Proceedings of the Nutrition Society (2018), **77**, 174–188 doi:10.1017/S0029665117004451
 First published online 25 January 2018

The Joint Winter Meeting between the Nutrition Society and the Royal Society of Medicine held at The Royal Society of Medicine, London on 6–7 December 2016

Conference on 'Diet, nutrition and mental health and wellbeing'
Symposium 4: Public health and nutrition strategies to promote good mental health

Tryptophan supplementation and serotonin function: genetic variations in behavioural effects

E. L. Gibson

Department of Psychology, University of Roehampton, London SW15 4JD, UK

The neurotransmitter serotonin has a role in affective disorders such as depression and anxiety, as well as sleep, cognitive function and appetite. This review examines the evidence that serotonin-related genotypes may moderate the behavioural effects of supplementation with the serotonin precursor amino acid L-tryptophan (TRP), on which synthesis of serotonin (or 5-hydroxytryptamine; 5-HT) depends. However, 95 % of serotonin is synthesised and used in the periphery, and TRP is also metabolised via non-5-HT routes such as the kynurenine pathway. Moreover, understanding of genotypes involved in regulation of serotonin raises questions over the generalisability of TRP effects on behaviour across individuals with varied serotonergic genotypes. To date, only differences between variants of the 5-HT transporter-linked promoter region (5-HTTLPR) have been investigated in relation to behavioural effects of TRP supplementation. Effects of 5-HTTLPR genotypes are usually compared between the alleles that are either high (L/L′) or low (S/S′) expressing of mRNA for the 5-HT transporter receptor. Yet, another key genetic variable is sex: in women, the S/S′ genotype predicts sensitivity to improved mood and reduced cortisol by TRP supplementation, during stressful challenges, whereas the L/L′ genotype protects against stress-induced mood deterioration. In men, the L/L′ genotype may confer risk of stress-induced increases in negative affect; there are insufficient data to assess effects on male S/S′ genotypes. However, better-powered studies to detect sex by genotype by stress by TRP interactions, as well as consideration of more genotypes, are needed before strong conclusions and recommendations for behavioural effects of TRP treatment can be reached.

Tryptophan supplementation: Serotonin and behaviour: 5-HT transporter-linked promoter region: Genetic polymorphism: Stress

Dietary tryptophan and the pathways to serotonin function

Serotonin, or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter in the central nervous systems (CNS) of the majority of animals, including human beings. Its synthesis depends on the supply of the essential amino acid, L-tryptophan (TRP), which cannot be biosynthesised by human beings and so must be obtained from dietary sources. Moreover, serotonin synthesis rate depends on the availability of the precursor TRP. The scope of this review is to consider recent findings from research involving effects of supplementing TRP supply on behaviour and their interaction with genetic susceptibility, including indirect evidence that TRP supplementation likely alters affective states via effects on central serotonin function.

An important consideration for understanding effects of TRP administration is that only about 5 % of endogenous serotonin is found in the brain; the

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HTTLPR, 5-HT transporter-linked promoter region; ATD, acute tryptophan depletion; CNS, central nervous systems; CSF, cerebrospinal fluid; LNAA, large neutral amino acids; MAO, monoamine oxidase; TDO, tryptophan 2,3-dioxygenase; TPH, TRP hydroxylase; TRP, L-tryptophan.

Corresponding author: E. L. Gibson, email l.gibson@roehampton.ac.uk

remainder is in the gut (about 90 %), principally released by enterochromaffin cells, and in peripheral tissue or in the blood, where it is taken up into blood platelets[1–3]. Indeed, the name serotonin derives from its discovery in blood 70 years ago and the observation that it caused contraction of vascular smooth muscle[4]; thus, one function of serotonin is to regulate local blood flow. This imbalanced distribution between brain and periphery needs to be borne in mind when considering the possible impact of dietary manipulation of central serotonin by TRP, and the potential influence of alternative metabolic pathways as well as probable moderating effects on these metabolic routes. These issues are considered further later; nevertheless, serotonin is a widely distributed and important CNS neurotransmitter, arising from neuronal cell bodies located in the higher and lower raphe nuclei of the brainstem, and acting at multiple receptor subtypes with a range of behavioural effects[5]. Serotonin's established importance in affective disorders and appetite, as well as sleep and cognition[6], make understanding who might benefit most from therapeutic use of TRP an important goal of research.

Metabolic pathways for L-tryptophan

As with other essential amino acids, TRP can contribute to hepatic biosynthesis of proteins; however, TRP is typically incorporated into proteins at only 1–2 % of total amino acids, making it the scarcest of amino acids in dietary proteins[3,7]. Nevertheless, if TRP is acutely deficient, incorporation into protein synthesis will contribute to a substantial lowering of plasma TRP levels[8,9]. However, in the absence of TRP deficiency, the majority of consumed TRP is metabolised via other pathways, including for synthesis of 5-HT, melatonin and niacin (vitamin B_3). Indeed, it has been estimated that only 1 % of dietary TRP is used for brain 5-HT synthesis[10]. TRP use for synthesis of niacin is via the oxidative kynurenine pathway, which has also been termed the TRYCAT pathway[11]. This pathway is becoming increasingly recognised as having important implications for health, including neuropsychiatric conditions such as depression[11,12]. A further route for TRP metabolism is via degradation by gut microbiota, which can lead to production of both positive and detrimental active metabolites, including quinolinic acid[1]; therefore, individual variation in the gut microbiome may have implications for TRP metabolism and thus brain health and psychological wellbeing[13].

The kynurenine, or TRYCAT, pathway involves an initial rate-limiting metabolism of TRP to kynurenine catalysed by the hepatic enzyme, tryptophan 2,3-dioxygenase (TDO), which can be induced by glucocorticoid hormones[14]. However, under inflammatory conditions, the extrahepatic enzyme, indole 2,3-dioxygenase becomes increasingly important in metabolising TRP to kynurenine, due to induction by pro-inflammatory cytokines[11]. These inductive influences on diversion of TRP metabolism away from 5-HT synthesis have been proposed as mechanisms underlying the link between stress, inflammation, deficient 5-HT function and depression[11,12].

The metabolism of TRP for the synthesis of 5-HT is catalysed by the rate-limiting enzyme, TRP hydroxylase (TPH), which converts TRP into 5-hydroxytryptophan. In turn, 5-hydroxytryptophan is decarboxylated to 5-HT by the enzyme aromatic amino acid decarboxylase. The key observation for this pathway is that TPH is not fully saturated by its substrate TRP under normal conditions, so that raising brain TRP levels could increase serotonin synthesis. However, brain TRP levels are buffered from plasma TRP by the blood–brain barrier: to be transported into the brain, TRP has to compete for uptake across the blood–brain barrier against other amino acids, in particular a group known as the large neutral amino acids (LNAA), especially the branched chain amino acids, leucine, isoleucine and valine, but also phenylalanine and tyrosine (the precursors for catecholamine, dopamine, adrenaline, noradrenaline, transmitter synthesis). Thus, the ratio of plasma or serum TRP to LNAA (TRP:LNAA) is recognised as the best peripheral biomarker of uptake of TRP into the brain[7]. Some 90 % of TRP in blood is typically bound to the blood protein albumin, and it is often assumed that the remaining free unbound fraction of TRP should be taken to be the best predictor of TRP entry across the blood–brain barrier. However, it has been shown that TRP binding to albumin is very labile, such that TRP can easily be released in cerebral circulation. Furthermore, TRP can be displaced from or prevented from binding to albumin by NEFA, which also bind readily to albumin[7,9]. Therefore, factors that alter NEFA levels in blood will affect the availability of free TRP for entry into the brain: for example, sympathetic activation by stress or exercise will induce lipolysis, increase plasma NEFA and so release more TRP from albumin. This acute stress-induced increase in availability of TRP for serotonin synthesis might contribute to the observation that even mild stress can increase 5-HT release in rat brain[15]. It also suggests that caution is required in interpreting correlations between single measures of plasma-free TRP and personality traits such as anxiety or aggression, as these may interact with the experimental procedure and perceived stressful nature of the study to modify TRP levels. In contrast, food or drink that stimulates insulin release, and so promotes uptake of NEFA into tissue, will tend to reduce the availability of free plasma TRP, but at the same time will remove competing LNAA from plasma into tissue[7]. Thus, measuring both free and total TRP may ensure better prediction of TRP entry into the brain and its behavioural associations[9,16].

However, 95 % of 5-HT is synthesised and used in the gut, blood and peripheral tissue[1,14]. Although the synthesis of 5-HT from TRP follows a similar biochemical path in brain and periphery, the form of the enzyme TPH, by and large, differs slightly between these regions; these isoforms are known as TPH1 and TPH2 respectively, indicating the order of characterisation[17,18]. To be precise, in the brain the principal isoform, TPH2, shown to depend on expression of a different gene form

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

from TPH1[18], was found to be highly expressed by measuring mRNA specific to the brainstem raphe nuclei, where brain serotonin is primarily synthesised, whereas TPH1 was found to be responsible for 5-HT synthesis, and ultimately melatonin, in the pineal gland[19] and gut[18]. However, this classification is oversimplified, as TPH1 mRNA has also been shown to be more highly expressed in the amygdala and hypothalamus than TPH2[20], although its precise role in those sites is uncertain.

Serotonin and behaviour

Serotonin has long been associated with several fundamental aspects of behaviour, including sleep, appetite, cognition, and social and emotional behaviours such as anxiety, depression, empathy and aggression[21,22]. These influences of serotonin on behaviour will be briefly reviewed prior to consideration of the impact of TRP supplementation and its interaction with 5-HT-related genotypes.

Early neurophysiological and lesion work on the function of CNS 5-HT demonstrated a clear role in regulating sleep[23], whereas the therapeutic use of monoamine oxidase (MAO) inhibitors (which prevent serotonin, and other monoamine, metabolism by the enzyme MAO), as well as development of tricyclic antidepressants (which inhibit synaptic reuptake of monoamine neurotransmitters), such as imipramine, to treat depression, led to the 'serotonin hypothesis' of depression, in which depression is seen primarily to result from a deficit in 5-HT function[24,25]. The theory expanded to consider a role for CNS 5-HT in associated clinical affective disorders as well as regulation of mood in healthy people[25]. However, this pharmacotherapeutic evidence was non-specific to serotonin, and ironically, notwithstanding the risk of oversimplifying neural bases to complex disorders, the best evidence for a major role for CNS 5-HT in control of affect has come from studies that manipulate TRP entry to the brain[26]. Furthermore, whilst recent studies combining neuroimaging with administration of selective serotonin reuptake inhibitors have also strengthened the evidence for a role for central 5-HT in depression[27], other evidence is emerging for the importance of peripheral metabolic pathways for TRP, including roles in inflammatory processes and melatonin synthesis, underlying major depression, seasonal affective disorder and bipolar disorder[1,11,12,28].

Central serotonin is known to be involved in cognitive function, especially memory, attention, decision making and information processing, as well as in the processing of emotionally relevant stimuli[26,29,30]. However, cognition and emotion, or affect, are not entirely separable, and are often strongly interdependent[31–33]. Emotions, via their neural substrates, influence memory and attention for example, and depression and anxiety are associated with cognitive impairments and biases that can contribute to the affective disorder and its maintenance[32,34].

Effects of acute tryptophan depletion

This review is mainly concerned with genetic susceptibility to effects of forms of TRP administration that may lead to increased serotonin synthesis in the brain; however, by way of comparison, and given the scientific influence, a brief overview is included of findings, and their implications, on deficits in central 5-HT induced by acute TRP depletion (ATD) methods[33,35]. ATD is usually induced by ingestion of amino acid loads devoid of the precursor amino acid TRP to suppress 5-HT synthesis, and can be preceded by a low-TRP diet for a few days[29]. This results in a substantial (e.g. >70 %) and rapid drop in plasma TRP, and TRP:LNAA ratio (>80 %) that may last 4–6 h[30,35]; similar effects have been found with a more palatable low-TRP collagen protein mixture[36], and more recently a gelatin-derived TRP-free protein/carbohydrate mixture has been used[9]. Moreover, the serotonin metabolite, 5-hydroxyindole acetic acid measured in cerebrospinal fluid (CSF) declined by about one-third at 12 h, after which measurements stopped[37]. ATD methods have provided the most consistent evidence for serotonergic involvement in cognition, including impairment of memory consolidation[38,39] and aspects of cognitive flexibility including learning[40] and decision-making[41]. Moreover, evidence in animal models is persuasive of opposing effects of both ATD and TRP supplementation on brain 5-HT[6,42–44].

In support of a key role for serotonin in affective disorders, ATD also alters emotional processing and regulation[45–47]. Reducing TRP access to the brain by ATD tends to mimic the cognitive biases seen in depressed populations, such as impaired memory for, attention to, or recognition of positive *v.* negative information including facial expressions[34,48,49]. However, positive effects of ATD on cognition, for example on decision making and focused attention have also been reported[50–52], albeit interacting with a history of depression[53]. One explanation has been that serotonin may affect 'hot' cognitive tasks that include an affective component, but not 'cold' cognitive tasks that do not obviously involve emotional stimuli[49].

Neuroimaging techniques show that activity of brain regions involved in emotion regulation such as the limbic system and prefrontal cortex is sensitive to ATD[46]. The evidence is consistent with a normally inhibitory role of serotonin on any tendency for negative emotional bias[54,55]. Importantly, family or personal history of depression, sex and at-risk genotypes, have been reported to moderate effects of ATD on brain activity to emotional stimuli[46,47,56].

Despite a history of use of anorexigenic drugs with serotonergic agonist activity such a d-fenfluramine[57], and reductions in food intake established for high doses of TRP[58], and thus an expectation that ATD might increase appetite, the few studies addressing this directly in human beings suggest little effect of ATD on appetite despite concurrent mood effects[59–61]. Two studies comparing ATD in women with bulimia nervosa *v.* healthy controls found conflicting results[60,62]: although both studies found increased negative affect in bulimic

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

women, only one reported increased energy intake in these women[62], although the other did find an increased desire to binge eat[60]. However, curiously, another study reported a concurrent increase in both nausea and hunger in healthy women[63]. These findings also need to be considered in the context of opposing relationships between depression and appetite across patients[64].

Two other behaviours that appear to be sensitive to serotonin depletion are aggression and impulsivity[33,65]. ATD has resulted in increased aggressive behaviour in the majority of studies where measured[33], and aggressive traits have correlated with plasma levels of TRP and CSF indices of serotonin turnover[65]. However, gene by environment interactions, including stressful life events, and sex differences, are likely to moderate findings[66,67], and a meta-analysis of associations between 5-HT function and aggression in human beings revealed only a weak negative relationship[68]. It may be that stronger associations will be found when genetic variants influencing serotonin function, such as in enzymes involved in synthesis and metabolism, or polymorphisms in transporter systems (see later), are taken into account[69,70]. Indeed, a key criticism put forward is the observation that ATD lowers TRP quite universally across participants, and yet the behavioural effects differ considerably depending on a propensity to dysfunction of mood or emotional regulation, or poor stress coping[9].

Effects of L-tryptophan administration and supplementation

In contrast to ATD, which is a research tool to investigate serotoninergic processes in human beings, and for which most effects are not beneficial, administration of TRP (and its first-stage metabolite, 5-hydroxytryptophan) has a long history of being studied for potential clinical benefit in depression, as well as for basic research, as a means to facilitate entry of TRP into the brain and thus elevate 5-HT synthesis and release[26,33]. The methods can vary from intravenous administration of TRP to oral supplementation of TRP, or use of TRP-rich proteins or peptide preparations, either acutely or chronically[26,29,71]. It is also possible to increase the TRP: LNAA ratio, and so enhance TRP entry across the blood–brain barrier, by feeding a carbohydrate-rich, very low-protein meal, since the rise in insulin removes more LNAA into surrounding tissue. This dietary method has been shown to benefit cognitive and emotional function, and reduce the cortisol response to stress, in more stress-prone, neurotic participants[72–75]. This mechanism has also been suggested to underlie dietary effects on mood and performance, such as calming after high-carbohydrate meals *v.* arousal after protein-rich meals[76,77]. Recently, using data from the US National Health and Nutrition Examination Survey for nearly 30 000 adults, dietary intake of TRP was found to be inversely associated with self-reported levels of depression, and positively related to sleep duration (more strongly in women; adjusted for protein intake)[78]. Thus, even in complex whole diets, TRP intake appears to provide psychological benefits.

TRP supplementation has been employed as a potential treatment for depression and sleep disturbance since the early 1960s[24,79], although a Cochrane Review of 108 trials (including for 5-hydroxytryptophan) for antidepressant effects in 2002 found that only two trials were of sufficient quality to be included[80]. Nevertheless, on that limited evidence, TRP was considered to be better than placebo in alleviating depression, at least in mild to moderately depressed people. Moreover, for more than a decade prior to that review, the US Food and Drug Administration had banned over-the-counter sales of TRP following an outbreak in 1989 of the harmful eosinophilia–myalgia syndrome in users of TRP supplements. The cause was eventually traced to impurities in TRP supplements from one Japanese manufacturer, after which the ban was lifted in 2001[10,26]. Thus, for at least five decades, TRP has been used pharmacologically, i.e. at daily doses sometimes well in excess of ten times the RDA (5 mg/kg) for this essential amino acid. There was early evidence for probable enhancement of brain 5-HT function: after 50 mg/kg TRP (3·5 g per 70 kg subject) was consumed in a milk drink, plasma TRP increased 8-fold, TRP in CSF increased 6-fold after 6–8 h, and the metabolite 5-hydroxyindole acetic acid increased almost 2-fold in CSF by 8 h, suggesting increased turnover of brain 5-HT[81]. This 2-fold increase in 5-HT turnover was replicated in a later study of CSF 5-hydroxyindole acetic acid changes, using 3 and 6 g TRP, with no further increase at the higher dose, although the level was sustained for longer, i.e. 12 *v.* 8 h[82].

In a review of potential side-effects, Fernstrom[26] concluded that such use of TRP appears to be largely safe from adverse events, although the evidence is limited and not systematic. There are some reports of symptoms such as nausea, tremor or dizziness when high doses are used (although these are also common symptoms reported in placebo-treated subjects). However, the greatest risk of side-effects occurs when TRP is combined with other drugs that enhance 5-HT availability, such as antidepressant selective serotonin reuptake inhibitors or MAO inhibitors: then a toxic 'serotonin syndrome' may occur that can include hyperthermia and coma[26]. A more common effect of high doses of TRP is fatigue or drowsiness, which has led to TRP being used to aid sleep, in which case sedation is not an unwanted side-effect[26]. However, a complication of oral TRP at higher doses is that it increases the release of several hormones, including growth hormone, cortisol and prolactin[83] (the latter thought to indicate increased central serotonin, and dopamine, activity). A recent study also reported that intragastric administration of 1·56 g TRP increased plasma cholecystokinin and glucagon-like peptide 1, as well as slowing gastric emptying[84]: although subjective appetite was not affected, it is likely that these mechanisms contribute to reduced food intake reported after higher doses of TRP[58]. Even so, food intake might be reduced merely due to TRP-induced drowsiness.

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

There is also concern that excess metabolism through pathways such as TRYCAT could lead to high levels of neuronally active metabolites such as kynurenic acid and quinolinic acid. However, a recent review did not find evidence for adverse side-effects via these routes, although it was acknowledged that more systematic research is needed[1]. Furthermore, it has been argued that the modest antidepressant effect of TRP loading is due to accelerated hepatic degradation of TRP in depressives, probably via stress-related neuroendocrine enhancement of the catabolic hepatic enzyme TDO[85].

As would be expected in a treatment with antidepressant potential, there is considerable evidence for beneficial effects of TRP on mood and social behaviour, and these findings have recently been reviewed[22,33]. There is some evidence that TRP can reduce aggression in schizophrenic patients[33], and reduce quarrelsomeness while increasing agreeableness in healthy participants with a tendency to irritability or aggression[22]. Thus, it has been proposed that serotonin may influence a basic drive to be social, and that modulation of serotonin can alter more complex social behaviours by affecting social behaviour along an agreeable-quarrelsome axis[33]. For example, there is evidence that TRP supplementation can promote prosocial behaviour in economic decision-making tasks[22]. Somewhat counterintuitively, a more recent study, in which 1 g TRP was given three times daily for 14 d to those with a family history of depression, found increased quarrelsomeness and reduced agreeableness (at home), but improved mood, compared with placebo[86]. This was interpreted as possibly reflecting the development of more control in social interactions at home.

Effects of L-tryptophan-rich protein preparations

Bearing in mind such concerns about loading with high doses of TRP as the single amino acid, in recent years methods have been developed to enhance TRP availability to the brain by administering TRP-rich dietary proteins: the most published example is the whey protein α-lactalbumin. The effects of this protein are usually compared with responses after ingestion of another protein, typically casein hydrolysate (another milk protein), which has lower levels of TRP but greater amounts of the competing LNAA[29].

Similarly to a high-carbohydrate meal, α-lactalbumin has been shown to enhance (or correct) serotonin function (indexed by prolactin release) and cognition, and to reduce cortisol release, in stress-prone (more anxious) participants[87,88]. α-Lactalbumin attenuated deficits in delayed memory in women suffering from premenstrual syndrome[89] and in recovered depressives and healthy subjects[90]. This TRP-rich protein also improved the perception of emotional faces within women[91]; however, effects on emotional face processing tend to be weaker than dosing with TRP alone[92].

Another TRP-rich protein that has been used for research in this area is a proprietary peptide product, which is an egg-white protein hydrolysate formulation that contains fewer competing LNAA (DSM Nutritional Products Ltd., Basel). This peptide, taken in drink form, has been shown to be more effective in raising plasma TRP:LNAA ratios than either α-lactalbumin or TRP alone[93,94]. Preliminary studies using a 12-g dose (0·66 g TRP) of this TRP-rich protein hydrolysate showed improved mood in all subjects and enhanced psychomotor and vigilance performance in individuals more resilient to stress[93,95]. This was supported by a functional MRI study in young women[96] which found that this dose improved mood acutely as well as increasing activation of brain limbic circuitry, especially medial cingulate gyrus, during a fear induction task. Conversely, during reward anticipation, activation of reward pathways was reduced. Effects on resting state connectivity were in line with modulation of brain regions involved in regulation of mood. Subsequently, lower doses were found to be effective in enhancing mood and positivity in emotional processing acutely (0·13 g TRP)[97], and chronically (0·07 g TRP for 19 d) in improving aspects of mood and sleep, as well as modest benefits to cognition, in middle-aged women, relative to a casein control treatment[98].

Role of genetics in moderating effects of L-tryptophan supplementation or challenge on serotonin-related behaviours

Gene polymorphisms involved in the metabolism of TRP and regulation of serotonin could have a substantial influence on behavioural effects of manipulations of TRP availability. There is potential for moderation of TRP effects by polymorphisms in each of the key enzymes influencing TRP metabolism and thus serotonin synthesis, i.e. TPH1, TPH2, TDO, indole 2,3-dioxygenase and also by polymorphisms of the MAO-A enzyme that metabolises central serotonin (Fig. 1). These various 5-HT-related polymorphisms may form an interactive system that determines the aetiology and prognosis of various forms of affective disorder[17,99–102]. However, the most evidenced serotonergic genetic influence on behaviour is the 5-hydroxytryptamine transporter-linked promoter region (5-HTTLPR) polymorphism of the serotonin transporter gene (SLC6A4)[103–104]. The recommended classification of 5-HTTLPR genotypes is a functional combination of variable number tandem repeats of short or long length of the gene promoter amplicon and SNP variants, L_A and L_G, where L_G is functionally equivalent to the short, and L_A to the long, variable number tandem repeats forms[103–104]. Effects of 5-HTTLPR genotypes are usually compared between the homozygous alleles that are either high (long variants; L/L′) or low (short variants but including L_A; S/S′) expressing of mRNA for the 5-HT transporter receptor.

Another important genetic factor in predicting serotonergic effects on behaviour is sex. Women are more susceptible to, and have higher heritability for, affective disorders (even allowing for sociocultural effects on presentation), may be more sensitive to stress, and tend to be more responsive to serotonin selective reuptake inhibitors

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

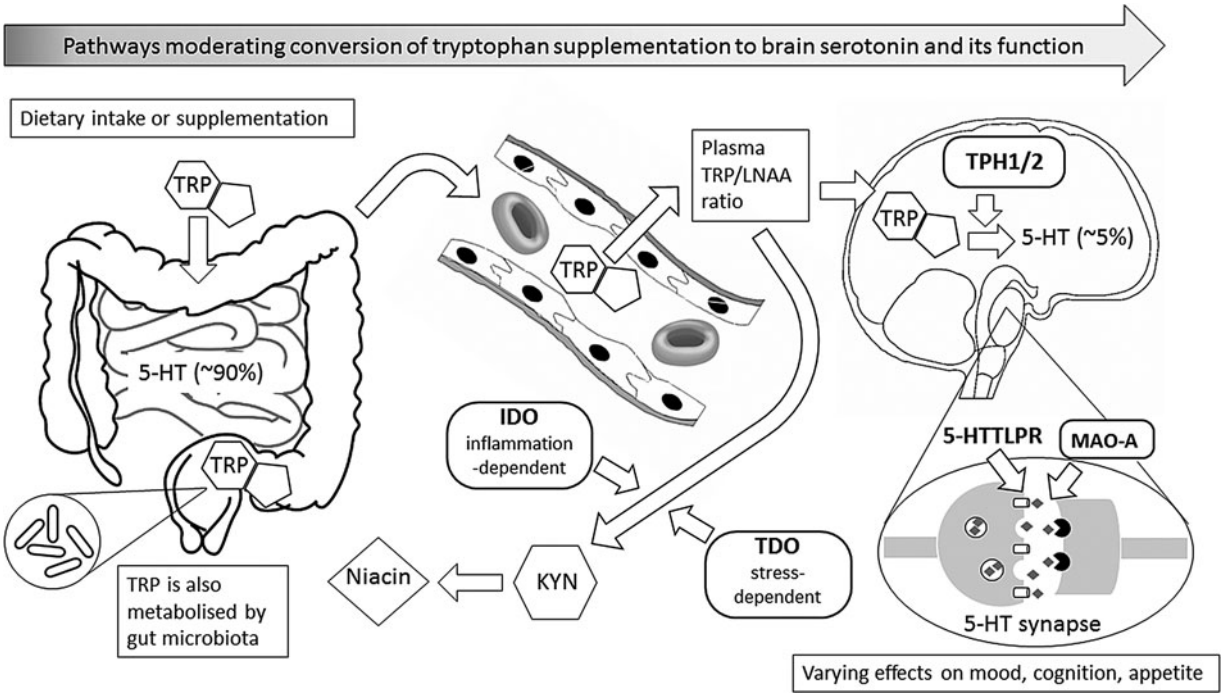

Fig. 1. This figure illustrates metabolic and other biochemical pathways in gut and blood that moderate the ability of supplementary L-tryptophan (TRP) to enter the brain as the precursor for the synthesis of brain serotonin (5-hydroxytryptamine (5-HT)), and thus to alter behaviour, especially mood, cognition and appetite. Rounded rectangles indicate enzymes involved in the various pathways. Thus, indole 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) are involved in the catabolism of TRP via the 'tryptophan catabolite' (TRYCAT) pathway, resulting in kynurenine (KYN) and then niacin formation. This could alter the TRP-to-large neutral amino acids (TRP/LNAA) ratio and thus TRP entry into the brain, where the enzyme tryptophan hydroxylase (TPH; present as either TPH1 or TPH2) is the rate-limiting step for conversion of TRP to 5-HT in serotonergic neurones. Action of 5-HT at the synapse can, in turn, be modified by the enzyme monoamine oxidase-A (MAO-A), and by the 5-HT transporter system that has functional genetic variants in the 5-HT transporter-linked promoter region (5-HTTLPR). Abbreviations in bold represent influences that have known functional genetic variants which may vary in their moderating effects; these, in turn, can interact with sex.

treatment[67]. Brain 5-HT synthesis rates are reportedly 50 % lower in women than men[105], and ATD causes greater lowering of mood in women than men[106]. In some studies, women also appear to be more sensitive to, or to benefit more from, TRP supplementation; indeed, some researchers chose to study women only for these reasons[97,98]. Furthermore, sex interacts with serotonergic gene polymorphisms in several systems, including 5-HTTLPR, TPH1, TPH2 and MAO-A[67,107], and these interactions can be further moderated by stress[108–111]. Therefore, the sex of participants needs to be considered when interpreting findings in this area.

Tryptophan administration and 5-hydroxytryptamine transporter-linked promoter region genotypes

Only a few studies have investigated whether these 5-HT- and TRP-related genotypes alter the effects of TRP loading (or challenge or supplementation), and these appear to be limited to a comparison of 5-HTTLPR genotypes: these studies are summarised in Table 1. In the earliest published study[108] to examine moderation of TRP loading by the 5-HTTLPR tri-allelic genotype, forty-one men and thirty-one women were infused intravenously with a high dose of TRP (100 mg/kg), while aspects of mood were assessed (Profile of Mood States). Far from improving mood, this procedure generally increased negative affect, but the effects were moderated by genotype and sex: in men, only those with the high-expressing L/L′ polymorphism showed increased negative mood, whereas in women, only the L/L′ group showed no increase in negative mood. This opposing interaction between sex and 5-HTTLPR genotype is in line with evidence based on the impact of social stressors on negative affect in adolescents[111]. However, sample sizes were small, especially in the S/S′ groups (seven men; nine women).

Using a far lower dose, and oral administration, Markus and Firk[112] examined potential interactions between acute TRP supplementation, stress and 5-HTTLPR genotype on mood, cortisol and cognition. They hypothesised that the TRP challenge would ameliorate the effects of stress on mood and cortisol in subjects homozygous for the tri-allelic S/S′ genotype compared to those with the L/L′ genotype. In a cross-over design, thirty student participants (sixteen S/S′-allele; fourteen L/L′-allele; only one man in each group) received either TRP (2 × 0·4 g) or placebo (lactose), prior to a stressful challenge, with baseline and post-stress measures of

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

Table 1. Summary of studies investigating interactions between L-tryptophan (TRP) supplementation or challenge and tri-allelic 5-HT transporter-linked promoter region (5-HTTLPR) genotypes on behaviour

Reference	Sample	Design and intervention	Measures	Main findings	Comments
Brummett *et al.*[108]	Healthy adults; thirty-one females, forty-one males; 54 % Mean ± SD age = 33·5 ± 9·1	Single blind. Overnight fast. TRP (10 mg/kg body weight) i.v. infusion. Saline infusion day 1, followed by TRP on day 2	Negative affect assessed by POMS prior to and 1 h after start of infusion.	Scores for Depression-Dejection increased 3-fold from pre- to post-TRP infusion for L/L′ males, but did not change for S/L or S/S′ males. In females, L/L′ scores did not change, but increased moderately for S/S′ genotypes	Small sample size for S/S′ groups (seven males, nine females). No saline infusion on same day as TRP. No significant effects on fatigue, anxiety and anger
Markus & Firk[112]	Twenty-eight female and two male students. Mean ± SD age = 19 ± 2	Double-blind cross-over design. Overnight fast. Oral TRP (2 × 0·4 g) or lactose placebo capsules, then stressful challenge (cold pressor and Serial-7 tasks in front of the camera)	POMS at baseline and post-stress. Cortisol in saliva	TRP reduced depression and fatigue scores, and increased vigour, only in S/S′ genotypes. No interaction with pre/post-stress	No stress-free condition. Single cortisol samples pre- and post-stress. No effect of TRP or genotype on cortisol
Markus & De Raedt[113]	Twenty-eight female students. Mean ± SD age = 19 ± 2	Double-blind cross-over design. Overnight fast. Oral TRP (0·8 g) *v.* cellulose placebo, then stressful challenge (cold pressor and Serial-7 tasks in front of the camera)	NAP using pictures with positive or negative valence – assesses tendency to inhibit negative emotional information. Positive and negative affect by questionnaire (PANAS)	TRP prevented the modest increase in negative affect seen after placebo for S/S′ but not L/L′ allele group. Stress weakened ability to inhibit negative information in S/S′ allele group but enhanced it in L/L′ group. No effect of TRP on this measure	No stress-free condition. Despite NAP being sensitive to stress and genotype, no effect of TRP on this measure
Markus *et al.*[114]	Forty-two female students (nineteen S/S′, twenty-three L/L′). High or low restrained eaters. Mean ± SD age = 19 ± 2	Double-blind cross-over design, counterbalanced for genotype and restraint level. TRP-rich protein hydrolysate drink (235 mg TRP) or placebo (casein hydrolysate), then stress: adapted	Baseline, pre- and post-stress measures of salivary cortisol (3 before stress, one after), mood (POMS), urge for food, snack food intake	No effect of TRP or genotype on stress-induced rise in cortisol. Stress increased anger in both TRP and placebo conditions, except for L/L′ group who did not increase anger after TRP. This same L/L′ group showed a reduced liking for high-fat sweet foods after stress in the TRP condition only. Overall, TRP reduced food intake *v.* placebo	No interactions with restrained eating, but this is not a good measure of emotional eating tendencies. Snack food intake during the study may have modified impact of TRP treatment, but note that L/L′ showed the greatest increase in plasma TRP: LNAA after TRP treatment *v.* placebo
Cerit *et al.*[126]	Twenty-two females, twenty-four males; approx. half of each were S/S′ or L/L′. Mean ± SD age = 20·4 ± 3	Double-blind between-subjects, stratified by genotype. Subchronic oral TRP (2·8 g/d as 7 × 0·4 g capsules taken morning, afternoon and evening) for 6 d, then TSST on day 7	Anxiety and depression (HADS); positive and negative affect (PANAS); tension, anxiety, sadness, annoyance by single-item MSS	No effects of TRP on mood/symptoms measures. Stress increased tension, anxiety and annoyance (MSS). No interactions with genotype. S/S′ group, not L/L′ group, showed higher stress-induced cortisol rise after placebo that was suppressed in TRP condition	Cortisol results suggest that S/S′ show greater stress responsiveness that in turn is reduced by TRP. Cortisol AUC not analysed. Sex analysed as a covariate, but significance not reported

Capello & Markus[123]	Ninety-nine female, nineteen male students; sixty in S/S′ and fifty-eight in L/L′ groups. Mean ± SD age = 24·0 ± 1·7	Double-blind between-subjects, stratified by genotype and neuroticism (N) trait (DPQ-N). Subchronic oral TRP (3 g/d as 2 × 0·5 g capsules taken three times/d) for 7 d, then stress (Maastricht Acute Stress Test) after lunch on day 8	Salivary cortisol (one baseline, two post-stress), mood (POMS), anxiety (state scale of STAI), appetite ratings, pre- and post-stress. Snack food intake after stress	Stress-induced rise in cortisol was reduced by TRP only in the S/S′ group. TRP treatment also reduced the stress-induced rise in anxiety (STAI) only in the S/S′ group. Negative affect (POMS) was increased by stress but not altered by genotype or treatment. For S/S′ only, high N subjects showed a stress-induced increase in appetite after placebo but not after TRP. Curiously, low N subjects ate more high-fat sweet snacks than did high N	Relatively large sample but not enough males to examine sex effects. Parallel effects of TRP in S/S′ subjects for cortisol, anxiety and appetite. Lunch intake, sex and BMI controlled for by covariance. Avoidance of high-fat sweet snacks in high N subjects may be related to health/weight concerns
Van Dalfsen & Markus[127]	S/S′ allele group: forty-six women, eleven men; L/L′ allele: forty-six women, eight men. Mean ± SD age = 23·9 ± 1·7	Double-blind between-subjects, stratified by genotype and neuroticism trait (median split on DPQ-N). Subchronic oral TRP (3 g/d as 2 × 0·5 g capsules taken three times/d) for 7 d	Prior to treatment: subjective sleep quality (1 month; adapted PSQI), neuroticism (DPQ-N), depression (BDI), (SLE; Dutch Life Events Questionnaire). During treatment: Daily Hassles Checklist. After treatment: PSQI sleep quality for 1 week	More neurotic participants had lower general sleep quality, unrelated to genotype, and also reported more SLE. Following treatment, only S/S′ genotype together with higher neuroticism was associated with poorer sleep quality for the placebo group, but with better sleep quality for the TRP-treated group	The main effect of neuroticism was stronger when BDI depression was not accounted for as a covariate. Sex and SLE were NS covariates

POMS, Profile of Mood States; NAP, Negative affect priming; PANAS, Positive and Negative Affect Schedule; TSST, Trier Social Stress Test; HADS, Hospital Anxiety and Depression Scale; MSS, Mood States Scale; DPQ-N, Dutch Personality Inventory; STAI, State and Train Anxiety Inventory; PSQI, Pittsburg Sleep Quality Index; BDI, Beck Depression Inventory; SLE, Stressful Life Events.

mood (Profile of Mood States) and salivary cortisol. The stressor consisted of repeated unpredictable cold pressor stress (hand on a 1·5°C cold plate) interspersed with a Serial-7 subtraction task (repeatedly subtracting 7 from a variable starting number), performed in front of a camera and researcher; errors were recorded. The design did not include a stress-free condition, and only a single baseline measure of cortisol, so interpretation of the observed decline in cortisol after the stress is difficult, as this decline is anyway typical for cortisol during the morning. However, neither TRP treatment nor genotype significantly altered this decline in cortisol. Nevertheless, the stressor caused mood to deteriorate, with increases in feelings of anger, depression and fatigue, but a decrease in vigour. A key finding of this study is that the TRP treatment reduced depression and fatigue, while increasing vigour, specifically in the S/S′ allele group only. However, these effects were pooled across stress condition, so presumably were not significantly altered by stress (data for a pre/post-stress × genotype × treatment interaction were not presented). Genotype also influenced performance on the subtraction task: the S/S′ group performed worse than the L/L′ group after placebo, but after TRP, performance was the same for both allele groups; again, this result was independent of stress. Even so, pre-stress results were not presented, so stress may have contributed somewhat to the findings. For example, the fact that the S/S′ group made more mistakes in the subtraction task under placebo may indicate that subjects with this genotype were not coping as well with the stressful aspect of the task: that this detriment was removed by TRP treatment strongly suggests it reflected suboptimal 5-HT function during a demanding task. It is also important to note that this sample consisted of twenty-eight women and only two men.

A subsequent report from this group[113] used the same stressor and TRP treatment to examine interactions of treatment, stress and 5-HTTLPR genotype on another measure of mood (Positive and Negative Affect Schedule) and attentional bias (inhibitory responses) to negative emotional stimuli. This bias was measured by reaction times to facial expressions varying in emotional valence and primed by previous stimuli of the same or opposite valence (negative affective priming). This study appears to have used the same participants as Markus and Firk[112] except excluding the two men (i.e. twenty-eight women). In the placebo condition, negative affect increased after stress only for the S/S′ genotype group, and furthermore this rise in negative mood was prevented by TRP treatment. For the negative affective priming task, there was an interaction between stress and genotype, such that S/S′ subjects showed faster responding to congruently than incongruently primed negative expressions after stress, an indicator of reduced inhibition to negative affective stimuli. The L/L′ group showed the opposite response, suggesting that this allele may confer some resilience to effects of stress on emotional processing. However, no effects of TRP treatment were found for this behaviour, although, as the authors point out, the study has a relatively small sample size and may be underpowered to detect three-way interactions of this sort.

Subsequently, Markus *et al.*[114], established a larger student cohort screened for 5-HTTLPR genotype, and studied nineteen female S/S′ and twenty-three female L/L′ homozygous allele groups, with about half of each group selected to be either high or low on restrained eating (Three Factor Eating Questionnaire[115]). This study investigated potential interactions between TRP treatment, 5-HTTLPR genotype, stress, restraint and emotional eating, in a double-blind placebo-controlled crossover design. Stress was elicited using a modified Trier Social Stress Test[116]; TRP challenge was accomplished using an egg-white protein hydrolysate enriched with TRP (4-g dose given as a 200-ml drink, containing 0·24 g TRP; DSM, Delft; see earlier), *v.* a casein hydrolysate placebo (0·03 g TRP). Blood samples were taken for amino acid analysis 90 min after consuming the drinks, and four salivary samples were taken during the study to assess cortisol levels. Interestingly, there was a significantly greater increase in plasma TRP:LNAA ratio following TRP treatment for the L/L′ group (70 % increase) compared with the S/S′ group (30 % increase). However, although stress resulted in a rise in cortisol, there were no significant effects of either TRP treatment, genotype or restrained eating on cortisol in this study. Mood generally deteriorated from before to after the stress; of particular interest, the increase in anger after stress occurred in all groups except the L/L′ genotype group who had received TRP supplementation, in whom there was no change in anger following stress.

Liking (pleasantness of taste) for a variety of foods of different sensory categories (sweet or savoury, low- or high-fat) was assessed using ratings of images of the foods. Only the high-fat sweet food liking ratings showed significant effects: in the L/L′ allele group, liking for high-fat sweet foods declined following stress only when given the TRP supplement, whereas there were no significant changes to liking ratings for the S/S′ allele group. Actual food intake was assessed by offering several snack foods (mini chocolate bars, pretzels and nuts) both before and after stress. The only significant result was a 38 % reduction in snack intake after TRP treatment (averaged across stress pre/post-measures); no effects of genotype, stress or restrained eating were seen. An overall appetite-suppressant effect of TRP may be expected, given that ATD tends to increase appetite[63], and higher doses of TRP (at least 2 g) have long been known to suppress appetite and reduce food intake by 10–20 %[58]; nevertheless, the dose of TRP effective here is considerably smaller (0·24 g), so the size of this effect is remarkable.

There are several intriguing findings in this study, not least the weaker increase in plasma TRP:LNAA ratio in the S/S′ subjects. The authors point out that this difference between genotypes is a unique finding, and speculate that it may be due to increased diversion of peripheral TRP to metabolism via the kynurenine pathway, due to induction of the hepatic TDO and peripheral indole 2,3-dioxygenase enzymes, which are known to be stress-sensitive[14]. However, direct evidence for such a mechanism reducing the TRP:LNAA ratio in S/S′ allele subjects after TRP supplementation is lacking. One

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

study that measured 5-HTTLPR genotypes and administered 50 mg/kg TRP did assess the plasma kynurenine: TRP ratio as an index of TDO activity; however, this was in male patients with alcohol use disorder, and the study did not assess behavioural effects of TRP[117]. Those patients who experienced 'blacked-out violent impulsive behaviour' during binge drinking showed a higher kynurenine:TRP ratio than those who did not, suggesting that less TRP would be available to the brain. Nevertheless, no differences were reported for 5-HTTLPR genotype subgroups, although sample sizes may have been too small (nine cases, nine alcohol-dependent controls, received oral TRP) for meaningful statistics in this pilot study, and polymorphisms in the enzymes themselves were not measured. This may be important as there is evidence for example that the TPH1 218AA polymorphism is a risk factor for alcoholism and bipolar disorder[118]. Anyhow, this impaired effect of TRP treatment on the plasma ratio in this S/S′ group[114] may explain the lack of behavioural effects seen for this group in this study, in contrast to some effects that were specific to the L/L′ genotype. Conversely, the most likely explanation for a lack of stress-induced, or emotional, eating is the probability that few of the participants had emotional eating tendencies. Participants were selected on the basis of scores on the Three Factor Eating Questionnaire restrained eating scale, which, unlike some items on the disinhibition or hunger scales of this questionnaire, does not explicitly assess emotional eating and is usually orthogonal to it. We have argued previously that cognitive restraint *per se* is not a good predictor of stress eating tendencies[119,120]. Furthermore, in a more recent study from this group, S/S′ allele subjects (both male and female) were shown to be more likely to eat sweet fatty foods after mild stress than L/L′ genotypes, an effect that was reduced by a sucrose preload[121]. However, in that study, there was no manipulation by TRP load. Another study from this group investigated whether examination stress would differentially affect appetite for these two genotype groups[122]: findings confirmed that the S/S′ genotype group was more likely to show stress-induced eating of sweet snacks, although again there was no manipulation of TRP.

Nevertheless, the interaction between genotype, stress, emotional eating and effects of subchronic TRP supplementation was investigated in mainly female participants (ninety-nine women, nineteen men) asked to self-administer 3 g TRP daily for 7 d (or placebo cellulose), before undergoing an acute stress test (repeated cold pressor and Serial-17 subtraction task known as the Maastricht Acute Stress Test)[123]. Changes in appetite ratings, snack intake, mood and cortisol were assessed. Subchronic TRP treatment reduced the cortisol response to stress only in the S/S′ allele group. Similarly, the TRP treatment resulted in a significantly less stress-induced increase in anxiety only in the S/S′ group, but independently of trait neuroticism. Stress increased rated appetite, but interestingly TRP reduced this increase specifically in S/S′ subjects who also scored highly on neuroticism. The parallels across these TRP by genotype interactions are notable. By comparison, the only significant finding reported for post-stress snack intake was a greater intake of sweet fatty snacks by the low neuroticism *v.* the high neuroticism group, perhaps due to health concerns in the latter group. The interaction of genotype with neuroticism on the stress-induced change in rated appetite is similar to the results of an earlier study in which mainly female participants with low or high trait anxiety were subjected to stress (mental arithmetic during loud noise) and treated acutely with either TRP-rich α-lactalbumin or casein[124]. Food liking and preference was assessed by responses to food images displayed via a computer program[125]. While appetite ratings increased for all groups after stress, both liking and preference for sweet foods increased specifically for highly anxious participants, and these increases were prevented by α-lactalbumin treatment, implying that the increased desire for sweet food induced by stress in high-anxious participants was related to impaired 5-HT function. However, in this study, genotypes were not measured. Moreover, in the case of actual eating[122], it seems that other factors influenced the behaviour, although differences in timing between stress and food intake could be involved, and in this subchronic treatment design, no treatment was given on the test day.

Another group also examined effects of a similar subchronic TRP treatment (2·8 g/d for 6 d) on responses to stress (Trier Social Stress Test) in relation to 5-HTTLPR genotype[126]. In this study, about half the participants were female (twenty-two women, twenty-four men), although sex was included as a covariate in analyses, rather than reporting interactions with sex. There was a clear interaction between stress, genotype and treatment on salivary cortisol: S/S′ allele subjects on placebo (cellulose) showed the largest rise in cortisol induced by the stress, supporting a stress sensitivity of this genotype, but this effect was substantially reduced by prior TRP treatment (even though no TRP was taken on the test day): the lower cortisol response in L/L′ participants was not further reduced by TRP. However, while mood deteriorated after the stress, this was not differentially influenced either by treatment or genotype, contrary to Capello and Markus[123].

Subsequently, a recent study investigated whether a similar subchronic treatment with TRP (3 g/d for 7 d) could benefit the quality of sleep, and whether this might depend on 5-HTTLPR genotype[127]. Thus, this study compared effects between S/S′ allele subjects (forty-six women, eleven men) and L/L′ allele subjects (forty-six women, eight men). Potential effects of neuroticism were investigated using a median split of questionnaire scores into high and low neuroticism groups. General sleep quality was assessed prior to treatment, then sleep quality after the week of treatment was measured for a further week. Higher neurotic participants tended to report lower general sleep quality, unrelated to genotype. However, following treatment, specifically S/S′ genotype together with higher neuroticism was associated with poorer sleep quality for the placebo group, but with better sleep quality for the TRP-treated group.

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

Finally, there is recent evidence of differential impact of 5-HTTLPR genotypes on mood changes during challenging tasks in the context of two intervention studies that had found beneficial effects of acute[97] and chronic[98] treatment with a TRP-rich egg-white protein hydrolysate (DSM) on mood, emotional processing and cognition in Caucasian women aged 45–65 years[128]. Participants were genotyped for the tri-allelic 5-HTTLPR polymorphism, and distributions of genotypes were in accordance with Hardy–Weinberg equilibrium (allele sample sizes; acute study: $SS/SL_G = 11$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 10$).

We planned to compare the two homozygous groups (SS/L_G (designated S/S′) *v.* L_AL_A (designated L/L′)) on behavioural outcomes; however, with several different treatment groups, cell sizes would be too small for meaningful analyses of treatment by genotype effects. Therefore, we examined outcomes on the pre-treatment baseline day, when the participants completed the same set of tests as during treatment, which allowed us to pool the outcome data for all participants within each genotype group. The series of cognitive and behavioural tests lasted for 3·5 h from the baseline (pre-test) mood measure to the final post-test mood measure, with 1 h of rest in between, so represented a challenging and potentially ego-threatening process for the participants. Furthermore, we compared pre-test to post-test changes only in those emotions that had proved responsive to subsequent TRP supplementation treatment. Specifically, these were wellbeing and fatigue in the acute study, and a positive feeling of high energy (stimulated, buzzing, impulsive) in the chronic study (emotions were derived by factor analyses of ratings on twenty-eight items presented on a computer, known as the Mental and Physical Sensations Scale). For the acute study, we found that well-being declined from pre- to post-test in the S/S′ group, but not in the L/L′ group, whereas fatigue increased significantly only for the S/S′ group. For the chronic study, high-energy mood increased from pre- to post-test for the L/L′ group, but did not change for the S/S′ group.

These differences in genotypes for mood changes during challenging and potentially stressful tasks are in line with evidence that the S/S′ genotype would confer greater risk of affective disorders such as anxiety or depression, or conversely a protective effect of the L/L′ allele, in women. Moreover, the known sensitivity of these changes in mood to TRP treatment supports mediation via changes in serotonin function.

Conclusions

The main theme emerging from the literature on TRP supplementation and genotypes is the observations of interactions between TRP and genotypes, sex and stress on changes in mood, cognition, cortisol and appetite. It is particularly important to consider the influence of a key ‘genotype’, sex. For example, in women, the 5-HTTLPR S/S′ genotype predicts sensitivity to improvements in the mood by TRP supplementation, especially during stressful challenges, whereas the L/L′ genotype tends to be protective against stress-induced mood deterioration and rise in cortisol, but may differ in sensitivity to TRP administration. In men, if anything, the L/L′ genotype confers risk of stress-induced increases in negative affect; however, there are insufficient studies with adequate power to detect sex × genotype × stress × TRP in the literature to draw strong conclusions.

Since the 5-HTTLPR genotypes may influence neurodevelopment and/or tonic 5-HT adaptive responsiveness at least as much as acute functioning of the brain serotonin system[103,129], it would be advantageous to assess extent of early life stress and/or stressful life events, as well as personality traits predictive of affective disorders, in studies of TRP effects on behaviour. However, when measuring multiple influences on behaviour, as well as sex differences, investigators need to ensure sufficiently large sample sizes to increase the likelihood of reliable findings[107]: routinely screening for genetic polymorphisms in suitable populations would be helpful.

There is a need to broaden studies on the potential benefits of TRP supplementation to include a greater range of serotonin-related genotypes, including enzymes involved in key metabolic pathways (Fig. 1). This may eventually lead to clear predictions as to who is likely to benefit most from this relatively simple nutrient-based treatment. Until then, although there is preliminary evidence that individuals with some genotypes, particularly the 5-HTTLPR S/S′ allele in women, may benefit from TRP supplementation as an aid to stress coping and emotional regulation including comfort eating, further research is needed before reliable recommendations can be made on targeted use of TRP treatment, or adjustment of dietary TRP intake, for beneficial behavioural outcomes.

Acknowledgements

The author grateful to the Nutrition Society, UK, and the Royal Society of Medicine, London, for inviting me to present a review of this topic at their Winter Conference on Diet, Nutrition and Mental Health and Wellbeing, December 2016.

Financial Support

The author has received research funding from DSM Nutritional Products Ltd., Basel, Switzerland.

Conflicts of Interest

The sponsors had neither influence on conducting the experiments beyond consultation over design, nor on statistical analyses. The sponsors did not contribute to this review.

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

Authorship

E. L. G. is solely responsible for authorship of this article.

References

1. Fernstrom JD (2016) A perspective on the safety of supplemental tryptophan based on its metabolic fates. *J Nutr* **146**, 2601S–2608S.
2. Jenkins TA, Nguyen JCD, Polglaze KE *et al.* (2016) Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients* **8**, 56.
3. Young SN & Teff KL (1989) Tryptophan availability, 5HT synthesis and 5HT function. *Prog Neuropsychopharmacol Biol Psychiatry* **13**, 373.
4. Watts SW, Morrison SF, Davis RP *et al.* (2012) Serotonin and blood pressure regulation. *Pharmacol Rev* **64**, 359–388.
5. Jacobs BL, Martin-Cora FJ & Fornal CA (2002) Activity of medullary serotonergic neurons in freely moving animals. *Brain Res Brain Res Rev* **40**, 45–52.
6. Fernstrom JD, Langham KA, Marcelino LM *et al.* (2013) The ingestion of different dietary proteins by humans induces large changes in the plasma tryptophan ratio, a predictor of brain tryptophan uptake and serotonin synthesis. *Clin Nutr* **32**, 1073–1076.
7. Fernstrom MH & Fernstrom JD (1995) Brain tryptophan concentrations and serotonin synthesis remain responsive to food consumption after the ingestion of sequential meals. *Am J Clin Nutr* **61**, 312–319.
8. Moja EA, Restani P, Corsini E *et al.* (1991) Cycloheximide blocks the fall of plasma and tissue tryptophan levels after tryptophan-free amino acid mixtures. *Life Sci* **49**, 1121–1128.
9. van Donkelaar EL, Blokland A, Ferrington L *et al.* (2011) Mechanism of acute tryptophan depletion: is it only serotonin? *Mol Psychiatry* **16**, 695–713.
10. Richard DM, Dawes MA, Mathias CW *et al.* (2009) L-Tryptophan: basic metabolic functions, behavioral research and therapeutic indications. *Int J Tryptophan Res* **2**, 45–60.
11. Maes M, Leonard BE, Myint AM *et al.* (2011) The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog Neuropsychopharmacol Biol Psychiatry* **35**, 702–721.
12. Anderson G, Jacob A, Bellivier F *et al.* (2016) Bipolar disorder: the role of the kynurenine and melatonergic pathways. *Curr Pharm Des* **22**, 987–1012.
13. Dinan TG & Cryan JF (2017) Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. *J Physiol* **595**, 489–503.
14. Badawy AA (2017) Kynurenine pathway of tryptophan metabolism: regulatory and functional aspects. *Int J Tryptophan Res* **10**, 1178646917691938.
15. Gibson EL, Barnfield AM & Curzon G (1996) Dissociation of effects of chronic diazepam treatment and withdrawal on hippocampal dialysate 5-HT and mCPP-induced anxiety in rats. *Behav Pharmacol* **7**, 185–193.
16. Pardridge WM & Fierer G (1990) Transport of tryptophan into brain from the circulating, albumin-bound pool in rats and in rabbits. *J Neurochem* **54**, 971–976.
17. Fukuda K (2014) Etiological classification of depression based on the enzymes of tryptophan metabolism. *BMC Psychiatry* **14**, 372–379.
18. Walther DJ & Bader M (2003) A unique central tryptophan hydroxylase isoform. *Biochem Pharmacol* **66**, 1673–1680.
19. Patel PD, Pontrello C & Burke S (2004) Robust and tissue-specific expression of TPH2 versus TPH1 in rat raphe and pineal gland. *Biol Psychiatry* **55**, 428–433.
20. Zill P, Büttner A, Eisenmenger W *et al.* (2007) Analysis of tryptophan hydroxylase I and II mRNA expression in the human brain: a post-mortem study. *J Psychiatry Res* **41**, 168–173.
21. Gyurak A, Haase CM, Sze J *et al.* (2013) The effect of the serotonin transporter polymorphism (5-HTTLPR) on empathic and self-conscious emotional reactivity. *Emotion* **13**, 25–35.
22. Steenbergen L, Jongkees BJ, Sellaro R *et al.* (2016) Tryptophan supplementation modulates social behavior: a review. *Neurosci Biobehav Rev* **64**, 346–358.
23. Jouvet M (1999) Sleep and serotonin: an unfinished story. *Neuropsychopharmacology* **21**, 24S–27S.
24. Coppen A, Shaw DM, Herzberg B *et al.* (1967) Tryptophan in the treatment of depression. *Lancet* **2**, 1178–1180.
25. Cowen PJ & Browning M (2015) What has serotonin to do with depression? *World Psychiatry* **14**, 158–160.
26. Fernstrom JD (2012) Effects and side effects associated with the non-nutritional use of tryptophan by humans. *J Nutr* **142**, 2236S–2244S.
27. Godlewska BR, Norbury R, Selvaraj S *et al.* (2012) Short-term SSRI treatment normalises amygdala hyperactivity in depressed patients. *Psychol Med* **42**, 2609–2617.
28. Pereira JC Jr, Pradella Hallinan M & Alves RC (2017) Secondary to excessive melatonin synthesis, the consumption of tryptophan from outside the blood-brain barrier and melatonin over-signaling in the pars tuberalis may be central to the pathophysiology of winter depression. *Med Hypotheses* **98**, 69–75.
29. Silber BY & Schmitt JA (2010) Effects of tryptophan loading on human cognition, mood, and sleep. *Neurosci Biobehav Rev* **34**, 387–407.
30. Young SN (2013) Acute tryptophan depletion in humans: a review of theoretical, practical and ethical aspects. *J Psychiatry Neurosci* **38**, 294–305.
31. Duncan S & Barrett LF (2007) Affect is a form of cognition: a neurobiological analysis. *Cognit Emotion* **21**, 1184–1211.
32. Rolls ET (2014) Emotion and decision-making explained: a precis. *Cortex* **59**, 185–193.
33. Young SN (2013) The effect of raising and lowering tryptophan levels on human mood and social behaviour. *Philos Trans R Soc Lond B Biol Sci* **368**, 20110375.
34. Mathews A & MacLeod C (2005) Cognitive vulnerability to emotional disorders. *Annu Rev Clin Psychol* **1**, 167–195.
35. Young SN, Smith SE, Pihl RO *et al.* (1985) Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology* **87**, 173–177.
36. Evers EA, Tillie DE, van der Veen FM *et al.* (2005) Effects of a novel method of acute tryptophan depletion on plasma tryptophan and cognitive performance in healthy volunteers. *Psychopharmacology* **178**, 92–99.
37. Williams WA, Shoaf SE, Hommer D *et al.* (1999) Effects of acute tryptophan depletion on plasma and cerebrospinal fluid tryptophan and 5-hydroxyindoleacetic acid in normal volunteers. *J Neurochem* **72**, 1641–1647.
38. McAllister-Williams RH, Massey AE & Rugg MD (2002) Effects of tryptophan depletion on brain potential

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

correlates of episodic memory retrieval. *Psychopharmacology* **160**, 434–442.
39. Riedel WJ, Klaassen T, Deutz NE *et al.* (1999) Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. *Psychopharmacology* **141**, 362.
40. Park SB, Coull JT, McShane RH *et al.* (1994) Tryptophan depletion in normal volunteers produces selective impairments in learning and memory. *Neuropharmacology* **33**, 575.
41. Rogers RD, Tunbridge EM, Bhagwagar Z *et al.* (2003) Tryptophan depletion alters the decision-making of healthy volunteers through altered processing of reward cues. *Neuropsychopharmacology* **28**, 153–162.
42. Crockett MJ, Clark L, Roiser JP *et al.* (2012) Converging evidence for central 5-HT effects in acute tryptophan depletion. *Mol Psychiatry* **17**, 121–123.
43. Gartside SE, Cowen PJ & Sharp T (1992) Effect of 5-hydroxy-L-tryptophan on the release of 5-HT in rat hypothalamus in vivo as measured by microdialysis. *Neuropharmacology* **31**, 9–14.
44. Hulsken S, Martin A, Mohajeri MH *et al.* (2013) Food-derived serotonergic modulators: effects on mood and cognition. *Nutr Res Rev* **26**, 223–234.
45. Cools R, Calder AJ, Lawrence AD *et al.* (2005) Individual differences in threat sensitivity predict serotonergic modulation of amygdala response to fearful faces. *Psychopharmacology* **180**, 670–679.
46. Biskup CS, Gaber T, Helmbold K *et al.* (2015) Amino acid challenge and depletion techniques in human functional neuroimaging studies: an overview. *Amino Acids* **47**, 651–683.
47. van der Veen FM, Evers EAT, Deutz NEP *et al.* (2007) Effects of acute tryptophan depletion on mood and facial emotion perception related brain activation and performance in healthy women with and without a family history of depression. *Neuropsychopharmacology* **32**, 216–224.
48. Harmer CJ, Rogers RD, Tunbridge E *et al.* (2003) Tryptophan depletion decreases the recognition of fear in female volunteers. *Psychopharmacology* **167**, 411–417.
49. Robinson OJ & Sahakian BJ (2009) A double dissociation in the roles of serotonin and mood in healthy subjects. *Biol Psychiatry* **65**, 89–92.
50. Riedel WJ, Sobczak S & Schmitt JA (2003) Tryptophan modulation and cognition. *Adv Exp Med Biol* **527**, 207–213.
51. Talbot PS, Watson DR, Barrett SL *et al.* (2006) Rapid tryptophan depletion improves decision-making cognition in healthy humans without affecting reversal learning or set shifting. *Neuropsychopharmacology* **31**, 1519–1525.
52. Booij L, Van der Does AJ, Haffmans PM *et al.* (2005) The effects of high-dose and low-dose tryptophan depletion on mood and cognitive functions of remitted depressed patients. *J Psychopharmacol* **19**, 267–275.
53. Sobczak S, Honig A, Nicolson NA *et al.* (2002) Effects of acute tryptophan depletion on mood and cortisol release in first-degree relatives of type I and type II bipolar patients and healthy matched controls. *Neuropsychopharmacology* **27**, 834–842.
54. Allen JJB, McKnight KM, Moreno FA *et al.* (2009) Alteration of frontal EEG asymmetry during tryptophan depletion predicts future depression. *J Affect Disord* **115**, 189–195.
55. Robinson OJ, Overstreet C, Allen PS *et al.* (2013) The role of serotonin in the neurocircuitry of negative affective bias: serotonergic modulation of the dorsal medial prefrontal-amygdala 'aversive amplification' circuit. *Neuroimage* **78**, 217–223.
56. Roiser JP, Levy J, Fromm SJ *et al.* (2012) Serotonin transporter genotype differentially modulates neural responses to emotional words following tryptophan depletion in patients recovered from depression and healthy volunteers. *J Psychopharmacol* **26**, 1434–1442.
57. Curzon G, Gibson EL & Oluyomi AO (1997) Appetite suppression by commonly used drugs depends on 5-HT receptors but not on 5-HT availability. *Trends Pharmacol Sci* **18**, 21–25.
58. Hill AJ & Blundell JE (1988) Role of amino acids in appetite control in man. In *Amino Acid Availability and Brain Function in Health and Disease*, pp. 239–248 [G Huether, editor]. Berlin: Springer.
59. Reilly JG, McTavish SF & Young AH (1997) Rapid depletion of plasma tryptophan: a review of studies and experimental methodology. *J Psychopharmacol* **11**, 381–392.
60. Kaye WH, Gendall KA, Fernstrom MH *et al.* (2000) Effects of acute tryptophan depletion on mood in bulimia nervosa. *Biol Psychiatry* **47**, 151–157.
61. Oldman AD, Walsh AE, Salkovskis P *et al.* (1994) Effect of acute tryptophan depletion on mood and appetite in healthy female volunteers. *J Psychopharmacol* **8**, 8–13.
62. Weltzin TE, Fernstrom MH, Fernstrom JD *et al.* (1995) Acute tryptophan depletion and increased food intake and irritability in bulimia nervosa. *Am J Psychiatry* **152**, 1668–1671.
63. Rieber N, Mischler D, Schumacher V *et al.* (2010) Acute tryptophan depletion increases experimental nausea but also induces hunger in healthy female subjects. *Neurogastroenterol Motility* **22**, 752–757, e220.
64. Simmons WK, Burrows K, Avery JA *et al.* (2016) Depression-related increases and decreases in appetite: dissociable patterns of aberrant activity in reward and interoceptive neurocircuitry. *Am J Psychiatry* **173**, 418–428.
65. Virkkunen M, Goldman D, Nielsen DA *et al.* (1995) Low brain serotonin turnover rate (low CSF 5-HIAA) and impulsive violence. *J Psychiatry Neurosci* **20**, 271.
66. Glick AR (2015) The role of serotonin in impulsive aggression, suicide, and homicide in adolescents and adults: a literature review. *Int J Adolesc Med Health* **27**, 143–150.
67. Perry LM, Goldstein-Piekarski AN & Williams LM (2017) Sex differences modulating serotonergic polymorphisms implicated in the mechanistic pathways of risk for depression and related disorders. *J Neurosci Res* **95**, 737–762.
68. Duke AA, Begue L, Bell R *et al.* (2013) Revisiting the serotonin-aggression relation in humans: a meta-analysis. *Psychol Bull* **139**, 1148–1172.
69. Antypa N, Serretti A & Rujescu D (2013) Serotonergic genes and suicide: a systematic review. *Eur Neuropsychopharmacol* **23**, 1125–1142.
70. Laas K, Kiive E, Maestu J *et al.* (2017) Nice guys: homozygocity for the TPH2 −703G/T (rs4570625) minor allele promotes low aggressiveness and low anxiety. *J Affect Disord* **215**, 230–236.
71. Schmitt JA, Wingen M, Ramaekers JG *et al.* (2006) Serotonin and human cognitive performance. *Curr Pharm Des* **12**, 2473–2486.
72. Markus R, Panhuysen G, Tuiten A *et al.* (2000) Effects of food on cortisol and mood in vulnerable subjects under controllable and uncontrollable stress. *Physiol Behav* **70**, 333.
73. Markus CR (2007) Effects of carbohydrates on brain tryptophan availability and stress performance. *Biol Psychol* **76**, 83.
74. Markus CR, Panhuysen G, Tuiten A *et al.* (1998) Does carbohydrate-rich, protein-poor food prevent a deterioration of mood and cognitive performance of stress-prone

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

subjects when subjected to a stressful task? *Appetite* **31**, 49–65.
75. Markus CR, Panhuysen G, Jonkman LM *et al.* (1999) Carbohydrate intake improves cognitive performance of stress-prone individuals under controllable laboratory stress. *Br J Nutr* **82**, 457–467.
76. Gibson EL & Green MW (2002) Nutritional influences on cognitive function: mechanisms of susceptibility. *Nutr Res Rev* **15**, 169–206.
77. Hoyland A, Lawton CL, Dye L *et al.* (2008) Acute effects of macronutrient manipulations on cognitive test performance in healthy young adults: a systematic research review. *Neurosci Biobehav Rev* **32**, 72–85.
78. Lieberman HR, Agarwal S & Fulgoni VL III (2016) Tryptophan Intake in the US Adult Population Is Not Related to Liver or Kidney Function but Is Associated with Depression and Sleep Outcomes. *J Nutr* **146**, 2609S–2615S.
79. Thomson J, Rankin H, Ashcroft GW *et al.* (1982) The treatment of depression in general practice: a comparison of L-tryptophan, amitriptyline, and a combination of L-tryptophan and amitriptyline with placebo. *Psychol Med* **12**, 741–751.
80. Shaw K, Turner J & Del Mar C (2002) Tryptophan and 5-hydroxytryptophan for depression. *Cochrane Database Syst Rev*, 1, CD003198, 1–17.
81. Eccleston D, Ashcroft GW, Crawford TB *et al.* (1970) Effect of tryptophan administration on 5HIAA in cerebrospinal fluid in man. *J Neurol Neurosurg Psychiatry* **33**, 269–272.
82. Young SN & Gauthier S (1981) Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. *J Neurol Neurosurg Psychiatry* **44**, 323–328.
83. Porter RJ, Gallagher P, Watson S *et al.* (2003) Elevated prolactin responses to L-tryptophan infusion in medication-free depressed patients. *Psychopharmacol* **169**, 77–83.
84. Meyer-Gerspach AC, Hafliger S, Meili J *et al.* (2016) Effect of L-tryptophan and L-leucine on gut hormone secretion, appetite feelings and gastric emptying rates in lean and non-diabetic obese participants: a randomized, double-blind, parallel-group trial. *PLoS ONE* **11**, e0166758.
85. Badawy AA (2013) Tryptophan: the key to boosting brain serotonin synthesis in depressive illness. *J Psychopharmacol* **27**, 878–893.
86. Hogenelst K, Schoevers RA & Aan Het Rot M (2015) The effects of tryptophan on everyday interpersonal encounters and social cognitions in individuals with a family history of depression. *Int J Neuropsychopharmacol* **18**.
87. Markus CR, Olivier B & de Haan EHF (2002) Whey protein rich in alpha-lactalbumin increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects. *Am J Clin Nutr* **75**, 1051–1056.
88. Markus CR, Olivier B, Panhuysen GEM *et al.* (2000) The bovine protein alpha-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. *Am J Clin Nutr* **71**, 1536–1544.
89. Schmitt JA, Jorissen BL, Dye L *et al.* (2005) Memory function in women with premenstrual complaints and the effect of serotonergic stimulation by acute administration of an alpha-lactalbumin protein. *J Psychopharmacol* **19**, 375–384.
90. Booij L, Merens W, Markus CR *et al.* (2006) Diet rich in alpha-lactalbumin improves memory in unmedicated recovered depressed patients and matched controls. *J Psychopharmacol* **20**, 526–535.
91. Attenburrow MJ, Williams C, Odontiadis J *et al.* (2003) Acute administration of nutritionally sourced tryptophan increases fear recognition. *Psychopharmacology* **169**, 104–107.
92. Scrutton H, Carbonnier A, Cowen PJ *et al.* (2007) Effects of alpha-lactalbumin on emotional processing in healthy women. *J Psychopharmacol* **21**, 519–524.
93. Markus CR, Firk C, Gerhardt C *et al.* (2008) Effect of different tryptophan sources on amino acids availability to the brain and mood in healthy volunteers. *Psychopharmacology* **201**, 107–114.
94. Mitchell ES, Slettenaar M, Quadt F *et al.* (2011) Effect of hydrolysed egg protein on brain tryptophan availability. *Br J Nutr* **105**, 611–617.
95. Markus CR, Verschoor E, Firk C *et al.* (2010) Effect of tryptophan-rich egg protein hydrolysate on brain tryptophan availability, stress and performance. *Clin Nutr* **29**, 610–616.
96. Kroes MC, van Wingen GA, Wittwer J *et al.* (2014) Food can lift mood by affecting mood-regulating neurocircuits via a serotonergic mechanism. *Neuroimage* **84**, 825–832.
97. Gibson EL, Vargas K, Hogan E *et al.* (2014) Effects of acute treatment with a tryptophan-rich protein hydrolysate on plasma amino acids, mood and emotional functioning in older women. *Psychopharmacology* **231**, 4595–4610.
98. Mohajeri MH, Wittwer J, Vargas K *et al.* (2015) Chronic treatment with a tryptophan-rich protein hydrolysate improves emotional processing, mental energy levels and reaction time in middle-aged women. *Br J Nutr* **113**, 350–365.
99. Oxenkrug GF (2010) Tryptophan-kynurenine metabolism as a common mediator of genetic and environmental impacts in major depressive disorder: the serotonin hypothesis revisited 40 years later. *Israel J Psychiatry Rel Sci* **47**, 56–63.
100. Qiu HM, Yang JX, Jiang XH *et al.* (2014) Upregulating serotonin transporter expression and downregulating monoamine oxidase-A and indoleamine 2, 3-dioxygenase expression involved in the antidepressant effect of sodium valproate in a rat model. *Neuroreport* **25**, 1338–1343.
101. Eisner P, Klasen M, Wolf D *et al.* (2017) Cortico-limbic connectivity in MAOA-L carriers is vulnerable to acute tryptophan depletion. *Hum Brain Mapp* **38**, 1622–1635.
102. Popova NK & Kulikov AV (2010) Targeting tryptophan hydroxylase 2 in affective disorder. *Expert Opin Ther Targets* **14**, 1259–1271.
103. Shinozaki G (2012) The integrated model of serotonin transporter gene variation (5HTTLPR) and the glial cell transporter in stress vulnerability and depression. *Med Hypotheses* **78**, 410–414.
104. Pergamin-Hight L, Bakermans-Kranenburg MJ, van Ijzendoorn MH *et al.* (2012) Variations in the Promoter Region of the Serotonin Transporter Gene and Biased Attention for Emotional Information: a meta-analysis. *Biol Psychiatry* **71**, 373–379.
105. Nishizawa S, Benkelfat C, Young SN *et al.* (1997) Differences between males and females in rates of serotonin synthesis in human brain. *Proc Natl Acad Sci U S A* **94**, 5308–5313.
106. Booij L, Van der Does W, Benkelfat C *et al.* (2002) Predictors of mood response to acute tryptophan depletion. A reanalysis. *Neuropsychopharmacology* **27**, 852–861.
107. Gressier F, Calati R & Serretti A (2016) 5-HTTLPR and gender differences in affective disorders: a systematic review. *J Affect Disord* **190**, 193–207.
108. Brummett BH, Muller CL, Collins AL *et al.* (2008) 5-HTTLPR and gender moderate changes in negative affect

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press

responses to tryptophan infusion. *Behav Genet* **38**, 476–483.
109. Kurrikoff T, Hiio K, Täht K *et al.* (2013) The 5-HTTLPR genotype and depressiveness link: contribution of aspects of environment and gender. *Psychiatry Res* **209**, 126–127.
110. McGuffin P, Alsabban S & Uher R (2011) The truth about genetic variation in the serotonin transporter gene and response to stress and medication. *Br J Psychiatry* **198**, 424–427.
111. Sjoberg RL, Nilsson KW, Nordquist N *et al.* (2006) Development of depression: sex and the interaction between environment and a promoter polymorphism of the serotonin transporter gene. *Int J Neuropsychopharmacol* **9**, 443–449.
112. Markus CR & Firk C (2009) Differential effects of tri-allelic 5-HTTLPR polymorphisms in healthy subjects on mood and stress performance after tryptophan challenge. *Neuropsychopharmacology* **34**, 2667–2674.
113. Markus CR & De Raedt R (2011) Differential effects of 5-HTTLPR genotypes on inhibition of negative emotional information following acute stress exposure and tryptophan challenge. *Neuropsychopharmacology* **36**, 819–826.
114. Markus CR, Verschoor E & Smeets T (2012) Differential effect of the 5-HTT gene-linked polymorphic region on emotional eating during stress exposure following tryptophan challenge. *J Nutr Biochem* **23**, 410–416.
115. Stunkard AJ & Messick S (1985) The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res* **29**, 71–83.
116. Kirschbaum C, Pirke KM & Hellhammer DH (1993) The 'Trier Social Stress Test' – a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* **28**, 76–81.
117. Vignau J, Soichot M, Imbenotte M *et al.* (2010) Impact of tryptophan metabolism on the vulnerability to alcohol-related blackouts and violent impulsive behaviours. *Alcohol Alcohol* **45**, 79–88.
118. Chen GL & Miller GM (2012) Advances in tryptophan hydroxylase-2 gene expression regulation: new insights into serotonin-stress interaction and clinical implications. *Am J Med Genet B Neuropsychiatr Genet* **159B**, 152–171.
119. Gibson EL (2012) The psychobiology of comfort eating: implications for neuropharmacological interventions. *Behav Pharmacol* **23**, 442–460.
120. Oliver G, Wardle J & Gibson EL (2000) Stress and food choice: a laboratory study. *Psychosom Med* **62**, 853–865.
121. Markus CR, Jonkman LM, Capello A *et al.* (2015) Sucrose preload reduces snacking after mild mental stress in healthy participants as a function of 5-hydroxytryptamine transporter gene promoter polymorphism. *Stress* **18**, 149–159.
122. Capello AEM & Markus CR (2014) Differential influence of the 5-HTTLPR genotype, neuroticism and real-life acute stress exposure on appetite and energy intake. *Appetite* **77**, 83–93.
123. Capello AE & Markus CR (2014) Effect of sub chronic tryptophan supplementation on stress-induced cortisol and appetite in subjects differing in 5-HTTLPR genotype and trait neuroticism. *Psychoneuroendocrinology* **45**, 96–107.
124. Verschoor E, Finlayson G, Blundell J *et al.* (2010) Effects of an acute alpha-lactalbumin manipulation on mood and food hedonics in high- and low-trait anxiety individuals. *Br J Nutr* **104**, 595–602.
125. Finlayson G, King N & Blundell J (2008) The role of implicit wanting in relation to explicit liking and wanting for food: implications for appetite control. *Appetite* **50**, 120–127.
126. Cerit H, Jans LA & Van der Does W (2013) The effect of tryptophan on the cortisol response to social stress is modulated by the 5-HTTLPR genotype. *Psychoneuroendocrinology* **38**, 201–208.
127. van Dalfsen JH & Markus CR (2015) Interaction between 5-HTTLPR genotype and cognitive stress vulnerability on sleep quality: effects of sub-chronic tryptophan administration. *Int J Neuropsychopharmacol* **18**, pyu057.
128. Gibson EL, Mohajeri MH, Wittwer J *et al.* (2016) Serotonin transporter genotype predicts changes in tryptophan sensitive moods in women. *Appetite* **101**, 222.
129. Outhred T, Das P, Dobson-Stone C *et al.* (2016) Impact of 5-HTTLPR on SSRI serotonin transporter blockade during emotion regulation: a preliminary fMRI study. *J Affect Disord* **196**, 11–19.

https://doi.org/10.1017/S0029665117004451 Published online by Cambridge University Press