

1 **Title of the article:** The Effects of Green Tea Amino Acid L-theanine Consumption on the Ability to
2 Manage Stress and Anxiety Levels: A Systematic Review

3 **Authors names with highest academic degrees:**

4 Jackson L. Williams (BHN Hons)[#], Julian M. Everett (MND)[#], Nathan M. D’Cunha (BHN Hons),
5 Domenico Sergi (PhD), Ekavi N. Georgousopoulou (PhD), Richard J. Keegan (PhD), Andrew J.
6 McKune (PhD), Duane D. Mellor (PhD), Nicola Anstice (PhD) and Nenad Naumovski (PhD)

7 **List of institutions:**

8 J. Williams*, J. Everett*, N. M. D’Cunha* R. Keegan, A.J. McKune*, N. Anstice* and N. Naumovski*
9 Faculty of Health, University of Canberra, Canberra, 2601, ACT, Australia

10 *Collaborative Research in Bioactives and Biomarkers (CRIBB) Group, University of Canberra,
11 Bruce, ACT, 2601, Australia

12 [#] These authors contributed equally to this manuscript

13

14 D. Sergi

15 Nutrition & Health Substantiation Group, Nutrition and Health Program, Health and Biosecurity,
16 Commonwealth Scientific and Industrial Research Organisation (CSIRO), Adelaide, SA, 5000,
17 Australia

18

19 E.N. Georgousopoulou

20 Australian National University Medical School, Australian National University, Canberra, ACT,
21 2605, Australia;

22 School of Medicine, University of Notre Dame Australia, Sydney, 2000, NSW, Australia

23

24 D.D. Mellor

25 Aston Medical School, Aston University, Birmingham, B4 7ET, U.K

26

27 N. Naumovski (Correspondence),

28 PO Box 5018, University of Canberra, Bruce, 2617, ACT, Australia. Telephone: +61 2 62068719;

29 Fax: +61 2 62015999. Email address: nenad.naumovski@canberra.edu.au

30

31

32

33

34

35 **ABSTRACT**

36 The green tea amino acid, L-theanine (L-THE) is associated with several health benefits, including
37 improvements in mood, cognition and a reduction of stress and anxiety-like symptoms. This systematic
38 review evaluated the effect of pure L-THE intake, in the form of orally administered nutritional
39 supplements, on stress responses and anxiety levels in human randomised controlled trials. Following
40 the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist, nine
41 peer-reviewed journal articles were identified where L-THE as a supplement was compared to a
42 control. Our findings suggest that supplementation of 200–400 mg/day of L-THE may assist in the
43 reduction of stress and anxiety in people exposed to stressful conditions. Despite this finding, longer-
44 term and larger cohort clinical studies, including those where L-THE is incorporated into the diet
45 regularly, are needed to clinically justify the use of L-THE as a therapeutic agent to reduce stress and
46 anxiety in people exposed to stressful conditions.

47

48 **Keywords:** L-theanine, stress response, anxiety, mental health, green tea, human trials

49

50 **Abbreviations**

51 L-THE L-Theanine

52 PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses

53 RCT Randomised Controlled Trial

54 HPA Hypothalamic Pituitary Adrenal

55 HR Heart Rate

56 HRV Heart Rate Variability

57 POMS Profile of Mood States

58 VAS Visual Analog Scale

59 STAI State-Trait Anxiety Inventory

- 60 BAI Beck's Anxiety Inventory
- 61 MAPS Mood, Alertness and Physical Symptoms Questionnaire
- 62 DASS Depression Anxiety and Stress Scale
- 63 VPT Visual Probe Task
- 64 VAMS Visual Analogue Mood Scale
- 65 HARS Hamilton Anxiety Rating Scale
- 66 sAA salivary Alpha-Amylase (sAA)
- 67 s-IgA Salivary Immunoglobulin A
- 68 BP Blood Pressure

69

70 **Introduction**

71 Stress and anxiety are two inter-related conditions that have a substantial impact on individuals,
72 communities and wider society, with 264 million people estimated to be living with anxiety disorders
73 in 2015 [1]. The terms '*stress*' or a '*stressor*' are commonly used to describe physical or psychological
74 responses to a perceived threat to homeostasis, as well as referring to a range of physiological
75 parameters necessary for survival. Additionally, these terms can also characterise an event, or series
76 of events, that cause a response, whether it be positive or negative [2]. In this paper, stress will be used
77 to describe the physiological response that is detrimental to an individual that in turn, elicits a
78 physiological and behavioural response.

79 Prolonged stress is related to a number of chronic conditions and diseases, including hypertension
80 [3], type 2 diabetes and coronary heart disease [4]. Furthermore, the effects of psychological stress are
81 associated with anxiety-related diseases, including eating disorders [5], irritable bowel syndrome [6]
82 and substance abuse [7]. The evaluation of the stress response, as well as the severity of anxiety, can
83 effectively be divided into two broad categories relating to L-THE; subjective psychological measures
84 using a variety of questionnaires and assessment tools [8-11]; and physiological responses such as

85 changes in blood pressure (BP), circulating hormones and salivary hormones, heart rate (HR), heart
86 rate variability (HRV) and autonomic nervous system reactivity [12-14]. In recent years, the use of
87 complementary therapies to treat anxiety and stress-related disorders has increased; with mixed
88 evidence surrounding their efficacy [15]. One such approach is the use of green tea, which has a long
89 history of being consumed to enhance relaxation [16].

90 The consumption of green tea in some of the most densely populated countries in the world,
91 such as China and Japan, accounts for around 20% of global consumption [17]. In recent years the
92 popularity, as well as the significant research interest that green tea has attracted, is typically attributed
93 to its favourable taste as well as numerous proposed health benefits including neuroprotection [18],
94 cholesterol-lowering properties [19-21], strong antioxidant capacity [22] whereby consumption is
95 associated with successful aging [23,24]. Among the many constituents found in green tea such as
96 polyphenols [25], flavonoids [26] and caffeine [27], L-Theanine (L-THE) in particular, has received
97 considerable interest in human trials [28,29].

98 L-THE was first isolated and identified in 1949 [30] as a water-soluble non-proteinogenic
99 amino acid predominantly found in the tea plant (*Camellia sinensis*), and responsible for the provision
100 of a unique taste similar to the savoury taste sensation that monosodium glutamate produces known as
101 'umami' [28,31]. According to the universal nomenclature of the International Union of Pure and
102 Applied Chemistry, L-THE is '2-amino-4-(ethylcarbamoyl) butyric acid' and it is referred to by many
103 different names including 'gamma-glutamylethylamide' and 'gamma-glutamyl-L-ethyl amide' [32]
104 reflecting the presence of glutamine, a conditionally essential amino acid found as a core unit in its
105 structure [29]. The theanine may occur as a racemic mix of its L- and D- enantiomers that compete for
106 absorption and urinary excretion with D- enantiomer reported to be metabolised at a faster rate while
107 L- being preferentially metabolised by the kidneys [29,33].

108 Studies in animal models have reported the decline of serum glucose, insulin and urea after the
109 administration of L-THE [34]. Therefore it was proposed that L-THE may be transported into systemic

110 circulation by sodium-glucose transport protein *1*; a mediator of glucose and galactose absorption
111 [34,35]. Furthermore, kinetic studies indicate that L-THE crosses the blood brain barrier via the amino
112 acid L transport-system as determined by the decrease of other L-system serum amino acids in its
113 presence [36].

114 The potential health benefits associated with the consumption of L-THE include improvements
115 in emotional status, quality of sleep [29,37], suppression of hypertension [38], and improvements in
116 mood and cognition [39]. Additionally, consumption of L-THE in combination with caffeine promotes
117 antioxidant and anti-inflammatory activity in the brain that may reduce the risk of cognitive
118 impairment [40]. The current evidence on the consumption of L-THE in humans and its effects on
119 stress and anxiety is equivocal. To date, the majority of evidence is based on animal research, which
120 has commonly used pure L-THE, and in combination with other bioactives such as caffeine [41] and
121 catechins that can potentially have synergistic and in some cases potentially antagonistic effects
122 [16,28].

123 To our knowledge, only one systematic review has investigated the effects of green tea
124 consumption on mood and cognition [39]. Nonetheless, it is important to note that this review
125 evaluated the effects of L-THE as well as a mixture of other tea components (catechins and caffeine)
126 rather than L-THE in its pure form. Therefore, this systematic review aimed to evaluate the effects of
127 L-THE consumption on anxiety levels and the stress response in humans. A secondary aim was to
128 provide insight into the potential mechanism of action that L-THE can pose and its possible therapeutic
129 benefits for reducing stress and anxiety.

130

131 **Methods**

132 *Design*

133 Standardised criteria for conducting and reporting systematic reviews of interventional studies was
134 followed based on the PRISMA 2009 statement and checklist [42]. This systematic review was

135 registered in an International Prospective Register of Systematic Reviews (PROSPERO); registration
136 number CRD42018104792.

137

138 *Eligibility criteria*

139 A PICOS (population, intervention, comparator, outcomes and setting) approach was used to structure
140 the systematic review and is as follows:

141 *Population:* Human populations of all ages, either healthy or with a diagnosis of anxiety and/or
142 depression were included.

143 *Intervention:* Randomised controlled trials (RCTs) or quasi-RCTs that reported the effects of L-THE
144 consumption on anxiety and stress outcomes were included. Trials were required to evaluate orally
145 administered L-THE alone or in combination with conventional therapy versus the same conventional
146 therapy alone. This criterion also included studies where quantifiable levels of L-THE were delivered
147 as part of a supplement or if it was a functional component of a supplement that may also contain other
148 compounds (i.e. food and drinks). Only studies with sample sizes of over 10 participants were included
149 to limit the influence of outliers and allow for a more robust analysis of the results.

150 *Comparator:* Using a placebo, active control (i.e. a pharmaceutical product) or other control in single
151 or double-blind human interventions.

152 *Outcomes:* Only studies that reported pre- and post-levels of stress and/or anxiety using at least one
153 validated psychological assessment method were included. The primary outcomes of interest included
154 levels of anxiety and/or stress determined using a variety of scales such as the Profile of Mood States
155 (POMS) [8], Visual Analog Scale (VAS) [9], State-Trait Anxiety Inventory (STAI) [10], and Beck's
156 Anxiety Inventory (BAI) [11]. Secondary outcomes were physiological responses including BP, skin
157 temperature, HR, HRV, cortisol and salivary immunoglobulin A (s-IgA) [29].

158 *Setting:* Any.

159 *Literature search*

160 Only studies that were published in their entirety in the English language in peer-reviewed journals
161 were included. Article selection was restricted to studies performed on humans (single- and double-
162 blind included) where an L-THE supplement was compared against a control.

163 Four electronic databases (PubMed, Scopus, Cochrane Library and Web of Science) were
164 searched for articles published from 1990 until October 2018. Articles were not selected prior to 1990
165 due to the difficulty in synthesis and low commercial availability of L-THE as a supplement prior to
166 this date. All retrieved articles were read in full, and where multiple publications referred to the same
167 study, only the latest publication was used [43]. Searches were re-run prior to submission (March
168 2019), with only one additional article included [44].

169

170 *Search terminology*

171 Search terms used were '*theanine*', '*L-theanine*', '*L-theanine AND stress*', '*L-theanine AND anxiety*',
172 '*gamma-glutamylethylamide*', '*gamma-glutamylethylamide AND stress*', '*gamma-*
173 '*glutamylethylamide AND anxiety*', '*gamma-glutamyl-L-ethyl amide*', '*gamma-glutamyl-L-ethyl*
174 '*amide AND stress*', '*gamma-glutamyl-L-ethyl amide AND anxiety*', '*N⁵-ethyl-L-glutamine*', '*N⁵-*
175 '*ethyl-L-glutamine AND stress*' and '*N⁵-ethyl-L-glutamine AND anxiety*'. In addition, articles that
176 included L-THE consumption and mood changes were also selected if the mood changes were
177 measured as either stress or anxiety or both.

178

179 *Study selection and data extraction*

180 Two authors (J.W. and J.E.) independently reviewed the titles and abstracts and extracted data in a
181 predefined table. The following information was extracted: the first author and year of publication;
182 mean age, sample size, experimental intervention (regimen); control intervention (regimen); stress
183 and/or anxiety outcome; and any adverse effects reported.

184 *Risk of bias assessment*

185 Risk of bias in the included studies was independently assessed by two authors (J.W. and J.E.)
186 following the Cochrane guidelines criteria [45]. The Cochrane guidelines assess the risk of bias based
187 on seven domains: random sequence generation, allocation concealment, blinding of participants and
188 personnel, blinding of outcome assessment, incomplete outcome data, and selective reporting bias.
189 Only articles with a low risk of overall bias were eligible for inclusion and any differences in opinion
190 were resolved through discussion with a third reviewer (N.N.).

191

192 *Data analysis*

193 Included studies were analysed based on the measures specific to the study design; the intervention
194 conducted; the sample size, age and gender of the participants; and various stress and anxiety
195 outcomes. The results were recorded based on the change in the measures from baseline to post-
196 consumption values and evaluated for effectiveness with the probability value ($p<0.05$) considered as
197 statistically significant. Due to the heterogeneity of the study design, setting, intervention and
198 outcomes, a meta-analysis was not conducted.

199

200 **Results**

201 *Study characteristics*

202 Initial search results identified 261 articles. Following removal of duplicates (n=40), reviews, letters
203 to the editor and articles not written in English (n=177), the number of articles was reduced to 44. Nine
204 articles [38,44,46-52] met the inclusion criteria (Figure 1). Seven studies recruited participants with
205 no known pre-existing mental health issues, while two studies used participants with an existing mental
206 health condition [44,49]. The total number of participants for all included studies was 270 with 134
207 males and 88 females (Table 1). Two studies were conducted in males only [46,47], and one study did
208 not provide the genders of its 48 participants [38].

209 *Study designs*

210 Eight studies were designed as randomised, double-blind, placebo-controlled trials [38,44,46-
211 49,51,52] while one study used a randomised, single-blind, placebo-controlled design [50]. Five of the
212 studies [46-48,51,52] used a crossover design with all the participants exposed to each of the
213 experimental treatments conditions. The other four studies [38,44,49,50] used a parallel design to
214 compare between an L-THE group and a placebo group. Seven of the studies compared L-THE to
215 placebo while the study by Lu *et al.* [48] compared L-THE against a prescribed anti-anxiety
216 pharmaceutical (Alprazolam; 1mg; Xanax[®], Pharmacia and UpJohn Ltd) as well as against placebo.
217 The study by White *et al.* [51] compared L-THE against a placebo in the form of a nutrient drink with
218 (active) and without (placebo).

219

220 *Treatment duration and doses*

221 In six studies [38,46-48,51,52], treatments were given acutely on the day in which various tests and
222 measurements were conducted. The other three studies were designed to test for chronic effects and
223 involved interventions over periods between seventeen days [50] to eight weeks [44,49]. In the acute
224 single day intervention trials, participants consumed 200 mg/day [46-48,52], while in the chronic trials,
225 participants consumed 400 mg/day of L-THE [49,50], and only one study used 450 and 900 mg/day.

226

227 *Outcomes*

228 Five studies included investigated the effects of L-THE on stress and anxiety [38,47,50-52] and four
229 studies investigated anxiety outcomes only [44,46,48,49]. The most commonly used scales to assess
230 changes in anxiety symptoms in the included studies was the STAI [38,46-48,50,51], VAS [47,50,52],
231 Hamilton Anxiety Rating Scale (HARS) [44,49], BAI [44,48], and POMS [52]. The Depression
232 Anxiety and Stress Scale (DASS) was used in one study [38]. Physiological data were assessed by HR

233 [38,46,47], BP [38,52], HRV [47], and skin temperature [52]. Biomarkers were measured in three
234 studies; s-IgA [47], salivary alpha-amylase (sAA) [50] and salivary cortisol [51].

235

236 *Risk of bias assessment*

237 The risk of bias was assessed for all included studies (Table 2). Overall, a 'Low' risk of bias was
238 observed across all domains. Some studies received an 'Unclear' classification due to insufficient
239 information provided in the articles. Only one study received a 'High' risk classification because this
240 study was single-blinded [50].

241

242 *L-THE and stress responses*

243 In total, five studies assessed stress responses [38,47,50-52]. In the study by Yoto *et al.* [52], the
244 participants performed a range of physical and mental tasks to test the acute effects of L-THE
245 consumption on the stress response. Following randomisation, the participants were required to
246 complete the POMS and VAS assessments, and baseline physiological measurements were taken
247 before they ingested either L-THE (200 mg), caffeine (100 mg) or a placebo. The participants were
248 then exposed to psychological stress load by auditory oddball target detection task (5 min) and
249 arithmetic mental task test (10 min) twice. This was followed by the cold pressor test (immersion of
250 hand in the slushy ice water) serving as an acute physical test. Participants were also analysed based
251 on the change in BP after the mental tasks while given the placebo: those with BP responses above
252 ('high-response') and below the mean ('low-response'). The 'high-response' group consuming 200
253 mg of L-THE had significantly lower systolic ($p=0.008$) and diastolic BP ($p=0.006$) in response to the
254 mental tasks, but not the cold pressor test compared to placebo. In the 'low-response' group, there
255 were no treatment effects in systolic or diastolic BP in the measurement periods.

256 The crossover trial by Kimura *et al.* [47] assessed the effects of L-THE (200 mg) on the stress
257 response (20 min arithmetic task) across four conditions: 1) at the start of the experimental procedure;

258 2) during the experimental procedure; 3) placebo; and 4) no treatment. The s-IgA and psychological
259 measures (STAI and VAS) were measured post-task while HR and HRV were measured continuously
260 through the experimental sessions. The results indicated a significant interaction between condition
261 and period for s-IgA ($p<0.01$), and s-IgA levels were higher under the placebo condition compared to
262 the other conditions. In addition, the stress task increased HR under the placebo condition ($p<0.05$)
263 while the L-THE conditions showed lower HR increments. The results of the HRV measurements
264 revealed that some aspects of the HRV analysis (LF/HF) in the placebo condition were significantly
265 higher ($p<0.05$) than other three conditions indicating more activation of the sympathetic nervous
266 system during acute stress.

267 The study by Rogers *et al.* [38] investigated the subjective, behavioural and physiological (BP
268 and HR) effects of L-THE and caffeine supplementation administered alone and/or in combination.
269 All participants received either a capsule of caffeine (250 mg) or placebo and drink containing L-THE
270 (200 mg) or no L-THE. Participants recorded their responses using the Mood, alertness and physical
271 symptoms (MAPS) questionnaire, BP and HR were assessed before and 40 min after the administration
272 of the treatments. The results indicated that the systolic and diastolic BP were higher after the caffeine
273 consumption than any other treatment combinations ($p<0.05$).

274 A study by Unno *et al.* [50] examined the effects of L-THE in pharmacy students during a
275 highly stressful period, the first week of pharmacy practice placement. Students were randomly
276 assigned L-THE twice daily (400 mg/day) or a placebo for 17 days, with the STAI performed before
277 and after their placement and sAA measured twice (morning and evening) daily. The results indicated
278 that the sAA was higher in the placebo group ($p=0.032$) while the L-THE group maintained the
279 baseline observed during the routine activities at the university. Interestingly, the STAI values were
280 not different between the two groups; however, the psychosocial stress was lower in L-THE group
281 ($p=0.020$) compared to placebo.

282 The crossover trial by White *et al.* [51] investigated the anti-stress effects of L-THE (200 mg)
283 administered in the form of an L-THE active nutrient beverage commercially available (NeuroBliss®)
284 in comparison to a placebo in 34 healthy participants (15 males, 19 females) [51]. This study carried
285 out a multi-tasking framework as a cognitive stressor and as a cognitive assessment. In total,
286 participants completed the task three times per visit (baseline, during and three hours post-
287 consumption). Stress, mood, fatigue and salivary cortisol were assessed prior to and following the
288 completion of each step of the cognitive stressor. The consumption of L-THE was associated with a
289 decrease in subjective stress responses ($p=0.003$) and reduced self-rated stress response ($p=0.006$)
290 measured 1 hr post-administration. No significant differences were reported in the subjective stress
291 response 3 hr after consumption; however, salivary cortisol levels were significantly lower in the L-
292 THE group ($p=0.047$) in comparison to placebo at this time point.

293

294 *Effect of L-THE on anxiety*

295 All studies [38,44,46-52] assessed the effects of L-THE on anxiety. The study by Ritsner *et al.* [49]
296 explored the effect of eight weeks of L-THE (400 mg) treatment on patients with schizophrenia or
297 schizoaffective disorders as an add-on to conventional antipsychotic therapy with anxiety levels
298 measured using the HARS. The consumption of L-THE was associated with a reduction in total HARS
299 score ($p=0.015$) with onset of improvement after the second week, and improvement in five of the
300 HARS items including anxious mood ($p=0.003$), tension ($p=0.021$), intellectual ($p=0.044$), muscular
301 ($p=0.012$) and sensory somatic complaints ($p=0.030$) in comparison to placebo group.

302 Lu *et al.* [48] compared the short term effects between the L-THE (200 mg), Alprazolam (1
303 mg; Xanax®, Pharmacia and UpJohn Ltd) and placebo on behavioural measures of anxiety in healthy
304 participants. There was no difference between L-THE and placebo on the STAI and BAI scores (All
305 p 's>0.05); however, L-THE reduced subjective anxiety in comparison with Alprazolam ($p<0.05$) and
306 placebo ($p<0.05$) on the 'Troubled' subscale of the VAMS. Also, VAMS-Tranquil scores were lower

307 for L-THE group compared to the other two treatments (All p 's >0.05). In addition, L-THE intake
308 reduced the total HARS score ($p=0.015$). Furthermore, the results indicated that neither L-THE nor
309 Alprazolam showed any acute anxiolytic effects under an electric shock model of anticipatory anxiety.

310 Higashiyama *et al.* [46] investigated the effects of L-THE (200 mg) consumption on attention
311 and reaction time responses in healthy university students with high and low anxiety propensities. The
312 consumption of L-THE resulted in a decrease in HR ($p=0.0016$) and an improved reaction time
313 response among high anxiety propensity participants compared to placebo. The STAI results indicated
314 time dependant decrement patterns of anxiety; however, no differences were observed between groups,
315 potentially attributing to the participants being familiar with the testing environment. Similarly, Rogers
316 *et al.* [38] assessed anxiety interactions in 48 university students using a visual probing task. The
317 consumption of L-THE slowed overall reaction time on the visual probe task, with social threat sub-
318 task reaction time ($p=0.046$), whereas no differences were observed in the physical threat reaction task
319 ($p=0.100$) compared to placebo. The authors did attempt to discuss the potential mechanisms of action
320 and reasons for not seeing anxiety lowering effects which were ascribed to participants being of a
321 healthy mental state.

322 The study by Kimura *et al.* [47] showed strong interactions between the perception of stress
323 and state anxiety ($p<0.01$). The STAI scores were higher during the mental arithmetic task period than
324 during the rest periods in the placebo group, but not in the other three conditions ($p<0.01$). The VAS
325 scores were also lower in both L-THE conditions than under the two control conditions ($p<0.01$).

326 In the study by White *et al.* [51] changes in brain alpha oscillatory activity, measured by
327 electroencephalography and using resting-state recordings, were related to the potential anti-stress
328 effects of L-THE supplementation. The results were higher in the L-THE treatment compared to the
329 placebo for the high anxiety trait groups ($p=0.019$, one-tailed), while no changes were observed in the
330 low anxiety group ($p>0.1$). It was suggested that the increase in resting alpha activity in the treatment
331 group did not reflect the mechanism that normalises alpha activity in individuals with high anxiety.

332 Instead, the L-THE treatment enhances alpha oscillatory level activity selectively in individuals that
333 self-reported higher levels of trait anxiety.

334 The most prolonged (treatment duration) study by Sarris *et al.* [44] was an eight-week phase II
335 randomised multicentre clinical trial in individuals ($n=46$) with generalised anxiety disorder. For the
336 first four weeks, participants received 225 mg L-THE twice daily. After that, participants who were
337 considered non-responders (based on HARS score), were prescribed double the original dose. Two
338 measures were used to assess anxiety symptoms (HARS; BAI); however, neither doses of L-THE
339 improved symptoms compared with placebo.

340 In the study by Unno *et al.* [50], there was an association between STAI and subjective stress
341 in the L-THE ($p=0.012$), but not in the placebo group. In the study by Yoto *et al.* [52] no differences
342 between the groups were observed for VAS scores; however, the scores were lower in the L-THE
343 group for tension-anxiety sub-scale of the POMS ($p=0.004$), representing a potential anxiolytic effect.

344

345 **Discussion**

346 The studies outlined in this systematic review indicate that L-THE supplementation has a potential
347 anti-stress effect and anxiety suppressive properties at levels between 200-400 mg/day. All included
348 articles demonstrate some strengths and indicators of quality, for instance, eight of the nine studies
349 were designed as randomised, double-blind, placebo-controlled trials [38,44,46-49,51,52] while one
350 study used a randomised, single-blind, placebo-controlled design [50]. However, according to the
351 Cochrane assessment of the risk of bias, there was a lack of clarity in some domains of bias present in
352 most studies.

353 Given the duration of the Ritsner *et al.* [49] study, there appear to be no side effects of taking
354 L-THE (400 mg/day) for 8 weeks. Furthermore, the study by Sarris *et al.* [44] with longer (10 weeks)
355 and higher L-THE intake (900 mg) reported some adverse events in both groups (placebo and L-THE),
356 but with no significant differences between groups. Considering that schizophrenia and schizoaffective

357 type of disorders are not acute types of mental health conditions, much longer L-THE trials may be
358 required to establish the potential time/concentration side effects. In addition, Ritsner *et al.* [49]
359 demonstrated benefits of L-THE through the amelioration of psychotic and anxiety-related symptoms
360 in contrast to the classical dopaminergic theory of psychosis [53].

361 Consumption of L-THE was also proposed to affect the physiological biomarkers of stress.
362 Elevated sAA is an indicator of autonomic nervous system excitation and was shown to be decreased
363 prior to stress exposure in healthy participants following consumption L-THE [50]. Additionally, a
364 previous study indicated that the consumption of L-THE was associated with improvements in sleep
365 quality [54], which can be regulated through the sAA levels where participants with lower sAA levels
366 had a longer sleep [50]. Once L-THE is consumed, it is absorbed within the intestinal lumen and
367 transported to a range of tissues, including the brain within 10-24 min [55]. Therefore, it can be
368 suggested that the results of the Unno *et al.* [50] study indirectly support the blood-brain-barrier
369 passing of L-THE to illicit its psychological effects [50,55]. Therefore, consumption of L-THE may
370 be an effective method for improvement in sleep quality without the increase in drowsiness.

371 The L-THE consumption may also suppress excessive glutamatergic tone, which is implicated
372 as a possible co-factor in increasing stress and its related anxiety responses [56,57]. The glutamatergic
373 transmission relies on L-THE-mediated inhibition of neuronal glutamine uptake which, in turn,
374 suppresses the conversion of glutamine to glutamate. Thus, the consequent decrease in glutamate
375 release from the pre-synaptic terminals induced by L-THE represents another plausible mechanism
376 contributing to the anti-stress and anti-anxiety effects (Figure 2) [50]. In addition, another mechanism
377 putatively responsible for the inhibition of glutamatergic transmission is underpinned by the ability of
378 L-THE to bind to the three glutamate receptor subtypes: Ar-methyl-D-aspartate (NMDA), amino-3-
379 hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainic acid (KA), thus competing with
380 glutamate [58]. Moreover, in murine models, it has been shown that L-THE intake suppresses the HPA
381 axis and behavioural depression [59], although the same mechanisms are yet to be demonstrated in

382 humans. Nevertheless, a study by Rogers *et al.* [38] suggested that L-THE may exhibit central
383 monoaminergic and glutaminergic effects suggesting that slowing the reaction time on the visual probe
384 task may also be attributed to the suppression of glutamate [50,58].

385 Although findings by Unno *et al.* [50] indicated that long-term use of L-THE could provide
386 'anti-stress' effects. However, there was no indication this finding was a direct result of the daily intake
387 of L-THE, a higher dose (400 mg), or a potential adaptive effect over several days [50]. Interestingly,
388 in the study by Yoto *et al.* [52], consumption of 200 mg of L-THE reduced subjective stress and BP,
389 but only in the short term. The study by Rogers *et al.* [38] indicated that L-THE could inhibit the
390 caffeine-induced rise in BP, which is accompanied by increased nervousness and considered as a
391 somatic symptom of anxiety. However, the DASS and STAI scores indicated participants were not
392 prone to anxiety or stress. It was further proposed that the initial mental state of the participants might
393 affect the reduction in anxiety caused by L-THE. However, this study speculates that tea is perceived
394 to be more relaxing, partly due to the reason that L-THE reduces alertness/arousal and anxiety [38].

395 The effectiveness of L-THE in the acute anxiety induced conditions was also compared against
396 the benzodiazepine alprazolam, a common pharmaceutical mediation used for the treatment of anxiety
397 and related disorders [48]. Overall, neither L-THE nor alprazolam treatment resulted in any anxiolytic
398 effects [48]. Despite this outcome, it seems that the intensity of anxiety was too great for L-THE to
399 provide an anxiolytic effect; however, L-THE had a greater effect in the resting state of healthy
400 individuals compared to participants with increased anxiety. Although alprazolam has shown some
401 inconsistencies with reducing anxiety under resting conditions [60], Higashiyama *et al.* [46]
402 highlighted that L-THE (200 mg) displayed positive effects relating to attentional performance as well
403 as reaction time in a cohort prone to high anxiety. This can potentially indicate that L-THE may not
404 be as effective in individuals that are not highly anxious. Furthermore, the results suggest a putative
405 mechanism of action highlighted by the increase in alpha waves as determined by
406 electroencephalogram [61]. This, in turn, supports the previous literature associating the relaxation

407 properties of L-THE with the increased synthesis of γ -aminobutyric acid, which plays a part in the
408 stress response pertinent to dopamine and serotonin increase in the brain [58,61]. The study by Sarris
409 *et al.* [44] of individuals with generalised anxiety disorder found no benefits using a high dose of L-
410 THE (450-900 mg), establishing that higher levels are potentially not beneficial in this type of a clinical
411 sample. This was the first study to investigate the use of L-THE for the treatment of anxiety and sleep
412 disturbance in generalised anxiety disorder and also the first to administer L-THE at doses higher than
413 400 mg/day. Although L-THE appeared to improve self-reported sleep satisfaction in participants with
414 generalised anxiety disorder, and insomnia symptoms in a sub-group of individuals with generalised
415 anxiety disorder, this preliminary pilot study found no evidence of any beneficial effects of L-THE
416 over the placebo in measures of anxiety and mood. Additionally, considering this trial used the highest
417 amount of L-THE in the included studies, it appears that understanding how high levels L-THE affects
418 the psychological response is still relatively unexplored.

419 Lastly, the study by White *et al.* [51] reported a decrease in the stress response post
420 consumption of a nutrient-based drink containing 200 mg L-THE in response to a cognitive stressor
421 task. The results indicated that the anti-stress response is attributed to a decrease in salivary cortisol
422 only in 3 hr post-dosage. The authors reported no differences in cognitive performance, although
423 posterior alpha oscillatory activity was found to be higher in participants exhibiting higher trait anxiety
424 participants but was not associated with the other anti-stress outcomes. This study was limited as the
425 active treatment that contained L-THE (200 mg) contained other ingredients: L-alpha
426 glycerylphosphorylcholine (25 mg), phosphatidylserine (1 mg) and micronized chamomile (10 mg)
427 that were unique to the active treatment and not the placebo. Although the beneficial effects of L-THE
428 were observed after post-consumption, the authors acknowledged that an additional arm of treatment
429 was needed to evaluate consumption of other ingredients in the nutrient drink as they may interact with
430 L-THE absorption.

431 Thus, in a setting where green tea consumption frequently occurs, as opposed to pure L-THE
432 consumption (4-8 cups/day; 150-200 mg), it is difficult to accurately measure the causative agent for
433 specific physiological and psychological outcomes, as green tea contains a number of polyphenolic
434 compounds (catechins) that might also interfere with the aforementioned health outcomes [16,24].
435 Indeed, studies have found tea drinkers have lower levels of depressive symptoms [62]; however, it is
436 difficult to identify the lead potential anti-depressive agent due to the other bioactive compounds in
437 green tea such as catechins. It is reported that the consumption of green tea in general or with other
438 constituents may affect the uptake of other compounds, such as vitamin B1 [63], interference of lipid
439 digestion due to green tea catechins [64] as well as the inhibiting effects L-THE shows in the presence
440 of caffeine [29]. Similarly, the presence of L-THE in the blood has previously been shown to decrease
441 the uptake of branched-chain amino acids, such as leucine, isoleucine and valine [36]; however, this
442 mechanism is yet to be elucidated in humans.

443

444 *Future directions*

445 Given the growing interest and prevalence of green tea bioactives consumption globally [23], future
446 human trials of L-THE consumption should be conducted over a more extended period (ten weeks or
447 longer) with the combination in identifying the therapeutic doses required for different population sub-
448 groups. The effect of L-THE in clinical practice focusing on the type of specific clinical population
449 may, in turn, affect cardiovascular outcomes with potentially beneficial outcomes. Currently, the
450 consumption of L-THE is associated the of a relaxed but alert state, providing the basis of L-THE use
451 as a nutraceutical with stress- and anxiety-reducing properties. Moreover, the use of L-THE with other
452 nutraceuticals (i.e. B-vitamins) may have positive synergistic effects on anxiety, stress, depressive
453 symptoms, and cognition [65,66]. Further, studies should be conducted to ascertain whether L-THE
454 binds to food matrixes before consumption as the health effects of L-THE may vary depending on the
455 type of food and/or compounds in which L-THE has been combined with.

456

457 **Conclusion**

458 The supplementation of L-THE in its pure form at dosages between 200–400 mg/day may help reduce
459 stress and anxiety acutely in people undergoing acute stressful situations, but there is insufficient
460 evidence to suggest it assists in the reduction of stress levels in people with chronic conditions.
461 However, the results of this study suggest that L-THE taken during times of heightened acute stress or
462 by individuals with a high propensity for anxiety and stress may exhibit beneficial properties via the
463 increased production of alpha waves and decrease of glutamate in the brain.

464

465 **Funding**

466 J Williams and N.M. D’Cunha are supported by an Australian Government Research Training Program
467 scholarship. N.M. D’Cunha is supported by a Dementia Australia Research Foundation PhD
468 scholarship. All other authors declare no funding sources.

469

470 **Acknowledgements**

471 Dr Paul Roach for providing initial feedback on the manuscript.

472

473 **References**

- 474 1. World Health Organization (2017) Depression and Other Common Mental Disorders: Global Health
475 Estimates. World Health Organization, Geneva, Switzerland
- 476 2. McEwen BS (2000) The neurobiology of stress: from serendipity to clinical relevance. *Brain Res*
477 886 (1-2):172-189
- 478 3. Pan Y, Cai W, Cheng Q et al. (2015) Association between anxiety and hypertension: a systematic
479 review and meta-analysis of epidemiological studies. *Neuropsych Dis Treat* 11:1121-1130

- 480 4. Hagstrom E, Norlund F, Stebbins A et al. (2018) Psychosocial stress and major cardiovascular
481 events in patients with stable coronary heart disease. *J Intern Med* 283 (1):83-92
- 482 5. Fitzsimmons EE, Bardone-Cone AM (2011) Coping and social support as potential moderators of
483 the relation between anxiety and eating disorder symptomatology. *Eat Behav* 12 (1):21-28
- 484 6. Myers B, Greenwood-Van Meerveld B (2009) Role of anxiety in the pathophysiology of irritable
485 bowel syndrome: importance of the amygdala. *Front Neurosci* 3:47
- 486 7. Durand MV, Barlow DH (2010) *Essentials of Abnormal Psychology*. Fifth edn. Wadsworth
487 Cengage Learning,
- 488 8. Spielberger CD (1972) Profile of Mood States. *Prof Psychol* 3 (4):387-388
- 489 9. Pfenning L, Cohen L, van der Ploeg H (1995) Preconditions for sensitivity in measuring change:
490 visual analogue scales compared to rating scales in a Likert format. *Psychol Rep* 77 (2):475-480
- 491 10. Hedberg AG (1972) State-Trait Anxiety Inventory. *Prof Psych* 3 (4):389-390
- 492 11. Dozois DJA, Dobson KS, Ahnberg JL (1998) A Psychometric Evaluation of the Beck Depression
493 Inventory-II. *Psychol assessment* 10 (2):83-89
- 494 12. Matthews KA, Woodall KL, Allen MT (1993) Cardiovascular reactivity to stress predicts future
495 blood pressure status. *Hypertension* 22 (4):479-485
- 496 13. Gorman JM, Sloan RP (2000) Heart rate variability in depressive and anxiety disorders. *Am Heart*
497 *J* 140 (4 Suppl):77-83
- 498 14. Nater UM, La Marca R, Florin L et al. (2006) Stress-induced changes in human salivary alpha-
499 amylase activity - associations with adrenergic activity. *Psychoneuroendocrino* 31 (1):49-58
- 500 15. McCabe D, Lisy K, Lockwood C et al. (2017) The impact of essential fatty acid, B vitamins,
501 vitamin C, magnesium and zinc supplementation on stress levels in women: a systematic review. *JBIS*
502 *Database System Rev Implement Rep* 15 (2):402-453. doi:10.11124/JBISRIR-2016-002965
- 503 16. Williams J, Sergi D, McKune AJ et al. (2019) The beneficial health effects of green tea amino acid
504 l-theanine in animal models: Promises and prospects for human trials. *Phytother Res* 3 (1)

- 505 17. Liu J, Xing J, Fei Y (2008) Green tea (*Camellia sinensis*) and cancer prevention: a systematic
506 review of randomized trials and epidemiological studies. *Chin Med* 3:12
- 507 18. Katergaris N, Dufficy L, Roach PD et al. (2015) Green Tea Catechins as Neuroprotective Agents:
508 Systematic Review of the Literature in Animal Pre-Clinical Trials. *AFTNS Open J* 1 (2):48-57
- 509 19. Bursill CA, Abbey M, Roach PD (2007) A green tea extract lowers plasma cholesterol by inhibiting
510 cholesterol synthesis and upregulating the LDL receptor in the cholesterol-fed rabbit. *Atherosclerosis*
511 193 (1):86-93
- 512 20. Bursill CA, Roach PD (2007) A green tea catechin extract upregulates the hepatic low-density
513 lipoprotein receptor in rats. *Lipids* 42 (7):621-627
- 514 21. Reto M, Figueira ME, Filipe HM et al. (2007) Chemical composition of green tea (*Camellia*
515 *sinensis*) infusions commercialized in Portugal. *Plant Foods Hum Nutr* 62 (4):139-144
- 516 22. Vuong QV (2014) Epidemiological evidence linking tea consumption to human health: a review.
517 *Crit Rev Food Sci Nutr* 54 (4):523-536
- 518 23. Naumovski N, Foscolou A, D'Cunha NM et al. (2019) The Association between Green and Black
519 Tea Consumption on Successful Aging: A Combined Analysis of the ATTICA and MEDiterranean
520 ISlands (MEDIS) Epidemiological Studies. *Molecules* 24 (10)
- 521 24. Crichton GE, Bryan J, Murphy KJ (2013) Dietary antioxidants, cognitive function and dementia--
522 a systematic review. *Plant Foods Hum Nutr* 68 (3):279-292
- 523 25. Naumovski N, Blades BL, Roach PD (2015) Food Inhibits the Oral Bioavailability of the Major
524 Green Tea Antioxidant Epigallocatechin Gallate in Humans. *Antioxidants* 4 (2):373-393
- 525 26. Yao LH, Jiang YM, Shi J et al. (2004) Flavonoids in food and their health benefits. *Plant Foods*
526 *Hum Nutr* 59 (3):113-122
- 527 27. Nehlig A, Daval JL, Debry G (1992) Caffeine and the central nervous system: mechanisms of
528 action, biochemical, metabolic and psychostimulant effects. *Brain Res Brain Res Rev* 17 (2):139-170

- 529 28. Vuong QV, Bowyer MC, Roach PD (2011) L-Theanine: properties, synthesis and isolation from
530 tea. *J Sci Food Agric* 91 (11):1931-1939
- 531 29. Williams J, Kellett J, Roach PD et al. (2016) L-Theanine as a Functional Food Additive: Its Role
532 in Disease Prevention and Health Promotion. *Beverages* 2 (13):1-16
- 533 30. Eschenauer G, Sweet BV (2006) Pharmacology and therapeutic uses of theanine. *Am J Health Syst*
534 *Pharm* 63 (1):26, 28-30
- 535 31. Cooper R (2012) Green tea and theanine: health benefits. *Int J Food Sci Nutr* 63 Suppl 1:90-97
- 536 32. Nobre AC, Rao A, Owen GN (2008) L-theanine, a natural constituent in tea, and its effect on
537 mental state. *Asia Pac J Clin Nutr* 17 Suppl 1:167-168
- 538 33. Desai MJ, Gill MS, Hsu WH et al. (2005) Pharmacokinetics of theanine enantiomers in rats.
539 *Chirality* 17 (3):154-162
- 540 34. Yan Q, Tong H, Tang S et al. (2017) L-Theanine Administration Modulates the Absorption of
541 Dietary Nutrients and Expression of Transporters and Receptors in the Intestinal Mucosa of Rats.
542 *Biomed Res Int* 2017:9747256
- 543 35. Scheid L, Ellinger S, Alteheld B et al. (2012) Kinetics of L-Theanine Uptake and Metabolism in
544 Healthy Participants Are Comparable after Ingestion of L-Theanine via Capsules and Green Tea. *The*
545 *Journal of Nutrition* 142 (12):2091-2096
- 546 36. Yokogoshi H, Kobayashi M, Mochizuki M et al. (1998) Effect of theanine, r-glutamylethylamide,
547 on brain monoamines and striatal dopamine release in conscious rats. *Neurochemical research* 23
548 (5):667-673
- 549 37. Turkozu D, Sanlier N (2017) L-theanine, unique amino acid of tea, and its metabolism, health
550 effects, and safety. *Crit Rev Food Sci Nutr* 57 (8):1681-1687
- 551 38. Rogers PJ, Smith JE, Heatherley SV et al. (2008) Time for tea: mood, blood pressure and cognitive
552 performance effects of caffeine and theanine administered alone and together. *Psychopha* 195 (4):569-
553 577

- 554 39. Camfield DA, Stough C, Farrimond J et al. (2014) Acute effects of tea constituents L-theanine,
555 caffeine, and epigallocatechin gallate on cognitive function and mood: a systematic review and meta-
556 analysis. *Nutr Rev* 72 (8):507-522
- 557 40. Cicero AFG, Fogacci F, Banach M (2018) Botanicals and phytochemicals active on cognitive
558 decline: The clinical evidence. *Pharmacol Res* 130:204-212
- 559 41. Haskell CF, Kennedy DO, Milne AL et al. (2008) The effects of L-theanine, caffeine and their
560 combination on cognition and mood. *Biol Psychol* 77 (2):113-122
- 561 42. Moher D, Liberati A, Tetzlaff J et al. (2009) Preferred reporting items for systematic reviews and
562 meta-analyses: the PRISMA statement. *J Clin Epidemiol* 62 (10):1006-1012
- 563 43. Clifton PM, Condo D, Keogh JB (2014) Long term weight maintenance after advice to consume
564 low carbohydrate, higher protein diets-a systematic review and meta analysis. *NMCD* 24 (3):224-235
- 565 44. Sarris J, Byrne GJ, Cribb L et al. (2018) L-Theanine in the Adjunctive Treatment of Generalised
566 Anxiety Disorder: A Double-Blind, Randomised, Placebo-Controlled Trial. *J Psychiat Res* 110:31-37
- 567 45. Higgins JPT, Altman DG, Sterne JAC (2011) Chapter 8: Assessing risk of bias in included studies.
568 In: Higgins J.P.T., S. G (eds) *Cochrane Handbook for Systematic Reviews of Interventions* Version
569 5.1.0 The Cochrane Collaboration,
- 570 46. Higashiyama A, Htay HH, Ozeki M et al. (2011) Effects of L-theanine on attention and reaction
571 time response. *J Funct Food* 3 (3):171-178
- 572 47. Kimura K, Ozeki M, Juneja LR et al. (2007) L-Theanine reduces psychological and physiological
573 stress responses. *Biol Psychol* 74 (1):39-45
- 574 48. Lu K, Gray MA, Oliver C et al. (2004) The acute effects of L-theanine in comparison with
575 alprazolam on anticipatory anxiety in humans. *Hum Psychopharm Clin* 19 (7):457-465
- 576 49. Ritsner MS, Miodownik C, Ratner Y et al. (2011) L-theanine relieves positive, activation, and
577 anxiety symptoms in patients with schizophrenia and schizoaffective disorder: an 8-week, randomized,
578 double-blind, placebo-controlled, 2-center study. *J Clin Psychiatry* 72 (1):34-42

- 579 50. Unno K, Tanida N, Ishii N et al. (2013) Anti-stress effect of theanine on students during pharmacy
580 practice: positive correlation among salivary alpha-amylase activity, trait anxiety and subjective stress.
581 *Pharmacol Biochem Behav* 111:128-135
- 582 51. White DJ, de Klerk S, Woods W et al. (2016) Anti-Stress, Behavioural and
583 Magnetoencephalography Effects of an L-Theanine-Based Nutrient Drink: A Randomised, Double-
584 Blind, Placebo-Controlled, Crossover Trial. *Nutrients* 8 (1)
- 585 52. Yoto A, Motoki M, Murao S et al. (2012) Effects of L-theanine or caffeine intake on changes in
586 blood pressure under physical and psychological stresses. *J Physiol Anthropol* 31:28
- 587 53. Helton SG, Lohoff FW (2015) Serotonin pathway polymorphisms and the treatment of major
588 depressive disorder and anxiety disorders. *Pharmacogenomics* 16 (5):541-553
- 589 54. Lyon MR, Kapoor MP, Juneja LR (2011) The effects of L-theanine (Suntheanine(R)) on objective
590 sleep quality in boys with attention deficit hyperactivity disorder (ADHD): a randomized, double-
591 blind, placebo-controlled clinical trial. *Altern Med Rev* 16 (4):348-354
- 592 55. Alteheld B, Scheid L, Stehle P et al. (2012) Kinetics of L-Theanine Uptake and Metabolism in
593 Healthy Participants Are Comparable after Ingestion of L-Theanine via Capsules and Green Tea. *J*
594 *Nutr* 142 (12):2091-2096
- 595 56. Yuen EY, Liu W, Karatsoreos IN et al. (2009) Acute stress enhances glutamatergic transmission
596 in prefrontal cortex and facilitates working memory. *Proc Natl Acad Sci U S A* 106 (33):14075-14079
- 597 57. Popoli M, Yan Z, McEwen BS et al. (2011) The stressed synapse: the impact of stress and
598 glucocorticoids on glutamate transmission. *Nat Rev Neurosci* 13 (1):22-37
- 599 58. Kakuda T, Nozawa A, Sugimoto A et al. (2002) Inhibition by theanine of binding of [3H]AMPA,
600 [3H]kainate, and [3H]MDL 105,519 to glutamate receptors. *Biosci Biotechnol Biochem* 66 (12):2683-
601 2686
- 602 59. Unno K, Iguchi K, Tanida N et al. (2013) Ingestion of theanine, an amino acid in tea, suppresses
603 psychosocial stress in mice. *Exp Physiol* 98 (1):290-303

604 60. Baas JM, Grillon C, Bocker KB et al. (2002) Benzodiazepines have no effect on fear-potentiated
605 startle in humans. *Psychopharmacology* 161 (3):233-247

606 61. Juneja R, Djong-Chi C, Tsutomu O et al. (1999) L-theanine—a unique amino acid of green tea and
607 its relaxation effect in humans. *Trends Food Sci Tech* 10 (6-7):199-204

608 62. Hintikka J, Tolmunen T, Honkalampi K et al. (2005) Daily tea drinking is associated with a low
609 level of depressive symptoms in the Finnish general population. *Eur J Epidemiol* 20 (4):359-363

610 63. Wang RS, Kies C (1991) Niacin, thiamin, iron and protein status of humans as affected by the
611 consumption of tea (*Camellia sinensis*) infusions. *Plant Foods Hum Nutr* 41 (4):337-353

612 64. Koo SI, Noh SK (2007) Green tea as inhibitor of the intestinal absorption of lipids: potential
613 mechanism for its lipid-lowering effect. *J Nutr Biochem* 18 (3):179-183

614 65. D’Cunha NM, Georgousopoulou EN, Dadigamuwage L et al. (2018) Effect of long-term
615 nutraceutical and dietary supplement use on cognition in the elderly: a 10-year systematic review of
616 randomised controlled trials. *Brit J Nutr* 119 (3):280-298

617 66. Barnett H, D’Cunha NM, Georgousopoulou E et al. (2017) Effect of Folate Supplementation on
618 Inflammatory Markers in Individuals Susceptible to Depression: A Systematic Review. *ERHM* 2:86-
619 100

620

621

622

623

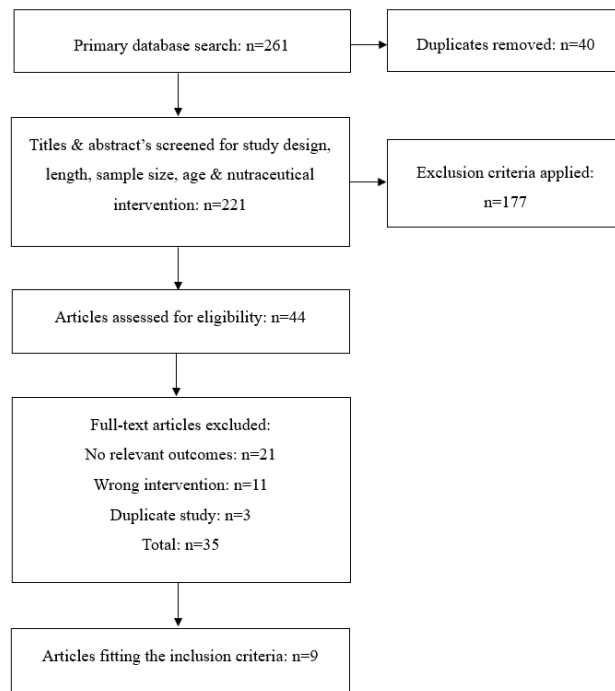
624

625

626

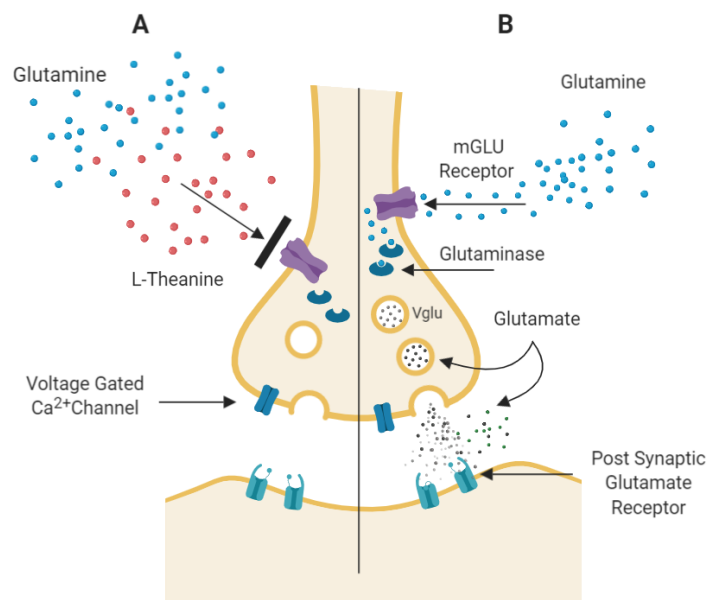
627

628



630

631 **Fig 1** PRISMA flowchart of search strategy and selection of relevant studies.



632

633 **Fig 2** L-THE may reduce the binding of glutamine to glutamate receptors (mGLU) inhibiting the
634 incorporation of extracellular glutamine into the neuron (A). This may prevent the formation of
635 vesicular glutamate (Vglu) decreasing the activation of post synaptic glutamate receptors (B).

636 **Table 1.** Summary of L-THE effects in human clinical trials

Author (Year)	Intervention and dosage	Sample size All (M; F)	Age (y) (Mean±SD)	Study design and duration of intervention	Stress / Anxiety task	Stress / Anxiety outcome	Stress results	Anxiety results
Higashiyama et al. (2011)	L-THE (200mg) in water Placebo	18 (18;0)	19±1	Randomized, placebo-controlled, double-blind intervention. Acute	Visual Attentional Task; Rapid Audio Response Task	STAI; HR	L-THE decreased HR (p=0.0016)	L-THE improved attention performance (p=0.0001) and reaction time (p=0.001) in healthy subjects prone to high anxiety
Kimura et al. (2007)	L-THE (200mg) after initiation L-THE (200mg)–after rest Placebo No treatment	12 (12;0)	21.5±1.4	Randomized, double-blind, placebo-controlled cross over design. Acute	Mental arithmetic task	STAI; VAS; HR; HRV; s-IgA	Placebo increased HR (p<0.05), HRV (LF/HF effect of period) (p<0.05), and elevated s-IgA (p<0.01) compared with L-THE and control	Placebo increased STAI (p<0.01) and VAS (p<0.01) score compared with L-THE and control
Lu et al. (2004)	L-THE (200mg) Alprazolam (1mg) Placebo	16 (12;4)	26.9±3.4	Randomized, double-blind, placebo-controlled, cross over design. Acute	Relaxed and anticipatory anxiety (AA) condition with electric shock	BAI; BDI-II; VAMS; STAI	NA	VAMS-Tranquil score lower for L-THE compared to placebo and Alprazolam (both p's<0.05). No differences in AA condition
Ritsner et al. (2011)	L-THE (400mg/day) Placebo	40 (9;31)	36.4±11.5	Randomised, double-blind, placebo-controlled design 8 weeks. Acute	Schizophrenia and Schizoaffective disorder patients	HARS	NA	L-THE reduced total HARS score (p=0.015)
Rogers et al. (2008)	Caffeine (250mg) + Placebo L-THE (200mg) + Placebo Caffeine (250mg) + L-THE (200mg) Placebo	48	20.5±2.0	Randomised, double blind, placebo-controlled cross over design. Acute	Ratings of mood, anxiety and alertness	MAPS; STAI; DASS; BP; HR, VPT	Caffeine alone increased systolic and diastolic BP compared with all other conditions (all p's<0.05)	L-THE slowed reaction time on the visual probe task social threat sub-task (p=0.046)

Sarris et al. (2018)	L-THE (225mg twice daily) Non-responders increased to 450mg twice daily at week 4 Placebo	46 (7;39)	Treatment: 40.7±15.0 Placebo: 32.2±9.3	Randomised, double-blind, placebo-controlled. 10 weeks (8-week trial, 2-week post-observation)	Anxiety, sleep quality and cognition	HARS; BAI; Penn State Worry Questionnaire	NA	No significant improvements in anxiety measures in the treatment group (p>0.05)
Unno et al. (2013)	L-THE (200mg twice daily) Placebo	20 (14;6)	22.4±0.2	Randomised, single-blind group comparison. Intervention 1 week prior to stressful condition and 10 days during.	Pharmacy practice task	STAI, VAS, sAA	Psychosocial stress lower in L-THE group (p=0.020) sAA lower in the L-THE group (p=0.032)	No significant corrections between STAI and sAA for L-THE (all p's>0.05)
White et al. (2016)	L-THE (200mg in a 430ml in a nutrient drink) Placebo	34 (15;19)	26.5±5.0	Randomised, placebo-controlled, double-blind crossover design. Acute	Multitasking framework	STAI, Bond-Ladder and Mood scales.	One hour post-dose subjective stress (p=0.003) and self-rated stress (p=0.006) levels were lower in L-THE group, and , cortisol was post-dose (p=0.047)	Posterior resting alpha activity was higher with L-THE in high trait anxiety group (p=0.019, one-tailed)
Yoto et al. (2012)	L-THE (200mg) + Placebo Caffeine (100mg) + Placebo Placebo only	16 (8;8)	22.8±2.1	Randomised, placebo-controlled, cross over design. Acute	Target detection test Arithmetic mental task Cold pressor test	BP, POMS, VAS	Decrease in systolic (p=0.008) and diastolic BP (p=0.006) after mental task in the high response groups.	Reduced Tension-Anxiety scores in POMS compared with placebo (p=0.004)

637

638

639

640

641

642 **Table 2.** Summary of risk of bias assessment of included studies

	Higashiyama et al. (2011)	Kimura et al. (2006)	Lu et al. (2004)	Ritsner et al. (2011)	Rogers et al. (2008)	Sarris et al. (2018)	Unno et al. (2013)	Yoto et al. (2012)	White et al. (2016)
Random sequence generation (selection bias)	Unclear	Low	Low	Low	Unclear	Low	Low	Low	Low
Allocation concealment (selection bias)	Low	Low	Low	Low	Low	Low	Low	Unclear	Low
Blinding of participants and personnel (performance bias)	Low	Low	Low	Low	Low	Low	High [†]	Unclear	Low
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Unclear	Unclear	Unclear	Unclear	Low	Low	High [†]	Unclear	Unclear
Blinding of outcome assessment (detection bias) (Mortality)	Low	Low	Low	Low	Low	Low	Low	Low	Low
Incomplete outcome data addressed (attrition bias)	Low	Low	Low	Low	Low	Low	Low	Low	Low
Selective reporting (reporting bias)	Low	Low	Low	Low	Low	Low	Low	Low	Low

643 [†] This study was single-blinded