

Pronounced Cognitive Deficits Following an Intravenous L-Tryptophan Challenge in First-Degree Relatives of Bipolar Patients Compared to Healthy Controls

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Cognitive impairment has repeatedly been described in bipolar disorders (BD). Serotonin (5-hydroxytryptophan; 5-HT) is possibly involved in these cognitive processes, more particularly in executive functions, learning, memory, and attention. The aim of this study was to investigate serotonergic vulnerability and its relation to cognitive functioning in healthy first-degree relatives of BD patients. We investigated the effects of an intravenous (i.v.) tryptophan (Trp) challenge and placebo on cognitive performance in 30 healthy first-degree relatives of bipolar patients (FH) and 15 matched controls in a double-blind crossover design. A distinction was made between relatives of type I BD patients (FH I) and type II BD patients (FH II). Performances on planning, memory, attention, and psychomotor tasks were assessed 3 h after Trp infusion. After Trp, planning and attention were impaired in FH subjects but not in controls. Independent of Trp, FH subjects showed cognitive deficits on memory, focused and divided attention, and psychomotor performance. FH I subjects showed more pronounced cognitive impairments than FH II and controls. In all groups, Trp impaired memory and psychomotor performance significantly. In conclusions, cognitive deficits in FH following Trp may reflect a central 5-HT vulnerability in frontal brain areas. Independent of Trp, cognitive deficits in FH provide evidence for a trait marker for BD.

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INTRODUCTION

In bipolar disorders (BD), cognitive deficits have been reported repeatedly (Coffman *et al*, 1990; Savard *et al*, 1980; Wolfe *et al*, 1987; Krabbendam *et al*, 2000). Manic patients showed impairments on planning and reaction time (RT) (Murphy *et al*, 1999). During remission, cognitive deficits may persist (van Gorp *et al*, 1998; Ferrier *et al*, 1999; Rubinsztein *et al*, 2000; Scott *et al*, 2000); euthymic BD patients showed impairments on visuospatial recognition, verbal and nonverbal memory and learning, retrieval of information from semantic memory and an increased RT on a planning task (Coffman *et al*, 1990; Ferrier *et al*, 1999; Rubinsztein *et al*, 2000). The fact that cognitive deficits persist during remission suggests that they constitute a trait marker in BD. Healthy first-degree relatives of BD patients (FH) provide a powerful design to investigate whether

cognitive deficits in BD reflect state or trait markers (Sobczak *et al*, 2000). Research on cognition in FH subjects who are free of psychopathology is new. In a previous study of our group, FH subjects showed impaired speed of information processing, on a planning task, and memory performance. Cognitive deficits seem to be more prevalent in relatives of patients with BD type I (FH I) compared to relatives of BD type II patients (FH II) (Sobczak *et al*, 2002c). There are no papers on cognition in FH subjects.

Serotonin (5-hydroxytryptophan; 5-HT) has been implicated in psychopathology of BD and has also been associated with cognitive performance, particularly learning and memory (Young *et al*, 1994; Riedel *et al*, 1999; Schmitt *et al*, 2000). Changes in cognitive performance following selective 5-HT modulations are important biological markers for brain 5-HT responsivity (Riedel *et al*, 2002). Serotonergic modulations can be achieved by either stimulation of the 5-HT system via 5-HT precursors, releasing agents, reuptake inhibitors and receptor agonists, or depletion of tryptophan, acute tryptophan depletion (ATD). ATD results in a transient decrease of central 5-HT. Acute 5-HT challenges showed impaired memory performance after 5-HT_{1A} challenge with ipsapirone (Riedel *et al*, 2002) and impaired psychomotor performance after 5-HT_{2C} challenge with m-CPP (Riedel *et al*, 2002) and i.v. Trp loading (Winokur *et al*, 1986). The relation between 5-HT

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functioning, biological vulnerability to BD, and cognition can be investigated using 5-HT challenge paradigms in FH subjects. Such a relation has been reported by a study of our group in which ATD impaired planning performance selectively in FH subjects. This suggests serotonergic vulnerability affecting frontal lobe areas (Sobczak *et al*, 2002c).

Intravenous L-Tryptophan (Trp) loading is a useful 5-HT challenge paradigm that can be applied to investigate cognitive effects following an overall stimulation of the brain 5-HT system.

The present study was designed to investigate 5-HT-related cognitive functioning following Trp challenge in FH subjects and healthy matched controls. To our knowledge, this is the first study on 5-HT challenge and cognition in FH subjects. The hypotheses were: Trp will induce more pronounced cognitive changes in FH subjects; FH subjects will show impaired planning and memory independent of Trp (implying a trait marker in BD); detrimental effects of Trp will be more pronounced in FH I; based on previous findings, Trp will improve frontal lobe mediated planning and attention whereas it will decrease memory and psychomotor performance independent of FH.

METHODS

Subjects

Subjects were family members and healthy matched controls. Family members were recruited via BD patients treated at the Department of Psychiatry, University Hospital Maastricht, via the consumer organization for manic-depressive patients and their families, and via advertisements in local newspapers. Healthy control subjects were also recruited via a newspaper advertisement and were free from any psychiatric family history.

FH subjects had at least one first-degree relative with a type I BD or type II BD diagnosis. All subjects were interviewed with an abbreviated version of the Family History Research Diagnostic Criteria (FHRDC) to assess FH (Endicott *et al*, 1975). Individual diagnoses (type I or type II BD, according to DSM IV) of the patients were verified via the patients' own psychiatrist.

A standardized psychiatric examination (Mini International Neuropsychiatric Interview; MINI) (Sheehan *et al*, 1994) was taken of all participants to examine the present psychiatric state according to DSM IV criteria. The Hamilton Depression Rating Scale (HDRS) (Hamilton, 1967), the Young Mania Rating Scale (YMRS) (Young *et al*, 1978), and the SCL-90 (Arrindell and Ettema, 1986) were used to verify the absence of depressive, manic, and general psychiatric symptomatology.

Physical health was assessed by means of a health questionnaire, a standard physical examination by a physician, and urine screening. The urine test (Combur[®]) included assessment of leukocytes, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, and erythrocytes. A Quick View, one-step pregnancy test (Quidel[®]) was carried out in female subjects to check for unsuspected pregnancy.

Exclusion criteria were current or past use of psychoactive medication (antidepressants, neuroleptics, and/or

Table 1 Demographic Characteristics. Mean \pm SE; * $p < 0.05$

Measure	FH	Controls
FH I	$n = 22$	
FH II	$n = 8$	
Women	$n = 20$	$n = 11$
Men	$n = 10$	$n = 4$
Family members		
Bipolar disorder	1.3	0.0
Age	41 ± 2.9	40 ± 3.7
IQ	117 ± 2.8	118 ± 2.6
BMI	25.3 ± 0.6	25.0 ± 0.7
SCL-90	102.2 ± 1.8	98.7 ± 1.8
YMRS	$0.7 \pm 0.2^*$	0.3 ± 0.2
HDRS	$1.3 \pm 0.3^*$	0.3 ± 0.2

anxiolytics) and lifetime psychiatric disorder including alcohol or drug abuse (all as indicated by the MINI), current active physical illness, lactation, and pregnancy.

Of the 47 subjects who participated, two did not complete the experiment for reasons unrelated to the study. The included subjects who completed the experiment were 30 FH (10 men and 20 women) and 15 controls (four men, 11 women). Table 1 shows the demographic characteristics of all subjects who completed the experiment. Relatives were children ($n = 19$), parents ($n = 9$), and siblings ($n = 2$). In all, 22 were FH I and eight were FH II subjects. The FH and control group did not differ significantly with respect to age, body mass index (BMI), intelligence quotient (IQ), and SCL-90 total score, but FH subjects scored significantly higher on baseline (HDRS) [$F(1, 43) = 4.30$, $p < 0.05$] and YMRS [$F(1, 43) = 4.71$, $p < 0.05$].

Of the female subjects, 21 were premenopausal and 10 were postmenopausal. A total of 13 women used oral contraceptives.

Each control subject was matched with two FH subjects with respect to sex, age, BMI, and IQ. Intelligence (IQ) was estimated using Groninger Intelligence Test (GIT) subtasks (Vocabulary, Mental Rotation, Mental Arithmetic, and Word Analogies) (Luteijn, 1966).

The Medical Ethics Committee of the University Hospital of Maastricht approved of the study. All participants signed informed consent.

Experimental Design and Treatment

The study was conducted according to a mixed between/within groups (3×2) design. The between-groups factor was FH with three levels: controls, FH I, and FH II. Treatment, the within-subjects factor, consisted of two levels: placebo and Trp loading. Trp and placebo were administered intravenously. The order of treatment was randomized. Administration was according to a double-blind, order-balanced crossover design. The two test days were spaced 3–5 days apart.

i.v. Trp loading. Trp infusions were prepared by dissolving 7.0 g Trp in 1000 cc water. Saline solution (NaCl) was added to ensure an isotonic solution ($\text{pH} = 7.4$). Each Trp solution

was sterilized by passage through a 0.22-mm polymer (Millipore) filter and tested for pyrogenicity and sterility. Saline solution (1000 cc) was given on a separate day as a placebo mixture. The 1000 cc solution was administered within 60 min. The appearances of solutions were identical.

Procedure

To eliminate response bias due to premenstrual symptoms, all premenopausal women were tested in the follicular phase of the menstrual cycle (Menkes *et al*, 1994; Rasgon *et al*, 2000).

On each test day, the subjects arrived at 9.00 am after an overnight fast. Immediately after arrival the i.v. cannula was inserted in a forearm vein. At 10.00 am (t_0), the saline solution, with or without Trp, was administered.

During the experiment, the subjects remained in the testing room, where they were allowed to do nonstrenuous activities. Subjects were allowed to drink decaffeinated coffee, herbal tea or water, and to eat protein-poor fruits like apples and oranges.

Blood samples for assessment of plasma Trp were taken at t_{-30} , t_{60} , t_{75} , t_{90} , and t_{105} (all time points t_X refer to $(-)$ X min (before)/after t_0). Cognitive assessment was completed at t_{180} .

Cognitive Assessments

The cognitive test battery took approximately 60 min to complete and consisted of tasks measuring planning, attention, psychomotor performance, and memory.

Planning

Computerized Tower of London (compu-TOL): Planning capacity was assessed by a modified version of the one-touch Tower of London (TOL) task (Owen *et al*, 1995; Sobczak *et al*, in press; Shallice, 1982). On a computer screen, two arrays of differently colored balls (red, yellow, and blue) on sticks were presented. The subject was requested to indicate the minimal number of steps necessary to rearrange the balls on the lower configuration to match the arrangement presented on the top half of the screen. The subjects had counted the number of moves and then respond by pressing the appropriate response button (2–5 steps) as quickly as possible. The complexity of the task is dependent on the minimal number of steps in which the rearrangement can be achieved: 2, 3, 4, and 5 steps. Performance was indicated by median RT as a function of the number of steps. The number of correct responses was checked for possible confounding of the RT results.

Secondary Outcome Variables

Attention

Go/NoGo Task (GONT): The GONT measures response readiness. Every second, one digit (0–9) was shown on a computer screen for 500 ms. The subject was asked to push a button as soon as he/she saw the digit except when he/she

saw a 'target-digit'. The number of correct responses and RT to the digits (distracters) were assessed. This task was aimed at measuring attention failures and was adopted after a version described by Robertson *et al* (1997).

Stroop Color Word Test (SCWT): The SCWT has often been used to test focused attention and response inhibition (Stroop, 1935). The test consisted of three reading cards (color names, color patches, color names printed in incongruously colored ink) displaying each 100 stimuli. The outcome variable was interference which denotes the percentage extra time needed to complete the third card relative to the average of the first and second cards: $(\text{time card III} / ((\text{time card I} + \text{time card II}) / 2)) * 100\%$ (Houx and Jolles, 1993).

Left/Right Choice Reaction Time (CRT): This is a parametric version of the well-known color-word response conflict task (Stroop, 1935). The words Left and Right are displayed either at the left or the right side of a computer screen. Response instructions are to respond quickly (by pressing a corresponding button) to the location of the word irrespective of its meaning. The output parameters are the RT and accuracy of responding as a function of task difficulty.

Dichotic Listening Task (DLT): The DLT measures both focused and divided attention for auditory stimuli (Kimura and D'Amico, 1989). Subjects received different auditory stimuli simultaneously through a headphone on each side. The test consisted of three subtasks, in which the subjects were instructed to focus to either the numbers presented in the left/right ear (focused attention) or both ears (divided attention) (Schmitt *et al*, 2000). Subjects were instructed to remember and identify the numbers on a computer screen. Performance on each subtask was calculated by the A' sensitivity measure (see VVLT).

Psychomotor performance

Motor Choice Reaction Time (MCRT): The MCRT measures sensorimotor speed (Houx and Jolles, 1993). The subjects held one button and were asked to press one of five other buttons located at equal distance from the hold button when lit. The subjects responded either to a single button (simple RT), to one of three buttons that lit up (three-choice RT), or to the right of the lit button (incompatible three-choice RT) as quickly as possible. Dependent variables were movement time (which indicates psychomotor RT) and initiation time (as a measure of information processing) as a function of task complexity.

Memory

The Visual Verbal Learning Test (VVLT): The VVLT measures short-term memory (STM) and long-term memory (LTM) performance (Schmitt *et al*, 2000; Lezak, 1995). The test consisted of a list of 30 monosyllabic words, which were presented in three trials on a computer screen. Each trial ended with a free verbal recall of the words (immediate recall: STM). The subject was requested to recall as many

words as possible without prior presentation 30 min after the third trial (delayed recall: LTM). A yes/no recognition test, consisting of 15 formerly learned words and 15 new but comparable words (distracters), was given after the delayed recall test (recognition). The RTs were recorded. Performance on the recognition task (LTM) was measured as $A' = 1 - 1/4 (fr/cr + (1 - cr)/(1 - fr))$, where cr is the proportion of correctly recognized words and fr is the proportion of falsely recognized (Pollack and Norman, 1964). Dependent variables were total number of correct words on immediate recall and delayed recall, A' and the median RT of correctly recognized target words as a measure of speed of retrieval from LTM.

Verbal Fluency Test (Fluency): The fluency test measures strategy-driven retrieval of information from semantic memory. The subjects are asked to report as many four-letter words with the same initial letter within one minute. The dependent variable is the number of correct reported words (Schmitt et al, 2000).

Mood, Hormones, and Physical Side Effects

Mood was assessed using the abbreviated Bimodal Profile of Mood States (POMS) (McNair et al, 1971). The severity of side physical effects was determined by a specially designed questionnaire (17 four-point items) (Sobczak et al, 2002a). Both were completed at 10.30 am (t_{30}), at t_{60} , t_{90} , and at t_{120} . Blood samples (to assess cortisol and prolactin) were taken at t_{60} , t_{75} , t_{90} , and t_{105} . The effects of Trp on mood, prolactin, and cortisol have been described elsewhere (Sobczak et al, 2002c).

Statistical Analysis

Outcome variables were analyzed using the General Linear Model (GLM) for repeated measures. The within-subjects factor was treatment (two levels: Trp or placebo); the between-subjects factor was family history (three levels: controls, FH I, or FH II). Univariate contrasts between groups were used to test the primary hypothesis of difference between controls and FH subjects (controls vs FH I+II) as well as the hypotheses of differences between controls and FH I and FH II relatives separately (controls vs FH I and controls vs FH II).

Mann-Whitney-U tests were used to test differences in side effects.

Relations between effects of Trp (determined as difference: Trp minus placebo) on cognitive measures and mood, peak prolactin and cortisol responses (determined as maximal response after Trp minus placebo) were evaluated by means of Pearson's product moment correlation.

Statistical analyses were performed with SPSS 9.0 for Windows.

RESULTS

Plasma Trp Levels

Plasma Trp concentrations [$F(1, 42) = 41.2$, $p < 0.01$] and ratio Trp:LNAAs [$F(1, 42) = 22.6$, $p < 0.01$] were significantly higher at baseline in the placebo condition. FH

subjects showed lower baseline plasma Trp [$F(1, 42) = 6.4$, $p < 0.05$] compared to controls. After correction for baseline differences, plasma Trp [$F(1, 42) = 978.7$, $p < 0.001$] and ratio Trp:LNAAs [$F(1, 42) = 502.3$, $p < 0.001$] increased significantly after i.v. Trp loading. Relative to baseline, the mean peak of plasma Trp and ratio Trp:LNAAs to i.v. Trp loading were 1049.4 mol/l, respectively 2.3. Following placebo, there was no change in plasma Trp and ratio Trp:LNAAs.

There were no effects of treatment order and FH on increase in Trp values after challenging. The values of plasma Trp are represented in Table 2.

Cognitive Assessments

Table 3 shows mean (\pm SE) of planning variables, separated for FH and control subjects.

Planning

compu-TOL: The three-way interaction of FH by number of steps by Trp on number of correct responses was significant [$F(3, 41) = 3.01$, $p < 0.05$], but there were no differences between FH I and FH II subjects. There was also a main effect of Trp [$F(1, 42) = 85.53$, $p < 0.01$] on median RT and a significant two-way interaction of number of steps by Trp on median RT [$F(3, 40) = 21.50$, $p < 0.01$]; median RT was increased significantly after Trp on the 3 and 4 steps problems but not on the 2 and 5 steps problems.

There were no effects of treatment order.

Table 4 shows mean (\pm SE) of other outcome variables, separated for FH and control subjects.

Table 2 Mean (\pm SE) of Plasma Tryptophan (Trp) and Ratio Trp:LNAAs (LNAAs: Valine, Leucine, Isoleucine, Tyrosine, Phenylalanine) after Placebo and Trp Challenge

	Trp		Trps LNAAs	
	FH	Controls	FH	Controls
Baseline (t_{-30})				
Placebo	65.7 (2.6)	79.8 (5.2)	0.14 (0.0)	0.15 (0.0)
Trp	56.4 (1.8)	61.0 (3.0)	0.12 (0.0)	0.11 (0.0)
t_{60}				
Placebo	49.6 (2.4)	60.3 (3.4)	0.11 (0.0)	0.11 (0.0)
Trp	1086.4 (56.6)	1107.5 (83.0)	2.40 (0.17)	2.27 (0.14)
t_{75}				
Placebo	50.0 (1.8)	57.7 (2.9)	0.11 (0.0)	0.11 (0.0)
Trp	879.7 (32.1)	862.9 (29.0)	2.00 (0.10)	1.92 (0.09)
t_{90}				
Placebo	48.1 (1.7)	55.1 (2.4)	0.10 (0.0)	0.11 (0.0)
Trp	828.7 (25.3)	828.2 (30.9)	1.94 (0.09)	1.86 (0.09)
t_{105}				
Placebo	48.5 (2.0)	55.1 (2.3)	0.10 (0.0)	0.11 (0.0)
Trp	797.2 (24.2)	786.5 (25.2)	1.88 (0.09)	1.79 (0.08)

Table 3 Mean (\pm SE) of the Primary Cognitive Outcome Measures (Planning). Results are Broken Down by Family History (Controls; C/FH I/FH II) and Treatment (Trp/Placebo; P).
* $p < 0.05$, $^{\text{ts}}p < 0.1$

Measure	Treatment	C (n = 15)	FH I (n = 22)	FH II (n = 8)
<i>Computerised Tower of London test (compu-TOL)</i>				
Correct responses (#)				
2 steps	Trp	9.2 (0.2)	8.9 (0.3)	8.6 (0.6)
	P	9.7 (0.2)	8.6 (0.4)	9.0 (0.5)
3 steps	Trp	9.0 (0.2)	9.2 (0.1)	9.0 (0.4)
	P	8.9 (0.4)	8.6 (0.4)	9.0 (0.3)
4 steps	Trp	7.2 (0.4)	7.4 (0.4)	7.9 (0.4)
	P	7.9 (0.4)	8.0 (0.4)	8.1 (0.9)
5 steps	Trp	8.2 (0.2)	7.0 (0.4)	7.0 (0.7)
	P	8.3 (0.4)	7.3 (0.5)	8.8 (0.5)
Median reaction time (msec)				
2 steps	Trp	4880 (262)	5425 (433)	6035 (761)
	P	5721 (693)	5899 (603)	5899 (875)
3 steps	Trp*	7170 (896)	7774 (772)	7885 (928)
	P	6802 (656)	7514 (690)	7166 (822)
4 steps	Trp*	9941 (808)	11155 (1201)	9798 (1163)
	P	8797 (738)	11547 (1111)	9554 (1256)
5 steps	Trp	13623 (1904)	17665 (1266)	17225 (2242)
	P	15463 (1970)	18347 (1899)	16641 (3024)

Attention

GONT: There was a significant interaction of Trp and FH on number of correct response inhibitions; after Trp FH subjects showed less correct response inhibitions [$F(1, 43) = 8.76$, $p < 0.01$] but controls did not. There were no effects on RT of correct responses nor did performance differ between treatment order or FH I and FH II subjects.

SCWT: The interference measure of the Stroop test did not show significant effects of FH, Trp, and treatment order.

CRT: On incompatible stimuli RT showed a significant interaction effect of Trp and FH (both FH I and FH) [$F(1, 43) = 3.98$, $p < 0.05$]; RT was impaired after Trp in FH but not in controls, and on number of correct responses this interaction was trend significant; FH subjects showed less correct responses after Trp. Independent of Trp, FH subjects tended to be slower on incompatible stimuli.

There were no significant effects of Trp and FH on accuracy or RT of compatible responses and there were no effects of treatment order.

DLT: FH subjects showed impaired performance on focused attention subtasks 'right' [$F(1, 43) = 7.24$, $p < 0.05$] and divided attention subtask 'both' [$F(1, 43) = 9.53$, $p < 0.01$] independent of Trp.

There were no main effects of Trp or treatment order. There were no differences between FH I and FH II.

MCRT: Movement time was increased after Trp [$F(1, 43) = 4.42$, $p < 0.05$] in FH and controls. There was a main effect of FH on movement time [$F(1, 43) = 5.09$, $p < 0.05$]; prolonged movement time in FH was due to prolonged movement time in FH I subjects [$F(2, 42) = 4.24$, $p < 0.05$].

There were no effects on initiation time and of treatment order.

Memory

VVL: Trp significantly impaired performance on delayed recall [$F(1, 43) = 7.76$, $p < 0.01$] and recognition A' [$F(1, 43) = 4.15$, $p < 0.05$]. Performance on the recognition task showed a significant interaction of Trp with treatment order [$F(1, 43) = 5.12$, $p < 0.05$]; subjects receiving Trp on the first test day showed an improvement in A' following placebo on the second test day, whereas subjects receiving placebo first were also better on the second test day. Following Trp, there was a trend towards impaired immediate recall performance [$F(1, 43) = 3.84$, $p < 0.1$].

Independent of Trp, performance on recognition A' was impaired in FH subjects [$F(1, 43) = 5.93$, $p < 0.05$], more specifically in FH I subjects [$F(2, 42) = 4.21$, $p < 0.05$]. There were no effects on median RT of the recognition task.

Fluency: There were no effects of Trp, FH, or treatment order on the number of correct reported words.

Mood, Hormones, and Physical Side Effects

Trp significantly increased feelings of anger [$F(1, 42) = 6.52$, $p < 0.05$], depression [$F(1, 42) = 187.92$, $p < 0.001$], fatigue [$F(1, 42) = 22.17$, $p < 0.01$], tension [$F(1, 42) = 6.06$, $p < 0.05$], and decreased feelings of vigor [$F(1, 42) = 12.12$, $p < 0.01$] on the POMS. Prolactin [$F(1, 41) = 30.17$, $p < 0.01$] and cortisol [$F(1, 41) = 10.86$, $p < 0.01$] increased significantly after Trp. Hormonal and mood responses to Trp did not differ at baseline and between FH groups and controls. In the Trp condition, significantly more side effects [$Z = -3.95$, $df = 1$, $p < 0.001$] were reported compared to the placebo condition. Reported side effects after Trp were

Table 4 Mean (\pm SE) of the Secondary Cognitive Outcome Measures (Attention, Psychomotor Speed, and Memory). Results are Broken Down by Family History (Controls; C/ FH I/FH II) and Treatment (Trp/Placebo; P). * $p < 0.05$, ^{ts} $p < 0.1$

Measure	Treatment	C (n = 15)	FH I (n = 22)	FH II (n = 8)
<i>Go/NoGo Task</i>				
RT (msec)	Trp	325 (10)	351 (13)	323 (18)
	P	331 (11)	342 (11)	307 (14)
No. correct (#)	Trp	8.8 (0.3)	8.1 (0.3)*	8.0 (0.5)*
	P	8.2 (0.3)	8.6 (0.3)	8.3 (0.5)
<i>Stroop Color Word Test</i>				
Interference (%)	Trp	41 (1)	41 (1)	37 (1)
	P	43 (1)	41 (1)	38 (2)
<i>Left/Right Choice Reaction Time</i>				
RT (msec)				
Incompatible	Trp	700 (18)	772 (26)*	683 (27)*
	P	712 (21)	732 (22)	661 (17)
Compatible	Trp	690 (17)	711 (16)	664 (27)
	P	686 (24)	688 (15)	661 (15)
No. correct (#)				
Incompatible	Trp	15.5 (0.2)	15.1 (0.2) ^{ts}	15.4 (0.3) ^{ts}
	P	15.3 (0.2)	15.5 (0.2)	15.4 (0.3)
Compatible	Trp	15.8 (0.1)	15.4 (0.2)	15.5 (0.3)
	P	15.9 (0.1)	15.5 (0.2)	15.6 (0.2)
<i>Dichotic Listening Task</i>				
A' (%)				
Right	Trp	94 (9)	89 (2)	79 (5)
	P	93 (1)	89 (2)*	89 (3)*
Both	Trp	86 (8)	83 (8)*	81 (2)*
	P	86 (8)	83 (7)*	82 (2)*
<i>Motor Choice Reaction Time</i>				
RT (msec)				
Simple	Trp	114 (6)	121 (7)	111 (6)
	P	110 (7)	127 (7)	105 (5)
Choice	Trp	125 (6)	131 (7)	116 (7)
	P	123 (7)	133 (6)	115 (6)
Incompatible	Trp	132 (9)	139 (8)	125 (11)
	P	130 (10)	137 (8)	119 (8)
Movement RT (msec)				
	Trp*	1046 (28)	1185 (40)	1087 (40)
	P	1032 (33)	1166 (37)*	1049 (30)
<i>Visual Verbal Learning Test</i>				
No. correct words (#)				
Immediate rec.	Trp ^{ts}	42.6 (2.8)	33.8 (2.6)	40.6 (4.5)
	P	42.1 (3.2)	38.8 (3.0)	41.0 (5.0)
Delayed rec.	Trp*	14.5 (1.5)	10.5 (1.3)	14.1 (2.4)
	P	15.1 (1.6)	12.8 (1.5)	16.1 (2.0)
Recognition A' (%)				
	Trp*	53 (9)	56 (5)	49 (8)
	P	58 (7)	60 (4)*	49 (6)
Recognition RT (msec)				
	Trp	647 (19)	833 (39)	729 (39)
	P	640 (19)	852 (42)	713 (24)
<i>Verbal Fluency Test</i>				
No. correct words (#)				
	Trp	12.9 (1.4)	11.0 (0.8)	12.1 (1.2)
	P	11.8 (1.0)	10.7 (1.0)	12.4 (1.5)

tiredness, nausea, drowsiness, dizziness, headache, dullness, feelings of weakness, loss of concentration, and diminished energy. Side effects did not differ between FH I, FH II, or control subjects.

Correlations of Behavioral, Physiological, and Cognitive Effects

Following Trp, decrease in vigor was positively correlated with decrease in delayed recall performance on the VVLT [$r = 0.39$, $p < 0.01$]. Detrimental effects of Trp on recognition A' showed a significant positive correlation with peak cortisol response following Trp [$r = 0.37$, $p < 0.05$].

There were no significant correlations between other POMS subscales, prolactin response, and effects of Trp on planning, attention and psychomotor performance. There were no significant correlations of total physical side effects and dependent variables on cognitive tasks.

DISCUSSION

This is the first study that investigated 5-HT vulnerability in FH subjects and cognitive functioning in FH following Trp challenge. These findings are in line with a previous study of our group on ATD in FH, highlighting an important relation between 5-HT, trait markers to BD, and cognition.

In sum, impaired planning performance and attention after Trp challenge was found in FH subjects but not in controls. There was no significant interaction of FH and Trp on memory and movement RT. Independent of Trp loading, FH subjects showed cognitive deficits on memory, psychomotor performance, focused and divided attention. Cognitive deficits on memory and psychomotor performance were more pronounced in FH I than in FH II. There was a main effect of Trp on memory and psychomotor performance; surprisingly, Trp impaired delayed recall memory performance and recognition.

Impaired performance in FH on planning and attention following Trp suggest central 5-HT vulnerability in frontal brain areas in these subjects (Dagher *et al*, 1999; Murphy *et al*, 1999; Owen *et al*, 1990; Rubinsztein *et al*, 2001). The findings on planning are in agreement with data of a previous study of our group, which showed pronounced planning deficits in FH following ATD (Sobczak *et al*, 2002c). Thus both a decrease and increase in central Trp may impair planning performance in FH. We speculate that in FH subjects the 5-HT system may be vulnerable to manipulations. Independent of the direction of 5-HT modulations, acute changes in central 5-HT may be regarded as a stressor and thereby disturb the equilibrium of 5-HT activity transiently resulting in cognitive impairments. This hypothesis is consistent with the kindling and stress sensitization theory of Post *et al* (Post and Weiss 1997), which includes adaptations in second messengers, genetic transcription, receptors, neurotransmitters, and neurons that may occur in response to stressors and remain present during remission of symptoms. In FH subjects, who have a biological vulnerability to BD, this stress sensitization may ultimately result in BD psychopathology.

After ATD, attention may be improved (Rosse *et al*, 1992; Schmitt *et al*, 2000; Sobczak *et al*, 2002c). We found impaired attention after Trp in FH, which is in agreement

with a linear negative association of 5-HT with (focused) attention.

Independent of Trp, FH subjects showed cognitive deficits on memory, movement time, and attention. Cognitive impairments have previously been described in FH and also in manic, depressed, and euthymic BD patients (Sobczak *et al*, 2002c; Rossi *et al*, 2000; Martinez-Aran *et al*, 2000; Ferrier *et al*, 1999; Murphy *et al*, 1999; Rubinsztein *et al*, 2000). The cognitive deficits in BD may be partially explained by abnormalities in functional brain structures and/or metabolism (Stoll *et al*, 2000; Manji *et al*, 2000; van Gorp *et al*, 1999; Coffman *et al*, 1990). As cognitive impairments are present in FH and persist during clinical remission in BD patients, they may be regarded as trait markers (Henry *et al*, 1973; van Gorp *et al*, 1998; Murphy *et al*, 1999; Coffman *et al*, 1990; Ferrier *et al*, 1999; Rubinsztein *et al*, 2000).

There was a tendency of FH I subjects to show more cognitive impairments compared to FH II and controls, which is in agreement with previous findings (Sobczak *et al*, 2002c). FH II subjects appear to be more vulnerable to ATD-induced mood changes (Sobczak *et al*, 2002a). This may suggest a biological distinction in FH I and FH II (Sobczak *et al*, 2002c). It is speculated that cognitive deficits in BD type I resemble those described in primary psychotic disorders, whereas BD type II patients show more characteristics of primary affective disorders (Dickerson *et al*, 2001; Krabbendam *et al*, 2000; Borkowska and Rybakowski, 2001).

The unexpected Trp-induced decrements in memory indicate impairments in consolidation affecting LTM performance. As there was a trend towards impaired immediate recall performance, the effects on LTM might probably be ascribed to impairments in STM. Deficits in STM have also been described following acute 5-HT challenges with ipsapirone (Riedel *et al*, 2002) and fenfluramine (Luciana *et al*, 1998) in healthy subjects. The detrimental effects of Trp on memory were comparative with those described following ATD (Sobczak *et al*, 2002c; Riedel *et al*, 1999; Schmitt *et al*, 2000; Rubinsztein *et al*, 2001). The fact that both an increase and decrease in Trp results in memory dysfunctions may suggest that there is an optimal 5-HT level for cognitive performance.

The Trp-induced impairment on movement time on MCRT is in agreement with results of Winokur *et al* (1986), who showed decreased motor performance on a symbol-copying test following i.v. Trp. Impaired RT was associated with sedation in the study of Winokur *et al* (1986) but not in our study.

Possible confounding factors that might have interfered with cognitive effects of Trp are physical side effects and changes in mood and cortisol release following Trp. Direct effects of 5-HT on vigilance and the conversion of Trp to melatonin may explain the sedative effects of Trp (Cooper *et al*, 1991; Schmitt *et al*, 2002). Correlation analyses showed sedation to be positively associated with impaired memory performance. There were no interfering effects of mood and physical side effects. There was a positive association of cortisol response following Trp and memory performance; a higher cortisol response was associated with lower Trp-induced impairments on recognition (see also Sobczak *et al*, 2002b). A possible memory-enhancing effect of acute

elevations of cortisol has been described before (Buchanan and Lovallo, 2001; Adler and Jajcevic, 2001). Thus, effects of Trp on memory might possibly have been influenced independently by both sedation and cortisol.

In conclusion, this study showed Trp-induced planning and attention impairments in FH subjects. These cognitive deficits suggest 5-HT vulnerability to BD psychopathology affecting frontal brain functions. Cognitive deficits in FH on memory, psychomotor performance, and attention independent of Trp provide evidence for a trait marker for BD. Independent of FH, the major findings of Trp on cognition were impaired memory and psychomotor performance. It is suggested that an acute change in central 5-HT following Trp may disturb an optimal 5-HT activity in frontal and temporal brain areas and hence cause cognitive deficits.

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