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The effects of high-dose and low-dose tryptophan depletion on mood and cognitive functions of remitted depressed patients

Linda Booij *Department of Psychology, Leiden University, Leiden, The Netherlands and Psychomedical Center Parnassia, The Hague, The Netherlands.*

A. J. Willem Van der Does *Department of Psychology, Leiden University and Department of Psychiatry, Leiden University Medical Center, Leiden, The Netherlands.*

P. M. Judith Haffmans *Psychomedical Center Parnassia, The Hague, The Netherlands.*

Wim J. Riedel *Department of Psychiatry, University of Cambridge, Cambridge, UK, Faculty of Psychology, Maastricht University, Maastricht, The Netherlands and GlaxoSmithKline, Translational Medicine and Technology, Cambridge, UK.*

Durk Fekkes *Departments of Psychiatry and Neuroscience, Erasmus University Medical Center, Rotterdam, The Netherlands.*

Marc J. B. Blom *Psychomedical Center Parnassia, The Hague, The Netherlands.*

Abstract

It has frequently been demonstrated that acute tryptophan depletion (ATD) induces a transient depressed mood in some patients who are in remission from depression. However, the effects of ATD on cognitive processes in remitted depressed patients have not been investigated. The aim of the present study was to investigate the effects of different extents of depletion on mood and cognitive tasks involving neutral and emotional stimuli. Twenty patients in remission or in partial remission from depression received ATD in a double-blind, crossover design. Mood was assessed at both sessions before, at +6.5 h and +24 h after depletion. Cognitive assessment in both sessions started at +4.75 h, and also before and after the whole procedure. The ATD mixtures induced the expected reductions of plasma tryptophan levels. High-dose ATD induced a depressive response in a subsample of patients and impaired the processing of positive information independent of mood change. Attention for neutral stimuli (Stroop interference) improved in a dose-dependent manner. ATD may affect mood and cognition via different pathways: one implicated in mood regulation and the processing of emotional information, and one for the processing of neutral information. The first pathway may be more important for discriminating vulnerability to impaired serotonin function. The comparison of the effects of high-dose and low-dose ATD is useful for those studies aiming to investigate the relationships among 5-HT, mood and cognition.

Keywords

catecholamine, cognition, depletion, depression, serotonin, tryptophan

Introduction

Depression impairs cognitive processes, including memory, attention, executive function and motor function (Austin *et al*., 2001). Although serotonin (5-hydroxytryptamine; 5-HT) neurotransmission plays an important role in the pathophysiology of depression (Maes and Meltzer, 1995), the relationship of 5-HT, depressed mood and cognitive processes remains poorly understood. In the acute tryptophan depletion (ATD) paradigm, 5-HT function is temporarily lowered by restricting the availability of its precursor L-Tryptophan (Trp) (Young *et al*., 1985). It has frequently been demonstrated that ATD temporarily induces a return of depressive symptoms in a subset of selective serotonin reuptake inhibitor (SSRI) treated remitted depressed patients (Delgado *et al*., 1990, 1999; Van der Does, 2001a; Booij *et al*., 2002, 2003). However, the effects of ATD on cognitive processes in these patients have not been studied.

In healthy samples, ATD had a selective negative effect on memory consolidation for verbal and non-verbal material (Park *et al*., 1994; Riedel *et al*., 1999; Schmitt *et al*., 2000; Sobczak *et al*.,

Corresponding author: Dr A. J. W. Van der Does, Department of Psychology, Leiden University, Wassenaarseweg 52, 2333 AK Leiden, The Netherlands. *Email*: vanderdoes@fsw.leidenuniv.nl

2002; Harrison *et al*., 2004), and a positive effect on focused attention as indicated by reduced Stroop interference or by improved dichotic listening performance (Park *et al*., 1994; Schmitt *et al*., 2000).

Of particular interest are ATD studies investigating the effects on emotional material because a mood-congruent bias has often been demonstrated in depression. For example, depressed patients are more likely to learn and retrieve negative than positive information (Burt *et al*.1995). Research with the Emotional Stroop test has shown that depression-related words cause more interference than neutral or positive words (Gotlib, 1984; Segal *et al*., 1995). Consistent with this, ATD in healthy volunteers impaired the processing of positive information (Murphy *et al*., 2002) and decreased the recognition of fearful faces (Harmer *et al*., 2003b) but left mood unaffected. The results of these studies suggest that cognitive tests may be more sensitive measures of ATD-induced depression than mood scales.

However, the absence of depressive response in healthy samples limits the use of these samples in investigating the associations among 5-HT, depressive symptoms and cognitive function. If cognitive changes are more sensitive markers for changes in 5-HT function than symptom, changes in cognitive function following ATD may occur in patients without a depressive response. Second, cognitive changes may already occur after moderate depletion, with no effect on symptoms.

It has been suggested that failures to replicate the mood effects of ATD may have been due to insufficient depletion (Spillmann *et al*., 2001; Van der Does, 2001b). Therefore, although all previous studies have used a placebo-controlled design, it has been recommended to compare the effects of two different dosages of ATD (100 g versus 25 g amino acids), aimed at reducing plasma Trp levels by 80–90% and 40–50%, respectively (Van der Does, 2001a).

The aim of the present study was to investigate the effects of moderate and strong reductions of Trp levels on mood and on cognitive tasks involving neutral and affective stimuli, in remitted depressed patients. The tested hypotheses were: (i) strong reductions of Trp levels, and not moderate reductions, will lead to a transient return of depressive symptoms and (ii) both moderate and strong reductions of Trp will lead to cognitive changes in patients with and without an ATD induced depressive response.

Materials and methods

Participants

Eligible patients were selected outpatients of a mood disorders clinic. Inclusion criteria were: age between 18 and 65 years; ongoing treatment with an SSRI or a serotonin noradrenaline reuptake inhibitor for at least 4 weeks, meeting DSM-IV criteria for depression in remission or partial remission, Hamilton Depression rating Scale (HRSD, 17-items) (Hamilton, 1960) lower than 15 (Frank *et al*., 1991). Exclusion criteria were: substance abuse within last 3 months, psychosis (lifetime), major physical illness, lactation, pregnancy. Diagnoses, demographic and clinical background variables were verified with the Structured Clinical Interview for DSM-IV (SCID-I) (First *et al*., 1995).

Amino acids

At each depletion session, patients received in randomized order either a high-dose (100 g) or a low-dose (25 g) ATD mixture. The composition of the 100-g mixture (aimed at reducing Trp levels by 90%) was similar to that reported by Delgado *et al*., 1990). The 25-g mixture consisted of the same amino acids (AAs) but at one quarter of the amount (Krahn *et al*., 1996). All AAs were mixed with cold water (4 °C) to a final volume of 300 ml. Liquid chocolate syrup was added and the mixture was served chilled to limit the unpleasant taste of some AAs. Patients were kept on a 24-h low-Trp diet (160 mg/day) before both sessions. The meals had an energy value of 2300 kCal and were prepared by dieticians. During the ATD sessions, water (de)caffeinated coffee, (herbal) tea, orange juice and protein-poor (< 0.05 g) cookies were allowed in standard amounts. Caffeinated coffee or tea was not allowed to be consumed for approximately 1 h before the cognitive tasks. The experimenter took care that the amount of caffeine consumed was not larger than usual. Patients had a low-Trp lunch 3 h after drinking the mixture (Riedel *et al*., 1999; Schmitt *et al*., 2000; Sobczak *et al*., 2002)

Instruments

Mood Symptoms were assessed with the 10-item Montgomery–Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979). Sleep items were omitted. The 17-item HRSD was also administered. Because the results were very similar, only the MADRS will be reported.

Cognition The cognitive tests took approximately 60 min. Parallel versions were used, except for the Stroop tasks and the Left/Right task.

Stroop Word Colour test

The Stroop test measures focused attention and response inhibition. Names of colors (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, colored patches were presented (Condition II). Finally, the names of colors 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 extra time needed for condition III relative to the average of conditions I and II.

Emotional Stroop test

An Emotional Stroop 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 colors as quickly as possible. The order of the word categories was randomized over the patients but fixed for each patient during all sessions. The order of the words within each category was randomized in all sessions.

Left/Right Choice RT

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 but to ignore its location, as fast as possible. The task consisted of two consecutive subtasks in which the stimulus interval differed (1000 ms fixed versus 500–1500 ms variable). Correct responses and RTs were registered.

Tower of London (TOL)

The TOL (Owen *et al*., 1995) is a planning task consisting of three colored 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 six moves). Correct responses and RTs were registered.

Letter Fluency

This test measures strategy-driven retrieval from semantic memory within a fixed time span. Participants were instructed to produce as many correct four-letter words with the same initial letter as possible within 1 min. Starting letters were H, M, R and L. The numbers of correct, nonsense and double-reported words were registered.

Abstract Patterns Recognition task (APRT)

The APRT (Rubinsztein *et al*., 2001) measures (speed of) retrieval 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.

Blood plasma

Venous blood was obtained (10 ml) using ethylenediaminetetraacetic acid tubes to determine total plasma Trp and the other large neutral amino acids (LNAA) phenylalanine, tyrosine, isoleucine, leucine and valine. Immediately after sampling, the blood was centrifuged for 20 min at 2650 g (maximum) and the plasma was stored at –65 °C. Quantitative amino acid analysis was performed by high-performance liquid chromatography (HPLC) as described previously (Fekkes *et al*., 1995). The concentrations 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) were measured in plasma by HPLC employing electrochemical detection (detection limit in plasma, 1 nM). Quantification was performed by measuring peak heights, and absolute concentrations were calculated using a combined external and internal standard (applying α-methyl 5-HT) method (Fekkes *et al*., 1997).

Procedure

After providing their written informed consent, participants were invited to a screening interview that included the SCID-I, HRSD, MADRS and an interview with a dietician. The cognitive tasks were also administered. The time between the intake and the first ATD session was approximately 1 week. During day 1 of each session, patients consumed the prepacked low-Trp meal. Patients came to the laboratory at 08.00 h or 09.00 h of day 2, after an overnight fast. Mood ratings and a blood sample were obtained (–1 h), followed by the ATD drink (0 h). For the next 4.5 h, patients remained in a private research room. Neutral videos and magazines were available. They completed the cognitive tasks at +4.75 h. A blood sample was taken at +6 h and mood was assessed at +6.5 h. Before participants went home, they received a sandwich or a Trp-enriched snack and were instructed to resume their regular meals. Mood ratings and a blood sample were taken the next morning. This procedure was repeated at least 4 days later; those who had received high-dose ATD received low-dose ATD and vice versa. The day after the second session, participants also completed the cognitive test battery (after the mood assessment). All patients were tested individually and were paid €115 for their participation.

Design

The study was conducted according to a randomized, double-blind, crossover design with two sessions, separated by at least 4 days. The hospital pharmacist took care of the randomization. To compare the effects of both low- and high-dose ATD with baseline levels, cognitive performance after ATD was compared with the mean of the first and the fourth administration of the neuropsychological tests.

Statistical analysis

Before analysis, all variables were examined for accuracy of data-entry, missing values and fit between their distributions and the assumptions of the statistical analyses. Clinical and demographic variables were investigated by means of chi-square tests and univariate General Linear Models (GLM). Differences in mood and cognitive performance between patients and controls were analysed by multivariate GLM.

The effects of the different doses of ATD on the outcome variables were analysed by GLM for repeated measures. For the MADRS, intervention (low-dose versus high-dose) and time (–1 h, +6.5 h and +24 h) were the within subjects factors. Intervention (baseline versus low-dose versus high-dose ATD) was used as within-subject factor for the cognitive measures. 'Baseline intervention' measures were defined as the average score obtained during the intake and post-intervention sessions. Contrast tests were used to investigate differences between specific interventions. *p*-values for contrast tests were corrected for multiple comparisons by dividing the *p*-value of 0.05 by the number of intervention

comparisons (baseline versus low-dose; baseline versus high-dose; low-dose versus high-dose), implicating that differences between interventions were tested at the 0.05/3 = 0.02 level of significance. To further investigate the relationship between depressive symptoms, cognitive function and 5-HT, post-hoc analyses were conducted by adding ATD depressive response ('responder' versus 'non-responder') as a between subjects variable, based on a clear division in magnitude of symptom change between the groups (see below). Second, the influences of minor mood changes were investigated by separate analyses entering delta MADRS scores [t +6.5 h – t (–1 h) following high-dose ATD] as a covariate.

There was no need to correct the p-value for the effects of ATD on depressive symptoms because these comparisons were planned, based on previous ATD studies in remitted depressed patients (Booij *et al*., 2002, 2003).

Results

Twenty-three participants entered the study (13 males, 10 females). Two patients (both males) dropped out after the first session; the first case due to a severe headache on the depletion day (after low-dose ATD) and the second case because of health problems after the first session (high-dose ATD) unlikely to be caused by ATD. These patients were not included in the analyses. The ATD mixtures were well tolerated; one female patient vomited approximately 10 min after ingestion of high-dose ATD, but she was able to complete both sessions. Because the reduction of plasma Trp levels in the high-dose condition was only marginally higher than in the low-dose condition in this participant (64% versus 55%) and much lower than the average reduction in the high-dose condition (see below), this patient was excluded from the analyses.

The clinical and demographic characteristics of the remaining, 20 patients are presented in Table 1. Two patients had very recently tapered off antidepressant medication. These patients were retained because unmedicated remitted depressed patients are also likely to respond to ATD (Smith *et al*., 1997; Booij *et al*., 2002).

Data screening

RTs of the TOL and the APRT were $\log_{10}$-transformed because of a non-normal distribution. Using Mahalonobis distances and standardized residuals criterion, one statistical outlier was detected on the MADRS (D^2 = 16.2; z = 3.2). This patient responded in the low-dose ATD condition and not after high-dose ATD. Another patient had an extreme increase in neutral Stroop interference levels after high-dose ATD (D^2 = 14.7; z = 3.05), and was a statistical outlier. One patient experienced a strong emotional reaction during the emotional Stroop task and interrupted the task. Another patient missed all 3- and 4-step problems of the TOL at intake and after the low-dose ATD session. Blood samples were missing for one patient at the post intervention day after both sessions. Analyses were conducted with and without statistical outliers. Cases with missing data were omitted separately by analysis.

Table 1 Clinical and demographic characteristics of the patient sample (n = 20)

Characteristic	Value
M/F	11/9
Age ± SD	48.7 ± 7.9
Education level	
High	n = 9
Medium	n = 7
Low	n = 3
Type of medication	
SSRI	n = 13 (two SSRI treatment free for 1 month)
SNRI	n = 7 (75–225 mg)
Diagnosis	
Subtype of last depressive episode:	
Not melancholic, atypic or catonic	n = 3
Melancholic	n = 11
Atypic	n = 6
Seasonal pattern	n = 2
Mean ± SD past episodes	4.8 ± 4.4 (range 1–16)
Single/recurrent	4/16
Partial remission	n = 13
Full remission	n = 7
Duration of remission(months) ± SD	5.9 ± 5.6 (range 1–24)

SSRI, Selective serotonin reuptake inhibitor; SNRI, serotonin noradrenaline reuptake inhibitor.

Biochemical effects

Both ATD mixtures significantly reduced total Trp and the Trp/LNAA ratio. Total Trp was significantly more reduced after a high than after low-dose ATD (mean ± SE change: –86.3 ± 1.2% versus –46.8 ± 3.2%). A similar pattern was observed for Trp/LNAA ratio (–93.3 ± 0.9% versus –42.2 ± 3.8%). The variance of reductions was small; for each patient, the reduction was approximately twice as large after a high-dose than after a low-dose.

Post-hoc analyses were conducted to investigate the effects of ATD on tyrosine levels. Unexpectedly, tyrosine levels and tyrosine/LNAA ratio increased significantly after a high-dose ATD (mean ± SE change: +214.0 ± 25.3% and +56.4 ± 14.5%), but not after a low-dose ATD (–2.0 ± 3.8% and +11.2 ± 3.6%). To further investigate the influence on dopamine (DA) and 5-HT function, HVA and 5-HIAA levels were measured post-hoc in the first 10 patients. At t(+6.5 h), high-dose ATD significantly decreased 5-HIAA levels compared to t(–1 h) (–50.3%) [$F(1,9) = 108.85$, $p < 0.001$]. There was no significant change of HVA levels (–23.6%) [$F(1,9) = 0.76$, not significant]. After omitting one patient who had extremely high HVA levels before depletion, the change in HVA levels was +3.3%.

Symptoms

The increase of MADRS scores at t(+6.5 h) was significantly higher after high-dose ATD than after low-dose ATD, as shown by a

Table 2 Effects of acute tryptophan depletion (ATD) on the biochemical outcome measures and mood (mean ± SE)

	Low-dose ATD			High-dose ATD		
	Pre	Post	+ 24h	Pre	Post	+ 24h
Total TRP	38.4 ± 1.5	20.4 ± 1.6	38.0 ± 1.6	39.4 ± 1.3	5.3 ± 0.5	35.6 ± 1.8
TRP/LNAA	8.7 ± 0.3	4.9 ± 0.3	7.2 ± 0.3	9.1 ± 0.3	0.6 ± 0.1	6.3 ± 0.3
5-HIAA				28.2 ± 1.9	14.1 ± 1.1	
Tyrosine	57.3 ± 2.9	56.2 ± 3.5	62.2 ± 4.9	58.0 ± 3.5	173.4 ± 11.5	67.0 ± 6.3
Tyrosine/LNAA	13.4 ± 0.5	14.9 ± 0.8	11.9 ± 0.6	13.6 ± 0.6	20.6 ± 1.4	11.9 ± 0.5
HVA				85.2 ± 25.9	65.1 ± 10.7	
MADRS	3.7 ± 0.9	3.7 ± 0.9	3.6 ± 0.9	4.6 ± 0.9	7.9 ± 1.8	3.4 ± 1.0

TRP, L-Tryptophan; LNAA, large neutral amino acids; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; MADRS, Montgomery–Asberg Depression Rating Scale.

Table 3 Mean ± SE of the cognitive tasks, broken down by intervention

Task	Baseline[a]	Low-dose ATD	High-dose ATD	Intervention effect
SCWT				
Condition I (ms)	498.5 ± 12.4	519.2 ± 10.6[b]	534.8 ± 11.6[b]	$F(2,38) = 5.52$, $p = 0.01$
Condition II (ms)	558.5 ± 16.9	550.6 ± 13.3	577.9 ± 16.4	$F(2,38) = 2.13$, $p = 0.13$
Condition III (ms)	789.2 ± 25.0	759.8 ± 22.0	762.7 ± 24.1	$F(2,38) = 1.80$, $p = 0.18$
Interference (%)	48.3 ± 3.3	42.4 ± 3.4[c]	34.7 ± 2.9[b]	$F(2,36) = 9.85$, $p < 0.001$
Emotional Stroop				
Positive words (ms)	707.1 ± 18.0	709.8 ± 19.9	717.2 ± 20.0	$F(2,36) = 0.22$, $p = 0.80$
Negative words (ms)	737.3 ± 21.2	724.0 ± 23.9	708.1 ± 21.6	$F(2,36) = 1.47$, $p = 0.24$
Neutral words (ms)	711.4 ± 16.2	706.6 ± 22.8	681.7 ± 18.1	$F(2,36) = 1.51$, $p = 0.23$
Positive Interference(%)	−0.5 ± 1.3	1.0 ± 1.9	5.3 ± 1.7[b]	$F(2,36) = 5.09$, $p = 0.01$
Negative Interference(%)	3.7 ± 2.0	2.7 ± 2.0	4.0 ± 2.0	$F(2,36) = 0.15$, $p = 0.86$
Left/Right task				
Congruent (ms)	659.1 ± 11.6	665.7 ± 16.2	660.0 ± 16.8	$F(2,38) = 0.02$, $p = 0.98$
Incongruent (ms)	682.9 ± 11.7	678.3 ± 18.6	679.9 ± 19.1	
Congruent (variable)	668.4 ± 12.8	664.3 ± 15.3	669.5 ± 17.3	$F(2,38) = 0.37$, $p = 0.69$
Incongruent(variable)	683.0 ± 12.1	689.0 ± 11.3	700.7 ± 15.6	
Word Fluency				
No. correct 0–30 s	7.5 ± 0.6	8.0 ± 0.5	9.0 ± 0.6[b]	$F(2,38) = 4.29$, $p = 0.02$
No. correct 0–60 s	12.2 ± 1.0	13.0 ± 1.1	12.2 ± 0.8	$F(2,38) = 0.63$, $p = 0.53$
Tower of London				
Percent correct				
2-step	87.1 ± 2.7	85.3 ± 3.5	84.2 ± 3.7	No. correct:
3-step	86.0 ± 3.7	85.8 ± 3.7	85.3 ± 2.5	$F(2,36) = 0.35$, $p = 0.71$
4-step	79.5 ± 3.7	75.8 ± 5.1	80.5 ± 3.3	
5-step	61.8 ± 4.9	63.7 ± 5.3	67.9 ± 5.9	
Reaction time (ms)				
2-step	5852 ± 410	6063 ± 564	5659 ± 429	Median reaction time:
3-step	7359 ± 615	6813 ± 639	7110 ± 697	$F(2,36) = 0.33$, $p = 0.72$
4-step	10310 ± 838	9456 ± 1133	9951 ± 954	
5-step	15836 ± 1549	15894 ± 2130	15338 ± 1742	
APRT				
Percent correct, STM	80.5 ± 2.2	83.1 ± 2.6	78.4 ± 2.8	$F(2,38) = 1.37$, $p = 0.26$
Percent correct, LTM	77.3 ± 2.5	78.1 ± 2.7	73.7 ± 3.8	$F(2,38) = 1.15$, $p = 0.33$
Reaction time, STM (ms)	2159 ± 131	2068 ± 92.4	2195 ± 171	$F(2,38) = 0.57$, $p = 0.57$
Reaction time, LTM (ms)	2078 ± 107	1966 ± 103	1890 ± 110	$F(2,38) = 1.67$, $p = 0.20$

[a]Baseline = mean(intake, post-intervention session). [b]Versus baseline; [c]25% versus 100% acute tryptophan depletion (ATD). SCWT, Stroop Word Colour test; APRT, Abstract Patterns Recognition task; STM, short-term memory; LTM, long-term memory.

significant interaction between time of rating and intervention [$F(2,36) = 7.32$, $p = 0.002$] (Table 2). Contrast test between t(+6.5 h) and t(–1 h) in the high-dose condition was not significant when the statistical outlier was included [$F(1,19) = 2.51$, $p = 0.13$] There were no baseline differences, nor was there any difference in mood between t(–1 h) and t(+24 h) in both conditions.

The MADRS change score data revealed two clearly separable groups: seven patients had a change of at least 6 points (range 6–12 points), and the remaining 13 patients had no change (range –1, 2 points). This clear division allowed us to conduct post-hoc analyses of the mood effect on cognitive performance by comparing 'responders' and 'non-responders', defining responders as those who had at least a 6-point MADRS increase (see below).

Cognition

The mean ± SE of all cognitive measures are presented in Table 3.

Cognitive performance at intake and learning effects To investigate potential learning effects on the cognitive tasks in the patient group, performance for the intake and post-intervention sessions was compared by paired t-tests or non-parametric tests. Improved performance was observed at the post-intervention session on all outcome measures of the TOL, except for the number of correct responses of the 2- and 4-step problems. A small learning effect was also found for the Left/Right task, but only during the fixed interval condition in both the congruent (677 versus 659 ms) and incongruent trials (691 versus 674 ms). Patients became slower during condition I of the Stroop Test (492 versus 509 ms). To investigate whether high-dose ATD could influence cognitive performance the next day, performances at intake and at the post-intervention session of patients that received high-dose ATD in the second session were compared with those that received high-dose ATD in the first session by multivariate GLM, entering 'order of intervention' as a between subjects variable. Patients who received high-dose ATD in the second session were faster on Cards I and II of the Neutral Stroop task at intake [Card I: $F(1,18) = 7.27$, $p = 0.01$; Card II: $F(1,18) = 9.90$, $p = 0.01$] and at the post-intervention session [Card I: $F(1,18) = 9.74$, $p = 0.01$; Card II: $F(1,18) = 6.47$, $p = 0.01$], indicating that the effects could not be due to the ATD mixture.

The suitability of taking the mean of the intake and the post-intervention sessions as a baseline measure was further checked by a repeated measures analyses, with 'session' (intake versus post-intervention session) as a within subjects factor and 'order' (high- versus low-dose ATD first) as a between subjects factor. There were no order–session interactions on any of the tests, indicating that the average score of these two sessions can be used reliably as a baseline score.

Effects of ATD on cognitive performance

Neutral Stroop task. Compared to baseline, ATD was associated with decreased interference levels in a dose-dependent manner. High-dose ATD significantly decreased interference levels compared to baseline [$F(1,18) = 13.41$, $p = 0.002$] and low-dose ATD [$F(1,18) = 13.58$, $p = 0.002$]. Low-dose ATD also decreased interference levels, but the difference with baseline levels was a statistically significant trend [$F(1,18) = 3.50$, $p = 0.08$]. An additional analysis including depressive response as a between subjects factor showed that responders were no more or less affected on the Stroop task than non-responders, neither were there any order effects. Inclusion of the statistical outlier revealed decreased interference levels in the low-dose condition [$F(1,19) = 4.88$, $p = 0.04$] and in the high-dose condition [$F(1,19) = 10.68$, $p = 0.004$] relative to baseline, with no difference between low-and high-dose [$F(1,19) = 1.68$, $p = 0.21$].

Emotional Stroop Task. High- but not low-dose ATD significantly increased interference levels for positive words [$F(1,18) = 15.84$, $p = 0.001$], which was independent of depressive response. ATD had no effect on interference levels for negative words. Post-hoc inclusion of the between-subjects factor 'responder versus non-responder' revealed a main effect of that variable [$F(1,17) = 8.91$, $p < 0.01$] with higher interference levels at baseline for the responders compared to the non-responders (3.7 ± 2.0% versus –0.2 ± 1.8%), but the presence of mood was not related to the extent of cognitive change following ATD. There were no order effects.

Other cognitive outcome measures. There were no main effects of ATD or interaction effects with depressive response on the Left/Right task or TOL. However, an additional analysis using gender as a between subjects factor showed that, relative to low-dose ATD, high-dose ATD improved performance in females but tended to impair performance in males [$F(2,34) = 7.11$, $p = 0.003$]. Changes relative to baseline were not significant.

On the Word Fluency, ATD had no effect on the number of words produced within 1 min. However, a more detailed analysis showed that, within the first 30 s, high-dose ATD significantly increased word production compared to the baseline and low-dose ATD. There were no effects of depressive response or ATD order. ATD had no effect on any of the outcome measures of the APRT.

Influence of minor mood changes on cognition. To investigate the influence of minor mood changes on ATD-induced cognitive performance, the GLM Repeated measures analyses on all cognitive outcome measures were rerun entering the symptom change during high-dose ATD session as a covariate, rather than using a categoric division of responders and non-responders. Overall, the results were very similar: when the responder × intervention interaction was significant (as described above), the significant results disappeared after ΔMADRS was entered as a covariate; when there was no responder × intervention interaction, entering the covariate did not change the results.

Biochemical correlates. Changes in mood or cognitive performance during the high-dose ATD session did not correlate significantly with percentage change of tryptophan, 5-HIAA, tyrosine or HVA. A trend was found for percentage change of 5-HIAA levels and changes of neutral Stroop interference ($r = 0.58$, $p = 0.08$).

Discussion

The present study was successful in creating two ATD dosages, lowering plasma Trp levels by 40–50% (25 g ATD; low-dose) and by 80–90% (100 g ATD; high-dose), as intended.

As expected, high-dose ATD produced a transient return of depressive symptoms in some participants, but not in all (Delgado *et al*., 1999; Spillmann *et al*., 2001). Low-dose ATD did not affect symptoms, despite a 46.8% reduction of Trp levels. The percentage of relapse after high-dose ATD (seven out of 20) is relatively low compared to Delgado *et al*. (1990) but similar to that reported in other studies in SSRI-treated remitted depressed patients (Bremner *et al*., 1997; O'Reardon *et al*., 2004). Patients in the study by Delgado *et al*. (1990) were clinically stable for approximately 4 weeks, whereas patients in the study by Bremner *et al*. (1997) and O'Reardon *et al*. (2004), as well as in the present study, had been in remission for approximately 6 months. As noted previously, differences in relapse rate may be related to the timing of the ATD procedure (Booij *et al*., 2002).

As hypothesized, cognitive changes occurred in patients with and without a depressive response, but the direction of change (improvement versus impairment) was dependent of the valence of stimuli used.

As in healthy volunteers, ATD improved focused attention for neutral stimuli (Park *et al*., 1994; Schmitt *et al*., 2000) and the changes in the present study were dose-dependent. Similarly, performance on working memory tasks (indicated by the TOL and fluency task) also improved slightly following ATD. Improved attentional performance following ATD may be due to the removal of 5-HT inhibiting actions on the DA system in the prefrontal cortex (Schmitt *et al*., 2000). High-dose ATD markedly increased tyrosine and the tyrosine/LNAA ratio, which are also involved in mood and cognition (Booij *et al*., 2003). This finding was unexpected because ATD effects on tyrosine levels have rarely been reported. However, a few other studies have reported rises in tyrosine levels (Carpenter *et al*., 1998; Klaassen *et al*., 1999). In healthy samples, tyrosine administration improved Stroop performance and working memory (Deijen *et al*., 1994), whereas acute phenylalanine tyrosine depletion (APTD) selectively impaired working memory performance (Harrison *et al*., 2004). However, low-dose ATD already tended to improve Stroop interference whereas catecholamine precursors remained largely unaffected. Furthermore, we found no relationship between changes in HVA or tyrosine and cognitive performance. Because tyrosine is also the precursor of noradrenaline, it is possible that improved attentional performance is partly related to enhanced noradrenaline function. It is recommended that the effects of ATD on levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) are measured, in addition to studying APTD and ATD in the same patients.

By contrast to the effects of ATD on neutral material, high-dose ATD increased interference for positive words. Patients became somewhat faster in the neutral and negative word condition, but needed more time to process positive information. Impaired emotional processing was also observed in those individuals who showed no depressive response, which is in agreement with the results of an ATD study in healthy volunteers by Murphy *et al*. (2002), who found slower responses to happy but not sad words. Another study found that a single SSRI administration in healthy volunteers facilitated the recognition of facial expressions of happiness and fear (Harmer *et al*., 2003a).

Because we found no changes in symptoms following low-dose ATD, but some changes in cognitive functioning, these cognitive changes may be more sensitive markers of 5-HT function than symptoms. One possibility is that cognitive changes mediate the relationship between 5-HT and mood However, the findings are also consistent with the idea that cognitive and symptomatic changes occur independently.

The differential effects on attention that were found in the present study (improvement in the processing of neutral information versus impairment in the processing of positive information) may be brought about by different mechanisms. At least two pathways modulate the ATD-induced effects on mood and cognition: the orbital/ventral medial and dorsolateral regions (Bremner *et al*., 1997; Smith *et al*., 1999). The effects of ATD in the ventral neural system may be different in vulnerable and non-vulnerable populations. Using positron emission tomography, a recent study identified different pathways for two emotional processing systems: 'hot' emotional processing (schematical processing; the actual emotional response) and 'cold' emotional processing (propositional processing; the analytical, rational mode) (Schaefer *et al*., 2003). The first was associated with increased activation of the ventromedial and the latter with activation of the anterolateral prefrontal cortex (Schaefer *et al*., 2003). The ventral system may be more closely implicated in 5-HT vulnerability to depression than the dorsolateral system. Imaging studies are needed to test this hypothesis further.

Methodological considerations

An important issue in neuropsychopharmacological studies concerns choosing the optimal design. In the present study, we compared the effects of two interventions with the mean performance during the first and fourth administration of the neuropsychological tests. This is a variant of the conventional pretest–post-test control group design (Neale and Liebert, 1986) but, in this case, the group variable refers only to treatment order. Unlike studies that did not measure of baseline performance during the intervention sessions (Park *et al*., 1994; Gallagher *et al*., 2003), learning effects were minimal or absent. A disadvantage of the present design is that expectancy effects during the 'baseline' sessions may be different from the effects during the depletion sessions. However, differences in cognitive performance between the intake session and the post-intervention session were minimal and, if any, these were observed on different tasks and/or in the opposite direction to those observed at the time that Trp levels were minimized. Moreover, there were no differences between those who received low- or high-dose ATD first. Furthermore, depletion improved performance on neutral tasks. If the test procedure influences cognitive performance negatively (because of expectancy, fatigue, etc.), baseline cognitive performance would have been overestimated,

resulting in smaller differences with the depletion sessions. In other words, the observed effects might have been bigger if a placebo condition had been concluded. Furthermore, a true placebo condition does not exist. Most ATD studies have used a control mixture containing 2.3–5.0 g Trp. This procedure causes a huge rise of Trp levels, varying from 10% to 500% (Weltzin *et al*., 1994; Klaassen *et al*., 1999). However, moderate increments (+45%) of Trp or Trp/LNAA ratio have been shown to affect cognitive performance and symptoms (Markus *et al*., 1998; Schruers *et al*., 2000; Markus *et al*., 2002). Studies making a direct comparison between cognitive performance and symptoms after a Trp-free and Trp-containing mixture may overestimate the difference in effect. Regarding the depressive response, the commonly used placebo procedures have only very rarely resulted in small symptom increases (Van der Does, 2001a), making it unlikely that the present study produced false positive results.

A limitation is that we did not include a control group that received ATD, which did not allow us to investigate the interaction between a history of depression and ATD. Thus, we do not know whether the effects we are looking at are due to ATD itself at different doses, or some interaction between ATD, diagnosis and treatment. On the other hand, most neuropsychological tasks used in the present study have also been used in ATD studies in healthy samples (Schmitt *et al*., 2000; Rubinsztein *et al*., 2001; Sobczak *et al*., 2002), allowing us to make indirect comparisons.

It could be argued that allowing caffeine consumption during the ATD sessions until 1 h before the cognitive tasks comprises a confounding factor because caffeine consumption could influence mood, cognition and catecholamine turnover (Nehlig, 1999; Smith, 2002). On the other hand, caffeine withdrawal probably also influences behaviour during the depletion sessions because withdrawal symptoms such as negative mood, anxiety and cognitive performance generally begin within 12–24 h, and are highest at 20–48 h after cessation (Nehlig, 1999), even in individuals with relatively low or moderate habitual caffeine consumption (Silverman *et al*., 1992; Nehlig, 1999; Smith, 2002). In the present study, cognitive performance during two active sessions was compared with the mean of two baseline, no depletion sessions, sessions in which it was not possible to keep rigid control on caffeine consumption for some hours before administration of the cognitive tasks. In addition, differences on mood, cognition and biochemical measures between the high- and low-dose ATD sessions were significant despite similar caffeine restrictions. Furthermore, if caffeine consumption affect symptoms, it probably occurs after excessive consumption and with the largest effect on anxiety levels (Smith, 2002), which remained unaffected. Hence, it is not very likely that the present effects are confounded by caffeine consumption.

To summarize, the present study has demonstrated that strong reductions induce cognitive changes in patients with and without a depressive response to ATD. Moderate reductions of Trp levels already induce slight cognitive changes, but not a depressive 'relapse'. The comparison of the effects of high-dose and low-dose ATD is useful for other studies aiming to investigate relationships among 5-HT, mood and cognition.

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