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Abstract Thirteen healthy subjects were subjected to tryptophan (TRP) depletion, lysine (LYS) depletion, and a placebo condition in a double blind cross-over study. The aim of the study was to test the specificity of psychological effects induced by TRP depletion. Subjects ingested a 100 g amino acid mixture with or without TRP or LYS. Six hours later, plasma TRP levels had decreased by 77% in the TRP depletion test and LYS levels by 51% in the LYS depletion condition. After 6 h of TRP depletion, subjects reported significantly more tiredness and lowering of mood, compared to subjects in the placebo group, and memory performance declined. After 6 h of LYS depletion, no significant differences in mood and memory compared to placebo were found. We conclude that the effects of TRP depletion on mood and memory are specific for the depletion of TRP and are not caused by the depletion of an amino acid per se. This supports the hypothesis that TRP depletion affects brain serotonin metabolism and not only brain protein metabolism in general.

Key words Tryptophan · Lysine · Amino acid · Mood · Memory

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Introduction

The tryptophan (TRP) depletion method is widely used to study the behavioural effects of reduced serotonergic metabolism in the brain. The aim of the procedure is to lower serotonin (5-hydroxytryptamine; 5-HT) levels in the brain by lowering 5-HT synthesis via depletion of its precursor, tryptophan. In animal studies, the efficacy of the method has been confirmed by measuring brain serotonin and 5-hydroxyindoleacetic acid levels (Young et al. 1989; Schaechter and Wurtman 1990). In humans, ingestion of 50–100 g of an amino acid mixture without TRP leads to a 75–90% reduction in plasma TRP within 4–6 h (Young et al. 1985; Benkelfat et al. 1994).

After TRP depletion, a significant reappearance of depressive symptoms occurred in remitted depressed patients (Delgado et al. 1990, 1991; Lam et al. 1996), an effect which disappeared within 24–48 h (Delgado et al. 1991). Several studies have shown that TRP depletion leads to a lowering of mood in normal subjects (Young et al. 1985; Smith et al. 1986; Ellenbogen et al. 1996) and particularly in subjects with a positive family history of depression (Benkelfat et al. 1994). Recovered depressed patients off drug treatment also show a significant mood lowering response to tryptophan depletion (Smith et al. 1997). Furthermore, TRP depletion in humans affects many psychological and physical functions such as cognition, nocturnal melatonin secretion, sleep, breathing patterns, memory and learning, anxiety, aggression, and food selection (Young et al. 1985,1988; Moja et al. 1989; Zimmermann et al. 1993; Benkelfat et al. 1994; Menkes et al. 1994; Park et al. 1994; Weltzin et al. 1994, 1995; Cleare and Bond 1995; Kent et al. 1996; Miller et al. 1996; Moeller et al. 1996).

In order to be considered an adequate challenge test for serotonergic functions, the TRP depletion method should be reliable, reversible, and specific. The first two issues have been addressed in other studies (Smith

et al. 1987; Moja et al. 1989; Delgado et al. 1990; Ellenbogen et al. 1996). In monkeys, Young et al. (1989) found that TRP depletion did not change the metabolism of other neurotransmitters like tyrosine and the catecholamines, whereas levels of tryptophan and 5-hydroxyindoleacetic acid in the cerebrospinal fluid were lowered. Thus, if the effects of TRP depletion are linked to neurotransmission in the brain, it is probably the serotonergic system that is affected. In that sense, there seems to be specificity.

Theoretically, TRP depletion has two major effects on brain metabolism: it reduces the synthesis of serotonin (Gessa et al. 1974; Moja et al. 1989), and the synthesis of proteins in general (Blazek and Shaw 1978; Cortamira et al. 1991). This latter effect is an aspecific effect that will also occur after depletion of any amino acid and could induce behavioural effects. We therefore questioned whether the effects of TRP depletion on mood and cognition could be mimicked by depletion of any amino acid. To our knowledge, no studies have addressed this question.

To study this aspect of the specificity of the TRP depletion method, normal subjects took part in three separate tests, in which they were given a mixture containing no TRP, a mixture containing no lysine (LYS), and a mixture containing both amino acids (placebo). We postulated that the behavioural effects of TRP depletion would not occur after LYS depletion. We assessed mood and memory measures, because earlier studies have demonstrated effects of TRP depletion on mood (Young et al. 1985; Smith et al. 1987; Benkelfat et al. 1994; Ellenbogen et al. 1996) and memory (Park et al. 1994) in normal subjects. Furthermore, both depressed mood and impaired memory are part of the depressive syndrome, which can be elicited by TRP depletion in subjects that are prone to depression (Delgado et al. 1990, 1991; Benkelfat et al. 1994; Lam et al. 1996).

Materials and methods

Subjects

Sixteen subjects were recruited by newspaper advertisements. Inclusion criteria were good physical and mental health (as assessed by medical history, and physical examination), no history of psychiatric disorder, no current axis I DSM IV diagnosis [as assessed by the Mini International Neuropsychiatric Interview (Sheehan et al. 1994)], and no use of psychotropic medication in the week before the start of the test. Both men and women in the age range 18–65 were allowed to participate.

Of the 16 subjects who entered the study, three subjects (all women, aged 20, 21, and 32 years) dropped out after the first session due to nausea and vomitting. Two of them had received the placebo mixture, and the third had ingested the TRP depletion mixture. The 13 subjects (three men, ten women) who completed the study had a mean age of 27 ± 7 years, and had a mean body mass index (kg/m^2) of 24 ± 5. Day of the menstrual cycle was recorded: while in the pre-menstrual week, two women received TRP depletion, two women received LYS depletion and four women receive placebo. Hence, the distributions of day of the menstrual cycle did not differ between treatment conditions in a manner that would augment an effect of TRP depletion or LYS depletion on mood or memory.

Procedure

The study was approved by the Medical Ethics Committee of the University Hospital Maastricht, The Netherlands, as part of a larger project evaluating the effects of TRP depletion on mood and memory in relation to a family history of depression. The results of that study are reported elsewhere (Van Praag et al. 1997). All subjects gave their written informed consent prior to their inclusion and were paid for their participation in the study. The subjects were informed that the study would investigate mood and memory in response to three different amino acid mixtures.

On three occasions (spaced 1 week apart), subjects underwent TRP depletion, LYS depletion, and placebo depletion in a randomized, double-blind fashion. On each test day, subjects arrived at the clinic at 0830 hours, after an overnight fast. Behavioural questionnaires (described later) were filled out by the subjects, and at 0900 hours (t_0) the subjects then ingested (as quickly as possible, but within 2 h) one of three amino acid mixtures (see Table 1): without TRP or LYS or with both amino acids present (placebo).

The LYS depletion mixture and the placebo amino acid mixture contained 3 g TRP. Each mixture also contained, 63g carbohydrates and 33 g fat in order to dissolve the amino acids. The mixtures were flavoured with artificial orange (test day 1) or apricot flavour (test day 2). On test day 3, the flavour (orange or apricot) was chosen by the subjects themselves. The three mixtures were of identical appearance.

For the 24 h following ingestion of the mixture, all subjects were kept on a diet containing 19 mg TRP and 80 mg LYS. This diet was identical for all three sessions. The diet consisted of six slices of bread with a total of 1.1 g protein, margarine, marmalade, two pieces of fruit, 200 g apple compote, tea, three glasses of lemonade, and mints (max. 50 g). At 7, 11 and 14 h after administration of the amino acid mixture on day 1, an additional 10 g of that day's amino acid mixture was ingested. The subjects stayed at the ward until after the behavioural (mood and memory) assessment at 1500 hours (t_6). They went home with their food supply and dietary instructions. A detailed description of all food and beverages consumed (time and amount) was kept by each subject, in order to encourage strict adherence to the diet. The next morning, the subjects returned at 9:00 a.m. (t_{24}) for the second delayed recall of the words learned at t_6. This was followed by a protein-rich meal to

Table 1 Composition of the placebo amino acid mixture. In the tryptophan depletion mixture, L-tryptophan was left out. In the lysine depletion mixture, L-lysine was left out

L-Alanine	5.5 g
L-Arginine	4.9 g
L-Cysteine	2.7 g
Glycine	3.2 g
L-Histidine	3.2 g
L-Isoleucine	8.0 g
L-Leucine	13.5 g
L-Methionine	3.0 g
L-Phenylalanine	5.7 g
L-Proline	12.2 g
L-Threonine	6.5 g
L-Tyrosine	6.9 g
L-Valine	8.9 g
L-Lysine	8.9 g
L-Serine	6.9 g
L-Tryptophan	3.0 g

compensate for possible deficiencies. Afterwards, they went home and resumed their normal food intake.

Blood samples were drawn at t_0, t_6 and t_{24} for determination of total plasma levels of TRP, LYS and all other amino acids.

Behavioural ratings

Behaviour was assessed by means of questionnaires administered at t_0, t_6 and t_{24}. Mood states were measured with the Dutch 30-item validated version of the Profile of Mood States Scale (POMS; McNair et al. 1988), von Zertssen's Mood Adjective Scale (Linden and Krautzig 1981), and a 100-mm Visual Analogue Scale (VAS) for depression. The POMS consists of five mood scales (depression, tension, vigor, hostility, and tiredness), each based on six items. Von Zerssen's Mood Adjective List consists of 28 bipolar mood states. Subjects are instructed to indicate which of the paired opposites (positive versus negative) best reflected their current mood state; if neither applied, subjects could fill an item in as ''neutral''. Positive, negative, and neutral scores are then summed over the 28 states, yielding three mood measures: Zer-pos, Zer-neg and Zer-neu. The VAS depression was rated 0 = ''no depression'' to 100 = ''maximum depression''.

A questionnaire concerning side effects was administered at t_0 and at 1.5, 3, 5 and 7 h after administration (these were averaged to comprise one t_6 measure) and at 24 h (t_{24}) after administration. The list contained the following 13 items, rated on 5 point scales (0 = ''not at all'' to 4 = ''very much''): dry mouth, dizziness, palpitations, headache, feeling cold, nausea, sleepiness, drowsiness, feeling warm, blurred vision, perspiration, tiredness, and abdominal discomfort.

Thirty word learning task

At t_6, 30 words were presented on a computer screen. Every 2 s, a new word was presented for 1 s. Following the presentation of all 30 words, subjects were asked to recall verbally as many words as possible (= immediate recall). The first immediate recall trial was followed by two more trials in which the same words were presented in the same order. After 30 min, they were asked to recall as many of the previously presented words as possible ($t_{6\rightarrow6}$). At t_{24}, subjects were asked to recall the words again ($t_{6\rightarrow24}$). The delayed recall trials at t_6 and t_{24} were followed by recognition trials in which subjects were to recognize 15 learned target words from 15 distractor words by means of pressing a yes/no button. Words were presented on a screen for 2000 ms or until the subject responded. Another 1000 ms elapsed before the next word appeared on the screen. Errors and reaction times were recorded.

Three different lists of 30 monosyllabic words were used, one per session, which were presented in a randomized order over the three sessions. Each list contained 18 nouns and 12 adjectives. The lists were comparable with regard to their level of abstraction and the affective tone of the words. All instructions were standardized and were read to the subjects from an instruction sheet.

One week before the experiment, the subjects practised the task with a separate list of words to reduce learning effects during the test.

Biochemical analysis

At t_0, t_6, and t_{24}, blood was withdrawn and immediately placed on ice and centrifuged within 30 min for 5 min at 1000 g at 4°C. An aliquot of 100 µl plasma was then mixed with 4 mg sulphasalicyl acid and frozen at –80°C until analysis (van Eijk et al. 1994). Analysis of total plasma TRP and LYS concentrations and those of other amino acids was carried out with high-performance liquid chromatography (van Eijk et al. 1993). In addition to total plasma TRP and LYS levels, the ratio of total plasma TRP versus the sum of the following five competing amino acids [the so-called Large Neutral Amino Acids (LNAAs)], valine, leucine, isoleucine, phenylalanine, and tyrosine (TRP/LNAAs), was determined. The same calculation was carried out with regard to LYS and its competitors arginine and ornithine [basic amino acids (BAAs)] (LYS/BAAs) (Oldendorf and Szabo 1976; Tews et al. 1981). These ratios provide an estimation of TRP and LYS uptake in the central nervous system (Fernstrom and Wurtman 1972).

Statistical analysis

All results are presented as means ± SD. Because the distribution curves of all questionnaire data (including the side effects) were skewed to the right, we used non-parametric tests to analyse the results. Wilcoxon's signed rank test was used to compare TRP versus placebo and LYS versus placebo effects at t_0 (baseline) and t_6. After Bonferroni-Holm correction, differences were considered significant if $P \leq 0.025$. Since there were no baseline differences between conditions, results at t_6 were evaluated as treatment effects. For the analysis of side effects, the sum scores for the six questionnaires completed after TRP depletion were compared with those after LYS and placebo depletion. Scores on the Word Learning tasks were normally distributed, so we used paired *t*-tests to compare TRP depletion versus the placebo condition and LYS depletion versus the placebo condition. Results from amino acid levels were evaluated using paired *t*-tests at t_6. Order effects over the word learning tasks were inspected by plotting the data. Order effects were not expected to occur over the mood questionnaires and were therefore not analysed.

Results

Plasma amino acids

TRP and LYS plasma levels and TRP/LNAA and LYS/BNAA ratios are listed in Table 2. The TRP plasma level at t_0 and at t_{24} did not differ between treatment conditions. The TRP plasma level at t_6 was lower in the TRP depletion condition ($t = 10.1$, $df = 11$, $P < 0.0001$). The LYS plasma level at t_0 did not differ between treatment conditions. The LYS plasma level at t_6 was lower in the LYS depletion condition ($t = 4.7$, $df = 11$, $P < 0.001$). The LYS plasma level at t_{24} was 10% lower in the LYS depletion condition ($t = 3.5$, $df = 11$, $P < 0.005$). The TRP/LNAA ratio at t_0 did not differ between treatment conditions. The TRP/LNAA ratio at t_6 was lower in the TRP depletion condition ($t = 8.6$, $df = 11$, $P < 0.001$). The TRP/LNAA ratio at t_{24} was lower in the TRP depletion condition ($t = 3.9$, $df = 11$, $P < 0.005$). The LYS/BNAA ratio at t_0 and at t_{24} did not differ between treatment conditions. The LYS/BNAA ratio at t_6 was lower in the LYS depletion condition ($t = 4.7$, $df = 11$, $P < 0.001$).

Table 2 Amino acid levels (ng/ml) and uptake ratios in the different conditions

Amino acid	Time	TRP depletion	LYS depletion	Placebo test
TRP	0	49.4 ± 10.8	50.0 ± 11.2	48.1 ± 10.2
	6	12.8 ± 4.8*	58.3 ± 18.0	53.7 ± 15.5
	24	41.6 ± 13.3	45.5 ± 6.5	47.7 ± 9.4
LYS	0	170.7 ± 41.2	178.4 ± 38.5	178.5 ± 44.2
	6	195.2 ± 48.8	89.4 ± 45.0*	169.7 ± 58.2
	24	210.4 ± 50.5	161.2 ± 43.7**	206.5 ± 50.8
Ratio	0	9.0 ± 1.6	9.0 ± 1.4	9.2 ± 1.4
TRP/LNAAs	6	1.9 ± 1.4*	8.3 ± 1.7	8.6 ± 3.1
	24	7.2 ± 1.1*	8.9 ± 1.0	8.8 ± 1.6
Ratio	0	1.1 ± 0.4	1.4 ± 0.4	1.4 ± 0.4
LYS/BNAAs	6	1.1 ± 0.3	0.7 ± 0.4*	1.5 ± 0.4
	24	1.6 ± 0.4	1.4 ± 0.3	1.5 ± 0.3

*$P < 0.001$ compared to placebo
**$P < 0.005$ compared to placebo

Table 3 Mean behavioural measurements ± SD at baseline and 6 h after the different amino acid mixtures

	Placebo		TRP depletion		LYS depletion	
	t_0	t_6	t_0	t_6	t_0	t_6
VAS-depression	3.2 ± 6.3	4.5 ± 13.0	2.7 ± 6.6	8.2 ± 18.2	2.2 ± 5.1	3.3 ± 7.0
POMS-tiredness	2.3 ± 4.1	2.2 ± 4.4	1.6 ± 2.9	3.9 ± 5.1*	1.9 ± 3.2	2.52 ± 3.6
POMS-depression	0.5 ± 1.0	0.2 ± 0.6	0.6 ± 1.3	1.5 ± 3.3	0.3 ± 0.9	0.5 ± 1.1
POMS-tension	1.2 ± 3.0	1.5 ± 3.9	0.8 ± 1.2	1.6 ± 2.3	2.3 ± 4.0	1.5 ± 3.8
POMS-hostility	0.7 ± 1.3	0.5 ± 1.1	0.5 ± 1.1	1.0 ± 1.8	0.7 ± 1.9	0.5 ± 1.0
POMS-vigilance	13.2 ± 6.0	13.7 ± 3.6	13.2 ± 4.7	12.1 ± 4.7	14.1 ± 5.4	12.9 ± 3.8
ZER-pos	25.2 ± 3.8	26.0 ± 2.7	25.4 ± 2.3	22.4 ± 4.8	25.2 ± 3.9	24.2 ± 3.3
ZER-neg	1.3 ± 1.9	1.2 ± 1.7	1.3 ± 1.3	3.3 ± 3.5*	1.7 ± 3.5	2.8 ± 0.9
ZER-neu	1.5 ± 2.7	0.8 ± 1.7	1.3 ± 1.4	2.3 ± 2.5	1.1 ± 1.9	1.0 ± 1.4

*$P < 0.025$ compared to placebo

Table 4 Mean recall and recognition sensitivity (%) and RT (ms) ± SD of learned words on the word learning task after the different amino acid mixtures

Treatment	Placebo	LYS depletion	TRP depletion
Total recall t_6	41.4 ± 10.0	42.2 ± 13.8	42.3 ± 10.7
1st Delayed recall $t_{6\rightarrow6}$	13.7 ± 5.9	14.3 ± 7.5	12.4 ± 6.4
2nd Delayed recall $t_{6\rightarrow24}$	12.0 ± 4.8	11.6 ± 7.6	9.0 ± 6.8*
Recognition $t_{6\rightarrow6}$	88.8 ± 12.9	90.5 ± 10.9	90.4 ± 7.0
Recognition $t_{6\rightarrow24}$	83.0 ± 12.2	81.2 ± 14.1	77.8 ± 23.9
Recogn. RT $t_{6\rightarrow6}$	573 ± 94	547 ± 72	587 ± 66
Recogn. RT $t_{6\rightarrow24}$	552 ± 103	534 ± 116	582 ± 105

*$P < 0.025$ versus placebo

Mood

Table 3 shows the mean behavioural measurements ± SD at baseline and 6 h after the different amino acid mixtures. Analysis of baseline ratings showed no significant differences between conditions. At t_6, increased tiredness was observed after TRP depletion compared to placebo as measured on the POMS-tiredness scale ($Z = -2.5$, $df = 12$, $P = 0.01$). Tiredness scores after TRP depletion also tended to be higher than after LYS depletion ($Z = -1.7$, $df = 12$, $P = 0.09$). Summed POMS-scores (in which the vigilance score was transformed according to $y = 20 - x$) were significantly higher after TRP depletion ($Z = -2.8$, $df = 12$, $P = 0.005$). Summed POMS-scores after TRP depletion also tended to be higher than after LYS depletion ($Z = -1.8$, $df = 12$, $P = 0.07$). Furthermore, a higher score on the total Negative Mood Score of Von Zerssen's Mood Adjective List ($Z = -2.4$, $df = 12$, $P < 0.025$) was seen after TRP depletion relative to placebo. There were no significant effects of LYS depletion compared to placebo on these measures. No other significant effects on mood were found.

Word learning task

Visual inspection of the data revealed no differences among subjects' word learning task performance on test days 1, 2 and 3. This suggests that there were no relevant order effects that could have modified the results. Memory task performance under the three treatment conditions is summarized in Table 4. No

Table 5 Mean side effects ± SD after the different amino acid mixtures

	Placebo	TRP	LYS
Stomach	1.2 ± 2.4	1.3 ± 3.0	1.1 ± 2.0
Dry mouth	2.3 ± 4.5	1.9 ± 3.0	1.2 ± 2.3
Feeling cold	1.3 ± 2.0	1.5 ± 3.3	1.3 ± 2.1
Feeling hot	1.2 ± 1.3	1.5 ± 1.8	1.8 ± 2.0
Dizziness	0.1 ± 0.3	0.7 ± 1.2	0.8 ± 1.7
Drowsiness	3.2 ± 5.2	3.6 ± 5.5	4.5 ± 6.1
Palpitations	0.0 ± 0.0	0.2 ± 0.4	0.1 ± 0.3
Headache	0.8 ± 1.2	1.1 ± 1.3	1.1 ± 1.9
Nausea	1.8 ± 2.1	2.9 ± 2.1	3.0 ± 2.8
Sleepiness	5.2 ± 4.2	4.3 ± 4.0	4.7 ± 5.2
Perspiration	0.6 ± 1.1	0.7 ± 1.2	0.6 ± 1.1
Blurred vision	1.5 ± 2.4	1.2 ± 2.1	1.2 ± 2.3
Tiredness	4.5 ± 4.6	3.8 ± 4.1	4.0 ± 5.1

significant differences in the immediate and first ($t_{6\rightarrow6}$) delayed recall of words were found after TRP or LYS depletion compared to placebo. However, there was a tendency that more words were recalled after LYS depletion than after TRP depletion ($t = 2.3$, $df = 12$, $P < 0.05$). No effects were found on recognition. In the second delayed recall test ($t_{6\rightarrow24}$), a significantly smaller number of learned words were recalled after TRP depletion than after placebo ($t = 2.7$, $df = 12$, $P < 0.025$). No significant differences in the second ($t_{6\rightarrow24}$) delayed recognition were found after TRP or LYS depletion compared to placebo. However, recognition RT tended to be faster after LYS depletion than after TRP depletion ($t = 2.4$, $df = 12$, $P < 0.05$).

Side effects

Scores of individual side effects per treatment are shown in Table 5. There were no statistically significant differences in side effects at t_0, t_6 and t_{24} reported after TRP depletion and LYS depletion compared to placebo and neither between TRP and LYS depletion.

Discussion

We compared effects of TRP depletion to those of LYS depletion in order to test the specificity of the TRP depletion method. We compared LYS and TRP depletion for the following reasons: first, LYS is an essential amino acid. Plasma LYS levels are more easily modified by a diet than those of a non-essential amino acid because the body is not able to produce LYS itself. Second, LYS and its derivatives are not involved in the production of any of the known neurotransmitters. Third, LYS is transported through the blood-brain barrier by a special carrier for basic amino acids, distinct from the carrier for TRP (Oldendorf and Szabo 1976). Although some overlap between these carriers exists, competition among amino acids for transport across the blood-brain barrier occurs mainly within the groups of amino acids carried by each of the systems. Thus, there is no evidence that LYS depletion will influence TRP uptake in the brain.

To the best of our knowledge, no studies have been conducted on lysine and mood or lysine and memory, probably because there is no theoretical support for such a relationship. The results of our study do not indicate that there is a relationship between lysine depletion and mood or memory.

Our results show that TRP depletion was followed by a significantly higher level of tiredness, together with a lowering of mood, and an impairment of consolidation of information in memory. No significant changes occurred after the placebo condition or after LYS depletion. Thus, the effects of TRP depletion are unlikely to have resulted from an aspecific inhibitory effect on protein metabolism in the brain.

The treatments were effective in causing depletion of TRP and LYS. The plasma depletion of LYS was however not as large as that of TRP (51% versus 74%), which is probably due to the relatively high levels of LYS in human blood compared to those of TRP. A failure to induce a severe lysine imbalance with a diet has also been observed in rats (Tews et al. 1981). Of course, a better comparison of effects would have been possible if the LYS depletion had been greater. Higher levels of ornithine and arginine in the amino acids mixture might lead to lower levels of LYS uptake in the brain by altering the plasma ratio of LYS/BNAAs.

Our assumption that LYS depletion would not influence TRP uptake in the brain was confirmed by the estimations of brain TRP uptake, as calculated from the TRP/LNAA ratios. This ratio was equal in both the placebo condition and the LYS depletion test. In general, ingestion of the amino acid mixtures cause few side effects (Table 5), although three subjects dropped out because of severe nausea and vomitting after the placebo mixture (two subjects) and the TRP depletion mixture (one subject). Because during the tests the side effects of the various challenges were comparable, our results cannot be attributed to side effects produced by TRP depletion.

The addition of a carbohydrate-fat mixture to the amino acid is a feature that distinguishes this TRP depletion method from previous studies, but this method was also used in the Riedel et al. (submitted) study. We chose to do so in order to provide sufficient nutrients to the subjects during a 24-h period. Carbohydrates when given without protein increase the rate of insulin, thus taking away several large neutral amino acids from the circulation (Fernstrom 1981), and hence might stimulate the uptake of TRP in the brain. However, it has also been argued that when proteins, or AA mixtures that stimulate protein synthesis, and carbohydrates are given simultaneously, the addition of carbohydrates does not alter TRP uptake in the brain (Fernstrom and Fernstrom 1995). Our data show that

in the TRP depletion condition, the uptake of TRP in the brain, as reflected by the TRP/ΣLNAA ratio, was diminished to a fairly low amount.

As to the effects of TRP-depletion on mood in this study, several remarks may be made. Despite the low number of subjects employed in the study, significant effects of TRP-depletion on the POMS summed score and on the POMS item tiredness were found. It may be noted here that ten of the 13 subjects were women and that eight of the 13 subjects had a positive family history for depression. As was mentioned previously, these factors contribute to the sensitivity of healthy subjects to the effects of TRP depletion (Benkelfat et al. 1994; Ellenbogen et al. 1996). We chose, however, not to consider these factors in the experimental design of the study because the total number of 13 subjects would render the design insensitive to those factors. The figures on sex and family history may, however, serve as circumstantial evidence to explain that in these healthy volunteers an effect of TRP-depletion on aspects of mood were found. Such effects were not found after LYS depletion and this can be interpreted as supporting the specificity hypothesis that the effects of TRP depletion on mood can be specifically attributed to lowered brain 5-HT. An effect of TRP depletion on tiredness has been reported earlier (Cleare and Bond 1995; Ellenbogen et al. 1996; Mc Dougle et al. 1996; Neumeister et al. 1997). A mood lowering effect of TRP depletion has also been found in several studies, as was mentioned in the introduction.

The effects of TRP depletion specifically on learning and memory in humans have been previously investigated by Park et al. (1994). This study showed an impairment of learning after ingestion of the TRP depletion mixture: after TRP depletion subjects needed more trials to learn correctly the spatial locations of abstract patterns and they made more errors in a paired association learning task. TRP depletion did not affect the initial number of correct associations made. Recognition memory was also not significantly affected. In a study designed similarly to the present one, our group investigated the effects of TRP depletion on learning and memory in 27 healthy volunteers. Briefly, the results showed no effects of TRP depletion on short-term memory performance (immediate recall) and neither on other cognitive parameters (short-term memory scanning, choice reaction time and visual search efficiency and -RT). TRP depletion specifically impaired delayed recall at 30 min ($t_{6\rightarrow6}$) and at 18 h ($t_{6\rightarrow24}$) after word learning. When a new word list was presented at (t_{24}), no differences in delayed recall 30 min after word list presentation were found between TRP depletion and placebo. Therefore it was concluded TRP depletion impairs consolidation of newly learned words, presumably in the 30 min after presentation. In the present study, we could only reproduce impaired delayed recall at 18 h ($t_{6\rightarrow24}$) after learning. An explanation for this may be that in the present study only 13 subjects were studied, whereas in the previous one 27 subjects were studied. As can be seen in Table 4, delayed recall scores after TRP depletion at t_6 were on average 1.3 lower than after placebo and 1.9 words lower than after LYS depletion, whereas in our previous study, using the same procedures and tests, the difference between TRP depletion and placebo was 2.3 words. The effects of TRP depletion at $t_{6\rightarrow24}$ were -3.0 words in the present study and -2.9 words in the previous study. Two explanations may be raised to account for the apparently different results. The first is one that attributes the apparently different results at t_6 to a problem of statistical power in the present study due to the small number of subjects. The second explanation is that the TRP depletion induced impairment of memory consolidation continues in the period between 30 min and 18 h after learning. The question would then become if we can exclude any external events that could be of explanatory value as to the observed difference in memory consolidation. The factors to exclude are mood and perhaps sleep. It is unlikely that mood effects explain impaired memory consolidation after TRP depletion, as previous studies (Park et al. 1994) have concluded that the observed learning and consolidation impairments were not mediated by mood. In the present study this is difficult to conclude, as the number of observations are low, but if lowered mood would explain impaired memory consolidation, then one would have to assume that this relationship is not momentary as they are not observed at the same moments in time. There is some evidence that TRP depletion alters sleep in a manner similar to those seen in depression (Bhatti et al. 1998; Voderholzer et al. 1998). Impaired sleep patterns may contribute to impaired delayed recall performance at the $t_{6\rightarrow24}$ assessment. Future studies could unravel this by including an assessment before sleeping, i.e. at $t_{6\rightarrow12}$.

Park et al. (1994) demonstrated that after TRP depletion subjects took more trials to learn to associate stimulus pairs. This can be taken as evidence for a relatively immediate effect (in terms of minutes) of TRP depletion on memory consolidation. Interestingly, TRP depletion impaired performance on a visual discrimination task only when TRP depletion was administered in the first session, that is when the procedure still had to be learned. When placebo was given in the first session, TRP depletion in the second session did not impair performance, which is another indication that TRP depletion impairs consolidation of newly learned material. The measures of short-term memory (storage), such as spatial working memory and the memory score in paired associate learning, were typically unaffected in the Park et al. (1994) study, so it may be concluded that the results of Park et al. (1994) and the present study are well in line, except for the lack of (significance of) a TRP depletion effect on the $t_{6\rightarrow6}$ delayed recall memory measure. Finally, the extended $t_{6\rightarrow24}$ measure may be a more sensitive procedure to

probe delayed recall, because it may be assumed that the memory trace becomes weaker over time and hence the task of recall and also recognition becomes more difficult at that time. In the case of the sensitivity of recognition performance as a measure for treatment-induced changes, this is clearly an advantage as the $t_{6\rightarrow6}$ measure of recognition efficiency may also be easily subjected to a ceiling effect.

Future studies of mood responses to TRP depletion should include depressed patients in remission, who are usually more sensitive to the effects of TRP depletion than normal subjects, and at least twice as many subjects with a more equal distribution of sex and age. Visual analogue scales should preferably be bipolar, to ensure a more normal distribution of results occurs so that more advanced statistics can be used.

In conclusion, the effects of TRP depletion in our study were specifically caused by TRP depletion and were not found after LYS depletion. The results of this study make general effects on protein metabolism an unlikely explanation for the effects of TRP depletion on mood and memory. Thus, our study provides evidence for the specificity of the TRP depletion method.

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