

# Low-Dose Tryptophan Depletion in Recovered Depressed Patients Induces Changes in Cognitive Processing Without Depressive Symptoms

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**Background:** Acute tryptophan depletion can induce a transient reappearance of depressive symptoms in recovered depressed patients. The neurochemical mechanism is thought to be impairment of brain serotonin neurotransmission, but the neuropsychologic mechanisms underlying the effect are unclear.

**Methods:** To assess whether low-dose tryptophan depletion can tease out the psychological mechanisms sensitive to substrate depletion in vulnerable subjects without inducing mood changes, a between-subjects randomized design was used. Recovered depressed patients ( $n = 24$ ) and healthy volunteers ( $n = 24$ ) were administered while fasting either a tryptophan-free or a control mixture, containing 31.2 and 33.2 g of amino acids, respectively. Objective and subjective ratings of mood were made before and 5 hours after ingestion; at the latter time point, cognitive and emotional processing were also assessed.

**Results:** Low-dose tryptophan depletion did not affect mood. Significant changes in emotional and cognitive processing occurred in the recovered depressed group, however, and to a lesser extent in the healthy volunteers. The profile of effects seen in the recovered patients suggested a return of the impairments seen in acute depression.

**Conclusions:** Our data suggest that low-dose tryptophan depletion permits investigation of the cognitive correlates of acute reductions in brain serotonin in populations vulnerable to depression and in healthy volunteers, without causing depressive symptoms.

**Key Words:** Cognitive function, depression, serotonin, tryptophan depletion

Acute dietary depletion of tryptophan (TRP), the amino acid precursor of serotonin, is a widely used method of lowering brain serotonin function in both healthy subjects and patients with psychiatric disorders. The usual technique involves oral administration of an amino acid mixture from which TRP has been omitted (for review, see Van der Does, 2001). This decreases the availability of TRP to the brain by two mechanisms, first by lowering plasma levels of TRP and second by increasing competition between TRP and other neutral amino acids for transport across the blood-brain barrier.

Studies of TRP depletion have identified a key role for serotonin in depression; both medicated and medication-free recovered depressed subjects have been reported to develop subjective and objective clinical depressive symptoms following consumption of a 100g TRP-free amino acid mixture (Delgado et al 1990; Smith et al 1997). A recent analysis of published studies suggest that a number of factors predict the mood-lowering effects of tryptophan depletion including recurrent depressive episodes, female gender, treatment with an selective serotonin reuptake inhibitor, and history of suicidal thoughts or attempts (Booji et al 2003). By contrast, healthy volunteers do not reliably experience clinical depressive symptoms following tryptophan depletion, although a subset of more vulnerable individuals may experience mild dysphoria (Van der Does et al 2001). The mechanisms by which TRP depletion produces depressive symptoms in recovered depressed subjects are of great interest but currently remain

unclear. Cognitive accounts of depression emphasize the importance of information processing biases in the onset of mood change and it is possible that TRP depletion could itself act through similar processes (Harmer et al 2003b). The conventional TRP-depleting mixtures poses problems in identifying an effect of this nature, however, because if it produces symptoms of acute depression this will itself produce changes in cognitive and emotional processing. Hence, it is difficult to disentangle the neuropsychologic effects of lowering brain serotonin activity from those associated with recurrence of depressive symptoms. Second, there are ethical difficulties in asking recovered depressed volunteers to undergo a procedure expressly designed to produce, albeit temporarily, the reexperience of depressive symptoms.

A lower dose of TRP-free amino-acid mixture might allow the dissociation of serotonin depletion's different effects. Previously, lower doses of amino acid mixture (50g or 31.5g) have been shown to reduce plasma TRP levels by over 65% (Moja et al 1988; Wolfe et al 1995; Young et al 1989). Furthermore, we found that a 50g TRP-free amino acid mixture failed to produce changes in mood in recovered subjects with bulimia nervosa (Oldman et al 1995), whereas a 100g mixture did (Kaye et al 2000; Smith et al 1999). Lower dose mixtures will produce less competition for brain entry, even if TRP levels in blood are reduced. Two additional studies, however, have found mood-lowering effects of low-dose mixtures. Moreno et al (1999) reported low mood following a 25g drink in recovered depressed patients, although this was not compared with a control mixture. Neumeister et al (2002) also found decreased mood following a 31.5g mixture in a subset of volunteers, depending on genotype and family history of depression.

The aim of our study was to explore the effects of a low-dose (31.2g) TRP mixture on cognition and emotional processing in a group of healthy volunteers and medication-free recovered depressed patients. Our hypothesis was that TRP depletion would, independent of changes in mood, induce acute cognitive changes and emotional biases characteristic of major depression and these effects would be greater in the recovered depressed patients.

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## Methods and Materials

### Participants

Twenty-four (14 women, 10 men) healthy volunteers and 24 (14 women, 10 men) euthymic volunteers with a history of depression gave informed consent to the study, which was approved by the Oxford Psychiatric Research Ethics Committee. On the basis of the structured clinical interview for DSM-IV, healthy volunteers were determined to be free of either current or past history of Axis I disorder, and euthymic participants were determined to have had one or more episodes of depression and currently be symptom free for at least 6 months. The duration of euthymia was determined through clinical interview, and ratings on the 17-item Hamilton Depression Rating Scale (maintained at < 8 by self-report for the previous 6-month period). Additionally, euthymic recovered depressives completed a Beck Depression Inventory (BDI) questionnaire at this initial interview and were included only if their scores fell below 7. All volunteers were screened for current physical illness and the presence of a family history of depression in a first-degree relative (although the latter was not an exclusion criterion for this study). The premenstrual week was avoided for the study period in female volunteers. Recovered depressed (RD) participants had been free of antidepressant medication for at least 3 months.

### Design and Mood State Ratings

Participants fasted overnight, arriving at the unit in the morning and were randomly allocated to double-blind treatment in a between-groups design with either a tryptophan-depleting (T-) or a control (T+) mixture. Subjective state was recorded in the morning before taking the mixture and 5 hours after its consumption using the following questionnaires: BDI (Beck et al 1961), a shortened version of Profile of Mood states (McNair et al 1992), and visual analogue scales (VAS) for the following dimensions: alert, afraid, happy, sad, guilty, tense, confused, and anxious. Additionally a modified 12-item Hamilton Depression scale (HAM-D), designed to be more suitable for detecting shorter-term changes in symptoms (Smith et al 1997) was completed before and 5 hours after the mixture. Venous blood samples were taken at the same time points to measure amino acid levels. Testing on a battery of psychological tests (described later) commenced 5 hours after mixture consumption. Baseline measures were not completed on these tests because for the most part they are not designed for repeated administration.

### Amino Acid Mixture

The tryptophan-depleting mixture consisted of L-isoleucine (4.2g), L-leucine (6.6g), L-lysine monohydrochloride (4.8g), L-methionine (1.5g), L-phenylalanine (2.9g), L-tyrosine (3.4g), L-threonine (3.0g), and L-valine (4.8g). The control mixture additionally contained 2g of tryptophan. Studies indicate that at least a 66% decrease in total plasma tryptophan occurs following a similar mixture (Moja et al 1988; Young et al 1989). The amino acids were suspended in tap water in identical beakers, and black-currant flavoring (SHS International, Liverpool, United Kingdom) was used to disguise the unpleasant taste.

### Biochemical Analyses

**Amino Acid Determination.** Blood samples were taken in lithium heparin tubes, centrifuged to obtain plasma and stored at -20°C. Total L-tryptophan concentrations were determined by an isocratic high-performance liquid chromatography (HPLC) method of analysis. Plasma proteins were removed by precipitation with a 3% trichloroacetic acid (TCA) and centrifugation at

10,000g. An aliquot of the supernatant (TCA) for total tryptophan or ultrafiltrate for free TRP was then diluted in mobile phase before injection onto the HPLC analytical column. Fluorescence end-point detection was used to identify tryptophan.

The large neutral amino acids levels were determined by reverse-phase gradient elution HPLC. Plasma proteins were removed by filtration and the ultrafiltrate diluted with .1 M HCL solution. The solution was made basic with an aliquot of sodium tetraborate solution (pH 10) before undergoing a derivatization reaction with o-phthalaldehyde reagent solution incomplete (Sigma P7914, Sigma-Aldrich, St. Louis, Missouri) reagent. The resulting solution was injected onto a HPLC analytical column for chromatographic separation. Fluorescence end-point detection was used to identify most of the primary amino acids.

Two volunteers from the T- recovered depressed group were omitted from all plasma analyses because of venepuncture failures.

### Psychological Tests

**Counting Stroop Task.** The number words "two," "three," "four," and "five" ( $n = 20$  trials) formed the incongruent condition and the animal words "cat," "dog," "mouse," and "bird" formed the neutral condition ( $n = 20$ ). Each stimulus was presented for 400 msec, followed by a fixation cross for 3 sec. Stimuli order was randomized with the exception that no more than two stimuli with the same number of words were presented consecutively. Subjects were required to indicate by a button press on a response box the number of words presented on the screen as quickly and accurately as possible.

**Emotional Counting Stroop Task.** Volunteers were asked to count the number of words in neutral ( $n = 20$ ) or aversive ( $n = 20$ ) sentences of 2–5 words. The two groups of words were matched for frequency, and, as far as possible, for the initial word, for example, "You are here" and "You are ugly." Results were analyzed in terms of average reaction time to each stimulus type and also as emotional interference scores, calculated as follows: emotional - neutral / neutral (e.g., Turken and Swick 1999).

**Emotional Memory Task.** Volunteers were presented with 66 characteristic words (e.g., amusing, discouraged), 33 of which were positive and 33 negative (taken from Bradley and Mathews 1983, presented for 5 sec each in a randomized order). Participants were asked to decide whether the word presented applied to them using a button response box. They were instructed to press the button as soon as they had made their decision and then to continue to look at the word until a new word appeared to replace it. Five minutes after the end of this task, volunteers were asked to recall as many words as possible, irrespective of the response they originally made.

The reaction time to classify negative and positive words was measured as well as negative bias in classification, that is, positive words not classified as personal characteristics, and negative words classified as personal characteristics. Recall was assessed by subtracting the number of words falsely recalled from those correctly recalled separately for positive and negative words.

**Auditory Verbal Learning Task.** The auditory verbal learning test (AVLT) assesses a number of different components of initial recall, learning, and delayed recall and recognition (Rey 1964). In the immediate recall phase, a 15-item word list was read to the participant five times, and after each trial, recall was tested. Immediate recall on a distracter list was then assessed, providing a short delay after which free recall of the original list was tested.

**Table 1.** Demographic and Clinical Information for Each Participant Group

	Healthy Control Subjects		Recovered Depressives	
	T–	T+	T–	T+
Age	37.4 (3.9)	39.8 (3.9)	38.8 (3.5)	37.4 (3.5)
Gender (M:F)	7:5	7:5	7:5	7:5
Weight (kg)	74.2 (3.3)	77.4 (4.3)	73.2 (4.1)	73.7 (3.4)
Verbal IQ	118 (1.8)	115 (2.8)	118 (1.9)	120 (1.6)
TRP/LNAA Ratio	.175 (.023)	.150 (.007)	.168 (.012)	.162 (.010)
Episodes ( <i>n</i> )	—	—	1.6 (.3), range 1–4	1.7 (.2) range 1–3
Family History of Depression	Y3, N9	Y1, N11	Y6, N6	Y7, N5 <sup>b</sup>
Period of Euthymia (months)	—	—	33.4 (16)	33.6 (8)

F, female; LNAA, large neutral amino acids; M, male; T–, tryptophan-depleting mixture; T+, control mixture; TRP, tryptophan.

Mean values with SEM in parentheses.

<sup>a</sup>Statistical comparison between recovered depressed and control groups.

<sup>b</sup>*p* < .05.

Fifteen minutes later, participants were tested for long delay free recall, followed by a recognition test, where they were asked to respond with “Yes” or “No” to each item on a list comprising the 15 targets plus 35 distractors.

Data were analyzed with respect to four variables; initial recall (average first recall trials for lists 1 and distracter list 2), learning over trials 2–5, delayed free recall (averaged across short and long-delay free recall), and delayed recognition. Signal detection equations were applied to the data from the recognition test to derive a measure of accuracy corrected for the participant's response tendency. The proportion of correctly recognized words (*cr*) and the proportion of falsely recognized (*fr*) constitute the nonparametric sensitivity measure:  $A' = 1 - 1 / 4(fr / cr + (1 - cr) / (1 - fr))$ .

**Emotion Potentiated Startle (EPS).** Picture stimuli from the International Affective Picture System (Lang et al 1998a) designed to elicit positive, negative, or neutral emotions were used (for more details, see Harmer et al 2003a). Stimuli were presented for 13 sec (intertrial interval of between 11 and 15 sec, mean 13 sec) in a quasi-random order. The eye-blink component of the startle response was recorded from the orbicularis oculi using electromyography (EMG startle response system, San Diego Instruments, San Diego, California). Acoustic probes were 50-msec, 95-dB bursts of white noise with a nearly instantaneous rise time and were delivered binaurally through headphones (delivered at 1.5, 4.5, or 7.5 sec following picture onset). Within each block of 21 pictures, probes were delivered on five of each trial type (neutral, positive, or negative) and three probes per block were given within the inter-trial interval. To habituate volunteers to the startle probes and to orient them to the procedure, volunteers viewed an introductory set of 9 neutral pictures and received 7 startle probes whilst a picture was on the screen and two within the ITI.

The EMG signals were filtered (low cutoff: .5 Hz; high cutoff: 100 Hz) and rectified. Eye-blink reflex magnitudes in mV were calculated by subtracting the amount of integrated EMG at reflex onset from the peak amplitude maximum amount of integrated EMG between 20 and 120 msec following probe onset. Trials with no perceptible eye-blink reflex were assigned a magnitude of zero and included in the analysis. Eye-blink reflexes with excessive noise during a 20-msec, prestartle baseline period were excluded. Of the 48 volunteers, 11 were not included in the analysis because of technical problems (*n* = 6), electrode interference (*n* = 4), or because they withdrew from the experiment. Numbers remaining in each group were as follows:

healthy T– = 7, T+ = 10; recovered depressives T– = 9, T+ = 11. Eye-blink reflex magnitudes were z-transformed within participants to minimize interparticipant variability.

**Facial Expression Recognition.** The facial expression recognition task featured the six basic emotions of happiness, surprise, sadness, fear, anger, and disgust, taken from the Pictures of Affect Series (Ekman et al 1976) These had been morphed between each prototype and neutral (Young et al 1997) to create different intensity levels of each expression in 10% steps. Four examples of each emotion at each intensity were presented and each face was also presented in a neutral expression, giving a total of 250 stimuli presentations.

These facial stimuli were presented on a computer screen (randomized order) for 500 msec and replaced by a blank screen. Volunteers made their responses by pressing a labeled key on the keyboard. Face recognition was analyzed using a signal detection analysis for target detection and bias (Green et al 1966; Grier 1971), which provides a measure of accuracy unconfounded by differences in response criterion and a measure of response bias.

## Statistical Methods

Results were analyzed using univariate or repeated-measures analysis of variance with reaction time, accuracy, or other test variables as the within-subjects measure and mixture type and personal history of depression as between-subjects measures. Statistically significant interactions were followed up using simple main effects analyses. Because gender did not interact with change in BDI or HAM-D scores and was matched across the groups, it was not included in the analyses.

## Results

### Participants: Demographic and Clinical Characteristics

The four groups were matched for age, verbal IQ (as assessed by the National Adult Reading Test), and gender (see Table 1). Significantly more of the recovered depressed participants had a self-reported family history of depression [ $F(1,44) = 6.5, p = .014$ ]. The T+ and T– groups were matched for number of episodes of depression and period of euthymia in the recovered depressed sample (Table 1). Baseline HAM-D, BDI, and total TRP and TRP/LNAA ratios were also not significantly different in the four groups (for mean baseline scores, see Table 2).

**Table 2.** HAM-D and BDI Mood Scale Ratings and Biochemical Analyses of Blood Plasma Before and 5 Hours After Administration of the TRP-depleting (T–) or Control Mixture (T+)

	Healthy Control Subjects				Recovered Depressive Subjects			
	T– Pre	T– Post	T+ Pre	T+ Post	T– Pre	T– Post	T+ Pre	T+ Post
Ham-D	.17 (.11)	.67 (.19)	.33 (.19)	.41 (.19)	1.00 (.41)	1.38 (.36)	.42 (.23)	.5 (.19)
BDI	1.5 (.56)	.58 (.33)	2.13 (.57)	2.25 (.80)	2.25 (.76)	1.75 (.68)	2.17 (.63)	1.63 (.53)
Total Tryptophan (nmol/mL)	12.64 (1.31)	5.52 (.57)	12.06 (.60)	19.81 (1.68)	12.53 (1.37)	3.27 (.56)	12.09 (.79)	15.92 (1.87)
Tryptophan/LNAA Ratio	.175 (.023)	.022 (.006)	.150 (.007)	.116 (.012)	.168 (.012)	.022 (.004)	.162 (.010)	.110 (.015)

BDI, Beck Depression Inventory; Ham-D, Hamilton Depression Rating Scale; LNAA, large neutral amino acids; TRP, tryptophan. Values represent means with SEM in parentheses.

### Changes in Plasma Tryptophan Levels

The TRP-free mixture, T–, significantly lowered both plasma TRP levels and the ratio of TRP to other large neutral amino acids compared with the control drink, T+, in both the patients and controls [Total TRP: time  $\times$  mixture  $F(1,42) = 115.7, p < .001$ ; TRP/LNAA: time  $\times$  mixture  $F(1,42) = 64.3, p < .001, ns$  interaction with history of depression; see Table 2].

### Objective and Subjective Ratings of Mood

Tryptophan depletion did not significantly affect objective (HAM-D) or subjective (BDI) ratings of depression in either the healthy control subjects or the recovered depressed group [Table 2; HAM-D: time  $\times$  mixture  $F(1,144) = 1.3, p = .3$ ; time  $\times$  mixture  $\times$  history  $F(1,44) = .04, p = .8$ ; BDI: main effect of history  $F(1,44) = .3, p = .6$ ; time  $\times$  mixture  $F(1,44) = 2.2, ns$ ; time  $\times$  mixture  $\times$  history  $F(1,44) = .25, ns$ ]. Profile of mood states was also unaffected by history or mixture (tension all  $ps > .4$ ; depression all  $ps > .13$ ; anger all  $ps > .58$ ; fatigue all  $ps > .25$ ; bewildered all  $ps > .6$ ; vigor all  $ps > .17$ ). No differences were seen on the VAS ratings when corrected for multiple comparisons. At the more lenient statistical threshold of  $p < .05$  per comparison, however, an effect of mixture type was seen on the “afraid” VAS rating, with the T– group showing a greater reduction in fear ratings irrespective of depressive history [main effect of mixture  $F(1,44) = 4.2, p = .046$ , history  $\times$  mixture  $F(1,44) = 1.1, p = .7$ ].

### Psychological Test Results

The effects of TRP depletion and history of depression on all of the tasks are summarized in Table 3.

**Behavioral Effects of TRP Depletion Evident Particularly in the Recovered Depressed Sample.** Emotion-potentiated startle: the average amplitude of startle responses were specifically

elevated following tryptophan depletion in the recovered depressed group [Figure 1 (right) between-subjects effect of depression history  $\times$  mixture;  $F(1,33) = 9.1, p = .005$ ]. Converting startle amplitudes to standard ( $Z$ ) scores revealed a potentiated response to negatively valenced pictures compared with positive and neutral pictures in each group; this was unaffected by mixture or history of depression [main effect of picture type  $F(2,66) = 4.7, p = .01$ , interaction with group or drink,  $ns$ , Figure 1 (left)]. Hence, startle responses were generally enhanced following TRP depletion in the recovered group irrespective of the picture stimuli presented.

Facial expression recognition: accuracy of facial expression recognition is shown in Figure 2. Tryptophan depletion specifically decreased the recognition of happy facial expressions in the recovered depressed group [overall interaction between history of depression  $\times$  facial expression  $\times$  mixture;  $F(6,264) = 2.5, p = .02$ , see Figure 2 for simple main effect analyses]. The opposite effect—increased happiness recognition—was found in the healthy control subjects (see Figure 2). There were no other significant main effects of mixture or mixture  $\times$  group interactions in this task (all comparison  $ps > .2$ ). There was, however, an overall group difference for the recognition of disgust, with the recovered depressed group showing enhanced recognition relative to the healthy control subjects [main effect of history of depression;  $F(1,44) = 5.0, p = .03$ ].

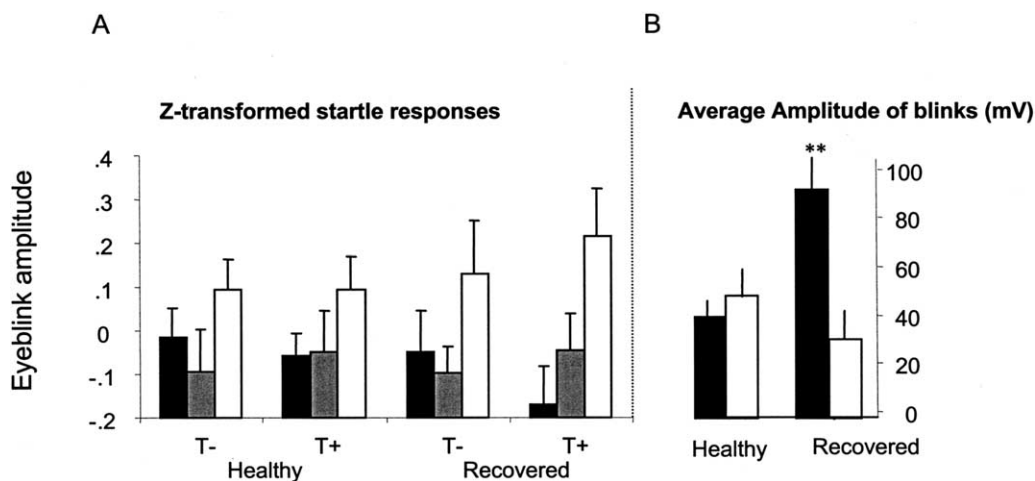
There was no overall effect of history of depression or mixture condition on bias [effect of mixture:  $F(6,264) = 1.2, p = .29$ ; effect of depression history:  $F = .49, p = .81$ ; interaction:  $F = 1.1, p = .38$ ] or on speed of correct responses [effect of mixture:  $F(6,264) = 1.1, p = .36$ ; effect of depression history:  $F = .7, p = .62$ ; interaction:  $F = .47, p = .83$ ].

Auditory-verbal learning task: Tryptophan depletion im-

**Table 3.** Summary of the Effects of Tryptophan Depletion and of Personal History of Depression on Performance of the Psychological Tasks Employed in This Study

Tasks	Effects of Tryptophan Depletion		
	Health Controls	Recovered Depressed	Effect of Depression History
Emotion-Potentiated Startle	—	Generalized elevation of startle response	—
Facial Expression Recognition	Enhanced recognition of happy facial expressions	Impaired recognition of happy facial expressions	Enhanced recognition of disgust expressions
AVLT (Memory)	—	Impaired initial recall memory	—
Emotional Stroop	Increased emotional interference score	Increased emotional interference score	—
Counting Stroop	—	—	—
Mood Congruent Memory	—	—	Lack of positive bias in recall of emotionally valenced words

AVLT, auditory visual learning test.



**Figure 1.** (A) Amplitude of startle responses (Z scores) while observing neutral (black), pleasant (gray), and unpleasant (white) pictures. (B) Raw amplitude of startle responses over all conditions following tryptophan depletion (black bars) and control mixture (white bars). Asterisks represent statistical significance of planned comparisons between T+ and T- mixture (\*\* $p < .01$ ). Values represent means  $\pm$  SEM.

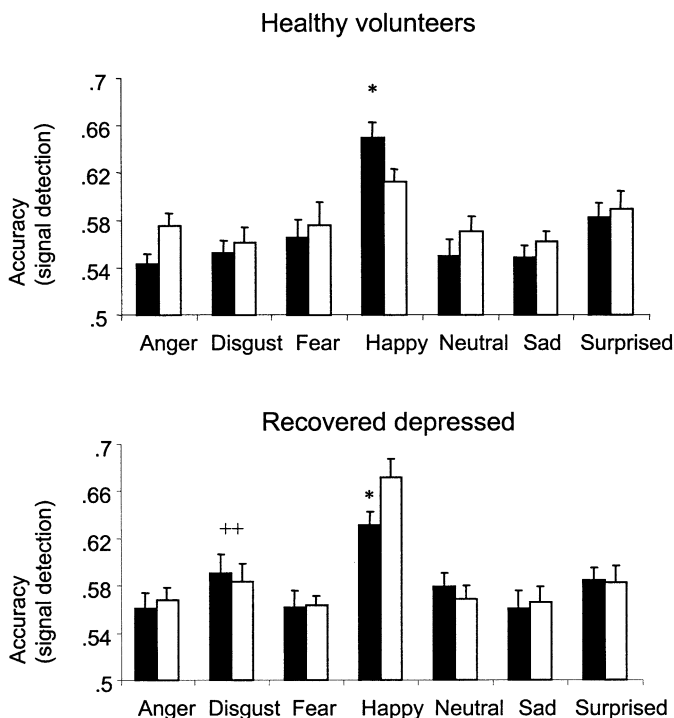
paired initial memory scores in the recovered depressed patients but not healthy control subjects [ $F(1,44) = 4.9, p = .03$ ; see Figure 3]. No effects were seen on learning or long-term memory consolidation on the auditory-verbal learning task (all  $ps \geq .2$ ).

**Behavioral Effect of TRP Depletion Evident in Both Healthy Volunteers and Unmedicated Recovered Depressives.** Counting Stroop task, neutral: Participants showed increased reaction times in the incongruent condition of this Stroop task (main effect of stimulus [ $F(1,44) = 25.1, p < .001$ ], but this was

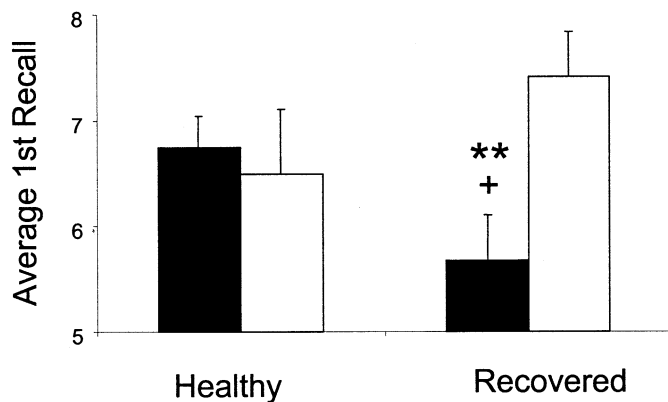
unaffected by depression history or mixture (all  $ps > .1$ ). Counting Stroop task, emotional: TRP depletion enhanced the emotional interference score irrespective of history of depression [main effect of mixture:  $F(1,44) = 4.5, p = .039, ns$ , interaction with depression history, see Figure 4]. No differences were seen in errors made (all  $ps > .6$ ).

**Behavioral Effect of History of Depression Independent of TRP Challenge**

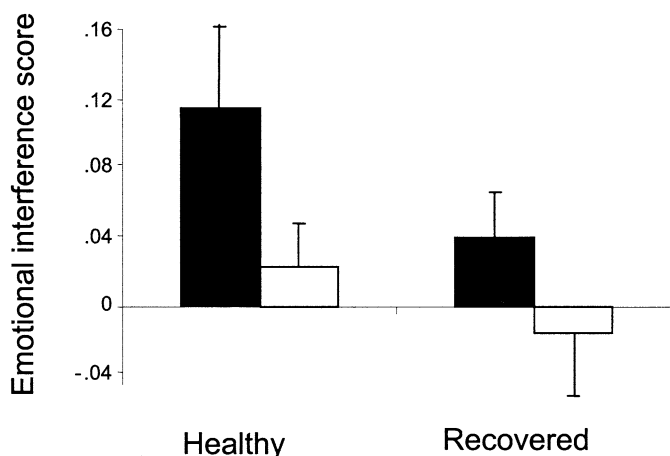
Emotional memory: There was no difference in the classification of positive or negative items as personally applicable (all  $ps > .1$ ). However, in the memory component of this task, recovered depressed volunteers recalled a lower proportion of positive words compared with the matched control subjects [main effect of depression history  $F(1,43) = 4.9, p = .03$ , see Figure 5]. This bias in recovered depressed subjects was unaffected by TRP depletion (all  $ps > .3$ ).



**Figure 2.** Facial expression recognition in healthy and recovered depressed volunteers following consumption of tryptophan free (black) or control (white) mixtures. Values represent means  $\pm$  SEM. Asterisks represent statistical significant of comparison between mixture types ( $p < .05$ ). Crosses represent statistical significance of planned comparisons between recovered depressed patients and the healthy control subjects ( $++ p < .01$ ).



**Figure 3.** Mean recall score for the first presentation of list 1 and list 2 AVLT following tryptophan depletion (black bars) or the control mixture (white bars). Values represent means  $\pm$  SEM. Asterisks represent statistical significance of comparison between T+ and T- (\*\* $p < .01$ ). Crosses represents statistical significance of planned comparisons between history of depression groups for the control (T+) condition (+  $p < .05$ ). AVLT, auditory visual learning test.



**Figure 4.** Emotional interference score in the emotional Stroop task in healthy and recovered depressed participants following consumption of T- (black bars) or T+ (white bars) mixture. Values represent mean emotional interference score  $\pm$  1 SEM.

## Discussion

### TRP Depletion in Recovered Depressed Subjects

The main findings of our study are that low-dose TRP depletion produces changes in some aspects of cognitive function and emotional processing comparable to those reported in major depression, but without a change in subjective mood. These effects were observed most consistently in the recovered depressed patients. Thus, following TRP depletion the recovered subjects, but not the healthy volunteers, demonstrated reduced recognition of happy facial expressions, increased startle responses, and reduced performance on recall memory all of which are well-characterized elements of the depressive-anxious cognitive profile. Both recovered depressed and healthy volunteers, however, showed an increased emotional Stroop effect following tryptophan depletion suggesting increased negative attentional bias in both vulnerable and nonvulnerable subjects.

Depression has been associated with decreased perception of happy facial expressions in a number of studies (e.g., Gur et al 1992; Rubinow and Post 1992; Surguladze et al 2004), and TRP depletion reproduced this effect in the recovered depressed subjects. Interestingly, TRP depletion had the opposite effect in the healthy volunteers who showed enhanced recognition of happy facial expressions relative to the control amino acid drink. Because this effect has not been reported in previous studies of tryptophan depletion in healthy volunteers (e.g., Harmer et al 2003b), it is possible that this difference is the result of a type 1 error. Alternatively, it may reflect the engagement of compensatory processes following low-dose tryptophan depletion in nonvulnerable patients. Opposing effects of citalopram have also been reported in recovered depressed and healthy volunteers (Bhagwagar et al 2004) supporting the idea of differential responses to serotonergic manipulations in individuals vulnerable to mood disorder.

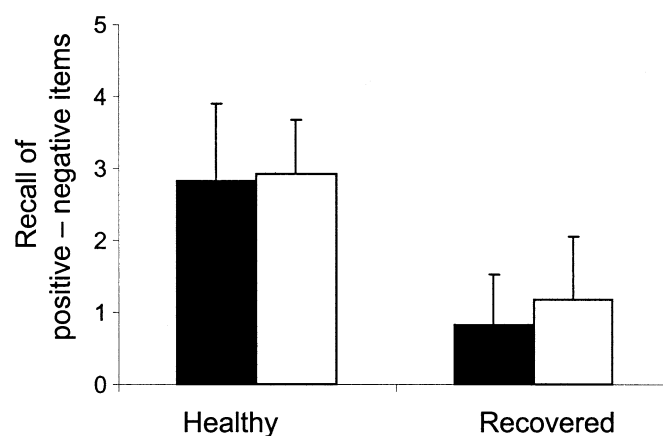
Abnormal elevation of startle responses has been demonstrated in anxiety and depression (Cuthbert et al 1994; Lang et al 1998b). Grillon (2002) has proposed that elevated baseline startle is an index of contextual fear with the enhanced startle responses in patient groups reflecting greater sensitivity to the negative context of the startle experiment. Our results suggest that serotonin depletion increases startle responses across all stimuli

conditions in the recovered depressed patients but not the healthy control group. Hence, TRP depletion appears to have returned the recovered depressed patients to a state seen in acutely depressed patients in the startle paradigm and again suggests that this group is more vulnerable to TRP depletion than volunteers with no history of psychiatric disorder.

A core cognitive symptom of depression is a global impairment of memory performance, which is ameliorated after successful serotonergic antidepressant treatment (Bondareff et al 2000; Keegan et al 1991). At least two studies of depressed patients point to a selective impairment in the initial recall phase of the AVLT (Query and Megran 1984; Sweeney et al 1989), although other examinations have shown impairments in all AVLT components (Austin et al 1992; Brown et al 1994). Tryptophan depletion impaired performance on the first-recall component of the AVLT in the recovered depressed group. Our results therefore suggest that we reproduced the performance deficit seen in depressed patients without altering recall in the healthy control subjects with low-dose TRP depletion. Although memory is globally impaired in depression, full-dose tryptophan depletion typically impairs delayed but not immediate learning in healthy people (Riedel et al 1999). These results therefore suggest that 31.2g tryptophan depletion does not simply induce a pattern of change seen in healthy volunteers, but at a lower threshold; rather, it induces changes in cognitive function more consistent with a depressive state.

### Effect of Low-Dose TRP Depletion Apparent in Both Subject Groups

Tryptophan depletion increased reaction time to emotional stimuli compared with neutral stimuli in the emotional counting Stroop task in both recovered depressed subjects and healthy volunteers, without affecting performance in the nonemotional Stroop task. The emotional Stroop task has been used to support the idea that depressed patients are more easily distracted by negative stimuli than healthy control subjects because negative constructs are highly accessible to them (Beck, 1967, 1976). Our results suggest that attentional bias toward negative self-referent emotional information, is increased by serotonin depletion in both vulnerable and nonvulnerable groups of volunteers. This



**Figure 5.** Mean difference ( $\pm$  SEM) between positive and negative characteristics recall score (number recalled minus false positives) following earlier classification of those characteristics in volunteers taking a T- (black bars) or T+ (white bars) mixture. There was a significant main effect of group in this analysis, with recovered depressed patients recalling a lower proportion of positive characteristics compared with the control volunteers [ $F(1,43) = 4.9$ ,  $p = .03$ ].

measure may therefore be particularly sensitive to increased negative processing found with compromised serotonin function.

### Effects of History of Depression

Increased negative processing in the affective memory task and in the perception of facial expressions was found in the recovered depressed patients yet was not affected further by TRP depletion. In the emotional memory task, recovered depressed volunteers recalled a higher proportion of negative words compared with healthy control subjects irrespective of TRP depletion, a pattern that has also been reported in acutely depressed patients (Bradley et al 1983). Because this bias appears to persist into periods of recovery from depression, it may represent a trait vulnerability marker. Enhanced recognition of facial expressions of disgust was also observed in our recovered depressed sample compared with the healthy control subjects. This phenomenon has been reported previously in euthymic medicated bipolar patients (Harmer et al 2002), indicating perhaps a general enhancement of disgust recognition in the euthymic phase of mood disorders. Enhanced recognition of fear has also previously been reported both in recovered unipolar (Bhagwagar et al 2004) and bipolar II (Lembke et al. 2002) depression, however; it seems likely that it reflects a generalized increase in negative perception in those vulnerable to mood disorder.

Despite these emotional processing biases, we did not find evidence for more generalized impairments in cognitive function in the recovered depressed patient group. These results therefore suggest that deficits of memory and psychomotor function reported to occur during depression may be state rather than trait factors (Elliot et al 1998). The absence of effect on memory performance is consistent with a recent study in remitted depressed patients (Weiland-Feilder et al 2004), although this study did report more pervasive deficits in sustained attention, which we did not assess.

### Tryptophan Depletion and Depression

Subjective ratings of mood or depression were not affected by low-dose tryptophan depletion in our study. A number of factors have been shown to increase the likelihood of low mood following tryptophan depletion, such as female gender, number of previous depressive episodes, and history of suicidal thoughts or actions (Booij et al 2002). The patients in this study may not have been particularly vulnerable to experiencing these symptoms because they included male and female participants and had a relatively low number of previous episodes of depression (average 2, range 1–4). Nonetheless, tryptophan depletion did induce a profile of cognitive changes consistent with a depressive state in these patients. These results suggest that psychologic task performance is more sensitive to low-dose tryptophan depletion than changes in mood.

Of course, with a higher-risk sample, this low-dose drink might also induce symptoms of depression. Thus, groups of healthy women with a family history of depression showed increased depression scores on the HAM-D after a 31.5g TRP-free mixture, and the serotonin transporter genotype influenced expression of depressive symptoms (Neumeister et al 2002). Genotype was not considered in our study, largely because of the lack of statistical power to detect differences in our small sample group. Future, larger-scale studies looking at the impact of family history and genotype on the emotional processing measures used here would therefore be of interest.

The control amino acid drink used in our study was similar in composition to previous studies looking at the effect of tryptophan

depletion in remitted depressed patients. Certain amino acids, such as histidine, which are involved in the synthesis of other key neurotransmitters, are not typically included in these amino acid mixtures. Future studies may wish to consider the involvement of histidine-histamine (and its interaction with tryptophan depletion) in mood and psychological function in at risk groups.

### Implications and Conclusions

The most challenging interpretation of these findings is that we have identified neuropsychological effects that start the cascade to a state of depression. A change in perceptual biases, reactivity, and immediate memory function could plausibly prime the return of a depressive state and were all selectively uncovered in the group with a history of depression. This occurs on an enduring background of impaired positive memory function and a bias to detect negative emotional expression in these participants. Another interesting possibility is that the changes we have identified are simply early or subtle indicators of depression itself that are more sensitive to detection than objective or subjective changes in mood. To distinguish between these possibilities will be an important task for applied cognitive neuroscience. In either case, the induction of mood relevant neuropsychologic changes can occur as a consequence simply of a selective neurochemical intervention. With appropriate replication, low-dose tryptophan depletion could offer a valuable tool for examining the consequences of abnormal serotonergic function on cognitive and emotional measures in vulnerable subjects and, potentially, the ability of such changes to be attenuated by pharmacologic or nonpharmacologic antidepressant treatments.

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