Acute Tryptophan Depletion Modulates Kynurenine Production: A Challenging Component of Cognitive Impairment in Brain-gut Axis Disorders

P.J. Kennedy[1,2], A. P. Allen[1,2], A. O'Neill[1], E.M.M. Quigley[1,3], J. F. Cryan[1,4], T. G. Dinan[1,2], G.Clarke[1,2]

[1]Alimentary Pharmabiotic Centre, [2]Department of Psychiatry, [3]Department of Medicine, [4]Department of Anatomy & Neuroscience, University College Cork, Cork, Ireland.

EMAIL: pj.kennedy@ucc.ie

University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Alimentary Pharmabiotic Centre

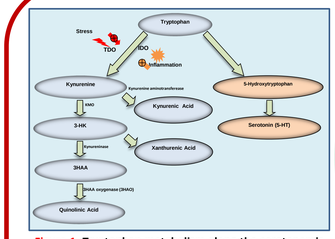


Figure 1. Tryptophan metabolism along the serotonergic pathway or kynurenine pathway. Tryptophan metabolism along the kynurenine pathway is dependant on the activity of indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO). The expression of TDO and IDO can be induced by stress (elevated glucocorticoids) or inflammatory cytokines, respectively.

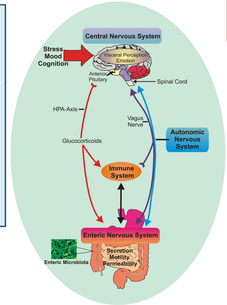


Figure 2: The brain-gut axis (1)

1. Introduction

- Acute tryptophan depletion (ATD) has commonly been used to examine the behavioral and cognitive consequences of challenging the serotonergic system.
- The specificity of ATD to modulate serotonergic activity alone has recently come under scrutiny (**1**) and the impact of ATD on kynurenine production, the predominant pathway of tryptophan metabolism (**Figure 1**), has yet to be determined.
- Manipulating kynurenine and downstream metabolites may modulate central nervous system glutamatergic and cholinergic signaling, key neurotransmitter systems in regulating cognitive function, in addition to affecting gastrointestinal symptomatology (**Figure 3**).
- Altered tryptophan metabolism along the kynurenine pathway (**2**) and impaired visuospatial memory (**3**) has been reported in Irritable bowel syndrome (IBS) a brain gut-axis disorder (**Figure 2**). However, if altered tryptophan metabolism along the kynurenine pathway underlies cognitive impairment in IBS is currently unknown.

2. Aims

- To determine if experimentally modulating tryptophan availability using acute tryptophan depletion (ATD) alters peripheral kynurenine production in patients with IBS.
- To investigate if modulating peripheral kynurenine production affects central measures of cognition, mood and arousal, in addition to GI symptomatology.

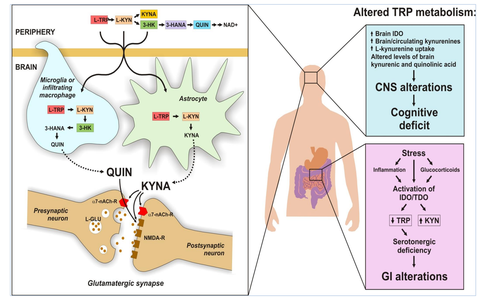


Figure 3. The potential effect of altered tryptophan metabolism along the kynurenine pathway on gastrointestinal symptoms and cognition in irritable bowel syndrome.

3. Methods

Study Design:

- Double blind, placebo controlled, crossover design.

Study Population:

- 9 female, Rome III positive IBS patients & 15 matched female healthy control participants (see **Table 1** for sample characteristics at baseline)
- All participants screened for psychiatric co-morbidity using the MINI Psychiatric interview
- Females not using contraceptive tested during follicular phase of cycle

Acute Tryptophan Depletion (ATD):

Participants consume a drink containing a number of amino acids but lacking tryptophan. As tryptophan competes with other amino acids to cross the blood brain barrier (BBB), experimentally reducing blood levels limits the amount crossing the BBB for further metabolism (see **Figure 4**).

Amino Acid Drink Composition:

- The ATD (**Trp-**) amino acid mixture (100 g; *Glanbia Nutritionals, Germany GmbH*) was based on a previously published composition (**6,7**)
- The control (**Trp+**) mixture had the addition of 3g of L-Tryptophan
- The total quantity of the amino acid mix was reduced by 20% to account for lower body weight of females.

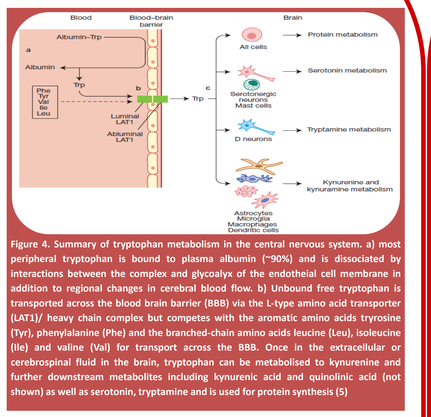
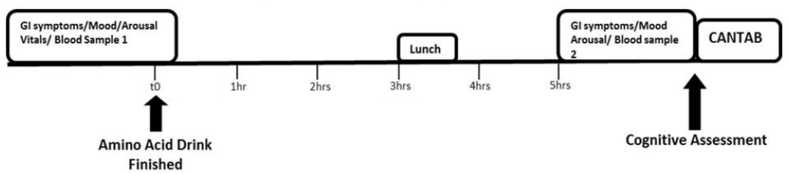


Figure 4. Summary of tryptophan metabolism in the central nervous system. a) most peripheral tryptophan is bound to plasma albumin (~90%) and is dissociated by interactions between the complex and glycoalyx of the endotheial cell membrane in addition to regional changes in cerebral blood flow. b) Unbound free tryptophan is transported across the blood brain barrier (BBB) via the L-type amino acid transporter (LAT1)/ heavy chain complex but competes with the aromatic amino acids tyrosine (Tyr), phenylalanine (Phe) and the branched-chain amino acids leucine (Leu), isoleucine (Ile) and valine (Val) for transport across the BBB. Once in the extracellular or cerebrospinal fluid in the brain, tryptophan can be metabolised to kynurenine and further downstream metabolites including kynurenic acid and quinolinic acid (not shown) as well as serotonin, tryptamine and is used for protein synthesis (5)

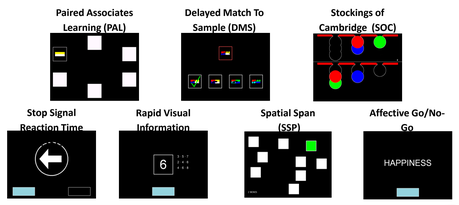
Experimental Day Timeline (separated by at least 7 days):



Measures:

- Plasma free and bound tryptophan/total kynurenine /kyn:trp ratio (**HPLC**).

Cognitive Function:



4. Results

Baseline Sample Characteristics

	Healthy Controls (n=14)	IBS (n=9)	p-value
Age	21.54 ± 0.45	22.78 ± 1.24	0.37
BMI	23.92 ± 2.84	24.12 ± 1.19	0.88
WAIS-R Full Scale IQ	106.28 ± 2.23	108.56 ± 2.16	0.48
Years of Education	16.93 ± 1.59	17.56 ± 0.84	0.47
Units of Alcohol per week	4.18 ± 1.01	3.44 ± 0.86	0.62
Hormonal Contraceptive Use (%)	12 (85.7%)	7 (77.8 %)	-
IBS Symptoms	25.92 ± 7.65	142.44 ± 18.76	<0.001***
STAI Trait	34.5 ± 2.28	34 ± 2.24	0.88
STAI State	27.36 ± 1.06	32.44 ± 2.71	0.057
BDI-II	4.5 ± 1.27	7.56 ± 2.19	0.21
PSS	11.93 ± 1.69	14.38 ± 1.74	0.36
PSQI	4.21 ± 0.72	6.11 ± 1.32	0.18

Table 1: Comparisons between IBS patients and healthy controls on demographic and clinical characteristics. Study participants were matched on the basis of age , IQ, years of education, body mass index (BMI) and units of alcohol consumed per week. Patients and controls did not significantly differ on state/ trait anxiety, depression, perceived stress, or sleep quality. Data are expressed as mean± SEM. Independent samples t-tests using IBM SPSS V20.0 were used to determine group differences. IBS, irritable bowel syndrome; WAIS-R, Wechsler Adult Intelligence Scale-Revised; STAI, State-Trait Anxiety Inventory; BDI-II, Beck Depression Inventory; PSS, Perceived Stress Scale; PSQI, Pittsburgh Sleep Quality Index.

ATD Modulates Plasma Kynurenine

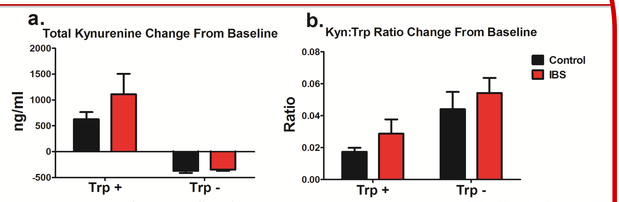


Figure 6. a. There was a significant main effect of treatment on plasma total kynurenine levels ($F(1, 21)= 46.601$, $p < 0.001$, $h_p^2= 0.689$), but no differential group effect ($F(1, 20)= 2.963$, $p= 0.101$, $h_p^2= 0.129$). Plasma total kynurenine significantly increased following the control (Trp+) drink ($p=<0.001$) and significantly decreased following the ATD (Trp-) drink ($p<0.001$). **b.** there was a significant main effect of treatment on the plasma kynurenine:tryptophan ratio (Kyn:Trp Ratio; $F(1, 21)= 10.403$, $p = 0.004$, $h_p^2 = 0.331$), but no differential group effect ($F(1, 21)= 1.992$, $p= 0.173$, $h_p^2 = 0.087$). The plasma Kyn:Trp ratio significantly increased following both Trp+ ($p<0.001$) and Trp- ($p<0.001$). The plasma Kyn:Trp ratio increased on average by 35.51% in healthy controls and 69.62% in patients with IBS following Trp+, and showed a 85.46% and 129.29 % increase in healthy controls and patients with IBS, respectively, following Trp-. Data are expressed as mean± SEM.

ATD Modulates Plasma Tryptophan

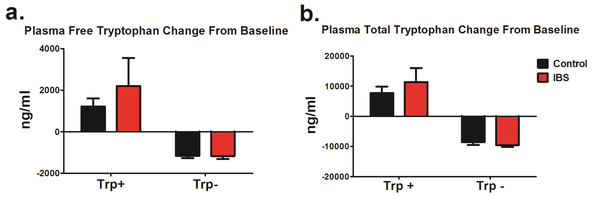


Figure 5. a. There was a significant main effect of treatment on plasma free tryptophan levels ($F(1, 21)= 20.618$, $p < 0.001$, $h_p^2= 0.495$) but no differential effect between patients with IBS and healthy controls ($F(1, 21)= 0.812$, $p= 0.378$, $h_p^2= 0.037$). Plasma free tryptophan significantly increased following the control (Trp+) drink ($p=0.008$) and significantly decreased following the ATD (Trp-) drink ($p<0.001$); **b.** There was a significant main effect of treatment on plasma total tryptophan levels ($F(1, 21)= 55.582$, $p < 0.001$, $h_p^2= 0.726$), but no differential group effect ($F(1, 21)= 0.506$, $p= 0.485$, $h_p^2= 0.024$). Plasma total tryptophan significantly increased following the control (Trp+) drink ($p=<0.001$) and significantly decreased following the ATD (Trp-) drink ($p<0.001$). Data are expressed as mean± SEM.

ATD Improves Visuospatial Memory in IBS

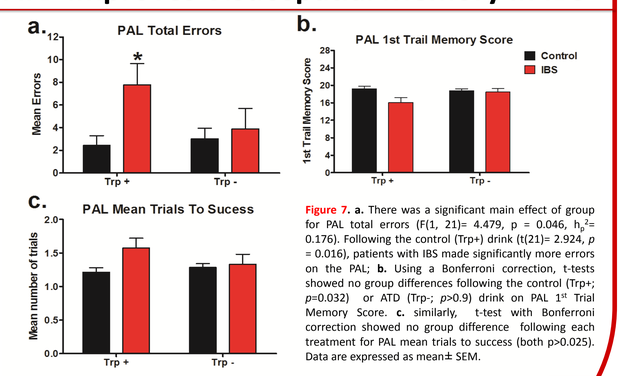


Figure 7. a. There was a significant main effect of group for PAL total errors ($F(1, 21)= 4.479$, $p = 0.046$, $h_p^2= 0.176$). Following the control (Trp+) drink ($t(21)= 2.924$, $p = 0.016$), patients with IBS made significantly more errors on the PAL; **b.** Using a Bonferroni correction, t-tests showed no group differences following the control (Trp+; $p=0.032$) or ATD (Trp-; $p>0.9$) drink on PAL 1st Trial Memory Score. **c.** similarly, t-test with Bonferroni correction showed no group difference following each treatment for PAL mean trials to success (both $p>0.025$). Data are expressed as mean± SEM.

ATD had no effect on additional cognitive, mood, arousal or GI symptom measures in patients with IBS or healthy controls

5. Conclusions

- Our results further question the specificity of ATD as an exclusively serotonergic challenge and suggest that via kynurenine production, this protocol may impact on cognition.
- The impact of ATD on glutamatergic and cholinergic neurotransmitter systems may lead to visuospatial memory impairments. However, this effect only emerged in our vulnerable IBS cohort who have pre-existing alterations in both kynurenine production and cognitive performance.
- These data have important implications for the current conceptual basis and specificity of the ATD protocol. Moreover, they provide some much needed insight to the central mechanisms underlying the cognitive neurobiology of IBS.

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