex and subgenual cingulate gyrus correlated best with symptomatic improvement. These findings were replicated in patients treated with paroxetine. Moreover, patients who responded while on placebo-treatment showed somehow similar but also distinct changes in brain metabolism compared to fluoxetine responders. This indicates that in neuroimaging studies, response and treatment effects may coincide, but both may also have their specific, distinguishable effects.

In recent fMRI studies, the increased activation of the amygdala to negative (sad, fearful, angry) and happy faces have been investigated after treatment of SSRIs (fluoxetine, sertraline, venlafaxine and bupropion). These studies rather consistently found decreased activation of the amygdala, insula and increased cortical activity after treatment. However, most patients in these studies were treatment responders at the end of the study. In contrast with previous PET-studies, none of the fMRI studies used a placebo-comparison measured twice, so no distinction between specific drug and response effects in fMRI can be made yet.

‡ Similar changes included increased metabolism in frontal, parietal and posterior cingulate, and decreased metabolism in subgenual cingulate gyrus. Distinct changes included no changes in subcortical brainstem, hippocampal, and caudate metabolism in placebo-responders.
(Pharmaco-)genetic effects relevant for antidepressants

Several genetic polymorphisms have been investigated in relation to treatment response to SSRIs. The polymorphism studied most is the SERT promoter gene (5-HTTLPR), for which a long (L) and a short (S) variant were identified, with a recently discovered functional tri-allelic variant (rs25531).29 The 5-HTTLPR is associated with the transcriptional activity of the SERT gene.30 Cells homozygous for the L-allele produce higher concentrations of SERT mRNA, and the rate of serotonin uptake by the transporter is >2-fold higher than in cells containing one or two copies of the S-allele. A meta-analysis of 15 studies showed a pooled association between the 5-HTTLPR-polymorphism and SSRI efficacy,31 with MDD patients with at least one L-allele having higher response rates to SSRIs. However, in a large sample of patients treated with citalopram (an SSRI), treatment response was not associated with the tri-allelic 5-HTTLPR-polymorphism.132;133 Furthermore, individuals carrying the S-allele experience increased adverse events after SSRI treatment,132;134 have elevated risk of depression in relation to life events,135 but also show increased amygdala reactivity to fearful stimuli.136;137 A large MRI-study in healthy controls showed associations of the 5-HTTLPR S/S polymorphism with unfavourable alterations in anatomy and function of the amygdala-cingulate feedback circuit.138 These findings strongly argue for an important role of the 5-HTTLPR-polymorphism in the development and functioning of emotional networks involved in MDD. Other pharmacogenetic associations with clinical response have been investigated, but will not be further addressed in this thesis.

Summary and questions addressed in this thesis

Major Depressive Disorder is a prevalent and disabling illness, which potentially recurs and may become a chronic disease. It is the second most common cause of disability worldwide, and has a 12-month prevalence of ±5.5%, with lifetime prevalences of 12-14% in males and 22-24% in females.3;5 MDD is associated with high direct treatment costs as well as indirect costs of loss of productivity and quality of life. Besides various forms of psychotherapy, pharmacotherapy with antidepressant drugs is often applied.3;5;21-25

Antidepressants are grouped in four different classes: Selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs) and a miscellaneous group including so-called dual action antidepressants. SSRIs, dual-action antidepressants and TCAs are used most often.39 Most antidepressants increase serotonergic and/or noradrenergic neurotransmission. When considered more precisely, different classes of antidepressants appear to have distinct additional pre- and postsynaptic effects.105;106 Secondary to increased serotonergic and/or noradrenergic neurotransmission, complex second messenger pathways are activated, which are only partly understood.107;108 Macroscopically, presumably relevant changes in neuronal networks following antidepressant treatment have been identified.99;101;104 Nevertheless, which changes are required and specific for the improvement of symptoms remains an enigma. Furthermore, the effects of increased neurotransmission might be affected by the genetic make-up of patients (i.e. the 5-HTTLPR polymorphism).29 Genetic polymorphisms appear to be associated with treatment effects,31 but might also influence the development and connectivity of the neuronal networks.33

Despite comparable efficacy of antidepressants, only 50% of the MDD-patients respond to the first antidepressant trial given, while fewer achieve full remission of symptoms.48;51 Therefore, insufficient response to the first antidepressant is a relevant clinical problem that challenges clinicians.

The clinician’s first dilemma is how to adequately and efficiently measure the changes in symptom severity, for which the further development and implementation of short, easy to use and valid clinician rated questionnaires would improve clinical decision making. The second dilemma is to time the change of the treatment initially started: this should not be changed too early, neither too late.70 The third dilemma is to balance helplessness, impaired functioning by the disease and (counter-) transference by ‘blaming the victim’, i.e. reproaching the patient of the moderate efficacy of antidepressants as ‘being unmotivated’ or ‘having a personality disorder’. 
When ‘the miracle doesn’t happen’, 5 strategies for non-response are possible options: prolongation of the initial trial, dose-escalation, switching, augmentation and combination of antidepressants. For all these options the current evidence is either equivocal or fragmentary. In order to develop evidence based treatment algorithms for the 50% of MDD-patients who turn out to be non-responsive to the first antidepressant trial, systematic overviews of the literature are required and gaps in the evidence-base need to be addressed by new research-projects.

More fundamentally, the perceived delayed onset of symptom improvement with antidepressants, their moderated efficacy, and their extensive effects in the brain beyond an increase in serotonergic or noradrenergic neurotransmission, summons the question what is the etiopathogenetic model for MDD. Or, more specifically, what is the evidence that corroborates the corner-stone of most antidepressant’s action: the monoamine hypothesis and the involvement of the serotonergic system as a causal explanation for MDD.

**Research projects underlying this thesis**

This thesis incorporates three research projects that were initiated and conducted by the Program for Mood Disorders of the Academic Medical Center (AMC). One additional project was initiated by the department of vascular medicine of the AMC.

First, a guideline-project was initiated with a grant from the Academic Medical Center (No SFA.07.012). Aim of this guideline project was to develop an evidence based guideline for non-response after the first SSRI.

Second, as a result of this guideline project, two grants were obtained from the Netherlands Organization for Health Research and Development (ZonMw), program Mental Health, education of investigators in mental health (Geestkracht-OOG; projects #100-002-001 and #100-002-002). These grants were applied to initiate the DELPHI-study (Dose-Escalation Legitimate? Pharmacology and Imaging studies in depression). The DELPHI-study was set up as a methodologically sound trial to study clinical effectiveness of dose-escalation as a strategy for non-response. As a novel extension to existent dose-escalation trials, we aimed to investigate the molecular neurobiological target of dose-escalation: the occupancy of the serotonin transporter (SERT) by paroxetine (a SSRI). This was done by the acquisition of two or three single photon emission computed tomography (SPECT) scans in a subgroup of drug-free patients of the total DELPHI-cohort.

A third project was started as an extension to the DELPHI-SPECT study. This third project could be planned after a grant from the Dutch Brain Foundation (Hersenstichting) was obtained (project #14F06.45). The DELPHI-fMRI aimed to investigate the neurobiological changes in brain activation by treatment with paroxetine in a functional Magnetic Resonance Imaging (fMRI) study. This study was superimposed on the SPECT-imaging of the last 22 drug-free patients participating in DELPHI.

The final fourth research project in this thesis was initiated after an almost fatal bleeding complication, which occurred in a patient who underwent surgery, and lost 12 litres of blood. This complication was afterwards attributed to fluoxetine use. This project was funded internally by mutual contributions of both participating AMC-departments.

**The questions addressed in this thesis**

1. **Is a short, easy to use clinician rated questionnaires as effective and precise as the routine Hamilton depression rating scale (HDRS)?**

   The HDRS is most frequently used as the golden-standardin clinical trials, but is probably too extensive to use in clinical practice. To facilitate clinical measurements of depression severity and objectivity for critical decision points, we reanalyzed the treatment-outcomes of two antidepressant-psychotherapy trials, which were performed by the Mentrum research group. We therefore investigated whether the effect-sizes of two 6-item subscales of the HDRS (17-items) – the Maier and Bech subscales – were comparable to the original HDRS in the measurement of depression severity and the sensitivity to measure changes. Furthermore, we investigated whether this comparability was stable across the full range of...
response to treatment, and across different treatments and for different baseline severity of depression. We also determined cut-off points for remission for these subscales compared to conventional HDRS definitions. See chapter 3.

2 What is the evidence for dose-escalation as a strategy for non-response to a first SSRI?
For this question, we performed a systematic review of the evidence for the dose response relationship for SSRIs in MDD. See chapter 4.

3 What is the evidence for switching antidepressants as a strategy for non-response to a first SSRI?
For this question, we performed a systematic review of the evidence for switching after failure of a first SSRI in MDD. Part of this study was a meta-analysis of three switch-studies. See chapter 5.

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?
For this question, we performed a systematic review of monoamine depletion studies reporting mood effects of depletion. As an extension of previous systematic reviews of monoamine depletion studies, we aimed to pool the results of the small-sized depletion studies, because they might not have detected small differences by a lack of power, and pooling would quantify the balance of positive versus negative studies. Therefore, we applied a pooling technique (modified from conventional meta-analyses of randomized controlled trial data and including an adjustment for small sample bias) to handle the statistically paired cross-over designs of these studies in formal, stratified meta-analyses. See chapter 6.

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?
Despite the fact that the working-mechanism of antidepressants supports the monoamine deficiency theory, the pathogenesis of MDD remains unclear. Therefore, differences in SERT availability in patients and healthy controls have been studied previously, with conflicting results. Additionally, significant effects on SERT availability have been reported for gender, smoking behavior, and season of scanning. Therefore, we analyzed the baseline SPECT-scans of the DELPHI-SPECT participants versus age and sex-matched healthy controls. Because our sample size was large, we were able to properly account for potential confounders and possible interactions, of which the multivariate effects are reported. See chapter 7.

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?
We performed this study because SSRI-response is likely associated with 5-HTTLPR polymorphisms, 5-HTTLPR polymorphisms might influence SERT availability (the target for SSRIs), and it is unclear how occupancy of the available SERTs is related to clinical response. Thus, we aimed to investigate the paroxetine treatment by genotype interaction regarding clinical response on the molecular level of SERT occupancy. We quantified the relation between SERT occupancy and clinical response, and studied how the 5-HTTLPR polymorphism affected this SERT occupancy-response relationship. We performed this study in the open phase of the DELPHI-SPECT study, when patients were treated with paroxetine 20 mg/day for 6 weeks. See chapter 8.

7 Is dose-escalation of paroxetine an effective clinical strategy for non-response in MDD?
The systematic review of dose-escalation (chapter 4), identified methodological flaws in previous dose-escalation trials. In this study we reevaluated the clinical efficacy of dose-escalation of paroxetine without these flaws, and, considering the molecular target of SSRIs, we also tested whether paroxetine dose-escalation increased SERT occupancy more than placebo dose-escalation. We therefore performed a 6 week, multicenter, randomized study in depressed patients not responding to 6 weeks of paroxetine at 20 mg/day. As a novel extension to previous clinical trials, and in order to elucidate the neurobiological basis for an
expected lack of benefit of dose-escalation, we included a SPECT imaging approach. Herewith, we quantified whether paroxetine dose-escalation increased SERT occupancy more than placebo dose-escalation. This enabled us to relate clinical findings to the neurobiological correlate of SERT occupancy. See chapter 9.

8 Does treatment with paroxetine normalize amygdala hyperactivation in MDD?
We initiated this study after the first reports of attenuated amygdala activation after treatment with sertraline. Thereafter several groups replicated a baseline hyperactivation of the amygdala but less consistently reported the attenuation of amygdala-hyperactivation after treatment. We therefore investigated whether: activation of the amygdala by (negative) facial expressions differed from healthy controls, this activation of the amygdala changed after 6 and 12 weeks of treatment with paroxetine, the activation of the amygdala and other brain areas merely changed by paroxetine treatment or in relation with clinical response, and whether dose-escalation of paroxetine in week 6 non-responders affected activations, compared to placebo-dose-escalation. For this study, we performed an fMRI study in 22 MDD patients who participated in the DELPHI-fMRI study. Patients were treated with paroxetine (20 mg/day followed by a randomized dose-escalation for non-responders) and were scanned at baseline, 6 weeks and 12 weeks of treatment. We obtained a baseline scan for 21 matched controls, to contrast baseline amygdala activation in MDD-patients. See chapter 10.

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 this study, we evaluated the effects of standard and increasing dosages of paroxetine on the bleeding tendency and hemostatic functions of platelets in patients who were drug-free before the start of paroxetine. In addition, we assessed whether these effects are modified by the 5-HTTLPR polymorphism. See chapter 11.

References


140. Dekker J, Molenaar PJ, Kool S, Aalst G van, Peen J, Jonghe F de. Dose-effect relations in time-limited combined psycho-
141. Maier W, Philipp M. Improving the assessment of severity of depressive states: a reduction of the Hamilton Depression 
142. Prien RF, Carpenter LL, Kupfer DJ. The definition and operational criteria for treatment outcome of major depressive 
60 Suppl 22: 29-34.
Collaboration; 2002.
measures of dopamine and serotonin transporter availability in healthy smokers and nonsmokers. Synapse. 2001; 41: 275-
284.
147. van Dyck CH, Malison RT, Seibyl JP, Laruelle M, Klumpp H, Zoghbi SS et al. Age-related decline in central serotonin 
148. Buchert R, Schulze O, Wilke F, Berding C, Thomasius R, Petersen K et al. Is correction for age necessary in SPECT or PET of 