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Enhanced Serotonin Transporter Function during Depression in Seasonal Affective Disorder

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Decreased synaptic serotonin during depressive episodes is a central element of the monoamine hypothesis of depression. The serotonin transporter (5-HTT, SERT) is a key molecule for the control of synaptic serotonin levels. Here we aimed to detect state-related alterations in the efficiency of 5-HTT-mediated inward and outward transport in platelets of drug-free depressed patients suffering from seasonal affective disorder (SAD). 5-HTT turnover rate, a measure for the number of inward transport events per minute, and tyramine-induced, 5-HTT-mediated outward transport were assessed at baseline, after 4 weeks of bright light therapy, and in summer using a case–control design in a consecutive sample of 73 drug-free depressed patients with SAD and 70 nonseasonal healthy controls. Patients were drug-naive or medication-free for at least 6 months prior to study inclusion, females patients were studied in the follicular phase of the menstrual cycle. All participants were genotyped for a 5-HTT-promoter polymorphism (5-HTTLPR) to assess the influence of this polymorphism on 5-HTT parameters. Efficiency of 5-HTT-mediated inward ($p = 0.014$) and outward ($p = 0.003$) transport was enhanced in depressed patients. Both measures normalized toward control levels after therapy and in natural summer remission. Changes in outward transport showed a clear correlation with treatment response ($\rho = 0.421$, $p = 0.001$). Changes in inward transport were mediated by changes in 5-HTT transport efficiency rather than affinity or density. 5-HTTLPR was not associated with any of the 5-HTT parameters. In sum, we conclude that the 5-HTT is in a hyperfunctional state during depression in SAD and normalizes after light therapy and in natural summer remission.

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INTRODUCTION

A basic tenet of the monoamine hypothesis of depression is that synaptic serotonin is low during major depressive episodes. Lowering of the serotonin precursor tryptophan by dietary tryptophan depletion causes a 30–60% decrease in brain serotonin content in animals and a transient reappearance of depressive symptoms in remitted patients with seasonal (Lam *et al*, 1996; Neumeister *et al*, 1997, 1998) and nonseasonal depression (Delgado *et al*, 1991; Smith *et al*, 1997). A decrease in synaptic serotonin in depression is further suggested by challenge studies with serotonergic agents (Levitan *et al*, 1998; Schwartz *et al*, 1997). Still, the literature proposes few explanations on how the supposed reduction in synaptic serotonin may arise.

The main mechanism controlling synaptic serotonin levels is Na^+/Cl-dependent reuptake into the presynaptic neuron via the 5-HT-transporter (5-HTT, serotonin transporter (SERT); Rudnick and Clark, 1993). Transport can be reversed by manipulating ion gradients (Pifl and Singer, 1999; Sitte *et al*, 2001) or by amphetamine-like substances such as tyramine (TYR; Hilber *et al*, 2005; Schuemann, 1960). The 5-HTT is expressed in the central nervous system (CNS) and peripheral tissues, including blood platelets. It is encoded by a single gene (Lesch *et al*, 1993; Ramamoorthy *et al*, 1993) regulated by a polymorphic promoter region (5-HTT-promoter polymorphism, 5-HTTLPR (Heils *et al*,

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1996; Lesch *et al*, 1996). Brain imaging techniques (positron emission tomography or single photon computed tomography, SPECT) allow for measurement of 5-HTT availability in the living human brain. While some studies suggest reductions in CNS 5-HTT availability in seasonal (Willeit *et al*, 2000) and nonseasonal depression (Malison *et al*, 1998; Parsey *et al*, 2006; Staley *et al*, 2006), recent studies failed to find altered 5-HTT binding (Meyer *et al*, 2004). An important limitation of current brain imaging techniques is that they are confined to quantification of 5-HTT binding sites but do not allow for functional assessments of transport processes.

Investigating maximal binding capacity (B_{max}) of radiolabeled ligands to platelet 5-HTTs is a classical research paradigm in biological psychiatry. Most studies did not screen for seasonality, and results vary, partly due to methodological differences. Studies using [^{3}H]imipramine suggest reduced 5-HTT expression during depression (Owens and Nemeroff, 1994), while binding of radiolabeled selective serotonin reuptake inhibitors seems to be unaltered (D'Hondt *et al*, 1994; Rosel *et al*, 1999) or increased (Neuger *et al*, 1999). A minority of studies (eg Franke *et al*, 2000, 2003; Neuger *et al*, 1999; Stain-Malmgren *et al*, 1998) investigated maximal transport velocity (V_{max}) in nonseasonal depression, with some of them (Neuger *et al*, 1999) reporting decreased V_{max} in female patients. Few investigated V_{max} in direct comparison to B_{max} (Nobile *et al*, 1999). A study in patients with seasonal affective disorder (SAD) showed reduced B_{max} of [^{3}H]paroxetine in patients with SAD, but no significant differences in V_{max} (Stain-Malmgren *et al*, 1998). To our knowledge, as of yet there is no study investigating pharmacologically induced, 5-HTT-mediated outward transport in depression.

This study investigated 5-HTT function in SAD, winter-type (Rosenthal *et al*, 1984), a subform of recurrent major depression (DSM-IV, 1994) where depressive episodes during fall/winter alternate with remission or hypomania during spring/summer. Bright light therapy (BLT) is a biologically active first-line treatment for SAD (Avery, 1998; Eastman *et al*, 1998; Lam *et al*, 2006; Lewy *et al*, 1998; Terman *et al*, 1998; Wirz-Justice, 1998). Since BLT, in contrast to pharmacotherapy, does not directly interfere with 5-HTT function, it offers the opportunity to investigate antidepressant response at the level of 5-HTT without interfering with measurements of 5-HTT parameters.

The two main functional parameters assessed in the present study are 5-HTT-mediated inward and outward transport. Inward transport was assessed by measuring 5-HTT turnover rates, which represent the number of transport events occurring at a 5-HTT molecule per min. Outward transport was assessed by measuring TYR-induced outward transport (E_{TYR}) of the 5-HTT-substrate [^{3}H]1-methyl-4-phenylpyridinium (MPP^+; Cesura *et al*, 1987; Javitch *et al*, 1985). With the exception of pharmacological events associated with stimulant use and abuse, we are not aware of any role for 5-HTT-mediated outward transport in the human brain. However, inward and outward transport provide information on the efficiency of 5-HTT-mediated transmembrane transport. These parameters were determined in drug-free patients with SAD and in healthy control subjects at baseline, after BLT, and in summer.

In view of the evidence for reduced serotonin levels during depression, and the antidepressant effect of 5-HTT blockade, we hypothesized that compared to healthy controls, patients with SAD would display higher 5-HTT turnover rates. Since outward transport is an *in vitro* probe for 5-HTT transport efficiency, we hypothesized that E_{TYR} will be enhanced in SAD patients. Our secondary hypothesis was that remission of depressive symptoms would be associated with a normalization of transport parameters toward control levels. In replication of earlier work (Greenberg *et al*, 1999), we further hypothesized that carriers of the 5-HTTLPR long allele would display increased V_{max} values and significant seasonal variations in V_{max}.

METHODS

Study Sample

The study and recruitment procedures were approved by the Ethics Committee of the Medical University Vienna (MUV). All participants gave written informed consent after full explanation of study procedures.

Patients were recruited at the Outpatient Clinic for SAD, Department of Biological Psychiatry, MUV, after self-referral or referral through their treating psychiatrists or general practitioners. Demographic and clinical information about longitudinal course of illness, previous treatment attempts, and other psychiatric diagnoses was obtained using a semi-structured interview based on DSM-IV (First *et al*, 1996), and by review of medical records and direct contact with previous psychiatrists. Patients were diagnosed by consensus of the authors using DSM-IV criteria for SAD. All patients were either drug-naive or free of psychotropic medication for at least 6 months prior to study inclusion. All study subjects underwent medical screening including physical examination, medical history, and routine hematology. Patients with a history of substance abuse 6 months prior to inclusion, or DSM-IV Axis I disorders other than SAD were not included into the study.

Healthy volunteers were recruited by newspaper advertisements and word of mouth. To avoid selection bias, information about precise study aims was withheld during pre-inclusion phase. The absence of any past or present DSM-IV Axis I disorder was ascertained using the Structured Clinical Interview for DSM-IV nonpatient version (First *et al*, 1996) administered by an experienced rater (NT) and the Structured Interview Guide for the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1967), Seasonal Affective Disorder Version (SIGH-SAD) (Williams *et al*, 1988) consisting of the HDRS and the supplement for atypical depressive symptoms. Control subjects had a negative family history for axis I disorders as ascertained by the family history screen (Weissman *et al*, 2000).

Patients and control subjects were given a German version of the Seasonal Pattern Assessment Questionnaire (Kasper, 1991) to assess the Global Seasonality Score (GSS). Control subjects with a GSS >6 were not included. Within each study year (fall to summer), patients and control subjects were matched as a group for gender and age. For every patient entering the study, a control subject was included within 2 weeks. To minimize variability intro-

duced by the influence of the menstrual cycle on 5-HTT function (Maswood *et al*, 1999), all premenopausal female patients had their visits in the follicular phase of their menstrual cycle. The entire study sample was of Caucasian origin.

Psychopathological Measurements

SIGH-SAD ratings were performed at baseline (first blood sampling), after 2 weeks, after 4 weeks BLT (second blood sampling), and within 4 weeks of 180 days after baseline visit during natural summer remission (third blood sampling). Only patients attaining a baseline SIGH-SAD score of 20 or more were included in the study. All psychopathological ratings were carried out by one and the same experienced psychiatrist (NT).

Bright Light Therapy

All participants were provided with standard BLT devices delivering white light in an intensity of 10 000 lux at a distance of approximately 60 cm. They were instructed to undergo 45 min of BLT shortly after arising from bed. To ensure compliance, all participants were asked to fill out a daily protocol on time and duration of their BLT sessions. Full remission was defined as a maximum posttreatment SIGH-SAD score of 8.

Pharmacological Measures

Blood withdrawal and sample handling. Blood samples (90 ml) were collected by venipuncture (EDTA tubes, 1% wt/vol in saline). All samples were anonymized and prepared for functional 5-HTT testing within 1 h from withdrawal. To control for possible circadian variations in 5-HTT function (Rausch *et al*, 2005), all blood samples were drawn between 8 and 9 a.m. throughout the study. All laboratory staff was fully blinded to the subject's status.

Platelet-rich plasma (PRP) was separated from blood cells by centrifugation (223*g*, Beckman-JS-21, 22°C) and diluted with CO_2-gassed Krebs–Henseleit buffer (KH; 6.92 g NaCl, 0.35 g KCl, 0.29 g $MgSO_4 \times 7H_2O$, 0.16 g KH_2PO_4, 2.1 g $NaHCO_3$, 2.1 g glucose per liter, pH = 7.4). Subsequently, PRP was used for uptake and superfusion experiments, and membranes were prepared for binding experiments. Platelets were isolated from supernatant by centrifugation (1470*g*, Sorvall-GLC-3 centrifuge, 15 min, swing-out rotor, 4°C). The supernatant was recovered as platelet-free plasma (PFP) and re-centrifuged using conditions as above. Platelet pellets were resuspended in KH containing 10% PFP and used in uptake and outward transport assays at 37°C. All experiments were done in triplicate determination.

Inward transport. For uptake assays, resuspended platelet solution (50 µl) was incubated for 3 min with various (5-HT) (0.03, 0.1, 0.3, 1.3, 10 µM unlabeled and [^{3}H]5-HT (Scholze *et al*, 2001); specific activity 21.5–25.8 Ci/mmol; constant (0.03 µM), 500 µl KH). Nonspecific uptake was determined at 10 µM serotonin in the presence of 1 µM paroxetine. Uptake was assessed by using a dilution technique with unlabeled 5-HT to reveal V_{max} and Km values (by recalculating and fitting the background-corrected uptake data to Michaelis–Menten kinetics, with c.p.m. values at the highest [5-HT] being 3–9 times over background). Uptake reaction was stopped as described above.

Binding experiments, turnover rates, and blood 5-HT content. For binding experiments, PRP was centrifuged (1659*g*, 15 min, Beckman-JS-21, swing-out rotor, room temperature), plasma was removed subsequently. Platelet pellets were stored at −80°C for up to 2 months. Pellets were resuspended in 3 ml of KH (without glucose) and centrifuged (19 510*g*, 10 min, 4°C) and resuspended in 1 ml ice-cold HME buffer (25 mM HEPES NaOH (pH 7.5), 2 mM $MgCl_2$, 1 mM EDTA) and centrifuged (19 510*g*, 10 min, 4°C). The pellet was resuspended in 0.5 ml HME (4°C) and subjected to a freeze-thaw cycle (liquid nitrogen) with subsequent sonication (40%, 20 pulses). Membranes were centrifuged (19 510*g*, 10 min, 4°C). Pellets were resuspended in KH (stored after snap freezing in liquid nitrogen, −80°C). Membranes were incubated in KH (500 µl, 22°C) containing [^{3}H]β-CIT ((1*R*,2*S*,3*S*,5*S*)-3-(4-iodophenyl)-8-[^{3}H]methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid; 0.01, 0.03, 0.1, 0.3, 1.3, 10 nM, specific activity 60– 80 Ci/mmol). Nonspecific binding was determined in the presence of 3 µM paroxetine. After 60 min, 1 ml ice-cold KH was added and filtration through Whatman GF/C-filters at 4°C (presoaked with 0.3% polyethyleneimine) performed. Protein concentration of platelet samples was measured with a Pierce BCA kit (Pierce, Rockford, IL); V_{max} and B_{max} values were normalized to the protein content of the platelet sample. To obtain turnover rates, V_{max} of [^{3}H]serotonin and B_{max} of [^{3}H]β-CIT were determined in separate, though parallel measurements. Blood 5-HT content in PRP and PFP was determined using an ELISA kit (RE 591 21; IBL Hamburg, Germany). In brief, a 50 µl sample was acylated, centrifuged, and incubated together with control plasma and standards into 96-well plates. A total of 50 µl 5-HT biotin and 50 µl anti-sera were mixed and incubated overnight (4°C). Enzyme conjugate and *p*-nitrophenyl-phosphate (PNPP) substrate solution were added and incubated for 1 h. The reaction was stopped with PNPP stop solution. Optical density was then measured using an Anthos Elisa plate reader at 405 nm.

Outward transport. Resuspended platelets were incubated with [^{3}H]MPP^+ for 1 h. In contrast to [^{3}H]5-HT, [^{3}H]MPP^+ is neither metabolized nor diffusible and thus gives a high signal-to-noise ratio (Scholze *et al*, 2001). Subsequently, platelet solution was re-centrifuged using conditions as described above, washed with prewarmed KH to remove residual radioactivity, and resuspended in KH. Aliquots were used in outward transport assays. Efflux was induced by addition of TYR, and to ensure the specific, 5-HTT-mediated nature of the efflux, by addition of the Na^+/H^+ ionophore monensin (E_{MON}) (Scholze *et al*, 2000), and a mixture of both ($E_{TYR/MON}$; final concentrations 100 and 1 µM, respectively). Reactions were stopped by addition of 1 ml ice-cold KH and immediate centrifugation (4°C, Sorvall-GLC-3, 1470*g*).

Data calculation for experimentally determined values. Data were adjusted for blank values and fitted to hyperbolic saturation isotherms (uptake, binding) using Prism

(GraphPad, San Diego, CA). Turnover rates were calculated by dividing V_{max} by B_{max} in each sample (1 per min).

Genotyping. Genomic DNA was extracted from leukocytes or whole blood according to standard procedures. Polymerase chain reaction amplification was performed by using the primers and procedures described by Cook *et al* (1997). PCR products were separated on 3% agarose gels and visualized by ethidium bromide staining.

Chemicals. Chemicals were obtained from following the companies: [^{3}H]MPP$^+$ and [^{3}H]serotonin, PerkinElmer Life Sciences Products (Boston, MA); [^{3}H]β-CIT, Amersham Biosciences Europe GMBH (Vienna, Austria); TYR, monensin and serotonin, Sigma-Aldrich Handels GmbH (Vienna, Austria); MPP$^+$, RBI/Sigma (Natick, MA); Paroxetine-HCl was a generous gift from GlaxoSmithKline Pharma GmbH, Vienna, Austria. All other chemicals were from commercial sources.

Statistical Analysis

Nonparametric tests were used since some of the variables did not meet assumption of normal distribution at some time points. Group differences between matched patients and control pairs and within-subject changes after BLT were assessed using Wilcoxon rank-sum tests and the corresponding 95% confidence intervals (CIs) in the location parameter. Circannual changes in 5-HTT parameters (before BLT, after BLT, summer) were tested for significance using Friedman tests for related variables. Nonparametric correlations were calculated between 5-HTT parameters and psychopathological ratings. Mann–Whitney *U*-tests were used to compare patients who remitted on BLT to BLT nonresponders. Wilcoxon signed-ranks tests were used for a group analysis of changes in 5-HTT parameters in BLT full-, partial-, and nonresponders. To exclude a significant influence of sex and age (Neuger *et al*, 1999; Staley *et al*, 2006) onto the main findings, an analysis of variance (ANOVA) was calculated for the two main outcome parameters before BLT using sex and age as covariates. Adjustment for multiple testing by the factor 2 was applied for the two main outcome measures (E_{TYR} and 5-HTT turnover rate), *p*-values below 0.025 were considered to be statistically significant.

RESULTS

Study Sample and Response to Treatment

Of 415 patients visiting at the outpatient clinic for SAD between fall/winter 1999 and spring 2002, 251 met DSM-IV criteria for SAD. Of these patients, 178 did not meet inclusion criteria because they were (1) taking psychotropic medication at inclusion or within 6 months prior to inclusion (136); (2) had relevant comorbid medical, neurological, or psychiatric conditions (31); (3) or were unwilling to consent to the study protocol (11). Data of 73 patients (49 females, 24 males, age 38.70 ± 13.1 years, mean ± SD) and 70 healthy controls (50 females, 20 males, age 39.23 ± 13.4 years) entered final analysis. Six patients dropped out before study visit 2, leaving 67 for analysis at week 4. Another 16 dropped out before visit 3, leaving 51 patients who completed the full protocol. Reasons for premature study termination were initiation of antidepressant drug therapy ($n = 18$), antihypertensive/antiaggregant therapy ($n = 1$), and withdrawal of consent ($n = 3$). All but two control subjects completed the full protocol.

After 4 weeks BLT, SIGH-SAD scores dropped from 25.71 ± 5.0 to 11.33 ± 6.9 (paired sample *t*-test: two-tailed $t = 14.8$, d.f. = 66, $p < 0.001$) in patients with SAD and, in line with earlier findings (Partonen and Lonnqvist, 2000),

Table 1 Alterations in Serotonin Transporter Inward and Outward Transport in Patients with SAD

	Baseline		After bright light therapy		Summer		Effects of time
	Mean ± SD	N[a]	Mean ± SD	N[a]	Mean ± SD	N[a]	p[b]
Tyramine-induced MPP+ Outward-transport (% baseline)							
Patients with SAD	52.33 ± 17.1	66	48.81 ± 14.6	58	49.20 ± 13.4	48	0.245
Healthy controls	42.56 ± 19.4	66	51.33 ± 17.8	67	48.28 ± 17.2	64	0.190
95% CI of group difference	17.2–3.4		3.2 to −8.3		7.0 to −5.0		
Between-group difference: (p[c])	0.003		0.33		0.69		
Serotonin transporter turnover rates (1/min)							
Patients with SAD	328.4 ± 367	61	244.4 ± 217	61	127.5 ± 78	43	<0.001
Healthy controls	192.5 ± 158	66	213.9 ± 202	63	177.4 ± 165	64	0.356
95% CI of group difference	234–38		105 to −44		−2 to −97		
Between-group difference (p[c])	0.014		0.44		0.04		

Abbreviations: MPP$^+$, [^{3}H]1-methyl-4-phenylpyridinium; SAD, seasonal affective disorder.
[a]Differences in group size between MPP$^+$ outward transport and 5-HTT turnover rates due to experimental failures; differences between baseline and other visits due to study dropouts.
[b]Friedman test for *n*-paired groups.
[c]Wilcoxon rank-sum test.

from 0.71 ± 1.2 to 0.44 ± 1.0 in healthy controls ($t = 2.29$, d.f. $= 69$, $p < 0.025$). In summer, SIGH-SAD scores dropped to 3.43 ± 4.1 in patients with SAD (week 4 *vs* summer: $t = 7.23$, d.f. $= 50$, $p < 0.001$) and to 0.18 ± 0.4 in controls ($t = 2.43$, d.f. $= 67$, $p < 0.018$).

Criteria for full remission were met by 27 patients after 4 weeks of BLT, another 19 patients showed reductions in SIGH-SAD scores of more than 50%. Twenty-one patients did not experience relevant changes in SIGH-SAD scores and were classified as BLT nonresponders.

Primary Outcome Measures

5-HTT turnover rates. Overall 5-HTT turnover rates were in good agreement with previously published data (Gu *et al*, 1994). At baseline, patients with SAD had significantly higher baseline 5-HTT turnover rates than healthy controls. No significant differences between patients and controls were seen after BLT or in summer. Turnover rates showed significant variation over the three time points in patients but not in controls (Table 1, Figure 1a). An ANOVA confirmed group differences between patients and controls ($F(1) = 8.228$, $p = 0.005$) but failed to show significant effects of sex ($F(1) = 2.565$, $p = 0.112$) or age ($F(1) = 0.124$; $p = 0.725$).

E_{TYR}. At baseline, patients with SAD displayed significantly higher E_{TYR} than control subjects. No significant group differences were seen after BLT and in summer. There were no significant fluctuations in E_{TYR} over the three time points in either patients or controls (Table 1, Figure 1b). An ANOVA performed to exclude significant effects of sex and age onto the findings confirmed group differences in E_{TYR} between patients and controls ($F(1) = 9.552$, $p = 0.002$) but failed to show significant effects of sex ($F(1) = 0.388$, $p = 0.535$) and age ($F(1) = 0.020$; $p = 0.889$).

Secondary Outcome Measures

[^{3}H]5-HT uptake velocity and [^{3}H]β-CIT-binding capacity. For a more detailed characterization of group differences and seasonal variation in 5-HTT turnover rates, separate *post hoc* analyses of [^{3}H]serotonin uptake and [^{3}H]β-CIT-binding experiments were performed.

V_{max}: Compared to healthy controls, patients had significantly elevated values for maximal [^{3}H]5-HT uptake velocity at baseline (mean V_{max} (pmol/μg/min) 5.76 ($n = 64$) *vs* 4.86 ($n = 68$), 95% CI_{diff} 1.78–0.21, Wilcoxon rank-sum test: $p = 0.008$). There were no significant differences in V_{max} after BLT (3.50 ($n = 63$) *vs* 2.98 ($n = 64$), 95% CI_{diff} 1.13–0.32, $p = 0.28$) or in summer (2.04 ($n = 43$) *vs* 2.58 ($n = 64$), 95% CI_{diff} 0.31–0.84, $p = 0.41$). V_{max} showed significant variation over the three time points in patients (Friedman's $\chi^2(2) = 21.714$, $p < 0.001$) and in healthy controls (Friedman's $\chi^2(2) = 17.803$, $p < 0.001$; Figure 1c).

No significant group differences were found in Km (Figure 1d), a correlate to the 5HT concentration at which the transport rate $V = V_{max}/2$ and thereby a measure for the apparent substrate affinity (mean Km (μM) baseline: 0.303 ($n = 64$) *vs* 0.232 ($n = 68$), 95% CI_{diff} 0.05–0.003, $p = 0.08$; after BLT: 0.335 ($n = 63$) *vs* 0.306 ($n = 64$), 95% CI_{diff} 0.07–0.01, $p = 0.11$; summer: 0.305 ($n = 43$) *vs* 0.232 ($n = 64$), 95% CI_{diff} 0.05–0.002, $p = 0.32$).

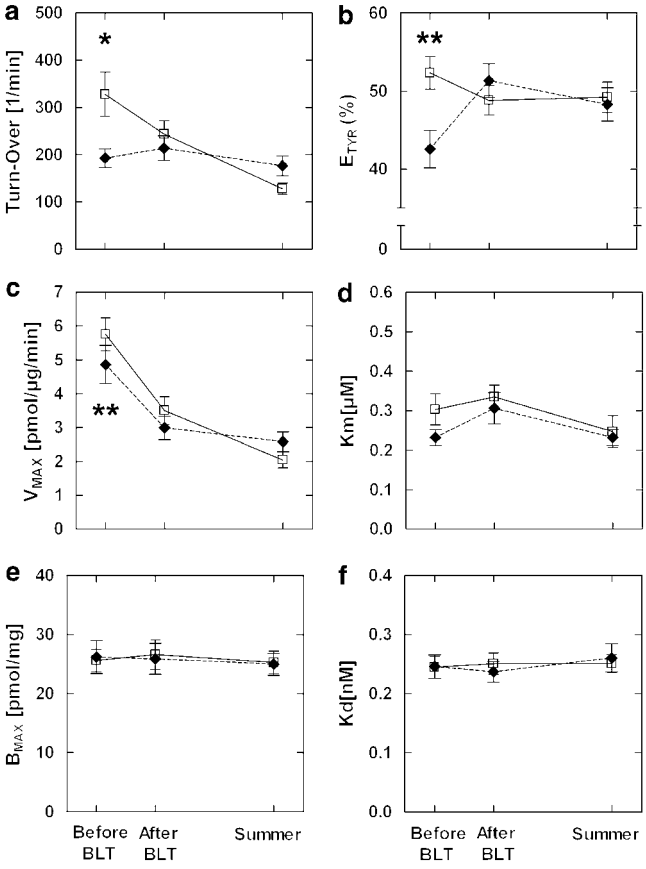

Figure 1 (a) Serotonin transporter (5-HTT) turnover rates in patients with SAD ($n = 61$ before bright light therapy, BLT) and healthy control subjects before and after 4 weeks of BLT and in natural summer remission (SAD: empty squares, $n = 61$ before BLT; healthy control subjects: filled diamonds, $n = 66$ before BLT). * Group comparison between patients with SAD and healthy control subjects before BLT; Wilcoxon rank-sum test, $p = 0.014$. (b) TYR-induced [^{3}H]MPP^+ outward transport (E_{TYR}) in platelets of patients with SAD and healthy subjects before and after 4 weeks of BLT and in summer (SAD: $n = 66$ before BLT; healthy control subjects: $n = 66$ before BLT; legend same as in graph (a)). ** Group comparison between patients with SAD and healthy control subjects before BLT; Wilcoxon rank-sum test, $p = 0.003$. (c) Maximal [^{3}H]serotonin uptake capacity (V_{max}) in platelets of patients with SAD ($n = 64$ before BLT) and healthy controls ($n = 68$ before BLT; legend same as in graph (a)). ** Group comparison before BLT; Wilcoxon rank-sum test, $p = 0.008$. (d) Km of [^{3}H]serotonin uptake in platelets of patients with SAD ($n = 64$ before BLT) and healthy controls ($n = 68$ before BLT; legend same as in graph (a)). (e) Maximal binding capacity (B_{max}) of the 5-HTT ligand [^{3}H]β-CIT in patients with SAD ($n = 63$ before BLT) and healthy controls ($n = 67$ before BLT; legend same as in graph (a)). (f) Kd of [^{3}H]β-CIT binding to platelets in patients with SAD ($n = 63$ before BLT) and healthy controls ($n = 67$ before BLT; legend same as in graph (a)).

B_{max}: There were no significant differences between patients and controls in maximal [^{3}H]β-CIT-binding capacity at any point in time (mean B_{max} (pmol/mg) baseline: 25.6 ($n = 63$) *vs* 26.1 ($n = 67$), 95% CI_{diff} 4.1–5.1; Wilcoxon rank-sum test: $p = 0.91$; after BLT: 26.6 ($n = 63$) *vs* 25.9 ($n = 65$), 95% CI_{diff} 3.8–4.2, $p = 0.84$; summer: 25.2 ($n = 44$) *vs* 24.9 ($n = 64$), 95% CI_{diff} 4.2–2.8, $p = 0.69$; Figure 1e). Likewise, no significant group differences were

found in 5-HTT affinity for [^{3}H]β-CIT (mean Kd (nM) baseline: 0.245 ($n=63$) *vs* 0.246 ($n=67$), 95% CI_{diff} 0.05–0.03, $p=0.77$; after BLT: 0.251 ($n=63$) *vs* 0.237 ($n=65$), 95% CI_{diff} 0.06–0.03, $p=0.52$; summer: 0.221 ($n=44$) *vs* 0.260 ($n=64$), 95% CI_{diff} 0.02–0.04, $p=0.38$; Figure 1f). B_{max} did not vary significantly over the three time points in patients (Friedman's $\chi^2(2)=4.512$, $p=0.105$) or healthy controls (Friedman's $\chi^2(2)=2.381$, $p=0.304$).

$E_{MON}/E_{TYR/MON}$: In both groups, magnitude of monensin-induced MPP^+ outward transport was similar to that induced by TYR. Combined TYR/monensin-induced outward transport was approximately 50% higher than either E_{TYR} or E_{MON}. This is in good agreement with a specific, carrier-mediated, saturable outward transport through the 5-HTT. Although effect sizes were somewhat smaller for E_{MON} and $E_{TYR/MON}$, the pattern of alteration was similar in all three groups of outward transport experiments. At baseline, E_{MON} was significantly elevated in patients compared to controls (mean E_{MON} (%): 55.95 ($n=66$) *vs* 49.06 ($n=66$), 95% CI_{diff} 13.9–3.4, Wilcoxon rank-sum test: $p=0.04$). $E_{TYR/MON}$ was trend-wise elevated (mean $E_{TYR/MON}$ (%): 75.67 ($n=68$) *vs* 70.54 ($n=65$), 95% CI_{diff} 9.1–0.1, $p=0.052$). There were no significant differences between patients and controls after BLT (mean E_{MON}: 52.96 ($n=58$) *vs* 55.66 ($n=66$), 95% CI_{diff} 3.1–9.0, $p=0.35$; mean $E_{TYR/MON}$: 76.58 ($n=57$) *vs* 74.28 ($n=67$), 95% CI_{diff} 5.5–1.3, $p=0.19$) or in summer (mean E_{MON}: 54.54 ($n=46$) *vs* 53.33 ($n=65$), 95% CI_{diff} 7.4–6.5, $p=0.98$; mean $E_{TYR/MON}$: 80.93 ($n=46$) *vs* 77.53 ($n=63$), 95% CI_{diff} 5.3–2.6, $p=0.55$).

Blood 5-HT levels: Blood 5-HT levels were a good reflection of 5-HTT efficiency measures, with highest platelet 5-HT levels in depressed patients with SAD, and low levels in summer. 5-HT content in PFP showed the opposite pattern. However, differences between patients and controls did not reach significance for PRP 5-HT content (mean PRP 5-HT content ($\mu g/\mu g_{Prot}$) baseline: 6.7 ($n=61$) *vs* 5.4 ($n=68$), 95% CI_{diff} −0.9–3.4, Wilcoxon rank-sum test: $p=0.08$; after BLT: 5.9 ($n=61$) *vs* 5.0 ($n=66$), 95% CI_{diff} −0.9–2.8, $p=0.44$; summer: 4.0 ($n=40$) *vs* 4.8 ($n=57$), 95% CI_{diff} −2.1–0.4, $p=0.51$) and PFP 5-HT content (mean PFP 5-HT content (ng/ml) baseline: 9.5 ($n=65$) *vs* 11.4 ($n=66$), 95% CI_{diff} −4.6–0.9, $p=0.24$; after BLT: 9.3 ($n=61$) *vs* 11.8 ($n=64$), 95% CI_{diff} −5.5–0.5, $p=0.12$; summer: 11.4 ($n=47$) *vs* 12.9 ($n=62$), 95% CI_{diff} −5.6–2.5, $p=0.31$). In good agreement with seasonal changes in 5-HTT transport efficiency, PRP 5-HT content showed significant seasonal fluctuations both in patients (Friedman's $\chi^2(2)=13.1$, $p=0.001$) and controls (Friedman's $\chi^2(2)=11.2$, $p=0.004$).

Response to Treatment

To assess a possible relationship between 5-HTT function and response to BLT, rank correlations were calculated between posttreatment SIGH-SAD scores and pre- and posttreatment E_{TYR} and 5-HTT turnover rates in patients with SAD. While no significant correlations were found between pretreatment 5-HTT parameters and posttreatment SIGH-SAD scores, both, posttreatment E_{TYR} (Spearman's ρ: 0.392, $p=0.002$, $n=58$) and 5-HTT turnover rates (Spearman's ρ: 0.296, $p=0.021$, $n=61$) correlated positively with depression ratings after BLT, with lower posttreatment E_{TYR} and lower 5-HTT turnover rates being associated with better response to BLT. There was also a significant correlation between relative reductions in SIGH-SAD scores calculated as ((pretreatment SIGH-SAD−posttreatment SIGH-SAD)/pretreatment SIGH-SAD) × 100) and relative changes in E_{TYR} (calculated as above; Spearman's ρ: 0.421, $p=0.001$, $n=58$), indicating greater E_{TYR} reductions in patients with better response to BLT. This was confirmed in a group analysis showing a drop in E_{TYR} in BLT responders (Wilcoxon signed-ranks test: $z=-2.0$, $p=0.046$, $n=24$), but not in partial ($z=-0.57$, $p=0.57$, $n=15$) or nonresponders ($z=-0.12$, $p=0.91$, $n=17$; healthy controls showed an increase in E_{TYR} after BLT; $z=-2.584$, $p=0.01$, $n=64$).

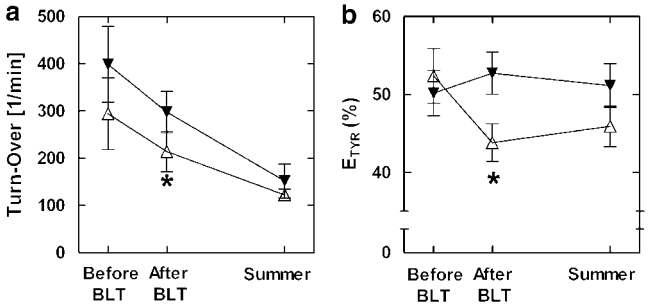

Figure 2 (a) Serotonin transporter (5-HTT) turnover rates in a subgroup of patients achieving full remission of depressive symptoms after 4 weeks of BLT (empty triangles; $n=26$ after BLT) and in BLT nonresponders (filled triangles; $n=19$ after BLT). * Group comparison after BLT; Mann–Whitney *U*-test, $p=0.022$. (b) TYR-induced [^{3}H]MPP^+ outward transport (E_{TYR}) in a subgroup of patients achieving full remission of depressive symptoms after BLT ($n=25$ after BLT) and in BLT nonresponders ($n=17$ after BLT; legend same as in graph (a)). * Group comparison after BLT; Mann–Whitney *U*-test, $p=0.029$.

Posttreatment 5-HTT turnover rates in full responders were lower than in BLT nonresponders (21.39 ± 21.4, $n=26$ *vs* 29.76 ± 18.9, $n=19$; Mann–Whitney *U*-test, $z=-2.298$, $p=0.022$; Figure 2a). Similar results were obtained for E_{TYR} (43.82 ± 11.9, $n=25$ *vs* 52.72 ± 11.8, $n=17$; Mann–Whitney *U*-test, $z=-2.178$, $p=0.029$; Figure 2b). No significant differences between remitters and nonresponders were found at baseline and in summer (Figure 2a and b; data not shown).

5-HTTLPR and 5-HTT Function

Genotype distribution did not deviate significantly from Hardy–Weinberg equilibrium in patients ($\chi^2(1)=0.24$, $p>0.05$) or controls ($\chi^2(1)=0.28$, $p>0.05$). In accordance with previous results (Johansson *et al*, 2003; Willeit *et al*, 2003), 5-HTTLPR genotype distribution did not differ significantly between patients with SAD and healthy controls (patients: *ll*: 22; *ls*: 38; *ss*: 13; controls: *ll*: 26; *ls*: 35; *ss*: 9; $\chi^2(2)=1.121$; $p=0.571$). An earlier study reported significant seasonal variations in serotonin uptake velocity in 5-HTTLPR *ll* homozygous subjects only (Greenberg *et al*, 1999). To replicate and extend this finding to other 5-HTT parameters, we performed analyses of variance separately in

patients and controls using E_{TYR}, 5-HTT turnover rates, B_{max}, V_{max}, and 5-HT levels in PRP and PFP as dependent variables and season (baseline *vs* summer) and the three genotypic groups as fixed factors. While the above-described influence of season on 5-HTT turnover rates and maximal 5-HT uptake velocity was confirmed in this analysis, the factor genotype (see also Lim *et al*, 2006) and the interaction terms genotype × season did not reach significance in any of the analyses (data not shown).

DISCUSSION

In this study, we provide, for the first time to the best of our knowledge, a comprehensive measurement of the forward and reverse 5-HTT transport modes in SAD. The present data show that 5-HTT turnover rates were elevated during depression, reduced toward control values after BLT, and slightly below control values in natural summer remission (Figure 1a). Likewise, TYR-induced outward transport of MPP^+ via the 5-HTT is elevated in platelets of depressed patients with SAD (Figure 1b). Patients and control subjects did not differ anymore in E_{TYR} after 4 weeks of BLT and in natural summer remission (Table 2).

Separate analysis of V_{max} and B_{max}, the two parameters used to determine 5-HTT turnover rates, showed that changes in serotonin uptake were mainly due to changes in V_{max} (Figure 1c). B_{max}, a measure for the amount of 5-HTT expressed in platelets, did not differ significantly between patients and control subjects at baseline, and it remained remarkably stable over all three time points in both groups (Figure 1e). Our methodology was not suited to discriminate 5-HTTs expressed on the platelet surface from intracellularly retained transporters. However, as shown by recent evidence, the preparation method used to produce platelet membranes reveals cell surface 5-HTTs rather than intracellularly retained 5-HTTs (Carneiro and Blakely, 2006). There were significant correlations between 5-HTT turnover numbers and E_{TYR} at baseline ($\rho = 0.271$, $p = 0.003$) but not at later time points (after BLT: $\rho = 0.106$, $p = 0.254$; summer: $\rho = -0.034$, $p = 0.737$). This suggests that efficiency of TYR uptake via the 5-HTT contributes to efflux measures, although not as the sole determinant.

Pretreatment 5-HTT turnover rates were highest in BLT nonresponders, somewhat lower in BLT responders, and lowest in healthy control subjects (Figures 1a and 2a). Patients who fully responded to treatment showed a drop in E_{TYR} after 4 weeks of BLT, while nonresponders showed virtually no change in E_{TYR} after BLT (Figure 2b). There were significant positive correlations between posttreatment SIGH-SAD scores and E_{TYR} and 5-HTT turnover rates, and significant positive correlations between changes in E_{TYR} and reductions in depression ratings after BLT. Before BLT, 5-HTT turnover numbers and E_{TYR} correlated significantly in patients achieving full remission ($\rho = 0.624$, $p = 0.002$), at a trend level in partial responders ($\rho = 0.482$, $p = 0.069$), while there was no correlation in nonresponders ($\rho = 0.083$, $p = 0.729$). The 5-HTTLPR polymorphism was not associated with any of the functional 5-HTT parameters, and in contrast to some (Greenberg *et al*, 1999; Patkar *et al*, 2003), but not other findings (Javors *et al*, 2005), our data failed to show an influence of 5-HTTLPR on seasonal variations in V_{max} or other 5-HTT parameters.

The factors influencing alterations in E_{TYR} in patients with SAD are currently unknown. With a posttreatment drop in BLT responders, an increase after BLT in healthy controls, and no change in BLT nonresponders (Figures 1b and 2b), changes in E_{TYR} may be the reflection of a compensatory process that BLT responders apply successfully, healthy controls do not need, and BLT nonresponders are not able to apply in response to physiological changes induced by exposure to BLT. However, elevated E_{TYR} in our study may well reflect the measured enhancement of inward transport capacity, since current models explaining outward transport also assume a stoichiometrical coupling of the two transport modes (Fischer and Cho, 1979; Seidel *et al*, 2005). With all likelihood, humoral factors in peripheral blood acting either directly on platelets or at their precursor cells, the megakcariocytes, may account for the changes in platelet 5-HTT function. Factors previously suggested to influence 5-HTT function are—among others—steroids, especially β-estradiol (Chang and Chang, 1999). Endogenous TYR may interact with the 5-HTT under physiological conditions as it has been shown to mediate physiological changes in serotonin-related functions (Nisimura *et al*, 2005), and the literature offers numerous reports on alterations in TYR metabolism (Hale *et al*, 1989; Harrison

Table 2 Schematic Summary of Main Study Findings

	Whole sample			Remitter			Nonresponder		
	Before BLT	**After BLT**	**Summer**	**Before BLT**	**After BLT**	**Summer**	**Before BLT**	**After BLT**	**Summer**
Turnover	↑	—	↓	—	—	—	↑↑	↑	—
E_{TYR}	↑	—	—	↑	↓	—	—	—	—
V_{max}	↑	—	—	—	—	—	↑	—	—
B_{max}	—	—	—	—	—	—	—	—	—

Turnover, number of inward transport events at serotonin transporter molecules.
E_{TYR}, tyramine-induced outward transport of MPP^+ relative to baseline.
V_{max}, maximal [^{3}H]serotonin uptake per minute.
B_{max}, maximal [^{3}H]β-CIT-binding capacity.
Arrows indicate significant alterations in functional serotonin transporter parameters relative to healthy control subjects; dashes indicate difference to controls not significant.

et al, 1984; Sandler *et al*, 1975) and its relation to treatment response (Hale *et al*, 1989; Stewart *et al*, 1988) in patients with depression. At a molecular level, transporter oligomerization (Seidel *et al*, 2005; Sitte *et al*, 2004) and transmembrane ion and voltage gradients (Hilber *et al*, 2005) have been shown to modulate 5-HTT efficiency. In addition, regulation of 5-HTT-conducting states depends on the interaction with an associated protein, syntaxin 1A (Quick, 2003). Carneiro and Blakely (2006) have noted changes in catalytic rates of 5-HTT following changes in 5-HTT association to another protein, Hic-5. It is left to future studies to investigate the relevance of these interactions for the pathogenesis of depression in SAD.

In line with the differential effects that associated proteins exert on the functional states of 5-HTT, it has been appreciated in recent years that efflux and influx appear to be distinct and separate functions of monoamine transporters: especially, regulation of efflux is specifically influenced by phosphorylation of the N terminus of biogenic amine transporters (Fog *et al*, 2006; Khoshbouei *et al*, 2004; Seidel *et al*, 2005). Moreover, mechanisms of catalytic activation and inactivation have recently been proposed for 5-HTT in platelets; these involve pathways relying on the activity of protein kinases C and G, p38 mitogen-activated protein kinase (MAPK), and protein phosphatase type 2A (Jayanthi *et al*, 2005; Carneiro and Blakely, 2006).

Function and surface expression of the closely related norepinephrine and dopamine transporters are influenced by partly pathogenic mutations (Hahn *et al*, 2005, 2003; Nass *et al*, 2005; Shannon *et al*, 2000). Sequence analysis of the human 5-HTT gene in recent years has revealed over 10 variants. One of these variants is a functional mutant (I425V) that leads to increased V_{max} but little change in substrate affinity (Kilic *et al*, 2003). In addition, a Gly56Ala substitution was found to be associated with altered catalytic activation in response protein kinase G/p38 MAPK activators (Prasad *et al*, 2005). It is left to future studies to determine whether some of these mutations play a role in altering 5-HTT function in SAD.

Our results bear notable similarity with results obtained in provocation studies in patients with SAD using mCPP (Garcia-Borreguero *et al*, 1995; Schwartz *et al*, 1997), an agent commonly referred to as 'nonspecific serotonin agonist'. These studies show enhanced hormonal and behavioral response to mCPP in depressed patients with SAD and normalization after BLT. Usually, these results are interpreted as an indication of compensatory upregulation in postsynaptic serotonin receptor function during depression and subsequent receptor normalization after BLT. However, besides being an agonist at serotonin receptors, mCPP—similar to TYR—elicits a nonexocytotic, 5-HTT-mediated exchange diffusion process (Gobbi *et al*, 2002; Rothman and Baumann, 2002). Hence, the enhanced mCPP response in SAD and its normalization after BLT could in part also be secondary to enhanced CNS 5-HTT function normalizing after successful BLT.

According to our data, seasonal changes and alterations in platelet 5-HTT function during depression in SAD are mediated by changes in 5-HTT transport efficiency rather than changes in the amount of 5-HTT protein. This is in contrast to two earlier studies in smaller samples who report either decreased (Stain-Malmgren *et al*, 1998) or increased (Smedh *et al*, 1999) B_{max} values during depression in SAD, and an increase (Stain-Malmgren *et al*, 1998) or decrease (Smedh *et al*, 1999) after BLT. In line with Ozaki *et al* (1994), we measured similar B_{max} values in patients and control subjects. Moreover, B_{max} remained stable over all three time points (see also Swiecicki *et al*, 2005). However, since all participants underwent BLT, and since measurements were limited to three time points, our study is not readily suited to assess naturally occurring circannual changes in 5-HTT B_{max}.

Using [^{123}I]β-CIT and SPECT, we have previously shown decreased midbrain 5-HTT availability in depressed patients with SAD (Willeit *et al*, 2000). It is unclear whether 5-HTT B_{max} data acquired in platelets can be expected to reflect the situation in the CNS (Malison *et al*, 1998; Rausch *et al*, 2005). Several additional regulatory processes, such as degradation or pruning of new synapses and *de novo* synthesis of 5-HTTs may contribute to the regulation of 5-HTT quantity in the CNS. In contrast to neurons, platelets are not equipped with protein synthesis machinery and are therefore unlikely to be capable of an adaptive response with regard to 5-HTT quantity. On the contrary, circulating humoral factors mediating an adaptive (or maladaptive) response in platelet 5-HTT function may at the same time be active in the CNS, where enhanced 5-HTT function would possibly lead to decreased synaptic serotonin levels. Platelet 5-HTT V_{max}—in contrast to B_{max}—has recently been shown to correlate with CNS 5-HTT V_{max}, provided that factors such as time of the day and gender are taken into account (Rausch *et al*, 2005). Matching procedures and blood withdrawals standardized for time of the day control these factors in the present study.

CONCLUSION

Reduced synaptic serotonin concentrations are a major tenet of the monoamine hypothesis of depression. Enhanced 5-HTT function as suggested by the present data may be an important mechanism contributing to low synaptic serotonin levels during depression, since it presumably leads to enhanced synaptic serotonin clearance. This mechanism is compatible with the known antidepressant effects of 5-HTT blockade in seasonal (Lam *et al*, 2006; Moscovitch *et al*, 2004) and nonseasonal (Axelrod and Inscoe, 1963) depression. Our data suggest that changes in 5-HTT efficiency are a meaningful and naturally occurring physiological process that is altered in patients with SAD, and they show that changes in 5-HTT efficiency occur parallel to changes in depressive symptoms. In view of the limitations of current techniques for the investigation of central nervous 5-HTT function, our data demonstrate the value of studying platelet 5-HTTs, and they underscore the importance of identifying mechanisms that control 5-HTT transport efficiency in humans.

DISCLOSURE/CONFLICT OF INTEREST

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