Manipulating serotonin function in depression
Merens, W.

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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
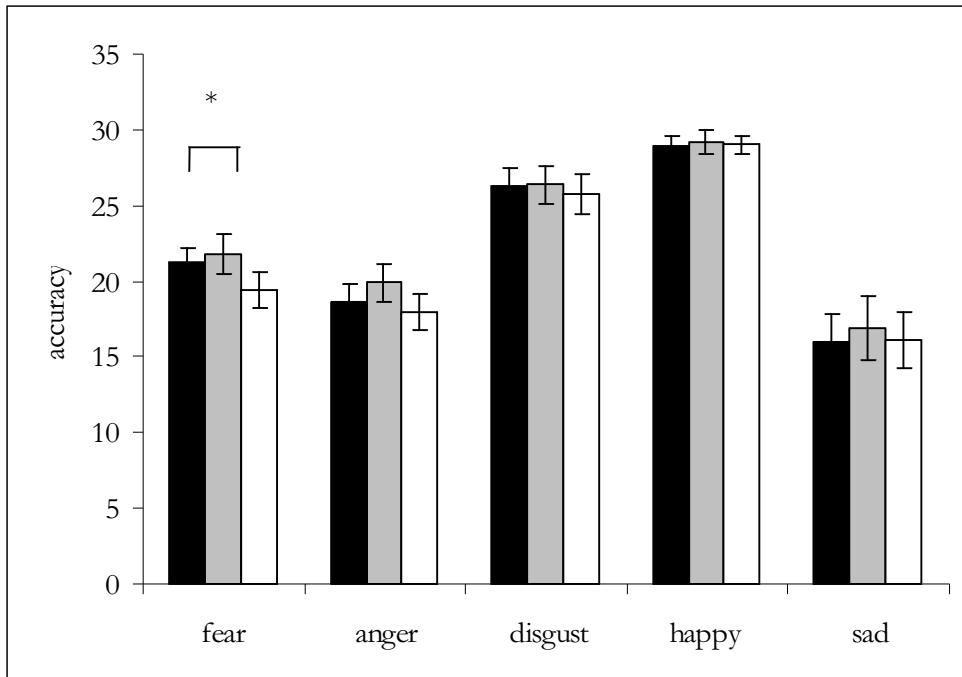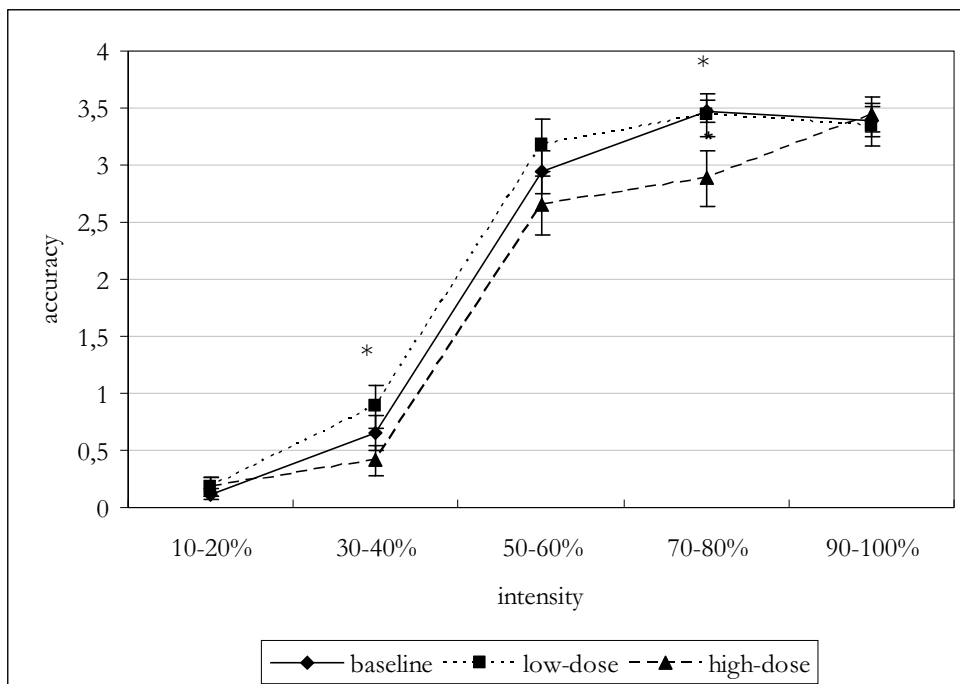
Figure 2. Fear accuracy

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).