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Effects of tryptophan loading on verbal, spatial and affective working memory functions in healthy adults

Monica Luciana, E. Darcy Burgund, Margit Berman and Karen L. Hanson

Department of Psychology, University of Minnesota, Minneapolis, Minnesota, USA.

Serotonin (5-HT) appears to modulate affective behaviours by providing a homeostatic threshold around which other transmitters respond. This general principle of activity should hold for other types of behaviour, including cognition, but has not been extensively examined. We hypothesized, based on past findings, that increased 5-HT would constrain prefrontally guided working memory functions that are mediated by catecholamine neurotransmitters. Healthy adults ingested amino acid compounds designed to deplete and load systemic tryptophan levels in a repeated-measures crossover design. Outcome variables included total plasma tryptophan, serum prolactin levels and self-report measures of mood, as well as measures of motor skill, attention, memory span and working memory for verbal, spatial and affective stimuli. Our findings indicate decrements in working memory for verbal and affective stimuli following tryptophan loading versus depletion, as well as subtle changes in vigilant attention and motor coordination. Implications for the aetiology and treatment of affective disorders and psychosis are discussed.

Key words: cognition; prefrontal; serotonin; tryptophan; working memory

Introduction

That monoaminergic neurotransmitters modulate emotional and cognitive states is generally accepted. The catecholamines modulate arousal levels, motivation towards rewards and goal-directed actions, as well as aspects of attention and cognitive functions such as working memory (Goldman-Rakic, 1987). Specifically, the experimental manipulation of dopamine (DA) transmission in the dorsolateral prefrontal cortex affects performance on spatial working memory tasks (Brozoski *et al*., 1979; Goldman-Rakic, 1987; Sawaguchi *et al*., 1988; Sawaguchi *et al*., 1990; Sawaguchi and Goldman-Rakic, 1991). Yet, the catecholamines do not act in isolation, and their activity is modulated by other neurochemicals, including serotonin (5-HT). Behavioural studies have demonstrated that 5-HT provides tonic inhibition over the facilitatory effects of DA with respect to a host of behaviours, including hyperlocomotion, DA-enhanced exploratory behaviour, intracranial self-stimulation and responses to incentive cues (Gerson and Baldessarini, 1980; Jenner *et al*., 1983; Leone *et al*., 1983; Hodges and Green, 1984; Leccese and Lyness, 1984; Gately *et al*., 1985; Depue and Spoont, 1986; Soubrie, 1986; Nakahara *et al*., 1989; Spoont, 1992; Howell *et al*., 1997). Moreover, there are overlapping DA and 5-HT receptor distributions in both limbic and cortical brain regions, and modulation of the ascending A10 DA pathways by 5-HT has been demonstrated (Goldman-Rakic *et al*., 1990; Hagan *et al*., 1993; Wang *et al*., 1995).

Whether these interactions influence cognitive, as well as limbic and striatal, functions has not been comprehensively studied. We hypothesized that 5-HT might regulate working memory in much the same way that it regulates other DA-modulated behaviours. We have demonstrated that pharmacological activation of D_2 DA receptors in normal humans facilitates spatial working memory (Luciana *et al*., 1992; Luciana and Collins, 1997) and, in addition, we reported that the same spatial working memory processes that were facilitated by bromocriptine (DA agonist) were impaired by fenfluramine (5-HT agonist) in the same group of healthy subjects (Luciana *et al*., 1998). Our finding of spatial working memory impairment using a 5-HT agonist supported the contention that 5-HT activation has a constraining influence over cognitive processes, particularly when those processes are tightly mediated by the activity in another dynamic system, as is the case with the influence of DA over spatial working memory. Whether this finding of serotonin-induced working memory impairment could be generalized to forms of working memory that involve affectively valenced stimuli and replicated through the use of other psychopharmacological agents was the focus of the current study.

A role for serotonin in working memory has not been systematically studied, but there are findings in the animal literature to support the notion that high serotonin impairs specific cognitive processes, including working memory, while low serotonin improves them (Fibiger *et al.* 1978; McEntee and Mair, 1980; Gray, 1982; Lalonde and Vikis-Freibergs, 1985; Altman and Normile, 1986; Winter and Petti, 1987; Altman and Normile, 1988; Barnes *et al*., 1990; Domeney *et al*., 1991; McEntee and Crook, 1991; Carli *et al*., 1992; Carli and Samanin, 1992; Pitsikas *et al*., 1993; Quartermain *et al*., 1993; Cole *et al*., 1994; Fontana *et al*., 1995).

Whether a similar agonist/antagonist relationship can be applied to humans is not known, although cognitive deficits have been noted in human volunteers following acute doses of 5-HT agonists (Grasby *et al.*, 1992; Weinstein *et al*., 1996; Robbins, 1997) and in psychiatric patients receiving selective serotonin reuptake inhibitors (Bartfai *et al.*, 1991; Abbruzzese *et al.*, 1993). In addition, the abuse of serotonergic drugs that deplete brain 5-HT (e.g. MDMA: 'Ecstasy') is associated with impaired recall of verbal information and with impulsive responding on tests of frontal lobe function (Morgan, 2000). These convergent lines of enquiry suggest that high levels of serotonin, whether experienced acutely or chronically, may impede informational flow in a manner that is detrimental to working memory and perhaps other higher order cognitive functions.

In the current study, the effects of both increased and depleted serotonin levels on cognitive function were examined within the same group of subjects through the use of dietary tryptophan manipulations. The manipulation of serotonin synthesis through tryptophan loading or depletion involves the ingestion of a high-protein amino acid drink that is balanced to either increase or decrease the likelihood that tryptophan will successfully compete with other large neutral amino acids for transport across the blood–brain barrier (Biggio *et al.*, 1974; Young *et al.*, 1985; Salomon *et al*., 1997). Rapid depletion of tryptophan using this technique has been employed in numerous studies of healthy adults and depressed patients (Young *et al*., 1985; Young *et al*., 1989; Delgado *et al.*, 1994; Park *et al.*, 1994; Cleare and Bond, 1995; Coull *et al*., 1995; Wolfe *et al*., 1995; Ellenbogen *et al*., 1996; Moeller *et al*., 1996; Bremner *et al*., 1997; Salomon *et al.*, 1997; Voderholzer *et al*., 1998; Delgado *et al.*, 1999). Following acute depletions of tryptophan, plasma tryptophan levels steadily decrease over the course of approximately 7 h. In studies involving nonhuman primates, this decrease has been associated with decreased brain 5-HT (Young *et al*., 1989). Additionally, changes in cerebrospinal fluid levels of the serotonin metabolite 5-HIAA following tryptophan depletion have been reported in human subjects (Carpenter *et al*., 1998; Williams *et al*., 1999).

Increases and decreases in brain 5-HT activity, including those achieved through tryptophan administration, also result in altered patterns of neuroendocrine function that can be observed through the secretion of several peripherally measured hormones, including cortisol, prolactin, and growth hormone (for reviews, see Murphy *et al.*, 1996; Van de Kar, 1996). In these studies, it has been concluded that acute dietary manipulations of tryptophan influence postsynaptic 5-HT receptor sensitivity. The remaining evidence linking dietary tryptophan manipulations to changes in central serotonin neurotransmission is provided by behavioural data.

There have been reports of lowered mood and increased anxiety in healthy and affectively disordered individuals after tryptophan depletion (Young *et al*., 1985; Benkelfat *et al*., 1994; Ellenbogen *et al*., 1996; Delgado *et al.*, 1999; Klaassen *et al*., *et al.*, 1999; Smith *et al*., 1999). Studies of cognitive changes in human subjects after tryptophan depletion have yielded a mixed pattern of findings. While researchers have found no effects of tryptophan depletion on executive function (LeMarquand *et al.*, 1998), others report that it affects the ability to form appropriate stimulus response associations (Coull *et al.*, 1995) and to learn visual discriminations (Park *et al.*, 1994; Rogers *et al.*, 1999). Deficits in memory consolidation and sensorimotor performance have also been reported (Coull *et al.*, 1995; Riedel *et al.*, 1999). Young *et al.* (1985) also reported that tryptophan depleted subjects experienced a lowered mood and demonstrated selective attention to dysphoric stimuli in the context of performing a cognitive task. None of the studies examining cognitive function following tryptophan depletion have included a tryptophan loading condition.

To examine the agonist and antagonist properties of serotonin in relation to cognitive function, we studied a group of healthy adults in a repeated measures design under acute tryptophan depletion (TD) and acute tryptophan loading (TL) conditions. Outcome variables included: (i) changes in peripheral arousal as indicated by pulse rate and blood pressure; (ii) changes in total plasma tryptophan levels; (iii) changes in serum levels of the neurohormones cortisol and prolactin; (iv) self-reported changes in measures of positive affect, negative affect and items related to side-effects associated with the tryptophan manipulation procedures; and (v) measures of cognitive functioning, including tests measuring psychomotor performance, vigilant attention, memory span, verbal fluency and working memory.

Methods

This study was approved by the Research Subjects' Protection Program at the University of Minnesota and by the Scientific Advisory Committee of the General Clinical Research Center (CRC). Nineteen adult participants, aged 18–31 years (mean 22.2 years, SD 4.5 years) completed the protocol. Subjects were scheduled for a pre-manipulation screening interview, during which a medical and psychiatric history was obtained. All participants were psychiatrically healthy, as determined by structured diagnostic interview (the Structured Clinical Interview for DSM-IV, patient version) (First *et al*., 1997). In addition, all participants were medically screened. Exclusions for pregnancy, oral contraceptive use during the previous 4 months, current medication use, menstrual irregularities, endocrinopathies, neurological disease and other relevant clinical conditions were applied. Individuals also completed a battery of self-report personality inventories, and baseline measures of all cognitive tests were administered. This session is subsequently referred to as the 'screening' session.

Following this initial screening, participants were scheduled for an additional 2 days of study at the University of Minnesota's General Clinical Research Center. Females were studied during the early to-mid-follicular phase (days 1–10) of the menstrual cycle. On each of these 2 days, participants were studied in a double-blind repeated-measures design, receiving either a tryptophan depletion or tryptophan loading procedure. Each tryptophan-manipulation session was separated in time by no less than 7 days, and sessions were counterbalanced between subjects. Prior to each tryptophan-manipulation session, individuals were required to observe a low-tryptophan diet that was prepared by the CRC's research dietician. The rationale behind this dietary restriction was to lower systemic tryptophan levels in order to equalize baseline values on each day of study and to induce maximal responsivity to each of the subsequent manipulations. This diet was consumed for 48 h prior to each session. Following the 48-h tryptophan-restricted diet, participants arrived at the CRC at 10.00 h for the tryptophan manipulation procedures. The protocol ran from 10.00 h to 18.15 h, beginning with the insertion of an intravenous catheter into the nondominant forearm of each participant shortly after his/her

arrival. Participants fasted from midnight the previous night with the exception of the amino acid mixture (provided at 10.30 h) and three 8 oz glasses of fruit juice at 12.00 h, 14.00 h and 16.00 h to prevent extreme hypoglycemia. Water was available upon request. Between 10.30 h and 18.15 h, blood was drawn for the determination of plasma total tryptophan, serum prolactin and serum cortisol. Additionally, participants completed hourly measures of mood state using the Positive and Negative Affect Schedule (PANAS) (Watson *et al*., 1988). The PANAS is a 20-item self-report rating scale that measures the degree (on a 5-point scale) to which individuals are currently experiencing items related to positive and negative affect.

The same 5-point scale was used to collect hourly ratings on the intensity of the following possible side-effects: bored, light-headed, hungry, fatigued, fidgety, dull, headache, cold, nauseous. Subjects remained recumbent throughout the day except from 14.30 h to 16.30 h when they completed a battery of cognitive tests.

Tryptophan depletion

The tryptophan depletion procedure was identical to that reported in several past studies (Young *et al*., 1985; Smith *et al.*, 1987; Delgado *et al.*, 1994; Cleare and Bond, 1995; Bjork *et al*., 1999; Delgado *et al.*, 1999). The amino acids used in this study were purchased from Ajinomoto USA, Inc. (Paramus, NJ, USA) At 10.30 h, subjects consumed a liquid amino acid mixture consisting of the following: L-alanine (5.5 g), L-glycine (3.2 g), L-histidine (3.2 g), L-isoleucine (8.0 g), L-leucine (13.5 g), L-lysine (8.9 g), L-phenylalanine (5.7 g), L-proline (12.2 g), L-serine (6.9 g), L-threonine (6.5 g), L-tyrosine (6.9 g) and L-valine (8.9 g). The amino acids, obtained in powdered form, were mixed with 300 ml of water that was then flavored with chocolate syrup. Subjects were instructed to drink the mixture as quickly as possible. Because of their unpalatable tastes, the following amino acids were placed in capsules that were ingested with the drink mixture: L-cysteine (2.7 g), L-arginine (4.9 g) and L-methionine (3.0 g). Hence the total protein content consumed was 100 g.

Tryptophan loading

The identical procedure was used, but the liquid mixture was supplemented with 10.3 g. of L-tryptophan (Young *et al.*, 1985; Cleare and Bond, 1995; Bjork *et al.*, 1999). Participants and experimenters were blind as to which mixture was being ingested on each day.

Pre-drink samples for baseline serum prolactin, cortisol, and total plasma tryptophan were obtained at 10.30 h, immediately prior to the ingestion of the protein drink (i.e. no more than 30 min after venapuncture and adoption of a recumbent position). Post-drink samples were obtained at 13.30 h, 15.30 h and 17.30 h for serum cortisol and prolactin. A single post-drink sample for plasma tryptophan was obtained at 15.30 h, because this time point (5 h post-ingestion) has been shown in prior studies to be the time of maximal plasma tryptophan depletion following the amino acid mixture (Young *et al.*, 1985; Smith *et al.*, 1987; Young *et al.*, 1989; Delgado *et al.*, 1994, 1999). Blood samples were immediately centrifuged and prepared for analysis. Serum cortisol and prolactin assays were conducted at the Fairview-University of Minnesota Hospital Laboratory. Tryptophan assays were performed at the Mayo Medical Laboratory (Rochester, MN).

Cognitive tests administered

A summary of cognitive tests and variables measured is presented in Table 1. To ensure that any observed tryptophan effects on working memory function were not due to influences on other cognitive abilities that contribute to working memory performance, we also measured vigilant attention, short-term memory span and psychomotor skill.

Working memory/executive function

Spatial working memory (Luciana et al., 1992; Luciana and Collins, 1997; Luciana et al., 1998)

On each of 48 trials, the participant viewed a fixation point in the centre of a computer monitor. During the viewing interval, a dot flashed in the periphery of the fixation point for 200 ms, after which both the dot and fixation point disappeared from view. The screen was masked for a delay interval (500 ms, 4 s or 8 s, randomly interspersed). A 'no delay' condition was also employed. Following the delay interval, the participant indicated the remembered location of the stimulus using a touch pen input device (FTG Data Systems, Inc., Stanton, CA, USA). Accuracy and response latency were recorded for each trial.

Affective working memory

This task utilized a modification of the delayed paired associates paradigm, which has been described by Milner (1995) as a sensitive index of frontal lobe dysfunction. In a previous study (Luciana and Collins, 1997), we used this paradigm with geometric designs as stimuli to measure nonspatial working memory. This variant of the task uses affective stimuli. On each of 96 trials, participants viewed a central '+' in the centre of a computer monitor. After 3 s, a face appeared. The face, presented in black and white, was a stimulus taken from the Ekman Pictures of Facial Affect that displayed one of seven affective states (neutral, happy, surprised, disgusted, fearful, angry or sad.). Type of affect and gender of the individual displayed were varied and unpredictable across trials. No stimulus was presented twice. Following stimulus presentation, the screen darkened for a delay interval of either 500 ms or 8 s. Afterwards, a second part of a face appeared comprising the eyes, nose or mouth of one of the Ekman faces. Individuals had to decide whether or not this second facial feature was an identical match (in terms of facial identity and affect) to that previously presented as the target stimulus. To indicate response selections, participants pressed 'yes' or 'no' buttons. Stimulus presentation and response time measurements were controlled through the use of the PsyScope software package (Cohen *et al*., 1993) and button box. Accuracy and response latency for each stimulus type were recorded. Examples of task stimuli are presented in Figure 1.

Verbal fluency (controlled word association test)

This test measured the ability to generate words within a lexical category, and has been used to infer expressive speech ability as well as frontal lobe function (Lezak, 1983). Participants were presented with a target letter (e.g. the letter 'F'), and told to generate as many words as possible beginning with that letter within a 1-min period. Three letters were used. Separate versions of the task were used for each experimental session. The number of words generated per letter is the primary variable of interest.

Table 1 Cognitive task performance across study conditions

Task variable	Screening	Loading	Depletion
Motor			
Finger tapping			
Average no. taps dominant hand	50.2 ± 7.9	50.4 ± 7.9	50.5 ± 8.4
Average no. taps nondominant hand	47.5 ± 7.7	48.1 ± 7.1	48.6 ± 7.2
Grooved pegboard			
Dropped pegs dominant hand	0.33 ± 0.84	0.67 ± 0.84	0.28 ± 0.46
Dropped pegs nondominant hand	0.22 ± 0.43	0.67 ± 1.03	0.50 ± 0.71
Time-to-completion dominant hand (sec)	65.90 ± 8.1	65.00 ± 8.7	62.30 ± 7.3
Time-to-completion nondominant hand (sec)	72.90 ± 9.4	75.90 ± 10.4	70.60 ± 9.5
Attention/concentration			
Letter cancellation task			
Time-to-completion (sec)	107.40 ± 20.6	101.80 ± 21.7	98.2 ± 16.1
Commission errors	0.21 ± 0.71	0.00 ± 0.00	0.0 ± 0.0
Omission errors	2.00 ± 2.4	0.50 ± 0.9	1.7 ± 1.9
Memory span			
No. of spatial items correctly recalled	7.42 ± 1.2	7.70 ± 1.0	7.63 ± 1.3
No. digits recalled in forward order	6.79 ± 1.4	7.16 ± 1.3	7.05 ± 1.6
No. digits recalled in backward order	5.73 ± 1.5	5.37 ± 1.5	5.95 ± 1.4
Working memory/executive function			
Verbal fluency			
Total number of words generated	47.0 ± 11.1	51.9 ± 12.1	50.3 ± 11.5
Spatial working memory			
Average error no delay (mm)	3.8 ± 2.0	4.4 ± 3.1	4.1 ± 3.2
Average error 500 ms delay	6.5 ± 1.6	6.8 ± 2.3	6.9 ± 3.7
Average error 4 s delay	8.6 ± 1.7	9.6 ± 3.1	9.9 ± 5.8
Average error 8 s delay	11.2 ± 3.3	11.5 ± 4.8	11.4 ± 4.7
Affective working memory			
Percent correct sad faces			
500 ms	84 ± 17	78 ± 18	76 ± 16
8 s	67 ± 17	53 ± 25	71 ± 14
Percent correct angry faces			
500 ms	79 ± 19	88 ± 13	90 ± 13
8 s	67 ± 13	61 ± 20	66 ± 14
Percent correct fearful faces			
500 ms	57 ± 13	64 ± 15	58 ± 15
8 s	66 ± 16	71 ± 19	76 ± 20
Percent correct disgusted faces			
500 ms	71 ± 18	70 ± 23	73 ± 18
8 s	70 ± 16	77 ± 21	77 ± 12
Percent correct happy faces			
500 ms	68 ± 14	76 ± 12	72 ± 15
8 s	64 ± 17	69 ± 19	66 ± 22
Percent correct surprised faces			
500 ms	64 ± 16	71 ± 13	74 ± 12
8 s	67 ± 18	73 ± 23	68 ± 20
Percent correct neutral faces			
500 ms	68 ± 15	74 ± 14	71 ± 15
8 s	64 ± 17	63 ± 20	64 ± 17

All values represent means ± one SD.

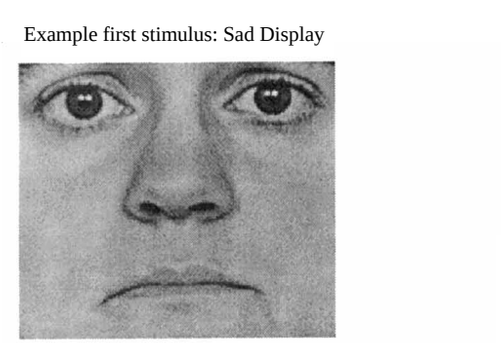

Figure 1 Sample stimuli from the Affective Working Memory Task. Individuals fixated on a central cue presented on a computer screen. A stimulus briefly appeared that was a photograph of a human face (see example first stimulus). Following delays of either 500 ms or 8 s, a second stimulus appeared, a photographed part of a face that was either a match or a nonmatch to the first stimulus. In order to be a correct match to the first stimulus, the identity and emotion displayed had to be the same

Table 2 Total plasma tryptophan, serum prolactin, and serum cortisol levels during each condition

	10.30 h	13.30 h	15.30 h	17.30 h
Total plasma tryptophan[a]				
Tryptophan loading	53.22 ± 9.44	–	551.4 ± 147.6	–
Tryptophan depletion	52.5 ± 10.9	–	7.7 ± 4.7	–
Serum prolactin levels[b]				
Tryptophan loading	12.6 ± 8.9	15.8 ± 10.2	11.1 ± 6.8	11.2 ± 6.6
Tryptophan depletion	12.5 ± 7.8	12.6 ± 6.6	9.8 ± 5.0	11.7 ± 7.4
Serum cortisol levels[b]				
Tryptophan loading	15.6 + 4.9	9.8 ± 3.8	14.0 ± 5.3	9.7 ± 3.4
Tryptophan depletion	15.4 ± 5.3	9.6 ± 2.0	13.0 ± 5.1	11.7 ± 5.7

[a]All values represent means ± SD expressed in µmol/l. According to the Mayo Medical Laboratory, where the assays were conducted, the normal reference range for individuals in this age group is 10–140µmol/l. [b]Values represent means ± SD. Prolactin levels are expressed in µg/l. Cortisol levels are expressed in µg/dl.

Short-term attention and memory span

Digit Span (Wechsler Adult Intelligence Scale, 3rd Revision)

This test measured immediate recall of auditory verbal information. A sequence of digits was presented to the subject, and s/he repeated the sequence after it was presented (digits forward). In a second condition, series' of digits were read, and participants repeated them in reverse order (digits backward). The number of digits correctly recalled in the proper sequence was recorded. An individual's forward digit span was regarded as a measure of immediate verbal memory and attention, while backward digit span was regarded as a measure of verbal working memory.

Spatial Span (Cambridge Neuropsychological Test Automated Battery)

This test measured immediate recall of visually presented nonverbal information, and is a nonverbal analogue of the digit span test, described above (Fray *et al.*, 1996). Participants, seated at a computer terminal, viewed arrays of squares on the screen. One by one, some of the squares 'lit up' in a sequence. Participants reproduced the sequence by touching the squares in the remembered sequence. The nonverbal memory span was recorded as the number of items that could be successfully remembered in the correct order.

Letter Cancellation Task (Lezak, 1983)

This task measured immediate attention and vigilance. Participants viewed a piece of paper on which was printed rows of capitalized letters. They were instructed to work row by row, crossing out all occurrences of the letters 'E' and 'C'. Time-to-completion and number of errors (errors of omission and commission) were recorded.

Motor speed/accuracy

Finger tapping test

This test measured motor speed. Participants tapped a key as many times as possible within 10-s periods. Three trials were administered for each hand, and the number of taps per trial was recorded.

Grooved pegboard test

This test is a measure of psychomotor skill. Participants were presented with a flat board containing rows of holes. They were also presented with small metal 'pegs' that fit into the holes on the board. The pegs are shaped so that one side is notched. The holes are differentially orientated so that each peg must be correctly manipulated in order to fit into them. Under timed conditions, participants used the pegs to fill the holes on the board using first the right hand, then the left hand. Accuracy and response latency were recorded.

Results

Data were analysed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA), version 9.0 for Windows. Repeated measures analyses of variance were used to evaluate the dependent variables across the depletion and loading conditions, with session order entered as a between-subjects variable. When significant interactions were found, paired *t*-tests were conducted post-hoc to determine the nature of those interactions. Alpha levels below 0.05 were considered statistically significant.

Effects on plasma tryptophan, serum prolactin, and cortisol levels (Table 2)

Changes in plasma tryptophan levels

Consistent with the findings of several other studies, tryptophan depletion resulted in a significant lowering of total plasma tryptophan levels relative to the day's pre-manipulation (10.30 h) baseline [$F(1,17) = 438.6$, $p < 0.000$]. This decrease did not differ according to whether the depletion session was experienced second or third in the study sequence [$F(1,17) = 2.32$, NS]. Similarly, tryptophan loading resulted in a highly significant elevation of plasma tryptophan relative to the day's baseline [$F(1,17) = 372.9$, $p < 0.000$] with no difference attributable to session order [$F(1,17) = 1.74$, NS]. Baseline tryptophan values did not significantly differ between days [$F(1,18) = 0.26$, NS].

Serum prolactin

In order to evaluate prolactin changes between conditions, each post-manipulation value (those obtained at 13.30 h, 15.30 h and 17.30 h) was subtracted from the day's pre-manipulation baseline (obtained at 10.30 h). Baseline values did not differ between sessions [$F(1,18) = 0.02$, NS]. These change scores were then compared in a repeated measures ANOVA with two levels of Condition (loading, depletion) and three levels of Time (change scores at 13.30 h, 15.30 h and 17.30 h). This analysis revealed no significant main effect of Condition [$F(1,18) = 0.74$, NS], a significant main effect of Time [$F(2,36) = 12.9$, $p < 0.000$], and a significant Condition–Time interaction [$F(2,36) = 5.03$, $p = 0.01$].

Post-hoc paired comparisons were conducted to investigate the nature of the interaction and indicated that there were significant differences between the TD and TL conditions at 13.30 h [$t(18) = 2.81$, $p = 0.01$] and at 15.30 h [$t(18) = 2.21$, $p < 0.05$]. These differences were not influenced by Session Order. Following tryptophan loading, there was a significant elevation in serum prolactin at 13.30 h [$t(18) = –2.29$, $p < 0.05$] but no significant change at 15.30 h [$t(18) = 1.13$, NS] or at 17.30 h [$t(18) = 1.17$, NS]. Following tryptophan depletion, there was a significant decrease in prolactin secretion at 15.30 h relative to the 10.30 h baseline [$t(18) = 2.10$, $p = 0.05$]. There were no changes from baseline at 13.30 h [$t(18) = –0.13$, NS] or at 15.30 h [$t(18) = 0.43$, NS].

Hence, with respect to their effects on prolactin secretion, each manipulation is performing in a manner that is consistent with the mild activation and inhibition, respectively, of central 5-HT.

Serum cortisol

Cortisol changes from baseline were similarly examined, and again there was no difference in baseline values across days [$F(1,18) = 0.11$, NS]. The analysis of $\Delta_{cortisol}$ values at the three time points revealed no significant main effect of Condition [$F(1,18) = 0.22$, NS], a significant main effect of Time [$F(2,36) = 12.2$, $p < 0.000$], but no significant Condition–Time interaction [$F(2,36) = 1.17$, NS]. As can be seen in Table 2, cortisol levels tended to rise in the afternoon of both days. Because the typical diurnal rhythm of cortisol secretion involves decreasing levels from 08.00 h to 16.00 h (Hellman *et al*., 1970; Krieger *et al*., 1971; Campbell *et al*., 1982), these increasing levels suggest that there was disruption of hypothalamic-pituitary-adrenal activity as a nonspecific consequence of the protocol.

Effects on cognition and working memory

Since tryptophan depletion and loading were equally likely to follow the screening session, significant differences between the two that cannot be attributed to order effects (whether each was second or third in the series of visits) represent conservative tests of the study's hypothesis. It was predicted that tryptophan loading, regardless of order of administration, would induce cognitive dysfunction relative to the depletion session. Means and standard deviations representing performance across all task variables are presented in Table 1. For each task, variables representing performance during each session were entered into a repeated measures analysis of variance with two levels of Condition (depletion and loading). Session Order (whether subjects received depletion prior to loading or vice versa) was entered as a between-subjects variable. When significant Condition effects or interactions were found, follow-up analyses were conducted to determine the nature of the findings.

Motor skill

Finger tapping test

The average number of finger taps for the dominant and nondominant hand were compared across the two conditions with Condition and Hand (dominant/nondominant) as the dependent variables. There was no significant main effect of Condition [$F(1,17) = 0.78$, NS]. There was a significant main effect of Hand [$F(1,17) = 5.29$, $p < 0.05$], but no interaction between Condition and Hand [$F(1,17) = 0.05$, NS]. There were no significant Order effects or interactions. Performance was faster with the dominant hand regardless of condition.

Grooved pegboard task

On the Grooved Pegboard task, time to completion was entered into a repeated measures ANOVA with two conditions (loading and depletion) and two levels of hand dominance (dominant versus non-dominant hand). Session order was entered as a between-subjects variable. This analysis revealed a main effect of Condition [$F(1,17) = 10.62$, $p < 0.01$], a main effect of hand Dominance [$F(1,17) = 43.01$, $p = 0.000$] but no significant Condition–Dominance interaction [$F(2,34) = 0.81$, NS]. There was no main effect of Order [$F(1,17) = 0.58$, NS] nor were there significant two-or three-way interactions between Session Order, Condition and Hand dominance. Completion time was faster in the depletion condition relative to the loading condition. This finding is not interpreted as motor slowing under the loading condition, because depletion also differed from the baseline screening [$F(1,17) = 8.12$, $p = 0.01$].

Number of dropped pegs was similarly examined, yielding a main effect of Condition [$F(1,16) = 6.49$, $p < 0.05$], no main effect of hand Dominance [$F(1,16) = 0.72$, NS], and no significant interaction between hand Dominance and Condition [$F(1,16] = 0.08$, NS]. There was no main effect of Session Order [$F(1,16) = 0.11$, NS] nor were there significant interactions between Session Order and Condition [$F(1,16) = 3.42$, $p < 0.10$] or between Session Order and Hand dominance [$F(1,16) = 2.71$, NS]. There were significantly more dropped pegs in the loading versus depletion conditions regardless of which hand was used. Loading was also different from the baseline screening [$F(1,16) = 5.92$, $p < 0.05$].

Thus, on the pegboard task, tryptophan depletion resulted in facilitation of motor behaviour that was observed as an increased speed of responding in the absence of changes in performance accuracy. Tryptophan loading did not influence motor speed, but resulted in lack of motor coordination.

Attention/concentration

On the biletter cancellation task, a repeated measures ANOVA was conducted on two conditions and two error types (omission versus commission) with Session Order as a between-subjects variable. This analysis yielded a main effect of Condition [$F(1,17) = 6.12$, $p < 0.05$], a main effect of Error type [$F(1,17) = 16.23$, $p = 0.001$] and a Condition–Error Type interaction [$F(1,17) = 6.12$, $p < 0.05$]. There was no main effect of Session Order [$F(1,17) = 0.07$, NS] nor was there a significant interaction between Condition and session Order [$F(1,17) = 1.10$, NS] or between Error type and session Order [$F(1,17) = 0.07$, NS]. The three-way interaction was not significant. With respect to the types of errors made by subjects, there were virtually no errors of commission recorded under any condition. Fewer errors of omission were made under the loading condition relative to the depletion condition. Loading, but not depletion, also differed from the baseline screening.

Examination of completion times revealed no difference between conditions [$F(1,17) = 2.77$, NS], although time-to-completion across conditions interacted with Session Order [$F(1,17) = 6.03$, $p < 0.05$]. Individuals who received loading after screening performed the task faster than those who received loading after depletion. However, performance under the depletion condition was uniform regardless of whether depletion occurred second or third in the session sequence.

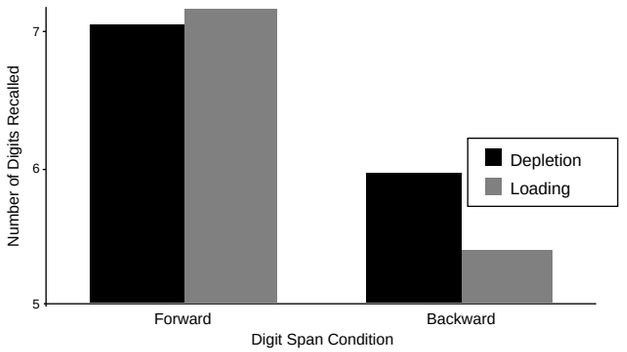

Figure 2 Digit Span performance for forward and backward trials on tryptophan depletion and tryptophan loading. The number of digits recalled in the correct sequence is indicated on the *y*-axis for each condition. Although digits backward is more difficult than digits forward under both conditions, there is a significant decrease in backward digit span under the influence of tryptophan loading

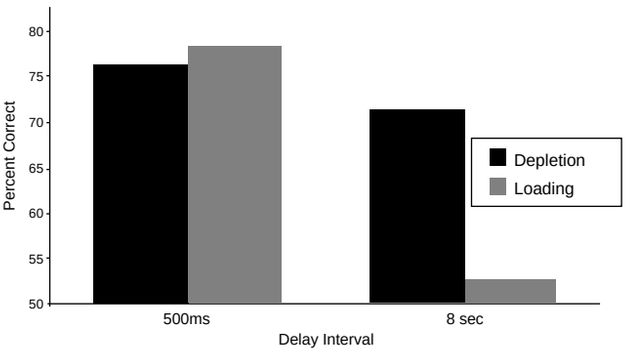

Figure 3 Percent correct trials in recalling faces with sad affective content is indicated on the *y*-axis for the tryptophan loading and depletion conditions under short (500 ms) and long (8 s) delay conditions. As indicated, there is a decrement in performance under the influence of tryptophan loading

Memory span

Spatial memory span

The conditions could not be distinguished based on length of memory span as measured by the spatial span test [$F(1,17) = 0.19$, NS]. There was no main effect of Session Order [$F(1,17) = 0.00$, NS], nor was there a significant interaction between Condition and Session Order [$F(1,17) = 0.01$, NS].

Verbal memory span (digit span)

On the digit span test, there were significant alterations in performance, as depicted in Figure 2. Forward and backward span were considered separately. The number of digits recalled in the forward direction did not differ between conditions [$F(1,17) = 0.13$, NS], and there was no significant main effect of session Order. There was no significant interaction between Condition and Order. The number of digits recalled in the backward direction was different between conditions [$F(1,17) = 4.73$, $p < 0.05$], with no significant main effect of Order [$F(1,17) = 0.44$, NS], nor a significant Condition–Order interaction [$F(1,17) = 0.24$, NS]. There were fewer digits recalled correctly in reverse order under the tryptophan loading condition. Loading also differed from the baseline screening. Because digits backward is considered to be a working memory task, this finding lends support to the notion that increased serotonin activity is associated with a worsening of working memory function.

Verbal fluency

The total number of words generated was compared across conditions, yielding no significant main effect of Condition [$F(1,17) = 0.50$, NS], no significant main effect of Session Order [$F(1,17) = 0.51$, NS], but a significant Condition–Order interaction [$F(1,17) = 13.35$, $p < 0.01$]. Participants who receiving depletion after loading produced more words (mean ± SD, 55.1 ± 11.1) than those who received depletion prior to loading (mean ± SD, 46.8 ± 10.9). This finding would be consistent with a practice effect across sessions. However, performance was equivalent under loading conditions regardless of whether loading was experienced before (mean ± SD, 51.5 ± 14.6) or after (mean ± SD, 52.1 ± 10.7) depletion.

Spatial working memory (dot location)

Accuracy scores across three delay intervals (500 ms, 4 s and 8 s) were entered into a repeated measures ANOVA, yielding no main effects of Condition [$F(1,16) = 0.16$, NS], a significant main effect of Delay [$F(2,32) = 36.05$, $p < 0.000$] but no significant Condition–Delay interaction [$F(2,32) = 0.18$), NS]. There were no significant main effects of Session Order or significant two- or three-way interactions between Session Order, Delay and Condition. Similar findings were observed in relation to response latency.

Affective working memory

Separate repeated measures ANOVAs were performed to consider how accuracy of working memory performance across two levels of Delay (500 ms and 8 s) varied across conditions as a function of the affective presentation of the stimulus (neutral, happy, surprised, fearful, disgusted, sad, angry). When the conditions were compared with each other, the only aspect of performance that distinguished the two was in the processing of sad affective content, which yielded a significant main effect of Condition [$F(1,17) = 10.40$, $p < 0.01$], a main effect of Delay [$F(1,17) = 7.93$, $p = 0.01$] and a trend towards a Condition–Delay interaction [$F(1,17) = 3.40$, $p < 0.10$]. There was no main effect of Session Order [$F(1,17) = 0.11$, NS]. There were no significant interactions between Condition and Session Order [$F(1,17) = 0.97$, NS], nor between Condition, Delay, and Session Order [$F(1,17) = 0.46$, NS]. As depicted in Figure 3, performance on loading was worse than on depletion, an effect that seems to be carried by performance on the long versus short delay trials. Performance was also worse under loading versus the screening session.

Notably, when the screening condition is examined in and of itself (see Table 1), the processing of sad affective content is highly accurate under both short (84% correct) and long (67% correct) delays. Indeed, during the screening session, sad faces were the easiest to recall relative to other affects under short delay conditions. However, relative to the short delay condition, they were also the stimuli that were maximally affected by the increase in delay interval, a pattern that appears to be enhanced under tryptophan loading conditions.

Effects on self-reported mood and intensity of side-effects

Both manipulations resulted in decrements in positive affect at all time points measured over the course of the study day (Luciana, 2000). Under loading conditions, level of negative affect was unchanged. On depletion, negative affect transiently decreased early in the afternoon (13.30 h), but was unchanged from baseline at all other time points. Thus, during the cognitive task administration interval, there were no significant changes in positive and negative affect that distinguished the two conditions. Side-effect intensity ratings were also examined for evidence that the impairments in cognition under the influence of tryptophan were attributable to general malaise. On tryptophan loading, the only rating to increase relative to the morning baseline during the cognitive task administration interval was an increase in self-report of headache. Anecdotally, several participants made comments that suggested the presence of mild dissociative symptoms including feeling 'spacey' or 'like I'm in a bubble'. On tryptophan depletion, the following items were reported as increased during the task administration interval: boredom and nausea. Side-effects were globally rated as more intense during tryptophan depletion versus loading, so it is surprising that cognition was not obviously impaired.

Discussion

To summarize, tryptophan loading and depletion resulted in altered levels of plasma tryptophan and serum prolactin that are consistent with mild activation and inhibition, respectively, of serotonin synthesis. The conditions were also distinct in terms of their effects on cognitive processes. In particular, we found evidence for disruption of working memory processes on the digit span and affective working memory tasks under 5-HT agonist conditions in this study. In our past study (Luciana *et al.*, 1998), we found evidence of spatial working memory impairment following fenfluramine administration to healthy adults. In the current study, spatial working memory was not affected, but tryptophan loading resulted in impaired digits backward performance and in an inability to maintain negative (sad) affective content in working memory. Additionally, tryptophan loading exerted a detrimental effect on fine motor coordination (grooved pegboard) as well as an enhancement of immediate vigilant attention, as exemplified by the letter cancellation task. These findings are consistent with the hypothesis of a restriction of informational flow under conditions of serotonin elevation leading to deficits in fine motor coordination, a narrowing of attentional resources and working memory impairments, particularly under conditions where processing demands are increased.

Differences in cognitive performance observed between the two tryptophan conditions do not appear to be due to distinctions that are related to side-effects, changes in peripheral arousal or to altered mood states. Both conditions resulted in decreased levels of positive affect at all time points recorded throughout the day. Similarly, ratings of side-effect intensity were increased early in the day (before the start of cognitive testing) and were more pronounced in the depletion versus loading condition. Although not discussed here, measures of autonomic arousal (changes in pulse and blood pressure) did not distinguish the two conditions during the cognitive task administration interval (Luciana, 2000). Moreover, the similar changes in cortisol secretion (rising values throughout the afternoon of each session) indicated that alterations in HPA activity that would be indicative of a stress response were equivalent across conditions.

Working memory is an executive function that involves several subprocesses, including the ability to engage a stimulus, to evaluate its motivational significance, to keep relevant information active while responses are being formulated across temporal delays and to accurately execute necessary responses. The tasks used in this study differentially recruit these various processes. The ability to attend to relevant stimuli is measured by virtually every task in the battery, although immediate attention is best captured by the letter cancellation task, spatial span, digits forward and by the 10-ms delay trials of the spatial and affective working memory tasks. Performance on these tasks was not impaired by tryptophan loading. Indeed, errors of omission were decreased on the letter cancellation task, suggesting relatively enhanced immediate focusing of attentional resources. Success on the short delay trials of the affective working memory task also indicates that the ability to recognize emotionally salient cues and respond to them accordingly is intact. Impaired digits backward performance, in the context of adequate forward digit span, is an indication that the ability to organize and maintain information while it is being manipulated (restated in reverse order) is compromised (Vanderploeg, 2000). Digit span impairments have been noted in schizophrenic patients and their first-degree relatives (Conklin *et al.*, 2000) leading to the question of whether this deficit might be due, in part, to relative overactivity in a subset of 5-HT projections. Notably, the atypical antipsychotic drugs appear to act via antagonism of 5-HT_2 receptors and lead to improvement in both positive and negative symptoms of the disorder and in executive functions that include fluency, attention and verbal working memory (Green *et al.*, 1997; Sharma and Hockler, 1998; Meltzer and McGurk, 1999).

Similarly, impaired working memory for spatial (past study: Luciana *et al.*, 1998) and affective (current study) stimuli under long (8 s) delay conditions suggests that high-serotonin manipulations are adversely impacting the ability to maintain motivationally salient information across time. Since the maintenance of information in working memory is modulated by catecholamine activity in the prefrontal cortex (Goldman-Rakic, 1987, 1988; Cohen and Servan-Schreiber, 1992; Luciana and Collins, 1997; Luciana *et al.* 1998), it may be that the threshold of activity in prefrontal neurons has been altered by increased serotonin. In the prefrontal cortex, the spines of cortical pyramidal neurons are the targets of dopaminergic afferents, as well as afferents from excitatory transmitters and inhibitory interneurons, forming a triadic structure within which neuronal excitability can be modulated (Goldman-Rakic, 1999). These pyramidal cells also receive afferent 5-HT projections (Goldman-Rakic *et al.*, 1990; Jakab and Goldman-Rakic, 1998), and 5-HT_{2A} receptors are evident in the proximal apical dendritic portions of these neurons. Furthermore, applications of 5-HT to pyramidal cells in layer V of the rat frontal cortex results in increases in the frequency and amplitude of excitatory postsynaptic potentials, suggesting that prefrontal efferent pathways are modulated by serotonin activity (Lambe *et al.*, 2000). This action may occur via the 5-HT_2 receptor system, involving both pre- and post-synaptic mechanisms that

likely result in the modulation of glutamate release (Marek and Aghajanian, 1998). Given that layer V pyramidal cells are those that project to an array of subcortical regions, including the monoamine-producing neurons in the midbrain, these findings converge to suggest that the memory fields of prefrontal neurons are regulated through interactions among the glutaminergic, catecholaminergic and serotonergic systems.

The possible regional specificity of the serotonin effects that we have observed warrants further study. Recent conceptualizations of prefrontal function have emphasized dissociations in information processing between dorsolateral and ventromedial regions. While spatial working memory has been associated with functional integrity of the dorsolateral prefrontal cortex (Goldman-Rakic, 1987, 1988), working memory for object cues (and faces in particular) may recruit regions of the frontal cortex that receive inputs from the ventral visual stream (Scalaidhe *et al*., 1999). The working memory tasks used in this battery would, in theory, differentially activate these regions, with the spatial working memory task activating primarily dorsolateral prefrontal cortex, and the facial affect working memory task activating primarily ventral/orbital prefrontal cortex. Whether the digit span task would be associated primarily with activity in one or the other region is unclear.

In addition to its role in working memory for object and facial cues, the ventral prefrontal region also seems to be critical for the ongoing maintenance of affective information, including mood states, in healthy and depressed individuals (Mayberg *et al.*, 1999). We speculate that our observed finding of impaired working memory performance for affective stimuli following tryptophan (serotonin) loading is due to constraint of neural activity by serotonin in ventral prefrontal regions. That serotonin influences metabolic activity in this region is supported by a recent PET study in which depressed patients were scanned before and after treatment with paroxetine. In treatment responders, there was a significant decrease in metabolic activity within ventral and orbitofrontal regions from pre- to post-treatment (Brody *et al*., 1999). The authors suggest that ventral prefrontal–subcortical circuitry may be implicated in the mediation of treatment responses to serotonin reuptake inhibitors in depressive disorders. Our findings would support their hypothesis in that increased serotonin disrupted working memory for negative facial affect which is mediated through ventral prefrontal circuitry. While we recognize that not all effects of psychoactive drugs are efficacious, these findings suggest that, in affectively disordered individuals, a mild working memory impairment might have some therapeutic potential. That is, if negative affective states cannot be maintained across time, then the subjective experience of sad affect might be increasingly short-lived, leading to a more regulated affective state and a decrease in the negative cognitive biases that characterize the disorder.

In conclusion, these findings lend further support to the hypothesis that increasing serotonin levels may lead to working memory impairments. In discussing significant findings from this study, we emphasize distinctions between the tryptophan loading and depletion conditions. These comparisons are not confounded by Session Order, since both were equally likely to follow the initial screening session. One potential criticism of our design is that cognitive task performance is generally vulnerable to practice effects across repeated sessions. If present, practice effects would lead to improvements in performance from the first to the second session and possibly to the third session as well. This potential confound would be of concern had we generally observed improvements in performance across sessions. Indeed, our finding of impaired working memory performance under tryptophan loading is all the more striking given that our design may have operated against that outcome. However, we do acknowledge that the interpretive clarity of our findings would be increased had we included a third condition, counterbalanced among the other two, in which individuals ingested a 'balanced' tryptophan mixture that exerted a neutral influence over tryptophan bioavailability (Young *et al.* 1985, 1989). Future studies using tryptophan manipulations to examine cognitive effects of changes in brain serotonin levels would ideally include all three conditions, repeated and counterbalanced within the same group of subjects. In addition, we acknowledge that this study includes a small sample and multiple statistical comparisons, which were not strictly controlled. Thus, these findings await replication. With these caveats in mind, if our finding of working memory impairment following increased serotonin activity can be replicated in a larger sample that includes affectively disordered and psychosis-prone individuals, then it would add to the growing body of literature on the manner in which 5-HT regulates cognition–emotion interactions.

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Address for correspondence

Monica Luciana
Department of Psychology
75 East River Road
University of Minnesota
Minneapolis, MN 55455
USA
Email: lucia003@tc.umn.edu

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