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# Manipulating serotonin function in depression

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# Manipulating serotonin function in depression

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# Manipulating serotonin function in depression

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in 1977

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| Overige leden: | Prof. dr. F.G. Zitman |
| | Dr. P.M.J. Haffmans |

***Het leven heeft misschien geen zin, maar ik toevallig wel***

*(Maarten van Roozendaal)*

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# 1

# Introduction

**Major depressive disorder**

Depressive disorder is one of the most disabling diseases in the world (Üstün et al., 2004). Lifetime prevalence rates of major depressive disorder are about 17% in the USA; one in six persons will have a diagnosable depression at some point in their lives (Blazer et al., 1994). However numbers may differ between countries (Hammen, 1997). More women than men are affected by depressive disorders worldwide (with a ratio of 2:1). The rates of onset and current depression are highest in late adolescence and early twenties (Hammen, 1997). The core symptoms of depression are low mood and anhedonia (the inability to gain pleasure from normally pleasurable experiences). Other symptoms may include a decreased appetite, difficulties sleeping, fatigue, feelings of worthlessness, diminished ability to concentrate and thoughts of death or suicide. Table 1 represents the diagnostic criteria for a Major Depressive Episode, as stated by the Diagnostic and Statistical Manual of Mental Disorders-IV (American Psychiatric Association, 1994).

The most effective treatments for depression are antidepressant medication, structured forms of psychotherapy (eg. cognitive behavioural therapy), or a combination of both. Selective serotonin reuptake inhibitors (SSRIs) form the most widely used pharmacological treatment for depression (Petersen et al., 2002).

Since rates of relapse and recurrence in depressive disorder are high (Judd, 1997), extensive research is done on the mechanisms that may play a role in the development and maintenance of depressive disorder. Vulnerability to depression may include cognitive, biological, psycho-social and genetic factors (Hammen, 1997). In terms of biological vulnerability to depression, neurotransmitter dysfunction (serotonin, dopamine, norepinephrine) is thought to play an important role (Maes & Meltzer, 1995). This thesis focuses on the

role that the neurotransmitter serotonin (5- hydroxy-triptamine; 5-HT) plays in depression, especially in mood and cognitive processing. The link between serotonin and two biological vulnerability factors (the cortisol response to stress and heart rate variability) associated with depression will also be investigated.

*The role of serotonin in depressive disorder*

The notion that a dysfunctional serotonergic system is involved in the pathophysiology of depression is supported by a wide range of experimental studies (Delgado et al., 1990; Maes & Meltzer, 1995). Abnormalities in the 5-HT system can occur at different levels: availability of the serotonin precursor tryptophan (Cowen et al., 1989), serotonin synthesis, release, reuptake or metabolism, or at the pre- or postsynaptic receptors (Cleare et al., 1998; Maes & Meltzer, 1995). This 'serotonergic vulnerability' may be caused by a variety of factors such as innate factors (genetic factors, family history, personality, gender, sex hormones); environmental factors (stress, immune system and cytokines, drug use), and bio-psychological interactions (Jans et al., 2007).

Some serotonin abnormalities are not only found in acutely depressed but also in remitted depressed patients and subjects with a family history of depression (Bhagwagar et al., 2006; Flory et al., 1998). This suggests that either a dysfunctional serotonin system or an increased sensitivity of the serotonin system is a trait abnormality in depression. However, not all depressed patients show all abnormalities in 5-HT function (Van Praag, 2004).

**Table 1.** The diagnostic criteria for Major Depressive Episode according to the DSM-IV

---

**A.** Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.

**(1)** depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful).

**(2)** markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others).

**(3)** significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.

**(4)** insomnia or hypersomnia nearly every day

**(5)** psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)

**(6)** fatigue or loss of energy nearly every day

**(7)** feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)

**(8)** diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)

**(9)** recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

**B.** The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

**C.** The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism).

---

The neurobiological basis of depression has been linked to the mechanism of action of antidepressant medication: serotonergic antidepressants increase brain serotonin function by inhibiting the re-uptake of the neurotransmitter serotonin (Blier & de Montigny, 1994; Delgado, 2000).

Since a direct measurement of serotonin in humans is problematic, research into serotonin function is based on indirect methods. Experimentally manipulating serotonin levels in humans makes it possible to study the role of serotonin function in depression and antidepressant action. In this thesis, two different interventions are used to manipulate serotonin activity in humans: acute tryptophan depletion and a diet enriched with the milk-whey alpha-lactalbumin.

**Manipulations of serotonin**

*Tryptophan depletion*

Serotonin synthesis depends on dietary intake of its precursor, the essential amino acid tryptophan. At the blood-brain barrier, tryptophan has to compete for entry with the other large neutral amino acids (LNAAs; tyrosine, phenylalanine, leucine, isoleucine and valine). Once tryptophan has entered the brain, it is synthesized in a rate limiting step by tryptophan-hydroxylase into 5-hydroxytryptophan (5-HTP) and then into serotonin. See Figure 1.

Acute tryptophan depletion (ATD) is a method to experimentally lower serotonin function by depleting the brain from its precursor tryptophan. This is done through administration of an amino-acid mixture devoid of tryptophan (Young et al., 1985). The most common ATD method involves a low-tryptophan diet during the 24 hours before the test session, followed by an overnight fast. In the morning of the test day, subjects are asked to consume a drink containing a 100g load of 15 amino-acids that does not contain

tryptophan (Bell et al., 2001), mostly mixed with water and artificial flavour. After five to six hours, ATD results in peak reductions of plasma tryptophan levels (+/- 70%) and ratio tryptophan/LNAA, which is an index of central 5-HT turnover, (+/- 90%). Therefore, ATD is a useful tool to investigate the effects of lowered serotonin function in humans. ATD results in significant behavioural effects, including a lowering of mood (Young et al., 1985), changes in cognitive performance (Park et al., 1994; Schmitt et al., 2000), increased impulsive behaviour (Young, 1986) and changes in sleep architecture (Bhatti et al., 1998). It is important to note that differential effects of ATD are found in healthy vs. depression vulnerable subjects. Mood effects of ATD are only found in remitted depressed patients taking SSRIs (Booij et al., 2002; Delgado et al., 1990) and in healthy subjects with a family history of depressive disorders (Benkelfat et al., 1994; Klaassen et al., 1999). Some of the other effects (on cognition, sleep) are not restricted to these groups, but may also occur in healthy volunteers.

The effects of ATD are usually compared to an amino acid mixture with tryptophan (Murphy et al., 2002; Park et al., 1994; Rubinsztein et al., 2001). This 'control' mixture generally results in a considerable but highly variable increase in plasma tryptophan and the tryptophan/LNAA ratio, thereby forming an active control condition instead of a placebo condition. This is especially undesirable when investigating subtle effects. An alternative was developed by Krahn et al. (1996): a quarter-strength amino acid mixture containing the same amino acids as the ATD mixture, again without tryptophan. The alternative was necessary because tryptophan was banned from the US market for several years after 1990. This mixture is also not a neutral control condition, but results in a predictable moderate reduction of the plasma tryptophan/LNAA ratio. Since this 'low-dose' mixture has been

found not to affect mood (e.g. Booij et al., 2005a), it allows for an investigation of the dose-response effects of lowered serotonin function and thus seems to be a better control condition for some research questions. Recently, different studies (Hayward et al., 2005; Munafò et al., 2006) have reported effects of a different low-dose tryptophan depletion method, using a mixture containing eight amino acids instead of the regular fifteen amino acids. The low-dose tryptophan depletion method is further discussed in Chapter 5.

*Dietary interventions*

Carbohydrate-rich diets have been found to increase the tryptophan/LNAA ratio and thus increase central serotonin function. This is due to a carbohydrate induced insulin response that stimulates the uptake of LNAA in skeletal muscles with the exception of tryptophan (Fernstrom & Wurtman, 1971). However, these increases are only found under rather extreme dieting conditions (Yokogoshi & Wurtman, 1986). Carbohydrate-rich, protein-poor diets have been found to increase the tryptophan/LNAA ratio by 42% compared to a control diet (Markus et al., 1998) and to prevent stress induced deterioration of mood and cortisol response but only in stress-vulnerable subjects (Markus et al., 1998). Also, carbohydrate intake improves cognitive performance of stress-vulnerable subjects under controllable laboratory stress (Markus et al., 1999). Overall, the effects of carbohydrate-rich diet seem to depend on factors such as the time of day, the type of task and the vulnerability of the population (Dye et al., 2000).

Another way to manipulate tryptophan levels is a diet rich in the milk-whey alpha-lactalbumin (Heine et al., 1996). Diet rich in alpha-lactalbumin leads to an increase in plasma tryptophan/LNAA ratio of 48% compared to a casein (placebo) diet (Markus et al., 2000), thereby raising brain serotonin

activity. Diet enriched with alpha-lactalbumin has been found to prevent stress-induced cortisol and mood response (Markus et al., 2000) and to improve cognitive performance (Markus et al., 2002), but again only in stress-vulnerable individuals.

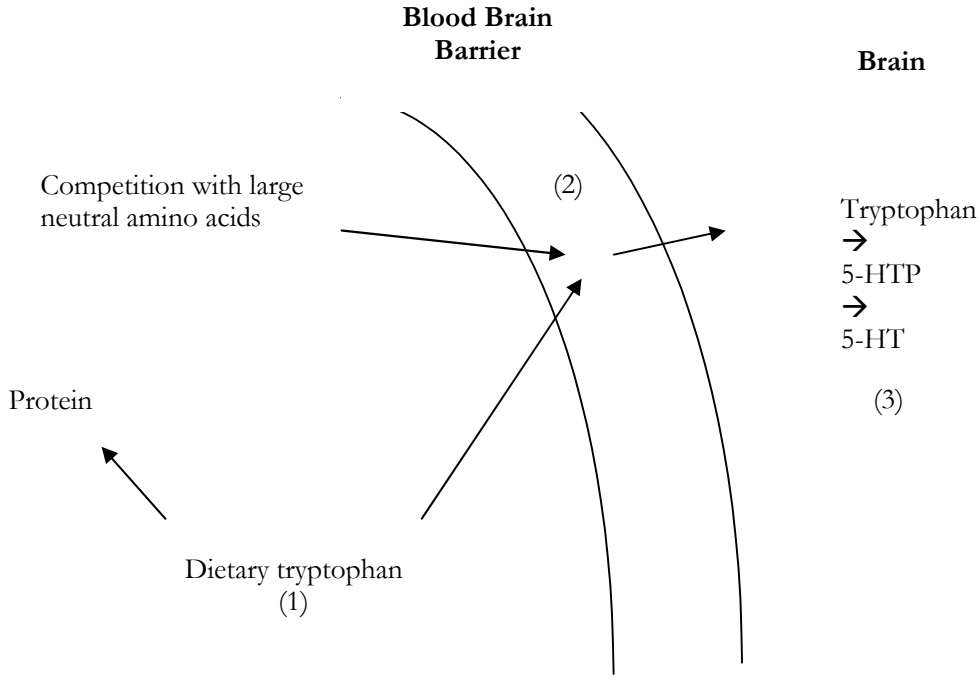
## Cognitive and biological vulnerability to depression

*Cognitive function*

Problems concentrating and making decisions are part of the diagnostic criteria of Major Depressive Disorder (American Psychiatric Association, 1994). Experimental research has shown that memory, learning, attention, motor function and problem solving may also be affected in depressed patients (Austin et al., 2001; Elliott, 1998; Weiland-Fiedler et al., 2004). In terms of impairments in emotional (as opposed to neutral) information processing, the recognition of facial expressions of emotions has been found to be affected in depressed patients (Bouhuys et al., 1999; Gur et al., 1992). Also an increased attentional bias for negative information (Williams et al., 1996) and an increased level of dysfunctional attitudes (Ingram et al., 1998) are found compared to healthy controls.

Some of these cognitive impairments persist into the euthymic phase; however research on cognitive impairments in recovered depressed patients has shown conflicting results (Paelecke-Habermann et al., 2005; Paradiso et al., 1997; Weiland-Fiedler et al., 2004). Recently, evidence indicates that persisting impairments may exist in the specificity of autobiographical memory (Spinhoven et al., 2006), the recognition of facial emotions (Bouhuys et al., 1999) and attentional bias (Williams et al., 1996).

**Figure 1.** Manipulating serotonin synthesis




Serotonin synthesis can be influenced at three levels: by restricting the dietary intake of tryptophan (1); by increasing the competition with the other large neutral amino acids (2); and by inhibition of tryptophan-hydroxylase which synthesizes tryptophan into 5-HTP (3). The ATD method described in this thesis is based on (1) and (2).

Some of these impairments are also related to risk of relapse (Bouhuys et al., 1999; Williams et al., 1996), suggesting that some aspects of emotional information processing may be vulnerability markers for depression.

Evidence from animal and human studies has linked serotonin to cognitive function, especially learning and memory (McEntee & Crook, 1991; Sirviö et al., 1995). ATD studies have supported these findings. In healthy volunteers, ATD selectively impairs learning (Park et al., 1994), memory retrieval and consolidation (Klaassen et al., 2002; Park et al., 1994; Riedel et al., 1999) and ATD improves attention in healthy samples (Schmitt et al., 2000) and patients (Booij et al., 2005a). Recently, interest has been paid to the effects of ATD on emotional information processing; ATD impaired the recognition of facial expressions of fear in female healthy volunteers (Harmer et al., 2003c) and increases emotional interference in both healthy and recovered depressed patients (Hayward et al., 2005; Munafò et al., 2006).

Research on the effects of antidepressant medication also supports the link between serotonin and cognition (Amado-Boccara et al., 1995; Harmer et al., 2003b; Thompson, 1991). Very brief (one day or one week) treatment with a serotonergic antidepressant causes selective changes in emotional information processing, in particular in the recognition of facial expressions of emotions, in both healthy volunteers and recovered depressed women (Bhagwagar et al., 2004; Harmer et al., 2003a; Harmer et al., 2004; Harmer et al., 2006a).

*Stress and cortisol*

Depressive episodes are often preceded by stressful life events (Brown et al., 1987; Kendler et al., 1999). Elevated cortisol levels, caused by stressful life events, may lower brain serotonin function and in turn lead to a depressed

state (Cowen, 2002). High cortisol levels may initially cause higher central nervous system turnover; however during continuous or frequent stress the availability of brain tryptophan and serotonin may diminish and vulnerability to depression may increase (Markus, 2003).

This makes cortisol an important biological mediator through which stress lowers serotonin function and thereby causes depression in vulnerable individuals. Cortisol is controlled by the hypothalamo-pituitary-adrenal (HPA) axis with which the central serotonergic system interacts. The finding of HPA-axis hyperactivity in depression appears to be consistent, although it is not found in all patients (Jans et al., 2007).

To study neuroendocrine dysfunction in depression, neuroendocrine challenge tests are used, such as d-fenfluramine (Cleare et al., 1998) or the dexamethasone/ corticotrophin releasing hormone (CRH) test (Baghai et al., 2002). Evidence from challenge studies indicates that depressed and remitted depressed patients show blunted neuroendocrine responses to drugs that stimulate serotonin turnover, suggesting decreased serotonin responsiveness (Bhagwagar et al., 2002a; Bhagwagar et al., 2002b; Flory et al., 1998; Riedel et al., 2002). These results indicate that blunted cortisol responses to a neuroendocrine challenge may be a vulnerability marker for depression.

*Heart rate variability*

Cardiovascular disease (CVD) is the leading cause of death in the United States (American Heart Association, 2006). Depression has been found to be an independent risk factor for CVD (see for a review Rugulies, 2002). Depression after a myocardial infarction also predicts mortality (Anda et al., 1993). Decreased heart rate variability (HRV) is a risk factor for CVD (Stein & Kleiger, 1999) and has also been associated with depression and may thus

underlie the increased risk of cardiovascular disease in depression (Gorman & Sloan, 2000; Grippo & Johnson, 2002; Musselman et al., 1998). HRV is a measure of autonomic regulation of the heart (Krantz & McCeney, 2002). HRV reflects the capacity of the autonomic nervous system to vary the intervals between consecutive heartbeats (Grippo & Johnson, 2002). Reductions in HRV are not exclusively related to depression (Agelink et al., 2002; Rechlin et al., 1994) but are also associated with generalized anxiety disorder (Thayer & Lane, 2000), impulse control disorders such as ADHD (Beauchaine et al., 2001), and alcoholism (Ingjaldsson et al., 2003). Negative results have also been found (Gehi et al., 2005). Serotonin dysfunction is suggested to play an etiological role in both depression and cardiac dysfunction (Grippo & Johnson, 2002), and may thus underlie the association between HRV and depression.

Considering the different vulnerability factors for depression that were discussed above and the fact that serotonin plays an important role in the pathophysiology and the treatment of depressive disorders, it would be interesting to investigate the specific role that serotonin plays in the cognitive and biological vulnerability to depression. Experimental manipulations of serotonin function may influence cognitive and/ or biological factors in individuals that are vulnerable to depression (e.g. remitted or recovered depressed patients). Since serotonin is linked to cognitive performance, the cortisol response to stress and heart rate variability, experimental changes in serotonin function may affect these processes, resembling the findings in depressed patients.

**Research aims**

This thesis will investigate the effects of three different serotonin manipulations (an alpha-lactalbumin enriched diet, low-dose ATD, high-dose ATD) on mood and cognitive processing in euthymic patients with a history of depressive disorder and healthy controls. The literature regarding a possible link between serotonin induced changes in mood and emotional information processing will also be discussed.

The first project that was carried out as part of the current thesis investigated the effects of an alpha-lactalbumin enriched diet, which increases serotonin activity, on mood and different aspects of neutral information processing in recovered depressed patients and healthy controls. The second project focussed on the effects of acute tryptophan depletion, which lowers serotonin function, on mood and neutral as well as emotional information processing in medicated remitted depressed patients. In addition to these two empirical studies, an overview of the literature is given on the effects of serotonin manipulations on mood and emotional information processing, to evaluate a possible link between serotonin induced changes in mood and emotional information processing. Apart from the effects of serotonin manipulations on cognitive processing, the link between serotonin activity and two different biological vulnerability factors for depression was also investigated. The first study additionally investigated the effects of alpha-lactalbumin on stress-induced cortisol response and the second study also looked at the effect of acute tryptophan depletion on heart rate variability.

**Outline of this thesis**

In Chapter 2, results of a study are reported in which remitted depressed patients are compared to healthy controls to investigate possible residual

cognitive impairments that persist into the euthymic phase. Chapter 3 will describe the effects of an alpha-lactalbumin enriched diet on cognitive performance in unmedicated recovered depressed patients and healthy controls. In Chapter 4 the effects of alpha-lactalbumin on mood and stress-induced cortisol response in unmedicated recovered depressed patients and healthy controls are reported. Chapter 5 describes the effects of low-dose and high-dose ATD on mood and neutral as well as emotional information processing in medicated remitted depressed patients. In Chapter 6, the effects of low-dose and high-dose tryptophan depletion on individual plasma tryptophan levels and the ratio tryptophan/LNAA will be discussed. In Chapter 7 the effects of ATD on heart rate variability in medicated remitted depressed patients are reported. A literature overview of studies investigating the effects of serotonin manipulations on emotional information processing and mood is given in Chapter 8. Also, evidence for a possible sequential link between serotonin induced changes in emotional information processing and mood is evaluated. Chapter 9 contains a summary and integration of the main findings, as well as methodological strengths and limitations, directions for future research and clinical implications of the findings reported in this thesis.

# 2

# Residual cognitive impairments in remitted depressed patients

W. Merens, L. Booij, A.J. W. Van der Does.
*Depression & Anxiety* (in press)

**Abstract**

Depressive disorders are associated with various cognitive impairments. Studies on whether or not these impairments persist into the euthymic phase have shown conflicting results, due to differences in test versions and in study samples. In the current paper we aimed to compare the cognitive performance of remitted depressed patients with that of age- and gender matched healthy volunteers across a wide range of cognitive domains. In two studies we found few differences on neutral as well as emotional information processing tests. The findings indicate that remitted depressed patients who use antidepressant medication still show an increased recognition of facial expression of fear compared to healthy controls. Patients also performed worse on a test of recognition of abstract visual information from long-term memory. No other residual cognitive impairments were found. These results indicate that most of the cognitive impairments associated with depression resolve with recovery through medication, even when recovery is incomplete. Considering the finding that remitted depressed patients have higher levels of cognitive reactivity future studies may investigate the possibility that these cognitive impairments have not resolved but have become latent, and may therefore easily be triggered by small changes in mood state.

**Introduction**

Problems concentrating and making decisions are part of the diagnostic criteria of major depressive disorder (American Psychiatric Association, 1994). Experimental research has shown that memory, learning, attention, motor function and problem solving may also be affected in depressed patients (Austin et al., 2001; Elliott, 1998; Weiland-Fiedler et al., 2004). The cognitive functions that are most impaired in depression are those which require effortful executive functioning, which is highly dependent on the prefrontal cortex (Elliott, 1998). Some studies have focused on impairments in emotional (as opposed to neutral) information processing in depressed patients. For example, the recognition of facial expressions of emotions has been found to be affected in depressed patients (Bouhuys et al., 1999; Gur et al., 1992). Also an increased attentional bias for negative information (Williams et al., 1996) and an increased level of dysfunctional attitudes (Ingram et al., 1998) are found compared to healthy controls.

Given the high risk of relapse in depression, it is important to investigate whether cognitive impairments persist into the euthymic phase and if so, whether these impairments may be predictive of depressive relapse. Research on cognitive impairments in recovered depressed patients has shown conflicting results. These conflicting results may be a function of differences in study sample, such as gender distribution, age, education level, residual depressive symptoms, medication status, and diagnosis. Marcos et al. found differences on tests measuring paired learning, immediate and delayed visual memory, delayed logical memory and block design between euthymic patients and healthy controls (Marcos et al., 1994). Part of the patient sample was medicated with imipramine, part of the sample was unmedicated at the time of study. The two groups consisted of both men and women and were equal in

age (mean ages 54 and 52 years) and education level. In another study, differences between depressed and non-depressed subjects on different memory tests (verbal memory, immediate and delayed recall, learning, retrieval) disappeared following imipramine treatment, but only in treatment responders. Improvement in depressive symptoms led to significant improvement in memory performance (Peselow et al., 1991). Again both groups were equal in age (mean 48-50 years), gender distribution (both men and women were tested) and level of intelligence. Paradiso et al. (1997) compared cognitive performance of patients with a –relatively chronic- history of unipolar and bipolar depressive disorder to that of age- (mean age 50-57 years) and education matched controls. Only male subjects were included and almost all patients were taking some form of psychotropic medication (benzodiazepines, tricyclics, trazodone). They found that euthymic unipolar patients performed worse on tasks measuring executive function (Trail Making B, Stroop CWT), visual-motor sequencing (Trail Making A), immediate memory (word-list memory test) and attention (digit symbols) compared to healthy controls. In another study, unmedicated male and female remitted depressed patients were impaired on tasks of rapid visual information processing (sustained attention), psychomotor speed and spatial working memory compared to healthy controls (Weiland-Fiedler et al., 2004). However, after correcting for residual depressive symptoms, only the difference in sustained attention remained significant. In this study mean ages were 36 and 38 years and all patients had been taking antidepressant medication in the past. These results were supported by another study that found medicated and unmedicated euthymic patients to be impaired in attentional and executive function (Paelecke-Habermann et al., 2005).

Regarding emotional information processing, persisting impairments have been found in the specificity of autobiographical memory (Spinhoven et

al., 2006), the recognition of facial emotions (Bouhuys et al., 1999) and attentional bias (Williams et al., 1996). Some of these impairments are also related to risk of relapse (Bouhuys et al., 1999; Williams et al., 1996).

Overall, depressed patients show cognitive impairments across a wide range of domains. Some of these impairments improve with clinical recovery, while others may persist into the euthymic phase. Some cognitive impairments may even be related to depressive relapse. However, following the results of Weiland-Fiedler et al. (2004), it remains questionable whether remitted depressed patients show any cognitive impairments in comparison to an adequately matched control group and, most importantly, when residual depressive symptoms are taken into account. The current study investigated cognitive performance in medicated, remitted depressed patients, who are expected to show relatively high levels of residual depressive symptoms, and two matched control groups. To cover a wide range of tests, two separate studies were undertaken. The two studies differed in the type of information processing that was assessed. Study 1 included mainly tests of emotional information processing; study 2 included tests that assessed neutral information processing. To check for possible differences between the study samples, both studies included a fluency test and a measure of attentional bias. No precise hypotheses were formed since the literature does not provide unequivocal results.

## Materials and Methods

### Study 1:

*Participants:*

*Patients:* As part of a larger study, two samples of remitted depressed patients were recruited from a Mood Disorders Program. Participants were male and female outpatients (of the Mood Disorders Program of Parnassia Psycho-medical Center, The Hague). Patients were at different stages in treatment, but were referred to the study only when their therapist thought they would meet criteria for remitted or recovered depression. Age limits were 18 to 65 years. Participants had to fulfill the following inclusion criteria: primary intake diagnosis of DSM-IV major depressive disorder; no longer fulfilling DSM-IV criteria for depression and Hamilton-17 scores lower than or equal to 15 (Frank et al., 1991); ongoing treatment with a selective serotonin reuptake inhibitor (SSRI) or selective serotonin and noradrenalin reuptake inhibitor (SSNRI) for at least four weeks; no history or current psychotic disorder; no substance abuse in the past 3 months, based on DSM-IV criteria; BMI equal or higher than 18; free of neuro-endocrine or neurological disease; no pregnancy or lactation (females). [1]

*Controls:* Healthy control participants were recruited through advertisements in local newspapers. Participants were matched to the patient group on age and gender. Inclusion criteria were: no mood disorders (lifetime); no first degree relatives with a mood disorder (lifetime); no history or current psychotic disorder; no substance abuse in the past 3 months, based on DSM-IV criteria; no use of psychotropic medication, free of neuro-endocrine or neurological disease.

[1] The patients in Study 1 are the same sample as in Merens et al. (Journal of Psychopharmacoly, in press); those in Study 2 are the same as in Booij et al. (2005), Journal of Psychopharmacoly 19, 267-275. The present data are slightly different, because in these two reports, baseline data were calculated on the basis of the screening session and a post-intervention session.

*Materials*

*Self-report:* The Beck Depression Inventory (Beck et al., 1996) is a self-rating scale that assesses the presence and severity of depressive symptoms. The Dutch version was used (BDI-II-NL, Van der Does, 2002b). The Dysfunctional Attitudes Scale (DAS, Weissman, 1979) assesses the level of dysfunctional attitudes. A 22 item version was used, based on the original form A. The Leiden Index of Depression Sensitivity (LEIDS) (Van der Does, 2002a) consists of 34 items and assesses the effects of dysphoric mood on cognitions ('cognitive reactivity').

*Depression severity:* The Hamilton Depression Rating Scale (HAM-D-17) was administered to patients to assess the severity of depressive symptoms (Hamilton, 1967).

*Cognition:* The cognitive test-battery took about 50 minutes to complete.

*Word Learning Test* (Saan & Deelman, 1986): A list of 15 unrelated, neutral words was presented on a tape. Immediate recall was tested after each of five consecutive presentations. After the fifth trial, subjects continued with a non-verbal task. Fifteen minutes later delayed recall was tested. Immediate recall performance was defined as the total of correct words remembered over the five trials. Delayed recall performance was defined as the number of correct words produced at delayed recall.

*Verbal fluency*: This task is a measure of strategy-driven retrieval from semantic memory within a fixed time span (Schmitt et al., 2000). Participants were instructed to produce as many correct four letter words as possible with the same initial letter within one minute. The starting letters were H, M, R or L; these were randomized over the participants. The total number of correct reported words was registered.

*Implicit Association Test:* The IAT is a sorting task that assesses implicit associations on the basis of reaction times (Egloff & Schmukle, 2002; Greenwald et al., 1998). This test is extensively used in social psychological research to assess stereotypes (Greenwald & Banaji, 1995). Participants are asked to sort stimuli representing four categories by pressing the appropriate key (each response key was assigned to two categories). If two categories are strongly related, the sorting task will be easier (i.e. faster RTs) when the categories share the same response key than when they share different response keys. We used an emotional and a neutral version of this task. Only median latencies for correct responses were included in the analyses. Reaction times to congruent (e.g. self and positive stimuli, insect and negative stimuli) and incongruent stimuli (e.g. self and negative stimuli, flowers and negative stimuli) were calculated.

*Dot-probe test*: This task measures attentional bias to emotional stimuli (MacLeod et al., 1986). Word pairs (threat words with neutral words and depression related words with positive words) were presented on a computer screen for 500 ms, one in the upper part of the screen and one below. Following the termination of that display, a dot appeared on the location of either word. Participants had to indicate the location of the dot by pressing a key. All word pairs were preceded by a white fixation cross for 500 ms. To control for possible outliers, only median latencies for correct responses were included in the analyses. Attentional bias was calculated by subtracting the RT for positive (neutral) words from the RT for depressive (threatening) words.

*Facial Expression Recognition test:* The facial expression recognition task, adapted from Harmer et al. (2003c), features examples of five basic emotions—happiness, sadness, fear, anger, and disgust (Ekman & Friesen, 1976). Emotional expression intensity was averaged between neutral (0%) and

emotional standard (100%) in 10% steps, providing a range of emotional intensities. Each emotion-intensity was presented by two examples (one male and one female face) in random order. Each face was presented on a computer screen for 500 ms. and immediately replaced by a blank screen. Participants made their response by pressing a labeled key, after which the next face appeared on the screen. They were instructed to respond as quickly and accurately as possible. Accuracy of recognition was calculated over the different intensity levels in five (20%) blocks. Reaction times for correct responses were calculated.

*Procedure*

*Patients:* After showing interest in taking part, all volunteers were given oral and written information about the study. Informed consent was obtained and participants who seemed to meet criteria were invited for the first session. During this session, the SCID-IV interview was administered to ensure patients no longer fulfilled criteria for MDD (First et al., 1995). Participants filled out all questionnaires and afterwards the cognitive tests were done. The session lasted two to three hours. Clinical background information was checked in medical records. The study was approved by an independent medical ethics committee (METIGG, Utrecht).

*Controls:* The healthy control subjects came in for one session in which the SCID-IV interview was administered to check the absence of mood disorders and other exclusion criteria. All questionnaires were filled out and the cognitive tests were performed during the same session, which lasted two to three hours.

**Study 2:**

In- and exclusion criteria, methods and procedures were identical to study 1. However, the DAS was not filled out and the LEIDS was only completed by patients and therefore not reported here.

*Cognition:* The cognitive tests took approximately 60 min.

*Verbal Fluency:* This test was identical to the fluency test in study 1.

*Stroop Colour Word test:* This test measures focused attention and response inhibition. Names of colours (red, yellow, blue and green) printed in black were presented one by one for a maximum of 1500 ms on a computer screen. Participants were instructed to read these words as fast as possible (Condition I). Next, coloured patches were presented (Condition II). Finally, the names of colours printed in an incongruent colour were presented and participants were instructed to name the colour of the ink (Condition III). Median reaction times (RTs) were recorded. Interference was defined as the extra time needed for condition III relative to the average of conditions I and II.

*Emotional Stroop test:* This test was used to assess attentional bias for emotional material. The stimuli were positive, neutral or depression-related words. Words printed in colour were presented consecutively on a computer screen. Participants were asked to name the colours as quickly as possible. The order of the word categories was randomized over the patients. The order of the words within each category was randomized.

*Left/Right Choice RT*: This test assesses motor speed and response inhibition as a function of task difficulty. The word 'left' or 'right' was presented in randomized order (1000 ms) either at the left or the right side of the screen. Participants were instructed to respond to the meaning of the word

but to ignore its location, as fast as possible. Correct responses and RTs were registered.

*Tower of London (TOL):* The TOL (Owen et al., 1995a) is a planning task consisting of three coloured balls (red, yellow and blue) placed on three sticks in various arrangements. Two arrangements were presented on the upper and lower half of the screen. The patient was instructed to indicate the minimal number of moves necessary to change the first arrangement into the second (two to five moves). Correct responses and RTs were registered.

*Abstract Patterns Recognition task (APRT):* The APRT (Rubinsztein et al., 2001) measures (speed of) recognition of non-verbal abstract information from short- and long-term memory. Sixteen abstract patterns were presented consecutively for 3000 ms, with 500 ms intervals. Participants were instructed to memorize the patterns. After three presentations of the complete series, two patterns were presented simultaneously; one that had been learned and a new pattern. Participants had to indicate as fast as possible which one had been previously presented. The recognition procedure was repeated after 35 min, during which verbal tasks were administered. Sensitivity measures (A' ) were calculated for the proportion of correctly recognized patterns, corrected for response tendency by the formula: A' = 1 – ¼ [fr/ cr + (1-cr) / (1– fr)], in which fr = the proportion of falsely recognized patterns and cr = proportion of correctly recognized patterns, following signal detection theory (Pollack & Norman, 1964).

*Statistical analysis*

Data were first screened for missing values, outliers, normal distributions and homogeneity of variance. Differences between patients and controls were analyzed with GLM ANOVA with Group as a fixed factor and BDI-II total

score as a covariate. Since matching for Level of education was unsuccessful in study 1, this variable was also entered as a covariate in the analyses of the cognitive measures from study 1. Data from the Facial Emotion Recognition task were analyzed with GLM repeated measures analysis with Emotion (happiness, sadness, fear, anger, and disgust) as a within-subjects factor and Group (controls vs. remitted depressed patients) as a between-subjects factor and BDI-II and Level of education as covariates. The TOL was also analyzed using GLM repeated measures with Steps (2, 3, 4, 5) as a within-subjects factor and Group as a between-subjects factor and BDI-II as a covariate. Data are reported as means ± standard deviations. All tests were corrected for multiple testing using Bonferroni corrections.

## Results

### Study 1:

*Data screening*

On the Facial Expression Recognition task, reaction time data were missing for one emotion in two control participants, one of whom did not recognize any sad faces correctly, the other did not recognize any angry faces correctly. On the Word Learning Test, data were missing for one control subject for the immediate recall, due to technical problems. One control subject was an outlier on the Word Learning Test as well as the IAT Neutral. Another control was an outlier on the Dot-probe test. Analyses were conducted with and without statistical outliers, however results were similar.

*Participants:*

Twenty healthy controls and nineteen remitted depressed subjects were included in the study. Participants were well matched on age and gender, however the control group had a higher level of education compared to the patient group ($\chi^2 = 10.6$, $p = .005$). Current comorbid diagnoses in the remitted depressed group were Social phobia (n = 1), Specific phobia (n = 2), chronic PTSD (n = 1) and Dysthymia (n = 4). Table 1 and 2 show clinical and demographical characteristics of both patients and controls of Study 1 and Study 2.

*Self report measures*

Recovered depressed patients scored higher on the BDI-II ($t(19.6) = -5.5$, $p < .001$) compared to controls. Patients also scored higher on the DAS ($t(37) = -3.7$, $p = .001$) and on some subscales of the LEIDS compared to the control group: Harm Avoidance ($t(37) = -6.6$, $p < .001$), Rumination ($t(37) = -9.6$, $p < .001$), Hopelessness ($t(37) = -2.2$, $p = .037$) and on the Total score ($t(37) = -4.2$, $p < .001$). Controls scored higher on Acceptance/Coping ($t(37) = 2.3$, $p = .026$) and Aggression ($t(37) = 2.2$, $p = .031$). When controlled for residual depressive symptoms, only the differences on the LEIDS Total score ($F(1,36) = 7.3$, $p = .010$), Rumination ($F(1,36) = 39.9$, $p < .001$) and Harm Avoidance ($F(1,36) = 16.5$, $p < .001$) remained significant.

**Table 1.** Characteristics of Study 1 and Study 2 (mean (SD))

| | **Study 1** | | | | |
|---|---|---|---|---|---|
| | Controls ($n$ = 20) | Patients ($n$ = 19) | | | |
| | | | **t** | **df** | ***p*** |
| Age (SD) | 47.7 (14.1) | 44.2 (13.0) | 0.8 | 37 | .426 |
| BDI-II | 1.4 (1.7) | 11.7 (8.0) | -5.5 | 19.6 | .000** |
| LEIDS totalscore | 24.7 (12.6) | 40.0 (9.7) | -4.2 | 37 | .000** |
| DAS | 58.8 (15.9) | 80.2 (19.8) | -3.7 | 37 | .001** |
| | | | **$\chi^2$** | **df** | ***p*** |
| M/F | 1/19 | 2/17 | 0.4 | 1 | .517 |
| Education level | | | 10.6 | 2 | .005** |
| - low | $n$ = 2 | $n$ = 7 | | | |
| - medium | $n$ = 8 | $n$ = 11 | | | |
| - high | $n$ = 10 | $n$ = 1 | | | |
| | **Study 2** | | | | |
| | Controls ($n$ = 21) | Patients ($n$ = 20) | | | |
| | | | **t** | **df** | ***p*** |
| Age | 44.1 (10.2) | 48.7 (7.9) | -1.6 | 39 | .114 |
| BDI-II | 5.2 (5.3) | 12.9 (10.1) | -3.0 | 28.4 | .006** |
| | | | **$\chi^2$** | **df** | ***p*** |
| M/F | 9/12 | 11/9 | 0.6 | 1 | .437 |
| Education level | | | 0.8 | 2 | .665 |
| - low | $n$ = 5 | $n$ = 3 | | | |
| - medium | $n$ = 6 | $n$ = 8 | | | |
| - high | $n$ = 10 | $n$ = 9 | | | |

BDI-II = Beck Depression Inventory, 2nd edition; LEIDS = Leiden Index for Depression Sensitivity; DAS = Dysfunctional Attitudes Scale
** $p < .010$

**Table 2.** Clinical characteristics of both patient groups (mean ± SD)

| | **Study 1** (*n* = 19) | **Study 2** (*n* = 20) |
|---|---|---|
| $HAM\text{-}D_{17}$ | 7.7 ± 3.6 [range 1-13] | 5.6 ± 3.8 [range 0-13] |
| Type of medication | | |
| - SSRI | *n* = 13 | *n* = 13 † |
| - SSNRI | *n* = 6 (150-375 mg) | *n* = 7 (75–225 mg) |
| Type of remission[1]: | | |
| - partial remission | n = 8 | n = 13 |
| - full remission | n = 11 | n = 7 |
| Duration of remission (months) ± SD | 13.1 ± 22.3 [range 1-102]†† | 5.9 ± 5.6 [range 1-24] |
| Number of episodes ± SD | 4.9 ± 4.1 [range 1-15] | 4.8 ± 4.4 [range 1-16] |
| Single / recurrent episode(s) | 2 / 17 | 4 / 16 |
| Diagnosis, subtype[2]: | | |
| - MDD, melancholic | *n* = 16 | *n* = 11 |
| - MDD, atypical | *n* = 1 | *n* = 6 |
| - MDD, seasonal pattern | - | *n* = 2 |
| - Not melancholic, atypical or catatonic | *n* = 2 | *n* = 1 |

HAM-D = Hamilton Rating Scale for Depression; SSRI = Selective Serotonin Reuptake Inhibitor; SSNRI = Selective Serotonin and Noradrenalin Reuptake Inhibitor; † two SSRI treatment free for 1 month; [1:] according to the criteria of Frank et al. 1991; †† this wide range is caused by one patient who had been recovered for over 8 years; without that patient the range is [1, 21]; [2:] subtype of most recent depressive episode

*Cognition*

See Table 3a for the cognitive tests of study 1.

*Facial Expression Recognition test:* Only a significant effect of Emotion ($F(3.9,137.6) = 10.3$ , $p < .001$) was found on the overall accuracy data, indicating that participants were better at recognizing certain emotions compared to others (see Figure 1). The main effect of Group was not significant ($F(1,35) = 1.5$, $p = .233$). Separate analyses per Emotion revealed a significant effect of Group ($F(1,35) = 5.5$, $p = .024$) for the recognition of fear, indicating that remitted depressed patients were better at recognizing facial expressions of fear compared to controls. Univariate analyses on fear accuracy per intensity level (in five 20% blocks) showed that the effect of Group was significant or borderline significant for all levels, except for the 30-40% intensity level: 10-20% $F(1,35) = 4.2$, $p = .049$; 30-40% $F(1,35) = 0.1$, $p = .788$; 50-60% $F(1,35) = 4.1$, $p = .049$; 70-80% $F(1,35) = 7.2$, $p = .011$; 90-100% $F(1,35) = 4.1$, $p = .051$ (see Figure 2). No significant main and interaction effects were found for the other emotions.

Regarding the reaction time data, a significant effect of Emotion was found ($F(2.7,88.0) = 4.1$, $p = .011$). The main effect of Group was not significant ($F(1,33) = 0.0$, $p = .834$). When analyzed per emotion, no significant effects of Group or Group x Emotion were found.

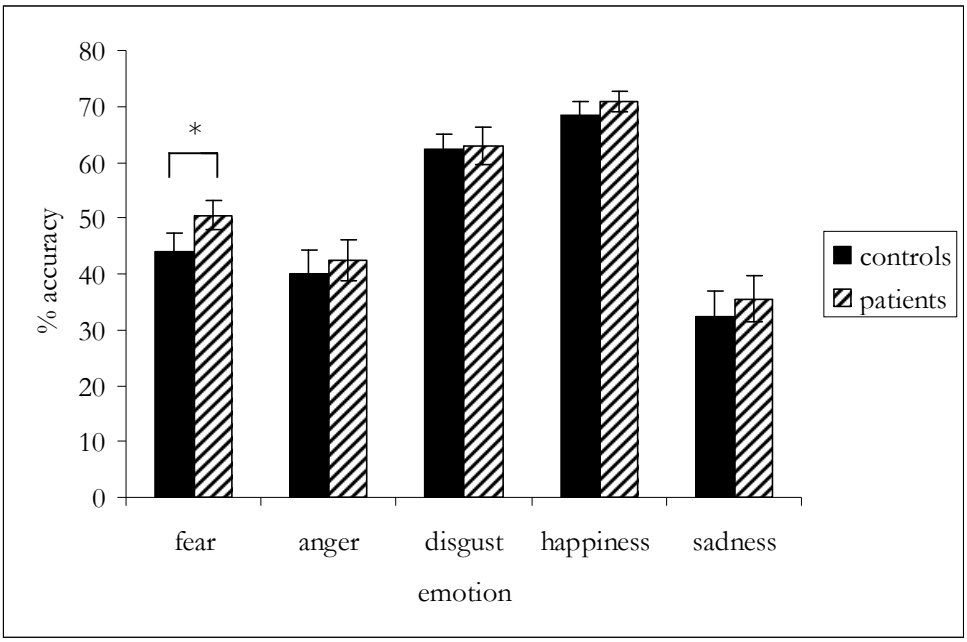
No other significant differences between the groups on cognitive performance were found in study 1.

**Table 3a.** Cognitive tests of Study 1, presented as means (SD)

| | **Controls** (*n* = 20) | **Patients** (*n* = 19) | ***F*** | **df** | ***p*** |
|---|---|---|---|---|---|
| **Verbal memory (WLT)** | | | | | |
| immediate recall # correct | 52.0 (9.0) | 49.6 (11.0) | 0.3 | 1,34 | .581 |
| delayed recall # correct | 11.1 (2.2) | 10.8 (2.7) | 1.3 | 1,35 | .260 |
| **Verbal Fluency** | | | | | |
| # correct | 12.4 (3.6) | 9.9 (3.5) | 0.03 | 1,35 | .868 |
| **IAT Neutral[2]** | | | | | |
| RT congruent (ms) | 685.7 (107.8) | 663.3 (126.4) | 2.4 | 1,35 | .134 |
| RT incongruent (ms) | 1139.8 (271.2) | 1049.0 (273.6) | 1.1 | 1.35 | .294 |
| **IAT Emotional** | | | | | |
| RT congruent (ms) | 828.6 (209.2) | 897.3 (304.2) | 0.1 | 1,35 | .717 |
| RT incongruent (ms) | 742.4 (111.8) | 847.4 (245.3) | 0.1 | 1,35 | .816 |
| **Dot-probe** | | | | | |
| AB depressive - positive (ms) | -2.3 (20.5) | -1.3 (22.9) | 0.0 | 1,35 | .904 |
| AB anxious - neutral (ms) | -1.4 (18.1) | -6.0 (16.5) | 0.4 | 1,35 | .524 |
| **FERT** | | | | | |
| **- Accuracy** | | | 1.5 | 1,35 | .233 |
| Anger | 1.6 (0.8) | 1.7 (0.6) | 0.5 | 1,35 | .505 |
| Fear | 1.8 (0.6) | 2.0 (0.5) | 5.5 | 1,35 | .024* |
| Sadness | 1.3 (0.8) | 1.4 (0.7) | 1.3 | 1,35 | .268 |
| Happiness | 2.7 (0.4) | 2.8 (0.3) | 0.2 | 1,35 | .669 |
| Disgust | 2.5 (0.5) | 2.5 (0.6) | 0.0 | 1,35 | .836 |
| **- Speed (ms)** | | | 0.0 | 1,33 | .834 |
| Anger | 1061.1 (344.2) | 1205.2 (305.4) | 0.1 | 1,34 | .783 |
| Fear | 1123.9 (525.6) | 1212.1 (464.9) | 0.3 | 1,35 | .578 |
| Sadness | 1459.6 (495.5) | 1514.2 (981.0) | 0.3 | 1,34 | .568 |
| Happiness | 805.1 (190.5) | 870.9 (233.6) | 0.0 | 1,35 | .999 |
| Disgust | 907.8 (263.1) | 1114.9 (691.3) | 0.4 | 1,35 | .542 |

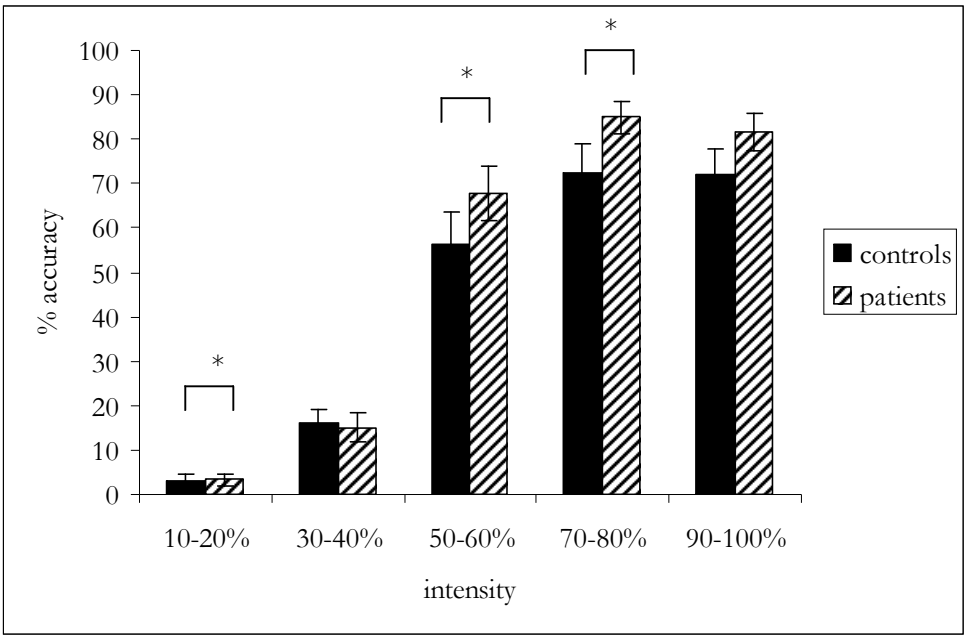
AB = attentional bias, FERT = Facial Expression Recognition Test, IAT = Implicit Attitudes Test, RT = reaction time, WLT = Word Learning Test, * $p < .05$, *F* values present the main effect of Group; [2]: analyses without one outlier are presented. All analyses were performed with Bonferroni corrections.

**Figure 1.** Facial emotion recognition for controls and remitted depressed patients (Mean ± SEM)




* $p < .05$

**Figure 2.** Fear accuracy over the different intensity levels (Mean ± SEM)




* $p < .05$

**Study 2:**

*Data screening:*

One patient missed all 4- and 5-step problems of the TOL. One control participant missed all 5-step problems of the TOL. Data for another control participant are missing for all positive words on the Emotional Stroop task. Cases with missing data were omitted separately by analysis. Outliers were found on the APRT, Stroop CWT, and Emotional Stroop test. Analyses were conducted with and without statistical outliers, however results were similar. The Verbal Fluency data were successfully log 10 transformed because of a non-normal distribution.

*Participants:*

Twenty-one controls and twenty remitted depressed patients were included in this study. The control group did not differ from the patient group in terms of gender, age and education level. Past comorbid diagnoses in the remitted depressed patient group were Panic disorder (n = 3, of whom one in partial remission), Social phobia (n = 1) and Anorexia nervosa (n =1).

*Self-report*

The remitted group had higher BDI-II scores compared to the control group ($F(1,39) = 9.19$, $p = .004$).

*Cognition*

See Table 3b for the cognitive tests of study 2.

*APRT:* A significant effect of Group was found for the recognition from long term memory (A'): $F(1,38) = 5.0$, $p = .030$. Patients appeared to

perform worse than controls at recognition of abstract visual information from long term memory.

**Discussion**

The current results indicate that medicated remitted depressed patients show an increased recognition of facial expressions of fear compared to healthy controls, even after statistical correction for differences in depressive symptoms. Also, patients scored higher on a self-report measure of cognitive reactivity and performed worse than controls at a task measuring recognition of abstract information from long term memory. No other residual cognitive impairments were found on a wide range of tests, despite the fact that the patients still suffered from residual depressive symptoms and were relatively chronic. The BDI-II scores of patients were higher than those of healthy controls, although both groups' scores were within the normal range (Van der Does, 2002b). These findings support the view that most cognitive deficits associated with depression are associated with clinical status, rather than a persisting vulnerability factor (Weiland-Fiedler et al., 2004). Some deficits may be more persistent however, and the higher cognitive reactivity scores suggest that the deficits may have become 'latent'.

A number of studies have shown that cognitive deficits may not be apparent when they are only assessed at 'resting' state (Lau et al., 2004). This implies that negative information processing biases may be rather easily activated by dysphoric mood states – either naturally occurring or induced in the laboratory. This process is called *cognitive reactivity*. Cognitive reactivity is an important vulnerability factor that is linked to depressive relapse (Segal et al., 2006).

**Table 3b.** Cognitive tests of Study 2, presented as means (SD)

| | **Controls** (*n* = 21) | **Patients** (*n* = 20) | ***F*** | **df** | ***p*** |
|---|---|---|---|---|---|
| **Verbal Fluency** | | | | | |
| # correct | 10.4 (3.9) | 12.1 (5.1) | 0.1 | 1,38 | .720 |
| **Stroop CWT** | | | | | |
| Condition I (ms) | 567.0 (76.9) | 552.5 (72.8) | 0.2 | 1,38 | .664 |
| Condition II (ms) | 487.5 (62.5) | 490.2 (56.0) | 0.1 | 1,38 | .784 |
| Condition III (ms) | 775.7 (156.4) | 792.0 (109.8) | 0.0 | 1,38 | .906 |
| Interference (%) | 47.3 (23.8) | 52.3 (16.7) | 0.1 | 1,38 | .774 |
| **Emotional Stroop Task** | | | | | |
| Negative words (ms) | 712.3 (88.2) | 749.6 (115.0) | 0.0 | 1,38 | .895 |
| Neutral words (ms) | 693.7 (91.4) | 722.5 (74.3) | 0.1 | 1,38 | .741 |
| Positive words (ms) | 702.1 (124.1) | 705.3 (83.4) | 0.1 | 1,37 | .729 |
| Interference negative (%) | 3.1 (9.2) | 3.7 (9.9) | 0.1 | 1,38 | .780 |
| Interference positive (%) | 1.7 (10.7) | -2.3 (6.7) | 1.1 | 1,37 | .295 |
| **Left/right task** | | | | | |
| Congruent (ms) | 634.9 (94.2) | 678.4 (58.9) | 0.9 | 1,38 | .353 |
| Incongruent (ms) | 652.0 (97.7) | 700.4 (54.6) | 2.0 | 1,38 | .168 |
| **Tower of London** | | | | | |
| **- % correct** | | | 0.3 | 1,36 | .584 |
| 2 steps | 88.1 (17.5) | 84.5 (16.7) | | | |
| 3 steps | 85.2 (19.4) | 78.5 (11.8) | | | |
| 4 steps | 72.9 (15.5) | 75.8 (21.2) | | | |
| 5 steps | 65.0 (24.0) | 54.7 (29.9) | | | |
| **- RT (ms)** | | | 0.1 | 1,36 | .812 |
| 2 steps | 5337.3 (1190.4) | 6733.6 (2001.4) | | | |
| 3 steps | 7359.3 (2424.0) | 8101.8 (3388.0) | | | |
| 4 steps | 10869.1 (3101.7) | 11902.9 (4482.1) | | | |
| 5 steps | 19407.5 (7191.4) | 17908.7 (8352.7) | | | |
| **APRT** | | | | | |
| A' STM (%) | 83.0 (9.7) | 78.3 (11.7) | 1.5 | 1,38 | .226 |
| A' LTM (%) | 80.5 (9.9) | 74.9 (14.2) | 5.0 | 1,38 | .030* |
| RT STM (ms) | 2164.2 (805.6) | 2308.0 (802.9) | 0.8 | 1,38 | .380 |
| RT LTM (ms) | 1976.4 (715.5) | 2107.9 (597.3) | 0.1 | 1,38 | .808 |

CWT = Colour Word Test; APRT = Abstract Visual Patterns Task; RT = reaction time; STM = short term memory; LTM = long term memory; *F* values represent the main effect of Group. All analyses were performed with Bonferroni corrections.

The finding of the current study that the difference between remitted depressed patients and controls in DAS scores became non-significant after controlling for residual symptoms is in line with Miranda et al. (1990) who have already shown that dysfunctional attitudes are mood-state dependent for subjects with a history of depression. The group differences on the LEIDS, which aim to measure reactivity of cognitions, remained significant after correction. The current findings therefore suggest that some of the other cognitive deficits might also be more easily triggered in remitted depressed patients than in never-depressed individuals. In line with our findings, Gemar et al. (2001) did not find any baseline differences when they studied implicit attitudes in formerly depressed and never depressed subjects. Only after a sad mood induction, a shift was found toward a negative evaluative bias in the formerly depressed group, again supporting the suggestion that cognitive impairments may become latently present following clinical recovery.

Interestingly, the finding that remitted depressed patients were better in recognizing fear indicates that facial expression recognition may be a scar and a persisting vulnerability factor for relapse to depression. Bhagwagar et al. (2004) also found increased recognition of fear in recovered depressed subjects relative to controls; however administration of a single dose of citalopram normalized this increased fear recognition. In contrast, our patients were already medicated for more than four weeks before entering the study. Bouhuys et al. (1999) found that increased perception of negative emotions is related to relapse, although the recognition of negative emotions decreased from the acute to the remitted phase. The conceptualization of fear recognition as a vulnerability marker was further supported in a study by Masurier et al. (2007) who found faster recognition of facial expressions of fear in female first-degree relatives of depressed patients compared to controls without a

family history of depression. Biases in the processing of emotional information may thus be a stable trait characteristic, even occurring before the onset of a first depressive episode (Leppänen, 2006; review).

Finally, the finding that the remitted depressed patients performed worse on a test measuring recognition from long-term visual memory is in line with previous studies which have shown persisting impairments in memory processes in euthymic patients (Marcos et al., 1994.)

In the current studies, remitted depressed patients were not impaired on tests measuring attentional bias. Studies in recovered depressed subjects mainly used the Stroop Colour Word task to measure attentional bias. Both Paradiso et al. (1997) and Trichard et al. (1995) found persisting impairments in Stroop performance in recovered depressed patients. Attentional bias is thought to be not only a symptom of depression, but also to be important in the development and maintenance of depressive disorders (Williams et al., 1996). Our results do not support this position, since no impairments were found on neutral and emotional Stroop interference as well as on attentional bias measured with the Dot-probe test. However, the literature on attentional bias in depression is contradictory, which may be explained by the differences in stimulus presentation- times (Mathews et al., 1996; Mogg et al., 1995). Studies using the Dot-probe test have found attentional biases in depression using relatively long stimulus presentations (1 sec or more) (Mogg et al., 1995). When stimuli are presented for shorter durations, results are mixed (Bradley et al., 1997; Mathews et al., 1996). Our stimulus presentation time of 500 ms. was probably not optimal to detect group differences.

One factor that might limit interpretation of the data is that patients were treated with serotonergic antidepressants when participating in the study.

Serotonergic antidepressants may have some sedative side effects, but these tend to wear off in the first two weeks of treatment (Amado-Boccara et al., 1995) and the effects on memory and psychomotor performance are of low intensity (Gorenstein et al., 2006; Thompson, 1991). In contrast, SSRIs have been found to positively affect neutral and emotional information processing acutely and after 7 to14 days (Bhagwagar et al., 2004; Harmer et al., 2002; Harmer et al., 2003a; Harmer et al., 2004; Harmer et al., 2006a). However, unmedicated recovered depressed patients also did not show any differences in neutral information processing compared to healthy controls (Booij et al., 2006a), although these groups did differ on cognitive reactivity (Merens et al., 2005). The latter studies used a considerable younger and less chronic sample however. How chronic SSRI use affects emotional processing is still unclear, so it may be possible that some cognitive impairments were remediated by SSRI treatment.

It also has to be considered that the lack of differences between groups in the current study may have been caused by insufficient statistical power. Sample sizes in both studies are relatively small and replication in larger samples is warranted. The fact that both patient groups were not completely asymptomatic only strengthens our conclusion that remitted depressed patients do not suffer from many cognitive impairments. Also, remission status (partial vs. full) did not affect the facial expression recognition data.

Future research may investigate the influence of clinical variables (chronicity, age of onset, treatment modality etc.) on cognitive performance of remitted depressed patients, to clarify possible mediating factors leading to cognitive impairment in depression. Finally, as cognitive function was not assessed during the acute phase of the depressive episode, it cannot be ruled out that we selected groups of remitted depressed patients who showed little

cognitive impairments even in a depressed state. However, this seems very unlikely since cognitive impairments in depression are common (Austin et al., 2001; Elliott, 1998) and both patients groups were relatively chronic.

# 3

# The effects of a diet enriched with alpha-lactalbumin on mood and cortisol response in unmedicated recovered depressed subjects and controls

W. Merens, L. Booij, C.R. Markus, F.G. Zitman, W. Onkenhout & A.J.W. Van der Does. (2005). *British Journal of Nutrition, 94,* 415–422

**Abstract**

Alpha-lactalbumin is a tryptophan-rich protein fraction. A diet enriched with alpha-lactalbumin increases the ratio of tryptophan to the other large neutral amino acids (LNAA), which may in turn increase brain serotonin content. In stress-vulnerable individuals, alpha-lactalbumin improved mood and attenuated the cortisol response after experimental stress. The aim of the present study was to investigate the effects of an alpha-lactalbumin-enriched diet on mood and stress response in recovered depressed subjects and healthy controls. Forty-three subjects (twenty-three recovered depressed and twenty healthy subjects) received alpha-lactalbumin and casein (placebo) on separate days, in a double-blind randomised crossover design. On both occasions, subjects underwent a stress test (an unsolvable mental arithmetic task with loud noise). The stress test affected mood in both conditions. Although the alpha-lactalbumin diet led to the expected rises in tryptophan and tryptophan/LNAA ratio, only minimal effects were found on mood and cortisol response to experimental stress. The results were the same for recovered depressed patients and controls. A one-day diet enriched with alpha-lactalbumin is not sufficient to prevent stress-induced mood deterioration or a cortisol response in unmedicated, recovered depressed subjects. Future studies may investigate the effects of longer-term diets or may investigate different samples (e.g. medicated patients).

**Introduction**

There is abundant evidence that serotonin (5-hydroxytryptamine; 5-HT) plays an important role in stress-related disorders such as major depression (Maes & Meltzer, 1995; Meltzer & Lowy, 1987). For instance, medications that augment 5-HT activity, such as selective serotonin reuptake inhibitors and monoamine oxidase inhibitors, are effective antidepressants (Blier & de Montigny, 1998; Nutt et al., 1999). Furthermore, experimental depletion of the 5-HT precursor L-tryptophan induces symptoms in depression-vulnerable subjects (patients in remission or family members of patients) but not in healthy subjects (Bell et al., 2001; Van der Does, 2001a). Conversely, tryptophan administration increases 5-HT synthesis in the brain in both human subjects and rats, and these effects are large enough to influence mood and behaviour (Young, 1996). For example, tryptophan decreased aggression and quarrelsome behaviour, and increased dominant behaviour, in healthy volunteers (Marsh et al., 2002; Moskowitz et al., 2001; Young & Leyton, 2002). In healthy females, tryptophan improved emotion recognition (Attenburrow et al., 2003). Tryptophan also has some therapeutic effect in mild to moderate depression, but it is not effective in more severe depression (Young, 1986; Young, 1996). It is clear, however, that boosting or depleting the 5-HT system has opposite effects on mood, although some of these effects are only observable in depression-vulnerable subjects.

Several studies have attempted to increase 5-HT concentrations through dietary interventions. Although selective serotonin reuptake inhibitors have fewer side-effects than other antidepressants, and are not toxic in overdose, a sizable number of patients do not tolerate them or fail to benefit from them (Fava, 2000). Survey data show that many patients prematurely stop their medications because of side-effects (Consumer Reports, 2004). So, if

effective and feasible, dietary interventions may comprise a viable addition or alternative to antidepressant medications. A carbohydrate-rich/protein-poor diet has been shown to increase the ratio of tryptophan/large neutral amino acids (LNAA). The diet prevented stress-induced mood deterioration and cortisol response in individuals vulnerable to stress (defined by high neuroticism scores; Markus et al., 1998). Carbohydrate-rich/protein-poor diets are, however, not healthy on a day-to-day basis (Christensen, 1997), and even a small amount of protein blocks the rise of tryptophan/LNAA.

Recently, an even larger effect on stress response was obtained with a diet containing tryptophan-rich alpha-lactalbumin protein. Alpha-lactalbumin has the highest tryptophan concentration of all protein fractions (Heine et al., 1996). This diet increased plasma tryptophan/LNAA by 43–48%, which is twice the augmentation found with a carbohydrate- rich/protein-poor diet (Markus et al., 2000; Markus et al., 2002), or after 7 d of daily tryptophan treatment (Chouinard et al., 1985). Alpha-lactalbumin had no side-effects, and improved mood and attenuated cortisol responses to stress in stress-vulnerable subjects (students with high neuroticism scores) but not in controls (low neuroticism; Markus et al., 2000). Orosco et al. (2004) found that alpha-lactalbumin had an anxiolytic effect in rats and increased 5-HT turnover in hippocampal areas. Since depression-vulnerable individuals are most likely to be affected by 5-HT manipulations, it seems worthwhile exploring the effects of alpha-lactalbumin in this group.

The present study was designed to investigate the effects of alpha-lactalbumin in depression-vulnerable individuals and in healthy, non-vulnerable controls. Based on previous research (Markus et al., 2000; Markus et al., 2002), we expected to find a protective effect of alpha-lactalbumin on stress-induced mood deterioration and cortisol response, rather than a direct effect on mood.

Furthermore, we expected these effects to occur only in the depression-vulnerable group.

## Materials and methods

*Subjects*

Eligible subjects were between 18 and 65 years of age and had either a history of a major depressive episode (patient group) or no history of mental disorder and no first-degree relative with a major depressive disorder (control group). Subjects were euthymic and were selected not on the basis of neuroticism scores (Markus et al., 2000; Markus et al., 2002) but on a history of depression. Approximately 60% of people with high neuroticism scores actually develop a depression (Gallagher, 1990; Kendler et al., 2004; Ormel et al., 2001). Since long-term studies have shown that up to 85% of recovered depressed patients experience a recurrence (Mueller et al., 1999), a past history of depression is a better marker of vulnerability.

In both groups, exclusion criteria were: current mental disorder; past psychotic disorder; substance abuse in the past 3 months or excessive dieting or binge eating, as defined by the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV; American Psychiatric Association, 1994). Furthermore, subjects in both groups had low levels of depressive symptoms (Montgomery Asberg Depression Rating Scale (MADRS score < 8), were not colour-blind or dyslexic, had been free of antidepressant medication for at least three months, had a normal body weight (BMI 18–27 kg/$m^2$), kept to a regular diet and were free of neuroendocrine or neurological disease. Female subjects were not pregnant or lactating, had a regular menstrual cycle or were postmenopausal. Pre-menopausal women who were not taking a contraceptive pill were tested during their mid-to-late follicular phase (days 4–10), whereas

pre-menopausal women taking a contraceptive pill participated during the period in which they actually took the pill. The groups were matched on gender, age and education level.

*Design*

The study was conducted according to a double-blind crossover design. Subjects were given balanced isoenergetic meals containing alpha-lactalbumin-enriched whey protein or casein protein (placebo). Treatment order was balanced over two sessions, separated by four weeks.

*Instruments*

Standardised, validated instruments were used to assess diagnosis, depressive symptoms, mood states and personality. The Structured Clinical Interview for DSM-IV (First et al., 1995) was used to verify diagnostic inclusion and exclusion criteria. Depressive symptoms were measured with the MADRS (Montgomery & Asberg, 1979), a ten-item interviewer-rating scale, and the self-rating Beck Depression Inventory (BDI-II; Beck et al., 1996; Van der Does, 2002b).

The Profile of Mood States (POMS; McNair et al., 1971; Wald & Mellenbergh, 1990) measures 'depression', 'anger', 'fatigue', 'tension' and 'vigor'. Neuroticism was measured to facilitate comparison with previous research (Markus et al., 2000; Markus et al., 2002); the Eysenck Personality Questionnaire (Eysenck et al., 1985) was used. Cognitive reactivity, a psychological vulnerability marker of depression, was also measured, using the Depressed States Checklist (DSC; Teasdale & Cox, 2001) and the Leiden Index of Depression Sensitivity (Van der Does, 2002a). The DSC consists of items that 'objectively' describe a depressed mood (e.g. sad, unhappy), and items that

also have a self-devaluative denotation (e.g. useless, a failure). Cognitive reactivity is the ratio of the endorsed self-devaluative and objective items (Teasdale & Cox, 2001). The Leiden Index of Depression Sensitivity is a self-rating scale of cognitive reactivity, with subscales 'hopelessness', 'rumination', 'harm avoidance', 'aggression', 'control/ perfectionism' and 'acceptance/coping' (Van der Does, 2002a).

*Procedure*

Depressed patients who had participated in a psychotherapy trial 1–2 years earlier were sent information and invited to participate. Subjects were also recruited through advertisements in a local newspaper, and through advertisements and leaflets at the local mental health centre and at several university buildings. Individuals who expressed interest were interviewed by telephone. If the inclusion and exclusion criteria appeared to be met, more information was sent, and people were invited for a screening session at the hospital, during which the Structured Clinical Interview for DSM-IV and MADRS was administered by trained clinical psychologists. Information about previous antidepressant treatments and the course and duration of depressive episodes was also obtained, and questionnaires were completed. Subjects gave informed consent to participate prior to filling out the questionnaires. The first session was scheduled for approximately 1 week after the screening session, or somewhat later depending on the menstrual cycle phase.

Subjects arrived at the laboratory at 9.00 am after fasting overnight. They were instructed not to use alcohol for 24 hours prior to the sessions and to arrive well rested. They received breakfast and lunch including either alpha-lactalbumin or casein. In the morning and afternoon, a battery of cognitive tests was administered, the results of which are reported elsewhere (Booij et al.,

2006a). During the day, two blood samples and four cortisol samples were taken. The session finished at around 3.30 pm, and subjects were instructed to resume their regular meals. The outline of sessions is shown in Table 1. Subjects were paid € 80 for participation. Subjects were tested individually at a clinical research unit of Leiden University Medical Centre.

**Table 1.** Scheme of the sessions

| **Time** (hrs) | | **Assessment** |
|---|---|---|
| 09.00 am | $t_{-1}$ | Arrival, BDI-II, DSC, POMS, blood sample |
| 10.00 | $t_{0}$ | Breakfast |
| 12.00 | $t_{2}$ | Lunch |
| 1.30 pm | $t_{3.5}$ | Cortisol (1), blood sample, POMS, DSC |
| 2.35 | $t_{4.5}$ | Cortisol (2), POMS, |
| 2.40 | | Stress Induction |
| 3.00 | $t_{5}$ | Cortisol (3), POMS, DSC |
| 3.10 | $t_{5.2}$ | Cortisol (4), Debriefing |

BDI-II = Beck Depression Inventory-II; DSC = Depressed States Checklist; POMS = Profile of Mood States

*Intervention*

The alpha-lactalbumin and casein diets used in the present study were identical to the diets used by Markus et al. (2000; 2002). Diets contained 1229 kJ with 5.2% energy as protein, 84.2% energy as carbohydrate and 10.6% energy as fat, composed of standard products (Netherlands Nutrition Centre). Each breakfast and lunch consisted of one slice of bread, 10 g butter, 15 g fruit jelly, 200 ml grape juice and a chocolate drink. The two diets were similar in

appearance and in macronutrient content with the exception of the composition of the chocolate-flavoured drink, for which the protein sources differed. Apart from breakfast and lunch, subjects were only allowed to drink water and one cup of tea or coffee.

The nutrient composition and amino acid profile of both chocolate drinks are displayed in Table 2. The amounts of tryptophan in the alpha-lactalbumin and casein diets were 12.3 g/kg (tryptophan/LNAA 8.7) and 9.5 g/kg (tryptophan/LNAA 4.7), respectively. The chocolate drink was prepared within 20 min before breakfast (first drink) and 20 min before lunch (second drink). To compensate for any taste differences, sugar was added to all the drinks (10 g; see Table 2). All meals were supervised to make sure that all foods were consumed.

**Table 2.** Composition of the chocolate drinks used in the alpha-lactalbumin and casein diets

| | **α-lactalbumin diet** | **casein diet** |
|---|---|---|
| **Composition** (g) | | |
| α-lactalbumin-enriched whey protein | 20 | 0 |
| Sodium caseinate | 0 | 20 |
| Cocoa | 3.5 | 3.5 |
| Granulated Sugar | 10 | 10 |
| Water | 200 | 200 |
| **Amino Acid Profile** (g/kg) | | |
| Isoleucine | 27.61 | 31.80 |
| Leucine | 47.56 | 59.31 |
| Phenylalanine | 20.80 | 32.24 |
| Tyrosine | 16.82 | 33.13 |
| Valine | 29.52 | 44.09 |
| Tryptophan | 12.32 | 9.51 |
| Tryptophan/LNAA (weight %) | 8.70 | 4.70 |

LNAA= large neutral amino acids

*Stress task*

An impossible mental arithmetic task, performed under noise stimulation, was used as an experimental stressor. Subjects were given eighteen successive 1 min trials, during which they had to do mental arithmetic under time constraints, while different levels of industrial noise (65, 70 or 80 dB) were presented through a headphone. Multiple choice questions were presented on a computer screen one at a time. A specified number of calculations (called the criterion) had to be solved correctly. Subjects were led to believe that they could control the intensity of noise by their performance. If they failed the criterion, the computer would set the noise level higher during the next trial; if, however, they met the criterion, they could choose the noise level themselves. Before the actual test, subjects were given two practice trials during which they had to solve a few calculations without noise and then with each of the three noise levels successively. The credibility of the task as well as motivation were enhanced by providing the subjects with constant on-screen feedback about the criterion in a particular trial, the number of calculations correctly solved and the time left for that trial.

Experimental stress was induced by manipulating the criterion so that all subjects continued to fail on each trial and thus could not choose the noise level for the next trial. The criterion was always set at one sum above what subjects could manage, as calculated from the average time per sum needed on the previous trial. This task has been demonstrated to be uncontrollable and to induce transient psychological and physiological stress (Markus et al., 1999; Markus et al., 2000; Markus et al., 2002; Peters et al., 1998). The stress task proved to be sensitive in crossover designs in studies by Markus et al. In addition, the test affects perceived stress as well as systolic and diastolic blood pressure (Peters et al., 1998).

*Salivary cortisol*

Cortisol samples were obtained with the Salivette sampling device (Sarstedt, Nümbrecht, Germany). With this procedure, saliva was collected in small cotton swabs and stored in special Salivette tubes at -30 °C until centrifugation. Saliva samples were centrifuged at 3000 rpm for 3 min at 20 °C. Cortisol concentrations were determined without extraction using a competitive chemiluminescence immunoassay.

A 20 µl sample of saliva was pipetted into microtitre plate wells coated with rabbit anti-cortisol antibodies. After the addition of 100 µl cortisol conjugated with horseradish peroxidase (enzyme conjugate), the mixture was incubated for 3 h at room temperature, and the cells were washed to stop the competition reaction. After addition of the chemiluminescence substrate solution containing peroxide and luminol, luminescence measurements were performed using a Berthold microtitre plate luminometer (Medical Laboratories, Mönchengladbach, Germany).

*Blood assessments*

Total tryptophan and its concentration ratio to LNAA (valine, isoleucine, leucine, tyrosine and phenylalanine) were determined by amino acid analysis. Blood was collected in 4 ml EDTA-containing tubes, and plasma was isolated after centrifugation at 2650 g at 20 °C for 20 min. For the determination of (total) tryptophan and LNAA, 400 µl plasma was deproteinised using an equal volume of 0.075 (w/v) sulphosalicylic acid in water, which concomitantly contained 382 µmol/l of the internal standard L-2,4-diamino- butyric acid (Fluka, Milan, Italy). The supernatant was isolated after centrifugation at 8000 g at 20 °C for 2 min, filtered through Millex 0.22 µm, 25 mm membrane filter units (Millipore, Billerica, MA, USA); 60 µl of this was analysed in 170 min by

means of ion-exchange chromatography and ninhydrin derivatisation on a Biochrom 20 automated amino acid analyzer (Pharmacia, Uppsala, Sweden) using standard conditions for physiological amino acid separation.

*Statistical analyses*

Group differences in demographic characteristics were examined with independent-sample t-tests. The effects of the interventions on cortisol response were analysed by means of repeated-measures ANOVA using general linear models. Within-subjects factors were Intervention (alpha-lactalbumin v. casein) and Time of assessment (before and after stress); the between-subjects factor was Group (recovered depressed v. controls). All mood scores were analysed using non-parametric tests (Wilcoxon and Mann–Whitney U). A test was considered to be significant when the *p* value was .05 or less. SPSS 11.5 (SPSS Inc., Chicago IL, USA) was used.

*Ethical considerations*

The study was approved by the Medical Ethics Committee of Leiden University Medical Center. Previous studies using casein or alpha-lactalbumin diets have shown that the procedure is safe, with no side-effects (Markus et al., 2000; Markus et al., 2002). Prior studies have used the stress induction task (Markus et al., 1999; Markus et al., 2000; Markus et al., 2002; Peters et al., 1998). There are no indications that the cognitive tasks or the stress task elicit the recurrence of depression.

## Results

*Data screening*

A very small number of missing values on items of the symptom questionnaires were replaced by the grand mean. No statistical outliers were detected. Twenty-two blood samples (13% of the total) were not available owing to difficulties with venepuncture. The amino acid levels and ratios were all normally distributed. Cortisol values and the scores on the mood subscales were not; these data were $\log^{10}$ transformed. Subscales of the POMS and DSC were analysed using non-parametric tests as the data were highly skewed and transformations were unsuccessful.

*Sample characteristics*

Fifty-seven individuals were invited for the screening session, eight of whom did not meet the inclusion and exclusion criteria. Three eligible subjects dropped out after the screening session, and another three dropped out after the first test day for various reasons. A total of forty-three subjects completed both test days. As shown in Table 3, there were no significant differences in age, education and gender distribution between groups. MADRS scores in both groups were low and did not differ significantly. The only significant difference was found on BDI-II scores at screening, with recovered depressed subjects having higher scores. However, BDI-II scores were low and well within the normal range in both groups. As shown in Table 4, the recovered depressed patient group had a mean age of depression onset at 19.9 years. The mean number of episodes was two. Approximately one-third had taken antidepressant medication in the past, and almost half had a first-degree relative with depression.

**Table 3.** Demographic and clinical characteristics (Mean ± SD)

| | **Recovered depressive** (*n* = 23) | **Healthy controls** (*n* = 20) | **Recovered vs. controls** | | |
|---|---|---|---|---|---|
| | | | ***t* / *Z*** | ***df*** | ***p*** |
| Age | 30.0 ± 9.7 | 27.0 ± 10.1 | .99 | 41 | .32 |
| Female % | 91.3 | 85 | ($\chi^2$) .41 | 1 | .52 |
| Education level[1] | 4.9 ± .8 | 5.1 ± .6 | -1.07 | 41 | .29 |
| BMI (kg/m$^2$) | 22.8 ± 2.5 | 21.7 ± 2.1 | 1.60 | 41 | .12 |
| MADRS | 1.3 ± 1.6 | 0.8 ± 1.6 | 1.03 | 41 | .31 |
| BDI-II | 4.4 ± 4.5 | 1.5 ± 2.2 | 2.83 | 41 | .008 |
| EPQ-R | | | | | |
| - Psychoticism | 3.2 ± 1.7 | 2.6 ± 1.3 | 1.29 | 41 | .20 |
| - Extraversion | 7.6 ± 3.4 | 9.7 ± 2.3 | -2.31 | 41 | .03 |
| - Neuroticism | 5.6 ± 2.3 | 2.6 ± 2.0 | 4.43 | 41 | < .001 |
| - Social desirableness | 5.7 ± 2.1 | 5.8 ± 2.1 | -.10 | 41 | .92 |
| LEIDS | | | | | |
| - Hopelessness | 4.6 ± 3.0 | 1.4 ± 1.0 | 4.76 | 41 | < .001 |
| - Aggression | 5.8 ± 3.9 | 3.1 ± 3.1 | 2.45 | 41 | .02 |
| - Perfectionism | 8.0 ± 4.7 | 4.1 ± 3.3 | 3.18 | 41 | .003 |
| - Harm avoidance | 9.5 ± 4.8 | 3.5 ± 2.6 | 5.23 | 41 | < .001 |
| - Rumination | 10.7 ± 4.9 | 5.6 ± 2.5 | 4.39 | 41 | < .001 |
| - Acceptance/coping | 2.2 ± 3.9 | 1.0 ± 1.7 | 1.38 | 41 | .18 |
| - Total score | 40.8 ± 18.9 | 18.5 ± 9.8 | 4.94 | 41 | < .001 |
| DSC | | | | | |
| - Affective | 26.5 ± 9.8 | 21.1 ± 5.0 | 1.79 | | .07 |
| - Self-devaluative | 22.0 ± 6.6 | 17.0 ± 2.6 | 2.42 | | .02 |

MADRS = Montgomery-Asberg Depression Rating Scale; BDI-II = Beck Depression Inventory-II; EPQ-R = Eysenck Personality Questionnaire-Revised; LEIDS = Leiden Index of Depression Sensitivity; DSC = Depressed States Checklist.
[1] On a scale of 1-6.

Significant baseline differences between the groups were found on the POMS depression ($Z = -2.21$, $p = .03$), POMS Fatigue ($Z = -2.06$, $p = .04$), POMS total ($Z = -2.31$, $p = .02$) and DSC self-devaluative ($Z = -2.42$, $p = .02$) subscales. Trends were found for POMS tension ($Z = -1.77$, $p = .08$) and DSC affective ($Z = -1.79$, $p = .07$). The recovered depressed subjects had higher scores on all these subscales. On the Eysenck Personality Questionnaire, groups differed on extraversion ($F(1,41) = 5.32$, $p = .03$) and neuroticism ($F(1,41) = 19.6$, $p < .001$). The neuroticism scores of the recovered depressed group were above average to high, according to published norms for Dutch populations (Sanderman et al., 1995). The two groups also differed on most subscales of the Leiden Index of Depression Sensitivity. After controlling for depressive symptoms (BDI-II), only the following subscales remained significant: neuroticism ($F(1,40) = 12.9$, $p = .001$), and the Leiden Index of Depression Sensitivity subscales of hopelessness ($F(1,40) = 13.6$, $p = .001$), control/perfectionism ($F(1,40) = 5.6$, $p = .02$), harm avoidance ($F(1,40) = 16.5$, $p < .001$), rumination ($F(1,40) = 10.5$, $p = .002$) and total ($F(1,40) = 13.9$, $p = .001$).

**Table 4.** Clinical characteristics of the recovered depressed subjects

| | **Mean** | **SD** |
|---|---|---|
| Age of onset (years) | 19.9 | 7.7 |
| Number of episodes | 2.0 | 0.9 |
| Antidepressant medication (past) | 34.8 % | |
| Antidepressant treatment (ever) | 78.3 % | |
| Family history (1st degree) of major depressive disorder | 47.8 % | |
| Current psychotherapy | 13 % | |

*Effect of dietary manipulations on amino acids*

As shown in Table 5, plasma tryptophan concentrations and tryptophan/LNAA did not differ at baseline between groups. Repeated measures analyses with Intervention and Time as within-subjects factors and Group as between-subjects factors on tryptophan/LNAA revealed a significant effect of Intervention ($F(1,29) = 84.3$, $p < .001$). Tryptophan/ LNAA increased significantly by 20.9% compared to baseline after the alpha-lactalbumin-diet and decreased by 30.0% after the casein diet. The relative rise of tryptophan/LNAA was 73.8% higher after alpha-lactalbumin than after casein. We also found significant effects of Time ($F(1,29) = 7.1$, $p = .013$) and of the Intervention x Time interaction ($F(1,29) = 193.3$, $p < .001$). There was no main effect of Group, nor any interaction effects involving Group.

**Table 5.** Plasma tryptophan (µmol/l) and tryptophan/LNAA ratio (Mean ± SD)

| | | **casein** | **alpha-lactalbumin** |
|---|---|---|---|
| Tryptophan | morning | 50.5 ± 11.2 | 50.8 ± 10.1 |
| | afternoon | 57.3 ± 8.9 | 87.9 ± 14.5 |
| Tryptophan/LNAA | morning | .11 ± .02 | .11 ± .02 |
| | afternoon | .08 ± .01 | .13 ± .02 |

*Effects of stress on cortisol*

Two cortisol samples were collected after the stress task, $t_5$ (directly after stress) and $t_{5.2}$ (10 minutes later). The highest cortisol level of these two was used in the analyses ($t_{max}$). Repeated measures analyses with Intervention and Time (before and after stress) as within-subjects factors, and Group as

between-subjects factor, on cortisol levels did not reveal the expected interaction of Intervention x Group x Time ($F(1,41) = 0.1$, $p = .71$). We did, however, find a significant main effect of Time ($F(1,41) = 9.7$, $p = .003$), reflecting an increase in cortisol levels after stress. No other main or interaction effects were found. Cortisol levels are shown in Table 6.

To check for any order of intervention effects, Order, instead of group, was included in the analysis as a between-subjects factor. No interaction effect of Order x Intervention was found ($F(1,41) = .01$, $p = .93$). A trend for an interaction effect of Intervention x Time x Order was found ($F(1,41) = 3.4$, $p = .07$), caused by the fact that cortisol did not rise after stress for subjects who had the alpha-lactalbumin diet on the second day. Alpha-lactalbumin did not have this protective effect for subjects who had that diet on the first day.

Although Markus et al. (1998; 2000) found cortisol responses in both men and women – using the same stressor – previous research with other stressors has shown that response may be dependent on gender, and for females on menstrual cycle and use of oral contraceptives (Kirschbaum et al., 1999). We therefore divided the total group of forty-three subjects into three groups: men ($n = 5$), women taking ($n = 23$) and women not taking ($n = 15$) hormonal contraceptives. This analysis showed significant interaction effects of Intervention x Group ($F(2,41) = 4.3$, $p = .02$), and of Time x Group ($F(2,41) = 3.8$, $p = .03$). Women taking contraceptives had no cortisol response to stress. Women not taking hormones had a small rise of cortisol on the casein day and not on the alpha-lactalbumin day, whereas men showed the reverse pattern. Because of the small groups and the opposite effects in men and women no further analyses were carried out.

*Effects of alpha-lactalbumin on mood*

To investigate any direct effects of alpha-lactalbumin on mood, we analyzed the differences in mood between the morning ($t_{-1}$) and the first afternoon rating ($t_{3,5}$). On the alpha-lactalbumin day, the POMS tension ($Z = -2.6$; $p = .008$) and DSC self-devaluative ($Z = -2.0$, $p = .04$) ratings decreased. A trend was found for POMS anger ($Z = -1.9$; $p = .06$). However, on the casein day, POMS anger ($Z = 2.9$; $p = .003$), depression ($Z = -2.8$; $p = .005$) and fatigue ($Z = -2.1$; $p = .035$), as well as both DSC scores, decreased. During the alpha-lactalbumin session, recovered patients had (almost) significantly larger decreases in DSC self-devaluative ($Z = -2.2$, $p = .03$) and POMS depression ($Z = -1.9$; $p = .06$) scores than controls. The difference in self-devaluative change scores between groups was also significant in the casein condition ($Z = -2.2$, $p = .03$). No other group differences were observed. Thus, mood improved slightly after both interventions. Only POMS depression scores tended to improve more in the recovered depressed group than in the control group on the alpha-lactalbumin day. However, this effect was due to a floor effect in the control group. So, as hypothesized, no direct effects of alpha-lactalbumin on mood were found.

**Table 6.** Cortisol (nmol/l) before ($t_{4.5}$) and after ($t_{max}$) stress (Mean ± SD)

| | **Casein** | | **Alpha-lactalbumin** | |
|---|---|---|---|---|
| | before stress | after stress | before stress | after stress |
| Healthy controls (*n* = 20) | 5.1 ± 6.4 | 5.9 ± 6.3 | 5.7 ± 5.1 | 5.4 ± 2.8 |
| Recovered depressed subjects (*n* = 23) | 5.2 ± 3.8 | 7.4 ± 7.0 | 4.5 ± 1.8 | 6.4 ± 6.4 |
| High neuroticism subjects (*n* = 27) | 5.1 ± 3.7 | 6.8 ± 6.6 | 4.2 ± 2.0 | 5.0 ± 4.1 |
| Low neuroticism subjects (*n* = 16) | 5.4 ± 7.1 | 6.5 ± 7.0 | 6.5 ± 5.3 | 7.6 ± 6.1 |

*Effects of stress on mood*

To investigate any protective effects of alpha-lactalbumin on the response to stress, scores on the POMS and DSC scales before and after stress were analysed. Mood scores are shown in Table 7. In both conditions, significant increases in DSC affective and self-devaluative scores were observed. In the alpha-lactalbumin condition, POMS anger, tension, depression and total scores increased, fatigue tended to increase and vigor decreased in response to stress. In the casein condition, similar changes were found for POMS anger, tension and total scores. A trend was found for depression in the casein condition. In the alpha-lactalbumin condition, recovered depressed subjects showed a significantly larger increase in self-devaluative scores compared with controls ($Z$ = -2.0, $p$ = .04). There were no differences between groups in POMS change scores in response to stress. To analyse the effect of Order on mood response to stress, Kruskal–Wallis tests were performed on mood change scores with order as the grouping variable. A significant effect of Order on vigour change score was found in both the recovered depressed group ($\chi^2$ =

5.8, $p$ = .016) and the control group ($\chi^2$ = 4.3, $p$ = .037), but only for casein. The decrease of vigour was smaller if casein was given on day 2, suggesting that the stressful nature of the task might have been slightly reduced on the second day. The same effect was found for the DSC, but then only in the controls. No other effects of order were found.

Results therefore showed that the stressor affected mood in both the alpha-lactalbumin and the casein condition. The effects were, however, much smaller than expected, and there were no differences between the two diets.

To explore the reasons for these unexpected findings, we also looked at possible effects of past antidepressant medication use, family history of depression and cognitive reactivity on cortisol response and on mood. Again, only significant effects of time were found. Previous research (Markus et al., 2000) using the same paradigm in a healthy population divided on the basis of neuroticism scores found effects of alpha-lactalbumin on mood and cortisol response. Therefore, we reallocated subjects to high- and low-neuroticism groups (median split; see Table 6). As in the previous analyses contrasting previously depressed and never- depressed subjects, no between-group differences in cortisol response were found. A significant interaction effect of Intervention x Group ($F$(1,41) = 7.9, $p$ = .007) and a main effect of Time ($F$(1,41) = 10.3, $p$ = .003) were again found. Further analyses indicated that the low-neuroticism group had higher cortisol levels on the alpha-lactalbumin day than on the casein day and that both groups showed an increase in cortisol level after stress.

**Table 7.** Mood before and after experimental stress (Mean ± SD)

| | **Casein diet** | | **Alpha-lactalbumin diet** | |
|---|---|---|---|---|
| | before stress | after stress | before stress | after stress |
| **Healthy controls** (*n* = 20) | | | | |
| POMS | | | | |
| Anger | 7.2 ± 0.4 | 8.4 ± 1.8 | 7.4 ± 1.1 | 8.6 ± 1.8 |
| Tension | 5.4 ± 0.7 | 5.9 ± 1.2 | 5.2 ± 0.4 | 6.3 ± 2.0 |
| Depression | 8.0 ± 0.0 | 8.2 ± 0.5 | 8.0 ± 0.0 | 8.2 ± 0.4 |
| Vigor | 15.5 ± 4.1 | 15.1 ± 4.3 | 15.3 ± 4.2 | 14.0 ± 4.1 |
| Fatigue | 7.6 ± 2.1 | 7.9 ± 1.8 | 7.5 ± 1.5 | 7.9 ± 1.8 |
| DSC Affective | 15.1± 0.7 | 15.7 ± 1.8 | 15.1± 1.1 | 16.2 ± 2.3 |
| DSC Self-devaluative | 14.0 ± 0.0 | 14.6 ± 1.1 | 14.0 ± 0.0 | 14.7 ± 1.4 |
| **Recovered depressed subjects** (*n* = 23) | | | | |
| POMS | | | | |
| Anger | 7.5 ± 1.4 | 8.7 ± 3.0 | 7.2 ± 0.5 | 8.3 ± 1.9 |
| Tension | 5.4 ± 0.6 | 6.8 ± 2.5 | 5.4 ± 0.8 | 6.8 ± 2.8 |
| Depression | 8.2 ± 0.5 | 8.5 ± 0.9 | 8.1 ± 0.4 | 8.5 ± 1.5 |
| Vigor | 11.7 ± 5.2 | 10.9 ± 4.1 | 12.5 ± 4.6 | 11.0 ± 3.9 |
| Fatigue | 8.2 ± 2.5 | 8.8 ± 2.4 | 8.5 ± 3.2 | 9.1 ± 3.4 |
| DSC Affective | 15.0 ± 1.0 | 16.4 ± 2.3 | 15.6 ± 2.0 | 17.0 ± 4.0 |
| DSC Self-devaluative | 14.0 ± 0.2 | 15.9 ± 3.4 | 14.1 ± 0.5 | 15.9 ± 2.4 |

POMS = Profile of Mood States; DSC = Depressed States Checklist

**Discussion**

This study used an alpha-lactalbumin-enriched diet to increase plasma tryptophan and tryptophan/LNAA ratio in recovered depressed subjects and controls. We hypothesised that alpha-lactalbumin would have a protective effect against stress, particularly in recovered depressed subjects. Although dietary alpha-lactalbumin led to the expected rises in tryptophan and tryptophan/LNAA, only minimal effects were found on mood and cortisol response to stress. The afternoon values of tryptophan/LNAA were 73.8% higher after the alpha-lactalbumin diet than after the casein diet, which is comparable to earlier findings (J.A.J. Schmitt et al., unpublished results). In previous studies, increasing tryptophan/LNAA by 43–48% (compared with casein) caused a decrease in the cortisol response to stress and reduced depressive feelings under stress in stress-vulnerable subjects (Markus et al., 2000). We found rather small effects of the stressor on cortisol and mood, and these changes were independent of group and diet. These finding are at odds with the experimenters' observations and subjects' comments, which indicated that the procedure was experienced as rather stressful and unpleasant. However, a trend of an order effect was found: alpha-lactalbumin had the expected protective effect on cortisol response if taken on the second day. Also, the change in vigor in response to the stress task was greater on the first day than on the second day, when the casein diet was given. However, no order effects were apparent for all other measures of mood. Nonetheless, the stressor may have been slightly more severe during its first presentation (when subjects were less prepared). It would follow that the protective effects of alpha-lactalbumin were insufficient on the first day. Markus et al. (2000), using the same stressor, found a protective effect of alpha-lactalbumin on stress-induced changes in mood and cortisol in students with high neuroticism

scores. There are a few possible explanations for our contradictory results. First, our recovered depressed subjects may have been 'too healthy'. This group's MADRS scores were below 8, they were not taking antidepressant medication, and they were relatively young, so few previous depressive episodes had occurred. Furthermore, the end of the last episode had been on average a little more than 2 years previously, also suggesting that our patient group was relatively healthy and stable. However, the expected group differences on personality (neuroticism) and markers of depressive vulnerability (cognitive reactivity) were observed, implying that the recovered group was, as a group, more vulnerable than the controls. The mean scores on these scales were above the norms for healthy subjects (Eysenck et al., 1985; Sanderman et al., 1995; Van der Does, 2005) but still not extremely high. Furthermore, this explanation is unlikely because Markus et al. found an effect of alpha-lactalbumin in a probably even healthier group. A second possible explanation is that the intervention was not strong enough. As will be reported separately elsewhere, alpha-lactalbumin did have an effect on cognitive function in both groups (Booij et al., 2006a). These findings suggest that the intervention did not fail, but a 1 day alpha-lactalbumin diet may be too weak also to affect mood and cortisol responses to stress in recovered patients. Future studies may investigate a higher dose or a longer duration of the diet. The protective effects of alpha-lactalbumin on cortisol response were also not observed, but this part of the study was hampered by the unexpectedly small effects of the experimental stressor. Previous studies using a different stressor – the Trier Social Stress-Task – have reported gender differences in cortisol response (Kirschbaum et al., 1999): men show the largest cortisol response, followed by women in their late luteal phase, women in the follicular phase and women taking oral contraceptives. The women in our sample were tested in

the late-follicular phase of the menstrual cycle (days 4–10) to minimise possible mood effects. For the purpose of measuring cortisol level, we might better have tested them at a later stage of the cycle. Detailed analyses of gender differences in cortisol response were hampered by the relatively small number of subjects in our sample and by the fact that only five men were included. On the other hand, no gender differences in cortisol response were found in previous studies that used the same stressor as the current study (Markus et al., 2000; Markus et al., 2002). Another issue often raised in stress research is the influence of context, including the person leading the experiment. In the Trier Social Stress-Task, the people conducting the experiment are instructed to be rejecting and 'unkind' to the participant, adding to the level of stress that is meant to be induced by the task. In our study, however, we used a computerised stress task. The instructions were also all presented by computer. The possibility cannot be excluded that the experimenters in the Markus et al. studies were more 'unkind' to the subjects than in the present study, implying that we may have missed the social component of the stressor. If both our results and those of Markus et al. are replicable, this implies that neuroticism and a history of depression are not similar concepts in terms of 5-HT vulnerability. Recent studies using the acute tryptophan depletion method found no relationship between neuroticism and the acute tryptophan depletion response (Stewart et al., 2002; Booij & Van der Does, unpublished results). Another finding from acute tryptophan depletion research is that a depressive response to this manipulation of 5-HT function is mostly found in selective serotonin reuptake inhibitor-treated patients (Delgado et al., 1999). The same may be true for the effects of a tryptophan-enriched diet. Alpha-lactalbumin possibly has a clearer effect in recovered depressed subjects taking selective serotonin reuptake inhibitors. In conclusion, alpha-lactalbumin increased

plasma tryptophan and tryptophan/LNAA in recovered depressed individuals and in healthy controls, but no direct or protective effects were found on mood or on stress-induced cortisol and mood responses. Since cognitive effects did occur (Booij et al., 2006a), future studies may investigate the effects of longer-term diets (e.g. 3 days) or may investigate different samples (e.g. medicated patients).

# 4

# Diet rich in alpha-lactalbumin improves memory in unmedicated recovered depressed patients and matched controls

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**Abstract**

Depression is associated with reduced brain serotonin (5-hydroxytryptamine; 5-HT) function and with cognitive dysfunctions. A diet rich in alpha-lactalbumin protein has been found to increase the ratio tryptophan /large neutral amino acids (tryptophan/ LNAA), and to improve cognitive functioning in individuals with high neuroticism scores. Since cognitive dysfunctions sometimes persist after remission of depression, the present study investigated the effects of alpha-lactalbumin-enriched diet on cognition in recovered depressed patients. Twenty-three recovered depressed patients and 20 healthy matched controls without a history of depression consumed meals rich in alpha- lactalbumin or casein protein in a double-blind crossover design. Mood, cognitive function and plasma amino-acids were assessed at both sessions before and after dietary intake. Alpha-lactalbumin protein had no effect on mood, but improved abstract visual memory and impaired simple motor performance. These effects were independent of history of depression. Supplements of alpha-lactalbumin may be useful for nutrition research in relation to age- or disease-related memory decline. The present findings should be further examined in different (e.g. medicated) samples. The long-term effects of alpha-lactalbumin should also be investigated.

**Introduction**

Depression is associated with impaired cognitive functioning (Austin et al., 2001). Impaired spatial and verbal memory has most frequently been reported (Burt et al., 1995: review). However, deficits in other domains are also common, including psychomotor skills, attention and executive functioning (Austin et al., 2001). Mild deficits often persist after remission (Paradiso et al., 1997), irrespective of residual symptoms (Weiland-Fiedler et al., 2004) or medication status (Paradiso et al., 1997).

The serotonin (5-hydroxytryptamine; 5-HT) system is important in the regulation of mood as well as cognitive functions. Selective serotonin reuptake inhibitors (SSRIs) are found to relieve depressive symptoms and to enhance memory function in humans and rats (McEntee & Crook, 1991). Conversely, experimental depletion of L-tryptophan (the precursor of serotonin) induces depressive symptoms in depression-vulnerable individuals (see for reviews: Booij et al., 2003; Van der Does, 2001a) and impairs long-term memory in healthy volunteers (Riedel et al., 1999; Schmitt et al., 2000). The effects of enhanced 5-HT activity on cognitive processes have frequently been investigated by using low protein diets that consist almost entirely of carbohydrates. These diets increase the amount of plasma tryptophan as compared to the other large neutral amino acids (ratio tryptophan/LNAA) that competes with tryptophan for uptake into the brain (Fernstrom & Wurtman, 1972). High-carbohydrate meals have been found to improve cognitive performance in both clinical and healthy populations, but the results are not consistent (see for reviews: Benton & Nabb, 2003; Dye et al., 2000; Gibson & Green, 2002). These inconsistent results may be related to the timing of intervention or the amount of carbohydrates consumed. Furthermore, individual differences in stress-vulnerability may be involved, as carbohydrates

improved mood and information processing in high stress-prone but not in low stress-prone healthy individuals (Markus et al., 1998). The use of carbohydrate-rich meals poses methodological difficulties, including lack of a placebo condition and expectancy effects (Dye et al., 2000; Gibson & Green, 2002; Spring et al., 1987). Besides, a very large amount of carbohydrates, and consequently high-caloric meals may be needed to produce biochemical and behavioural changes, making implementation in a regular diet undesirable.

A different method to enhance tryptophan availability involves using alpha-lactalbumin protein (Markus et al., 2000; 2002). Alpha-lactalbumin protein has the highest L-tryptophan concentration of all protein fractions (Heine et al., 1996). A diet enriched with alpha-lactalbumin increased the ratio plasma tryptophan/LNAA by 46–48% in healthy volunteers, as compared to casein (placebo). This effect is two times higher than the effect generally found after a carbohydrate- rich diet (Markus et al., 2000), or after 7 days of daily treatment with L-tryptophan (Chouinard et al., 1985). Alpha-lactalbumin improved mood and information processing, and attenuated stress-induced cortisol-responses in stress-vulnerable subjects (non-patients with high neuroticism scores) but not in controls (low neuroticism scores) (Markus et al., 2000; 2002). These data suggest that enhancing 5-HT function through diet may be particularly beneficial for vulnerable individuals under high levels of stress and may improve stress coping.

In the present study we were particularly interested in investigating the cognitive effects of alpha-lactalbumin in recovered depressed patients and matched controls. Since cognitive dysfunctions may persist after remission, we expected to find residual cognitive impairments at baseline in recovered depressed patients relative to controls. Furthermore, we expected that alpha-lactalbumin would improve cognitive function compared to a casein diet, and

that the effects of alpha-lactalbumin would be more pronounced in recovered depressed patients than in controls. We have previously reported that alpha-lactalbumin did not change mood or cortisol response following laboratory stress in recovered depressed patients or controls (Merens et al., 2005). However, cognitive effects in the absence of mood effects have regularly been reported after manipulations of neurotransmitters, e.g. tryptophan depletion (Booij et al., 2005a; 2005c) or a single dose of an antidepressant (Harmer et al., 2002; 2003a).

## Methods and materials

*Participants*

Twenty-three recovered depressed patients (21 females and two males) and 20 controls (17 females and three males) participated in the study. Some of the patients were former participants of a randomized psychotherapy trial, whereas additional patients and all controls were recruited via advertisements at Leiden University or in local newspapers. Inclusion criteria were: age between 18 and 65; meeting DSM-IV criteria for history of depression (patient group only); free of antidepressant medication for at least 3 months; no history of psychiatric disorders and having no first-degree relative of major depressive disorder (control group only); no current psychiatric disorder; Montgomery-Asberg Depression Rating Scale lower than eight (Montgomery & Asberg, 1979) and a Body Mass Index (BMI in $kg/m^2$) above 18. Exclusion criteria were: substance abuse within last 3 months, psychosis (lifetime), major physical illness, lactation, pregnancy and excessive dieting or binge eating. Diagnoses, demographic and clinical background variables were verified by means of the Structured Clinical Interview for DSM-IV (SCID-I) (First et al., 1995).

*Composition of the diet*

At each session, participants received a carbohydrate-rich breakfast and lunch. The energy intake per session totalled 294 kcal (1229 kJ), of which 21% was from fat, 75% as carbohydrates and 5% as protein. Each breakfast and lunch consisted of one slice of bread, 10 g butter, 15 g fruit jelly, grape juice (200 ml) and a chocolate drink. The two diets were identical with the exception of the composition of a chocolate drink in which the protein sources differed. The chocolate drink in the alpha-lactalbumin diet contained a whey-protein fraction rich in alpha-lactalbumin (containing 12.3 g/kg tryptophan; tryptophan/LNAA ratio of 8.7) and the chocolate drink in the control diet contained sodium caseinate (containing 9.5 g/kg tryptophan, tryptophan/LNAA ratio of 4.7). The composition and preparation of the chocolate drinks were similar in appearance and macronutrient composition as in Markus et al. (2000; 2002). Other nutrients or drinks were not allowed until the end of session, except for water during the whole day and one cup of coffee or tea without milk +1.25 hours after breakfast.

*Instruments*

*Mood:* Changes in mood were measured using the Dutch shortened paper and pencil version of the Profile of Mood States questionnaire (POMS) (McNair et al., 1971; Wald & Mellenbergh, 1990). The POMS comprises five different subscales for mood. The subscale Anger (range: 0–28), Depression (range: 0–32), Fatigue (range: 0–24) and Tension (range: 0–24) refer to a negative mood state, whereas the subscale Vigour (range: 0–20) concerns a positive mood.

*Personality:* Neuroticism (N) was measured with the shortened Eysenck Personality Questionnaire (EPQ-RSS)(Eysenck & Eysenck, 1991). The Dutch translation (Sanderman et al., 1995) was used, which has different norms from the original (about 1.5 points lower). According to the manual, the mean N score of the general population is around four. Norms for psychiatric patients are not available; however we found a mean N score of 6.4 (S.E. 0.55) in a recent study of 39 (partially) remitted depressed outpatients (who were in treatment and who had a mean of 4.4 past episodes of depression) (Van der Does & Booij, 2005).

*Cognition:*

*Sternberg Memory Scanning Task*

The computerized Memory Scanning Task is based on the information processing model of Sternberg, who distinguishes scanning and non-scanning stages of information processing (Sternberg, 1969). The Memory Scanning Task consisted of three trials, corresponding to a set of two, three or six consonants respectively. In each trial, the set of letters is presented in the middle of the screen, and the participant was instructed to memorize them ('memory' set). After memorization, 90 letters in each trial are presented on the screen in a random order one by one for 1500 ms each at an interval range of 500 ms. Fifty percent of the letters presented belonged to the memory set and 50% did not. Participants were instructed to push on the 'yes' button if the letter presented belonged to the memory set of that condition and on the 'no' button when it did not. Reaction times and number of errors for each condition were the outcome measures of this test.

*Abstract Patterns Recognition Task (APRT)*

The APRT modelled after Rubinsztein et al. (2001) measures (speed of) retrieval of non-verbal abstract information from short-term memory (STM) and long-term memory (LTM). Sixteen abstract patterns were presented consecutively for 3000 ms, with 500 ms intervals. Participants were instructed to memorize the patterns. After three presentations of the complete series, two patterns were presented simultaneously; one that had been learned and a new pattern. Participants had to indicate as fast as possible which one had been previously presented. The recognition procedure was repeated after 35 minutes, during which verbal tasks were administered. Sensitivity measures (A') were calculated for the proportion of correctly recognized patterns, corrected for response tendency by the formula: A' = 1-1/4(fr/cr + (1-cr)/(1-fr)), in which fr = the proportion of falsely recognized patterns and cr = proportion of correctly recognized patterns (signal detection theory; Pollack & Norman, 1964).

*Stroop Colour Word Task (SCWT)*

The Stroop test measures focused attention and response inhibition. Names of colours (red, yellow, blue, green) printed in black were presented one by one for a maximum of 1500 ms on a computer screen. Participants were instructed to read these words as fast as possible (Condition I). Next, coloured patches were presented (Condition II) of which the name of the colour had to be named. Finally, names of colours printed in an incongruent colour were presented and participants were instructed to name the colour of the ink (Condition III). Median reaction times (RTs) and errors were recorded. Interference was defined as the percentage of extra time needed for condition III relative to the average of conditions I and II.

*Left/right choice reaction time*

This task was used to assess motor speed and response inhibition as a function of task difficulty. The word 'left' or 'right' was presented in randomized order either at the left or the right side of the screen. Participants were instructed to respond to the meaning of the word while ignoring its location, as fast as possible. The task consisted of two consecutive subtasks in which the stimulus interval differed (1000 ms fixed vs. 500–1500 ms variable). Correct responses and RTs were registered.

*Tower of London (TOL)*

The TOL modelled after Owen et al. (1995b) is a planning task consisting of three coloured balls (red, yellow and blue) placed on three sticks in various arrangements. Two arrangements were presented on the upper and lower half of the screen. The patient was instructed to indicate the minimal number of moves necessary to change the first arrangement into the second (two to five moves). Correct responses and RTs were registered.

*Blood plasma*

A blood sample was obtained (10 ml) using EDTA tubes to determine total plasma tryptophan and the other large neutral amino acids (tyrosine, phenylalanine, isoleucine, leucine and valine). Immediately after sampling, the blood was centrifuged for 20 minutes at 2650 gmax and the plasma was stored at -20 °C. Quantitative amino acid analysis was performed by an ion-exchange chromatography on a Biochrom 20 automated amino acid analyser (Pharmacia) as described elsewhere (Merens et al., 2005).

*Design and procedure*

The study was conducted according to a randomized double-blind crossover design with two experimental sessions. One week before the first experimental session, after receiving oral and written information about the study and providing written informed consent, potential participants were invited to a screening interview that included the Structured Clinical Interview for DSM-IV (SCID-I) (First et al., 1995), Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979), the Beck Depression Inventory-II (BDI-II) (Beck et al., 1996) and a medical examination. The SCID and the MADRS interviews were conducted by trained clinical psychologists. In addition, the cognitive test battery was administered.

During the experimental sessions, participants came to the laboratory at 8 or 9 am (-1h) after an overnight fast. Baseline mood was measured with the POMS and a first blood sample was obtained, followed by administration of cognitive tasks. The cognitive test battery took 45 minutes. Next, participants ate breakfast, including a drink containing either tryptophan-rich alpha-lactalbumin or casein (0 h). Lunch, at +2 h, also contained either an alpha-lactalbumin or casein drink. A second blood sample was taken at +3.5 h, followed by the POMS and cognitive tests at +3.75 h. Participants were tested individually, and remained in a private research room between assessments.

The order of presentation of the alpha-lactalbumin and casein diets was counterbalanced. Both experimental sessions were separated by exactly four weeks. Pre-menopausal women not taking a contraceptive pill were tested during their mid-to-late follicular phase (days 4–10), whereas pre-menopausal women taking the contraceptive pill participated during the period in which they actually took the contraceptive pill. All participants were paid €80 for

participation. The study was approved by the Medical Ethics Committee of Leiden University Medical Centre.

*Statistical analysis*

Prior to analysis, all variables were examined for accuracy of data-entry, missing values and fit between their distributions and the assumptions of the statistical analyses. Group differences in demographic characteristics, baseline mood ratings and cognitive performance were examined by means of chi-square statistics and multivariate analyses of variance (MANOVA) by using the General Linear Model (GLM: SPSS 11.5 for Windows, SPSS Inc, Chicago). RTs of the TOL and Left/Right task were $\log^{10}$ transformed prior to analysis. POMS scores were analysed with nonparametric statistics because transformations were unsuccessful, as shown by visual inspection and Shapiro Wilk statistics (Stevens, 1996). The effects of the interventions on cognitive tests and biochemical outcome measures were analysed by separate repeated measure multivariate analyses of variance, using Intervention (alpha-lactalbumin vs. casein) and Time (before vs. after intervention) as within-subjects factors and Group (recovered depressed patients vs. controls) as between-subjects factor. Thus Intervention x Time and Intervention x Time x Group interactions reflected the main effects of interest. Level of difficulty was added as a within-subjects factor for the Memory Scanning Task (three levels) and Tower of London (four levels); whereas the Left/Right tasks consisted of an additional level 'condition' (congruent vs. incongruent). Significant results revealed by these procedures were further examined by post hoc tests. Although we counterbalanced for order of intervention, we first conducted analyses with Order of intervention as a covariate. However, Order of

intervention did not contribute to any of the effects. All statistics were evaluated at a significance level of 5%.

## Results

*Data screening/drop-outs*

Of a total of 49 participants who were included, 43 (23 recovered depressed patients; 20 controls) completed the study. Three recovered depressed patients were included but decided not to participate. Three patients dropped out after the first session; the first case due to nausea (after alpha-lactalbumin), the second case dropped out because of feeling uncomfortable with venapunction during the first session (casein); the third one could not be contacted to schedule the second session on time (casein). These patients were left out of all analyses. Due to a computer failure, data of the cognitive tasks during the screening session for one control patient were lost. For one patient, data of the Memory Scanning task during the screening session were missing. TOL data during the screening session were missing for another patient. Morning assessments of the Left/Right task in the casein condition were unavailable for one patient. Twenty-two of the 172 blood samples (13%) were missing because of difficulties with the venapunction. Cases with missing data were omitted separately by analysis.

*Participants, baseline cognitive performance*

Clinical and demographic characteristics are shown in Table 1. Recovered depressed patients ('recovered MDD') did not differ from the participants with no history of depression ('controls') in terms of gender distribution, age and education level, indicating that matching was successful. BDI-II scores were higher in the patient group than in controls ($F(1,41) = 7.31$; $p = .01$); however,

BDI-II scores are low and well within the normal range in both groups. There were no group differences on any of the cognitive tasks conducted during the screening session or in the morning sessions of both conditions. Controlling data for group differences in BDI-II scores did not change these results.

*Dietary effects on amino acids*

Repeated measures analyses for tryptophan/LNAA with Intervention and Time as within-subjects factors and Group as between-subjects factors revealed a main effect of Intervention ($F(1,29) = 84.39$; $p < .001$), Time ($F(1,29) = 7.08$; $p = .01$) and a significant interaction between Intervention and Time ($F(1,29) = 193.28$; $p < .001$). Tryptophan/ LNAA increased significantly by 20.9% compared to baseline after the alpha-lactalbumin-diet and decreased by 30.0% after the casein diet. After alpha-lactalbumin, the tryptophan/LNAA ratio was 71.5% higher than after casein. After alpha-lactalbumin, tryptophan levels increased 77.5% relative to baseline and were 54.0% higher than after casein ($p < 0.001$). There were no group or baseline differences in plasma tryptophan or ratio tryptophan/LNAA. Repeated measures analyses for tyrosine/LNAA revealed a main effect of Intervention ($F(1,29) = 22.51$; $p < .001$), Time ($F(1,29) = 8.14$; $p = .01$) and a significant interaction between Intervention and Time ($F(1,29) = 123.59$; $p < .001$). Compared to baseline levels, tyrosine/ LNAA decreased 11.5% in the alpha-lactalbumin condition and increased 28.1 % in the casein condition. After casein, the tyrosine/LNAA ratio was 35.8% higher than after alpha-lactalbumin.

**Table 1.** Characteristics of the investigated sample

| | **Recovered MDD** ($n$ = 23) | **Controls** ($n$ = 20) |
|---|---|---|
| Mean age in years ± SD | 29.96 ± 9.7 | 26.95 ± 10.1 |
| Female | $n$ = 21 | $n$ = 17 |
| Body Mass Index (BMI) (kg/m² ± SD) | 22.84 ± 2.5 | 21.69 ± 2.1 |
| Eduction level High[1] / Medium[2] / Low [3] | 1 / 14 / 7 | 1 / 12 / 7 |
| MADRS | 1.30 ± 1.6 | 0.80 ± 1.6 |
| BDI-II | 4.43 ± 4.5 | 1.45 ± 2.2 |
| Number of previous episodes | 2.00 ± 0.9 | |
| - Single episode | $n$ = 7 | |
| - Multiple episodes | $n$ = 15 | |
| Age of onset first episode ± SD | 19.91 ± 7.7 | |
| History of treatment: | | |
| - SSRI | $n$ = 1 | |
| - Psychotherapy | $n$ = 7 | |
| - SSRI + Psychotherapy | $n$ = 5 | |
| - Alternative treatment | $n$ = 3 | |
| - Spontaneous recovery | $n$ = 7 | |

[1] Higher vocational education, university; [2] Secondary education, medium and higher level or senior secondary vocational education; [3] Primary education, secondary education lower level. MADRS = Montgomery Asberg Depression Rating Scale; BDI-II = Beck Depression Inventory – 2nd edition; SSRI = selective serotonin reuptake inhibitor.

*Mood*

POMS depression scores (mean ± SE) in the alpha-lactalbumin session changed from 0.74 ± 0.29 to 0.17 ± 0.14 for the recovered MDD group. The mean ± SE for the control group in the alpha-lactalbumin session on that scale was 0.00 ± 0.00 both before and after intervention. POMS depression scores in the casein condition changed from 1.00 ± 0.47 to 0.17 ± 0.10 for the recovered MDD group and from 0.30 ± 0.16 to 0.00 ± 0.00 for the control group. Non-parametric tests did not reveal a significant Group or Intervention effect on any POMS subscale.

*Cognition*

Multivariate analysis of variance revealed an intervention x time interaction for the outcome measures of the APRT ($F(4,38) = 3.06$; $p = .03$). Further univariate tests revealed significant Intervention x Time interactions for the STM measures A' ($F(1,41) = 5.99$; $p = .02$) and RT ($F(1,41) = 4.07$; $p = .05$) and also for LTM–RT ($F(1,41) = 4.49$; $p = .04$), but not for LTM–A' ($F(1,41) = 0.08$; $p = .78$) (Figures 1 and 2). Alpha-lactalbumin diet improved the number of correctly recognized abstract pictures and improved speed of recognition from STM and LTM, but there were no group differences, as shown by nonsignificant Intervention x Time x Group interactions for STM–A' ($F(1,41) = 2.04$; $p = .16$), STM–RT ($F(1,41) = 0.53$; $p = .47$) and LTM–RT ($F(1,41) = 2.26$; $p = .14$). The interaction for LTM–A' was statistically a trend ($F(1,41) = 3.08$; $p = .09$). To further explore the effects as shown in Figures 1 and 2, separate analyses were conducted for the recovered MDD and control group. These should be interpreted with caution however because of an absence of an interaction with group in the primary analyses of interest. In the recovered MDD group, significant Intervention x Time interactions were

found for STM–A' ($F(1,22) = 9.09$; $p = .006$), LTM–RT ($F(1,22) = 4.64$; $p = .04$), and a trend for LTM–A' ($F(1,22) = 2.89$; $p = .10$). The Intervention x Time interaction for STM–RT was not significant ($F(1,22) = 0.70$; $p = .41$), whereas this interaction was the only significant interaction in the control group ($F(1,19) = 5.07$; $p = .04$).

For the Left/Right task, there was a multivariate significant Intervention x Time x Group effect ($F(2,39) = 4.12$; $p = .02$), due to a group difference in the subtask with variable stimulus intervals ($F(1,40) = 7.78$; $p = .01$). Separate analysis for the recovered MDD group and the control group showed that controls became faster after alpha-lactalbumin condition relative to casein condition, whereas the reverse pattern occurred in the recovered MDD group. Using a similar double multivariate repeated measures design, the number of errors increased in the alpha-lactalbumin condition relative to casein condition, as shown by an Intervention x Time interaction effect ($F(2,39) = 3.47$; $p = .02$). Univariate tests showed that this was true for the fixed interval subtask ($F(1,40) = 6.87$; $p = .01$). There were no higher order interactions with group.

On the Memory Scanning Task, there was an overall multivariate main effect of memory set on total reaction time ($F(2,40) = 81.34$; $p < .001$) and number of errors ($F(2,40) = 18.33$; $p < .001$), indicating that the time to respond and the number of errors increased when the memory set becomes larger. A similar pattern was found for the TOL, with slower responses ($F(3,36) = 173.53$; $p < .001$) and a higher number of errors ($F(3,36) = 43.85$; $p < .001$) as the task became more difficult.

There were no Intervention x Time, Intervention x Time x Group on the Memory Scanning Task, Stroop Task and TOL (Table 2).

**Figures 1 and 2.** Mean change (SE) in percentage of correctly recognized patterns (Figure 1, above) and median RT (ms) for correctly recognized patterns (Figure 2, below) for the Abstract Patterns Recognition Task as a function of group and intervention. Scores are changes relative to baseline performance (post – pre-intervention).

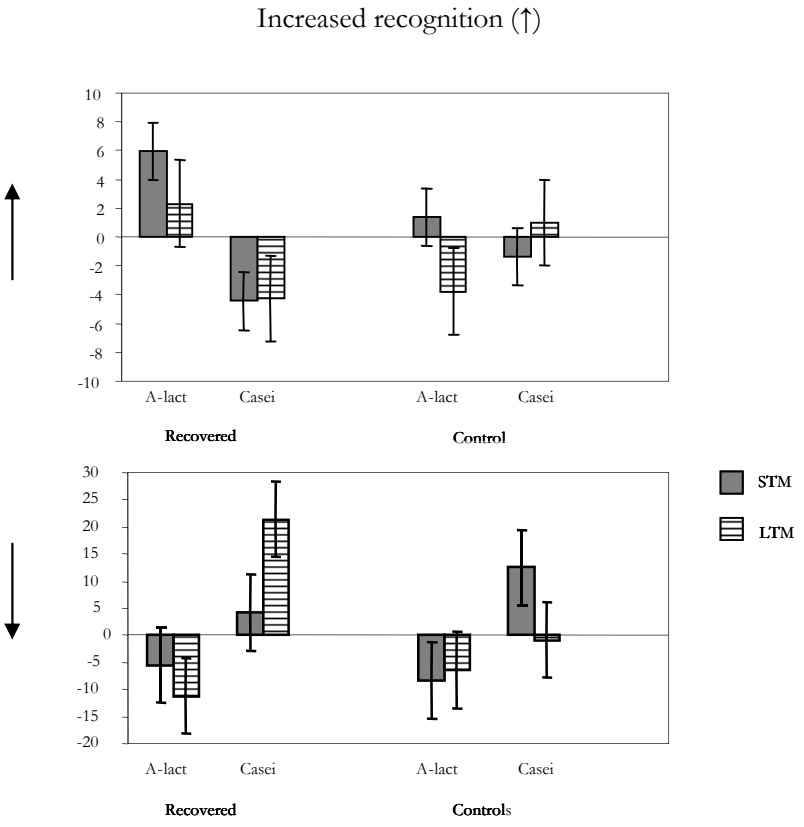

**Table 2.** The means (SE) of the cognitive tasks as a function of group, time and intervention

| | | **Recovered MDD** (*n* = 23) | | **Controls** (*n* = 20) | |
|---|---|---|---|---|---|
| Cognitive test | Diet | before diet | after diet | before diet | after diet |
| **Memory Scanning** | | | | | |
| **- % correct** | | | | | |
| 2 letters | α-lac | 97.6 (0.5) | 98.0 (0.4) | 97.8 (0.3) | 97.1 (0.4) |
| | casein | 98.1 (0.4) | 98.4 (0.3) | 96.9 (0.5) | 97.2 (0.3) |
| 3 letters | α-lac | 98.1 (0.5) | 97.9 (0.5) | 97.3 (0.4) | 96.8 (0.5) |
| | casein | 97.9 (0.5) | 98.0 (0.4) | 96.3 (0.5) | 97.2 (0.5) |
| 6 letters | α-lac | 95.9 (0.8) | 94.3 (1.3) | 94.2 (1.1) | 92.8 (1.2) |
| | casein | 95.7 (0.8) | 94.4 (1.1) | 93.0 (0.8) | 91.4 (2.1) |
| **- RT(ms)** | | | | | |
| 2 letters | α-lac | 464 (13) | 465 (13) | 468 (13) | 456 (14) |
| | casein | 456 (10) | 463 (13) | 465 (15) | 467 (15) |
| 3 letters | α-lac | 489 (11) | 489 (13) | 503 (12) | 492 (13) |
| | casein | 491 (15) | 480 (14) | 516 (15) | 496 (15) |
| 6 letters | α-lac | 560 (18) | 567 (21) | 580 (19) | 599 (29) |
| | casein | 570 (21) | 566 (20) | 583 (19) | 594 (25) |
| **SCWT** | | | | | |
| Condition I (ms) | α-lac | 476 (10) | 488 (15) | 478 (14) | 463 (13) |
| | casein | 473 (9) | 489 (12) | 468 (13) | 454 (12) |
| Condition II (ms) | α-lac | 563 (15) | 557 (15) | 529 (15) | 515 (13) |
| | casein | 557 (13) | 554 (14) | 537 (14) | 523 (13) |
| Condition III | α-lac | 726 (21) | 696 (23) | 699 (21) | 660 (18) |
| (ms) | casein | 720 (21) | 710 (22) | 701 (24) | 652 (20) |
| Interference (%) | α-lac | 39.5 (1.9) | 32.9 (2.5) | 38.6 (2.2) | 35.0 (2.0) |
| | casein | 40.0 (2.9) | 36.1 (2.8) | 39.6 (3.6) | 33.3 (2.7) |
| **Left/Right Task** | | | | | |
| Congruent (ms) | α-lac | 600 (19) | 573 (21) | 607 (21) | 567 (18) |
| | casein | 604 (18) | 573 (13) | 597 (21) | 564 (16) |
| Incongruent (ms) | α-lac | 602 (18) | 603 (19) | 624 (25) | 588 (16) |
| | casein | 599 (17) | 584 (17) | 608 (23) | 586 (20) |
| Congruent | α-lac | 619 (17) | 613 (15) | 629 (17) | 588 (17) |
| (variable) | casein | 625 (14) | 607 (17) | 618 (20) | 609 (18) |
| Incongruent | α-lac | 622 (16) | 624 (18) | 627 (20) | 594 (14) |
| (variable) | casein | 637 (16) | 604 (16) | 617 (19) | 606 (18) |

**Table 2.** *(continued)*

| Cognitive test | Diet | **Recovered MDD** (*n* = 23) | | **Controls** (*n* = 20) | |
|---|---|---|---|---|---|
| | | before diet | after diet | before diet | after diet |
| **TOL % correct** | | | | | |
| 2 step | α-lac | 88.6 (2.9) | 89.1 (3.0) | 92.2 (2.7) | 93.3 (2.9) |
| | casein | 86.8 (3.9) | 88.6 (3.2) | 90.5 (2.3) | 92.2 (2.5) |
| 3 step | α-lac | 89.5 (2.6) | 89.1 (3.3) | 89.4 (1.7) | 88.9 (3.5) |
| | casein | 91.4 (2.6) | 92.7 (1.6) | 89.4 (2.2) | 91.7 (2.6) |
| 4 step | α-lac | 77.3 (4.2) | 78.6 (3.8) | 86.1 (3.3) | 83.9 (3.1) |
| | casein | 81.8 (3.3) | 79.5 (3.7) | 81.7 (3.2) | 84.4 (2.4) |
| 5 step | α-lac | 74.1 (4.2) | 74.5 (3.9) | 72.8 (3.9) | 80.0 (3.7) |
| | casein | 75.0 (3.3) | 75.5 (3.7) | 68.3 (4.1) | 72.2 (3.6) |
| **TOL RT (ms)** | | | | | |
| 2 step | α-lac | 4113 (280) | 3513 (158) | 4444 (465) | 3784 (234) |
| | casein | 3861 (235) | 3627 (251) | 4273 (320) | 3710 (244) |
| 3 step | α-lac | 4766 (290) | 4621 (1299) | 5457 (325) | 5235 (299) |
| | casein | 4931 (323) | 5007 (326) | 5649 (608) | 4834 (310) |
| 4 step | α-lac | 6936 (492) | 6690 (407) | 8095 (490) | 7210 (431) |
| | casein | 6670 (343) | 6372 (327) | 8008 (609) | 7006 (442) |
| 5 step | α-lac | 10819 (786) | 10010 (864) | 13384 (1305) | 12365 (1139) |
| | casein | 10915 (703) | 10584 (855) | 11547 (922) | 11398 (874) |

α -lac = alpha-lactalbumin condition; SCWT = Stroop Colour Word Task; TOL = Tower of London; RT = reaction time

**Discussion**

The aim of this study was to investigate whether increased tryptophan availability after alpha-lactalbumin diet affects cognitive function, particularly in recovered depressed patients. The alpha-lactalbumin diet increased plasma tryptophan/LNAA ratio (21% increase from morning to afternoon; afternoon ratio 71.5% higher than in the placebo condition). Memory performance after alpha-lactalbumin improved in both groups, and no other reliable effects were found.

*Baseline cognitive performance in recovered depressed patients vs. controls*

Unexpectedly, no baseline differences in cognitive performance were found between recovered depressed patients and controls. Using the same cognitive tests, our previous study in remitted depressed patients showed impaired abstract long-term visual memory at baseline relative to controls (Booij et al., 2005a). This result is also in contrast with other studies that found residual cognitive impairments in short-term memory, attention and executive functioning in recovered depressed patients (e.g. Paradiso et al., 1997). One likely explanation for this difference might be that the recovered MDD group in the present study was much younger (30.0 years) than in our previous study (48.7 years) or in Paradiso et al. (55.9 years), with about one third of the individuals having experienced a single episode during late adolescence rather than multiple episodes. Cognitive impairments in the recovered phase may be more severe in patients with recurrent episodes than in those with a single episode (Kessing, 1998).

*Dietary effects on cognitive performance*

The alpha-lactalbumin diet improved abstract visual memory in both recovered depressed patients and controls. More specifically, alpha-lactalbumin improved recognition and speed of retrieval from short- and long-term abstract visual memory. Overall, there was no interaction with group. There were no effects on the TOL, Memory Scanning Task and Stroop Task, indicating that alpha-lactalbumin did not change the encoding phase, working memory, perception or general motor speed. Thus, alpha-lactalbumin may specifically affect memory consolidation in an early phase.

This effect mirrors the consolidation deficit that was found after lowering 5-HT function by tryptophan depletion in healthy volunteers (Riedel et al., 1999; Rubinsztein et al., 2001) and is in line with improved memory after a single dose of citalopram in healthy volunteers (Harmer et al., 2002). Alpha-lactalbumin also improved abstract visual memory in females with premenstrual symptoms (Schmitt et al., 2005). However, it is important to mention that the latter study did not include a control group. Hence, the present study showed that the beneficial effects on memory are not limited to individuals vulnerable to 5-HT related mental disorders. This is of interest because of the fact that the mean age of the participants in the present study was 28.5 years, while cognitive processes usually start to decline around 45–50 years (Hedden & Gabrieli, 2004: review). Thus abstract visual memory improved even though performance was uncompromised by aging or psychiatric symptoms.

Results might also be (partly) explained by impaired memory in the casein condition. Without a non-intervention control group, these possibilities cannot be separated. Nevertheless, the change of tryptophan/LNAA ratio in the casein condition was comparable to what is usually observed after a

balanced meal (Fischer et al., 2003; Spring et al., 1987), which justifies the use of casein as a placebo procedure (Schmitt et al., 2005).

Alpha-lactalbumin impaired motor performance, as shown by an increased number of errors in the fixed interval condition of the Left/Right task, irrespective of group. These results are consistent with previous studies finding decreased performance on simple RT tasks after 5-HT stimulation in patients and in healthy volunteers (Riedel et al., 2002; Sobczak et al., 2003). As intervention had no effect on the number of errors in the more difficult versions of the Left/Right task (variable time intervals) or on working memory tasks (Tower of London/Memory Scanning Task), this suggests that alpha-lactalbumin impaired cognitive performance when the task was relatively easy and monotonous, possibly due to the sleep-inducing properties of alpha-lactalbumin (Markus et al., 2005; Minet-Ringuet et al., 2004).

Complex interactions were found on the variable time interval condition of the Left/Right task. RTs improved in the control group after alpha-lactalbumin and remained unchanged after casein. Conversely, in the recovered MDD group, RTs improved after casein but did not change after alpha-lactalbumin. We have no clear explanation for this finding. In the Left/Right task, both task uncertainty (incongruent vs. congruent trials) and time uncertainty (fixed vs. variable stimulus time intervals) were manipulated. Alpha-lactalbumin had no differential effect on the congruent and incongruent trials, a finding consistent with the lack of effect on interference levels on the Stroop Colour Word Task in the present study. Thus, 5-HT did not affect performance in conditions of task uncertainty. No previous studies investigated the effects of 5-HT and/or depression on motor speed as a function of time uncertainty.

The lack of effect on the Memory Scanning Task found in the present study contrasts with the results of Markus et al. (1998; 2002), who reported improved information processing in individuals with high neuroticism scores. Life events and neuroticism are risk factors for major depression, and individuals with high neuroticism have a greater risk of major depression in response to a stressful life event (Kendler et al., 2004). Individuals with high neuroticism may benefit more from an alpha-lactalbumin diet because a high amount of 5-HT activity is required to cope with stress, thereby increasing the risk of a shortage in brain 5-HT concentrations, which increases the risk of depression (Markus et al., 2002). However, about 60% of the individuals with high neuroticism scores actually became depressed (Ormel et al., 2001). In addition, neuroticism has been shown to be stable over time in adulthood (Kendler et al., 2004). In the present study, most recovered depressed patients had been treated. They may also have coping mechanisms developed through effective treatment, which may reduce the detrimental effects of stress. The neuroticism (N) scores of the recovered depressed group in the present study were above average to high (mean ± SD: 5.64 ± 2.3, range: 2–10), while the control group had low-average scores (mean ± SD: 2.60 ± 2.0, range 0–8). These group differences are statistically and clinically significant but seem smaller than in Markus et al. (2002), who used a different measure and selected students with N scores in the lowest and highest quartile of a large subject pool. The results of the present study suggest that high neuroticism and history of depression are different concepts in terms of 5-HT vulnerability.

Mood remained unaffected, which is consistent with the finding that single administration of SSRIs ameliorated emotional processing in healthy volunteers, without changing mood (e.g. Harmer et al., 2003a). These findings support our suggestion that cognitive markers may be more sensitive makers

for changes in 5- HT function than mood or symptom scales (Booij et al., 2005a).

*Effect of diet on plasma amino acids and serotonin*

The increases in tryptophan and ratio tryptophan/LNAA were within similar range as in Schmitt et al. (2005) and Markus et al. (2000; 2002). However, the composition of breakfast and lunch used in Markus et al. (2000; 2002) and Schmitt et al. (2005) contained about three times more calories and two times more carbohydrates than in the present study, whereas the composition of the alpha-lactalbumin or sodium caseinate containing chocolate drink were identical. Thus, the present study demonstrated that alpha-lactalbumin is able to raise tryptophan levels without necessarily ingesting an excessive amount of carbohydrates, suggesting that alpha-lactalbumin might be relatively easy to implement within a regular diet. A study, reporting an increase of 16% relative to baseline using an amount of 12g alpha-lactalbumin combined with a regular meal, supports this notion (Beulens et al., 2004).

However, as we assessed total tryptophan concentration and not free tryptophan, it must be acknowledged that we do not know how much tryptophan following alpha-lactalbumin actually reaches the brain. Plasma tryptophan, however, circulates in two forms: either bound to plasma albumin proteins (80–90%) or free (10–20%). It has been argued that only free-circulating tryptophan controls the uptake of plasma tryptophan into the brain, whereas others have suggested that total tryptophan (plasma free and bound levels) is the most decisive factor. Separate studies have shown that both increases in total plasma tryptophan (initiated, for instance, by immobilization stress or carbohydrate consumption) and increases in free tryptophan (initiated, for instance, by physical stress or fasting) may lead to an increase in brain

tryptophan and serotonin activity (Chaouloff, 1993). Moreover, total- and free tryptophan are very closely related, also following an alpha-lactalbumin diet (Attenburrow et al., 2003). In animals, administration of alpha-lactalbumin increased baseline extracellular 5-HT in the hypothalamus, indicating that alpha-lactalbumin not only enhance 5-HT synthesis but also its release (Orosco et al., 2004). In humans, increases of 20–40% in ratio tryptophan/LNAA led to significant increases in peripheral markers of 5-HT activity, including cortisol and prolactin (Anderson et al., 1990b; Kaye et al., 1988; Markus et al., 2000).

Changes in mood and cognitive performance might also be related to raised plasma tyrosine levels, as catecholamines are involved in mood and cognitive processes as well (Booij et al., 2003: review). The effects on catecholamine precursor levels in the alpha-lactalbumin condition are probably negligible – in fact, the tyrosine/LNAA ratio decreased slightly. The rise in the casein condition was higher, which is not surprising as this diet contained less tryptophan and twice the amount of tyrosine (Markus et al., 2000; Markus et al., 2002). No other alpha-lactalbumin experiments have reported tyrosine/LNAA ratios. In healthy samples, tyrosine administration improved Stroop performance and working memory (Deijen & Orlebeke, 1994), however we found no intervention effect on working memory tasks. Similarly, a memory consolidation deficit was induced by ATD and not by Acute Phenylalanine Tyrosine Depletion (APTD), whereas a working memory deficit was induced by APTD and not by ATD (Harrison et al., 2004). High-dose ATD (100 g) markedly decreases tryptophan levels and 5-hydroxyindoleacetic acid (5-HIAA), but also induces an increase (50%) of tyrosine/LNAA levels – however, homovanillic acid (HVA) remains unaffected, measured either in cerebrospinal fluid (Carpenter et al., 1998) or plasma (Van der Does & Booij,

2005). Nevertheless, it is recommended to investigate the biochemical specificity further by combining alpha-lactalbumin- enriched diets with monoaminergic depletion paradigms and to develop alternative placebo procedures.

In conclusion, diet enriched with alpha-lactalbumin enhanced memory, irrespective of history of depression. Mood and other cognitive functions remained unaffected. As 5-HT activity is reported to decline with aging (McEntee & Crook, 1991), the present findings could be further examined in older samples. The long-term effects of alpha-lactalbumin should also be investigated.

# 5

# The effects of experimentally lowered serotonin function on emotional information processing and memory in remitted depressed patients

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**Abstract**

It has been well documented that acute tryptophan depletion (ATD) induces symptoms in remitted depressed patients treated with an SSRI. ATD also has effects on cognition, both in patients and in healthy samples. The exact nature of ATD-induced cognitive changes in depression remains unclear. It is also unclear whether cognitive effects can be induced through partial ('low-dose') depletion. The aim of this study is to investigate the differential effects of low-dose and high-dose ATD on emotional information processing and mood in remitted depressed patients. Eighteen remitted depressed patients received high-dose and a low-dose ATD in a randomized, double-blind, within-subjects crossover design. Mood was assessed before and after administration of the depletion drink. Five hours after administration, patients conducted tests measuring neutral and emotional information processing. High-dose ATD increased depressive symptoms and induced a temporary depressive 'relapse' in half of the patients. High-dose ATD also decreased the recognition of fear and impaired learning and memory retrieval. The impaired learning occurred only in mood-responders. Low-dose ATD had no effects on mood but speeded the recognition of facial expressions of disgust. Accurate recognition of sad faces at baseline was associated with mood response to ATD. High-dose ATD leads to changes in memory and in the recognition of negative facial expressions in SSRI-treated remitted depressed patients. The effect of low-dose ATD on mood and cognition seems to be quite limited. Emotional information processing at baseline predicts mood-response to ATD.

**Introduction**

Acute tryptophan depletion (ATD) is a useful tool to investigate the effects of lowered serotonin function in humans (Young et al., 1985). With this procedure plasma tryptophan is temporarily reduced by 70-90%. Central serotonin function is also affected (Carpenter et al., 1998; Nishizawa et al., 1997). ATD results in a temporary depressive 'relapse' in 50-60% of remitted depressed patients taking serotonergic antidepressants (Van der Does, 2001a). In healthy individuals only small mood effects are found in subjects with a family history of affective disorders (Benkelfat et al., 1994; Klaassen et al., 2002).

ATD also has cognitive effects. In healthy volunteers, ATD selectively impairs learning (Park et al., 1994), memory retrieval and consolidation (Klaassen et al., 2002; Park et al., 1994; Riedel et al., 1999) and ATD improves attention in healthy samples (Schmitt et al., 2000) and patients (Booij et al., 2005a). Recently, interest has been paid to the effects of ATD on emotional information processing. ATD impaired the recognition of facial expressions of fear in female healthy volunteers, but not in males (Harmer et al., 2003c).

Three studies investigated the effects of 'low-dose ATD', which involves administering fewer and/or smaller amounts of amino acids compared to 'high-dose ATD'. In both healthy and recovered depressed individuals, an increased negative attentional bias was found following low-dose ATD, in absence of any effects on mood (Hayward et al., 2005). Also, in recovered depressed patients low-dose ATD reduced the recognition of happiness, increased startle response, impaired initial memory of neutral words (Hayward et al., 2005), and increased processing of social threat cues (Munafò et al., 2006). Booij et al. (2005a), however, found only minor effects of low-dose ATD on cognition in remitted depressed patients. In that study, selective

attention for neutral stimuli improved following high-dose and low-dose ATD in a dose-dependent manner, and the processing of positive information was impaired only following high-dose ATD (Booij et al., 2005a). A limitation of this study is that the cognitive test battery consisted mainly of neutral stimuli, which may explain the differences with Hayward et al. (2005) and Munafò et al. (2006). It is also possible, however, that differences in the low-dose procedures are responsible. The traditional (high-dose) ATD procedure consists of 15 amino acids; amounting to 102.5g. Booij et al. used the Krahn et al. (1996) procedure as their low-dose condition, which consists of a 25% amount of the same 15 amino acids (25.7 g). Although developed as a placebo, this procedure reduces plasma tryptophan levels by 40-50% (Van der Does, 2001a). Hayward et al. and Munafò et al. however, used 8 of these 15 amino acids in approximately 50% amount (a total of 31.2 g). Another difference is that in Booij et al. (2005a) patients kept a low-tryptophan diet the day before the ATD sessions. Calculations using the data provided by Hayward et al. reveal that their low-dose mixture resulted in an 87% decrease in the ratio of tryptophan to large neutral amino acids (LNAA). This effect is similar to the decrease found following high-dose depletion in other studies. The low-dose mixture in Booij et al. (2005a) resulted in a decrease of only 42% in the tryptophan/LNAA ratio. Hence, it may be doubted whether the studies by Hayward et al. and Munafò et al. may be viewed as low-dose ATD studies (Merens & Van der Does, 2007).

The present study is the first to investigate the effects of low-dose vs. high-dose ATD on emotional information processing, and aims to answer three questions:

1. Do low-dose and high-dose ATD have differential effects on emotional information processing and memory in remitted depressed patients?

2. Does ATD exert a specific effect on (a certain form of) emotional information processing or is the effect generalized over different aspects of emotional information processing?
3. Is there a relationship between emotional information processing and changes in mood?

To investigate this, different cognitive tests were used to assess neutral (memory) as well as emotional information processing (negative attentional bias, facial expression recognition, implicit attitudes) in remitted depressed patients. Two different ATD dosages were used to investigate a possible dose-response relationship. This study extends on previous work by combining two ATD dosages to test the effects on emotional information processing and memory in remitted depressed patients and by investigating the link between emotional information processing and changes in mood. Based on previous research we hypothesized that high-dose ATD would increase symptoms in a subgroup of patients, but no mood effects of low-dose were expected (Booij et al., 2005a). We also hypothesized that both high-dose and low-dose ATD would affect attentional bias and facial expression recognition (Harmer et al., 2003c; Hayward et al., 2005; Munafò et al., 2006) and that the cognitive effects of low-dose ATD would be strongest in participants with a depressive response to high-dose ATD.

## Methods

*Participants*

Participants were outpatients of the Mood Disorders clinic of a psychiatric hospital. Inclusion criteria were 18 to 65 years; primary DSM-IV diagnosis of major depressive disorder; no longer fulfilling DSM-IV criteria for depression; Hamilton Rating Scale for Depression (HAM-D-17) scores below 16; ongoing

treatment with a Selective Serotonin Reuptake Inhibitor (SSRI) or a Selective Serotonin-Noradrenalin Reuptake Inhibitor (SSNRI) for at least four weeks; no psychosis (lifetime); no substance abuse in the past 3 months (DSM-IV criteria); (above-) normal weight (BMI > 18 kg/$m^2$); free of neuro-endocrine and neurological disease; no pregnancy or lactation.

*Design*

The study was conducted according to a double-blind, randomized crossover design.

*Composition of the Amino-Acid (AA) mixtures*

The high-dose AA mixture consisted of fifteen amino acids (102.5 g) (Delgado et al., 1990). The low-dose mixture consisted of the same amino acids but at one quarter strength (25.7 g) (Krahn et al., 1996). The amino acids were given in drink form by mixing the amino acid powders with water to a final volume of 300 ml. To compensate for the unpleasant taste of the mixture, chocolate syrup was added and the mixtures were served chilled.

*Materials*

*Symptoms*

Symptoms were assessed using the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979) and the Brief Anxiety Scale (BAS) (Tyrer et al., 1984). A self-rating list of physical symptoms was administered, consisting of 48 items rated on a five-point scale. State versions of all questionnaires were used during the depletion sessions. Because changes in weight or sleep do not occur during the course of an ATD session, these items were omitted.

*Cognition*

*Facial Expression Recognition Test:* This task, adapted from Harmer et al. (2003c), features examples of five basic emotions—happiness, sadness, fear, anger, and disgust (Ekman & Friesen, 1976). Emotional expression intensity was morphed between neutral (0%) and emotional standard (100%) in 10% steps, providing a range of emotional intensities. Each emotion-intensity was presented twice (one male and one female face) in random order on a computer screen for 500 ms. and immediately replaced by a blank screen. Participants responded by pressing a labelled key, after which the next face appeared. They were instructed to respond as quickly and accurately as possible. Accuracy and speed of recognition were recorded.

*Word Learning Test* (Saan & Deelman, 1986): A list of 15 unrelated, neutral words was presented from a tape (e.g. flower, store). Immediate recall was tested after each of five consecutive presentations. After the fifth trial, subjects continued with a non-verbal task. Fifteen minutes later delayed recall was tested. Different versions were used at each session in a randomized order. Immediate recall performance was defined as the total of correct words remembered over the five trials. Retrieval was defined as the number of correct words produced at delayed recall. Incorrect responses were also registered. Consolidation was defined as the number of correct words at delayed recall compared to the fifth trial.

*Verbal fluency*: Participants were instructed to produce as many correct four letter words with the same initial letter as possible within one minute. The starting letters were H, M, R or L (Schmitt et al., 2000) and the order of these letters was randomized over the assessments. The total number of correct and incorrect words was calculated.

*Dot probe test*: This task measures attentional bias to emotional stimuli (MacLeod et al., 1986). Word pairs were presented on a computer screen for 500 ms, one in the upper part of the screen and one below. Following the termination of that display, a dot appeared on the location of either word. Participants had to indicate the location of the dot by pressing a key. All word pairs were preceded by a white fixation cross for 500 ms. Subjects were first run through six practice trials. The main experiment consisted of trials of neutral words paired with threat-related words and trials of depression-related words paired with positive words. The neutral and threat-related words were taken from Lavy et al. (1993) and the depression and positive words from McCabe et al. (2000). To control for possible outliers, median latencies for correct responses were used in the analyses; incorrect responses and responses on practice trials were excluded. Attentional bias was calculated by subtracting the RT for positive (neutral) words from the RT for depressive (threatening) words. Attentional bias is associated with faster reaction times if the dot replaces the negative word compared to trials where the dot replaces the neutral or positive word.

*Implicit Association Test:* The IAT is a sorting task that assesses implicit associations on the basis of reaction times (Egloff & Schmukle, 2002; Greenwald et al., 1998). This test is extensively used in social psychological research to assess stereotypes (Greenwald & Banaji, 1995). Participants are asked to sort stimuli representing four categories by pressing the appropriate key (each response key was assigned to two categories). If two categories are strongly related, the sorting task will be easier (: faster RTs) when the categories share the same response key than when they share different response keys. We used an emotional and a neutral version of this task. In the neutral version, negative and positive words were presented, which were paired

with flower- and insect words. RTs to congruent (e.g. flower words paired with positive words) and incongruent (e.g. insect words paired with positive words) trials were calculated. The emotional version was identical to the neutral version except for the stimuli: self-related words were paired with negative words and other-related words with positive words and vice versa. The negative and positive words were taken from McCabe et al. (2000); the neutral words were selected by Lavy et al. (1993) and the self and other words were taken from Egloff & Schmukle (2002) and were previously used by De Jong (2002). To control for possible outliers, median latencies for correct responses were included in the analyses; incorrect responses were excluded. Reaction times to congruent (e.g. self and positive words) and incongruent stimuli (e.g. self and negative word pairs) were calculated.

The *Dysfunctional Attitudes Scale* (DAS) (Weissman, 1979) is a self-rating scale and assesses the level of dysfunctional attitudes, which is a measure of cognitive reactivity. Two comprised 11-item versions, based on the original form A, were used in randomized order.

*Biochemical measures*

To determine total plasma tryptophan concentrations and the ratio total tryptophan/ large neutral amino acids (LNAA), venous blood was obtained (10ml) in ethylene-diamine-tetra-acetic-acid tubes. After sampling, the blood was centrifuged for 20 minutes at $2650g_{max}$. Plasma was stored at – 65°C until quantitative amino acid analysis by high-performance liquid chromatography took place (as described by Fekkes et al., 1995).

*Procedure*

After showing interest in taking part, all volunteers were given oral and written information about the study. Written informed consent was obtained and participants who seemed to meet inclusion criteria were invited for a screening session. During the screening session, the Structured Clinical Interview for DSM-IV (First et al., 1995), HAM-D17 and MADRS were administered. Participants filled out the personality questionnaires. A clinical psychologist or a trained research assistant conducted the interviews. At screening the cognitive tasks were done for the first time. The day before each ATD session, participants kept a low-protein diet (Booij et al., 2005a). Participants arrived at 9 am at the laboratory after fasting overnight. They were instructed not to drink alcohol 24 hours prior to the sessions and to arrive well rested. After arrival, baseline symptom measures were taken, followed by a blood sample. Next, participants ingested the AA mixture within half an hour. Participants remained in the research room until 5 pm. Neutral music and magazines were available. Participants were not allowed to sleep. Water, (de)caffeinated coffee and (herb) tea were allowed in standard amounts. Three hours after ingestion of the AA mixture, participants were served a protein-poor lunch (Booij et al., 2005a; Riedel et al., 1999). At $t_{4.5}$ the cognitive tasks were administered, which took approximately 50 minutes. Next, a blood sample was obtained and symptoms and side-effects were assessed ($t_{6.5}$). Before the participants went home at 5 pm, they received a snack to speed up normalization of tryptophan levels. The procedure was repeated approximately seven days later; participants who had first received the 100% strength mixture now received the 25% strength mixture and vice versa. The morning after each session, patients returned to the lab where symptoms were assessed and a blood sample was obtained. The day after the second ATD session, participants performed the

cognitive tests a final time (post-intervention session). The study was approved by an independent medical ethics committee (METIGG, Utrecht). Participants were tested individually. Participants were paid €115 for participation.

*Statistical analysis*

The effects of ATD on symptoms and cognitive performance were analyzed separately using General Linear Models (GLM) for repeated measures. Symptom ratings were analyzed with Intervention (low-dose vs. high-dose) and Time (pre-depletion vs. post-depletion vs. the next day) as within-subject factors. Cognitive performance was analyzed with Intervention (baseline vs. low dose vs. high-dose) as within-subject factor. In those instances in which the assumption of homogeneity of covariance in repeated measured analysis was violated, as assessed with Mauchly's Test of Sphericity, Huynh-Feldt corrected p-values were used (Field, 2005). Main effects were compared using Bonferroni corrections to adjust for multiple comparisons. Results are reported as means and standard deviations.

**Results**

*Data screening*

All data were screened for accuracy of data-entry, missing values and the assumptions of multivariate data-analysis. Data were missing for one patient at the post-intervention session following the second day, due to an emotional reaction to the high-dose depletion drink. After having contacted the patient by telephone the next morning, she reported to be less emotional but she was still very tired and did not want to come to the lab. For the MADRS and BAS, data for this patient were replaced by the outcome of a regression equation

based on her mood in the afternoon, because we knew her symptoms had not returned to baseline the next morning (when X = mood in the afternoon, A = constant, b = unstandardized coefficient and Y = mood the next morning: Y = A + b . X). The missing biochemical measures were replaced by the sample mean. The MADRS, BAS and side-effect data were log transformed due to non-normal distributions. All transformations were successful.

*Participants*

Twenty patients were included. One patient withdrew after the screening session due to family problems. One patient dropped out after the first day (high-dose depletion) due to physical and mood complaints following that session. These complaints had disappeared the day after, however she decided to withdraw participation. These patients were excluded from analyses, leaving a total of eighteen participants. Five participants vomited after high-dose ATD; one of them also vomited after low-dose ATD. One patient could only finish 75% of the drink on both days. All these cases were retained. Clinical and demographical characteristics of the patients are shown in Table 1.

**Table 1.** Clinical and demographical characteristics ($n$ = 18)

| | **Mean (SD)** |
|---|---|
| M / F | 2 / 16 |
| Age (years) | 44.4 (13.3) |
| Type of medication | |
| - SSRI [1] | $n$ = 12 |
| - SSNRI [2] | $n$ = 6 |
| Type of remission | |
| - partial remission | $n$ = 7 |
| - full remission | $n$ = 11 |
| Duration of remission, partial or full (months) | 13.4 (22.9) |
| Age of onset of first depressive episode | 26.5 (15.6) |
| Number of previous episodes (incl. latest) | 4.8 (4.2) [range 1-15] |
| Single / recurrent | 2 / 16 |
| Family History of Depression (1st degree) | $n$ = 12 |
| BMI | 25.5 (3.4) |
| HDRS (at screening) | 7.8 (3.7) |

SSRI = Selective Serotonin Reuptake Inhibitor; SSNRI = Selective Serotonin and Noradrenalin Reuptake Inhibitor; [1] citalopram 20-60 mg, fluoxetine 40 mg, paroxetine 10-40 mg , sertraline 100 mg; [2] venlafaxine 150 mg- 375 mg; BMI = Body Mass Index; HDRS = Hamilton Depression Rating Scale

*Effects on plasma tryptophan levels*

In the low-dose condition, mean plasma tryptophan levels decreased with 60.3% ± 13.3 from 42.8 ± 5.1 µmol/l to 17.3 ± 6.9 µmol/l. In the high-dose condition, plasma tryptophan levels decreased with 84.0% ± 11.0 from 41.9 ± 7.1 µmol/l to 6.7 ± 4.4 µmol/l. The plasma tryptophan/LNAA ratio decreased with 58.5% ± 15.9 in the low-dose condition (from 10.4 ±1.6 to 4.3 ± 1.6) and with 91.4% ± 8.7 in the high-dose condition (from 10.0 ± 1.6 to .82 ± .80). Repeated measures analysis on the tryptophan/LNAA ratio revealed significant effects of Intervention ($F$(1,17) = 59.7, $p$ < .001), Time ($F$(1.64,27.81) = 266.0, $p$ < .001) and Intervention x Time ($F$(2,34) = 28.9, $p$ <

.001). Both interventions resulted in a significant decrease in plasma tryptophan and the tryptophan/LNAA ratio and the decrease was larger after high-dose than after low-dose ATD. Since a sizable number of patients had vomited following the high-dose AA mixture, all analyses were run twice, both with and without those five patients. All effects regarding the plasma tryptophan and tryptophan/LNAA ratio remained significant with 13 patients.

*Side effects*

Physical complaints increased in the high-dose but not in the low-dose condition and levels were back to normal the next morning (Intervention $F(1,16) = 5.0$, $p = .039$; Time $F(2,32) = 17.0$, $p < .001$; Intervention x Time ($F(2,32) = 5.7$, $p = .008$). The main effects remained significant for $n = 13$, however the interaction effect became a trend ($F(2,22) = 2.9$, $p = .079$). Significant Intervention x Time interactions were found for individual items indicating a decreased appetite ($F(1.28,20.41) = 8.1$, $p = .006$), increased nausea ($F(2,32) = 14.4$, $p < .001$), and sweaty hands ($F(2,32) = 4.1$, $p = .027$). Symptoms were back to baseline the next morning.

*Effects on symptoms*

Depressive symptoms increased significantly in the high-dose condition from 5.3 ± 5.4 to 10.8 ± 8.1, but not in the low-dose condition (from 3.7 ± 4.0 to 3.6 ± 4.2). All scores were back to baseline the next morning (Intervention $F(1,17) = 17.3$, $p = .001$; Time $F(2,34) = 4.6$, $p = .018$; Intervention x Time $F(2,34) = 3.9$, $p = .030$). High-dose ATD induced a brief 'depressive relapse', defined as an increase in MADRS score of 6 points or more (Booij et al., 2005a) in nine patients. Relapses occurred in four of twelve SSRI users and five of six SSNRI users, indicating mood response to be equally distributed over

SSNRI and SSRI users. These patients will be referred to as mood-responders. When relevant, analyses were re-run including Mood-response as a between-subject factor. Anxiety symptoms also increased following high-dose ATD but not following low-dose ATD (Intervention $F(1,17) = 2.9$, $p = .105$, Time $F(2,34) = 3.2$, $p = .053$, Intervention x Time: $F(2,34) = 4.6$, $p = .017$). The effects of ATD on symptoms remained the same when the mood data were re-analysed, excluding the five patients who vomited. Of the five patients who vomited, two were mood-responders.

*Learning effects, baseline measures and order effects*

To detect possible learning effects, screening and post-intervention assessments were compared for all cognitive tests. No learning effects were found on the Dot-probe and Word Learning Test. On the VF, patients produced more correct words post-intervention compared to screening ($t(16) = -4.5$, $p < .001$). On the IAT neutral, patients were faster to respond to congruent ($t(16) = 2.6$, $p = .021$) as well as incongruent stimuli ($t(16) = 4.8$, $p < .001$) at post-intervention compared to screening. This was also true for the IAT emotional (congruent stimuli $t(16) = 4.1$, $p = .001$; incongruent stimuli $t(16) = 2.8$, $p = .012$). Patients were better at recognizing sadness ($t(16) = 2.8$, $p = .012$), anger ($t(16) = 4.3$, $p = .001$), disgust ($t(16) = 2.4$, $p = .029$) and fear ($t(16) = 3.4$, $p = .004$) at the post-intervention session compared to screening. Patients were also faster in recognizing the different emotions at post-intervention compared to screening ($p$ values ranging from .000 to .051). To control for these effects, a baseline cognitive performance score was calculated by taking the mean score of the screening session and the post-intervention session (the day after the second depletion session). This procedure is in lieu of a baseline measure at the morning of each ATD session (cf. Booij et al.,

2005a). Repeated measures analyses were done with Intervention (baseline vs. low dose vs. high-dose) as within-subject factor.

Effects of Order of administration (low-dose ATD first vs. high-dose ATD first) were investigated by including Order as a between subjects factor in the analyses. Only on the IAT emotional, an interaction effect of Intervention x Order was found for incongruent stimuli ($F(2,32) = 5.7$, $p = .007$) indicating that patients who received the low-dose first, became faster to respond to incongruent stimuli following both low-dose and high-dose. Patients who received the high-dose first became slower to respond to incongruent stimuli following high-dose ATD.

*Effects on neutral information processing*

*Word Learning Task*

*Immediate recall:* An Intervention effect was found for the number of correct ($F(2,34) = 4.1$, $p = .026$) and incorrect ($F(1.24,21.07) = 6.0$, $p = .018$) responses. Post-hoc contrast tests indicated fewer correct responses after high-dose depletion compared to baseline ($F(1,17) = 4,9$, $p = .040$) and low-dose depletion ($F(1,17) = 5.4$, $p = .033$). Also, more incorrect responses were given following high-dose depletion compared to baseline (main effect of Intervention $F(1,17) = 8.2$, $p = .033$) and low-dose ATD (a trend for an Intervention effect: $F(1,17) = 3.9$, $p = .066$).

*Retrieval:* An Intervention effect was found for the number of correct ($F(1.45,25.58) = 9.8$, $p = .002$) and incorrect ($F(1.56,26.58) = 4.2$, $p = .035$) words at +15 min. Contrast tests revealed that the number of correct words decreased after high-dose ATD compared to baseline ($F(1,17) = 14.5$, $p = .001$) and low-dose depletion ($F(1,17) = 8.4$, $p = .010$). The number of incorrect responses increased following high-dose compared to baseline (effect

of Intervention $F(1,17) = 5.6$, $p = .031$) and low-dose (a trend of an Intervention effect $F(1,17) = 4.3$, $p = .055$).

*Consolidation:* Main effects of Intervention ($F(1.29,21.99) = 6.7$, $p = .012$) and Time ($F(1,17) = 21.2$, $p < .001$) were found. The interaction effect of Time x Intervention was not significant ($p > .1$). Subjects recalled fewer words at delayed recall compared to immediate recall (5th trial), but there were no differences between the conditions. When data were re-analysed with $n = 13$, results were equal.

*Verbal Fluency:* The number of correct and incorrect words were analysed separately with Intervention as a within-subjects factor. No effects of high-dose and low-dose depletion were found on any of the verbal fluency measures ($p > .10$).

*Effects on emotional information processing*

*Facial Emotion Recognition Test*

*Accuracy:* Overall accuracy data were analyzed with Intervention and Emotion as within-subject factors. Subjects were better at recognizing certain emotions compared to others (main effect of Emotion $F(4,68) = 24.1$, $p < .001$) and they performed differently over the interventions (main effect of Intervention $F(2,34) = 4.1$, $p = .026$) (see Figure 1). When re-analysed with $n = 13$, the Intervention effect became a trend ($F(2,24) = 3.0$, $p = .071$) but the effect of Emotion remained significant ($p < .001$). Separate analyses were done for each emotion with Intervention and Intensity (in five 20% blocks) as within-subject factors. Decreased fear recognition was found following high-dose ATD (Intervention $F(2,34) = 4.1$, $p = .025$; Intensity $F(3.12,52.96) = 233.1$, $p < .001$; Intervention x Intensity $F(8,136) = 2.0$, $p = .052$) as compared to low-dose ($F(1,17) = 6.2$, $p = .023$) and baseline ($F(1,17) = 6.3$, $p = .023$).

The Intervention effect was significant for the 30-40% ($F$(1.53,25.99) = 3.9, $p$ = .044) and 70-80% ($F$(2,34) = 4.5, $p$ = .018) intensity levels. The higher the intensity of the emotion, the better it was recognized. No difference in fear recognition between low-dose depletion and baseline was found (see Figure 2). Repeating the analysis with 13 patients, the Intervention effect was no longer significant for the 30-40% intensity level ($F$(1.4,16.5) = 1.6, $p$ = .230) but remained significant for the 70-80% intensity level ($F$(2,24) = 3.7, $p$ = .039). No effects of ATD on the recognition of the other emotions were found.

*Speed*: Analyses for the reaction times were done with Intervention and Emotion as within-subject factors. Main effects of Intervention ($F$(2,34) = 7.2, $p$ = .002) and Emotion ($F$(2.38,40.44) = 8.4, $p$ < .001) revealed that ATD affected speed of recognition and that subjects were faster in recognizing certain emotions compared to others. Separate analyses per emotion showed that subjects became faster at recognizing disgust following ATD (main effect of Intervention $F$(1.36,23.07) = 4.0, $p$ = .048). Contrast tests showed that low-dose ATD speeded the recognition of disgust compared to baseline ($F$(1,17) = 10.4, $p$ = .005). High-dose also speeded the recognition of disgust compared to baseline, although this was a trend ($F$(1,17) = 4.2, $p$ = .056). When data were re-analysed with $n$ = 13, the overall Intervention effect disappeared ($F$(1.3,15.9) = 2.3, $p$ = .146), however the difference between low-dose ATD and baseline remained significant ($F$(1,12) = 6.9, $p$ = .022).

The results of the other cognitive tests are shown in Table 2.

**Figure 1.** Facial expression recognition

Facial expression recognition at baseline (dark bars), following low-dose depletion (grey bars) and high-dose depletion (white bars). Values represent means ± 1 SEM. *Asteriks* represent statistical significance of the comparisons, * $p < .05$

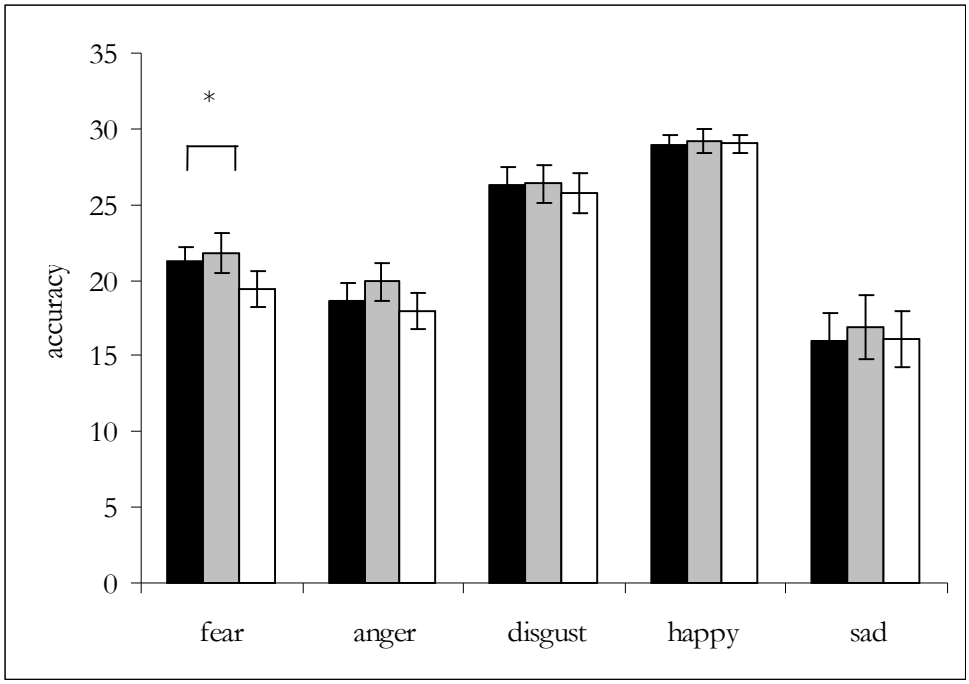
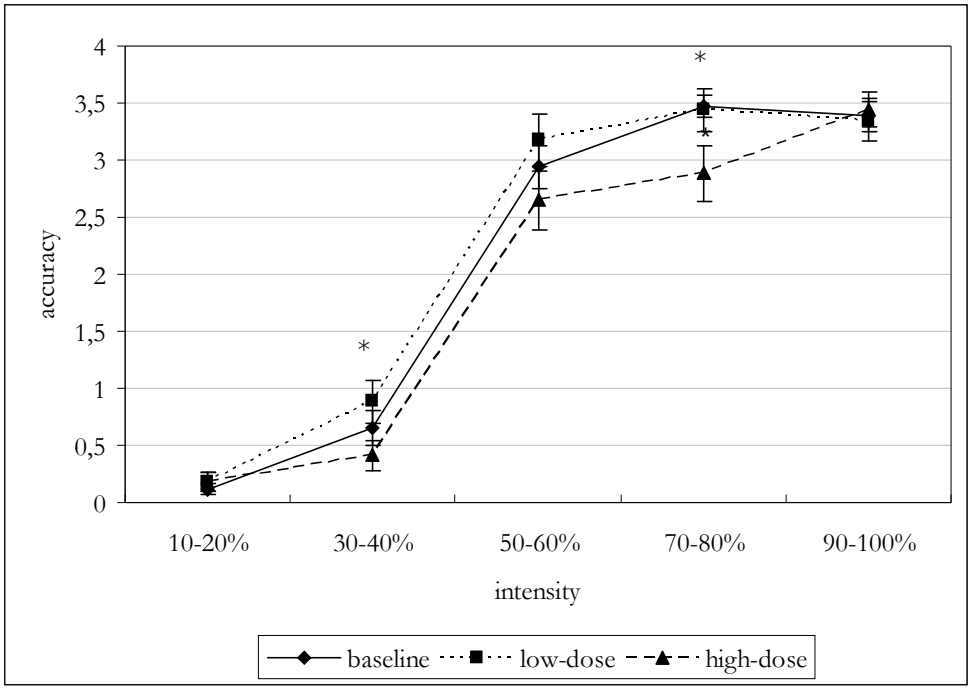


**Figure 2.** Fear accuracy

Fear accuracy (number of correctly recognized faces) per 20% intensity block at baseline, following low-dose depletion and high-dose depletion. Values represent means ± 1 SEM. *Asteriks* represent statistical significance of comparisons, * $p < .05$



*Dot-probe Task:* Attentional bias scores were calculated for depressive and threatening stimuli. One patient appeared to be an outlier, due to extremely slow responses. Analyses on the attentional bias score, with Intervention as a within-subjects factor, were therefore performed with and without this patient. No effects of low-dose and high dose ATD on attentional bias were found ($p > .10$).

*Implicit Association Test*

*Neutral:* Median latencies for congruent and incongruent word pairs were calculated (e.g. flower and positive vs. insect and positive). One outlier was found due to a large number of incorrect responses; analyses were performed with and without this subject. Analysis with Intervention and Stimulus-type (congruent vs. incongruent) was performed on the reaction time data. The IAT-effect (Greenwald et al., 1998) was found, indicating that subjects were faster to respond to congruent stimuli compared to incongruent stimuli (main effect of Stimulus-type $F(1,17) = 87.9$, $p < .001$). This effects was also significant for $n = 13$. No effect of ATD on the IAT effect was observed.

*Emotional:* Two outliers were found; one made a large number of errors, the other was extremely slow to respond. Analyses were performed on median response latencies, with and without the outliers, using Intervention and Stimulus-type as within-subjects factors. Latencies for congruent and incongruent stimuli were calculated. No IAT effect was found indicating that subjects were equally fast to respond to congruent (self and positive) and incongruent (self and negative) stimuli. No effect of low-dose or high-dose depletion was found ($p > .10$).

*Dysfunctional Attitudes Scale:* DAS scores were analysed with Intervention as a within-subjects factor. No effect of low-dose or high-dose depletion on DAS scores was found.

**Table 2.** Cognitive tests

| | Baseline | Low-dose | High-dose | *F* | df | *p* |
|---|---|---|---|---|---|---|
| **Dot-probe**[1] | | | | | | |
| RT depressive (ms) | 514.8 (70.6) | 519.0 (97.3) | 526.5 (81.8) | 0.6 | 16 | .569 |
| RT anxious (ms) | 531.5 (78.7) | 524.1 (91.3) | 528.4 (88.3) | 0.1 | 16 | .874 |
| RT positive (ms) | 519.5 (69.6) | 513.8 (84.7) | 523.2 (82.8) | 0.3 | 16 | .752 |
| RT neutral (ms) | 535.7 (87.3) | 524.7 (90.4) | 533.2 (84.5) | 0.3 | 16 | .727 |
| AB depressive-positive | -4.7 (13.3) | 5.2 (25.7) | 3.3 (20.7) | 1.0 | 16 | .384 |
| AB anxious-neutral | -4.3 (13.8) | -0.6 (16.9) | -4.8 (25.5) | 0.3 | 16 | .763 |
| **Word Learning Task** | | | | | | |
| immediate recall correct[2] | 47.9 (11.6) | 47.7 (9.7) | 43.0 (11.7) | 4.1 | 17 | .026* |
| immediate recall false | 1.0 (1.3) | 2.0 (2.2) | 4.3 (4.8) | 6.0 | 17 | .018* |
| delayed recall correct | 10.7 (2.8) | 10.4 (3.4) | 8.3 (3.7) | 9.8 | 17 | <.001** |
| delayed recall false | 0.3 (0.5) | 0.3 (0.5) | 1.0 (1.2) | 4.2 | 17 | .035* |
| **Verbal Fluency** | | | | | | |
| # correct responses | 10.9 (3.2) | 9.8 (4.0) | 10.3 (4.6) | 1.4 | 17 | .271 |
| # false responses | 0.5 (0.8) | 0.3 (0.6) | 0.3 (0.6) | 0.3 | 17 | .732 |
| **IAT Neutral** | | | | | | |
| RT congruent (ms) | 651.6 (102.4) | 638.8 (93.4) | 639.8 (98.0) | 0.6 | 17 | .537 |
| RT incongruent (ms) | 1005.1 (226.0) | 998.0 (213.0) | 1007.9 (249.1) | 0.1 | 17 | .923 |
| **IAT Emotional** | | | | | | |
| RT congruent (ms) | 851.3 (252.0) | 827.1 (205.0) | 826.1 (233.7) | 0.8 | 17 | .443 |
| RT incongruent (ms) | 817.7 (206.9) | 799.9 (214.0) | 822.0 (254.8) | 0.4 | 17 | .584 |
| **DAS-11** | 38.9 (8.8) | 35.7 (7.6) | 36.5 (9.4) | 2.9 | 34 | .069 |

Values represent means with SD in parentheses. AB = attentional bias, RT = reaction time, IAT = Implicit Attitudes Test, * p < .05, ** p < .01, [1] Dot-probe data are reported for *n* = 17, excluding one outlier, [2] total of trial 1-5

*Differences between mood-responders and non-responders*

At screening, mood-responders had a higher level of depressive symptoms compared to non-responders (MADRS score 12.3 ± 4.6 vs. 5.7 ± 5.0, $t(16) = 3.0$, $p = .009$). Furthermore, mood-responders were better at recognizing sadness than non-responders (main effect of Mood-response $F(1,16) = 6.6$, $p = .021$). When corrected for residual depressive symptoms, the effect of Mood-response became a trend ($F(1,15) = 3.6$, $p = .077$). The increased accuracy was not at the expense of the speed of recognition: an overall interaction effect of Emotion x Mood-response was found for speed of facial expression recognition ($F(5,80) = 4.0$, $p = .003$): mood-responders were faster at recognizing sadness (main effect of Mood-response $F(1,16) = 4.8$, $p = .043$) and tended to be faster at recognizing fear ($F(1,16) = 3.5$, $p = .080$). However, when corrected for baseline depressive symptoms, these effects disappeared ($F(1,15) = 0.9$, $p = .338$; $F(1,15) = 0.5$, $p = .479$). A trend for an interaction effect of Intervention x Mood-response was found for the number of correct responses at immediate recall ($F(2,32) = 3.2$, $p = .056$); mood-responders recalled more correct words at baseline compared to non-responders (52.1 ± 12.8 vs. 43.8 ± 9.1) but this difference was not significant (main effect Mood-response $F(1,17) = 2.5$, $p = .132$). Separate analyses for mood-responders and non-responders revealed that immediate recall performance decreased after high-dose ATD in mood-responders (main effect of Intervention $F(2,16) = 6.1$, $p = .011$) but not in non-responders (main effect of Intervention $F(2,16) = 0.1$, $p = .911$). Analyses with $n = 13$ revealed equal results.

*Effects of type of antidepressant medication*

Twelve patients were taking an SSRI at the time of study: citalopram 20-60 mg ($n = 5$); paroxetine 10-40 mg ($n = 5$); sertraline 100 mg ($n = 1$); fluoxetine 40

mg ($n$ = 1). Six patients were taking the SSNRI venlafaxine, 150-375 mg. The effect of type of medication (SSRI vs. SSNRI) on the different outcome measures was investigated by including type of medication as a between-subjects factor in the repeated measures analyses. Differences were found between patients taking an SSRI and patients taking an SSNRI in that the latter group experienced more physical symptoms (main effect of Medication $F(1,15) = 5.0$, $p = .041$), more depressive symptoms ( main effect of Medication $F(1,16) = 5.7$, $p = .029$) and more anxiety symptoms (main effect of Medication $F(1,16) = 8.5$, $p = .010$). However these effects were independent of ATD.

A considerable overlap was found between SSNRI use and mood-response: the majority of patients taking an SSNRI were mood-responders (five out of six), compared to one third of the patients taking an SSRI (four out of twelve).

**Discussion**

This study confirms that the effects of ATD on psychiatric symptoms depend on the extent of depletion. High-dose ATD led to a marked increase of symptoms, whereas low-dose ATD did not (91% vs. 59% reduction of plasma tryptophan/LNAA ratio). Although 59% reduction of tryptophan is thought to be sufficient to decrease brain serotonin synthesis (Biggio et al., 1974; Young et al., 1989), low-dose ATD may still be used as a placebo procedure, if the study focuses on symptoms. Furthermore, this study showed that ATD affects memory and facial expression recognition, as had previously been found in healthy subjects. Another new finding is that baseline emotional information processing, in particular the recognition of facial expressions of sadness, predicts mood-response to ATD. A number of other information processing measures have recently been found to predict depressive response

to ATD: cognitive reactivity (Booij & Van der Does, 2007); emotional Stroop interference (Booij et al., 2005a) and low heart rate variability, which is associated with impaired prefrontal functioning (Booij et al., 2006b).

*Differential effect of low-dose and high-dose ATD*

We observed few effects of low-dose ATD, since only the speed of disgust recognition was affected. While two recent studies presented stronger effects of low-dose ATD (Hayward et al., 2005; Munafò et al., 2006), the low-dose mixtures in these two studies resulted in larger decreases of tryptophan and the tryptophan/LNAA ratio and may therefore not be comparable to our low-dose mixture (Merens & Van der Does, 2007). Furthermore, these studies compared 'low-dose' ATD to a tryptophan containing mixture which increased tryptophan and tryptophan/LNAA ratio (by 48% and 27% respectively). This active control mixture may have increased the probability of finding an interaction effect. In the current study, low-dose ATD resulted in a 58.5% decrease of tryptophan/LNAA.

Several studies have now reported that ATD increases emotional Stroop interference (attentional bias) in healthy subjects and in recovered depressed patients (Booij et al., 2005a; Evers et al., 2006; Hayward et al., 2005; Munafò et al., 2006). However, the current study failed to find an effect of ATD on attentional bias as measured with the dot-probe test. This difference may be due to the different tests to measure attentional bias: the dot-probe test is a measure of spatial attention, whereas performance on the Stroop test reflects several other processes. Another reason may be that we did not use the optimal stimulus presentation times. Studies using the dot-probe test have found attentional biases in depression using relatively long stimulus presentations (1 sec or more) (Mogg et al., 1995). When stimuli are presented

for shorter durations, results are mixed (Bradley et al., 1997; Mathews et al., 1996). In the current study stimuli were presented for only 500 ms., which is the most often used procedure, but which in retrospect may explain the lack of effect on this measure. However, the dot-probe has advantages in that it circumvents the possibility of response bias interpretations, since it requires a response to a neutral stimulus (dot). Also, it allows for an investigation of both a facilitating and a possible impairing effect of the emotional stimulus on the detection of the dot, depending on the position of the emotional word relative to the dot (MacLeod et al., 1986).

*Specific or generalized effect of ATD on information processing*

High-dose ATD decreased the recognition of facial expressions of fear, which was not accompanied by faster or slower responses to fear. The recognition of facial expressions of disgust was speeded following both low-dose and high-dose ATD. This unexpected effect may represent a mood-congruent effect of ATD, which may be related to the unpalatable taste of the AA mixtures, but also to the self-declarative nature of depressive cognition.

The effect on fear recognition is in line with Harmer et al. (2003c) who found a similar effect in healthy females, but not in males. Since our sample included only two males, it is unclear whether this gender effect was replicated. Even though these effects are consistent, they are in contrast to the expected effects of lowered serotonin function and the symptomatic effects of ATD. Studies investigating the acute effects of other serotonin manipulations have also found effects on emotion recognition. In healthy subjects, both citalopram and tryptophan increased the recognition of fear (Attenburrow et al., 2003; Harmer et al., 2003a). However, in remitted depressed patients elevated fear recognition was normalized following citalopram administration (Bhagwagar et al., 2004).

Subchronic treatment with citalopram or tryptophan decreased the recognition of negative facial expressions in healthy volunteers (Harmer et al., 2004; Murphy et al., 2006). Both the direction of the effect of ATD on facial expression recognition and the differential effects of SSRI administration on fear recognition in different samples remain to be fully explained.

In contrast with our previous findings, ATD had no effect on verbal fluency, which measures retrieval from semantic memory (Booij et al., 2005a). Effects of ATD on learning, memory retrieval and consolidation have been observed in healthy individuals (Park et al., 1994; Riedel et al., 1999; Schmitt et al., 2000). In remitted depressed patients, ATD was found to decrease memory for positive words (Booij et al., 2005c). ATD also impaired short-term memory, in recovered but not in healthy subjects, and without affecting learning or long-term memory consolidation (Hayward et al., 2005). In the present study high-dose ATD impaired learning and retrieval in remitted depressed patients, which is in line with results in healthy subjects. However, the impaired learning following high-dose ATD occurred mainly in mood-responders. In contrast to previous studies, memory consolidation was not affected.

Our results give no indication for a general effect of ATD on emotional information processing. Only facial expression recognition was affected by ATD; attentional bias and implicit attitudes were unaffected. Future research should direct attention to the effects of ATD on different aspects of emotional information processing to clarify whether effects are specific for certain aspects of emotional information processing. Although the patients in the current study were repeatedly exposed to emotional stimuli, habituation to these stimuli does not seem to be an explanation for the absence of any effects on the IAT and dot-

probe test. The observed effects of ATD on the recognition of fearful facial expression do not support this explanation. Also, we compared the effects of ATD to a baseline measure that was based on the mean of the screening and final (post-intervention) session. If learning or habituation effects occur, the average of these sessions would be the best estimate of the baseline (the 2nd and 3rd sessions were low- and high-dose ATD in randomized order).

*Relationship between emotional information processing and serotonergic vulnerability*

Mood response to ATD was related to faster and more accurate recognition of sadness and faster recognition of fear, although when corrected for residual depressive symptoms only the effect on the accuracy of sadness recognition remained, and became statistically a trend. The sample of the current study was too small to correct for clinical and demographic factors that might mediate the relationship between cognitive processing and mood response to ATD. However, the relation between recognition of sadness and mood response to ATD was not entirely related to the level of residual depressive symptoms since the effect became a trend and did not disappear when corrected for residual depressive symptoms. These results are in line with research in symptomatic and remitted depressed patients, which showed that a bias towards the perception of negative faces is a vulnerability factor for depressive relapse and that negative mood increases this negative perceptual bias (Bouhuys et al., 1999; Gur et al., 1992).

Differential results were found for SSNRI and SSRI users; however these were mostly independent of ATD. These differences may be due to an overlap with mood-response since five out of six SSNRI users were mood-responders.

*Suggestions for future research*

Facial expression recognition has been found to be related to serotonin function. However, more research on the direction of this association is needed. Since our study failed to find an effect of ATD on attentional bias, ATD studies comparing different tests to measure attentional bias may shed light on the link between serotonin and attentional bias in depression. Also, more studies on the effects of ATD on the different aspects of memory in remitted depressed patients are needed since evidence in this field mainly involves healthy subjects.

*Limitations*

The small sample size of the current study is a limitation. Furthermore, the relatively large number of patients who vomited following the depletion mixture warrants replication of the results in a larger sample. We carried out all analyses twice (with and without the five patients who vomited in response to the AA mixture), and found only a few minor differences in results. The biochemical data, when analysed for all patients, showed a significant decrease in tryptophan levels, supporting the view that despite the vomiting, the amino-acids were well absorbed. We also compared the decreases in plasma tryptophan and tryptophan/LNAA ratio of the five patients who vomited to the patients who did not. No significant differences were observed ($p$ values > .08); therefore we decided to include the patients who vomited in the overall analyses. Since the present study was a continuation of our previous experiments, using the same batch of amino acids, and the same design, procedures, recruitment and setting, it is unclear what has caused the differences in tolerability. Another limitation is the absence of a placebo condition. However, a true placebo does not exist. Studies have used mixtures

containing between 2.3g and 5g tryptophan. This procedure leads to increases in tryptophan and the tyrptophan/LNAA ratio which in turn may affect cognitive performance and symptoms (Markus et al., 1998; Markus et al., 2002). Including such a placebo mixture may thus lead to overestimations of the effects of ATD. A possible solution for future studies would be to use the novel ATD method of administrating a natural collagen mixture with low-tryptophan content (Lieben et al., 2004), since the placebo condition of this method seems to have no effect on tryptophan concentrations in humans (Evers et al., 2005).

# 6

# Plasma tryptophan levels following low-dose and high-dose tryptophan depletion

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ATD has been found to markedly decrease plasma tryptophan levels and the ratio tryptophan/LNAA, depending on the amount and composition of the amino acid mixture, whether it is combined with a low-tryptophan diet and on the time at which plasma samples are taken. In this chapter, different 'low-dose' ATD methods are discussed. Also, the inter-individual variations in plasma tryptophan levels following high-dose and low-dose ATD are reported.

**Low-dose tryptophan depletion**

Acute tryptophan depletion (ATD) is a popular method to investigate the effects of lowered serotonin function in humans. Acute tryptophan depletion induces a temporary depressive 'relapse' in 50-60% of remitted depressed patients treated with serotonergic antidepressants. In healthy individuals, ATD has no or minor mood effects, but cognitive effects have been found in both healthy and recovered depressed individuals (Booij et al., 2003).

The magnitude of the reduction of plasma tryptophan concentrations following ATD depends on the amount and composition of the amino acid mixture (Young et al., 1989) and whether or not a pre-test low tryptophan diet is included. It has been suggested that a threshold exists that needs to be exceeded before any behavioural effects occur, since studies in which the plasma tryptophan reduction was lower than 70% generally do not find any symptomatic effects (Van der Does, 2001b). However, depression-congruent effects on sleep architecture have been observed at moderate tryptophan reductions (Bhatti et al., 1998). The placebo procedure developed by Krahn et al. (1996) may be suitable as a low-dose ATD procedure (Van der Does, 2001a). Since this procedure reduces plasma tryptophan concentrations by 40-50%, and has been found not to affect mood (Booij et al., 2005a), it allows for the investigation of possible dose-response effects.

Booij et al. (2005a), using the Krahn et al. method as low-dose ATD, found that ATD had a dose-dependent effect on selective attention (Stroop colour-word interference) in remitted depressed patients, but no other cognitive effects of low-dose ATD were observed. Merens et al. (in press) observed no effects of low-dose ATD on attention, memory, and accuracy of emotion recognition in remitted depressed patients (Merens et al., in press). Two recent papers have reported much stronger effects of low-dose ATD. Hayward et al. (2005) found that low-dose ATD had no effects on mood ratings in unmedicated recovered depressed subjects, but that it increased the emotion-potentiated startle reflex, impaired recognition of happy faces and initial recall memory and increased emotional Stroop interference. Some cognitive effects were also observed in healthy controls. Munafò et al. (2006) reported that low-dose ATD slightly increased self-rated depressive symptoms in medicated recovered depressed patients and also increased Stroop interference for social threat words.

The low-dose mixture used by Hayward et al. (2005) and Munafò et al. (2006) consisted of eight amino-acids (31.2g), whereas the Krahn et al. (1996) procedure consists of 15 amino-acids (25.7g). We calculated the plasma tryptophan reductions obtained by Hayward et al. (2005), and found that low-dose ATD decreased plasma tryptophan levels by 73.9% in recovered depressed patients. The tryptophan/large neutral amino acids (LNAA) ratio decreased by 86.9%. This suggests that Hayward et al. (2005) studied *high-dose* ATD rather than low-dose. The reductions cannot be calculated from the report by Munafò et al. (2006), but this study used the same procedure and partly the same sample. Viewing these studies as high-dose ATD studies resolves the inconsistencies with the studies by Booij et al. (2005a) and Merens et al. (in press). It also explains the symptomatic effects in the Munafò et al.

study. However, high-dose ATD would be expected to have increased symptoms in Hayward et al.'s paper. This may be explained by the fact that patients in this study had a relatively low number of previous episodes, which predicts a weaker response to ATD (Booij et al., 2002).

There is no generally accepted definition of high-dose or low-dose ATD. Hayward et al. (2005) presented their study as a low-dose ATD study on the basis of the amount of amino acids used. However, peripheral biochemical measures indicate that this study may be considered a high-dose ATD study. In our view, the term low-dose should reflect the decrease of plasma tryptophan concentrations and not the amount and content of the ATD mixture. Future research should carefully consider which terminology is used to prevent misinterpretation and biochemical data should be reported in detail.

**Inter-individual variations in plasma tryptophan levels following ATD**

The regularly used ATD mixture containing fifteen amino acids, weighing approximately 100g (also called 'high-dose' ATD) usually results in a temporary decrease in plasma tryptophan levels of 70-90%. Peak reductions in plasma levels are found 5 to 7 hours after ingestion of the amino-acid mixture (Delgado et al., 1990; Young et al., 1985). This decrease in plasma tryptophan levels following ATD is thought to be sufficient to significantly decrease brain serotonin synthesis (Biggio et al., 1974; Young et al., 1989). Since tryptophan competes with the other large neutral amino acids (LNAA) at the blood-brain barrier, the ratio of tryptophan/LNAA is suggested to be an important index of serotonin turnover (Biggio et al., 1974; Fernstrom & Wurtman, 1972).

In our ATD study, which is reported in Chapter 5 and 7, high-dose ATD led to a 91% reduction of plasma tryptophan/LNAA ratio, compared to a 59% reduction following low-dose ATD (: a mixture of fifteen amino acids at

a quarter strength of the regular 100g mixture). Since a sizable number of patients had vomited or not finished the AA mixture, we inspected the individual decreases in plasma tryptophan and the tryptophan/LNAA ratio following both low-dose and high-dose ATD (see Table 1). Especially the low-dose mixture resulted in a large variability in the reductions in the tryptophan/LNAA ratio: three patients showed a large decrease (79% - 86%) in the plasma tryptophan/LNAA ratio, equivalent to the decreases found in the high-dose condition. Three other patients showed a relatively small decrease in the tryptophan/LNAA ratio following low-dose ATD (33% - 37%). These six patients all deviated from the mean decrease following low-dose (59% ± 16) with more than one standard deviation.

Following high-dose ATD, three patients showed a smaller decrease than expected in plasma tryptophan/LNAA ratio (71% - 81%). For two of these patients, this may be attributed to the fact that they vomited following the high-dose mixture. However, three other patients that vomited following the high-dose mixture did not show a smaller than expected decrease in plasma levels.

The inter-individual variations in the decreases of plasma tryptophan/LNAA ratio were larger than in previous studies (Booij et al., 2005a; Booij et al., 2006b). Unfortunately, studies usually do not report individual decreases in plasma tryptophan levels, so not much is known about their variations or the origin of these variations. One factor that could play a role in inter-individual differences in tryptophan levels following ATD is gender. Men have a higher rate of serotonin synthesis compared to women and the biochemical effects of ATD are greater in women compared to men (Nishizawa et al., 1997). The two men that were included in our study did show relatively small decreases in tryptophan/LNAA ratio following low-dose

ATD, but the biochemical response to high-dose ATD in men was similar to women's.

The large variations in tryptophan levels may be partly explained by the fact that several participants vomited. Five out of eighteen patients that were included in our study, vomited in response to high-dose ATD, one of whom also vomited in response to low-dose ATD. Four of these patients were able to complete the study. One patient dropped out after the high-dose ATD session (which was the second session for her) because of an emotional reaction to the depletion drink. One patient was excluded from the analyses because she dropped out after the first session (high-dose ATD) due to physical and mood complaints following that session (she also vomited in response to the high-dose mixture).

Since the present study was a continuation of previous experiments, using the same batch of amino acids, and the same design, procedures, recruitment and setting, it is not certain what has caused the differences in tolerability. However, ATD mixtures are consistently reported to be highly unpalatable and nauseating (Reilly et al., 1997). There are rather large differences in the reported tolerability of ATD in the literature. Some studies do not report any somatic side-effects, other studies may report side effects following both the tryptophan depletion drink and the control mixture (e.g. Rubinsztein et al., 2001; Schmitt et al., 2000; Spillman et al., 2001); other studies report low, but varying numbers of patients that vomited following the ATD mixture (e.g. Anderson et al., 2003; Booij et al., 2005a; Riedel et al., 1999; Spillman et al., 2001).

Despite a clear overall difference between low-dose and high-dose ATD in the present study, in one third of the patients the biochemical effects

were out-of-range following low-dose, and in three out of eighteen patients following high-dose.

**Table 1.** Individual decreases in tryptophan/LNAA ratio following low-dose and high-dose ATD (%)

| Patient | low-dose | high-dose |
|---|---|---|
| 1 | -37.41 | -88.19 |
| 2 | -32.73 | -93.57 |
| 3 | -50.81 | -96.14 |
| 4 | -72.75 | -97.72**a** |
| 5 | -59.36 | -94.63 |
| 6 | -67.11 | -97.59**b** |
| 7 | -71.93 | -98.65 |
| 8 | -60.98 | -96.11 |
| 9 | -85.98 | -98.81 |
| 10 | -49.35 | -80.79 |
| 11 | -50.84 | -97.25 |
| 12 | -48.89 | -96.19 |
| 13 | -79.94 | -96.91 |
| 14 | -79.22 | -87.42**c** |
| 15 | -33.83 | -96.12 |
| 16 | -47.87 | -86.43 |
| 17 | -67.52**d** | -70.55**e** |
| 18 | -55.72 | -72.91**f** |

**a**: vomited five hours after ingestion of the high-dose mixture
**b**: vomited within 15 minutes after ingestion of the high-dose mixture
**c**: vomited one hour after ingestion of the high-dose mixture
**d**: vomited one and a half hour after ingestion of the low-dose mixture
**e**: vomited ten minutes after ingestion of the high-dose mixture
**f**: vomited one and a half hour after ingestion of the high-dose mixture

We underlined the values that differed from the mean decrease in plasma tryptophan/LNAA by more than one standard deviation: low-dose: M = 58.5% SD = 16.0; high-dose: M = 91.4% SD = 8.7.

# 7

# The effects of acute tryptophan depletion on heart rate variability in remitted depressed patients

W. Merens, A.J. W. Van der Does, C.A. Swenne

**Abstract**

Low heart rate variability (HRV) has been found to be associated with depressive disorder. Recent findings indicate that experimentally lowering serotonin function leads to reduced HRV and increased impulsivity in remitted depressed patients with a history of suicidal ideation (Booij et al., 2006b). Also, symptom effects of ATD correlated with low HRV at baseline. These findings suggest that low HRV may be mediated by serotonin function in a specific group of depression vulnerable patients. Fourteen remitted depressed patients received high-dose and a low-dose ATD in a randomized, double-blind, within-subjects crossover design. Mood was assessed before and after administration of the depletion drink. Five hours after administration, attentional bias was measured. HRV was measured during rest and during the attentional test. ATD led to the expected decreases in plasma tryptophan levels. High-dose ATD increased depressive and anxiety symptoms in half of the patients. High-dose ATD increased heart rate compared to baseline, but no statistically significant effects on HRV were found. At baseline, patients with low HRV had higher attentional bias scores for threat-related stimuli compared to patients with high HRV. Also, mood response to ATD was higher in patients who showed a decrease in HRV following ATD. Unfortunately, a replication of the differential effects in patients with and without a history of suicidal ideation could not be performed, due to unequal sample sizes. The direction of effects of ATD on HRV in patients with a history of suicidal ideation was the same as in a previous study. More research is needed on the role serotonin plays in HRV in depressive disorder. Especially the specific role of impulsivity and suicidal ideation should be further investigated. Our findings do suggest that low HRV may be related to mood and poor affective processing through changes in serotonin function.

**Introduction**

Heart rate variability (HRV) is a measure of autonomic regulation of the heart (Krantz & McCeney, 2002). HRV reflects the capacity of the autonomic nervous system to vary the intervals between consecutive heart beats (Grippo & Johnson, 2002). Reductions in HRV are related to depression (Agelink et al., 2002; Rechlin et al., 1994), however, negative results have also been found (Gehi et al., 2005). Low HRV is also associated with generalized anxiety disorder (Thayer & Lane, 2000), impulse control disorders such as ADHD (Beauchaine et al., 2001), and alcoholism (Ingjaldsson et al., 2003).

Depression has been found to be an independent risk factor for cardiovascular disease (CVD) (see for a review Rugulies, 2002). Cardiovascular disease is the leading cause of death in the United States (American Heart Association, 2006). Depression after a myocardial infarction also predicts mortality (Anda et al., 1993). Decreased HRV is a risk factor for CVD (Stein & Kleiger, 1999) and has also been associated with depression and may thus underlie the increased risk of cardiovascular disease in depression (Gorman & Sloan, 2000; Grippo & Johnson, 2002; Musselman et al., 1998).

Serotonin dysfunction is suggested to play an etiological role in both depression and cardiac dysfunction (Grippo & Johnson, 2002), and may thus underlie the association between HRV and depression. This has been supported by several studies. Kellett et al. showed changes in cardiac function following serotonin depletion in rats (2005). In a study with depressed patients, resting heart rate and systolic blood pressure decreased following 21 days of treatment with the selective serotonin reuptake inhibitor (SSRI) nefazodone, but no effects on HRV were found (Agelink et al., 2001). Glassman et al. concluded that treatment with the SSRIs paroxetine, fluoxetine and sertraline either had no effects or was beneficial for depressed patients with heart disease

(Glassman et al., 1998). SSRIs may also have a beneficial effect on cardiac function and improve HRV in patients with panic disorder (Gorman & Sloan, 2000). The evidence described above indicates that HRV is a risk factor for cardiovascular disease. Depression is linked to cardiovascular disease and is associated with lowered HRV. Serotonin is implicated in depression and may also play a role in HRV. Therefore, manipulating serotonin function (through SSRI administration or by another means) may affect HRV.

Acute tryptophan depletion (ATD) is a useful tool to investigate the effects of lowered serotonin function in humans. ATD leads to a rapid lowering of plasma tryptophan levels. ATD results in a temporary depressive 'relapse' in 50-60 % of recovered depressed patients treated with an SSRI (Van der Does, 2001a). In healthy subjects, mood effects of ATD are only found in subjects with a family history of depression (Klaassen et al., 2002). Cognitive effects of ATD have been found in both healthy subjects and in recovered depressed subjects. ATD selectively impairs learning (Park et al., 1994), memory retrieval and consolidation (Klaassen et al., 2002; Park et al., 1994; Riedel et al., 1999) in healthy volunteers and ATD improves attention in healthy samples (Schmitt et al., 2000) and patients (Booij et al., 2005a; Evers et al., 2006). Also, ATD may increase emotional Stroop interference (Booij et al., 2005a; Evers et al., 2006; Hayward, 1995) in recovered depressed patients.

Besides the link between serotonin and cognitive processing, autonomic nervous system regulation as manifested in cardiac variability may be related to both attentional regulation and affective processing, since decreased HRV is a marker for low parasympathetic activation and prefrontal hypoactivity (Thayer & Brosschot, 2005). HRV in particular has been shown to be related to attentional control and to emotional regulation, low HRV being related to poor affective processing (Thayer & Lane, 2000). Further evidence

indicates that HRV is related to cognitive flexibility (Johnsen et al., 2003). The prefrontal cortex is necessary for many tasks involving executive function. The level of resting HRV seems to be related to executive functioning. Subjects with high HRV were found to perform better on executive tasks and working memory tasks compared to subjects with low HRV (Hansen et al., 2003; Hansen et al., 2004).

Since serotonin may underlie the link between HRV and depression, and HRV may in turn be related to cognitive processing, it would be interesting to investigate the effects of lowered serotonin function on HRV and cognitive performance in depression.

Recently, the effects of ATD on HRV were investigated in a sample of remitted depressed patients (Booij et al., 2006b). Selective effects were found in remitted depressed patients with a history of suicidal ideation; high-dose ATD reduced HRV during rest, increased impulsivity (as measured by the Continuous Performance test) and increased anxiety symptoms in this subgroup of patients. Also, symptom effects of ATD correlated with low HRV at baseline (Booij et al., 2006b). The authors suggested that reduced HRV in depression may thus be limited to patients who are prone to display impulsive or aggressive behaviour.

The first objective of the current study is to replicate these findings using a different test of attention, to investigate whether the effects of ATD are mainly linked to impulsivity or to attention and anxiety. If serotonin is indeed implicated in HRV, lowering serotonin function may affect (: decrease) HRV. It is interesting to investigate whether the effects on HRV will be limited to those patients who experience a mood response following ATD or if the effect will be generalized to all patients. We evaluated the effects of ATD on mood, cardiac function and attentional bias to depressive and threatening

information in medicated remitted depressed patients with and without a history of suicidal ideation. ATD affects attentional bias, as measured with the Stroop task (Booij et al., 2005a; Evers et al., 2006; Hayward et al., 2005; Munafò et al., 2006). However, since the Stroop task is used as an experimental stressor in cardiac activity research (Renaud & Blondin, 1997), we chose another measure of attentional bias; the Dot-probe test (MacLeod et al., 1986) to investigate how mental workload may affect the influence of ATD on HRV. Since mental effort is known to lower HRV measures (Johnsen et al., 2003; Van Roon et al., 2004), the workload task may strengthen the lowering effect of ATD on HRV. We hypothesized that ATD would increase symptoms in a subgroup of patients. Furthermore, we expected low HRV to be related to the symptomatic effect of ATD and to a decreased attentional function following ATD. We hypothesized that the effects of ATD on attentional bias would be strongest in patients with a history of suicidal ideation.

Research has identified several predictors of response to ATD in remitted depressed patients, such as chronicity of depression (Booij et al., 2002) and cognitive reactivity (Booij & Van der Does, 2007). The study of Booij et al. (2006b) showed that low HRV predicted a stronger response to ATD in SSRI treated remitted depressed patients. The second aim of the current study is therefore to find support for the predictive role of low HRV in mood response to ATD.

## Methods

*Participants*

Participants were outpatients of the Mood Disorders clinic of a psychiatric hospital. Inclusion criteria were age between 18 to 65 years; primary DSM-IV diagnosis of major depressive disorder; no longer fulfilling DSM-IV criteria for

depression; Hamilton-17 scores below 16; ongoing treatment with an SSRI or selective serotonergic and noradrenergic reuptake inhibitor (SSNRI) for at least four weeks; no psychotic disorder (lifetime); no substance abuse in the past 3 months (DSM-IV criteria); BMI above 18 kg/$m^2$; free of neuro-endocrine or neurological disease; no pregnancy or lactation.

*Design*

The study was conducted according to a double-blind, randomized crossover design.

*Composition of the amino-acid mixtures*

The high-dose amino-acid (AA) mixture consisted of fifteen amino acids (102.5 g) (Young et al., 1985). The low-dose mixture consisted of the same amino acids but at one quarter strength (25.7 g) (Krahn et al., 1996). The amino acids were given in drink form by mixing the amino acid powders with water to a final volume of 300 ml. To compensate for the unpleasant taste of the mixture, chocolate syrup was added and the mixtures were served chilled.

*Instruments*

*Symptoms*

The Hamilton depression rating Scale (HDRS) (Hamilton, 1967) and the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979) were administered at screening. Symptoms were assessed during the test sessions using the Comprehensive Psychopathological Rating Scale (CPRS) (Goekoop et al., 1991), an observer-rating scale that includes the MADRS and the Brief Anxiety Scale (BAS) (Tyrer et al., 1984). Because

changes in weight or sleep do not occur during the course of an ATD session, these items were omitted.

*Attentional bias*

The Dot-probe task measures attentional bias (MacLeod et al., 1986). Word pairs were presented one by one on a computer screen for 500 ms, one in the upper part of the screen and one below. Following the termination of that display, a dot appeared on the location of either word. Participants had to indicate at which location the dot was placed by pressing a key. The duration of the presentation of the dot on the screen was variable since the dot disappeared and a new screen appeared as soon as a response was given. Each trial started with a white fixation cross for 500 ms. Subjects were first run through six practice trials. The main experiment consisted of trials of neutral words paired with threat-related words and trials of depression-related words paired with positive words. Only median latencies for correct responses were included in the analyses to reduce the influence of possible outliers. Attentional bias was associated with faster reaction times if the dot replaces the negative word compared to trials where the dot replaces the neutral or positive word. Attentional bias is calculated as the mean reaction time for depressive (or threat related) words minus the mean reaction time for positive (or neutral) words.

*Biochemical analyses*

To determine total plasma tryptophan concentrations and the ratio total tryptophan/ large neutral amino acids (LNAA), venous blood was obtained (10ml) in EDTA tubes. After sampling, the blood was centrifuged for 20 minutes at $2650g_{max}$. Plasma was stored at – 65°C until quantitative amino acid

analysis by high-performance liquid chromatography took place (as described by Fekkes et al., 1995).

*Cardiac activity*

Heart rate and HRV were measured by the VU-AMS device (version 4.6. TD-FPP, Vrije Universiteit, Amsterdam, the Netherlands). This device has been used extensively and details of its characteristics have been published elsewhere (AMS; Klaver et al., 1994). In the present study the electrocardiogram signal was recorded using disposable pre-gelled Ag-AgCL electrodes (ConMed, New York, USA) that were placed at the jugular notch of the sternum, 4 cm under the left nipple and at the lateral right side. The device detects the R-wave of the electrocardiogram and records the time in milliseconds (with one millisecond resolution). Using this three electrode configuration inter-beat intervals were generated from which HRV was computed using software from the Leiden University Medical Center (Bootsma et al., 1994). Apart from heart rate (HR), different time domain measures of HRV were taken: the standard deviation of the inter-beat interval (SD), the coefficient of variation, CVr (SD/ inter-beat interval), the square root of the mean squared differences of successive inter-beat intervals (RMSSD) and the percentage of successive normal interval differences greater than 50 ms (PNN50). Frequency domain measures included low frequency power (LF: 0.07-0.14 Hz) which is associated with both parasympathetic and sympathetic modulation and high frequency power (HF: 0.14-0.40 Hz) which is a measure of centrally mediated cardiac vagal control (Berntson et al., 1997). HF variations are variations in HR related to the respiratory frequency (also called respiratory sinus arrhythmia: RSA). Also, the LF/HF ratio was calculated, which is thought to reflect the relative balance of sympathovagal influences on the heart (Bootsma et al., 1994). We chose to

include both time and frequency domain measures of HRV since studies on depression and HRV usually investigate both (e.g. Rechlin et al., 1994; Van der Kooy et al., 2006), although studies on the effect of workload or mental stress on HRV focus mostly on frequency measures (Hjortskov et al., 2004; Van Roon et al., 2004).

*Procedure*

After showing interest in taking part, all volunteers were given oral and written information about the study. Participants who seemed to meet inclusion criteria were invited to a screening session during which informed consent was obtained. During the screening session, the SCID-IV interview (First et al., 1995), HDRS and MADRS were administered. A clinical psychologist or a trained research assistant conducted the interviews. At screening the cognitive tasks were done for the first time. The day before each ATD session, participants kept a low-tryptophan diet (Booij et al., 2005a).

The scheme of the test sessions is presented in Table 1. Participants arrived at 9.00 am at the laboratory after fasting overnight. They were instructed not to use alcohol 24 hours prior to the sessions and to arrive well rested. After arrival (t-1), blood pressure was assessed; baseline mood measures were taken, followed by a blood sample. Next, participants ingested the AA mixture within half an hour (t0). Participants remained in the research room until 5.00 pm. Neutral music and magazines were available. Participants were not allowed to sleep. Water, (de)caffeinated coffee and (herb) tea were allowed in standard amounts. Three hours after ingestion of the AA mixture, participants were served a protein-poor lunch (Booij et al., 2005a; Riedel et al., 1999). During the hour preceding the cognitive and cardiac measures, patients were not allowed to smoke or to drink caffeinated coffee or tea. At around

2.15 pm, the electrodes were placed and the AMS device was connected to the computer. Also, blood-pressure was assessed. Patients performed a neutral distraction task for 5 minutes ('resting' period): they were given a Swedish text and were instructed to cross each letter *a*. It was explained that this was intended as a simple baseline task and that there were no demands concerning time or accuracy. At 2.30 pm (t4.5) the cognitive tasks were performed which lasted about 50 minutes. Thereafter, the electrodes were removed, a blood sample was obtained and the symptom interview and questionnaires were administered (t6.5). Before the participants went home, again the blood-pressure was assessed and they received a snack to speed up normalization of tryptophan levels. The procedure was repeated approximately seven days later; participants who had first received the 100% strength mixture now received the 25% strength mixture and vice versa. The morning after each session, patients returned to the lab where symptoms were assessed as well as blood pressure and a blood sample was obtained. The day after the second ATD session, cardiac activity was assessed while participants again performed the cognitive tests (post-intervention session). This study was approved by an independent medical ethics committee (METIGG, Utrecht). Participants were tested individually and were paid € 115,-.

**Table 1.** Scheme of the sessions

| Time | Assessment | |
|---|---|---|
| 9.00 am | Arrival, CPRS, blood pressure | (t-1) |
| 9.50 | Blood sample | |
| 10.00 | Ingestion AA mixture | (t0) |
| 1.00 pm | Lunch | |
| 2.30 | Cardiac assessment, blood pressure & cognitive tests | (t4,5) |
| 4.00 | Blood sample | |
| 4.30 | CPRS, blood pressure | (t6,5) |
| 5.00 | End of session | |

AA = amino acid; CPRS = Comprehensive Psychopathological Rating Scale

*Statistical analysis:*

The effects of tryptophan depletion on symptoms and Dot-probe performance were analyzed separately using General Linear Models (GLM) repeated measures analyses. Symptom ratings were analyzed with Intervention (low-dose vs. high-dose) and Time (pre-depletion vs. post-depletion vs. the next day) as within-subject factors. Dot-probe performance was analyzed with Intervention (baseline vs. low-dose vs. high-dose) as within-subject factor. Baseline cognitive performance was calculated by taking the mean score of the screening session and the post-intervention session (the day after the second depletion session). This procedure is in lieu of a baseline measure at the morning of each ATD session and corrects for any learning effects that may occur over the course of four assessments (Booij et al., 2005a). The post-intervention HRV assessment was taken as a baseline for cardiac activity. Cardiac activity measures were analyzed separately using Intervention (baseline,

low-dose, high-dose ATD) and Period (resting vs. Dot-probe) as within-subject factors. Since cardiac activity may be influenced by age, gender and smoking, the effect of these variables were checked separately. The effect of order of administration on cardiac activity and Dot-probe performance was also analyzed. In those instances in which the assumption of homogeneity of covariance in repeated measured analysis was violated, as assessed with Mauchly's Test of Sphericity, Huynh-Feldt corrected p-values were used (Field, 2005). Bonferroni corrections were used to adjust for multiple comparisons.

**Results**

*Data screening*

All data were screened for accuracy of data-entry, missing values and the assumptions of multivariate data-analysis. The MADRS and BAS data were not normally distributed; hence a logarithmic transformation was performed. PNN50, LF, HF and LF/HF ratio were $\log^{10}$ transformed because of skewed distributions. All transformations were successful.

*Participants*

Twenty patients were included. One patient withdrew after the screening session due to family problems. One patient dropped out after the first day (high-dose depletion) due to physical and mood complaints following that session. These complaints had disappeared the day after, however she decided to withdraw participation. One patient was excluded from analyses due to a possible lack of sinus rhythm. Data were missing for one patient at the post-intervention session following the second day, due to an emotional reaction to the high-dose depletion drink. After having contacted the patient by telephone the next morning, she reported to be less emotional but she was still very tired

and did not want to come to the lab. For two patients cardiac data were missing due to technical problems. Analyses were therefore performed on fourteen patients. Four patients vomited after high-dose ATD; one patient also vomited after low-dose ATD. One patient could only finish 75% of the drink on both days. All these cases were retained. Analyses with and without those patients did not show any differences in results. Clinical and demographical characteristics of the subjects are shown in Table 2.

**Table 2.** Characteristics of the sample ($n$ = 14)

| | **Mean (SD)** |
|---|---|
| Age | 42.9 (12.0) |
| Males/Females | 1/ 13 |
| Number of smokers | 8 |
| Type of medication: SSRI/SSNRI | 9 / 5 |
| Number of previous episodes | 5.4 (4.7) |
| Duration of remission (months) | 15.3 (25.7) |
| Single/recurrent episodes | 2 / 12 |
| Partial/ full remission | 5 / 9 |
| SI+/ SI- | 11 / 3 |
| MADRS | 9.1 (5.8) |
| HDRS | 7.4 (3.8) |

SSRI: selective serotonin reuptake inhibitor, SSNRI: selective serotonin noradrenalin reuptake inhibitor, SI: history of suicidal ideation, MADRS = Montgomery-Asberg Depression Rating Scale, HDRS = Hamilton Depression Rating Scale

*Effects on amino-acid levels*

In the low-dose condition, mean plasma tryptophan concentrations decreased by 58.3% from 43.0 ± 5.1 μmol/l to 18.1 ± 5.7 μmol/l. In the high-dose condition plasma tryptophan levels decreased by 82.2% from 41.4 ± 6.9 μmol/l to 7.3 ± 4.7 μmol/l. The plasma tryptophan/LNAA ratio decreased by

56.2% in the low-dose condition (from 10.2 ± 1.5 to 4.4 ± 1.4) and by 89.9% in the high-dose condition (from 9.9 ± 1.6 to 1.0 ± 0.9). Significant effects of Intervention ($F(1,13) = 30.8$, $p < .001$), Time ($F(2,26) = 225.9$, $p < .001$) and Time x Intervention ($F(2,26) = 34.0$, $p < .001$) were found for the tryptophan/LNAA ratio. Both interventions resulted in a significant decrease in plasma tryptophan and the tryptophan/LNAA ratio and the decrease was larger after high-dose than after low-dose ATD.

*Symptoms*

Depressive symptoms increased significantly in the high-dose condition from 4.1 ± 3.5 to 10.0 ± 6.8, but not in the low-dose condition (from 4.4 ± 4.1 to 3.1 ± 4.1). All scores were back to baseline the next morning (Intervention $F(1,13) = 13.9$, $p = .003$; Time $F(2,26) = 4.0$, $p = .030$; Intervention x Time $F(2,26) = 6.7$, $p = .004$). High-dose ATD induced a depressive relapse (defined as an increase in MADRS score of 6 points or more) in seven of fourteen patients. These patients will be referred to as mood-responders. Anxiety symptoms also increased following high-dose ATD but not following low-dose ATD (Time x Intervention: $F(2,26) = 8.1$, $p = .002$). Side-effects of the tryptophan depletion drinks are reported elsewhere (Merens et al., in press).

*The effect of ATD on cardiac activity*

Analyses with Intervention (low-dose vs. high-dose) and Time (morning (t-1) vs. beginning of the afternoon (t4,5) vs. end of the afternoon (t6,5) vs. next morning (t+24h)) on blood pressure revealed no effects of ATD on blood pressure. Only a Time effect was found for the diastolic blood pressure ($F(3,39) = 4.1$, $p = .012$) indicating a decrease in diastolic BP from morning to

the beginning of the afternoon ($F(1,13) = 14.2$, $p = .002$) during both interventions.

Analyses with Intervention and Period (rest vs. Dot –probe) revealed effects of Intervention ($F(2,26) = 4.3$, $p = .025$) and Period ($F(1,13) = 14.2$, $p = .002$) on heart rate. Heart rate increased following high-dose ATD compared to baseline ($F(1,13) = 7.9$, $p = .015$) and low-dose ($F(1,13) = 7.4$, $p = .018$). Heart rate was higher during rest than during the Dot-probe (M ± SEM: 78.2 ± 2.2 vs. 76.0 ± 2.1). No significant effect of ATD on any of the HRV measures was found.

Eleven patients reported to have suffered from suicidal ideation (SI+) in the past, while three patients did not (SI-). Due to these unequal sample sizes, analyses using suicidal ideation as a between subjects factor could not be performed. Hence, analyses on HR and HRV measures were done for the eleven SI+ patients only. Again significant effects of Intervention ($F(2,20) = 6.9$, $p = .005$) and Period ($F(1,10) = 8.6$, $p = .015$) were found on HR. HR increased following high-dose ATD compared to baseline and was higher during rest than during Dot-probe. Also an interaction effect of Intervention x Period ($F(2,20) = 6,9$, $p = .005$) was found. Post-hoc contrast tests showed that low-dose ATD decreased HR slightly during rest, but increased HR during the Dot-probe ($F(1,10) = 7.9$, $p = .018$). HR increased following high-dose compared to baseline during both periods ($F(1,10) = 6.5$, $p = .029$).

For RMSSD, also an interaction effect of Intervention x Period was found ($F(2,20) = 5.4$, $p = .013$). Post hoc tests indicated that following low-dose ATD, RMSSD decreased during the Dot-probe but increased during rest ($F(1,10) = 6.2$, $p = .032$). RMSSD and PNN50 both decreased following high-

dose ATD compared to baseline; however these changes were not significant. See Table 3 for the cardiac measures per intervention for the SI+ group.

Analyses on HR and HRV were rerun to check for possible influences of smoking, order of administration and age. The effect of gender could not be investigated since only one male was included in the sample. Regarding HR, the Intervention effect was no longer significant when age was included as a covariate, ($F$(2,24) = 0.1, $p$ = .907), and the effect of Period became a trend ($F$(1,12) = 4.2, $p$ = .062); however no main effect of age was present ($F$(1,12) = 0.7, $p$ = 431). Order did not have an effect on HR ($F$(1,12) = 0.02, $p$ = .904), neither did smoking ($F$(1,12) = 0.8, $p$ = .388). Also, no effects were found of order or smoking on any of the HRV measures. When age was included as a covariate in the repeated measures of LF, a main effect of age was found ($F$(1,12) = 9.2, $p$ = .010), indicating that LF was lower for older patients. For RMSSD, an effect of Period ($F$(1,12) = 5.8, $p$ = .033) and an interaction effect for Period x Age ($F$(1,12) = 5.9, $p$ = .032) were found when age was included as a covariate; RMSSD was lower during rest than during the Dot-probe. The same effects were found for PNN50 (Period $F$(1,12) = 5.1, $p$ = .044; Period x Age $F$(1,12) = 5.1, $p$ = .043); PNN50 was lower during rest than during the Dot-probe when age was included as a covariate.

*Attentional bias*

Attentional bias scores were calculated for depression- and threat-related stimuli. One patient appeared to be an outlier, due to extremely slow responses. Analyses were therefore performed with and without this patient. Using repeated measures with Intervention as within-subject factor, no effects of low-dose and high dose ATD on attentional bias were found ($p$ > .10).

Order of administration (low-dose first vs. high-dose first) did not have an effect on Dot-probe performance.

*Relationship between HRV and Dot-probe performance*

Patients with low HRV levels during rest at baseline (median split) had higher attentional bias scores for threat-related words at baseline than patients with high HRV. The difference was significant for the ratio LF/HF (-18.1 vs. 7.4 ms; $t(12) = -3.1$, $p = .009$). Statistical trends were found for SD (-14.6 vs. 4.0 ms: $t(12) = -2.0$, $p = .075$), PNN50 (-17.7 vs. 1.6 ms; $t(12) = -1.9$, $p = .078$), and CVr (-14.6 vs. 4.0 ms: $t(12) = -2.0$, $p = .075$).

*Relationship between HRV and clinical variables*

The change in HRV (Δ) from baseline to high-dose ATD was calculated (for RMSSD, PNN50, SD, CVr, LF, HF, and ratio LF/HF separately). Scores were divided into groups of patients that showed either an increase or a decrease in HRV following high-dose ATD. The degrees of freedom for the different tests may vary because patients whose HRV levels remained unchanged ($\Delta = 0$) following ATD were left out of the analyses. Mood-response was higher in patients who showed a decrease in HRV following ATD. This was significant for LF ($t(12) = 2.2$, $p = .050$) and a trend for RMSSD ($t(11) = -2.1$, $p = .061$). Patients with a decrease in HRV following ATD were older compared to patients showing an increased HRV. This was significant for RMSSD ($t(11) = -2.5$, $p = .030$) and PNN50 ($t(9) = -2.3$, $p = .048$).

**Table 3.** Means (SD) of the cardiac measures per intervention for patients with a history of suicidal ideation (SI+) ($n$ = 11)

| | **Baseline** | **Low-dose** | **High-dose** | ***F*** | ***p*** |
|---|---|---|---|---|---|
| HR (beats/min) | | | | 6.9 | .005** |
| - rest | 73.9 (7.5) | 71.9 (6.0) | 80.1 (9.6) | | |
| - dot-probe | 71.5 (7.9) | 72.8(7.4) | 76.8 (8.0) | | |
| SD IBI (ms) | | | | 1.1 | .338 |
| - rest | 32.1 (8.7) | 35.5 (12.9) | 31.4 (7.7) | | |
| - dot-probe | 33.3 (11.5) | 35.1 (11.6) | 31.0 (9.3) | | |
| CVr (%) | | | | 0.4 | .647 |
| - rest | 3.9 (1.1) | 4.2 (1.3) | 4.2 (1.0) | | |
| - dot-probe | 3.9 (1.3) | 4.3 (1.5) | 4.0 (1.3) | | |
| RMSSD (ms) | | | | 1.7 | .209 |
| - rest | 18.9 (7.5) | 20.7 (8.5) | 15.4 (4.5) | | |
| - dot-probe | 20.4( 9.6) | 18.2 (8.6) | 16.6 (4.7) | | |
| PNN50 | | | | 2.1 | .143 |
| - rest | 2.6 (4.2) | 3.6 (5.9) | 0.8 (1.5) | | |
| - dot-probe | 4.0 (5.7) | 2.7 (4.3) | 1.1 (1.8) | | |
| LF ($ms^2$) | | | | 0.2 | .816 |
| - rest | 319.6 (181.3) | 410.2 (388.9) | 409.7 (244.6) | | |
| - dot-probe | 347.9 (181.1) | 335.4 (257.1) | 375.9 (254.2) | | |
| HF ($ms^2$) | | | | 0.2 | .829 |
| - rest | 288.7 (182.4) | 417.9 (374.6) | 285.4 (177.1) | | |
| - dot-probe | 316.1 (220.0) | 307.3 (210.0) | 241.3 (162.6) | | |
| LF/HF ratio | | | | 0.6 | .558 |
| - rest | 1.9 (2.5) | 1.1 (0.9) | 2.0 (2.4) | | |
| - dot-probe | 1.7 (2.1) | 1.3 (0.6) | 1.6 (1.2) | | |

The original data are reported. Analyses on the PNN50, LF, HF, LF/HF ratio were done using log transformed variables. ** $p < .01$
$F$ = main effect of Intervention in the repeated measures analysis, df = (2,20)

*Relationship between baseline HRV and mood response to ATD*

A trend was found for the correlation between baseline PNN50 during rest and the change in MADRS following high-dose ATD; $r = .497$, $p = .070$. No other correlations were found between baseline HRV and mood response to ATD. Patients with relatively low (below median) HRV levels did not have a depressive response to ATD more often compared to patients with high HRV levels.

**Discussion**

As reported elsewhere (Merens et al., in press), the current findings confirm that high-dose ATD affects mood in remitted depressed patients, whereas low-dose does not affect mood (Booij et al., 2005a; Spillman et al., 2001). Half of the patients were mood-responders in response to high-dose ATD. The reported decreases in plasma tryptophan levels and the ratio tryptophan/LNAA following ATD are also in line with expectations. Previous research using low-dose and high-dose ATD found similar changes in tryptophan levels (Booij et al., 2005a).

High-dose ATD increased heart rate both during rest and during the Dot-probe test. This is in line with previous findings that also showed an effect of ATD on HR (Booij et al., 2006b). The finding that HR was higher during rest than during the Dot-probe test is in line with the finding that HR decreases during tests that require sustained attention (Swenne et al., 1995), although HR has been found to increase during the Stroop task (Renaud & Blondin, 1997). In that study however, the Stroop task was suggested to act as an experimental stressor.

However, although the effects of ATD on the HRV measures in the SI+ group were in the same direction as in our previous study, none of these

changes was statistically significant. Unfortunately, a replication of the differential effects in patients with and without a history of suicidal ideation was not possible due to a low number of patients without such a history.

At baseline, low HRV was related to a higher attentional bias for threat-related words. This is in line with previous research that found a higher attentional bias for threat-related words in low HRV patients with dental anxiety compared to high HRV patients (Johnsen et al., 2003). Our results also follow the findings that low HRV is related to poor affective information processing (Thayer & Lane, 2000).

In the total group of remitted depressed patients, a decreased HRV following high-dose ATD was related to a stronger mood-response compared to an increased HRV. This suggests that patients who are sensitive to changes in serotonin in the sense that they show a mood response following ATD, may also respond to ATD with a lowered HRV.

In contrast to expectations, low PNN50 at baseline was not related to an increased mood response to ATD (Booij et al., 2006b) but to a decreased mood response. This correlation however did not reach significance.

*Limitations and suggestions for future research:*

The current findings are limited by the small number of patients. Replication in larger samples is therefore warranted. Also, we did not select for a history of suicidal ideation when recruiting patients for this study.

We assessed HRV during rest and during a Dot-probe test. The choice of the cognitive tests was based on the possible link between serotonin function and attentional bias. Other tests that may be more specifically linked to cardiac function, such as the Stroop test or other tests of executive function, should also be investigated in future studies. A strength of the Dot-probe test

should also be mentioned. The inter-stimulus-intervals of the task were variable between and within individuals due to variable response times (a new stimulus was presented as soon as a response was given). The HF component of HRV is sensitive to task-induced signal repetition, but by using this version of the Dot-probe no task related peak in HF was formed and the HF component of HRV was not influenced (Mulder, 1992).

In addition to the effects of acute tryptophan depletion, it would also be interesting to investigate the effects of other serotonin manipulations on heart rate variability, to clarify the exact mechanisms underlying the link between serotonin and cardiac variability. Previous studies have found effects of SSRI treatment on cardiac activity in depressed patients (Agelink et al., 2001; Bär et al., 2004; Glassman et al., 1998) however not much is known about the acute effects of SSRI administration on HR and HRV in depression vulnerable subjects. Kemp et al. investigated the effects of acute SSRI administration in healthy subjects (Kemp et al., 2004). Compared to placebo, citalopram suppressed differences in heart rate associated with the viewing of pleasant and unpleasant images. Also, the electrophysiological activation to unpleasant images was attenuated following citalopram, while the activation to pleasant images was potentiated.

In summary, the present study could not establish a link between serotonin function, HRV and impulsivity. However our findings do indicate that low HRV may be related to mood and poor affective processing through changes in serotonin function.

# 8

# The effects of serotonin manipulations on emotional information processing and mood

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**Abstract**

Serotonin is implicated in both mood and cognition. It has recently been shown that antidepressant treatment has immediate effects on emotional information processing, which is much faster than any clinically significant effects. This review aims to investigate whether the effects on emotional information processing are reliable, and whether these effects are related to eventual clinical outcome. Treatment-efficiency may be greatly improved if early changes in emotional information processing are found to predict clinical outcome following antidepressant treatment. This is a review of studies investigating the short-term effects of serotonin manipulations (including medication) on the processing of emotional information, using PubMed and PsycInfo databases. Twenty-five studies were identified. Serotonin manipulations were found to affect attentional bias, facial emotion recognition, emotional memory, dysfunctional attitudes and decision making. The sequential link between changes in emotional processing and mood remains to be further investigated. The number of studies on serotonin manipulations and emotional information processing in currently depressed subjects is small. No studies yet have directly tested the link between emotional information processing and clinical outcome during the course of antidepressant treatment. Serotonin function is related to several aspects of emotional information processing, but it is unknown whether these changes predict or have any relationship with clinical outcome. Suggestions for future research are provided.

**Introduction**

Major depressive disorder is one of the most disabling diseases in the world (Üstün et al., 2004). Of the people who have been treated for a depressive episode, 50% will get depressed again. This percentage increases to a 90% chance of a future depression, having experienced three depressive episodes in the past (Judd, 1997). The most effective treatments for depression are antidepressant medication, structured forms of psychotherapy (eg. cognitive behavioural therapy), or a combination. Selective serotonin reuptake inhibitors (SSRIs) form the most widely used pharmacological treatment for depression (Petersen et al., 2002). The monoamine theory of depression states that monoamine levels, such as serotonin, are low in the brain during untreated depressive episodes, but no explanation had been found for how monoamine loss occurs (Maes & Meltzer, 1995). Recently, Meyer et al. (2006) found evidence that elevated monoamine oxidase (MAO-A) density may be the primary monoamine-lowering process during depression.

Serotonergic antidepressants increase brain serotonin function by inhibiting the re-uptake of the neurotransmitter serotonin (5- hydroxy-triptamine; 5-HT) (Blier & de Montigny, 1994). Research has indicated that 80% occupancy of the serotonin transporter (5-HTT) may be necessary for the therapeutic effect of SSRIs to occur (Meyer et al., 2004). One puzzling factor in the treatment with SSRIs is the delay in onset of action. A recent meta-analysis (Taylor et al., 2006) suggests that treatment with SSRIs may lead to clinical improvement as early as the end of the first week. Although this is faster than is commonly assumed, there is still a considerable delay between the biochemical effects of SSRI administration and symptomatic improvement. One possible explanation for this delay in onset is a process called 'receptor desensitization'. All SSRIs enhance the activity of the 5-HT1a autoreceptor via

the blockade of the 5-HT transporter in the raphe nuclei (Blier & de Montigny, 1998). After two to three weeks, 5-HT transmission is increased in the brain because of a normalized firing rate in the presence of sustained 5-HT reuptake blockade and because the terminal 5-HT autoreceptor is desensitised. The autoreceptors normally have an inhibitory effect on the amount of serotonin that is released per impulse. After long-term administration of an SSRI, this inhibitory effect is lifted (Blier & de Montigny, 1998).

Evidence for this receptor desensitisation-hypothesis comes from studies investigating the effects of the 5-HT1a/β-adrenoreceptor antagonist pindolol in combination with SSRI treatment. Pindolol blocks the 5-HT1a autoreceptor on the cell body of 5-HT neurons to prevent the initial decrease in firing activity of these neurons at the start of SSRI treatment. This process mimics the desensitisation of the 5-HT1a autoreceptor, which occurs after about 2 weeks of SSRI treatment. Pindolol has been found to accelerate the therapeutic effect of SSRIs (Ballesteros & Callado, 2004; Perez et al., 1997) although results have been conflicting (Berman et al., 1997; Moreno et al., 1997).

*Cognitive effects of antidepressant treatment*

A recently proposed alternative explanation for the delay in therapeutic response following treatment with antidepressants is that cognitive changes, which may occur within hours after administration, mediate the antidepressant response (Amado-Boccara et al., 1995; Harmer et al., 2003b). Very brief (one day or one week) treatment with a serotonergic antidepressant caused selective changes in emotional information processing, in particular changes in the recognition of facial expressions of emotions (Bhagwagar et al., 2004; Harmer et al., 2004). It may take a number of days or weeks for these changes to build

up to a clinical (mood) effect (Harmer et al., 2003a). This hypothesis receives indirect support from research showing that experimental lowering of serotonin may also lead to cognitive changes in the absence of changes in mood (Hayward et al., 2005; Murphy et al., 2002; Park et al., 1994).

In the psychological treatment of depression the emphasis lies on reducing negative cognitions and information processing biases. The research on the immediate cognitive effects of antidepressant treatment suggests that similar effects may occur in pharmacotherapy (Harmer et al., 2003b). This idea remains speculative but is the basis for recent studies on the acute cognitive effects of antidepressants and hypotheses about their relation to a clinical response (Bhagwagar et al., 2004; Harmer et al., 2002; Harmer et al., 2003a; Harmer et al., 2003b). To test this idea, cognitive functions should be measured in patients before starting on an SSRI and for the first few weeks of treatment, to investigate whether any changes in emotional information processing are related to subsequent mood response. If clinical outcome can be predicted on the basis of short-term changes in emotional processing, earlier switching to another antidepressant may become feasible. However, such a study seems premature considering how little is known of the relation between cognitive and mood effects following SSRI treatment.

SSRIs may regulate emotional information processing by activating plasticity processes via brain-derived neuroptrophic factor (BDNF) in neural networks associated with mood regulation. Serum BDNF is suggested to influence plasticity. Structural alterations in neuronal plasticity occur in patients with mood disorders (Sheline et al., 1999). Supporting this notion are findings that indicate baseline levels of serum BDNF in depressed patients were significantly lower than those of controls (Gonul et al., 2005). However, after eight weeks of SSRI treatment serum BDNF levels had increased significantly

and differed no longer from those of controls. The exact timeline of the changes in BDNF is of yet unknown but seems to resemble that of clinical response and may thus be slower than the process of receptor desensitisation. Also a post-mortem study found increased BDNF expression in hippocampal regions at the time of death in patients treated with antidepressants compared to untreated patients (Chen et al., 2001). It was therefore concluded that antidepressants may normalize hippocampal levels of this important neuroprotective factor. Animal studies showed that chronic stress and chronic antidepressant treatment are associated with long-lasting changes in BDNF gene expression in the hippocampus (Tsankova et al., 2006). Since the hippocampus contributes to altered mood in depression and to cognitive function (Duman, 2004), a possible link between SSRIs, emotional processing and plasticity is suggested.

The aim of the present paper is to review the available evidence concerning the hypothesis that cognitive changes mediate the effects of antidepressant treatment. This hypothesis is based on three assumptions. The first assumption is that serotonin manipulations (in particular the administration of antidepressants) have reliable effects on emotional information processing. The second is that these effects are also observed in currently depressed patients starting antidepressant treatment. The third assumption is that the cognitive effects of antidepressants predict clinical outcome. No studies have yet addressed the third assumption. We will therefore focus our review on the first two assumptions, and summarize the studies that measured short-term effects of serotonin manipulations on emotional information processing and mood and the effects of pharmacotherapy on emotional information processing and mood in depression. We will first briefly summarize the cognitive dysfunctions that are

associated with depression, which is necessary to assess whether any cognitive changes induced by serotonin manipulations are clinically relevant. In addition, different aspects of emotional information processing related to depression will be discussed.

*Neutral and emotional information processing in depression*

Cognitive dysfunctions (eg. dysfunctions in the processing of neutral as opposed to emotional information) associated with depressive disorders have been subject of many studies (Deuschle et al., 2004; Elliott, 1998; Paradiso et al., 1997). Clear conclusions on the exact cognitive dysfunctions are difficult to draw because of the large differences among studies in subject samples, methods and design. Also, most tests are linked to a number of different cognitive domains, making it difficult to clarify the primary deficit when performance is impaired (Austin et al., 2001). Cognitive dysfunctions that are often impaired in depression include memory, learning, attentional set-shifting, psychomotor speed, sustained attention, and complex problem solving (Austin et al., 2001; Weiland-Fiedler et al., 2004). Effortful executive tasks, which are related to the functioning of the prefrontal cortex, are most often impaired (Elliott, 1998). Cognitive impairments are also found in recovered depressed patients; however these effects are usually statistically non-significant after controlling for residual depressive symptoms, except for attentional deficits (Paelecke-Habermann et al., 2005; Weiland-Fiedler et al., 2004).

Apart from dysfunctions in the processing of neutral information, dysfunctions in emotional information processing have also been studied extensively. The earliest studies investigated a mood-congruent memory bias: depressed patients have a better memory for disorder-related information

(Blaney, 1986; Bower, 1981; Teasdale, 1983), and impaired memory for positive information (Matt et al., 1992).

Depressed patients also have difficulties in retrieving *specific* autobiographical memories (Brittlebank et al., 1993; Kuyken & Dalgleish, 1995; Williams & Scott, 1988). This deficit may even persist into the euthymic phase (Peeters et al., 2002) and predicts depressed mood after a stressful life event (Van Minnen et al., 2005).

Compared to healthy volunteers, depressed patients are impaired in the recognition of facial expressions of emotions. Both a general deficit in the recognition of emotions as well as emotion-specific impairments have been found (Bouhuys et al., 1999; Gur et al., 1992; Persad & Polivy, 1993; Rubinow & Post, 1992). A recent study showed subtle impairments in discrimination accuracy as well as a bias away from the identification of happy faces (Surguladze et al., 2004).

Murphy et al. (2001) investigated decision making in manic and depressed patients and healthy controls using a gambling task. Both patient groups showed delayed deliberation times and altered betting strategies, however only the manic patients were impaired in the quality of their decisions. In the depressed and the control group, the subjects who were slowest were also the ones that made suboptimal decisions.

Depressed patients have been found to show an attentional bias for negative stimuli. This bias is thought to be not only a symptom of depression, but also to be important in the development and maintenance of depressive disorders (Williams et al., 1996). The literature on attentional bias in depression is contradictory, which may be explained by the fact that different measures of attentional bias (e.g. the Stroop and the dot-probe test) are not necessarily inter-correlated and may even assess different constructs (Gotlib et al., 2004).

Higher-order cognitive processes in depression are also distorted: depressed patients hold negative schemas about oneself, the world and the future (Beck, 1976). These dysfunctional cognitions can be assessed with the Dysfunctional Attitudes Scale (DAS) (Beck, 1976; Weissman, 1979). In acutely depressed patients, DAS scores are significantly increased compared to healthy controls; however they normalize following clinical recovery. When negative mood increases, the level of dysfunctional attitudes has been found to increase more in recovered depressed patients than in healthy controls (Ingram et al., 1998). This increase in DAS scores in response to relatively small deteriorations of mood has been labelled cognitive reactivity (CR). Higher CR scores have been associated with an increased risk of relapse in recovered depressed subjects (Segal et al., 2006).

Overall, depressed patients show impairments in several cognitive domains, such as memory, learning, attentional set-shifting, psychomotor speed, sustained attention, planning, inhibitory control and problem solving. Impairments in emotional information processing include the recognition of facial expressions of emotions, attentional bias toward negative material, an over-general autobiographical memory, an increased level of dysfunctional attitudes and impaired decision making. Only some of these impairments are detectable in remitted depressed patients.

As noted above, the framework of this review is the hypothesis that cognitive changes mediate the antidepressant response. Because of a lack of studies directly testing this hypothesis, we will review studies 1) on the short-term effects of serotonin manipulations on emotional information processing and mood in healthy and depression-vulnerable individuals and depressed

patients, and 2) on the changes in emotional information processing and mood in depressed patients starting antidepressant treatment.

**Methods**

A literature search was performed with PubMed and PsycInfo databases (1966-2006) using the following key words: serotonin, depression, emotional information processing, cognition, SSRI, and tryptophan. We also searched reference lists of papers that seemed suitable to review. Twenty-five studies were identified through this combined search strategy to be eligible for inclusion in this review. The first study was published in 1994 and the most recent studies were published in 2006. Studies were divided into four classes on the basis of the manipulation that was used:

- four studies on short-term effects of administration of a serotonergic antidepressant (SSRI)
- two studies using a serotonin-receptor agonist or antagonist
- three studies using dietary manipulations (e.g. tryptophan loading)
- sixteen studies using acute tryptophan depletion (ATD)

**Results**

All studies including their subject sample, methods, outcome measures and results are shown in Table 1.

**Table 1.** Effects of serotonin manipulations on emotional information processing and mood

| Authors | Sample | Intervention | Outcome measures | Results |
|---|---|---|---|---|
| *SSRI studies* | | | | |
| Harmer et al. (2003a) | *n* = 25 healthy women | Citalopram (10 mg) vs. saline (5 ml); single, intravenous administration | - Facial expression recognition task<br>- VAS scales, BFS | A better and faster recognition of fear and happiness was found after citalopram. No effects on mood were found. |
| Bhagwagar et al. (2004) | *n* = 20 unmedicated euthymic women with a history of MDD<br>n = 20 healthy women | Citalopram (10 mg) vs. saline; single, intravenous administration | - Facial expression recognition task<br>- VAS scales | Citalopram decreased recognition of fear in recovered depressed subjects and increased fear recognition in healthy subjects; citalopram increased subjective anxiety more in recovered depressed women. |
| Harmer et al. (2004) | *n* = 42 healthy volunteers | Citalopram (20 mg/day) vs. reboxetine (8 mg/day) vs. placebo; 7 days, oral administration | - Facial expression recognition task<br>- Emotional categorization task<br>- Emotional memory<br>- Emotion potentiated startle<br>- STAI, BDHI, BDI, PANAS, SASES, BFS | Citalopram and reboxetine reduced recognition of anger and fear and increased recall of positive material. Citalopram abolished the emotional startle response to negative stimuli. Reboxetine decreased subjective feelings of hostility and increased energy. |

**Table 1.** (*continued)*

| Authors | Sample | Intervention | Outcome measures | Results |
|---|---|---|---|---|
| Harmer et al. (2006a) | *n* = 24 healthy volunteers | citalopram (20 mg/day) vs. placebo; 7 days, oral administration | - Masked facial expression recognition task<br>- Facial expression recognition task<br>- STAI, BDHI, BDI, BFS, VAS scales | Citalopram attenuated the neural response to fear, decreased the recognition of fearful facial expressions and reduced subjective hostility. |
| *5-HT receptor agonist / antagonist studies* | | | | |
| Meyer et al. (2003) | 1) *n* = 29 healthy volunteers | d-fenfluramine (0.3 mg/kg) vs. clonidine (1.4 μg/kg) ; single intravenous administration | - Dysfunctional attitudes scale<br>- VAS scales | DAS scores decreased after administration of d-fenfluramine compared to placebo. |
| | 2) *n* = 22 unmedicated depressed subjects; *n* = 18 subjects with a history of self-harm; *n* = 29 healthy volunteers | | - Dysfunctional attitudes scale<br>- cortex 5-HT2 receptor binding potential using [18F]setoperone PET<br>- HAM-D | Depressed subjects with high DAS scores had higher 5-$HT_2$ BP compared to healthy subjects. |
| Harmer et al. (2006b) | *n* = 24 healthy volunteers | Ondansetron (12 mg) vs. lactose; single, oral administration | - Facial expression recognition task<br>- Emotional categorization task<br>- Emotional memory<br>- Emotion potentiated startle<br>- STAI, VAS scales | Ondansetron diminished the emotion potentiated startle. No effects were found on mood, facial expression recognition or emotional memory. |

**Table 1.** *(continued)*

| Authors | Sample | Intervention | Outcome measures | Authors |
|---|---|---|---|---|
| *Tryptophan augmentation studies* | | | | |
| Luciana et al. (2001) | *n* = 19 healthy volunteers | Tryptophan loading (single administration) vs. ATD | - Digit span/ spatial span[a]<br>- Letter cancellation task<br>- Spatial working memory<br>- Affective working memory<br>- Verbal fluency<br>- Finger tapping test<br>- Grooved pegboard test<br>- PANAS | Both tryptophan loading and ATD decreased positive affect. ATD increased motor performance. Tryptophan decreased motor coordination and verbal and affective working memory (only negative content) and increased immediate attention. |
| Murphy et al. (2006) | *n* = 38 healthy volunteers | Tryptophan (1 g 3x/day) vs. placebo; 14 days, oral administration | - Facial expression recognition task<br>- Emotion potentiated startle<br>- Attentional probe task<br>- Emotional categorization<br>- Emotional memory<br>- Dysfunctional attitudes scale<br>- PANAS, STAI, BDHI, BDI, BFS | Tryptophan increased the recognition of happiness, decreased the recognition of disgust in females, reduced attentional vigilance to negative words and decreased baseline emotional startle response. No effects on mood or dysfunctional attitudes were found. |

**Table 1.** *(continued)*

| Authors | Sample | Intervention | Outcome measures |
|---|---|---|---|
| *ATD studies* | | | |
| Park et al. (1994) | *n* = 12 healthy men | ATD vs. placebo | - Spatial working memory test<br>- Tower of London<br>- Visual discrimination test (attentional set shifting)<br>- Paired associated learning<br>- Pattern and spatial recognition test<br>- Rapid visual information processing<br>- Autobiographical memory test<br>- VAS scales | ATD selectively impaired learning and retrieval. No effects on mood were found. |
| Coull et al. (1995) | *n* = 12 healthy volunteers | ATD vs. placebo[b] | - Emotional selective attention task | ATD reduced reaction times to incompatible stimuli, but not to compatible stimuli. (independent of emotional valence). No effects on focused attention were found. |
| Rogers et al. (1999a) | *n* = 31 healthy volunteers[c] | ATD vs. placebo | - Decision making task | ATD impaired decision making: the tendency to choose the least probable outcome and deliberation times were increased. |

**Table 1** *(continued)*

| Authors | Sample | Intervention | Outcome measures | Results |
|---|---|---|---|---|
| Rubinsztein et al. (2001) | *n* = 30 healthy volunteers | ATD vs. placebo | - Affective go/no-go task<br>- Visual pattern recognition task<br>- POMS, VAS scales | ATD impaired 'maintenance of set' and visual delayed recognition. No effect on mood was found. |
| Klaassen et al. (2002) | *n* = 16 healthy volunteers (FH+)[1]<br>*n* = 11 healthy volunteers (FH-) | ATD vs. placebo | - Visual verbal learning test (positive and neutral words)<br>- POMS | ATD lowered mood in FH+ subjects and impaired delayed recall of neutral and positive words but not negative words in all subjects. |
| Murphy et al. (2002) | *n* = 12 healthy women | ATD vs. placebo | - Probability reversal task<br>- Affective go/no-go task<br>- Tower of London<br>- HAM-D, VAS scales | ATD slowed the processing of happy material and slowed responding in a visual discrimination and reversal learning task. No effects on planning or mood were found. |
| Anderson et al. (2003) | *n* = 28 healthy volunteers | ATD vs. placebo | - Gambling task | No effect of ATD on probabilistic choice was found. |
| Harmer et al. (2003c) | *n* = 38 healthy volunteers | ATD vs. placebo | - Facial expression recognition task<br>- PANAS | ATD impaired recognition of fear only in women. No effects on reaction times or mood were found. |

**Table 1.** *(continued)*

| Authors | Sample | Intervention | Outcome measures | Results |
|---|---|---|---|---|
| Rogers et al. (2003) | *n* = 18 healthy volunteers | ATD vs. placebo | - Decision making task | ATD impaired decision making. |
| Booij et al (2005c) | *n* = 20 medicated remitted depressed patients | High-dose vs. low-dose ATD | - Self-referent adjectives encoding and recall task<br>- MADRS, BAS, CPRS, BDI-II, PANAS | High-dose ATD decreased the consistency of positive trait ratings and decreased immediate recall of positive words in mood-responders. |
| Booij et al. (2005a) | *n* = 20 medicated remitted depressed patients | High-dose vs. low-dose ATD | - Tower of London<br>- Stroop task (neutral and emotional)<br>- Abstract patterns recognition task<br>- Letter fluency<br>- Left/right choice reaction time<br>- MADRS | High-dose ATD impaired the processing of positive information, independent of mood change. ATD improved attention for neutral information in a dose-dependent manner. High-dose ATD increased depressive symptoms in a subgroup of patients. |
| Evers et al. (2005) | *n* = 15 healthy volunteers | ATD vs. placebo | - Probabilistic reversal learning task<br>- Visual verbal learning test (positive and neutral words)<br>- Abstract pattern learning task<br>- VAS version of POMS | ATD increased reaction times for delayed recognition on verbal learning. No effects on mood were found. |

**Table 1.** *(continued)*

| Authors | Sample | Intervention | Outcome measures | Results |
|---|---|---|---|---|
| Hayward et al. (2005) | *n* = 24 unmedicated recovered depressed patients<br>*n* = 24 healthy volunteers | Low-dose ATD vs. placebo | - Stroop task (neutral and emotional)<br>- Emotional memory task<br>- Auditory verbal learning task<br>- Emotion potentiated startle<br>- Facial expression recognition task<br>- BDI, POMS, VAS scales, HAM-D | Low-dose ATD did not affect mood. ATD produced an elevation of the startle response, impaired recognition of happiness, impaired initial recall memory and increased emotional interference in the recovered depressed group. In healthy subjects, ATD enhanced recognition of happiness and increased emotional interference. |
| Talbot et al. (2006) | *n* = 32 healthy volunteers | ATD vs. placebo | - Decision making task<br>- ID/ED attentional set-shifting task<br>- VAS scale | ATD improved decision-making. No effects on risk taking, speed of decision making, set-shifting, reversal learning or mood were found. |
| Evers et al. (2006) | *n* = 15 healthy women | ATD vs. placebo | - Stroop task (neutral and emotional)<br>- POMS | ATD increased interference for negative words and decreased interference on the neutral Stroop. No effect on mood was found. |

**Table 1.** *(continued)*

| Authors | Sample | Intervention | Outcome measures | Results |
|---|---|---|---|---|
| Munafò et al. (2006) | *n* = 24 medicated recovered depressed patients<br>*n* = 24 unmedicated recovered depressed patients<br>*n* = 24 healthy volunteers | Low-dose ATD vs. placebo | - Stroop task (emotional)<br>- BDI, POMS, VAS scales, HAM-D | ATD increased the processing of social threat cues and self-rated depression only in medicated recovered depressed patients. |

BAS = Brief Anxiety Scale, BDI = Beck Depression Inventory, BDHI = Buss-Durkee Hostility Inventory, BFS = Befindlichkeits Scale, CPRS= Comprehensive Psychopathological Rating Scale, HAM-D = Hamilton Rating Scale for Depression, MADRS = Montgomery-Asberg Rating Scale for Depression, MDD = Major Depressive Disorder, PANAS = Positive and Negative Affect Scale, POMS = Profile of Mood States, SASES = Social Adaption Self Evaluation Scale, STAI = State-Trait Anxiety Inventory, VAS = Visual analogue scale, [1] FH = Family history of major affective disorder,
[a] Neuroendocrine measures were also taken but are not reported here, [b] The effects of clonidine, diazepam and haloperidol were also studied but are not reported here, [c] Amphetamine users, opiate users, frontal subjects and ORB-PFC patients were also included but these results are not reported here.

*Selective serotonin reuptake inhibitor studies*

It has long been known that antidepressant medications have effects on cognitive function. For instance, a review by Thompson (1991) shows a wide range of effects of antidepressants on memory. The effects varied from improvement to no effect to impairment, and there was limited evidence for specific effects of different antidepressants. In patients with memory impairments, SSRIs possibly had a positive effect on memory (Thompson, 1991). Amado-Boccara et al. (1995) also reviewed the effects of antidepressants on –neutral- cognitive performance. They concluded that serotonergic antidepressants either had no effect on reaction time tasks and psychomotor functioning or caused small improvements. The authors noted that the acute effects differed from the effects on the middle and long- term. For most antidepressants a tolerance effect occurs after a week or two. In depressed individuals who often suffer from impairments in memory and concentration, a normalization of cognitive function accompanies mood improvement following long-term administration of an SSRI.

More recent research has focused on the acute effects of SSRIs on the processing of emotional, in contrast to neutral, information (Bhagwagar et al., 2004; Harmer et al., 2003a). This seems to yield more consistent findings. Harmer et al. (2003a) investigated the effects of intravenous administration (over 30 minutes) of 10 mg citalopram or saline (placebo) on mood and information processing in healthy women. Participants completed the assessments 30 minutes after the end of the infusion. Compared to placebo, participants were better and faster at recognizing facial expressions of happiness following citalopram. Contrary to expectation, the recognition of fearful facial expressions also improved. No immediate effects of citalopram on mood were observed. Bhagwagar et al. (2004) compared the effects of

citalopram on facial expression recognition in women with and without a history of depressive episodes. A single dose of citalopram or placebo was administered intravenously and effects were measured 30 minutes after the infusion. The women with a history of depression showed a better recognition of fear at baseline compared to healthy women, which was normalized following administration of citalopram. This effect was opposite to the effect seen in the healthy women, in whom the recognition of fear increased following citalopram. Subjective anxiety levels were increased after citalopram in both groups, although this effect tended to be stronger in the participants with a history of depression. These results suggest that a negative cognitive bias may persist into the euthymic phase and that this cognitive vulnerability is sensitive to antidepressant administration. The authors suggested that the effect of the antidepressant on the processing of fear (increasing vs. decreasing) may be dependent on baseline levels of fear processing. This however was not tested and no other studies have reported baseline levels of facial emotion recognition to be of influence on the response to serotonergic modulation.

Harmer et al. (2003a) suggest that changes in the processing of emotional information may occur independently of changes in mood. Also, there appear to be significant differences between the acute effects of SSRI administration and the effects of repeated ('sub-chronic') SSRI administration. A seven-day administration of citalopram (20 mg/day) or the selective norepinephrine reuptake inhibitor (SNRI) reboxetine (8 mg/day) in healthy volunteers resulted in a decreased recognition of anger and fear, and an increased memory for positive material (Harmer et al., 2004). Citalopram also abolished the increased startle response to negative stimuli. Mood and anxiety were not significantly affected. Another study also found decreased recognition

of fear after a seven-day administration of citalopram (20 mg/day) compared to placebo, which was accomplished by a reduced neural response to fear measured by functional Magnetic Resonance Imaging (fMRI). Citalopram also reduced subjective hostility (Harmer et al., 2006a). A single dose of an SSRI however resulted in increased levels of fear recognition in healthy subjects (Harmer et al., 2003a). These effects of SSRIs on the processing of fearful facial expressions are mirrored in the initial increase of symptoms of anxiety during the early phase of SSRI treatment before these and other symptoms eventually decrease (Bagdy et al., 2001; Humble & Wistedt, 1992). However, a single dose of an SSRI not only increased fear recognition but also the recognition of happiness (Harmer et al., 2003a). This might indicate that serotonin manipulations have a general effect on perception rather than a specific effect on the recognition of certain –positive or negative- emotions. The fact that serotonin affects a positive and negative emotion in the same direction, may also indicate that there are two separate processes. On the one hand an effect of SSRIs on fear recognition, which reverses from acute to repeated administration. On the other hand there is a positive effect of SSRIs on affective processing (i.e. on the recognition of happy facial expressions, emotional memory and attentional bias) which is seen very early with antidepressant drug administration and is still observed after 1 week of treatment. It remains to be seen which of these effects is related to clinical improvement, if any.

*Conclusion*

Short-term administration of an SSRI increases the processing of fear and happiness in healthy individuals and decreases the processing of fear in recovered depressed women. Sub-chronic administration of an SSRI in healthy

subjects results in opposite effects compared to acute administration, e.g. a decreased recognition of anger and fear. Mood is affected by SSRI administration but only in recovered depressed patients and independent of emotional information processing.

*Serotonin receptor agonist / antagonist studies*

Challenge procedures using 5-HT receptor agonists and antagonists have been widely used. Apart from neuro-endocrine effects, cognitive effects have also been investigated, but rarely. Riedel et al. (2002) found that the 5-$HT_{2c}$ agonist metachlorophenylpiperazine (m-CPP), but not the 5-$HT_{1a}$ agonist ipsapirone selectively increased depression and tension in depressed patients. Ipsapirone however impaired immediate recall in controls but improved immediate recall in depressed patients. m-CPP impaired signal detection efficiency in controls but not in patients in a visual search task and impaired reaction times in both groups on a choice reaction time task. These results support the hypothesis that depression is associated with 5-$HT_{1a}$ receptor desensitisation and 5-$HT_{2c}$ receptor sensitisation.

Meyer et al. (2003) investigated the effects of *d*-fenfluramine vs. clonidine (an $\alpha_2$ receptor agonist, as a placebo) on dysfunctional attitudes (measured with the DAS) and 5-$HT_2$ receptor binding potential (BP) in the cortex. Three different groups were studied: unmedicated depressed patients, patients with a history of self-injurious behaviour outside of a depressive episode (all with an Axis II diagnosis of Borderline Personality Disorder) and healthy controls. The rationale for this study was based on the finding that suicide victims with and without major depressive episodes have elevated levels of 5-$HT_2$ receptor density in the prefrontal cortex (Arango et al., 1990; Hrdina et al., 1993). Since higher levels of dysfunctional attitudes are found in

depressed patients with and without suicidal tendencies, the authors hypothesized that 5-$HT_2$ BP and dysfunctional attitudes may be related. Results showed that dysfunctional attitudes improved significantly more after *d*-fenfluramine compared to clonidine in healthy subjects. No effects on subjective mood were found. In a second experiment, dysfunctional attitudes were found to be strongly related to 5-$HT_2$ BP in all cortex brain regions in depressed patients. This was not true for patients with a history of self-harm and healthy controls. A subgroup of depressed patients with high DAS scores had greater 5-$HT_2$ BP in all investigated brain regions compared to healthy controls. These results indicate a relationship between serotonin and the level of dysfunctional attitudes. Also, depressed patients with high levels of dysfunctional attitudes showed low levels of 5-HT agonism and higher 5-$HT_2$ BP compared to healthy subjects. In the patients with self-injurious behaviour, cortex 5-$HT_2$ BP was unrelated to the levels of dysfunctional attitudes. The authors concluded that a deregulated neuromodulation of serotonin seems to play an important role in the pathophysiology of dysfunctional attitudes. This might indicate that SSRI-treatment affects the same cognitive processes which are also the focus of cognitive therapy. Simons et al. (1984) have found that antidepressant therapy and cognitive therapy resulted in similar changes on various measures, including dysfunctional attitudes. Again, the results of this study suggest that cognitive processes are sensitive to changes in serotonin function. Recently, more evidence was found to support the link between 5-HT receptor BP and dysfunctional attitudes (Bhagwagar et al., 2006). Recovered depressed patients demonstrated elevated cortical 5-$HT_{2A}$ receptor BP compared to healthy volunteers and 5-$HT_{2A}$ BP correlated positively with DAS scores only in recovered depressed patients. However, future research is needed since no other studies have looked at the acute reactivity of

dysfunctional attitudes following manipulations of serotonin function, e.g. soon after initiating SSRI treatment. Also, the link between 5-$HT_2$ BP and dysfunctional attitudes was only significant in a small subgroup of depressed patients (those with a high level of dysfunctional attitudes). No link between cortex 5-$HT_2$ BP and DAS scores in patients with chronic self-harm behaviour was found. The authors suggested that the extreme psychological factors playing a role in borderline personality disorder may obscure the relationship between dysfunctional attitudes and serotonin. Also, other serotonin abnormalities that do not influence 5-$HT_2$ BP may play a role.

One study investigated the effects of a serotonin receptor antagonist on emotional processing (Harmer et al., 2006b). Healthy volunteers were given either 12 mg ondansetron (a $5HT_3$ antagonist) or placebo orally. The effects on facial emotion recognition, emotional categorization and memory and emotion potentiated startle were assessed as well as mood. Results indicated that ondanstron reduced the emotion potentiated startle, especially in response to negative pictures. Contrary to the effects of acute SSRI administration, no effects were found on facial emotion recognition. Also no effects were found of ondansetron on mood or emotional memory. These results suggest a role for $5HT_3$ receptors in some elements of fear processing. However other measures of emotional processing, for example the recognition of fearful facial expressions, may depend on different mechanisms; for example more interpretative strategies than the emotion potentiated startle, which is a more automatic process (Harmer et al., 2006b).

*Conclusion*

Increasing serotonin function by administration of *d*-fenfluramine, results in decreased levels of dysfunctional attitudes in healthy subjects. Also,

high serotonin receptor binding potential is associated with more dysfunctional attitudes in depressed individuals, but not in healthy subjects and borderline patients with a history of self-harm. These results are not confounded by any effects on mood. A study on the effects of ondansetron suggests a role for $5HT_3$ receptors in other, more automatic aspects of fear processing. $5HT_2$ and $5HT_3$ receptors may thus play separate roles in the effects of serotonergic manipulations on mood and cognition.

*Tryptophan augmentation studies*

Increasing serotonin function by raising plasma levels of its precursor tryptophan can be achieved with different methods. Tryptophan loading studies mostly administer L-tryptophan intravenously or orally. Plasma tryptophan levels can also be elevated through administration of alpha-lactalbumin, a milk whey rich in tryptophan (Heine et al., 1996). Both methods have proven to be effective in raising plasma tryptophan levels and the ratio of tryptophan to the large neutral amino acids (LNAAs). Compared to casein (placebo), a diet rich in alpha-lactalbumin was found to increase the plasma tryptophan/LNAA ratio by 73.8% (Merens et al., 2005). Alpha-lactalbumin improved memory scanning in healthy, stress-vulnerable subjects compared to placebo (Markus et al., 2002) and improved abstract visual memory and slowed simple motor performance, without affecting mood in both recovered depressed patients and healthy controls (Booij et al., 2006a). Attenburrow et al. (2003) investigated the effects of an 80% tryptophan powder derived from milk whey (approximately 1.8 g tryptophan) on facial expression recognition in healthy females. This resulted in enhanced recognition of fear and happiness compared to placebo. These results are similar to the effects of acute SSRI administration on recognition of fear and happiness (Harmer et al., 2003a).

The results are also in line with the effects of a fourteen days intervention with tryptophan in healthy volunteers by Murphy et al. (2006) who found that the recognition of happiness was increased and the recognition of disgust was decreased, but only in females. Tryptophan administration also decreased attentional vigilance toward negative stimuli as well as baseline emotional startle response. Mood was not affected, neither was the level of dysfunctional attitudes (Murphy et al., 2006).

Luciana et al. (2001) compared the effects of tryptophan loading with the effects of tryptophan depletion on cognitive functioning, mood and neuroendocrine measures (prolactin and cortisol) in healthy participants. Tryptophan loading impaired working memory performance of negative stimuli and fine motor coordination and improved immediate vigilant attention. Both tryptophan loading and depletion resulted in a slight decrease of positive affect, leading the authors to conclude that the observed changes in cognitive function were not related to any changes in mood. However, because of a lack of a placebo condition, the results of this study may be inflated.

*Conclusion*

In healthy subjects enhancement of the serotonin precursor may lead to impaired affective working memory but also to enhanced attention and recognition of fear and happiness. Sub-chronic effects include an increased recognition of happiness and a decreased recognition of disgust as well as a decreased processing of negative stimuli. These cognitive changes seem to be unrelated to changes in mood. Tryptophan loading seems to have a broader range of effects on cognitive performance compared to the effects of receptor-agonist and -antagonist administration.

*Acute tryptophan depletion studies*

Experimental depletion of the serotonin precursor tryptophan has been found to decrease plasma tryptophan levels by 70-90% in 5-6 hours (Young et al., 1989). Acute tryptophan depletion (ATD) is therefore a useful tool to investigate the effects of lowered serotonin function in humans. ATD results in a temporary depressive relapse in 50-60% of recovered depressed patients taking serotonergic antidepressants (Delgado et al., 1990; Van der Does, 2001a). In healthy subjects, only small mood effects are found and particularly in subjects with a family history of affective disorders (Ellenbogen et al., 1999; Klaassen et al., 2002). In acutely depressed patients, ATD did not worsen symptoms on the test day, however a delayed effect was observed the next morning in a minority of patients (improvement and worsening) (Delgado et al., 1994). A delayed mood improvement was also found in depressed patients treated with venlafaxine (Booij et al., 2005b). The effects of ATD on mood are congruent with the expected effects of lowered serotonin function. One question we aim to answer in this paper is whether the same is true for the cognitive effects following ATD.

Tryptophan and cognitive performance are associated in a complex manner: differential results are observed across cognitive functions and subject groups. Experimental manipulations of tryptophan seem to affect temporal and frontal cognitive functions (memory consolidation and working memory for example) in opposite ways. ATD affects the processing of neutral information in healthy subjects; it especially impairs memory consolidation and improves focussed attention (Harrison et al., 2004; Riedel et al., 1999; Schmitt et al., 2000). These effects have been repeatedly reported and support a specific role for serotonin in memory and learning and not in executive (frontal-lobe) functions. However, because of the variation in cognitive tests used, drawing

strong conclusions on the effects of ATD on cognitive function remains problematic.

*Effects of ATD in healthy subjects*

A wide range of studies have been done on the cognitive effects of ATD in healthy participants but only a few reported effects of ATD that were dependent of the emotional valence of the stimuli. Murphy et al. (2002) found slowed responses to happy but not sad target words in the affective go/no-go task following ATD in healthy female volunteers. ATD also slowed responding in a visual discrimination and reversal learning task, in absence of a change in mood. These findings were in line with an earlier study that found increased reaction times for happy but not sad targets in depressed patients compared to healthy controls (Murphy et al., 1999). Rubinsztein et al. however did not find a differential effect of ATD on target valence using the same affective go/no-go task (Rubinsztein et al., 2001).

Harmer et al. (2003c) used a facial expression recognition task to test the effects of ATD in healthy subjects. Two competing hypotheses were formulated by the authors. The first stated that since administration of an SSRI resulted in increased fear recognition, ATD should result in *decreased* fear recognition (Harmer et al., 2003a). However, since acute administration of an SSRI can have the paradoxical effect of decreasing synaptic serotonin levels through activation of auto-receptors, ATD may also result in *increased* recognition of fear. Results showed that, ATD decreased the recognition of fear, but only in females. The speed of recognition of fear was slowed in all subjects. No effects on mood were found. These results are in line with the finding that increased serotonin function increases the processing of fear. As a possible explanation for the specific effects in women, the authors noted that

the effects of ATD on mood are also greater in women, possibly resulting from greater reductions in serotonin synthesis following ATD in women compared to men (Ellenbogen et al., 1996; Nishizawa et al., 1997). One methodological remark should be made regarding this study. The effects of ATD on the accuracy of facial emotion recognition may have been even more complex. The interaction effect of emotion x drink in females was not significant ($p$ = 0.1). However, separate analyses per emotion revealed a significant effect of ATD on fear recognition. In males the interaction effect of Emotion x Drink was also not significant ($p$ = 0.13). However, no separate analyses were performed in this group, suggesting that a significant effect of ATD on one of the emotions in males may have been overlooked. Looking at the graphic display of the accuracy data, ATD may have increased the recognition of anger and surprise in males.

Park et al. used a variety of neutral tests and found that ATD selectively impaired learning and retrieval in healthy male volunteers. However, no effect was found on the specificity of autobiographical memory (Park et al., 1994). Coull et al. (1995) found that, using an emotional selective attention task, ATD reduced reaction times to incompatible stimuli but this effect was independent of the emotional valence of the distractor words. Evers et al. found that speed of delayed recognition on a verbal learning task was slowed following ATD, but again no differential effect was found for word valence (Evers et al., 2005). In another study ATD improved performance on a neutral Stroop task but increased the interference for negative words in an emotional Stroop task (Evers et al., 2006). Hayward et al. also found an increased negative attentional bias in healthy subjects in response to ATD, together with an enhanced recognition of happiness (Hayward et al., 2005). These results are in

line with result from studies in depressed patients, showing an emotional processing bias towards negative stimuli (e.g. Murphy et al., 1999).

*Effects of ATD on decision making*

Rogers et al. (1999a) used a gambling task to investigate the effect of ATD on decision making in healthy subjects. Results showed that tryptophan depleted subjects had an increased tendency to choose the least probable outcome and a trend toward increased deliberation times compared to the placebo group. These impairments partly resembled the performance of amphetamine abusers and patients suffering from damage to the orbitofrontal-prefrontal cortex. Results from animal and human studies indicate that the difficulty with decision making in chronic amphetamine abusers may result from altered serotonergic modulation of the ventral PFC and its connected structures (Groenewegen et al., 1997; Hotchkiss & Gibb, 1980).

In a recent study on decision making by Rogers et al. (2003), a novel version of the gambling task was used that allows for an examination of separate mechanisms that play a role in decision making: the magnitude of expected gains and losses and the probability to which these outcomes are delivered. ATD resulted in an impaired capacity to discriminate between magnitudes of expected gains, associated with different choices. ATD was not associated with altered discrimination between the magnitudes of expected losses, or altered discrimination with the relative probability with which these positive or negative outcomes occurred. Risk-averse and risk-seeking bias were also unaffected by ATD. The authors concluded that decreased serotonin function leads to altered modulation of cortical and subcortical regions that mediate important aspects of associative learning. They suggested that decision making may be influenced by monoaminergic systems, possibly involving

changes in the modulation of the prefrontal cortex and the limbic-striatal circuit.

Another study investigated the effect of ATD on decision making, using the same gambling task as Rogers et al. and ID/ED attentional set shifting in healthy subjects (Talbot et al., 2006). Contrary to previous results, ATD *improved* decision making (subjects chose the more likely outcome more often following ATD compared to placebo). There was no effect of ATD on set-shifting, reversal learning, risk taking, impulsivity or mood. The contradiction between these results and the results of the Rogers et al studies (1999a; 2003) was suggested to be due to possible influences of trait characteristics of the individuals tested, such as aggression and genetic factors associated with a family history of alcoholism. Also, genetically determined variations in the effects of ATD on ventral PFC function (e.g. decision making) caused by, for example, serotonergic polymorphisms were suggested to play a role. Finally, there were differences between the studies in the exclusion of hormonal contraception, the permission of smoking and small differences in age and IQ (Talbot et al., 2006).

Anderson et al. (2003) studied the effects of ATD on an imaginary gambling task in healthy subjects. No effects of ATD were found on the performance on this task, but the imaginary nature of the gambling task raises the question of whether this test is comparable to computerized gambling tests.

These studies emphasize the need for further investigations of the role of serotonin in decision making. Other factors possibly confounding the contradictory results should also be investigated: e.g. characteristics of the subject sample such as age and gender; differences in design –between- or within-subjects- and task difficulty.

In healthy volunteers with and without a family history of affective disorders (FH+ and FH-) the effect of ATD on memory bias and mood was investigated (Klaassen et al., 2002). ATD led to a significant lowering of mood (at 6 hours after ingestion of the drink), especially in the FH+ subjects. ATD impaired the recall of neutral words (6 hours after ingestion of the ATD drink) and positive words (at +24 hours), but not of negative words in all subjects. There was no association between mood and affective memory bias. However, ATD did impair delayed recall for neutral words more in mood-responders (all FH+ subjects) than in non-mood responders. The authors suggested that depressed mood did not seem to mediate the cognitive disturbance. Also, since only delayed recall was affected, ATD seems to selectively influence consolidation and not retrieval.

*Effects of ATD in remitted and recovered depressed subjects*

Booij et al. (2005a) investigated the effects of a full strength tryptophan depletion drink compared to a quarter strength mixture (high-dose vs. low-dose) on mood and cognitive function in medicated remitted depressed patients. The low-dose tryptophan depletion mixture consists of the same amino acids as the high-dose mixture, but at quarter strength following the method of Krahn et al. (1996). Most studies included in this review use a tryptophan containing amino acid mixture as a placebo (Murphy et al., 2002; Park et al., 1994; Rubinsztein et al., 2001) which causes a marked but highly variable increase of tryptophan levels. This means that most studies use an active condition rather than a neutral control condition, which is important when investigating subtle effects. A low-dose tryptophan mixture is also not a neutral procedure but results in a predictable moderate reduction of the tryptophan/LNAA ratio. Since the low-dose mixture has been found not to

affect mood (Booij et al., 2005a), it allows for an investigation of the dose-response effects of lowered serotonin function and thus is a better control condition for some research questions.

Following high-dose ATD, a subgroup of patients experienced a temporary depressive 'relapse' (: responders) (Booij et al., 2005a). The processing of positive material was impaired independent of mood change, whereas in another study in healthy subjects ATD impaired attention for negative words (Evers et al., 2006). Attention for neutral stimuli improved in a dose-dependent manner in all patients, which is in line with the effects of ATD in healthy subjects (Evers et al., 2006). The authors suggested that ATD may affect mood and cognition through different pathways; one implicated in mood and the processing of emotional information and one implicated in the processing of neutral information. In another study, high-dose ATD decreased immediate recall of positive words in responders but not in non-responders. Also, high-dose ATD tended to decrease the consistency of positive trait ratings compared to low-dose ATD, but again only in responders (Booij et al., 2005c). Another study investigated the effects of ATD in unmedicated recovered depressed patients and healthy controls (Hayward et al., 2005). The recovered depressed subjects showed an elevated startle response, impaired short-term memory, increased negative attentional bias and impaired recognition of happy facial expressions. Independent of the effects of ATD, the recovered depressed subjects showed enhanced recognition of disgust compared to healthy controls and a lack of a positive bias in the recall of emotionally valenced words. No changes in mood were found following the low-dose tryptophan depletion.

Munafò et al. (2006) studied the effects of ATD on the processing of social threat cues in medicated and unmedicated recovered depressed subjects

and healthy controls. Compared to a control drink, the tryptophan depletion mixture increased the processing of social threat cues on an Emotional Stroop Task in the medicated recovered depressed subjects, as shown by an increased interference. In this group, also a small but significant increase in self-rated depression scores was found. The tryptophan depletion mix did not affect mood and emotional processing in healthy volunteers and unmedicated depressed subjects. The authors explained the effects by suggesting that ATD may remove the neurochemical support of continued medication usage among this group. Also, the two recovered depressed groups may differ in their underlying vulnerability to decreases in serotonin function. Important to note is that the medicated patient group in this study was not selectively treated with SSRIs but with different kinds of serotonergic antidepressants (eg. also tricyclic antidepressants); however patients taking selective noradrenergic medication were not included.

*Conclusion*

ATD affects a broad range of cognitive functions, both in healthy volunteers and in remitted depressed patients. However, not all functions are affected and there are between-group differences in effects. In healthy subjects, ATD affects facial emotion recognition, attention for neutral stimuli and attentional bias, in absence of any mood effects. ATD seems to impair decision making in healthy subjects, however results are conflicting. ATD lowered mood in FH+ subjects and impaired the recall of neutral and positive words, but not of negative words in both FH- and FH+ subjects. In recovered depressed subjects, ATD increased depressive symptoms and affected the processing of positive material; attention for neutral stimuli; startle response, initial memory, attentional bias; the recognition of happy facial expressions and

the processing of social threat cues. No relationship between changes in mood and emotional information processing was found, except for the decreased recall of positive words following ATD in remitted depressed patients.

**Discussion**

The framework of this review is the theory that cognitive changes mediate the clinical effects of antidepressants. Three assumptions underlying this theory were formulated.

The first assumption is that serotonin manipulations (including the administration of antidepressants) have reliable effects on cognition. In this context, the following aspects of emotional functioning have been investigated:

Results from several studies suggest a role for serotonin in the *recognition of facial expressions of emotions*, especially fear. These results have been found across a range of different procedures, however the effects on fear processing appear to be dependent on the length of treatment: In healthy subjects, an acute increase in serotonin function elevated the recognition of fear and happiness (Attenburrow et al., 2003; Bhagwagar et al., 2004; Harmer et al., 2003a), after seven days the recognition of fear and anger was decreased (Harmer et al., 2004; Harmer et al., 2006a), and after fourteen days the recognition of happiness was increased and the recognition of disgust was decreased in females (Murphy et al., 2006). The effects on fear processing also appear to be affected by vulnerability to depression: in recovered depressed individuals, a decreased recognition of fear was found following a single SSRI administration (Bhagwagar et al., 2004), which is contrary to the increased fear recognition found in healthy volunteers. In line with expectations, the effects of ATD seem to be opposite to those of acute SSRI- and tryptophan administration: ATD decreased the recognition of fear in healthy females but

not in men (Harmer et al., 2003c) and impaired the recognition of happiness in both healthy and recovered depressed subjects (Hayward et al., 2005). The reported differences between acute vs. sub-chronic administration and between different samples appear to be important and need further investigation since the underlying mechanisms are still unclear.

Different studies investigated the effects of ATD on *attentional bias* using the Stroop test. Studies differed in the type of test (colour naming or counting) and type of stimuli (neutral and emotional; masked or unmasked) that were used. Despite these differences, the results are rather consistent. Attention for neutral stimuli is improved by ATD in both healthy women and recovered depressed subjects, as shown by a decreased interference for incongruent stimuli (Booij et al., 2005a; Evers et al., 2006). However, ATD has been found to increase attentional bias for negative stimuli in both healthy and recovered depressed subjects (Evers et al., 2006; Hayward et al., 2005; Munafò et al., 2006). Only in medicated recovered depressed subjects, this was accompanied by an increase in subjective depression (Munafò et al., 2006). One study found an increased interference for positive material in recovered depressed subjects following ATD, independent of mood change (Booij et al., 2005a). Following fourteen days of tryptophan administration, a reduced attentional vigilance to negative words was found (Murphy et al., 2006). Overall, lowered serotonin function is found to improve attention for neutral stimuli and to increase attentional bias toward emotional stimuli in both healthy and recovered depressed subjects. Increased serotonin function may have the opposite effect.

Several studies have shown that serotonin manipulations affect *emotional memory* in healthy and recovered depressed individuals. Lowered serotonin function impairs the memory of positive material; increased

serotonin function improves the memory for positive material and impairs the memory for negative material. (Evers et al., 2005; Harmer et al., 2004; Klaassen et al., 2002; Luciana et al., 2001). Only one study found a link between serotonin induced changes in mood and recall for positive material (Booij et al., 2005c). However some studies reported negative results (Harmer et al., 2006b; Hayward et al., 2005).

Serotonin is suggested to play a role in *decision making* in healthy individuals, but results have been contradicting, perhaps due to methodological differences (Anderson et al., 2003; Rogers et al., 1999a; Rogers et al., 2003; Talbot et al., 2006).

Interestingly, higher serotonin receptor binding potential was associated with unfavorable scores on *dysfunctional attitudes* in healthy volunteers, currently depressed (Meyer et al., 2003) and recovered depressed patients (Bhagwagar et al., 2006). Fourteen days of tryptophan administration in healthy subjects had no effect on dysfunctional attitudes (Murphy et al., 2006).

The effect of serotonin manipulations on attentional bias is most robust. The role serotonin plays in the processing of facial expressions of fear and other emotions however needs further investigation since differences exist in the effects of acute vs. sub-chronic administration and between different samples. These differences appear to be important however the underlying mechanisms are still unclear. Therefore, future research, preferably from different research groups, will need to verify the exact mechanisms with which serotonin is linked to the recognition of certain facial expressions and the direction of this association. The findings of a link between serotonin receptor binding potential and dysfunctional attitudes seem very promising for future research on the biological correlates of emotional information processing.

Serotonin is also suggested to affect decision making and emotional memory; however more research is needed to clarify the role serotonin plays in these cognitive processes. In particular, it is unclear whether these effects are limited to certain serotonin manipulations and/ or certain study samples. The effect of serotonin on the specificity of autobiographical memories has only been investigated in healthy individuals and remains uncertain.

The second assumption stated that the cognitive effects of serotonin manipulations are also observed in currently depressed subjects starting antidepressant treatment. Only one study on the effects of SSRIs on emotional information processing in currently depressed patients could be found (Fava et al., 1994). However, this study did not assess acute and short-term cognitive changes but only performed pre- and post treatment assessments. It is therefore not suitable for our review. Evidence from other studies also indicates that dysfunctional attitudes decrease following antidepressant treatment, however these studies did not primarily investigate selective serotonergic antidepressants, but mainly cognitive therapy and tricyclic antidepressants (Beevers et al., 2003; DeRubeis et al., 1990; Peselow et al., 1990; Simons et al., 1984). These results suggest that similar cognitive changes occur following cognitive as well as pharmacological treatment. Of note is that these studies have only looked at dysfunctional attitudes and not at the more automatic aspects of emotional information processing. It may well be that early in treatment, changes in for example attentional bias occur, which may relate to subsequent clinical improvement. Future research should investigate the effects of different antidepressant treatments on various forms of emotional information processing.

Some indirect evidence also exists on the link between serotonin and emotional information processing in depressed patients. An fMRI study in depressed patients found increased left amygdala activation in response to masked emotional faces, especially fearful faces. This was normalized following an eight week treatment with the antidepressant sertraline (Sheline et al., 2001). Since the amygdala plays a central role in the processing of emotions, especially fear, these results suggest that serotonergic antidepressants may exert their effects in part by normalizing dysfunctional emotional processing.

The third assumption stated that the cognitive effects of antidepressants predict clinical response. No studies were found that investigated this assumption. Fava et al. (1994) found that the decreased level of dysfunctional attitudes following eight weeks of fluoxetine treatment was unrelated to symptomatic improvement. However, since only pre- and post treatment assessments were obtained, the temporal and causal relationship between cognitive changes and subsequent symptom change could not be explored, which makes this study unsuitable to investigate the third assumption. Simons et al. did find a link between cognitive changes and symptomatic improvement, and this was true for both cognitive therapy and tricyclic antidepressants (Simons et al., 1984). However, this study is also less suitable since it used the same design as Fava et al. and selective serotonergic antidepressants were not investigated. Various other studies looked at the mechanisms of change of pharmacotherapy compared to cognitive therapy (DeRubeis et al., 1990; Hollon et al., 2005; Rush et al., 1981). These studies have found that changes in cognition do not relate to subsequent changes in symptoms among pharmacotherapy patients, although substantial cognitive changes do occur. The authors explain this by the fact that in

pharmacotherapy, cognitive changes are not accompanied by the use of cognitive and behavioural strategies (e.g. meta-cognitive monitoring) (DeRubeis et al., 1990). However, besides cognitive measures that assess the goals of cognitive therapy (e.g. dysfunctional attitudes) other forms of emotional processing (e.g facial expression recognition, attentional bias) may also be relevant to study during treatment. Since dysfunctional attitudes have been found to be linked to serotonin receptor binding potential (Bhagwagar et al., 2006; Meyer et al., 2003), other forms of emotional processing may as well have serotonin-related correlates in the brain. In order to investigate the causal relationship between cognitive changes and subsequent symptomatic improvement, mood and (different types of) emotional information processing should be assessed regularly during the first two months of antidepressant treatment.

The evidence discussed in this review is limited by the absence of studies in currently depressed patients. The large variety of cognitive tests makes it hard to draw definite conclusions. Most studies did not report power analyses and almost all studies are based on small sample sizes. However, the quality of the reviewed studies in terms of the methodology is satisfactory. Studies are comparable since most studies used a between-group design and matched their groups for age, gender and IQ or level of education. One study only included men (Park et al., 1994) whereas other studies only included women (Attenburrow et al., 2003; Bhagwagar et al., 2004; Evers et al., 2006; Harmer et al., 2003a; Murphy et al., 2002), thereby possibly influencing results since serotonin synthesis differs between females and males (Nishizawa et al., 1997).

Regarding the quality of the decision making studies, two inconsistencies should be noted. First, Anderson et al. (2003) used an imaginary gambling task instead of a computerized test, which may have caused the negative results. Second, despite of using the same gambling task, Talbot et al. (2006) found that ATD *improved* decision making in contrast to the *impaired* decision making found by Rogers et al. (1999a; 1999b). The authors suggested a few explanations for this inconsistency (see Results section under *Effects of ATD on decision making*), however both studies seem to be set out equally well, and further replications are necessary

The ATD studies differed in design (parallel or cross-over) and the exact depletion method used (amount of amino acids, amount of tryptophan added to the control mixture, adherence to a low-tryptophan diet before the session). These differences may have influenced results, for example in the case of control mixtures in ATD studies that increased plasma tryptophan levels, thereby increasing the contrast between ATD and the control procedure. Some studies used low-dose ATD as a control mixture (Booij et al., 2005a; Hayward et al., 2005; Munafò et al., 2006). However, the studies of Hayward et al. and Munafò et al. reported large decreases in plasma tryptophan following their low-dose and may better be referred to as high-dose ATD (Merens & Van der Does, 2007).

Although the hypothesis that cognitive effects of antidepressants predict clinical outcome is as of yet unsupported, there are promising directions for future research. Serotonin is certainly involved in various aspects of emotional information processing. However, a link between serotonin induced changes in mood and changes in emotional information processing has not been established. To fully explore this link, future research should be directed at currently depressed patients. Furthermore, studies should include

immediate as well as sub-chronic and long-term measurements of different aspects of emotional information processing to clarify a possible mediating role of cognitive changes in clinical outcome, as well as the pattern of change. Different cognitive domains are worth investigating, especially attentional bias, facial emotion recognition, decision making, dysfunctional attitudes and emotional memory. Studies should combine various outcome measures to investigate the effects of antidepressant treatment on different forms of emotional information processing. Subjective and objective mood measures should be obtained in order to make comparison between studies in healthy and (recovered) depressed patients possible. Also, apart from serotonergic antidepressants, other antidepressant treatments may be included to investigate possible common effects on emotional information processing and their underlying mechanisms.

# 9

# Discussion

The focus of this thesis was to investigate the effects of serotonin manipulations on mood and on neutral as well as emotional information processing in remitted and recovered depressed patients. In this discussion, first a summary will be given of the main findings. Then, the main findings of this thesis will be integrated and further discussed. Also, methodological considerations are reported as well as directions for future research and the clinical implications of the findings.

**Summary of main findings**

In Chapter 2, two studies were described that investigated neutral as well as emotional information processing in medicated, remitted depressed patients and healthy controls matched on age and gender. A wide range of cognitive functions was assessed, e.g. verbal and non-verbal memory, attentional bias, planning, facial expression recognition and response inhibition. The findings indicated that remitted depressed patients show an increased recognition of facial expression of fear compared to healthy controls. No other residual cognitive impairments were found. The results suggest that generally, cognitive impairments associated with depression tend to resolve with symptomatic improvement. However, specific impairment in certain aspects of emotional information processing may persist into the euthymic phase.

In Chapter 3, the effects of an alpha-lactalbumin-enriched diet on mood and cognitive performance were investigated in unmedicated recovered depressed patients and matched healthy controls. The alpha-lactalbumin diet increased the plasma tryptophan/LNAA ratio with 21% from morning to afternoon; the afternoon ratio was 73.8% higher in the alpha-lactalbumin condition compared to the placebo condition. The alpha-lactalbumin diet had no effect on mood, but improved abstract visual memory and impaired simple

motor performance. These effects were independent of history of depression. Alpha-lactalbumin did not change the encoding phase, working memory, perception or general motor speed. Thus, alpha-lactalbumin may specifically affect memory consolidation in an early phase. The memory effect of alpha-lactalbumin is in line with other studies that show a link between serotonin and memory processes (Riedel et al., 1999; Schmitt et al., 2000; Sirviö et al., 1995). Supplements of alpha-lactalbumin may be useful for nutrition research in relation to age- or disease-related memory decline. However, the present findings should be further examined in different samples and also, the long-term effects of alpha-lactalbumin should be investigated. The results of this study also support the suggestion that cognitive markers may be more sensitive makers for changes in 5- HT function than mood or symptom scales (Booij et al., 2005a).

In Chapter 4, the effects of an alpha-lactalbumin-enriched diet and a casein (placebo) diet on mood and stress response were investigated in the same sample of unmedicated recovered depressed subjects and healthy controls as has been described in Chapter 3. During both diets, subjects underwent a computerized stress task, which affected mood in both conditions. Although the alpha-lactalbumin diet led to the expected rises in plasma tryptophan and tryptophan/LNAA ratio, only minimal effects were found on mood and cortisol response to experimental stress. The results were the same for recovered depressed patients and controls. We concluded that a one-day diet enriched with alpha-lactalbumin is not sufficient to prevent stress-induced mood deterioration or a cortisol response in unmedicated, recovered depressed subjects.

In Chapter 5, the effects of high-dose and low-dose ATD on mood and neutral as well as emotional information processing in remitted depressed

patients were reported. High-dose ATD increased depressive symptoms and induced a temporary depressive 'relapse' in half of the patients. High-dose ATD also decreased the recognition of fear and impaired learning and memory retrieval. The impaired learning occurred only in mood-responders. Low-dose ATD had no effects on mood but speeded the recognition of facial expressions of disgust. Furthermore, accurate recognition of sad faces at baseline was associated with mood response to ATD. We concluded that the effect of low-dose ATD on mood and cognition seems to be quite limited. Also, facial expression recognition at baseline may predict mood-response to ATD.

In Chapter 6, the different doses (high-dose vs. low-dose) of acute tryptophan depletion were discussed. The magnitude of the reduction of plasma tryptophan levels following ATD depends on the amount and composition of the amino-acid mixture and whether a pre-test low-tryptophan diet is included. The regular 'high-dose' ATD consists of fifteen amino acids (100 g) and leads to reductions in plasma levels of 70-90% in five to six hours (Young et al., 1989). In the literature, different low-dose mixtures are studied. Sometimes the term ' low-dose' is based on the amount of amino acids in the mixture, however using a mixture containing eight amino acids (31.2g), may decrease the plasma tryptophan/LNAA ratio by 87%, which is similar to the decrease found following regular ATD (Hayward et al., 2005; Munafò et al., 2006). We used a low-dose mixture consisting of the same amount of amino acids as the regular ATD mixture (fifteen), but at quarter strength (25.7 g). This mixture reduced plasma tryptophan levels by 59%. In this chapter also the inter-individual variations in plasma tryptophan levels following ATD are discussed. Not much is known about the magnitude of inter-individual variations in plasma tryptophan levels; however we found some large

deviations from the mean decrease in the tryptophan/LNAA ratio following both low-dose and high-dose ATD. This could only be partly explained by the fact that several patients threw up. Since the individual variations in the degree of plasma tryptophan depletion can be substantial, the term low-dose is probably best used to describe an ATD method that uses substantially less amino-acids compared to the conventional mixture (Cowen et al., 2007).

In Chapter 7, the effects of ATD on heart rate variability were tested in remitted depressed patients. High-dose ATD increased heart rate both during rest and during the Dot-probe test. This is in line with previous findings that also showed an effect of ATD on heart rate (Booij et al., 2006b). However, although the effects of ATD on HRV in patients with a history of suicidal ideation were in the expected direction, none of these changes was statistically significant. Unfortunately, a replication of the differential effects in patients with and without a history of suicidal ideation (Booij et al., 2006b) was not possible due to a low number of patients without such a history. Our findings indicated that a low HRV may be related to mood and poor affective processing through changes in serotonin. However, the relation between HRV and serotonin function should be further investigated using larger samples.

In Chapter 8, the literature on the acute and short-term effects of serotonin manipulations on mood and emotional information processing was reviewed. The hypothesis on which this chapter was based is that cognitive changes may mediate the symptomatic response to antidepressants. Because of a lack of studies directly testing this hypothesis, we focused on the short-term effects of serotonin manipulations on emotional information processing and mood in healthy and depression-vulnerable individuals and on the changes in emotional information processing and mood in depressed patients starting antidepressant treatment. Twenty-five studies were identified. Manipulations

were divided into four classes: SSRI administration, serotonin receptor agonist or antagonist administration, tryptophan loading and tryptophan depletion. Serotonin manipulations were found to have reliable immediate (time range: one hour - 14 days) effects on attentional bias, facial emotion recognition, emotional memory, dysfunctional attitudes and decision making. This review was limited by the lack of studies directly testing the changes in emotional information processing in depressed patients starting antidepressant treatment. Therefore, the sequential link between serotonin induced changes in emotional processing and mood remains to be further investigated.

**Integration of main findings**

In this section of the Discussion, first a few comments will be made on persisting cognitive impairments in recovered depressed patients. Second, the link between serotonin function and neutral as well as emotional information processing will be discussed. Third, the findings related to cortisol response to stress and heart rate variability will be evaluated. Also a few comments will be made about the effects of ATD on plasma tryptophan levels and somatic symptoms. Finally, the specificity of the ATD response will be discussed.

*Persisting cognitive impairments in euthymic depressed patients*

In addition to investigating the effects of serotonin manipulations on cognitive performance in depression-vulnerable patients, we also compared the cognitive performance of these patients to that of healthy controls in two separate studies. Characteristics of the two patient samples are outlined in Table 1.

The level of residual cognitive impairment in remitted/recovered depressed patients is known to be associated to factors such as age, severity of residual depressive symptoms, number of previous episodes and medication

status (Elliott, 1998; Kessing, 1998; Weiland-Fiedler et al., 2004). However, despite the fact that our patient samples differed on these important factors - the medicated remitted depressed patients were older, more chronic and suffered more residual depressive symptoms compared to the unmedicated recovered depressed patients- no differences in cognitive performance were found between the recovered/remitted patients and healthy controls, except for an increased fear recognition in the medicated remitted patient group. The lack of residual cognitive impairments in euthymic formerly depressed patients, especially with regard to the processing of neutral information, is strengthened by the fact that in the two studies combined, a wide variety of cognitive functions was assessed. Unfortunately no direct comparison of cognitive functioning between the two patient samples was possible, since different tests were administered in the two studies. However, since performance across a wide range of tests assessing neutral as well as emotional information processing was evaluated, we may conclude that cognitive impairments improve substantially with symptomatic recovery, which is in line with some previous studies (Elliott, 1998; Weiland-Fiedler et al., 2004), although results have been mixed (Paradiso et al., 1997). The fact that we did find a difference in the recognition of facial expression of fear between remitted depressed patients and controls is in line with other findings (Bhagwagar et al., 2004) and may point to a persistent impairment in certain types of emotional information processing.

**Table 1.** Demographic and clinical characteristics of the two patient samples

| | **Recovered depressed patients** (*n* = 20) | **Remitted depressed patients** (*n* = 18) |
|---|---|---|
| Age (years) | 30.0 (9.7) | 44.4 (13.3) |
| Gender (M/F) | 2 / 21 | 2 / 16 |
| MADRS | 1.3 (1.6) | 9.0 (5.8) |
| Partial/ full remission | 1 / 22 | 7 / 11 |
| Number of previous episodes | 2.0 (0.9) [range 1-4] | 4.8 (4.2) [range 1-15] |
| Single/recurrent | 7 / 16 | 2 / 16 |
| Medication status | No current antidepressant medication | SSRI (*n* = 12) SSNRI (*n* = 6) |

Values are presented as means (SD). MADRS = Montgomery-Asberg Depression Rating Scale; SSRI = selective serotonin reuptake inhibitor; SSNRI = selective serotonin and noradrenalin reuptake inhibitor

*Neutral and emotional information processing*

In this thesis, we examined the effects of two different serotonin manipulations on various vulnerability factors for depression. The main outcome measure in this thesis was cognitive performance; the processing of neutral and emotional information. Both the alpha-lactalbumin enriched diet and acute tryptophan depletion had effects on cognitive processing. Although the effects of alpha-lactalbumin on plasma tryptophan and the tryptophan/LNAA ratio are smaller than the effects following ATD (the alpha-lactalbumin diet increased the tryptophan/LNAA ratio with 21% compared to a decrease of 59% following low-dose and 91% following high-dose ATD), both manipulations had an effect on memory.

Alpha-lactalbumin improved recognition and speed of retrieval from short- and long-term abstract visual memory in both recovered depressed patients and healthy individuals. The memory effect of alpha-lactalbumin is in line with studies finding improved memory performance following increased

serotonin function (Harmer et al., 2002; Schmitt et al., 2005), although our study indicated that the memory effect of alpha-lactalbumin is not restricted to individuals vulnerable to depression. Our results are also mirrored in the impaired memory consolidation following ATD in healthy volunteers (Riedel et al., 1999; Rubinsztein et al., 2001). In this thesis, ATD impaired verbal memory (learning and retrieval) in remitted depressed patients. The effect of ATD on memory is in line with the results in healthy and depression-vulnerable individuals (Booij et al., 2005c; Hayward et al., 2005; Park et al., 1994; Riedel et al., 1999; Schmitt et al., 2000). However, in contrast to previous studies, memory consolidation was not affected by ATD in our study.

The literature shows that serotonin manipulations have reliable effects on different forms of emotional information processing. In Chapter 8, four classes of serotonin manipulations were reviewed: SSRI administration; administration of a serotonin agonist or antagonist; tryptophan augmentation and acute tryptophan depletion and the effects in healthy and depression-vulnerable individuals were evaluated. Evidence indicates that the recognition of facial expressions of emotions, attentional bias, emotional memory, decision making and the levels of dysfunctional attitudes are affected by changes in serotonin function.

The effect of serotonin manipulations on attentional bias is most robust. The role serotonin plays in the processing of facial expressions of fear and other emotions however needs further investigation since differences exist in the effects of acute vs. sub-chronic administration and between different samples. These differences appear to be important however the underlying mechanisms are still unclear. Therefore, future research, preferably from different research groups, will need to verify the exact mechanisms with which

serotonin is linked to the recognition of certain facial expressions and the direction of this association. The findings of a link between serotonin receptor binding potential and dysfunctional attitudes seem very promising for future research on the biological correlates of emotional information processing. Serotonin is also suggested to affect decision making and emotional memory; however more research is needed to clarify the role serotonin plays in these cognitive processes. In particular, it is unclear whether these effects are limited to certain serotonin manipulations and/ or certain study samples. The effect of serotonin on the specificity of autobiographical memories has only been investigated in healthy individuals and remains uncertain.

Our findings from the ATD study support the link between serotonin function and facial expression recognition. First, ATD affected the recognition of facial expressions of emotions: low-dose ATD speeded the recognition of facial expressions of disgust and high-dose ATD decreased the recognition of facial expressions of fear. Although we did not assess the effects of alpha-lactalbumin on emotional information processing, evidence suggests that in healthy individuals the recognition of facial expressions of fear is increased by a diet enriched with alpha-lactalbumin (Attenburrow et al., 2003). Also, a fourteen day diet enriched with tryptophan (1g 3x/day) resulted in increased recognition of happiness and decreased recognition of disgust in healthy females (Murphy et al., 2006). The fact that we found a decreased recognition of fear following ATD is in line with results in healthy females (Harmer et al., 2003c). Different serotonin manipulations (ATD, SSRI administration) are found to affect facial expression recognition in healthy as well as recovered depressed individuals (Bhagwagar et al., 2004; Harmer et al., 2003b; Harmer et al., 2003c; Harmer et al., 2004; Hayward et al., 2005). The effect on the recognition of *fearful* facial expressions seems most robust. However, the

direction of the effect of serotonin on facial expression recognition seems to depend on the timing of the administration (acute vs. repeated) and on the studied population, suggesting that there may be two separate processes involved. On the one hand an effect of SSRI administration on fear recognition, which reverses from acute to repeated administration. This is mirrored in the effects of SSRI administration on symptoms; anxiety symptoms may first increase at the start of SSRI treatment, followed by an eventual decrease in anxiety and other symptoms. On the other hand there is a positive effect of SSRIs on affective processing (: on the recognition of happy facial expressions, emotional memory and attentional bias) which is seen very early after administration of a single dose and is still observed after one week of treatment.

Secondly, our results indicate that mood-response to ATD may be related to better and faster recognition of facial expressions of sadness at baseline. The direction of the impairment in facial expression recognition (increased vs. decreased) seems to vary: our finding is opposite to results of a study on the effects of ATD in healthy female volunteers with a family history of depression: a stronger mood response to ATD was associated with a *less* accurate recognition of negative facial emotions and a stronger right amygdala response to intense fearful faces compared to happy faces (Van der Veen et al., 2007). However, in line with our findings, this suggests that performance (and brain activation) associated with facial expression recognition partly depend on the effect of ATD on mood.

Thirdly, when compared to healthy controls, remitted depressed patients showed an increased recognition of facial expressions of fear. These findings suggest that the recognition of facial expression may be a persisting vulnerability factor, related to serotonin vulnerability. However what is unclear

is whether this impaired recognition of facial expressions is limited to the recognition of certain emotions or evolves around negative emotions in general.

It is important to note that besides serotonin, other neurotransmitters, e.g. nor-epinephrine, are also related to the recognition of facial expressions of emotions (Harmer et al., 2003b; Harmer et al., 2004)

*Cortisol response to stress*

Contrary to expectations, we did not find a protective effect of an alpha-lactalbumin enriched diet on stress-induced cortisol response in recovered depressed patients. Although this may be largely explained by the small effect of the stressor on cortisol, other possible explanations also need to be noted. Firstly, previous research did find a protective effect of alpha-lactalbumin on stress-induced cortisol, however only in healthy individuals with high neuroticism scores (Markus et al., 2000). High levels of neuroticism are known to predict depressive disorders (Kendler et al., 2006), however our findings suggest that high-neuroticism and a history of depression may be two different concepts in terms of serotonin vulnerability. Secondly, although evidence may point out that remitted depressed patients show blunted neuroendocrine responses to drugs that stimulate serotonin turnover (Bhagwagar et al., 2002a; Bhagwagar et al., 2002b; Flory et al., 1998), the effect of a diet rich in alpha-lactalbumin may be weaker compared to a challenge using citalopram, d-fenfluramine or intravenous tryptophan. Also important to note is that although the prolactin response to citalopram was blunted in recovered depressed patients, the cortisol response was not, suggesting that some aspects of HPA-axis dysfunction (in this case the blunted cortisol response) may be state markers of depression (Bhagwagar et al., 2002b).

*Heart rate variability*

The possible link between serotonin and HRV has recently received more attention. Research has indicated that treatment with serotonergic antidepressants may have a beneficial effect on HRV in panic disorder patients and possibly also in depressed patients, although the results are mixed (Agelink et al., 2001; Glassman et al., 1998; Gorman & Sloan, 2000). Further evidence comes from a recent more experimental study in which ATD was found to affect HRV in medicated remitted depressed patients with a history of suicidal ideation (Booij et al., 2006b). We have tried to replicate these findings. However, due to unequal sample sizes, the analyses on differences between remitted depressed patients with and without a history of suicidal ideation could not be performed. Since little research has been done on the link between experimental changes in serotonin and HRV, no conclusion can be drawn as of now about a possible link between changes in serotonin and changes in HRV. The proposed mechanism of impulsivity as a mediator between serotonin and HRV (Booij et al., 2006b) seems promising, but requires further investigations especially in specific patient samples, e.g. patients suffering from impulse control disorders. It may be that HRV is affected by changes in serotonin levels, but only when measured over a longer period of time; it may also be that serotonin function is linked to cardiac function –heart rate and blood pressure-, but not to HRV per se (Agelink et al., 2001). Overall, more research will need to be done on the possible link between serotonin and HRV, in healthy as well as depression-vulnerable patients.

*The effects of ATD on plasma tryptophan levels and somatic symptoms*

It is not possible to measure central changes in serotonin in humans directly (Anderson et al., 1990a). Therefore blood plasma amino acid levels were obtained in both of our studies, giving a peripheral measure of serotonin function. Because tryptophan competes with the other essential amino acids for entry at the blood-brain-barrier, relative tryptophan depletion is thought to occur in the central nervous system (CNS) following ATD that parallels the changes measured in blood. Animal studies have confirmed that the oral ATD methods are able to lower CNS levels of tryptophan, serotonin and 5-hydroxyindoleatic acid (5-HIAA), the major metabolite of serotonin in the cerebrospinal fluid (CSF) (Carpenter et al., 1998). Using lumbar punctures to sample CSF continuously, Carpenter et al. found that CSF tryptophan levels and plasma tryptophan levels were highly correlated, suggesting that ATD indeed results in substantial declines in central serotonin turnover (Carpenter et al., 1998).

Recently, different studies have investigated the effects of a low-dose tryptophan depletion mixture on plasma tryptophan levels (Booij et al., 2005a; Hayward et al., 2005; Munafò et al., 2006). However, the effects of low-dose ATD on plasma tryptophan levels seem to differ largely. Hayward et al. and Munafò et al. used the same mixture containing eight amino acids (31.2 g), which resulted in an 87% decrease of the ratio tryptophan/ LNAA. Our low-dose mixture contained fifteen amino acids (25.7 g), which decreased the ratio tryptophan/LNAA with 59%. Although both mixtures were named low-dose based on the amount and composition of the amino-acid mixture, results indicate that the effects on plasma tryptophan levels may be in the range of those following regular (or 'high-dose') ATD. The behavioural effects also differed between these studies, which may be partly explained by the

differences in the effects on plasma tryptophan levels. We therefore suggest that the percentage decrease in plasma tryptophan levels should always be considered when reporting results of ATD.

When examining the individual decreases in plasma ratio tryptophan/LNAA following both low-dose and high-dose ATD, we discovered large inter-individual variations (see Table 1 in Chapter 6). Especially low-dose ATD may result in both small (33 - 37%) as well as large (79 – 86%) decreases in plasma tryptophan/LNAA levels, the last resembling the effects of high-dose ATD (mean decrease of 91%). Since individual plasma tryptophan levels are never reported in the literature, not much is known about the variability in tryptophan levels in other ATD studies. It seems however important to investigate individual variations in the effects of ATD on plasma tryptophan levels in future studies, since the behavioural effects of ATD may depend on the effect of ATD on plasma tryptophan levels.

The large variations in post-ATD tryptophan levels in our study may be partly explained by the fact that a relatively large number of patients vomited in response to the ATD mixture. The unpleasant taste of ATD mixtures has been widely reported, as well as side-effects that may occur after ingestion of amino acid mixtures, such as nausea, dizziness and vomiting (Delgado et al., 1990; Lam et al., 1996). Since no lasting or serious side-effects of ATD were ever reported, the procedure is deemed safe. However, symptom provocation studies have been criticized for ethical reasons, especially in the USA (Miller & Rosenstein, 1997). Booij et al. reported the results of an evaluation of the ATD procedure by patients who underwent both low-dose and high-dose ATD (Booij et al., 2005c). The results indicated that individuals did not regret participating and despite the provocation of symptoms and the fact that the study had no direct benefit, some participants still experienced

personal advantages. However the number of patients experiencing side-effects was much higher in our sample compared to Booij et al. (2005c). Only one patient out of twenty-one in the Booij et al. study vomited following high-dose, compared to five out of eighteen in our study. Although the total number of side-effects decreased after both mixtures in the Booij et al. study, scores on 'nausea' and 'feel sick' increased following both conditions. In our study the total number of side-effects increased following high-dose, but not following low-dose ATD. Significantly increased scores were found for 'decreased appetite', 'increased nausea' and 'sweaty hands'. Symptoms were back to baseline the next morning, suggesting that indeed no lasting side effects are inflicted by ATD. Since we used the same batch of amino acids and the same design and procedure as Booij et al., it is not certain what has caused the difference in tolerability.

*Specificity of the ATD response*

There has been debate on whether ATD induces a true depressive relapse or whether the effects of ATD are attributable to an increase in physical symptoms of discomfort with the amino acid drink (Lam & Yatham, 2003). Booij et al. (2005c) concluded that ATD is a specific model of depressive relapse. The authors based this on a specific increase in depressive symptoms (MADRS and HDRS) and a lack of effect on the PANAS Negative scale and the Brief Anxiety Scale (BAS). In contrast to Booij et al., our findings showed an increase in depressive symptoms (MADRS and HDRS) as well as anxiety symptoms (BAS) and physical symptoms in response to high-dose ATD. Therefore, our results seem to disagree with the concept of ATD as a specific model for depressive relapse. However, the fact that we found an effect of ATD on anxiety symptoms is not surprising since serotonergic projections play

a role in both depression and anxiety disorders (Graeff et al., 1996). Also, serotonergic medication such as SSRIs appears to be effective in treating depressive as well as anxiety disorders (Vaswani et al., 2003). ATD studies have also been performed in anxiety disorder patients (Bell et al., 2001). The literature indicates that ATD increases anxiety and panic only when combined with a panicogenic challenge in healthy controls (Klaassen et al., 1998) and in untreated panic disorders patients (Miller et al., 2000). In medicated social anxiety patients ATD seems to reverse the therapeutic effects of SSRIs (Argyropoulos et al., 2004). ATD however does not appear to exacerbate symptoms in OCD (Bell et al., 2001). These studies indicate that ATD may be a useful tool to investigate the role of serotonin function in some of the anxiety disorders.

**Methodological considerations**

*Design*

Both the alpha-lactalbumin and the ATD study were conducted in a randomized double-blind crossover within-subjects design. Therefore we did not have the problem of unknown group differences which is inherent to a between-subjects design. However, the downside of a within-subjects design is the fact that it is vulnerable to the effects of repeated testing. In the ATD study, patients performed the cognitive tests four times in total (at screening, once during the afternoon of each of the two sessions and at a post-intervention session). The two sessions were separated by approximately a week. We calculated a baseline measure as a comparison for the two test sessions, based on the first and last assessment to adjust for the possible learning effects regarding the cognitive measures. In the alpha-lactalbumin study another procedure was used. The cognitive tests were first practiced at

the screening session. Thereafter, both groups performed the cognitive tests twice during each test session: in the morning before the alpha-lactalbumin or casein drink, and in afternoon. The test sessions in this study were separated by four weeks.

Secondly, in contrast to the alpha-lactalbumin study, we did not test the effects of ATD in healthy controls. However, evidence shows that our finding with regard to the effect of ATD on facial expression recognition is in line with results in healthy volunteers (Harmer et al., 2003c). This seems to suggest that this effect does not represent some form of depression vulnerability. However, our finding of a link between sad facial expression recognition and mood-response was not found in healthy volunteers. Therefore, an increased baseline level of sadness recognition may represent increased depression vulnerability.

*Statistical power*

Another concern is the generalizability of the results, because of the small sample sizes in both studies. The ATD study included eighteen patients and the alpha-lactalbumin study included twenty patients and twenty-three healthy controls. These sample sizes are quite common in challenge studies, but replication of the results is warranted. However, since we used different inclusion criteria for the ATD and alpha-lactalbumin study, the patient samples of the two studies differed: unmedicated vs. medicated, recovered depressed patients vs. remitted depressed patients, history of single vs. multiple depressive episodes, etc. Also, the results of two experimental manipulations with opposite effects on serotonin function (increased vs. decreased) were coupled in this thesis. We feel both of these characteristics increased the scope of our results and strengthened the findings reported in this thesis.

The results from the ATD - heart rate variability study were limited by the fact that no equal subgroups of patients with and without a history of suicidal ideation could be formed. Therefore, the intended replication of the Booij et al. study (2006b) could not be performed. Our results do not support their finding that serotonin plays a role in HRV, however the small sample and unequal subgroups cause us to be careful about drawing conclusions from that study.

*Literature review*

One of the strengths of this thesis is the fact that in addition to two experimental studies, a literature review was performed on the effects of serotonin manipulations on emotional information processing and mood. Hereby we have provided a broader context to the current results and the possible clinical implications of the link between serotonin and emotional information processing. Also, the literature review provided us with a means to comprehensively discuss the status quo of the current research on the effects of serotonin manipulations, emotional information processing and depression.

Our literature review is not the first but the last chapter (Chapter 8) of this thesis and was based on a hypothesis that was formed while doing the alpha-lactalbumin and ATD studies and studies by others: do serotonin induced changes in emotional processing lead up to symptomatic recovery in depressed patients starting antidepressant treatment? Since this hypothesis has not been studied directly, we decided to review the existing literature on the effects of serotonin manipulations on emotional information processing and mood. The review thus preludes part of the discussion since all evidence for serotonin induced changes in emotional information processing and mood are

discussed and evidence for a possible link between mood and emotional information processing was evaluated in Chapter 8.

*Information processing*

We included a wide range of cognitive tests in both the ATD and alpha-lactalbumin study. In Chapter 3 and 4 on the effects of alpha-lactalbumin, only findings related to neutral information processing were reported. We did include a test of emotional information processing in that study (an emotional version of the Stroop task) however alpha-lactalbumin did not affect performance on that test (Markus, personal communication, March 2007). Since the cognitive tests differed between the two studies, no indirect comparison could be made between the effects of ATD and alpha-lactalbumin. However, we used a wide range of tests in both studies, and therefore we covered a large domain of cognitive functioning, adding to the generalizibility of the results.

*Cortisol response to stress*

Regarding the effect of alpha-lactalbumin on stress-induced cortisol response, two remarks should be made. First, the results were limited by the fact that only minimal increases in mood and cortisol were found following the stress task. It may well be that the computerized stress-task we used, was not stressful enough since the induced stress posed no social-evaluative threat (Dickerson & Kemeny, 2004), thereby minimizing the cortisol response and negatively influencing the possible effect of alpha-lactalbumin on cortisol. Secondly, to minimize possible fluctuations in mood, we tested the women taking part in the alpha-lactalbumin study in their mid-to-late follicular phase or during the period they were actually taking the pill. This however may have

been problematic in case of the cortisol assessments. Since the cortisol response to stress may be dependent on gender, and for females on menstrual cycle or oral contraceptives (Kirschbaum et al., 1999), a possible gender effect may have confounded the effects of alpha-lactalbumin on cortisol response to stress. Since our subgroups of men and women (on and off oral contraceptives, or post-menopausal) were too small, we could not further investigate this. However, an alternative explanation for the lack of effect of alpha-lactalbumin on stress induced mood- and cortisol response is that a one day alpha-lactalbumin diet may be too weak to affect these responses in recovered depressed patients.

**Directions for future research**

*Diet enriched with alpha-lactalbumin*

Our findings suggest that an alpha-lactalbumin enriched diet has effects on cognitive performance; however a one day alpha-lactalbumin diet may be too weak also to affect mood and cortisol responses to stress in recovered patients. Future studies may investigate a diet enriched with a higher dose of alpha-lactalbumin or a longer duration of the diet. Since our results are contrary to previous findings (Markus et al., 2000), future research should investigate whether our results and Markus et al.'s can be replicated. If so, neuroticism and history of depression may not be equal constructs in terms of serotonergic vulnerability.

We did not find an effect of alpha-lactalbumin on mood in unmedicated recovered depressed patients, which is comparable to the effect of ATD: a depressive response to ATD mainly occurs in SSRI treated patients (Booij et al., 2003). Therefore, it may well be that alpha-lactalbumin has a more

pronounced effect in recovered depressed patients taking an SSRI as opposed to unmedicated patients.

The biochemical specificity of alpha-lactalbumin enriched diets should be further investigated by combining alpha-lactalbumin enriched diets with monoamine depletion paradigms. Also, different placebo procedures should be developed since the casein diet resulted in an increase in tyrosine, which may affect cognitive performance (Deijen & Orlebeke, 1994).

*ATD*

Our results support the fact that ATD is a useful tool to investigate the link between serotonin and emotional processing in medicated remitted depressed patients. However, some important issues have come up that need to be further investigated.

First of all, the term low-dose ATD is used for different ATD dosages that may have very variable effects on plasma tryptophan levels. More research is needed to construct the optimal low-dose, which does not affect mood and is significantly different compared to the original high-dose ATD. Second, the experience of patients undergoing ATD should be systematically investigated to prevent unethical research strategies. Third, ATD may also be a useful tool to investigate the role of serotonin in anxiety disorders and anxiety symptoms in clinical populations. Fourth, more research is needed on the direction of the link between serotonin and facial expression recognition in healthy as well as depression vulnerable subjects. Fifth, the finding of a link between facial expression recognition and mood response to ATD seems promising with regard to research into vulnerability to serotonin and depression.

Recent findings have indicated that future studies should focus on investigating the possible mediating effect of the polymorphism at the

serotonin transporter linked polymorphic region (5-HTTLPR) in the effects of ATD. ATD impaired verbal recall in healthy volunteers homozygous for the s allele at the 5-HTTLPR, while episodic memory was unimpaired in the ll genotype group (homozygous for the l allele) (Roiser et al., 2007). Mood was unaffected in both groups. Thus, this polymorphism may be a moderating factor in the link between ATD and cognitive performance.

*Emotional information processing*

Our findings support the relevance of emotional information processing in the research on the role of serotonin in depression. The role serotonin plays in the processing of facial expressions of fear and other emotions needs further investigation since differences exist in the effects of acute vs. repeated administration and between different samples. The findings of a link between serotonin receptor binding potential and dysfunctional attitudes seem very promising but also require further investigation. More research is also needed on the role serotonin plays in decision making and emotional memory. In particular, it is unclear whether these effects are limited to certain serotonin manipulations and /or certain study samples.

There is of yet no direct evidence for a link between serotonin induced changes in mood and emotional information processing, however there are promising directions for future research. The effects of different antidepressant treatments on various forms of emotional information processing should be investigated to verify possible common effects on emotional information processing and their underlying mechanisms. Since studies on the effects of serotonin on mood and emotional information processing in currently depressed patients are lacking, future research should focus on clinical populations. Furthermore, future research should include immediate as well as

sub-chronic and long-term measurements of different aspects of emotional information processing to clarify a possible mediating role of cognitive changes in clinical outcome, as well as the pattern of change.

*Biological vulnerability markers*

Our results suggest that experimental manipulations of serotonin using either the ATD method or a diet enriched with alpha-lactalbumin, may not be suitable to investigate cortisol response to stress or heart rate variability. It is however important to note that very little research has been done investigating the effects of these methods on biological factors. The main focus has been to investigate the role of serotonin in cognitive processing, and our results support the notion that this may be the most fruitful scope of this kind of research.

Regarding the effect of alpha-lactalbumin on cortisol response to stress, more research is warranted on the specific effects of alpha-lactalbumin on neuro-endocrine measures. Evidence from both animal and human studies indicates that serotonin plays little role in control of basal cortisol release but is important in release of cortisol in response to stress (Porter et al., 2007). It therefore seems important to further investigate the specific mechanisms underlying this association.

**Clinical implications**

Much is still unknown about the role serotonin plays in the development, maintenance and treatment of depressive disorders. This thesis has elucidated the relevance of emotional information processing in investigating the link between serotonin and depression. Although the research reported in this thesis was fundamental in nature, some remarks can be made about the clinical

relevance of the reported findings. First of all, the research on serotonin function in depression may bring us closer to optimal treatment for depressed patients. The literature on the effects of serotonin manipulations on mood and emotional information processing shows us that certain aspects of emotional information processing are related to serotonin function. Whether this association is involved in the therapeutic response to antidepressants needs to be further investigated. However, serotonin challenge studies such as ATD and the administration of an alpha-lactalbumin enriched diet provide us with important knowledge about the possible mechanisms underlying antidepressant action. Our finding that mood response to ATD was related to the recognition of sad facial expressions, suggests that some aspects of emotional information processing may be predictors of sensitivity to changes in serotonin. Therefore, neurotransmitter manipulation seems to be a useful method to study individual vulnerability and to gain insight into the relationship between cognition, serotonin and mood.

Although alpha-lactalbumin might be relatively easy to implement within a regular diet (Beulens et al., 2004), our results do not provide evidence for a particular clinical relevance of an alpha-lactalbumin diet in individuals with a history of depressive episodes, although a positive effect on memory was observed. Such a diet may be more relevant to stress-prone individuals (with high neuroticism scores) who are protected from the adverse effects of a stressor by an alpha-lactalbumin diet (Markus et al., 2000). Since we did find an effect of alpha-lactalbumin on memory in both healthy and recovered depressed individuals, an alpha-lactalbumin diet may be relevant for aging populations (McEntee & Crook, 1991), although the long-term effects should be further investigated.

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# Samenvatting

**Het manipuleren van de serotonine functie bij depressies**

Een depressie is een van de meest invaliderende stoornissen ter wereld. Ongeveer een op de zes mensen in Amerika krijgt op enig punt in zijn/ haar leven een depressie, de precieze prevalentie wisselt per land. Meer vrouwen dan mannen worden depressief (ratio 2:1). De meeste nieuwe gevallen van een depressie komen voor gedurende de late adolescentie en in de jonge volwassenheid. De kernsymptomen van een depressie zijn een sombere stemming en anhedonie (niet in staat zijn plezier te beleven). Daarnaast kan er sprake zijn van een verminderde eetlust, slaapproblemen, vermoeidheid, gevoelens van waardeloosheid, verminderd concentratievermogen en gedachtes over de dood en suïcide.

De meest effectieve behandelingen van een depressie zijn antidepressieve medicatie, gestructureerde vormen van psychotherapie (bijvoorbeeld cognitieve gedragstherapie) of een combinatie van beide. Selectieve serotonine heropname remmers (SSRIs) vormen de meest gebruikte farmacologische behandeling voor depressies.

Aangezien het terugvalpercentage bij depressies erg hoog is, wordt er veel onderzoek gedaan naar de mechanismen die mogelijk een rol spelen in de ontwikkeling en het voortbestaan van een depressie. Kwetsbaarheid voor depressies kan gelegen zijn in cognitieve, biologische, psycho-sociale en genetische factoren. Het functioneren van verschillende neurotransmitters (serotonine, dopamine, norepinefrine) speelt een belangrijke rol met betrekking tot de biologische kwetsbaarheid voor depressies.

Van serotonine is bekend dat het een belangrijke rol speelt bij het ontstaan van depressies. Verstoringen in het serotonine-systeem kunnen voorkomen op verschillende niveaus in zowel acute als in herstelde depressieve patiënten en in mensen met een familiegeschiedenis van depressies. Deze

neurobiologische basis voor depressies is verbonden met het werkingsmechanisme van antidepressieve medicatie: serotonerge antidepressiva verhogen de serotonine functie in de hersenen door de heropname van serotonine te verhinderen. Onderzoek naar serotonine is gebaseerd op indirecte methodes, aangezien een directe meting van serotonine in mensen problematisch is. Het experimenteel manipuleren van serotonine niveaus maakt het mogelijk de rol van serotonine in depressies en de werking van antidepressiva te onderzoeken.

Het doel van dit proefschrift is om de effecten van twee verschillende serotonine manipulaties (acute tryptofaan depletie en een dieet verrijkt met het melkwei alfa-lactalbumine) op stemming en het verwerken van neutrale en emotionele informatie te onderzoeken in herstelde en herstellende depressieve patiënten. Het verband tussen serotonine en twee biologische kwetsbaarheidmaten voor depressies (de cortisol respons op stress en hartritmevariabiliteit) wordt ook onderzocht.

In Hoofdstuk 2 worden twee onderzoeken beschreven waarin gekeken werd naar het verwerken van neutrale en emotionele informatie door herstelde depressieve patiënten die medicatie gebruikten en gezonde controles, welke overeenkwamen wat betreft leeftijd en geslacht. Verschillende cognitieve functies werden gemeten, bijvoorbeeld verbaal en non-verbaal geheugen, aandachtsbias, plannen, gezichtsherkenning en respons inhibitie. De resultaten lieten een verhoogde herkenning van angstige gezichten zien bij herstelde depressieve patiënten vergeleken met controle proefpersonen. Er werden geen andere resterende cognitieve beperkingen gevonden. De resultaten wijzen er op dat cognitieve beperkingen die gepaard gaan met een depressie in de meeste gevallen verdwijnen als de depressieve symptomen afnemen. Specifieke

beperkingen in bepaalde aspecten van emotionele informatieverwerking blijven mogelijk bestaan nadat de patiënt hersteld is.

Alfa-lactalbumine is een proteïne rijk aan tryptofaan. Een dieet dat verrijkt is met alfa-lactalbumine verhoogt de ratio van tryptofaan tot de andere 'large neutral amino acids' (LNAAs), waardoor vervolgens het serotonine niveau in de hersenen stijgt. In Hoofdstuk 3 en 4 worden de effecten van een dieet verrijkt met alfa-lactalbumine op stemming, cortisol respons op stress en cognitief functioneren onderzocht. Drieëntwintig herstelde depressieve patiënten en twintig gezonde controles ontvingen alfa-lactalbumine en caseïne (placebo) op verschillende dagen, volgens een dubbelblind gerandomiseerd cross-over design. Op beide dagen ondergingen de proefpersonen een stresstaak. Stemming, cognitief functioneren en plasma aminozuren werden gemeten voor en na inname van de maaltijden.

Het alfa-lactalbumine dieet verhoogde de ratio tryptofaan/LNAA met 21% van de ochtend tot de middag; de middag ratio was 73.8% hoger in de alfa-lactalbumine conditie vergeleken met de placebo conditie. Het alfa-lactalbumine dieet had geen effect op stemming maar het verbeterde het abstract visueel geheugen en het verstoorde eenvoudig motorisch functioneren. Deze effecten waren in beide groepen aanwezig. Alfa-lactalbumine had geen effect op de opslagfase ('encoding'), het werkgeheugen, waarneming of algemene motorische snelheid. Mogelijk heeft alfa-lactalbumine een specifiek effect op geheugen-consolidatie in een vroeg stadium. Het effect van alfa-lactalbumine op het geheugen komt overeen met de resultaten van andere onderzoeken die een verband tussen serotonine en geheugenprocessen hebben aangetoond. Alfa-lactalbumine supplementen zouden nuttig kunnen zijn voor voedingsonderzoek met betrekking tot leeftijd- of ziekte gerelateerd

geheugenverlies. De huidige resultaten dienen echter nader onderzocht te worden in verschillende populaties en de lange termijn effecten van alfa-lactalbumine moeten ook onderzocht worden. De resultaten van dit onderzoek ondersteunen het idee dat cognitieve factoren mogelijk sensitiever zijn voor veranderingen in de serotonine functie dan stemming.

De gecomputeriseerde stresstaak beïnvloedde de stemming negatief in beide condities. Hoewel het alfa-lactalbumine dieet tot de verwachte verhoging in plasma tryptofaan en ratio tryptofaan/LNAA leidde, werden er slechts minimale effecten gevonden op stemming en de cortisol respons op de stresstaak. De effecten in de herstelde depressieve patiënten verschilden niet van de effecten in de controle groep. Een dieet verrijkt met alfa-lactalbumine gedurende één dag is dus niet voldoende om de door stress geïnduceerde verslechtering in stemming of de cortisol respons tegen te gaan.

Acute tryptofaan depletie (ATD) is een methode om de serotonine functie bij mensen tijdelijk te verlagen. Dit gebeurt door toediening van een aminozuurdrankje waarin tryptofaan ontbreekt. Onderzoek heeft veelvuldig bewezen dat ATD depressieve symptomen induceert in herstellende depressieve patiënten die een SSRI slikken. ATD beïnvloed ook het cognitief functioneren, zowel in patiënten als in gezonde groepen. De exacte oorsprong van de door ATD geïnduceerde cognitieve veranderingen bij depressies is vooralsnog onduidelijk. Ook onbekend is of er cognitieve veranderingen kunnen plaatsvinden na partiële (lage dosering) depletie.

In Hoofdstuk 5, 6 en 7 worden de resultaten besproken van een onderzoek naar de effecten van een lage en een hoge dosering tryptofaan depletie op stemming, neutrale en emotionele informatieverwerking en hartritmevariabiliteit in herstellende depressieve patiënten die serotonerge

antidepressiva gebruiken. Achttien herstellende depressieve patiënten ontvingen een hoge dosis en een lage dosis ATD in een dubbelblind, gerandomiseerd, binnen-proefpersoon cross-over design. Stemming werd gemeten voor en na de inname van het depletiedrankje. Vijf uur na inname voerden de patiënten een reeks cognitieve tests uit. Tijdens deze tests werden hartritme en hartritmevariabiliteit gemeten.

De hoge dosering ATD (vijftien aminozuren, 102.5 gram) verlaagde de ratio tryptofaan/LNAA met 84%. De lage dosering (op een kwart sterkte van de hoge dosering: vijftien aminozuren, 25.7 gram) verlaagde de ratio tryptofaan/LNAA met 59%. De hoge dosering ATD verhoogde het aantal depressieve symptomen en zorgde voor een tijdelijke terugval bij de helft van de patiënten. De hoge dosis verminderde de herkenning van angstige gezichten en verstoorde het leervermogen en ophalen van informatie uit het geheugen ('retrieval'). Het verstoorde leervermogen kwam slechts voor bij de patiënten met een stemmingsrespons. De lage dosering had geen effect op stemming maar versnelde de herkenning van gezichtsuitdrukkingen van walging. De juiste herkenning van verdrietige gezichten bij baseline hield verband met de stemmingsrespons op ATD. Hoge dosering ATD verhoogde het hartritme tijdens rust en tijdens de aandachtstaak. ATD had echter geen significant effect op hartritmevariabiliteit in patiënten met een geschiedenis van suïcidaliteit. Een lage hartritmevariabiliteit houdt mogelijk wel verband met stemming en verstoorde verwerking van affectieve informatie door veranderingen in de serotonine functie. Er is echter meer onderzoek nodig naar de relatie tussen hartritmevariabiliteit en serotonine in grotere groepen.

In Hoofdstuk 6 worden de effecten van de verschillende ATD doseringen op plasma tryptofaan en de ratio tryptofaan/LNAA besproken.

Ook worden de inter-individuele verschillen in plasma tryptofaan in reactie op de verschillende ATD doseringen nader bekeken.

In Hoofdstuk 8 wordt de literatuur besproken over de acute en korte-termijn effecten van serotonine manipulaties op stemming en emotionele informatieverwerking. De hypothese waarop dit hoofdstuk gebaseerd is, is dat cognitieve veranderingen mogelijk de symptomatische respons op antidepressiva mediëren. Omdat er geen onderzoeken zijn die deze hypothese direct getest hebben, hebben we ons gericht op de korte-termijn effecten van serotonine manipulaties op emotionele informatieverwerking en stemming. We hebben ons hierbij beperkt tot onderzoek bij gezonde personen en personen die kwetsbaar zijn om depressief te worden. Ook hebben we literatuur over de veranderingen in emotionele informatieverwerking en stemming in depressieve patiënten die starten met antidepressiva gezocht. Er werden vijfentwintig onderzoeken geïdentificeerd. Er werden vier groepen manipulaties onderscheiden: toediening van een SSRI, toediening van een serotonine receptor agonist of antagonist, tryptofaan toevoeging en tryptofaan depletie. Serotonine manipulaties bleken betrouwbare snelle (tijdsrange: 1 uur- 14 dagen) effecten te hebben op aandachtsbias, de herkenning van emoties uit gezichten, emotioneel geheugen, disfunctionele attitudes en besluitvorming. Er is echter meer onderzoek nodig naar het verband tussen door serotonine geïnduceerde opeenvolgende veranderingen in emotionele informatieverwerking en stemming, vooral in depressieve patiënten.

Hoofdstuk 9 bevat een algemene discussie van de resultaten die vermeld worden in dit proefschrift. De bevindingen wijzen op een effect van alfa-lactalbumine op cognitief functioneren, hoewel een dieet gedurende een dag te

zwak is om ook een effect op stress-geïnduceerde stemming en cortisol te bewerkstelligen in herstelde depressieve patiënten. Toekomstig onderzoek gericht op een hogere dosis alfa-lactalbumine of een langere duur van het dieet is gewenst.

De resultaten van het ATD onderzoek ondersteunen de bewering dat ATD een nuttige methode is om het verband tussen serotonine en emotionele informatieverwerking te onderzoeken in herstellende depressieve patiënten die antidepressiva gebruiken. Er is echter meer onderzoek nodig naar de optimale lage dosering, die geen effect heeft op stemming en significant verschilt van de originele hoge dosering. Ook dient de richting van het verband tussen serotonine en de herkenning van emoties uit gezichten verder onderzocht te worden in gezonde personen maar ook in personen die kwetsbaar zijn voor een depressie. De bevinding dat de herkenning van emoties uit gezichten verbonden is met de stemmingsrespons op ATD lijkt veelbelovend met betrekking tot onderzoek naar kwetsbaarheid voor serotonine en depressies.

Onze bevindingen ondersteunen de relevantie van emotionele informatieverwerking in het onderzoek naar de rol die serotonine speelt in depressies. De rol die serotonine speelt in de herkenning van angst en andere emoties uit gezichten verdient meer onderzoek aangezien er verschillen zijn in de effecten van acute vs. herhaalde toediening en tussen verschillende groepen. Er is tot op heden geen bewijs gevonden voor een verband tussen door serotonine opgewekte veranderingen in stemming en emotionele informatieverwerking maar er zijn veelbelovende bevindingen gedaan voor toekomstig onderzoek. Toekomstig onderzoek moet zich richten op klinische populaties en zal zowel acute als meer lange-termijn metingen moeten bevatten van verschillende vormen van emotionele informatieverwerking.

Experimentele manipulaties van serotonine d.m.v. ATD of alfa-lactalbumine zijn mogelijk niet geschikt om de cortisol respons op stress en hartritmevariabiliteit te onderzoeken. Er is echter maar weinig onderzoek gedaan naar de effecten van deze methodes op biologische kwetsbaarheidfactoren.

Het onderzoek naar de serotonine functie in depressies brengt ons mogelijk dichter bij de optimale behandeling voor depressieve patiënten. Sommige aspecten van emotionele informatieverweking lijken gerelateerd aan de serotonine functie. Of dit verband een rol speelt in de therapeutische respons op antidepressiva moet verder onderzocht worden. Onderzoek m.b.v. serotoninemanipulaties zoals ATD en alfa-lactalbumine biedt ons belangrijke kennis over de mogelijke mechanismen die een rol spelen in de werking van antidepressiva. Onze bevinding dat de stemmingsrespons op ATD gerelateerd is aan de herkenning van verdrietige gezichten, lijkt te wijzen op het feit dat sommige aspecten van emotionele informatieverwerking mogelijk voorspellers zijn van een gevoeligheid voor veranderingen in serotonine.

# Dankwoord

Eindelijk is het dan zo ver; ik mag beginnen aan het leukste deel van dit proefschrift.
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# Curriculum Vitae

Wendelien Merens, born on April 30 1977 in Delft, the Netherlands, completed secondary education (Gymnasium) at the Sint Stanislas College in Delft in 1995. She studied Psychology at Leiden University, graduating in Clinical and Health Psychology in May 2001. In 2001-2002, she worked as a Research Fellow at the Depression Clinical and Research Program at Massachusetts General Hospital/ Harvard Medical School in Boston. Upon return in the Netherlands, Wendelien was employed as a counselor by HSK in The Hague before starting her PhD at Leiden University in March 2003. When her PhD position ended, she held a part-time teaching position at Leiden University until September 2007. She also worked part-time at GGZ Leiden in Oegstgeest at the Department for Personality Disorders. In January 2008 she will start clinical training (Gz-opleiding) at the Center for Eating Disorders 'Ursula' in Leidschendam.

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