Universiteit
Leiden
The Netherlands

Manipulating serotonin function in depression

Merens, W.

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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.