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# Effects of tryptophan depletion in fully remitted patients with seasonal affective disorder during summer

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## ABSTRACT

**Background.** Deficiencies in brain serotonin function are believed to play an important role in the pathophysiology of seasonal affective disorder/winter type (SAD). However, no direct evidence has been reported so far that lowered brain serotonin activity causes the symptoms of SAD.

**Methods.** We studied 11 SAD patients who had suffered recurrent winter depressive episodes of SAD and were fully recovered and off treatment during the summer. In a randomized, balanced, double-blind crossover design patients received two amino acid beverages, one containing tryptophan and the other containing no tryptophan but otherwise identical. Behavioural ratings and plasma total and free tryptophan concentrations were assessed at baseline before administration of the amino acid beverages and at several time points afterwards.

**Results.** The tryptophan-free amino acid beverage induced significant decreases of plasma total and free tryptophan levels and both levels increased during sham depletion (condition × time interaction: $P < 0{\cdot}001$). Tryptophan depletion, but not sham depletion caused a transient return of depressive symptoms (condition × time interaction: $P < 0{\cdot}001$).

**Conclusions.** The present study demonstrates that SAD patients in remission during the summer are vulnerable to a return of depression when depleted of tryptophan. This finding supports the importance of serotonergic mechanisms in the pathophysiology of SAD.

## INTRODUCTION

Seasonal affective disorder, winter pattern (SAD), is a condition characterized by the annual reoccurrence of depressive episodes during fall/winter alternating with spring/summer euthymia or hypomania (Rosenthal *et al*. 1984). During recent years there has been interest in which role brain serotonin (5-hydroxytryptamine, 5-HT) systems play in the pathophysiology of SAD (Kasper *et al*. 1996).

Human hypothalamic 5-HT concentrations (Carlsson *et al*. 1980) vary seasonally with lowest levels during winter. Serotonergic mechanisms might be involved in the pathophysiology of some of the so-called atypical symptoms of SAD. Patients with SAD frequently report seasonal changes of appetite and food preference with subsequent weight gain during winter (Rosenthal *et al*. 1987*a*). Carbohydrate ingestion has been reported to energize SAD patients but sedates normal controls (Rosenthal *et al*. 1989). This may represent a behavioural–biochemical feedback loop for compensating a putative abnormality in brain serotonergic function in SAD patients (Fernstrom & Wurtman, 1971).

Further evidence for the involvement of serotonergic systems in SAD can be inferred from the aberrant hormonal (prolactin, cortisol, growth hormone) responses to the administration of the predominantly 5-$HT_2$C receptor agonist meta-chlorophenylpiperazine (m-CPP)

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and the 5-$HT_1$D agonist sumatriptan in untreated SAD patients, which are normalized following light treatment and during summer (Garcia-Borreguero *et al.* 1995; Yatham *et al.* 1997). Another study showed blunted plasma ACTH and norepinephrine responses after administration of m-CPP in SAD patients compared with healthy controls across light-treated condition and untreated condition (Schwartz *et al.* 1997). Abnormal behavioural responses, the most notable being 'activation–euphoria' after application of m-CPP before, but not after, light therapy or during summer, are confined to SAD patients only and are not found in healthy controls (Joseph-Vanderpool *et al.* 1993; Jacobsen *et al.* 1994; Schwartz *et al.* 1997). Serotonergic compounds have been found to be effective in the treatment of SAD (Jacobsen *et al.* 1989; O'Rourke *et al.* 1989; Blashko, 1995; Lam *et al.* 1995). The assessment of cellular serotonergic function showed inconsistent results. Platelet imipramine binding sites have been found to be reduced in SAD patients relative to normal controls and light therapy was reported to increase their number (Szádóczky *et al.* 1991). In contrast, the evaluation of platelet [$^3$H]paroxetine binding to characterize the platelet 5-HT transporter, the 5-HT-stimulated $Ca^{2+}$ response to measure 5-$HT_2$ receptor function in platelets, and measurements of platelet 5-HT content in SAD patients and healthy controls before and after light therapy showed no evidence for platelet serotonergic abnormalities in SAD (Ozaki *et al.* 1994).

The tryptophan depletion (TD) paradigm has been developed to investigate the role 5-HT plays in the pathophysiology of psychiatric disorders and pharmacological and non-pharmacological treatment modalities (Neumeister *et al.* 1997*a*). The administration of an amino acid mixture lacking tryptophan has been shown to induce transient significant decreases of plasma total and free tryptophan levels, which are predicted to cause a reduction in brain 5-HT function since availability of tryptophan in the brain is a rate-limiting step in the synthesis of 5-HT (Gessa *et al.* 1974; Moja *et al.* 1989; Young *et al.* 1989; Delgado *et al.* 1990). Findings from a positron-emission-tomography study in humans using $^{11}$C-$\alpha$-methyl-tryptophan showed a significant reduction of brain 5-HT synthesis after TD (Nishizawa *et al.* 1997).

Healthy controls with a multigenerational family history for major affective disorder reported a greater reduction in mood after TD than healthy controls without a positive family history (Benkelfat *et al.* 1994). Drug-free healthy male subjects experience a mild lowering of mood induced by TD (Young *et al.* 1985; Smith *et al.* 1987). The effects of TD on mood in healthy women are inconsistent since one study (Zimmerman *et al.* 1993) but not another (Oldman *et al.* 1994) reports an increase of depressive symptoms after TD. Interestingly, TD does not induce depressive symptoms in healthy controls treated with fluoxetine (Barr *et al.* 1997), which is in sharp contrast to the TD-induced depressive relapse in formerly depressed patients on selective 5-HT re-uptake inhibitors (Delgado *et al.* 1990).

Our group has been involved in two earlier TD studies to probe serotonergic systems in the pathophysiology of SAD and the mechanism of action of light therapy (Neumeister *et al.* 1997*b*, *c*). We have previously reported on the behavioural responses induced by TD in SAD patients during winter in both untreated and bright light-treated conditions. In this paper we have investigated whether the depressiogenic effects of TD are present in fully remitted SAD patients who were off treatment during the summer. A positive result would suggest persistence of serotonergic vulnerability despite symptomatic improvement.

## METHOD

### Patients

Eleven patients (9 females and 2 males) from our out-patient clinic for SAD were invited to participate in a study investigating the effects of short-term TD in remitted SAD patients during the summer. Before entering the protocol all patients were given oral and written explanations of the study design, which has been approved by the Ethics Committee of Vienna University, and then gave written informed consent for participation. The informed consent form included the information that a transient exacerbation of depressive symptoms may occur during the study procedures. The study period lasted from June until the first week in September 1996. The average age of the subjects was $38{\cdot}5 \pm 11{\cdot}5$ years (mean $\pm$ S.D.). All patients met the Rosenthal

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criteria for SAD (Rosenthal *et al*. 1984), and the diagnostic criteria for major depressive disorder ($N = 3$) or bipolar II disorder ($N = 8$) with seasonal pattern, as defined by the DSM-IV (American Psychiatric Association, 1994). The age at onset of SAD in these patients was $26{\cdot}5 \pm 8{\cdot}0$ years (mean $\pm$ S.D.), the patients reported $10{\cdot}1 \pm 7{\cdot}1$ (mean $\pm$ S.D., range 3–20) previous depressive episodes. At the time of the intervention all participants were fully remitted from depression; four of them reported elevated mood. During the preceding winter all participating subjects had suffered from a depressive episode, for which 6 patients had received light treatment. Of the remaining 5 patients, 3 subjects were untreated during the winter, and 2 received antidepressant medications which they dropped at the latest in March. Screening with routine physical and laboratory examinations, including blood and urine analysis and ECG ensured that all patients were medically healthy. Patients with a history of substance abuse were ineligible to participate. All patients were drug-free for at least 4 months before entering the study.

### Mood ratings

The assessments of mood used the Structured Interview Guide for the Hamilton Depression Rating Scale, Seasonal Affective Disorders Version (SIGH-SAD) (Williams *et al*. 1988). The SIGH-SAD is composed of the 21-item version of the HDRS (Hamilton, 1967) and eight additional items which have been shown to be of particular relevance for SAD (Rosenthal *et al*. 1987*b*). The items concerning sleep, diurnal variation, eating and weight change were omitted since they cannot be meaningfully assessed during the test sessions. For clarity, only total scores of the modified version of the SIGH-SAD are presented in this paper. All patients were remitted for at least 4 weeks when entering the study period. Criteria for remission included a SIGH-SAD score not greater than 12 and having a score less than 8 on the 21-item version of the HDRS. All ratings were performed by an experienced psychiatrist who was blind to the treatment sequence (N. P.-R.).

### Tryptophan depletion procedure

The study used a randomized, balanced, double blind, crossover design. TD was induced by oral administration of a solution of amino acids without tryptophan in the morning of the test day. During sham depletion the amino acid beverage was supplemented with 2·3 g tryptophan. The description of the amino acid beverages is described elsewhere (Delgado *et al*. 1990). The patients and the investigator who administered the amino acid beverage (A.N.) were not able to distinguish the tryptophan-free from the tryptophan-supplemented beverage. The TD session and the sham depletion session were separated by 6–9 days. We decided not to provide the patients with a 24 h low-tryptophan diet before ingestion of the amino acid drink since we tried to keep the burden for the participating patients as low as possible. From a scientific point of view, we did not expect this to result in a significantly greater lowering of plasma TD. Blood samples for the assessment of changes in plasma total and free tryptophan levels, as well as behavioural ratings were taken on day 1 at 8.30 a.m., before ingestion of the amino acid beverages (9.00 a.m.), and 5 and 7 h, after ingestion of the beverages. Patients were allowed to drink water during day 1 but did not eat until about 5.00 p.m. After the final ratings and the blood samples were obtained on day 1 all patients were provided with a low monoamine diet, which they were allowed to take home with them and eat at their regular meal time. Patients were then asked to return to the clinic in the morning of day 2 at 8.30 a.m. At this time point behavioural ratings and a blood sample were taken and immediately afterwards the patients returned to unrestricted dietary intake.

All patients were clinically assessed as being well enough to return home at the end of each depletion procedure. We ensured that the patients were with a family member or close relative. Moreover, it was ensured that patients were able to contact one of the investigators by telephone and in person at any time after discharge if needed.

### Biochemical methods

Blood samples were immediately centrifuged at room temperature and 5000 ***g***. Concentrations of total plasma tryptophan were assessed using high-performance liquid chromatography (HPLC) with fluorometric detection (Anderson *et al*. 1981). For determination of free plasma tryptophan concentrations an ultrafiltrate of plasma through an anisotropic, hydrophilic

ultrafiltration membrane system (Amicon, Witten, Germany) was obtained and subjected to a HPLC with fluorometric detection (Anderson *et al.* 1981).

### Statistical analysis

Changes in plasma total and free tryptophan levels as well as behavioural changes were assessed with a three-way repeated measures analysis of variance (ANOVA) with order of sessions as grouping variable and two intra-subject factors: treatment (TD *v.* sham depletion) and time. The repeated measures ANOVA was Greenhouse–Geisser corrected and corrected *P* values are reported. For clarity, uncorrected degrees of freedom are shown. Results were considered significant when $P < 0{\cdot}05$. Significant interactions revealed by the ANOVA were further examined with Bonferroni corrected paired *t* tests (2-tailed) to determine when significant changes occurred. The adjusted $\alpha$ level for the conducted paired *t* tests is 0·01274. Depressive symptoms exacerbation after ingestion of the amino acid beverage was defined as at least a 50% increase from the day 1, 8.30 a.m. SIGH-SAD score and an HDRS (21-item version) total score of 12 or more. Results are presented as means ± S.D. Analyses were performed using the SPSS/PC, V.7.0.

## RESULTS

### Changes in plasma total and free tryptophan levels

Measurements of plasma total and free tryptophan concentrations at baseline before ingestion of the amino acid beverages disclosed insignificant differences between the TD condition (total tryptophan: 53·2 ± 6·7 μmol/l; free tryptophan: 7·6 ± 1·6 μmol/l) and the sham depletion condition (total tryptophan, 51·0 ± 11·1 μmol/l; free tryptophan, 6·9 ± 1·2 μmol/l). As seen in Fig. 1 TD induced significant decreases of both plasma total and free tryptophan levels, whereas both plasma levels increased during sham depletion (ANOVA treatment × time interaction: total tryptophan, $F(3, 27) = 63{\cdot}60$, $P < 0{\cdot}001$; free tryptophan, $F(3, 27) = 41{\cdot}86$, $P < 0{\cdot}001$). During TD nadir values were found 5 h after ingestion of the amino acid beverage (total tryptophan, 7·3 ± 2·8 μmol/l, 86·3% decrease, $t = 23{\cdot}91$, df = 10, $P < 0{\cdot}001$; and free tryptophan, 0·9 ± 0·6 μmol/l, 88·2% decrease, $t = 14{\cdot}94$, df = 10, $P < 0{\cdot}001$). During sham depletion, both levels increased with peak

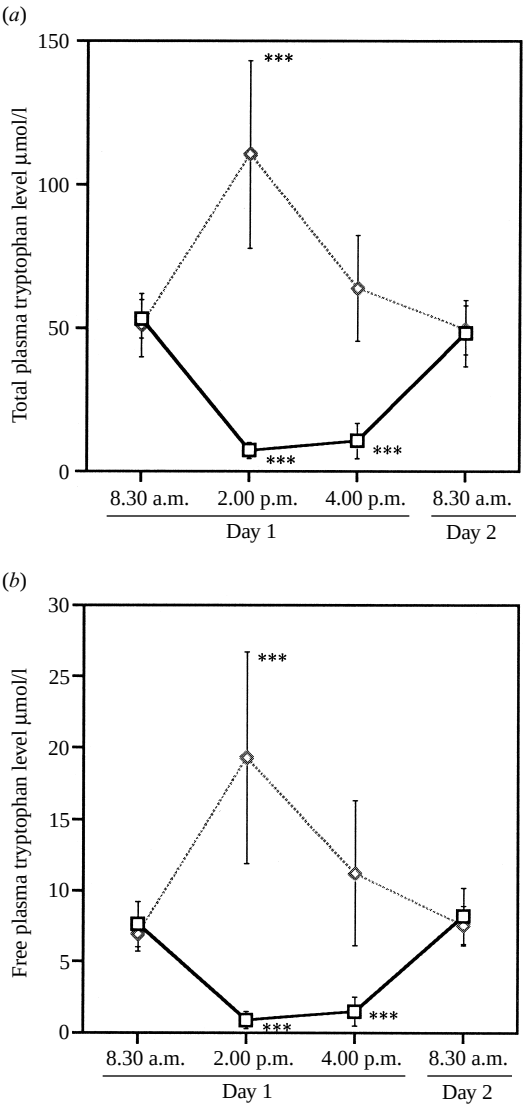


FIG. 1. The effects of tryptophan depletion (□—□) and sham depletion (◇ ·····◇) on plasma total (*a*) and free (*b*) tryptophan levels in fully remitted patients with seasonal affective disorder during summer. During tryptophan depletion plasma total and free tryptophan levels were significantly (*** $P < 0{\cdot}001$, paired *t* test, 2-tailed, Bonferroni-corrected) decreased 5 and 7 h after ingestion of the tryptophan-free amino acid beverage at day 1, 8.30 a.m. During sham depletion plasma total and free tryptophan levels were significantly (*** $P < 0{\cdot}001$, paired *t* test, 2-tailed, Bonferroni-corrected) increased 5 h after ingestion of the tryptophan-supplemented amino acid beverage. Values are means ± S.D.

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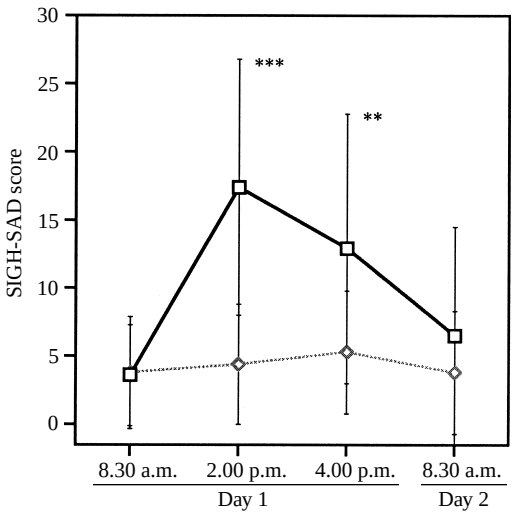


FIG. 2. Depression scores (means ± S.D.) on a modified version (see text) of the Structured Interview Guide for the Hamilton Depression Rating Scale, Seasonal Affective Disorders Version (SIGH-SAD). Tryptophan depletion (□—□), but not sham depletion (◇ ····· ◇) caused a return of depressive symptoms in remitted patients during summer (*** $P < 0{\cdot}001$, ** $P < 0{\cdot}01$, both compared with day 1, 8.30 a.m., paired $t$ test, 2-tailed, Bonferroni-corrected).

plasma concentrations 5 h after ingestion of the tryptophan-supplemented amino acid beverage (total tryptophan, $110{\cdot}5 \pm 32{\cdot}6$ $\mu$mol/l, $t = -5{\cdot}34$, df = 10, $P < 0{\cdot}001$; and free tryptophan), $19{\cdot}3 \pm 7{\cdot}4$ $\mu$mol/l, $t = -5{\cdot}09$, df = 10, $P < 0{\cdot}001$). Twenty-four hours after ingestion of the amino acid beverages the plasma tryptophan concentrations were returned to baseline again with no between-group differences (TD – total tryptophan, $48{\cdot}2 \pm 11{\cdot}6$ $\mu$mol/l free tryptophan, $8{\cdot}2 \pm 2{\cdot}0$ $\mu$mol/l, sham depletion – total tryptophan, $49{\cdot}4 \pm 8{\cdot}6$ $\mu$mol/l, free tryptophan, $7{\cdot}5 \pm 1{\cdot}4$ $\mu$mol/l.

### Behavioural changes

The ANOVA assessing the change of depressive symptoms in fully remitted SAD patients in summer during TD and sham depletion showed significant main effects of condition ($F(1,9) = 27{\cdot}67$, $P < 0{\cdot}001$), and time ($F(3,27) = 13{\cdot}29$, $P < 0{\cdot}001$). The interaction of condition and time of sampling was significant ($F(3,27) = 13{\cdot}92$, $P < 0{\cdot}001$). As seen in Fig. 2 paired $t$ tests showed significant increases from total SIGH-SAD scores obtained before administration of the amino acid beverage (day 1, 8.30 a.m.) compared with SIGH-SAD scores 5 h (day 1, 2.00 p.m.; $t = -5{\cdot}15$, df = 10, $P < 0{\cdot}001$), and 7 h (day 1, 4.00 p.m.; $t = -3{\cdot}58$, df = 10, $P = 0{\cdot}005$) after ingestion of the tryptophan depleting beverage. No significant changes of depression scores were found after administration of the tryptophan-supplemented amino-acid beverage. Eight of the 11 patients met the depressive relapse criteria during TD, no patient relapsed during sham depletion ($\chi^2 = 9{\cdot}63$, df = 1, $P < 0{\cdot}01$, Yates' corrected). In the morning of the next day (day 2, 8.30 a.m.) 6 of the 8 patients were fully recovered again, 2 patients still showed clinically relevant increases of depression scores, but returned to baseline levels within the following 48 h. There was no significant interaction between order by treatment by time indicating that the sequence of testing did not influence the clinical outcome. There was no apparent relationship between the effects of TD on plasma total and free tryptophan levels and changes in the SIGH-SAD total scores.

## DISCUSSION

In the present study TD but not sham depletion induced a transient return of depressive symptoms in the majority of fully remitted patients with SAD off treatment during the summer. Comparing the behavioural effects of TD and sham depletion in winter before and after light therapy, and in summer significant differences were found. TD disrupts the antidepressant effects of bright light therapy in patients with SAD (Lam *et al.* 1996; Neumeister *et al.* 1997*b*). Based on the finding that TD reverses the antidepressant effects of serotonergic, but not noradrenergic antidepressants (Delgado *et al.* 1991), researchers have reasoned that light therapy mediates the antidepressant effects probably involving serotonergic mechanisms. No significant behavioural changes occurred during TD or sham depletion before initiation of light therapy when SAD patients were symptomatically depressed in the winter (Neumeister *et al.* 1997*c*). Comparing the behavioural effects of TD in light therapy-remitted SAD patients during winter and naturally remitted patients during summer it is noteworthy that in the summer the onset of the depressive relapse was earlier and the behavioural effects were more short-lived.

Considering the behavioural effects of TD in

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SAD patients in untreated and light-treated conditions and during summer it can be hypothesized that a serotonergic disfunction plays a key role in the pathophysiology of SAD and that light therapy may compensate for the underlying deficit probably involving serotonergic mechanisms. However, it has to be acknowledged that the present study cannot demonstrate the specificity of this abnormality for SAD since the study does not include control groups of non-seasonal depressives or healthy controls. Interestingly, those subjects who reported elated mood before ingestion of the tryptophan depleting aminoacid beverage became depressed to the same extent as the patients who were euthymic at baseline. It has to be acknowledged however that we did not assess their hypomanic state at baseline using standardized rating instruments and thus cannot absolutely exclude the possibility that their elated mood could have affected both baseline and later mood ratings.

The findings of the present study are consistent with results from two recent studies in non-seasonal depressives (Moreno *et al*. 1996; Smith *et al*. 1997), showing that TD induced transient depressive relapses in fully recovered patients who were not taking antidepressant medications. However, one study using a similar methodology reported no behavioural effects of TD (Leyton *et al*. 1997). The inconsistencies between the noted studies could be explained by the fact that different patient populations have been studied implicating that certain subgroups of depressed patients may be specifically vulnerable to changes in brain 5-HT function. Smith and Moreno and their colleagues studied patients with histories of severe depressive episodes including suicidal thoughts or histories of suicide attempts and patients with a co-morbidity of bulimia nervosa. In contrast, none of the patients in the study by Leyton *et al*. was reported to have had a history of persistent suicidal ideation or suicide attempts during their depressive episodes. It is noteworthy that the patients in our study had no history of suicidality either and also no other axis I diagnosis beside MDD or bipolar II disorder based on DSM-IV criteria. However, it can be speculated that patients with SAD may be specifically susceptible to changes in brain 5-HT function since SAD patients are also believed to be vulnerable to the decrease of brain 5-HT content naturally occurring during winter (Carlsson *et al*. 1980). The question of whether the time since the last depressive episode affects behavioural responses to TD in recovered patients off therapy remains unanswered.

We assessed only plasma tryptophan concentrations and not the other large amino acids (LAA), which compete with tryptophan in the same carrier system for uptake into the brain (Oldendorf, 1973). It was shown that changing the ratio between tryptophan and the LAA as it occurs during TD induces changes in insulin and glucagon metabolism that affect tryptophan uptake and may possess behavioural effects of their own (Baldessarini, 1984). We did not measure these two and other hormones and thus cannot exclude the possibility that other factors besides lowering tryptophan availability also had some influence on the behavioural changes that were found in our study. Moreover, it is noteworthy that we did not find significant correlations between biochemical changes and behavioural measurements. Considering the complexity and the multiple interconnections between various brain neurobiological systems it can be speculated that other neurobiological systems besides the serotonergic may have some effects on the regulation of mood as well.

The use of TD in vulnerable subjects does raise ethical issues. Our informed consent form stated explicitly that during the study a transient recurrence of depressive symptoms may occur. All participating patients were assessed as being well enough to return home after each depletion session and they could reach one of the investigators at any time after discharge if needed. Participants had further clinical assessments the day after discharge. Although the majority of patients experienced a return of the depressive symptomatology during TD none of them needed to contact the investigators after having left the clinic.

Taken together decreased brain 5-HT function induced by TD evoked typical depressive symptoms in fully remitted patients with SAD who were off treatment. Thus, it can be concluded that patients with previous episodes of winter depression remain vulnerable to alterations in brain 5-HT function. Moreover, our data support hypotheses that 5-HT plays a key role in the pathophysiology of SAD.

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