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Tryptophan depletion decreases the recognition of fear in female volunteers

Received: 15 October 2002 / Accepted: 6 January 2003 / Published online: 4 April 2003
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Abstract *Rationale:* Serotonergic processes have been implicated in the modulation of fear conditioning in humans, postulated to occur at the level of the amygdala. The processing of other fear-relevant cues, such as facial expressions, has also been associated with amygdala function, but an effect of serotonin depletion on these processes has not been assessed. *Objective:* The present study investigated the effects of reducing serotonin function, using acute tryptophan depletion, on the recognition of basic facial expressions of emotions in healthy male and female volunteers. *Methods:* A double-blind between-groups design was used, with volunteers being randomly allocated to receive an amino acid drink specifically lacking tryptophan or a control mixture containing a balanced mixture of these amino acids. Participants were given a facial expression recognition task 5 h after drink administration. This task featured examples of six basic emotions (fear, anger, disgust, surprise, sadness and happiness) that had been morphed between each full emotion and neutral in 10% steps. As a control, volunteers were given a famous face classification task matched in terms of response selection and difficulty level. *Results:* Tryptophan depletion significantly impaired the recognition of fearful facial expressions in female, but not male, volunteers. This was specific since recognition of other basic emotions was comparable in the two groups. There was also no effect of tryptophan depletion on the classification of famous faces or on subjective state ratings of mood or anxiety. *Conclusions:* These results confirm a role for serotonin in the processing of fear related cues, and in line with previous findings also suggest greater effects of tryptophan depletion in female volunteers. Although acute

tryptophan depletion does not typically affect mood in healthy subjects, the present results suggest that subtle changes in the processing of emotional material may occur with this manipulation of serotonin function.

Keywords Tryptophan · Fear · Females · Amygdala · Serotonin

Introduction

Serotonergic processes have been implicated in the processes underlying fear and anxiety, although the nature of this relationship is far from clear. Acute pharmacological manipulations which increase 5-HT function typically increase anxiety or fear responses in the laboratory in a variety of paradigms such as the elevated plus maze, open field test and social interaction test (see Borsini et al. 2002 for a review), while 5-HT receptor antagonists have been reported to have the opposite effect (e.g. Bagdy et al. 2001; Zhang et al. 2001; Martin et al. 2002). Consistent with this, extracellular levels of 5-HT are observed to increase during fear, elicited through presentation of an aversive conditioned stimulus (Hashimoto et al. 1999) or during inescapable tail shock (Amat et al. 1998).

In line with these animal investigations suggesting an anxiogenic role for serotonin, volunteer studies have also found that blockade of 5-HT₂ receptors decreased skin conductance response during an aversive conditioning paradigm (using ritanserin: Hensman et al. 1991 and nefazodone: Silva et al. 2001). Using a different paradigm, we have observed that acute administration of the selective serotonin re-uptake inhibitor (SSRI) citalopram (10 mg, IV) increased the recognition of fear cues from facial expression (Harmer et al. 2003). Hence, a role for serotonin in the processing of fear-relevant stimuli appears to hold true for humans as well as non-human animals.

In contrast to these pre-clinical studies, the clinical utility of the SSRI in the treatment of anxiety suggests

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that enhancing serotonin over the longer term may be anxiolytic. To resolve this conflict, the dual 5-HT defence hypothesis suggests that 5-HT facilitates conditioned anxiety by acting on forebrain structures such as the hippocampus and amygdala, but inhibits unconditioned panic or defense responses through actions in the dorsal periaqueductal gray (Graeff et al. 1997). While conditioned fear is suggested to underlie the development of generalised anxiety (GAD), the unconditioned processes are believed to be at the root of panic disorder. The efficacy of SSRI following repeated treatment in GAD may therefore relate to “down-stream” effects such as desensitisation of post-synaptic receptors (Deakin 1991) or alterations in intra-cellular signalling (Manji et al. 2001).

An alternative explanation for these differences between clinical efficacy and experimental findings is that the preclinical studies typically focus on the effects of acute administration of SSRIs. Acute SSRI administration can have the paradoxical effect of decreasing synaptic serotonin levels through activation of autoreceptors in the raphe. As such, previous studies, including our own (Harmer et al. 2003), may have examined the consequences of reduced, rather than increased, serotonin release. Under this interpretation, serotonin would appear to be anxiolytic, with the therapeutic effects of SSRI only becoming apparent following desensitisation of autoreceptors with repeated administration.

The current study therefore further investigated the role of serotonin in the processing of fear cues using the facial expression recognition model. Acute tryptophan depletion (ATD) was used to assess the effects of an acute decrease in serotonin in healthy volunteers. ATD involves the administration of a mixture of essential amino acids without tryptophan, thereby decreasing the availability of tryptophan to the brain through two potential mechanisms; increased protein synthesis (lowering plasma tryptophan levels) and increased competition for transport across the blood-brain barrier (see Reilly et al. 1997 for a review). If the effect of acute SSRI administration relates to a paradoxical decrease in serotonin function, then tryptophan depletion should also increase recognition of fearful facial expressions. However, if, as postulated, the previously reported effect of citalopram (Harmer et al. 2003) was a consequence of increased serotonin function, then tryptophan depletion should decrease the recognition of fearful facial expressions.

Materials and methods

Subjects

Thirty-eight healthy volunteers (20 males and 18 females), aged between 20 and 35 years, took part in this investigation. Subjects with any history of psychiatric (assessed using structured clinical interview for DSM IV) or significant physical illness were excluded. All subjects gave written informed consent and the local ethics committee approved the protocol.

Volunteers were randomized (double-blind) to receive an amino acid mixture lacking tryptophan (T-) or a balanced amino acid drink (BAL) as a control. These two groups were matched in terms of age (mean age 24.5 ± 0.9 versus 23.4 ± 0.9 years, respectively) and verbal IQ measured with the national adult reading test (NART: Nelson and O'Connell 1978) (114.4 ± 1.8 and 113.6 ± 1.4). Eleven of the 20 males and nine of the 18 females were randomised to the T-condition.

Amino acid mixtures

The composition of the T- mixture for male and female subjects, respectively, was L-alanine (5.5 g, 4.58 g); L-arginine (4.9 g, 4.08 g); L-cysteine (2.7 g, 2.25 g); glycine (3.2 g, 2.25 g); L-histidine (3.2 g, 2.67 g); L-isoleucine (8.0 g, 6.67 g); L-leucine (3.5 g, 11.25 g); L-lysine monohydrochloride (11.0 g, 9.17 g); L-methionine (3.0 g, 2.5 g); L-phenylalanine (5.7 g, 4.75 g); L-proline (12.2 g, 10.17 g); L-serine (6.9 g, 5.75 g); L-threonine (6.5 g, 5.42 g); L-tyrosine (6.9 g, 5.75 g); L-valine (8.9 g, 7.42 g). The BAL mixture also contained tryptophan (2.3 g, 1.92 g). The amino acids were suspended in tap water, which was flavoured with lime in order to disguise the unpalatable taste of the mixture.

Stimuli

The facial expression recognition task featured six basic emotions, happiness, surprise, sadness, fear, anger and disgust, taken from the Pictures of Affect Series (Ekman and Friesen 1976). These had been morphed between each prototype and neutral using techniques described by Young et al. (1997). Briefly, this procedure involved taking a variable percentage of the shape and texture differences between the two standard images 0% (neutral) and 100% (full emotion) in 10% steps. Four examples of each emotion at each intensity were given (total of ten individuals). Each face was also given in a neutral expression, making a total of 250 stimuli presentations.

The non-emotional classification task featured famous faces taken from six categories: actor, comedian, politician, sportsperson, TV presenter and musician. A total of 40 faces within each category were presented. There were also ten presentations of non-famous faces, as a reference category broadly equivalent to the neutral classification in the emotion recognition task.

In both tasks, the face stimuli were presented on a computer screen for 500 ms and immediately replaced by a blank screen. Volunteers made their responses by pressing a labelled key on the keyboard. Volunteers were asked to respond as quickly and as accurately as possible.

Subjective state ratings

Subjective state was assessed using the positive and negative affective mood scales (PANAS) in 16 BAL and 14 T- volunteers (PANAS; Watson et al 1988) at baseline and at +5 h after consumption of the amino acid drinks. This information was used to assess the relationship between the effects of rapid tryptophan depletion on facial expression processing and any possible effects on the mood of the volunteers.

Total tryptophan determination

Venous 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 HPLC method of analysis. Plasma proteins were removed by precipitation with 3% trichloroacetic acid (TCA) and centrifugation at 10,000 g. An aliquot of the supernatant (TCA) for total tryptophan was then diluted in mobile phase before injection onto the HPLC analytical column. Fluorescence end-point detection was used to identify tryptophan.

Procedure

All volunteers followed a low protein diet (<20 g total) on the day before the study and then fasted overnight. Volunteers attended the laboratory at 8.30 a.m. on the day of the study. Blood samples (15 ml) were taken at this time to obtain baseline levels of total plasma tryptophan. Volunteers then drank an amino acid drink over a 60-min period. None of the volunteers reported any side effects beyond transitory nausea. Volunteers were also given a low protein (<2 g total) lunch at mid-day. Five hours after consumption of the amino acid drink (+5 h), a second blood sample was taken in order to assess reductions in total plasma tryptophan. All volunteers then completed the facial expression and facial identity tasks in a randomised order.

Analysis

Given evidence that tryptophan depletion may have greater effects on 5-HT synthesis in women than in men (Nishizawa et al. 1997), performance on all tests was analysed using a split-plot analysis of variance with drink and gender as between-groups factor and facial expression or occupation as the within-subjects factor. Significant interactions were analysed further using analysis of variance for each gender and completed using simple main effect analyses.

Results

Subjective state

Ratings of positive mood decreased over the experimental day in both groups [main effect of time of rating; $F(1,26)=6.2$, $P=0.02$], but this was not affected by group [group×time $F(1,26)=1.6$, $P=0.2$] or by gender [group×time×gender $F(1,26)=0.7$, $P=0.4$]. There was no effect on negative mood [time $F(1,26)=0.6$, $P=0.5$; group×time $F(1,26)=0.00$, $P=0.9$; group×time×gender $F(1,26)=0.2$, $P=0.7$]. Hence, tryptophan depletion exerted no specific effects on mood in this group of healthy volunteers.

Total tryptophan

Administration of the tryptophan-free mixture decreased levels of plasma total tryptophan relative to volunteers receiving balanced amino acid drink at 5 h [time×group interaction $F(1,30)=30.0$, $P<0.001$]. However, this fall in tryptophan was not significantly affected by gender [group×time×gender: $F(1,30)=0.05$, $P=0.8$]. Mean change in the female volunteers was -10.2 (± 0.8) compared to $+10.4$ (± 1.7), while male volunteers showed a mean change of -11.7 (± 0.7) compared to $+8.6$ (± 1.2) in the T- relative to BAL condition. Absolute total tryptophan concentrations at 5 h were also not significantly different in the two genders, falling to 2.3 ± 0.3 and 2.2 ± 0.2 in the male and female volunteers, respectively [$F(1,33)=0.1$, $P=0.7$; group×gender $F(1,33)=1.2$, $P=0.6$].

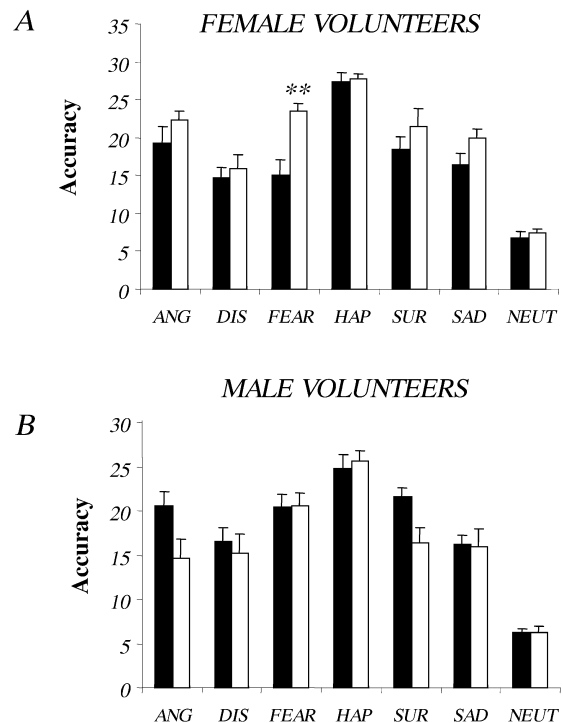


Fig. 1 Facial expression recognition in female (A) and male (B) volunteers following consumption of the tryptophan-free amino acid mixture (dark bars) or the balanced mixture of amino acids (light bars). Values represent means \pm 1 SEM. Asterisks represent statistical significance of the comparisons: *** $P<0.01$

Facial expression recognition

Accuracy

There was a significant interaction between drink×gender [$F(6,204)=10.1$, $P=0.003$] and a marginal effect of drink×gender×facial expression [$F(6,204)=1.9$, $P=0.077$], suggesting that tryptophan depletion exerted different effects in male versus female volunteers. Analysis of these two sets of volunteers separately suggested that tryptophan depletion affected facial expression recognition in females [Fig. 1A: emotion×drink: $F(6,96)=2.1$, $P=0.1$] but not males [Fig. 1B: emotion×drink: $F(6,108)=1.7$, $P=0.13$]. In female volunteers, tryptophan depletion selectively impaired recognition of fearful facial expressions [gender×drink for fear: $F(1,34)=7.5$, $P=0.01$; female T- versus BAL: $t=8.3$, $df=16$, $P=0.003$]. Such a trend was not apparent in the male volunteers (male T- versus BAL: $t=0.1$, $df=18$, $P=0.9$). This difference in the female volunteers was maintained in a signal detection analysis which controls for the influence of different criterion for responding with the label “fear” [target sensitivity: drink×gender: $F(1,34)=8.5$, $P=0.006$]. Mean target sensitivity for fear was 0.55 ± 0.01 versus 0.62 ± 0.01 in the T- versus BAL female volunteers ($t=4.2$, $df=16$, $P=0.001$) and 0.59 ± 0.01 versus 0.58 ± 0.01 for the two male groups ($t=0.2$, $df=18$, $P=0.8$). Inclusion of subjective state ratings (change in

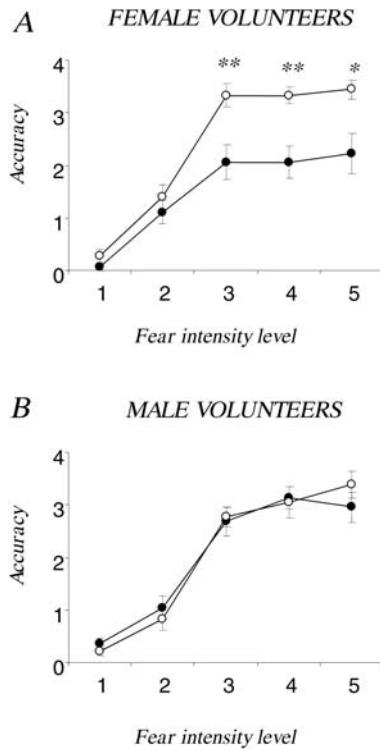


Fig. 2 Fear recognition over the different intensity levels used in the task for female (A) and male (B) volunteers. *Dark bars*: following the tryptophan free amino acid drink; *light bars*: following the BAL amino acid drink. Values represent means±1 SEM. Asterisks represent statistical significance of the comparisons: * $P<0.05$, ** $P<0.01$

positive and negative mood) as co-variables did not abolish this significant difference in the female volunteers [$F(1,11)=15.2$, $P=0.003$].

Recognition of the fearful facial expressions was analysed over the different morphed intensity levels used in this task (summed into 20% blocks). Overall, there was an interaction between drink and gender [$F(1,34)=7.5$, $P=0.01$]. Analysis of the female volunteers separately revealed that tryptophan depletion particularly affected the recognition of high levels of fearful facial expressions, with accuracy levels reaching a lower plateau than those receiving the control mixture [Fig. 2A: drink×intensity: $F(4,64)=3.7$, $P=0.008$, see Fig. 2 for simple main effect analyses]. There was no effect on recognition of fearful faces in the male volunteers even in this more detailed analysis [Fig. 2B: drink×intensity: $F(4,72)=0.9$, $P=0.5$; main effect of drink $F(1,18)=0.0$, $P=0.9$].

Speed

In the overall analysis of variance, there was no significant effect of tryptophan depletion in terms of reaction time of correct identifications [Fig. 3: main effect of drink $F(1,34)=0.0$, $P=0.9$; drink×emotion $F(6,204)=1.6$, $P=0.2$; drink×emotion×gender $F(6,204)=$

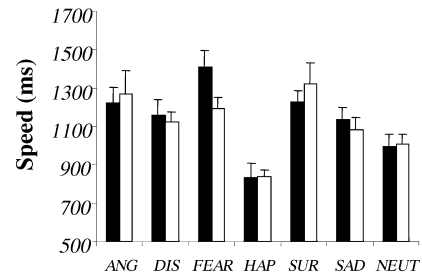


Fig. 3 Speed (ms) of correct identifications in the facial expression recognition task. *Dark bars*: following the tryptophan free amino acid drink; *light bars*: following the BAL amino acid drink. Values represent means±1 SEM

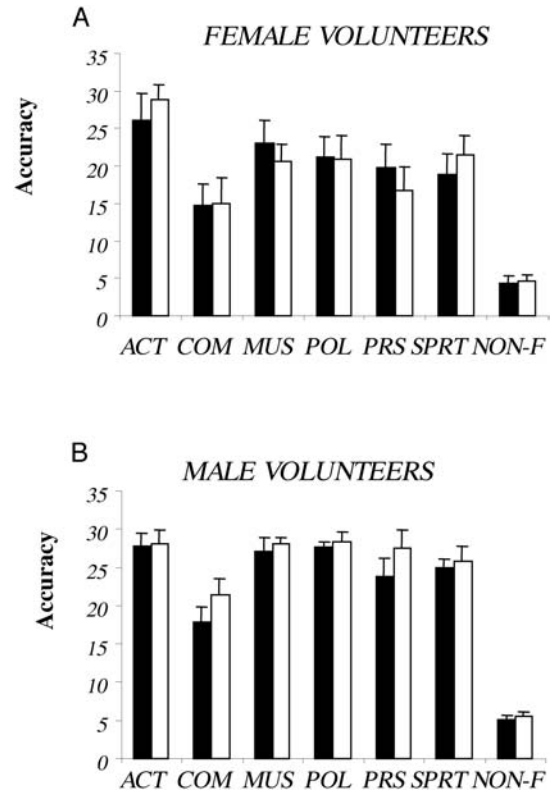


Fig. 4 Correct identifications in the famous face classification task for the female (A) and male (B) volunteers. *Dark bars*: following the tryptophan free amino acid drink; *light bars*: following the BAL amino acid drink. Values represent means±1 SEM

1.9, $P=0.09$]. However, individual comparisons were computed to ensure that differences in accuracy reported above were not the result of a speed-accuracy trade-off. The only significant difference between the two groups was for fear. Volunteers receiving the T- mixture were significantly slower to identify facial expressions of fear [$F(1,34)=2.0$, $P=0.05$] and this did not interact significantly with gender [$F(1,34)=0.4$, $P=0.5$], indicating that there may also be some level of impaired fear processing in the male volunteers. Further, this slowing of reaction time to identify facial expressions of fear suggests that the

impaired recognition found after tryptophan depletion was not the result of impulsive responses.

Non-emotional classification

Tryptophan depletion did not affect non-emotional facial judgements [Fig. 4: drink $F(1,34)=0.2$, $P=0.7$; drink \times category $F(6,204)=0.5$, $P=0.8$; drink \times gender $F(1,34)=0.2$, $P=0.7$; or drink \times category \times gender $F(6,204)=1.3$, $P=0.3$]. Reaction time was also unaffected by tryptophan depletion or by gender (all comparisons $P>0.6$). These results suggest that ATD does not have generalized effects on response selection or reaction time, thereby confirming the specific effect of this manipulation on the categorisation of fearful facial expressions of emotion.

Discussion

Results from this study suggest that acute tryptophan depletion decreased the recognition of fearful facial expressions in healthy females. However, male volunteers were relatively unaffected by this manipulation. These deficits in fear processing occurred in the absence of more general changes in the processing of semantic information associated with faces or in the processing of other facial expressions of emotion. These data add to previous findings suggesting a role for serotonin in processing fear-related cues, and further suggest that acute decreases in serotonin are associated with decreased fear processing in humans.

The present difference in the recognition of emotional state following tryptophan depletion occurred in the absence of significant changes in subjective mood and was not affected by inclusion of mood ratings as covariates. Although ATD can induce depressive relapse in patients with previous history of mood disorder (Smith et al. 1997), it does not typically affect mood in healthy volunteers with no family history of depression (Salomon et al. 1997; for review, see Van der Does 2001). These results suggest that shifts in the processing of emotional information can occur independently, or at a lower threshold, than overt differences in mood.

The present results are in line with previous results suggesting a role for serotonin in fear-related processes. In humans, the effects of serotonergic manipulations on conditioned fear has been assessed using an aversive conditioning procedure in which skin conductance responses to a stimulus associated with a loud noise are recorded. Typically, agents which block 5-HT₂ receptors (ritanserin: Hensman et al. 1991; nefazodone: Silva et al. 2001) or decrease serotonin tone (buspirone: Hellewell et al. 1999) have been reported to decrease conditioned fear responses. These effects have been suggested to represent modulation of serotonin function with the amygdala (Graeff et al. 1997), given the wealth of data highlighting the importance of the amygdala in conditioning and fear responses in humans and non-human animals (reviewed

by Maren 2001; Davis and Whalen 2001). Consistent with this role, the amygdala has also been found to be involved in the processing of fearful facial expressions. Patients with lesions to the amygdala show reduced identification of fearful faces (Adolphs et al. 1994, 1999) and functional neuroimaging studies have reported activation of the amygdala in response to fearful face stimuli in healthy volunteers (e.g. Morris et al. 1996). Hence, the present modulation of fearful facial identification may relate to a common role for serotonin in the processing of fear relevant cues within the amygdala and associated circuits. We have previously reported that acute administration of the SSRI citalopram (Harmer et al. 2003) and also of 2 g of tryptophan (unpublished observations) increased the recognition of fearful faces. The present study indicates that depletion of serotonin produced the opposite pattern in female volunteers, suggesting a reciprocal role for serotonin in the processing of fear relevant cues.

The relationship between acute manipulations of serotonin and processing fear relevant cues suggests an "anxiogenic" role for serotonin. These results appear counterintuitive, given the therapeutic efficacy of SSRI in anxiety disorders such as GAD and panic disorder (Kent et al. 1998). However, there appear to be marked differences between the effects of acute versus repeated treatment with SSRI. Initial treatment with SSRI often increases generalized anxiety and agitation before therapeutic effects are seen in these disorders (Kent et al. 1998). Consistent with this postulated difference between acute and repeated treatment, we have recently found that sub-chronic (7 days) treatment with citalopram decreased the recognition of fearful (and other negative) facial expressions in healthy male and female volunteers (Harmer et al. 2002). The mechanism responsible for this reversal is unknown, but may relate to the differential modulation of a common neurobiological substrate such as the amygdala. Such effects could result from specific changes within the serotonin system (such as desensitization of 5HT₂ receptors) or through more distal effects on other neurochemical or neural systems, including changes in intracellular signalling (Manji et al. 2001). Nevertheless, the current results suggest that the facial expression processing may provide an appropriate paradigm to examine the effects of acute versus repeated treatment with serotonergic agents on fear-relevant processing.

In our previous study, the administration of citalopram not only increased the recognition of fearful facial expressions but also the identification of facial expressions of happiness (Harmer et al. 2003). This facilitation in the recognition of happiness was postulated to represent an early action of SSRI on positive social perception, which may be important in their therapeutic actions. However, in the present study, the converse effect of decreased processing of happiness was not found following ATD. Further investigation is required to assess whether the recognition of happy facial expressions would be decreased by tryptophan depletion in patients recovered from depression, or in those who have a

positive family history for mood disorder, where abrupt decreases in serotonin may be more likely to affect the functioning of neural systems important in the regulation of mood and positive affect (Van der Does 2001).

Although similar reductions in plasma tryptophan were achieved in both sexes, the effects of ATD on accuracy of fear recognition was only seen in the female volunteers. Previous studies have also reported larger effects of ATD on mood in female volunteers (Ellenbogen et al. 1996), which may be the consequence of greater reductions in serotonin synthesis, as assessed using radiolabelled alpha-methyl-L-tryptophan with positron emission tomography (Nishizawa et al. 1997). Dieting also has differential effects on responses mediated by the serotonergic system in males and females. Endocrine responses to tryptophan infusion are facilitated in women not in men, and plasma tryptophan levels are lower after 2 weeks of calorie restriction (Goodwin et al. 1987; Goodwin et al. 1990). The finding that responses to identify fearful facial expressions were slower in the T- group irrespective of gender, suggests that male volunteers may still show some effects of tryptophan depletion, but to a lesser extent than the females. Future studies are needed to confirm the generality of these effects of serotonin manipulations on fear processing in male volunteers, to assess whether the present finding is related to the dynamics of tryptophan depletion or to acute manipulations of serotonin function in general.

In summary, the present results suggest that acute decreases in serotonin lead to impairments in the processing of fearful facial expressions in healthy females. These results complement previous findings suggesting enhanced fear perception following acute increases in serotonin levels through SSRI administration or tryptophan administration. The relationship between acute and chronic effects of serotonergic manipulations and their relevance to the therapeutic efficacy of SSRI in anxiety disorders remains to be fully unravelled.

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