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7 8 9	Tryptophan supplementation and serotonin function: genetic variations in behavioural effects
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30 Abstract

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The neurotransmitter serotonin has a role in affective disorders such as depression and anxiety, as 32 well as sleep, cognitive function and appetite. This review examines the evidence that serotonin-33 related genotypes may moderate the behavioural effects of supplementation with the serotonin 34 precursor amino acid tryptophan (TRP), on which synthesis of serotonin (or 5-hydroxytryptamine; 35 5-HT) depends. However, 95% of serotonin is synthesised and used in the periphery, and TRP is 36 also metabolised via non-5-HT routes such as the kynurenine pathway. Moreover, understanding of 37 38 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 39 40 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 41 42 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: 43 44 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-45 induced mood deterioration. In men, the L/L' genotype may confer risk of stress-induced increases 46 in negative affect; there are insufficient data to assess effects in male S/S' genotypes. However, 47 better powered studies to detect sex by genotype by stress by TRP interactions, as well as 48 consideration of more genotypes, are needed before strong conclusions and recommendations for 49 50 behavioural effects of TRP treatment can be reached.

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Dietary tryptophan and the pathways to serotonin function

Serotonin, or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter in the central 55 56 nervous systems (CNS) of the majority of animals including human beings. Its synthesis depends on supply of the essential amino acid, l-tryptophan (TRP), which cannot be biosynthesised by 57 58 human beings and so must be obtained from dietary sources. Moreover, serotonin synthesis rate depends on availability of the precursor TRP. The scope of this review is to consider recent 59 findings from research involving effects of supplementing TRP supply on behaviour and their 60 interaction with genetic susceptibility, including indirect evidence that TRP supplementation likely 61 62 alters affective states via effects on central serotonin function.

An important consideration for understanding effects of TRP administration is that only 63 about 5% of endogenous serotonin is found in the brain; the remainder is in the gut (about 90%), 64 65 principally released by enterochromaffin cells, and in peripheral tissue or in the blood, where it is taken up into blood platelets ^(1; 2; 3). Indeed, the name serotonin derives from its discovery in blood 66 70 years ago and the observation that it caused contraction of vascular smooth muscle ⁽⁴⁾; thus, one 67 function of serotonin is to regulate local blood flow. This imbalanced distribution between brain 68 and periphery needs to be borne in mind when considering the possible impact of dietary 69 manipulation of central serotonin by TRP, and the potential influence of alternative metabolic 70 71 pathways as well as probable moderating effects on these metabolic routes. These issues are considered further below; nevertheless, serotonin is a widely distributed and important CNS 72 73 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⁾. 74 75 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 76 goal of research. 77

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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 B₃). Indeed, it has

been estimated that only 1% of dietary TRP is used for brain 5-HT synthesis ⁽¹⁰⁾. TRP use for 86 synthesis of niacin is via the oxidative kynurenine pathway, which has also been termed the 87 'tryptophan catabolite' pathway (TRYCAT)⁽¹¹⁾. This pathway is becoming increasingly recognised 88 as having important implications for health, including neuropsychiatric conditions such as 89 depression ^(11; 12). A further route for TRP metabolism is via degradation by gut microbiota, which 90 can lead to production of both positive and detrimental active metabolites, including quinolinic acid 91 92 ⁽¹⁾; therefore, individual variation in the gut microbiome may have implications for TRP metabolism and thus brain health and psychological wellbeing ⁽¹³⁾. 93

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 (IDO) 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 synthesis of 5-HT is catalysed by the rate-limiting enzyme, 101 tryptophan hydroxylase, which converts TRP into 5-hydroxytryptophan (5-HTP). In turn, 5-HTP is 102 decarboxylated to 5-HT by the enzyme aromatic amino acid decarboxylase. The key observation 103 for this pathway is that TRP hydroxylase (TPH) is not fully saturated by its substrate TRP under 104 normal conditions, so that raising brain TRP levels could increase serotonin synthesis. However, 105 brain TRP levels are buffered from plasma TRP by the blood brain barrier (BBB): to be transported 106 107 into the brain, TRP has to compete for uptake across the BBB against other amino acids, in 108 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 109 110 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 111 uptake of TRP into the brain ⁽⁷⁾. Some 90% of TRP in blood is typically bound to the blood protein 112 albumin, and it is often assumed that the remaining free unbound fraction of TRP should be taken to 113 be the best predictor of TRP entry across the BBB. However, it has been shown that TRP binding 114 to albumin is very labile, such that TRP can easily be released in cerebral circulation. Furthermore, 115 TRP can be displaced from or prevented from binding to albumin by free fatty acids (FFA), which 116 also bind readily to albumin ^(7; 9). Therefore, factors that alter FFA levels in blood will affect 117 118 availability of free TRP for entry into the brain: for example, sympathetic activation by stress or

119 exercise will induce lipolysis, increase plasma FFA and so release more TRP from albumin. This acute stress-induced increase in availability of TRP for serotonin synthesis might contribute to the 120 observation that even mild stress can increase 5-HT release in rat brain ⁽¹⁵⁾. It also suggests caution 121 is required in interpreting correlations between single measures of plasma free TRP and personality 122 traits such as anxiety or aggression, as these may interact with the experimental procedure and 123 perceived stressful nature of the study to modify TRP levels. In contrast, food or drink that 124 125 stimulates insulin release, and so promotes uptake of FFA into tissue, will tend to reduce availability of free plasma TRP, but at the same time will remove competing LNAA from plasma 126 into tissue ⁽⁷⁾. Thus, measuring both free and total TRP may ensure better prediction of TRP entry 127 into brain and its behavioural associations ^(9; 16). 128

However, 95% of 5-HT is synthesised and used in the gut, blood and peripheral tissue ^(1; 14). 129 Although the synthesis of 5-HT from TRP follows a similar biochemical path in brain and 130 periphery, the form of the enzyme TPH by and large differs slightly between these regions; these 131 isoforms are known as TPH1 and TPH2 respectively, indicating the order of characterisation ^(17; 18). 132 To be precise, in the brain the principal isoform, TPH2, shown to depend on expression of a 133 different gene form from TPH1⁽¹⁸⁾, was found to be highly expressed by measuring mRNA specific 134 to the brainstem raphe nuclei, where brain serotonin is primarily synthesised, whereas TPH1 was 135 found to be responsible for 5-HT synthesis, and ultimately melatonin, in the pineal gland ⁽¹⁹⁾ and gut 136 ⁽¹⁸⁾. However, this classification is oversimplified, as TPH1 mRNA has also been shown to be more 137 highly expressed in the amygdala and hypothalamus than TPH2 ⁽²⁰⁾, although its precise role in 138 those sites is uncertain. 139

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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 inhibitors
(which prevent serotonin, and other monoamine, metabolism by the enzyme monoamine oxidase;
MAO), as well as development of tricyclic antidepressants (which inhibit synaptic reuptake of

151 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 152 function ^(24; 25). The theory expanded to consider a role for CNS 5-HT in associated clinical 153 affective disorders as well as regulation of mood in healthy people ⁽²⁵⁾. However, this 154 pharmacotherapeutic evidence was non-specific to serotonin, and ironically, notwithstanding the 155 risk of oversimplifying neural bases to complex disorders, the best evidence for a major role for 156 CNS 5-HT in control of affect has come from studies that manipulate TRP entry to the brain ⁽²⁶⁾. 157 Furthermore, whilst recent studies combining neuroimaging with administration of selective 158 159 serotonin reuptake inhibitors (SSRI) 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 160 161 for TRP, including roles in inflammatory processes and melatonin synthesis, underlying major depression, seasonal affective disorder and bipolar disorder ^(1; 11; 12; 28). 162

163 Central serotonin is known to be involved in cognitive function, especially memory, 164 attention, decision making and information processing, as well as in the processing of emotionally 165 relevant stimuli ^(26; 29; 30). However, cognition and emotion, or affect, are not entirely separable, and 166 are often strongly interdependent ^(31; 32; 33). Emotions, via their neural substrates, influence memory 167 and attention for example, and depression and anxiety are associated with cognitive impairments 168 and biases that can contribute to the affective disorder and its maintenance ^(32; 34).

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Effects of acute tryptophan depletion

This review is mainly concerned with genetic susceptibility to effects of forms of TRP 171 administration that may lead to increased serotonin synthesis in the brain; however, by way of 172 comparison, and given the scientific influence, a brief overview is included of findings, and their 173 174 implications, on deficits in central 5-HT induced by acute TRP depletion (ATD) methods (Young 2013; Young et al. 1985). ATD is usually induced by ingestion of amino acid loads devoid of the 175 176 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 177 TRP/LNAA ratio (>80%) that may last 4-6 hours ^(30; 35); similar effects have been found with a 178 more palatable low-TRP collagen protein mixture ⁽³⁶⁾, and more recently a gelatin-derived TRP-free 179 protein/carbohydrate mixture has been used ⁽⁹⁾. Moreover, the serotonin metabolite, 5-180 hydroxyindole acetic acid (5-HIAA) measured in cerebrospinal fluid (CSF) declined by about one-181 third at 12 hours, after which measurements stopped ⁽³⁷⁾. ATD methods have provided the most 182 consistent evidence for serotonergic involvement in cognition, including impairment of memory 183

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; 43; 44).

In support of a key role for serotonin in affective disorders, ATD also alters emotional 187 processing and regulation ^(45; 46; 47). Reducing TRP access to the brain by ATD tends to mimic the 188 cognitive biases seen in depressed populations, such as impaired memory for, attention to, or 189 recognition of positive vs. negative information including facial expressions ^(34; 48; 49). However, 190 positive effects of ATD on cognition, for example on decision making and focused attention have 191 also been reported ^(50; 51; 52), albeit interacting with history of depression ⁽⁵³⁾. One explanation has 192 been that serotonin may affect "hot" cognitive tasks that include an affective component, but not 193 "cold" cognitive tasks that do not obviously involve emotional stimuli ⁽⁴⁹⁾. 194

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-200 fenfluramine ⁽⁵⁷⁾, and reductions in food intake established for high doses of TRP ⁽⁵⁸⁾, and thus an 201 expectation that ATD might increase appetite, the few studies addressing this directly in human 202 beings suggest little effect of ATD on appetite despite concurrent mood effects ^(59; 60; 61). Two 203 studies comparing ATD in women with Bulimia Nervosa vs. healthy controls found conflicting 204 results ^(60; 62): though both studies found increased negative affect in bulimic women, only one 205 reported increased energy intake in these women ⁽⁶²⁾, although the other did find an increased desire 206 to binge eat ⁽⁶⁰⁾. However, curiously, another study reported a concurrent increase in both nausea 207 and hunger in healthy women ⁽⁶³⁾. These findings also need to be considered in the context of 208 opposing relationships between depression and appetite across patients ⁽⁶⁴⁾. 209

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 below), 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⁾.

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Effects of TRP administration and supplementation

In contrast to ATD, which is a research tool to investigate serotoninergic processes in human 224 beings, and for which most effects are not beneficial, administration of TRP (and its first-stage 225 226 metabolite, 5-HTP) 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 227 synthesis and release ^(26; 33). The methods can vary from intravenous administration of TRP to oral 228 supplementation of TRP, or use of TRP-rich proteins or peptide preparations, either acutely or 229 chronically ^(26; 29; 71). It is also possible to increase the TRP/LNAA ratio, and so enhance TRP entry 230 across the BBB, by feeding a carbohydrate-rich, very low-protein meal, since the rise in insulin 231 removes more LNAA into surrounding tissue. This dietary method has been shown to benefit 232 cognitive and emotional function, and reduce the cortisol response to stress, in more stress-prone, 233 neurotic participants ^(72; 73; 74; 75). This mechanism has also been suggested to underlie dietary effects 234 on mood and performance, such as calming after high-carbohydrate meals vs. arousal after protein-235 rich meals (76; 77). Recently, using data from the US National Health and Nutrition Examination 236 Survey for nearly 30,000 adults, dietary intake of TRP was found to be inversely associated with self-237 238 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 239 psychological benefits. 240

241 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 5-242 243 HTP) 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 244 in alleviating depression, at least in mild to moderately depressed people. Moreover, for more than 245 a decade prior to that review, the US Food and Drug Administration had banned over-the-counter 246 247 sales of TRP following an outbreak in 1989 of harmful eosinophilia-myalgia syndrome in users of TRP supplements. The cause was eventually traced to impurities in TRP supplements from one 248

Japanese manufacturer, after which the ban was lifted in 2001 ^(10; 26). Thus, for at least 5 decades, 249 TRP has been used pharmacologically, i.e. at daily doses sometimes well in excess of 10 times the 250 RDA (5 mg/kg) for this essential amino acid. There was early evidence for probable enhancement of 251 brain 5-HT function: after 50 mg/kg TRP (3.5 g per 70 kg subject) was consumed in a milk drink, 252 253 plasma TRP increased 8-fold, TRP in cerebrospinal fluid (CSF) increased 6-fold after 6-8 hours, and the metabolite 5-HIAA increased almost two-fold in CSF by 8 hours, suggesting increased turnover 254 of brain 5-HT⁽⁸¹⁾. This two-fold increase in 5-HT turnover was replicated in a later study of CSF 5-255 HIAA changes, using 3 g and 6 g TRP, with no further increase at the higher dose, although the level 256 was sustained for longer, i.e. 12 hours vs. 8 hours ⁽⁸²⁾. 257

In a review of potential side effects, Fernstrom ⁽²⁶⁾ concluded that such use of TRP appears 258 to be largely safe from adverse events, although the evidence is limited and not systematic. There 259 are some reports of symptoms such as nausea, tremor or dizziness when high doses are used (although 260 these are also common symptoms reported in placebo-treated subjects). However, the greatest risk 261 of side-effects occurs when TRP is combined with other drugs that enhance 5-HT availability, such 262 as antidepressant serotonin selective reuptake inhibitors (SSRI) or MAO inhibitors (MAOI): then a 263 toxic 'serotonin syndrome' may occur that can include hyperthermia and coma ⁽²⁶⁾. A more common 264 effect of high doses of TRP is fatigue or drowsiness, which has led to TRP being used to aid sleep, in 265 which case sedation is not an unwanted side-effect ⁽²⁶⁾. However, a complication of oral TRP at 266 higher doses is that it increases release of several hormones including growth hormone, cortisol and 267 prolactin⁽⁸³⁾ (the latter thought to indicate increased central serotonin - and dopamine - activity). A 268 recent study also reported that intragastric administration of 1.56 g TRP increased plasma 269 cholecystokinin and glucagon-like peptide 1 (GLP-1), as well as slowing gastric emptying ⁽⁸⁴⁾: 270 271 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 272 273 due to TRP-induced drowsiness.

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⁾. 281 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 282 been reviewed ^(22; 33). There is some evidence that TRP can reduce aggression in schizophrenic 283 patients ⁽³³⁾, and reduce quarrelsomeness while increasing agreeableness in healthy participants with 284 a tendency to irritability or aggression ⁽²²⁾. Thus, it has been proposed that serotonin may influence 285 a basic drive to be social, and that modulation of serotonin can alter more complex social behaviours 286 by affecting social behaviour along an agreeable-quarrelsome axis ⁽³³⁾. For example, there is evidence 287 that TRP supplementation can promote prosocial behaviour in economic decision-making tasks ⁽²²⁾. 288 289 Somewhat counterintuitively, a more recent study, in which 1 g TRP was given 3 times per day for 14 days to those with a family history of depression, found increased quarrelsomeness and reduced 290 agreeableness (at home), but improved mood, compared to placebo ⁽⁸⁶⁾. This was interpreted as 291 292 possibly reflecting development of more control in social interactions at home.

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Effects of TRP-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 to 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). Alpha-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 perception of emotional faces with in 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
 LNAAs (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 an fMRI study in young women 314 315 ⁽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 316 reward anticipation, activation of reward pathways was reduced. Effects on resting state 317 connectivity were in line with modulation of brain regions involved in regulation of mood. 318 Subsequently, lower doses were found to be effective in enhancing mood and positivity in 319 emotional processing acutely (0.13 g TRP) $^{(97)}$, and chronically (0.07 g TRP for 19 days) in 320 improving aspects of mood and sleep, as well as modest benefits to cognition, in middle-aged 321 women, relative to a casein control treatment ⁽⁹⁸⁾. 322

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

327 Gene polymorphisms involved in metabolism of TRP and regulation of serotonin could have a substantial influence on behavioural effects of manipulations of TRP availability. There is potential 328 329 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, IDO, and also by polymorphisms 330 331 of the monoamine oxidase A (MAO-A) enzyme that metabolises central serotonin (Figure 1). These various 5-HT-related polymorphisms may form an interactive system that determines the 332 aetiology and prognosis of various forms of affective disorder (17; 99; 100; 101; 102). However, the most 333 evidenced serotonergic genetic influence on behaviour is the 5-hydroxytryptamine transporter-334 linked promoter region (5-HTTLPR) polymorphism of the serotonin transporter gene (SLC6A4) 335 ^(103; 104). The recommended classification of 5-HTTLPR genotypes is a functional combination of 336 variable number tandem repeats (VNTR) of short or long length of the gene promoter amplicon and 337 single nucleotide polymorphism (SNP) variants, L_A and L_G, where L_G is functionally equivalent to 338 the short, and L_A to the long, VNTR forms ^(103; 104). Effects of 5-HTTLPR genotypes are usually 339 compared between the homozygous alleles that are either high (long variants; L/L') or low (short 340 variants but including L_A; S/S') expressing of mRNA for the 5-HT transporter receptor. 341

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 SSRI 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; 109; 110; 111). Therefore,
the sex of participants needs to be considered when interpreting findings in this area.

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TRP administration and 5-HTTLPR genotypes

355 Only a few studies have investigated whether these 5-HT- and TRP-related genotypes alter 356 the effects of TRP loading (or challenge or supplementation), and these appear to be limited to comparison of 5-HTTLPR genotypes: these studies are summarised in Table 1. In the earliest 357 published study ⁽¹⁰⁸⁾ to examine moderation of TRP loading by the 5-HTTLPR tri-allelic genotype, 358 41 men and 31 women were infused intravenously with a high dose of TRP (100 mg/kg), while 359 aspects of mood were assessed (Profile of Mood States; POMS). Far from improving mood, this 360 361 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, 362 363 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 364 social stressors on negative affect in adolescents ⁽¹¹¹⁾. However, sample sizes were small, especially 365 in the S/S' groups (7 men; 9 women). 366

Using a far lower dose, and oral administration, Markus and Firk⁽¹¹²⁾ examined potential 367 interactions between acute TRP supplementation, stress and 5-HTTLPR genotype on mood, cortisol 368 and cognition. They hypothesised that the TRP challenge would ameliorate the effects of stress on 369 mood and cortisol in subjects homozygous for the tri-allelic S/S' genotype compared to those with 370 the L/L' genotype. In a cross-over design, 30 student participants (16 S/S'-allele; 14 L/L'-allele; 371 372 only one man in each group) received either TRP (2 x 0.4 g) or placebo (lactose), prior to a stressful challenge, with baseline and post-stress measures of mood (POMS) and salivary cortisol. The 373 374 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 375 376 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 377 378 the observed decline in cortisol after stress is difficult, as this decline is anyway typical for cortisol

during the morning. However, neither TRP treatment nor genotype significantly altered this 379 decline in cortisol. Nevertheless, the stressor caused mood to deteriorate, with increases in feelings 380 of anger, depression and fatigue, but a decrease in vigour. A key finding of this study is that the 381 TRP treatment reduced depression and fatigue, while increasing vigour, specifically in the S/S' 382 allele group only. However, these effects were pooled across stress condition, so presumably were 383 not significantly altered by stress (data for a pre/post-stress x genotype x treatment interaction were 384 not presented). Genotype also influenced performance on the subtraction task: the S/S' group 385 performed worse that the L/L' group after placebo, but after TRP, performance was the same for 386 387 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 388 389 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 390 was removed by TRP treatment strongly suggests it reflected suboptimal 5-HT function during a 391 demanding task. It is also important to note that this sample consisted of 28 women and only 2 392 393 men.

A subsequent report from this group ⁽¹¹³⁾ used the same stressor and TRP treatment to 394 examine interactions of treatment, stress and 5-HTTLPR genotype on another measure of mood 395 396 (Positive and Negative Affect Schedule; PANAS) and attentional bias (inhibitory responses) to negative emotional stimuli. This bias was measured by reaction times to facial expressions varying 397 398 in emotional valence and primed by previous stimuli of the same or opposite valence (Negative Affective Priming; NAP). This study appears to have used the same participants as Markus and 399 Firk ⁽¹¹²⁾ except excluding the two men (i.e. 28 women). In the placebo condition, negative affect 400 increased after stress only for the S/S' genotype group, and furthermore this rise in negative mood 401 was prevented by TRP treatment. For the NAP task, there was an interaction between stress and 402 403 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 404 stimuli. The L/L' group showed the opposite response, suggesting that this allele may confer some 405 resilience to effects of stress on emotional processing. However, no effects of TRP treatment were 406 found for this behaviour, though, as the authors point out, the study has a relatively small sample 407 size and may be underpowered to detect three-way interactions of this sort. 408

Subsequently, Markus, Verschoor and Smeets ⁽¹¹⁴⁾ established a larger student cohort
screened for 5-HTTLPR genotype, and studied 19 female S/S' and 23 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, TFEQ⁽¹¹⁵⁾). This study investigated potential interactions 412 between TRP treatment, 5-HTTLPR genotype, stress, restraint and emotional eating, in a double-413 blind placebo-controlled crossover design. Stress was elicited using a modified Trier Social Stress 414 Test ⁽¹¹⁶⁾; TRP challenge was accomplished using an egg white protein hydrolysate enriched with 415 TRP (4-g dose given as a 200-ml drink, containing 0.24 g TRP; DSM, Delft; see above), versus a 416 casein hydrolysate placebo (0.03 g TRP). Blood samples were taken for amino acid analysis 90 417 minutes after consuming the drinks, and four salivary samples were taken during the study to assess 418 cortisol levels. Interestingly, there was a significantly greater increase in plasma TRP/LNAA 419 420 following TRP treatment for the L/L' group (70% increase) compared to the S/S' group (30% increase). However, although stress resulted in a rise in cortisol, there were no significant effects of 421 either TRP treatment, genotype or restrained eating on cortisol in this study. Mood generally 422 deteriorated from before to after the stress; of particular interest, the increase in anger after stress 423 occurred in all groups except the L/L' genotype group who had received TRP supplementation, in 424 whom there was no change in anger following stress. 425

Liking (pleasantness of taste) for a variety of foods of different sensory categories (sweet or 426 savoury, low- or high-fat) was assessed using ratings of images of the foods. Only the high-fat 427 sweet food liking ratings showed significant effects: in the L/L' allele group, liking for high-fat 428 429 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 430 431 offering several of 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 432 across stress pre/post measures); no effects of genotype, stress or restrained eating were seen. An 433 overall appetite-suppressant effect of TRP may be expected, given that ATD tends to increase 434 appetite ⁽⁶³⁾, and higher doses of TRP (at least 2 g) have long been known to suppress appetite and 435 reduce food intake by 10-20% ⁽⁵⁸⁾; nevertheless, the dose of TRP effective here is considerably 436 smaller (0.24 g), so the size of this effect is remarkable. 437

There are several intriguing findings in this study, not least the weaker increase in plasma TRP/LNAA 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 IDO 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 study that measured 5-HTTLPR genotypes and administered 50 mg/kg TRP did 445 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⁾. 446 Those patients who experienced "blacked-out violent impulsive behaviour" during binge drinking 447 showed a higher kynurenine: TRP ratio than those who did not, suggesting that less TRP would be 448 available to the brain. Nevertheless, no differences were reported for 5-HTTLPR genotype 449 subgroups, although sample sizes may have been two small (9 cases, 9 alcohol-dependent controls, 450 received oral TRP) for meaningful statistics in this pilot study, and polymorphisms in the enzymes 451 themselves were not measured. This may be important as there is evidence for example that the 452 TPH1 218AA polymorphism is a risk factor for alcoholism and bipolar disorder ⁽¹¹⁸⁾. Anyhow, this 453 impaired effect of TRP treatment on the plasma ratio in this S/S' group (114) may explain the lack of 454 behavioural effects seen for this group in this study, in contrast to some effects that were specific to 455 the L/L' genotype. On the other hand, the most likely explanation for a lack of stress-induced, or 456 emotional, eating is the probability that few of the participants had emotional eating tendencies. 457 Participants were selected on the basis of scores on the TFEQ restrained eating scale, which, unlike 458 some items on the disinhibition or hunger scales of this questionnaire, does not explicitly assess 459 emotional eating and is usually orthogonal to it. We have argued previously that cognitive restraint 460 per se is not a good predictor of stress eating tendencies (119; 120). Furthermore, in a more recent 461 462 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 463 preload ⁽¹²¹⁾. However, in that study, there was no manipulation by TRP load. Another study from 464 this group investigated whether examination stress would differentially affect appetite for these two 465 genotype groups ⁽¹²²⁾: findings confirmed that the S/S' genotype group were more likely to show 466 stress-induced eating of sweet snacks, though again there was no manipulation of TRP. 467

Nevertheless, the interaction between genotype, stress, emotional eating and effects of 468 469 subchronic TRP supplementation was investigated in mainly female participants (99 women, 19 men) asked to self-administer 3 g TRP per day for 7 days (or placebo cellulose), before undergoing 470 an acute stress test (repeated cold pressor and serial-17 subtraction task known as the Maastricht 471 Acute Stress Test; MAST)⁽¹²³⁾. Changes in appetite ratings, snack intake, mood and cortisol were 472 assessed. Subchronic TRP treatment reduced the cortisol response to stress only in the S/S' allele 473 group. Similarly, the TRP treatment resulted in significantly less stress-induced increase in anxiety 474 only in the S/S' group, but independently of trait neuroticism. Stress increased rated appetite, but 475 interestingly TRP reduced this increase specifically in S/S' subjects who also scored highly on 476 neuroticism. The parallels across these TRP by genotype interactions are notable. By comparison, 477

478 the only significant finding reported for post-stress snack intake was a greater intake of sweet fatty snacks by the low neuroticism vs. the high neuroticism group, perhaps due to health concerns in the 479 latter group. The interaction of genotype with neuroticism on stress-induced change in rated 480 appetite is similar to the results of an earlier study in which mainly female participants with low or 481 high trait anxiety were subjected to stress (mental arithmetic during loud noise) and treated acutely 482 with either TRP-rich α -lactalbumin or case in ⁽¹²⁴⁾. Food liking and preference was assessed by 483 responses to food images displayed via a computer program ⁽¹²⁵⁾. While appetite ratings increased 484 for all groups after stress, both liking and preference for sweet foods increased specifically for high 485 486 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 487 impaired 5-HT function. However, in this study, genotypes were not measured. Moreover, in the 488 case of actual eating ⁽¹²²⁾, it seems that other factors influenced the behaviour, although differences 489 in timing between stress and food intake could be involved, and in this subchronic treatment design, 490 no treatment was given on the test day. 491

Another group also examined effects of a similar subchronic TRP treatment (2.8 g/day for 6 492 days) on responses to stress (TSST) in relation to 5-HTTLPR genotype ⁽¹²⁶⁾. In this study about half 493 the participants were female (22 women, 24 men), although sex was included as a covariate in 494 495 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 496 497 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 498 day): the lower cortisol response in L/L' participants was not further reduced by TRP. However, 499 while mood deteriorated after the stress, this was not differentially influenced either by treatment or 500 genotype, contrary to Capello and Markus⁽¹²³⁾. 501

Subsequently, a recent study investigated whether a similar subchronic treatment with TRP 502 (3 g/day for 7 days) could benefit quality of sleep, and whether this might depend on 5-HTTLPR 503 genotype $^{(127)}$. Thus, this study compared effects between S/S' allele subjects (46 women, 11 men) 504 and L/L' allele subjects (46 women, 8 men). Potential effects of neuroticism were investigated 505 using a median split of questionnaire scores into high and low neuroticism groups. General sleep 506 quality was assessed prior to treatment, then sleep quality after the week of treatment was measured 507 for a further week. Higher neurotic participants tended to report lower general sleep quality, 508 unrelated to genotype. However, following treatment, specifically S/S' genotype together with 509

higher neuroticism was associated with poorer sleep quality for the placebo group, but with bettersleep quality for the TRP-treated group.

Finally, there is recent evidence of differential impact of 5-HTTLPR genotypes on mood 512 changes during challenging tasks in the context of two intervention studies that had found beneficial 513 effects of acute ⁽⁹⁷⁾ and chronic ⁽⁹⁸⁾ treatment with a TRP-rich egg-white protein hydrolysate (DSM) 514 on mood, emotional processing and cognition in Caucasian women aged 45-65 years ⁽¹²⁸⁾. 515 Participants were genotyped for the tri-allelic 5-HTTLPR polymorphism, and distributions of 516 genotypes were in accordance with Hardy-Weinberg equilibrium (allele sample sizes; acute study: 517 $SS/SL_G = 11$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$, $L_AL_A = 13$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$; chronic study: $SS/SL_G = 13$, $SL_G/SL_GL_A = 36$; chronic study: $SS/SL_G = 13$; 518 10). 519

We planned to compare the two homozygous groups $(SS/L_G [designated S/S'] vs. L_A L_A$ 520 521 [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 522 examined outcomes on the pretreatment baseline day, when the participants completed the same set 523 of tests as during treatment, which allowed us to pool the outcome data for all participants within 524 each genotype group. The series of cognitive and behavioural tests lasted for 3.5 hours from the 525 baseline (pre-test) mood measure to the final post-test mood measure, with one hour of rest in 526 527 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 528 responsive to subsequent TRP supplementation treatment. Specifically, these were well-being and 529 fatigue in the acute study, and a positive feeling of 'high energy' (stimulated, buzzing, impulsive) in 530 531 the chronic study (emotions were derived by factor analyses of ratings on 28 items presented on a computer, known as the Mental and Physical Sensations Scale). For the acute study, we found that 532 well-being declined from pre- to post-test in the S/S' group, but not in the L/L' group, whereas 533 fatigue increased significantly only for the S/S' group. For the chronic study, 'high energy' mood 534 increased from pre- to post-test for the L/L' group, but did not change for the S/S' group. 535

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.

[Table 1 about here]

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Conclusions

The main theme emerging from the literature on TRP supplementation and genotypes is the 545 observations of interactions between TRP and genotypes, sex and stress on changes in mood, 546 cognition, cortisol and appetite. It is particularly important to consider the influence of a key 547 'genotype', sex. For example, in women, the 5-HTTLPR S/S' genotype predicts sensitivity to 548 549 improvements in 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, 550 but may differ in sensitivity to TRP administration. In men, if anything, the L/L' genotype confers 551 risk of stress-induced increases in negative affect; however, there are insufficient studies with 552 adequate power to detect sex x genotype x stress x TRP in the literature to draw strong conclusions. 553

Since the 5-HTTLPR genotypes may influence neurodevelopment and/or tonic 5-HT 554 adaptive responsiveness at least as much as acute functioning of the brain serotonin system (103; 129), 555 556 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. 557 However, when measuring multiple influences on behaviour, as well as sex differences, 558 investigators need to ensure sufficiently large sample sizes to increase the likelihood of reliable 559 findings ⁽¹⁰⁷⁾: routinely screening for genetic polymorphisms in suitable populations would be 560 561 helpful.

562 There is a need to broaden studies on the potential benefits of TRP supplementation to 563 include a greater range of serotonin-related genotypes, including enzymes involved in key metabolic pathways (Figure 1). This may eventually lead to clear predictions as to who is likely to 564 benefit most from this relatively simple nutrient-based treatment. Until then, although there is 565 preliminary evidence that individuals with some genotypes, particularly the 5-HTTLPR S/S' allele 566 in women, may benefit from TRP supplementation as an aid to stress coping and emotional 567 regulation including comfort eating, further research is needed before reliable recommendations can 568 be made on targeted use of TRP treatment, or adjustment of dietary TRP intake, for beneficial 569 570 behavioural outcomes.

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897 <u>Table 1</u>: Summary of studies investigating interactions between TRP supplementation or challenge and tri-allelic 5-HTTLPR genotypes on

898 behaviour

Reference	Sample	Design and intervention	Measures	Main findings	Comments
Brummett et al. (2008) ⁽¹⁰⁸⁾	Healthy adults; 31 females, 41 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 Profile of Mood States (POMS) prior to and 1 hr 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 (7 males, 9 females). No saline infusion on same day as TRP. No significant effects on fatigue, anxiety and anger.
Markus & Firk (2009) ⁽¹¹²⁾	28 female and 2 male students. Mean \pm SD age = 19 \pm 2.	Double-blind cross- over design. Overnight fast. Oral TRP (2 x 0.4 g) or lactose placebo capsules, then stressful challenge (cold pressor and Serial-7 tasks in front of 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 (2011) (113)	28 female students. Mean ±SD age = 19 ±2.	Double-blind cross- over design. Overnight fast. Oral TRP (0.8 g) vs. cellulose placebo, then stressful challenge (cold	Negative affect priming (NAP) using pictures with positive or negative valence – assesses tendency to inhibit negative emotional information. Positive	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	No stress-free condition. Despite NAP being sensitive to stress and genotype, no effect of TRP on this measure.

		pressor and Serial-7 tasks in front of camera).	and negative affect by questionnaire (Positive and Negative Affect Schedule; PANAS).	enhanced it in L/L' group. No effect of TRP on this measure.	
Markus et al. (2012) ⁽¹¹⁴⁾	42 female students (19 S/S', 23 L/L'). High or Low restrained eaters. Mean \pm SD age = 19 \pm 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 Trier Social Stress Test (TSST).	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 reduced liking for high-fat sweet foods after stress in the TRP condition only. Overall, TRP reduced food intake vs. 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 greatest increase in plasma TRP/LNAA after TRP treatment vs. placebo.
Cerit et al. (2013) ⁽¹²⁶⁾	22 females, 24 males; approx. half of each were S/S' or L/L' . Mean \pm SD age = 20.4 \pm 3.	Double-blind between-subjects, stratified by genotype. Subchronic oral TRP (2.8 g/day as 7 x 0.4 g capsules taken morning, afternoon and evening) for 6 days, then TSST on day 7.	Anxiety and depression (Hospital Anxiety and Depression Scale; HADS); positive and negative affect (PANAS); tension, anxiety, sadness, annoyance by single-item Mood States Scale (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 (2014) (123)	99 female, 19 male, students; 60 in S/S' and 58 in L/L' groups. Mean \pm SD age = 24.0 \pm 1.7.	Double-blind between-subjects, stratified by genotype and neuroticism (N) trait (Dutch Personality Inventory; DPQ-N). Subchronic oral TRP (3 g/day as 2 x 0.5 g capsules taken 3 times/day) for 7 days, then stress (Maastricht Acute Stress Test) after lunch on day 8.	Salivary cortisol (one baseline, two post- stress), mood (POMS), anxiety (state scale of State and Train Anxiety Inventory), 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 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 body mass index controlled for by covariance. Avoidance of high- fat sweet snacks in high N subjects may be related to health/weight concerns.
Van Dalfsen & Markus (2015) (127)	S/S' allele group: 46 women, 11 men; L/L' allele: 46 women, 8 men. Mean \pm SD age = 23.9 \pm 1.7.	Double-blind between-subjects, stratified by genotype and neuroticism trait (median split on DPQ-N). Subchronic oral TRP (3 g/day as 2 x 0.5 g capsules taken 3 times/day) for 7 days.	Prior to treatment: subjective sleep quality (1 month; adapted Pittsburg Sleep Quality Index, PSQI), neuroticism (DPQ-N), depression (Beck Depression Inventory, BDI), Stressful Life Events (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 not significant covariates.

899 Figure 1 caption:

900 This figure illustrates metabolic and other biochemical pathways in gut and blood that moderate the ability of supplementary tryptophan (TRP)

- 901 to enter the brain as the precursor for synthesis of brain serotonin (5-HT), and thus to alter behaviour, especially mood, cognition and appetite.
- 902 Rounded rectangles indicate enzymes involved in the various pathways. Thus, IDO and TDO are involved in catabolism of TRP via the
- 903 'TRYCAT' pathway, resulting in kynurenine (KYN) and then niacin formation. This could alter the 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
- 906 5-HT transporter system that has functional genetic variants in the 5-HT transporter-linked promoter region (5-HTTLPR). Abbreviations in bold
- 907 represent influences that have known functional genetic variants which may vary in their moderating effects; these in turn can interact with sex.
- 908 Other abbreviations: LNAA, large neutral amino acids; IDO, indole 2,3-dioxygenase; TDO, tryptophan 2,3-dioxygenase.



Figure 1