

Effects of Tryptophan Depletion in Drug-Free Depressed Patients Who Responded to Total Sleep Deprivation

Alexander Neumeister, MD; Nicole Praschak-Rieder, MD; Barbara Hefselmann, MD; Oliver Vitouch, MS; Manfred Rauh, MD; Arnd Barocka, MD; Johannes Tauscher, MD; Siegfried Kasper, MD

Background: There is some evidence that sleep deprivation (SD) might exert its antidepressant properties by involving serotonergic mechanisms. We investigated the effects of short-term tryptophan depletion (TD) on depressed patients who responded to a single night of total SD.

Methods: Drug-free depressed inpatients (n=30) were randomly assigned to either TD or sham depletion. Tryptophan depletion was induced by a 24-hour low-tryptophan diet (day 1) followed the next morning by ingestion of a tryptophan-free amino acid mixture (day 2). During sham depletion, the diet and the amino acid beverage were supplemented with tryptophan. Sleep deprivation was performed from day 1 until day 2. Only SD responders received the amino acid beverage the morning after SD. Behavioral ratings and total and free plasma tryptophan levels were obtained before and after the test sessions.

Results: Twenty-two of 30 patients showed a favorable outcome after SD. As predicted, TD significantly lowered total and free plasma tryptophan levels, whereas both levels increased during sham depletion. No acute effects on mood were observed during the day after SD in either treatment group. Unexpectedly, TD, but not control testing, prevented the depressive relapse after the recovery night in most of the patients.

Conclusions: Tryptophan depletion did not reverse the antidepressant effects of SD, but it prevented the relapse beyond a night of recovery sleep. These findings suggest that SD does not act via a single monoamine-related mechanism, but they allow the assumption that TD may induce neurochemical alterations that transiently improve depression.

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THE NEUROTRANSMITTER serotonin (5-hydroxytryptamine [5-HT]) is believed to be involved in the pathophysiology of depressive illness. Lower plasma tryptophan levels,^{1,2} reduced cerebrospinal fluid 5-hydroxyindoleacetic acid levels,³ and decreased platelet 5-HT uptake⁴ indicate diminished 5-HT function in depressed patients. Neuroendocrine challenge studies⁵⁻¹⁰ and brain imaging studies using positron emission tomography¹¹ comparing depressed patients with healthy controls suggest decreased brain 5-HT responsiveness in depression. Moreover, the attenuated hypothermic response following 5-HT_{1A} receptor activation with ipsapiron in unipolar depressed patients¹² supports the hypothesis of serotonergic dysregulation. Brain 5-HT_{2A} receptor density was significantly increased in unmedicated depressed patients¹³ and suicide victims,^{14,15} but not in healthy controls or depressed patients who had recovered with medication.¹³ These data support the importance of serotonergic mechanisms not only in the pathogenesis

of depression, but also in the mechanisms of antidepressant treatments.^{16,17}

Tryptophan depletion (TD), induced by a low-tryptophan diet and a tryptophan-free amino acid mixture, appears to be useful to investigate serotonergic mechanisms in depression. In humans, TD induces a rapid and substantial lowering of total and free plasma tryptophan levels with the lowest tryptophan concentrations 5 hours after ingestion of the amino acid beverage,¹⁸ which would be predicted to cause a reduction in brain 5-HT function because availability of tryptophan in the brain is a rate-limiting step in the synthesis of 5-HT.¹⁹ Tryptophan depletion had moderate mood-lowering effects on a group of healthy male subjects whose depression scale scores were in the high normal range.¹⁸ Subjects with a multigenerational history of major affective disorder show a greater reduction of mood after TD.²⁰ Few acute effects of TD on mood were observed in a group of depressed, symptomatic, unmedicated patients when tryptophan was depleted, but those patients who later responded to a pharmacological treatment experienced an

From the Department of General Psychiatry (Drs Neumeister, Praschak-Rieder, Hefselmann, Tauscher, and Kasper) and the Institute of Psychology (Mr Vitouch), Vienna University, and the Departments of Psychiatry (Dr Barocka) and Pediatrics (Dr Rauh), University of Erlangen, Vienna, Austria.

PATIENTS AND METHODS

PATIENTS

Thirty depressed inpatients (**Table**) participated (6 men and 24 women; mean±SD age, 42.6±15.4 years). On admission to the study, a semistructured clinical interview based on DSM-IV⁴⁰ criteria was administered to determine the nature of present and past psychiatric disorders. Twenty patients had a lifetime diagnosis of major depressive disorder, 6 had a history of bipolar I disorder, and 4 had a history of bipolar II disorder. Comorbid lifetime diagnoses were a history of alcohol abuse (subject had not abused alcohol during the 12 months prior to the study), dysthymic disorder, anxiety disorder, and eating disorder in 1 patient each. All participants had normal blood and urine tests and physical examination results, including normal electrocardiogram results. Female patients underwent a serum pregnancy test. Subjects with a history of substance abuse in the past 6 months and those with medical and neurological disorders were excluded. Eighteen patients were drug free for at least 7 days and 12 patients had never been treated with psychiatric medications. Previous medications of the patients are listed in the Table. Patients who had been previously treated with fluoxetine were excluded from the study. All patients were informed about the study design, which was approved by the Ethics Committee of Vienna University, Vienna, Austria, and written informed consent was obtained.

BEHAVIORAL RATINGS

Behavioral ratings included a modified version of the Hamilton Depression Rating Scale (HDRS) (21-item version).⁴¹ The items concerning sleep, weight loss, and diurnal variation were omitted because they cannot be meaningfully assessed during the day after SD. Only total scores of the modified version of the HDRS are presented. The self-report inventory consisted of a German-language version of the Profile of Mood States,⁴² and a visual analog scale assessing the quality of sleep during the recovery night, which was completed the morning after the recovery night. Objective ratings were obtained by an experienced psychiatrist (N.P.-R.), who was unaware of SD and blinded to challenge type. All scales were administered at baseline (day 1, 8:30 AM), the morning after SD (day 2, 8:30 AM), 5 and 7 hours after intake of the amino acid beverages, and the morning after the recovery night (day 3, 8:30 AM).

SLEEP DEPRIVATION

Total SD (39 hours) was performed from day 1, 7 AM until day 2, 10 PM. During the SD period the patients were free to walk in their room and a medical student closely observed them to ensure compliance. Positive response to SD was defined by at least a 40% decrease of the HDRS total score in the morning after SD (day 2, 8:30 AM) compared with the baseline HDRS score. Patients who did not respond to SD (n=8) did not continue the study protocol after the rating at day 2, 8:30 AM, and their data were not used for statistical purposes. After final behavioral ratings and blood samples were obtained, all patients were treated with a selective 5-HT reuptake inhibitor (paroxetine) with an individual dosage regimen.

TRYPTOPHAN DEPLETION PROCEDURE

The study used a balanced, controlled parallel-group design. Assignment to test condition—either TD or sham depletion—was random and double-blind. Each test sequence consisted of 3 consecutive days: TD was induced by a 24-hour, 160-mg/d, low-tryptophan diet with placebo capsules taken 3 times daily (day 1), followed the next morning (day 2, 9 AM) by ingestion of a tryptophan-free 15-amino acid beverage. During sham depletion, the diet and the amino acid beverage were supplemented with capsules containing 500 mg of tryptophan taken 3 times daily and 2.3 g of tryptophan, respectively. The description of the diet and the composition of the amino acid beverage are documented elsewhere.¹⁶ Blood samples for assessments of total and free plasma tryptophan levels were obtained together with the behavioral examinations. Patients returned to a standardized diet on day 2 at about 5 PM and to unrestricted food intake in the morning of test day 3, after final blood samples and behavioral ratings had been obtained.

BIOCHEMICAL METHODS

Blood samples were immediately centrifuged for 10 minutes at room temperature and 5000g. Total plasma tryptophan levels were assessed using high-performance liquid chromatography with fluorometric detection. For determination of free plasma tryptophan levels, an ultrafiltrate was obtained by centrifuging 1 mL of plasma in an Amicon Centrifuge Filter (Amicon, Witten, Germany) with an anisotropic, hydrophilic ultrafiltration membrane. Tryptophan in the ultrafiltrate was measured by high-performance liquid chromatography with fluorometric detection.⁴³

STATISTICAL ANALYSIS

Biochemical changes and behavioral measures of SD responders were analyzed by a 3-way repeated-measures analysis of variance (ANOVA) using patient group (drug-naïve patients and those patients treated with medications before the washout period) and challenge type (TD vs sham depletion) as between-subject factors and time as a within-subject factor. One important question of the data analysis focused on whether drug-naïve patients reacted differently to the procedure than previously treated patients. In the ANOVA this was manifested primarily in the interaction of patient group × challenge type × time. The interaction of challenge type × time demonstrated the effects of TD in the sample. Deviations from sphericity were adjusted using the Greenhouse-Geiser ϵ test and corrected *P* values for all ANOVAs are reported. Significant interactions were further examined with Bonferroni-corrected paired and unpaired *t* tests. To meet a global α level of .05, the adjusted α level for the conducted paired *t* tests is .006. Depressive symptom exacerbation after ingestion of the amino acid beverage was defined as an at least 50% increase from the day 2, 8:30 AM HDRS total score and a HDRS score of 16 or more. Data, which are presented as mean±SD, were considered significant when *P*<.05. We used SPSS/PC, version 7.0, statistical software for data analysis.⁴⁴

Demographic and Clinical Characteristics

Patient No./ Sex/Age, y	Diagnosis, DSM-IV*	No. of Previous Depressive Episodes	Previous Medication (mg/d)	Drug-free Period, d	Current Depressive Episode, d
Tryptophan-Depletion Group					
1/F/25	BPI	3	Clomipramine (100)	180	60
2/M/23	MDD	2	Drug-naive	...	60
3/F/30	MDD	0	Drug-naive	...	90
4/F/29	MDD	3	Amitriptyline (150)	14	70
5/F/35	MDD	1	Doxepin (100)	10	30
6/M/70	MDD	1	Drug-naive	...	21
7/F/56	BPI	8	Alprazolam (4)	21	100
8/F/24	BPI	1	Drug-naive	...	14
9/F/24	MDD	0	Alprazolam (2)	21	80
10/M/33	BPII	1	Drug-naive	...	14
11/F/54	MDD	1	Drug-naive	...	20
Sham-Testing Group					
1/F/43	BPII	0	Amitriptyline (125)	14	20
2/M/69	MDD	0	Drug-naive	...	40
3/F/25	BPII	2	Doxepin (150)	21	40
4/F/40	BPII	1	Drug-naive	...	60
5/F/33	MDD	2	Drug-naive	...	21
6/F/53	BPI	2	Clopentixol (25)	28	80
7/F/50	MDD	3	Paroxetine (30)	180	30
8/F/55	MDD	5	Citalopram (20)	300	120
9/F/24	MDD	1	Drug-naive	...	20
10/F/40	MDD	5	Amitriptyline (150)	28	120
11/M/59	MDD	4	Propenthidyl (80)	14	200
Sleep-Deprivation Nonresponders					
1/F/69	MDD	5	Clomipramine (75)	180	20
2/F/53	BPI	5	Paroxetine (30)	180	60
3/F/54	MDD	0	Doxepin (100)	21	30
4/F/59	MDD	0	Drug-naive	...	30
5/F/26	MDD	2	Diazepam (10)	14	40
6/F/50	MDD	6	Paroxetine (20)	60	50
7/M/29	MDD	0	Drug-naive	...	180
8/F/41	BPI	4	Citalopram (20)	300	120

*MDD indicates major depressive disorder; BPI, bipolar I disorder; and BPII, bipolar II disorder. Ellipses indicate not applicable.

antidepressant effect the day after TD.²¹ Tryptophan depletion reversed the antidepressant response in formerly depressed patients experiencing a remission of symptoms, a phenomenon closely associated with the concomitant administration of selective 5-HT reuptake inhibitors²² and light therapy.^{23,24}

It is well known that a single night of total sleep deprivation (SD) exerts a rapid and dramatic, albeit usually short-lasting, improvement of mood.²⁵ Several lines of evidence suggest that SD induces a variety of changes in neurobiological variables. Neuroendocrinological examination results indicate increased activity of the hypothalamic-pituitary-thyroid axis²⁶⁻³⁰ with SD. Baseline nonsuppression of cortisol release after dexamethasone administration normalized to a suppressor status in SD responders.^{31,32} Enhanced dopaminergic activity could be hypothesized to be responsible for the decline of prolactin levels after SD.²⁸ Moreover, SD responders showed lower baseline cerebrospinal fluid homovanillic acid levels as compared with nonresponders.^{33,34} The decline of methoxyhydroxyphenylethylglycol levels in the cerebrospinal fluid among SD responders, compared with an increase among nonresponders,^{33,34} indicates changes in noradrenergic functioning during SD, which might be related to the clinical response to the treatment. Indirect

evidence suggests the involvement of serotonergic mechanisms in SD. A blunted prolactin response to fenfluramine was shown to predict a positive antidepressant response to SD.³⁵ The increased prolactin response to intravenous tryptophan after SD compared with a night of undisturbed sleep suggests enhanced 5-HT function, a finding that was confined to female patients only.³⁶ Antidepressants,²⁵ especially those that act primarily on 5-HT systems,^{37,38} or bright light therapy³⁹ prolong the improvement of mood after SD, suggesting a synergism possibly involving serotonergic mechanisms.

We investigated the effects of TD in drug-free depressed patients in remission after a night of total SD. We tested the hypothesis that enhanced serotonergic function mediates the immediate antidepressant effects of SD and expected TD to acutely reverse the post-SD antidepressant effect.

RESULTS

BIOCHEMICAL EFFECTS

Tryptophan depletion reduced total and free plasma tryptophan levels when compared with sham depletion (challenge type \times time: total tryptophan, $F[4,72]=107.50$, $P<.001$; free tryptophan, $F[4,72]=52.45$, $P<.001$). Peak

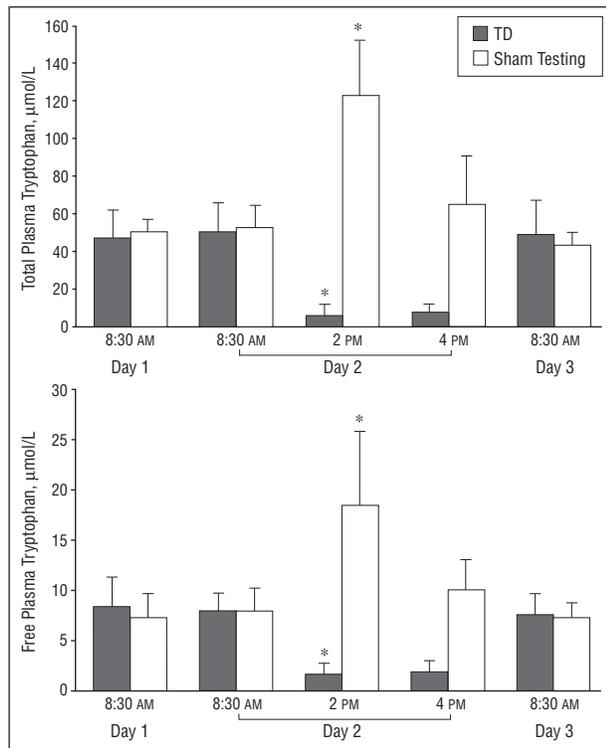


Figure 1. Mean±SD total (top) and free (bottom) plasma tryptophan concentrations during tryptophan depletion (TD) and sham depletion. Peak effects of the depletion procedures were found 5 hours after ingestion of the beverages. All tests are Bonferroni-corrected paired *t* tests (2-tailed), compared with day 1, 8:30 AM. Asterisks indicate $P < .001$.

effects were observed 5 hours after administration of the amino acid beverage (**Figure 1**). Baseline plasma tryptophan levels before beginning the diet did not differ significantly between the TD group (total tryptophan, 49.9 ± 12.8 µmol/L; free tryptophan, 8.1 ± 3.0 µmol/L) and sham depletion group (total tryptophan, 50.3 ± 6.0 µmol/L; free tryptophan, 6.9 ± 2.3 µmol/L). During TD, total plasma tryptophan levels were reduced to 7.6 ± 5.5 µmol/L (85% decrease; $t = 11.23$, $df = 10$, $P < .001$). Sham depletion led to a significant increase of total plasma tryptophan levels (124.2 ± 29.5 µmol/L; $t = -7.80$, $df = 10$, $P < .001$). Free plasma tryptophan levels showed similar directions of changes during TD (1.2 ± 1.0 µmol/L, 85% decrease; $t = 8.03$, $df = 10$, $P < .001$) and sham depletion (18.3 ± 7.3 µmol/L; $t = -5.73$, $df = 10$, $P < .001$). After the recovery night, the tryptophan levels returned to baseline with no significant between-group differences (TD group: total tryptophan, 52.0 ± 15.8 µmol/L; free tryptophan, 7.5 ± 1.8 µmol/L; sham depletion group: total tryptophan, 44.2 ± 5.5 µmol/L; free tryptophan, 7.1 ± 1.6 µmol/L). The previous medication status of the study patients did not influence the outcome.

BEHAVIORAL EFFECTS

Mood

The ANOVA used to analyze changes of HDRS total scores during TD and sham depletion disclosed nonsignificant main effects of patient group and challenge type, but a significant main effect of time ($F[4,72] = 59.38$, $P < .001$), and

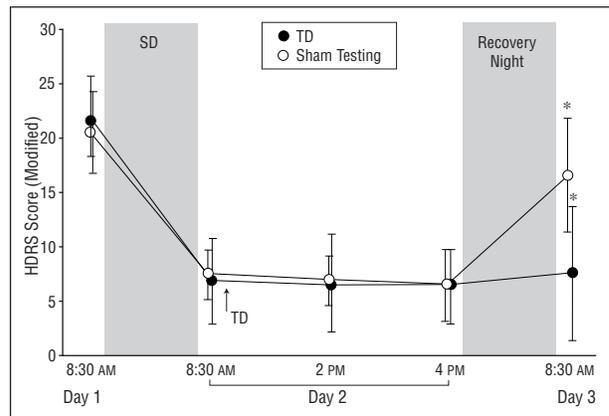


Figure 2. Scores (mean±SD) on a modified version of the Hamilton Depression Rating Scale (HDRS, 21-item version) of depressed patients who responded to a single night of total sleep deprivation (SD). Tryptophan depletion (TD) or sham depletion was performed in the morning after SD. No significant changes of mood occurred on the day of SD (day 2). After the recovery night, patients in the sham depletion group, but not those in the TD group, showed a significant increase of the HDRS total score (asterisks, $P < .001$, Bonferroni-corrected paired *t* test, 2-tailed, compared with day 2, 4 PM). The between-group difference was also significant at this time point ($P = .001$, unpaired *t* test).

a significant challenge type \times time interaction ($F[4,72] = 6.66$, $P < .01$) (**Figure 2**). The unpaired *t* test showed no significant difference between both treatment groups in the morning after SD and before administration of the beverages. No significant changes in mood were observed during day 2 in the TD group and the sham depletion group. In the morning after the recovery night, HDRS total scores were significantly increased in those patients who received the tryptophan-supplemented amino acid beverage, compared with the scores at day 2, 4 PM ($t = -4.69$, $df = 10$, $P < .001$). In contrast, HDRS scores were not significantly changed in the TD group after the recovery night ($t = -0.63$, $df = 10$, $P = .54$). The between-group difference was also significant ($t = -3.84$, $df = 20$, $P = .001$). The evaluation of whether drug-naïve subjects reacted differently compared with those patients who had received medications before the washout period showed a nonsignificant interaction of patient group \times challenge type \times time ($F[4,72] = 0.50$, $P = .61$). Nine (82%) of 11 patients experienced a depressive relapse after a night of recovery sleep during sham depletion compared with 1 (9%) of 11 during TD ($\chi^2 = 8.98$, $df = 1$, $P < .01$; Yates correction). The improvement of mood in the TD group did not persist for longer than 4 days after ingestion of the amino acid beverage and all patients became fully symptomatic again within this period. Eight patients in the TD group and 6 patients in the sham depletion group responded to treatment with paroxetine (defined as $\geq 50\%$ improvement on the HDRS after 8 weeks).

Self-rating Scales

The changes in the depression subscale of the Profile of Mood States (**Figure 3**) showed a nonsignificant main effect of challenge type ($F[1,18] = 1.47$, $P = .24$), a significant effect of time ($F[4,72] = 34.71$, $P < .001$), and a trend towards significance for the challenge type \times time interaction ($F[4,72] = 3.39$, $P = .08$). Challenge type \times time

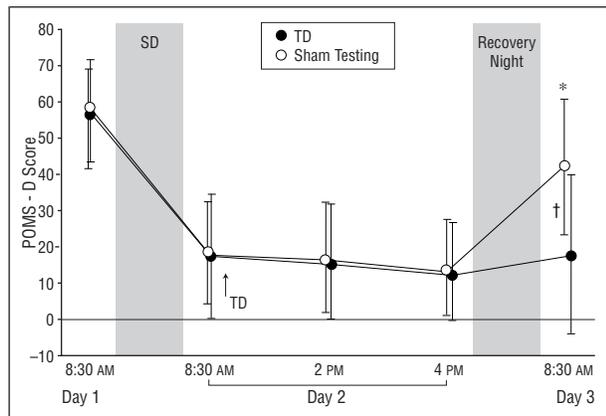


Figure 3. Scores (mean±SD) on the depression subscale of the Profile of Mood States (POMS-D)⁴² of sleep deprivation (SD) responders. No acute behavioral changes were found after intake of the amino acid beverage. After the recovery night, those patients who received the tryptophan-supplemented beverage showed an increase in POMS-D scores (asterisks, $P < .001$, Bonferroni-corrected paired t test, 2-tailed), statistically significantly different from the tryptophan depletion (TD) group (dagger, $P < .01$, unpaired t test).

interactions of the subscales of activity, fatigue, and moroseness were not significant.

Patients' self-ratings on a visual analog scale assessing the quality of sleep during the recovery night did not reveal significant between-group differences.

COMMENT

Short-term TD did not reverse the antidepressant effect of total SD in depressed patients, but it unexpectedly prevented the relapse after 1 night of recovery sleep. If the antidepressant effect of SD was explained by an increase of serotonergic activity alone, then TD would have been believed to rapidly reverse this effect. Therefore, we conclude that the temporary effect of SD does not depend on stable short-term levels of brain 5-HT, and it appears likely that nonserotonergic mechanisms may also be involved in the antidepressant effect of SD.

The mechanisms involved in the TD-induced prolongation of the SD-related improvement of depression warrant some discussion. Tryptophan depletion may have a direct, but delayed, effect on brain 5-HT systems, which could result in a supersensitivity within this system after a period of tryptophan restriction.^{45,46} Alternatively, the transiently diminished availability of 5-HT at 5-HT autoreceptors may increase neuronal 5-HT synthesis and release, which consequently down-regulates postsynaptic 5-HT receptors. Another possibility is that TD-induced changes in the metabolism of melatonin⁴⁷ have prevented the relapse after the recovery night, involving a mechanism possibly similar to that of SD. Also, TD may have influenced other brain neurotransmitter systems, neuropeptides, or second or third messenger systems that may have mediated the prevention of relapse in the TD group. Moreover, it has to be considered that the reduced availability of tryptophan, combined with the supplementation of the large amino acids competing for the same carrier system that transports them across the blood-brain barrier,^{48,49} induces changes in the metabolism of insulin and glucagon and fatty free amino ac-

ids,⁵⁰ which may interfere with the behavioral changes found in our study.

An overall issue in this and prior studies using the TD paradigm is that the administration of the tryptophan-supplemented amino acid beverage leads to significant increases of plasma tryptophan levels, which makes it an active control. Supplementation with tryptophan may interfere with catecholaminergic metabolism⁵¹ and may induce hypothalamic-pituitary-adrenal axis activity, influencing the central noradrenalin and 5-HT control.⁵² Moreover, the question arises whether the increase in plasma tryptophan levels reflects an increase in brain tryptophan and 5-HT content. It has to be considered that brain tryptophan concentrations do not depend only on plasma tryptophan levels, but also on the concentrations of the large amino acids. Animal experimental studies¹⁹ and studies in humans^{53,54} indicate that the increase of total and free plasma tryptophan levels after administration of a tryptophan-supplemented amino acid mixture is not accompanied by an increase of brain tryptophan or brain 5-HT levels. The plasma ratio of tryptophan and large amino acids after ingestion of a tryptophan-supplemented amino acid beverage, comparable to the one we used, suggests that some decrease occurred in tryptophan availability at the carrier, and also possibly a decline in brain tryptophan concentrations.^{53,54} Nevertheless, the depletion of brain tryptophan must have been much greater in that group of patients receiving the tryptophan-free amino acid beverage. Thus, our control treatment to TD was conservative and can be assumed to be a reasonable control because most subjects relapsed after the recovery night, which is the general pattern of mood changes following SD,²⁵ whereas the TD subjects behaved unusually by remaining well.

One important question, raised by the fact that most of our patients were receiving medication prior to entering the study, is whether there were effects of previous drug treatments on the results, because it has been found that antidepressant treatments influence brain 5-HT function and receptors.⁵⁵ However, an analysis of our data showed that it was unlikely that previous medications influenced the results of our study.

Our study replicates findings of a previous study,²¹ which showed that TD had delayed antidepressant effects in those unmedicated depressed patients proving to be antidepressant-responsive. Although both studies were open trials and used different antidepressant medications, the delayed improvement of mood after TD in some depressed patients suggests that these patients might improve with medications that enhance serotonergic neurotransmission.

When one considers the results from the literature and our own findings with regard to the 5-HT hypothesis of the biological mechanism underlying the antidepressant effect of SD, it seems unlikely that changes in the 5-HT system alone mediate the clinical effects of SD and the subsequent outcome. Nevertheless, better understanding the biological processes that induce the antidepressant effects of SD, and the mechanisms of TD that prolong the antidepressant effects of SD, could help to improve the treatment of depressed patients.

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