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The Effect of Tryptophan Depletion on Brain Activation Measured by Functional Magnetic Resonance Imaging during the Stroop Test in Healthy Subjects

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Summary

We investigated the role of serotonin in cognitive activation of the frontal cortex. The serotonergic system was affected by the administration of an amino acids mixture without tryptophan (tryptophan depletion). In a placebo-controlled double-blind cross-over study with 20 healthy volunteers, we tested the hypothesis that a tryptophan (serotonin) decrease affects the activation of prefrontal cortex by the Stroop test. Cognitive brain activation was evaluated by functional magnetic resonance imaging (fMRI). Tryptophan depletion decreased the plasma tryptophan level up to 90 % for five hours after the tryptophan-free drink had been consumed when compared with the same mixture with tryptophan ($p \leq 0.0001$). Tryptophan depletion did not affect the Stroop test performance. We compared fMRI activation in both conditions (tryptophan depletion and placebo) with plasma tryptophan levels as the covariates. The tryptophan depletion increased the activation (fMRI signal) in the bilateral mediofrontal cortex, anterior cingulate and left dorsolateral prefrontal cortex. The present findings allow the postulate that serotonergic medial forebrain and cingulum bundle pathways play a role in the activity of cortical structures involved in Stroop test processing.

Key words

Serotonin • Tryptophan depletion • fMRI • Stroop test • Cognition

Introduction

Deficits in cognitive functions are the crucial symptoms of a variety of neuropsychiatric disorders such as dementia, mental retardation, attention deficit disorder and schizophrenia. The relationship between cognitive test performance and functional morphological abnormality has been reported and the majority of findings confirm the relationships between cognitive dysfunction and the frontal forebrain (Williamson *et al.* 1989, Volz *et al.* 1997, Cabeza and Nyberg 2000, Loeb and Poggio 2002, Wolf *et al.* 2003). The role of various

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neuromediators in different domains of cognition is less understood. The prefrontal neuronal networks are targets of the forebrain monoaminergic (dopamine, noradrenaline and serotonin) and cholinergic projections. The function of these chemical ascending systems is often referred to as neuromodulatory. The term neuromodulation is taken to mean the enhancement, reduction, prolongation or curtailment of information processing by activity within these systems; often with only their minor participation in computations of the target neuronal systems (Robbins 2000). Some advances in psychopharmacology indicate that serotonin (5-HT) plays an important role in cognition (Altman and Normile 1988). The role of serotonin in cognition is supported by the cognitive enhancing effect of some atypical antipsychotics. Schizophrenic patients have clinically significant cognitive deficits (Goldberg *et al.* 1993) and atypical antipsychotic drugs show superior clinical efficacy, particularly against cognitive dysfunction (Horáček 2000, Kennedy *et al.* 2001). Atypical antipsychotic drugs such as clozapine, olanzapine, quetiapine, ziprasidone, risperidone show significantly greater $5HT_{2A}$ than D_2 antagonism. $5HT_{2A}$ antagonism hypothetically plays an important role in the improvement of cognitive deficits by these drugs (Meltzer and McGurk 1999) and chronic treatment with atypical antipsychotic drugs induces down-regulation of $5\text{-}HT_{2A}$ receptors (Gray and Roth 2001).

With respect to these findings we focused on the role of serotonin in cognition. The aim of the study was to determine the possible role of serotonin activity in the cognitive performance and brain activation that mediate function in the Stroop test. The Stroop test (Stroop 1935) is one of the most commonly used multiple-condition test of prefrontal functions. The original Stroop test was designed to establish competing response tendencies within the study and to assess the subject's ability to suppress interfering stimuli. In this interference condition, the subject is given color words which are printed in an incongruent ink color (i.e. the word "red" printed in blue ink). The subject is asked to report the ink color and ignore the word's semantic meaning by suppressing the tendency to read the color word. The interference generated in the Stroop test is often viewed as a general index of cognitive flexibility, attention and executive functions which depend on the prefrontal cortex (Graf *et al.* 1995, Uttl and Graf 1997). The advantage is that the Stroop test is relatively unaffected by test-retest situations (MacLeod and Mathews 1991).

In our experiment we influenced the serotonergic system by a tryptophan depletion technique. This method has been used increasingly as a tool for studying brain serotonergic systems in humans and in animals (Bel and Artigas 1996, Klaassen *et al.* 1999, Fadda 2000, Fadda *et al.* 2000). Tryptophan depletion is induced by the administration of an amino acid mixture without tryptophan. This amino acid mixture causes a decline in serotonin synthesis and turnover, as well as a probable decrease in the neuronal release of this amine (Moja *et al.* 1989, Klaassen *et al.* 1999). The technique of tryptophan depletion is based on the evidence that tryptophan conversion to serotonin through 5-hydroxy-tryptophan is catalyzed by the enzyme tryptophan hydroxylase. The activity of tryptophan hydroxylase is a rate-limiting step in the synthesis of serotonin (Gessa *et al.* 1975). The tryptophan-free diet consists of a large neutral amino acid (LNAA) mixture, with the exception of tryptophan. The LNAA competes with tryptophan for a single blood-brain barrier transporter. Therefore, the administration of TRP-free mixture leads to a decrease of brain tryptophan and consequently to a decrease of serotonin synthesis (Miller *et al.* 1992, Bell *et al.* 2001). In humans tryptophan levels decreased during acute tryptophan depletion with the maximum decrease within 5 hours after the tryptophan-free amino acid drink has been ingested (Harper *et al.* 1970, Delgado *et al.* 1990, Benkelfat *et al.* 1994).

Cognitive brain activation was evaluated by functional magnetic resonance imaging (fMRI). Functional magnetic resonance imaging is a non-invasive technique enabling the measurement of regional cerebral activation and thus elaborating dynamic functional maps of brain activity with uniquely high spatial (1.3 x 2.0 mm) and temporal (dynamic events lasting for 3-8 s) resolution compared to other functional brain-imaging methods. The technique is reported to detect regional changes in oxygenation of hemoglobin detectable in T2*-weighed images. These changes of signal intensity are related to local changes in perfusion as a result of neuronal activation in a given area. The method is referred to as a blood oxygen-level-dependent (BOLD) technique (Ogawa *et al.* 1992) and represents a versatile tool for studying regional brain activity during cognitive testing.

In a placebo-controlled double-blind cross-over study with 20 healthy volunteers, we tested the hypothesis that the tryptophan decrease affects the activation of the prefrontal cortex by the Stroop test.

Methods

Subjects and study design

Twenty right-handed healthy volunteers (10 males and 10 females) were investigated in this study. The mean age was 22.8±2.3 years (19-28 years, mean ± S.D), and the average education was 16.1±1.6 years (14-18 years). The family and previous medical history of any psychiatric disorders was evaluated, along with a structured interview and physical examination and biochemistry investigation to exclude any mental or somatic disorders.

The study was carried out in a double-blind design controlled by a placebo (amino acid mixture with tryptophan). The subjects were randomized into two groups, with one group beginning the procedure with inactive treatment on the first and second day, followed by active treatment on the third and fourth day. The order was reversed in the second group. During the first day of inactive treatment the subjects were on a normal diet. On the second day the placebo amino acid control solution was administered at 7:30 h and contained all amino acids (below) including tryptophan 2.3 g. Five hours later the subjects underwent neurocognitive testing and fMR investigation, and blood samples for tryptophan levels were collected. During the third day, the low tryptophan diet was administered with an amount of tryptophan not exceeding 160 mg, with 48 g of proteins and 10 500 kJ per day. On the fourth day the tryptophan-free drink was administered at 7:30 h. The amino acid drink consisted of 15 amino acids without tryptophan (Delgado *et al.* 1990, 1994): 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. g, L-lysine monochloride 11.0 g, L-methionine 3.0 g, L-phenylalanine 5.7 g, L-proline 12.2 g, L-serine 6.9 g, L-threonine 6.9 g, L-tyrosine 6.9 g, L-valine 8.9 g (L-threonine was obtained from Flamma S. P. A., amino acids from SHS Liverpool). L-methionine, L-cysteine and L-arginine were administered in separate capsules due to their unpleasant taste. Subjects were allowed to drink a non-sweet beverage, but did not ingest food during this procedure (Delgado *et al.* 1990, 1994). Five hours later the subjects again underwent neurocognitive testing, fMR investigation and the collection of blood samples to determine the tryptophan level.

Stroop test performance measure

Because head movement from fully vocalizing responses cause artifacts within the scanner, we used behavioral data acquired physically outside the scanner to investigate the changes in performance of the Stroop task. In the present study, a standard Stroop Color-Word task (Daniel 1983, Golden 1975) was administered to every subject five hours after the administration of a tryptophan-free drink or an inactive placebo. The Stroop test included three subtests. In the Color Naming subtest, the subjects were asked to report the color of randomly sequenced color rectangles. In the Word Reading subtest, the subjects were asked to read color words randomly printed in black ink, establishing a response set to reading color words (i.e. “red,” “green,” “blue”). In the Interference subtest, the subjects were given color words printed in an incongruent ink color (i.e. the word “red” printed in blue ink). The subjects were asked to report the ink color and ignore the word’s semantic meaning by suppressing the tendency to read the color word. All sections of the test were timed, and the time to complete each section is a dependent variable of interest.

Stroop task activation during fMRI and image acquisition

Subjects were scanned in a supine position on a padded scanner couch in a dimly-illuminated fMR room. Stimuli were projected onto a screen by a computer and a LCD projector. The projection screen was secured vertically within the magnet bore at leg level after the subject had been positioned. Head movement was minimized by a forehead strap. Subjects viewed the color words through a tilted mirror placed directly in front of their heads and detected the color of the words presented on a video monitor. A modified Stroop task was presented in blocks of active or resting conditions. In the resting condition all the words were printed in colors congruent to the meaning of the word (e.g. the word "blue" was displayed in the color blue). In the active condition all words were color names incongruent to the color presented (e.g., the word "blue" was displayed in red color). The experimental active (incongruent) condition alternated with the control (congruent) condition, and four blocks of each condition were performed. Each condition was presented in blocks lasting 56 s, with 24 presentations of words per block and an inter-stimulus interval (ISI) of 7 s. Sixty four T2*-weighed volume images were acquired during each measurement on a 1.5-T Siemens Magnetom Vision Unit (Siemens, Erlangen Germany) using a single-shot gradient echo EPI sequence (TR = 7 s, TE = 54 ms, flip angle = 90 °) in 27 oblique slices of 4 mm thickness each.

The matrix size was 128 x 128, with a voxel size of 1.8 x 1.8 x 4 mm.

fMRI data analysis

The data analysis was performed using Statistical Parametric Mapping (SPM99, http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab (Mathworks, USA). As a pre-processing step, the EPI images were realigned to the first one and all volumes were re-sliced with sinc interpolation. Mean images were created to estimate the normalizing parameters and then the normalized images were written using these parameters. The EPI volumes were transformed into the same stereotactic space so that comparisons among subjects, or a multi-subject analysis, were possible. To improve the signal to noise ratio and to compensate for the anatomical variability, all volumes were smoothed with a full width at half the maximum of 6 mm isotropic Gaussian kernel. SPM99 analysis was performed to find regions that had a significant change in BOLD signal during the active (incongruent) condition compared to the rest (congruent) condition, for all individual subjects in first level of analysis. The hemodynamic response was modeled with a boxcar function convolved with a hemodynamic response function. Confounds of global signal changes were removed by applying a high pass filter (cut-off cycle was 128 s). The results (contrasts) of individual evaluations in both conditions (tryptophan depletion and placebo) were compared in the second level of SPM99 analysis with plasma tryptophan level as covariate (model of 2 conditions and 1 covariate, SPM99). By the use of two sample T-tests we also compared the activation between males and females in the second level of analysis. P-values ≤ 0.001 uncorrected for multiple comparisons at a single voxel level at each cluster level were used with a minimum of five voxels over the threshold. P-values ≤ 0.05 corrected for multiple comparisons were identified for cluster level statistics. In congruence with the *a priori* hypothesis that serotonin activity (tryptophan level) affects the cognitive activation of the frontal cortex, the total number of voxels for the correction was defined as the entire frontal cortex volume (S.V.C.-small volume correction). S.V.C. was defined as the brain volume anterior from the central sulcus.

Statistics

Normal distribution in Stroop subtests and plasma tryptophan levels was tested by means of the Kolmogorov-Smirnov test with the Dallal and Wilkinson approximation to the Lilliefors' method. Because the data failed the normality test, we used non-parametric tests. The Wilcoxon matched paired test was used to compare the plasma tryptophan level. The analyses of gender differences, demographic data, comparisons of Stroop subtests between both conditions (tryptophan depletion and placebo) and with the Czech population norms (Daniel 1983) were performed by the Mann Whitney test.

HPLC determination of total plasma tryptophan levels

The reverse phase chromatographic analysis was accomplished on Separon SGX C18 with a column length of 15 cm and i.d. 3.0 mm (Tessek, Czech Republic) using isocratic elution (Dionex pump P580A and a Rheodine injector with a 20 μl sample loop) and fluorimetric detection at 300 nm for excitation and 340 nm for emission (Shimadzu RF-535). The mobile phase consisted of a phosphate buffer (0.01 M KH_2PO_4, 0.0025 M 1-heptane sulfonic acid, pH 4.5) – methanol (70: 30, v/v); at a flow rate of 1.2 ml/min a complete separation of TRP and 5-fluoro-dl-tryptophan (internal standard) and other amines were obtained within 5 min. The linear relationship has been found between peak-areas and concentrations of total TRP between 0.05 and 50 g/ml, $r = 0.999$. Intra-assay precision for duplicate determinations was 1.4 % and inter-assay reproducibility was 3 %.

Table 1. The results in three subtests of the Stroop test in conditions of tryptophan depletion (TRP **–**) and the control condition (TRP +). There were no significant differences between both conditions (Mann Whitney test).

	Color Naming		**Word Reading**		**Interference Condition**	
Group	**TRP –**	**TRP +**	**TRP –**	**TRP +**	**TRP –**	**TRP +**
Mean (s)	50.07	48.31	59.2	56.77	90.87	81.15
S.D.	7.878	10.05	6.405	11.44	15.61	13.64

Results

The influence of tryptophan depletion on tryptophan plasma levels and Stroop test performance

Tryptophan depletion decreased the total plasma tryptophan level five hours after the tryptophan-free drink was consumed (2.05±1.50 μg/ml) and was compared with the control amino acid mixture with tryptophan (18.95± 6.94 μg/ml, W= –190, p≤ 0.0001). We did not find any significant difference in the Stroop test outcome in the tryptophan-free and placebo condition (Table 1). The results in all Stroop subtests were better in our sample than in the Czech population norms (Colors p≤ 0.00001, Words p≤ 0.005, Interference p≤ 0.00001)

The influence of tryptophan depletion on Stroop test fMRI activation

The contrasts of individual fMRI evaluations in both conditions (tryptophan depletion and placebo) were compared in the second level of SPM99 analysis with plasma tryptophan levels as the covariates. The height threshold was T = 3.54 and extent threshold consisted of 5 or more voxels (k = 5). We found that in right hemisphere tryptophan depletion increased the fMRI signal (p≤0.001, Table 2) in the medial frontal gyrus (BA 10), middle frontal gyrus (BA 6), superior frontal gyrus (BA 8), inferior frontal gyrus (BA 9, 45) and anterior cingulate (BA 33). In the left hemisphere the fMRI signal was increased in the inferior frontal gyrus (BA 47), medial frontal gyrus (BA 10) and superior frontal gyrus (BA 6, 10). Using the multiple comparisons correction for the whole frontal cortex (S.V.C.) the left inferior frontal gyrus and the right medial frontal gyrus exceeded the threshold for p≤ 0.05 (Table 2, Fig. 1). We did not find any gender differences in fMRI activation.

Table 2. The increase of the BOLD fMR signal associated with Stroop test activation in tryptophan depletion compared with the placebo condition, and the tryptophan plasma levels as the covariate. The results indicate that there is a higher fMR signal in tryptophan depletion compared with the control (placebo) condition. The p-values for all voxels exceeding the height threshold (T=3.54) and extent threshold of 5 or more voxels (k=5) are lower than 0.001 before correction for multiple comparisons. The voxels with p-value ≤ 0.05 with correction for the all voxels in frontal cortex (S.V.C. - small volume correction) are marked by an asterisk (*). The statistical significance for the whole model (set-level) which indicates the chance of finding this or a greater number of clusters in the search is p = 0.003.

Cerebral region	BA	L or R	x, y, z			k_E
Inferior Frontal Gyrus *	47	L	-24	12	-16	96
Medial Frontal Gyrus *	10	R	6	52	0	313
Medial Frontal Gyrus	10	R	10	56	6	
Medial Frontal Gyrus	10	R	16	60	2	
Medial Frontal Gyrus	10	R	8	38	-8	
Medial Frontal Gyrus	10	L	-4	56	6	
Inferior Frontal Gyrus	47	L	-32	32	-10	41
Middle Frontal Gyrus	6	R	34	-2	50	27
Superior Frontal Gyrus	8	R	14	46	48	28
Superior Frontal Gyrus	10	L	-10	66	24	8
Superior Frontal Gyrus	6	L	-16	34	56	21
Middle Frontal Gyrus	8	L	-34	34	48	5
Anterior Cingulate	33	R	4	20	14	9
Inferior Frontal Gyrus	45	R	62	16	18	5
Inferior Frontal Gyrus	9	R	62	14	22	

R, right hemisphere; L, left hemisphere; BA, Brodmann's Area; x, y, z, coordinates of the Talairach space for each maximum; k_E, is the number of voxels exceeding the threshold by 5 or more.

Discussion

The 90 % decrease of plasma tryptophan after tryptophan depletion in our sample is in accordance with previous studies which also indicated the physiological effect of tryptophan depletion (Benkelfat *et al.* 1994). In

rodents, the TRP-free mixture induced serotonin depletion in the brain and influenced various forms of animal behavior such as pain sensitivity, sexual behavior, aggressiveness and cognition (Fadda *et al.* 1974, 2000, Fadda 2000, Miller *et al.* 1979, 1992, Moja and Benedetti 1996, Blokland *et al.* 2002). We did not find any difference in the Stroop test performance in tryptophan depletion and placebo conditions. The results of other human studies that focused on cognition are also inconsistent. In healthy volunteers, Gallagher *et al.* (2003) detected the improvement only of simple motor speed/attention following acute tryptophan depletion with a minimal effect on the other cognitive domains. Other studies in non-clinical populations confirmed a decreased cognitive ability after tryptophan depletion (Park *et al.* 1994, Luciana *et al.* 2001, Rubinsztein *et al.* 2001, Rogers *et al.* 2003). In schizophrenic subjects, plasma tryptophan decrease improved the performance in Stroop subtests (Rosse *et al.* 1992) and impaired the executive functions (Golightly *et al.* 2001). The lack of influence by tryptophan depletion on Stroop test performance in our sample supports the previous finding that the impact of tryptophan depletion on the mood and cognition is dependent on the individual vulnerability of the serotonergic system. For example, the decrease of 5-HT activity is characteristic for the depression and subjects with a depressive disorder in the past (Benkelfat *et al.* 1994, Hughes *et al.* 2002, Murphy *et al.* 2002) or in the family history (Riedel *et al.* 1999, 2002, Sobczak *et al.* 2002) are highly sensitive to tryptophan depletion compared with the controls. In our study we included only healthy volunteers without personal or familial history of depression or other psychiatric disorders. Moreover, the good Stroop test performance in our sample (performed by a younger average age group with a high level of training) would make the subjects even less sensitive to the serotonergic manipulation.

In previous neuroimaging studies the Stroop test activated the right or bilateral cingulate cortex, left dorsolateral prefrontal cortex and anterior lateral prefrontal cortex (Bench *et al.* 1993, George *et al.* 1997, Pardo *et al.* 1990, Taylor *et al.* 1997). In our study we found that tryptophan depletion increases fMRI activation in the bilateral mediofrontal cortex, anterior cingulate and left dorsolateral prefrontal cortex (Table 2, Fig. 1). These findings are in anatomical accordance with the above mentioned studies and indicate that tryptophan depletion increases the activation in the same areas as are involved in Stroop test processing. In recovered depressed subjects it was proven that tryptophan depletion was associated with diminished neural activity (^{15}O-PET) in the ventral anterior cingulate, orbitofrontal cortex and caudate nucleus regions (Smith *et al.* 1999). These regions partially overlap with our results, but the effect of decreased activation in depressed patient is in contrast with the increased activation in our sample. This discrepancy supports the role of individual serotonergic sensitivity in the effect of tryptophan depletion.

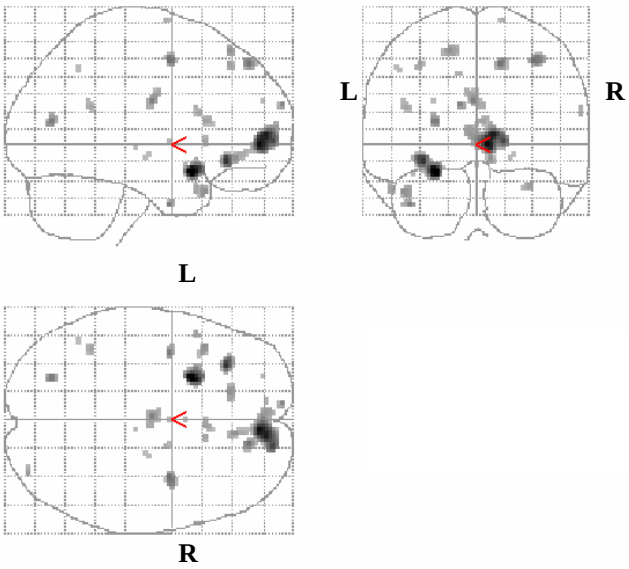

Fig. 1. The areas with higher BOLD fMR signal associated with the Stroop test activation in the condition of tryptophan depletion compared with control condition and with tryptophan level as the covariate (height threshold T=3.54, extent threshold k≥5, p≤ 0.001 uncorrected). R - right hemisphere; L - left hemisphere.

Our findings of an increased BOLD fMR signal in the mediofrontal, dorsolateral frontal cortex and cingulate regions during tryptophan depletion reflect both Stroop test-specific activation and the distribution of serotonin neurons in brain pathways. Serotonergic neurons in mammals are localized in the raphe nuclei and in the extraraphe reticular 5-HT cell groups. The large multipolar extensive ramification implies that the 5-HT neurons innervate and modulate multiple target networks in a coordinated fashion (Baumgarten and Lachenmayer 1985, Jacobs *et al.* 1990). The serotonergic medial forebrain bundle serves as the major ascending 5-HT pathway for the telencephalon specifically in the frontal, piriform and insular cortices, and also with moderate density to the occipital, entorhinal, orbitofrontal and infralimbic regions (Azmitia and Segal 1978). The cingulum bundle of the medial forebrain bundle is

involved in modulation of the anterior cingulate cortex. The 5-HT projections to the cortex exhibit a crude somatotropic arrangement with clustering of neurons in discrete spatial parts of the dorsal raphe nuclear complex and significant proportions of collateralized neurons which innervate functionally related cortical target areas. These arrangements support the modulatory role of the dorsal raphe in different types of information processing (Van Bockstaele *et al.* 1993, Gonzalo-Ruiz *et al.* 1995). Respecting the arrangement of the serotonergic system, our findings allow the suggestion that the serotonergic medial forebrain pathways and cingulum bundle play a role in the activity of cortical structures involved in Stroop test processing.

With reference to the our finding that tryptophan depletion influences fMRI activation but not Stroop test performance it is possible to speculate that the neuropsychological effect would be more pronounced in populations with affected 5-HT system such as depressive or schizophrenic patients. Another explanation is that the increased activity in the frontal cortex detected by fMRI compensates the cognitive processing deficit induced by the decrease of 5-HT and the psychological effect would be undetectable in this case.

Our fMRI data support the hypothesis that a serotonin blocking mechanism underlies the cognitive enhancing effect of atypical antipsychotic drugs with 5-HT_2 (and D_2) blocking properties. This effect would be mediated by the activation of the frontal brain regions involved in cognition.

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References

ALTMAN HJ, NORMILE HJ: What is the nature of the role of the serotonergic nervous system in learning and memory: prospects for development of an effective treatment strategy for senile dementia. *Neurobiol Aging* **9**: 627-638, 1988.

AZMITIA EC, SEGAL M: An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J Comp Neurol* **179**: 641-667, 1978.

BAUMGARTEN HG, LACHENMAYER L: Anatomical features and physiological properties of central serotonin neurons. *Pharmacopsychiatry* **18**: 180-187, 1985.

BEL N, ARTIGAS F: Reduction of serotonergic function in rat brain by tryptophan depletion: effects in control and fluvoxamine-treated rats. *J Neurochem* **67**: 669-676, 1996.

BELL C, ABRAMS J, NUTT D: Tryptophan depletion and its implications for psychiatry. *Br J Psychiatry* **178**: 399-405, 2001.

BENCH CJ, FRITH CD, GRASBY PM, FRISTON KJ, PAULESU E, FRACKOWIAK RS, DOLAN RJ: Investigations of the functional anatomy of attention using the Stroop test. *Neuropsychologia* **31**: 907-922, 1993.

BENKELFAT C, ELLENBOGEN MA, DEAN P, PALMOUR RM, YOUNG SN: Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiatry* **51**: 687-697, 1994.

BLOKLAND A, LIEBEN C, DEUTZ NE: Anxiogenic and depressive-like effects, but no cognitive deficits, after repeated moderate tryptophan depletion in the rat. *J Psychopharmacol* **16**: 39-49, 2002.

CABEZA R, NYBERG L: Imaging cognition II: An empirical review of 275 PET and fMRI studies. *J Cogn Neurosci* **12**: 1-47, 2000.

DANIEL J: *Stroop Test.* (in Slovak) *Psychodiagnostic and Didactic Tests***,** Bratislava, 1983.

DELGADO PL, CHARNEY DS, PRICE LH, AGHAJANIAN GK, LANDIS H, HENINGER GR: Serotonin function and the mechanism of antidepressant action. Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch Gen Psychiatry* **47**: 411-418, 1990.

DELGADO PL, PRICE LH, MILLER HL, SALOMON RM, AGHAJANIAN GK, HENINGER GR, CHARNEY DS: Serotonin and the neurobiology of depression. Effects of tryptophan depletion in drug-free depressed patients. *Arch Gen Psychiatry* **51**: 865-874, 1994.

FADDA F: Tryptophan-free diets: a physiological tool to study brain serotonin function. *News Physiol Sci* **15**: 260-264, 2000.

FADDA F, BIGGIO G, LIGUORI G: Binding of tryptophan to different serum proteins in humans and in rats. *Experientia* **30**: 635-637, 1974.

FADDA F, COCCO S, STANCAMPIANO R: A physiological method to selectively decrease brain serotonin release. *Brain Res Brain Res Protoc* **5**: 219-222, 2000.

GALLAGHER P, MASSEY AE, YOUNG AH, MCALLISTER-WILLIAMS RH: Effects of acute tryptophan depletion on executive function in healthy male volunteers. *BMC Psychiatry* **3**: 10, 2003.

GEORGE MS, KETTER TA, PAREKH PI, ROSINSKY N, RING HA, PAZZAGLIA PJ, MARANGELL LB, CALLAHAN AM, POST RM: Blunted left cingulate activation in mood disorder subjects during a response interference task (the Stroop). *J Neuropsychiatry Clin Neurosci* **9**: 55-63, 1997.

GESSA GL, BIGGIO G, FADDA F, CORSINI GU, TAGLIAMONTE A: Tryptophan-free diet: a new means for rapidly decreasing brain tryptophan content and serotonin synthesis. *Acta Vitaminol Enzymol* **29**: 72-78, 1975.

GOLDBERG TE, GREENBERG RD, GRIFFIN SJ, GOLD JM, KLEINMAN JE, PICKAR D, SCHULZ SC, WEINBERGER DR: The effect of clozapine on cognition and psychiatric symptoms in patients with schizophrenia. *Br J Psychiatry* **162**: 43-48, 1993.

GOLDEN CJ: The measurement of creativity by the Stroop Color and Word Test. *J Pers Assess* **39**: 502-506, 1975.

GOLIGHTLY KL, LLOYD JA, HOBSON JE, GALLAGHER P, MERCER G, YOUNG AH: Acute tryptophan depletion in schizophrenia. *Psychol Med* **31**: 75-84, 2001.

GONZALO-RUIZ A, LIEBERMAN AR, SANZ-ANQUELA JM: Organization of serotoninergic projections from the raphe nuclei to the anterior thalamic nuclei in the rat: a combined retrograde tracing and 5-HT immunohistochemical study. *J Chem Neuroanat* **8**: 103-115, 1995.

GRAF P, UTTL B, TUOKKO H: Color- and picture-word Stroop tests: performance changes in old age. *J Clin Exp Neuropsychol* **17**: 390-415, 1995.

GRAY JA, ROTH BL: Paradoxical trafficking and regulation of 5-HT_{2A} receptors by agonists and antagonists. *Brain Res Bull* **56**: 441–451, 2001.

HARPER AE, BENEVENGA NJ, WOHLHUETER RM: Effects of ingestion of disproportionate amounts of amino acids. *Physiol Rev* **50**: 428-558, 1970.

HORÁČEK J: Novel antipsychotics and extrapyramidal side effects. Theory and reality. *Pharmacopsychiatry* **33 (**Suppl 1): 34-42, 2000.

HUGHES JH, GALLAGHER P, YOUNG AH: Effects of acute tryptophan depletion on cognitive function in euthymic bipolar patients. *Eur Neuropsychopharmacol* **12**: 123-128, 2002.

JACOBS BL, WILKINSON LO, FORNAL CA: The role of brain serotonin. A neurophysiologic perspective. *Neuropsychopharmacology* **3**: 473-479, 1990.

KENNEDY A, JAIN S, VINOGRADOV S: Atypical antipsychotics for schizophrenia: Their collective role and comparative. *Formulary* **36**: 500-517, 2001.

KLAASSEN T, RIEDEL WJ, DEUTZ NE, VAN SOMEREN A, VAN PRAAG HM: Specificity of the tryptophan depletion method. *Psychopharmacology (Berl)* **141**: 279-286, 1999.

LOEB C, POGGIO GF: Neural substrates of memory, affective functions, and conscious experience. *Adv Anat Embryol Cell Biol* **166**: 1-111, 2002.

LUCIANA M, BURGUND ED, BERMAN M, HANSON KL: Effects of tryptophan loading on verbal, spatial and affective working memory functions in healthy adults. *J Psychopharmacol* **15**: 219-230, 2001.

MACLEOD C, MATHEWS A: Biased cognitive operations in anxiety: accessibility of information or assignment of processing priorities? *Behav Res Ther* **29**: 599-610, 1991.

MELTZER HY, MCGURK SR: The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. *Schizophr Bull* **25**: 233-255, 1999.

MILLER BL, PACHTER JS, VALZELLI L: Brain tryptophan in isolated aggressive mice. *Neuropsychobiology* **5**: 11-15, 1979.

MILLER HL, DELGADO PL, SALOMON RM, LICINIO J, BARR LC, CHARNEY DS: Acute tryptophan depletion: a method of studying antidepressant action. *J Clin Psychiatry* **53** (Suppl): 28-35, 1992.

MOJA EA, BENEDETTI F: Sexual behavior in male and female rats after a tryptophan-free amino acid mixture. *Adv Exp Med Biol* **398**: 89-91, 1996.

MOJA EA, CIPOLLA P, CASTOLDI D, TOFANETTI O: Dose-response decrease in plasma tryptophan and in brain tryptophan and serotonin after tryptophan-free amino acid mixtures in rats. *Life Sci* **44**: 971-976, 1989.

MURPHY FC, SMITH KA, COWEN PJ, ROBBINS TW, SAHAKIAN BJ: The effects of tryptophan depletion on cognitive and affective processing in healthy volunteers. *Psychopharmacology (Berl)* **163**: 42-53, 2002.

OGAWA S, TANK DW, MENON R, ELLERMANN JM, KIM SG, MERKLE H, UGURBIL K: Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci USA* **89**: 5951-5955, 1992.

PARDO JV, PARDO PJ, JANER KW, RAICHLE ME: The anterior cingulate cortex mediates processing selection in the Stroop attentional conflict paradigm. *Proc Natl Acad Sci USA* **87**: 256-259, 1990.

PARK SB, COULL JT, MCSHANE RH, YOUNG AH, SAHAKIAN BJ, ROBBINS TW, COWEN PJ: Tryptophan depletion in normal volunteers produces selective impairments in learning and memory. *Neuropharmacology* **33**: 575-588, 1994.

RIEDEL WJ, KLAASSEN T, DEUTZ NE, VAN SOMEREN A, VAN PRAAG HM: Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. *Psychopharmacology (Berl)* **141**: 362-369, 1999.

RIEDEL WJ, KLAASSEN T, SCHMITT JA: Tryptophan, mood, and cognitive function. *Brain Behav Immun* **16**: 581-589, 2002.

ROBBINS TW: Chemical neuromodulation of frontal-executive functions in humans and other animals. *Exp Brain Res* **133**: 130-138, 2000.

ROGERS RD, TUNBRIDGE EM, BHAGWAGAR Z, DREVETS WC, SAHAKIAN BJ, CARTER CS: Tryptophan depletion alters the decision-making of healthy volunteers through altered processing of reward cues. *Neuropsychopharmacology* **28**: 153-162, 2003.

ROSSE RB, SCHWARTZ BL, ZLOTOLOW S, BANAY-SCHWARTZ M, TRINIDAD AC, PEACE TD, DEUTSCH SI: Effect of a low-tryptophan diet as an adjuvant to conventional neuroleptic therapy in schizophrenia. *Clin Neuropharmacol* **15**: 129-141, 1992.

RUBINSZTEIN JS, ROGERS RD, RIEDEL WJ, MEHTA MA, ROBBINS TW, SAHAKIAN BJ: Acute dietary tryptophan depletion impairs maintenance of "affective set" and delayed visual recognition in healthy volunteers. *Psychopharmacology (Berl)* **154**: 319-326, 2001.

SMITH KA, MORRIS JS, FRISTON KJ, COWEN PJ, DOLAN RJ: Brain mechanisms associated with depressive relapse and associated cognitive impairment following acute tryptophan depletion. *Br J Psychiatry* **174**: 525-529, 1999.

SOBCZAK S, RIEDEL WJ, BOOIJ I, AAN HET RM, DEUTZ NE, HONIG A: Cognition following acute tryptophan depletion: difference between first-degree relatives of bipolar disorder patients and matched healthy control volunteers. *Psychol Med* **32**: 503-515, 2002.

STROOP JR: Studies of interference in serial verbal reaction. *J Exp Psychol* **1**: 643-662, 1935.

TAYLOR SF, KORNBLUM S, LAUBER EJ, MINOSHIMA S, KOEPPE RA: Isolation of specific interference processing in the Stroop task: PET activation studies. *Neuroimage* **6**: 81-92, 1997.

UTTL B, GRAF P: Color-Word Stroop test performance across the adult life span. *J Clin Exp Neuropsychol* **19**: 405-420, 1997.

VAN BOCKSTAELE EJ, BISWAS A, PICKEL VM: Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. *Brain Res* **624**: 188-198, 1993.

VOLZ HP, GASER C, HAGER F, RZANNY R, MENTZEL HJ, KREITSCHMANN-ANDERMAHR I, KAISER WA, SAUER H: Brain activation during cognitive stimulation with the Wisconsin Card Sorting Test – a functional MRI study on healthy volunteers and schizophrenics. *Psychiatry Res* **75**: 145-157, 1997.

WILLIAMSON PC, KUTCHER SP, COOPER PW, SNOW WG, SZALAI JP, KAYE H, MORRISON SL, WILLINSKY RA, MAMELAK M: Psychological, topographic EEG, and CT scan correlates of frontal lobe function in schizophrenia. *Psychiatry Res* **29**: 137-149, 1989.

WOLF H, JELIC V, GERTZ HJ, NORDBERG A, JULIN P, WAHLUND LO: A critical discussion of the role of neuroimaging in mild cognitive impairment. *Acta Neurol Scand* Suppl 179: 52-76, 2003.

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