

Cognitive effects of acute tryptophan depletion in the healthy elderly

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Background: Studies investigating the cognitive effects of serotonin depletion, using the technique of acute tryptophan depletion (ATD) by dietary means, have generally suggested that ATD impairs delayed verbal recall and recognition. In two previous studies in the elderly, this result has not been replicated and ATD impaired working memory. These results may be susceptible to type II error but a similar testing schedule in the individual studies allows data to be pooled in a larger analysis.

Methods: Data from two separate double-blind placebo-controlled studies of the effects of ATD on cognitive function in the elderly were combined. In one study, a low dose and in the other a high dose of amino acids was used. In a repeated measures analysis of variance, the effects of ATD and the interaction of this with the other factors (age, gender and dose) on cognitive measures was examined.

Results: Data from 31 healthy subjects aged between 60 and 81 years were analysed. There were no main effects of ATD or consistent interactions between ATD and age, gender or dose. There were significant interactions between ATD, gender and dose. When tryptophan depleted, females having the higher dose drink had reduced scores on Digit span and immediate recall on the Rey Auditory Verbal Learning Test.

Conclusion: The enlarged data set did not confirm an overall effect of ATD on working memory or on delayed word recall but does suggest an effect of ATD on encoding or registration in the subgroup of females receiving a higher strength drink.

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Introduction

The technique of acute tryptophan depletion (ATD) has been used to study the role of the serotonin (5-HT) system in cognitive function, both in healthy younger subjects and in groups of patients with conditions involving abnormalities of the 5-HT system. Although there are several methods available, this technique typically involves administration of a balanced amino acid drink, which is lacking in tryptophan (TRP). By this means, peripheral TRP levels are reduced by 70–80% within 5–7 h (1) and central 5-HT synthesis is also reduced (2).

Studies in healthy volunteers consistently suggest that ATD impairs delayed recall and recognition of verbal material in particular, with little consistent evidence of impairment of executive function secondary to ATD and in some studies even

improvement in focused attention and executive function [see (3) for review]. Studies that have found evidence of executive deficit secondary to ATD have found specific impairment in functions subserved by ventral and medial prefrontal areas (3). In contrast, our studies in elderly subjects have not replicated impairment in memory consolidation but have found impairment in executive function and working memory (4,5).

Clearly these inconsistencies may have arisen for a number of reasons.

- (a) Most of the studies are small (15–20 subjects) giving rise to the possibility of type II errors. Such errors may be made more likely by the increased variability in cognitive function in elderly compared with younger groups.
- (b) Different neuropsychological testing batteries with tasks, which may differ in sensitivity have

been used in different studies. Sensitivity may also differ depending on the age group being studied. We previously hypothesised that a likely reason for our finding of impairment in executive function and working memory tasks following ATD in the elderly groups was that performance on these tasks is reduced in the elderly (6) and is therefore more susceptible to pharmacological manipulations such as ATD.

- (c) Groups may differ in their sensitivity to the effects of ATD. For example, age-related changes in the 5-HT system may result in greater vulnerability to ATD challenge in the elderly. It is also possible that this may be more relevant in females given the reduction in oestrogen secretion seen at menopause and the effects of oestrogen on 5-HT function and cognitive function (7). Furthermore, females may have a higher turnover of 5-HT and a greater degree of reduction in 5-HT synthesis during ATD (2).
- (d) Studies vary according to the size and exact composition of the amino acid used. The degree of 5-HT depletion may, therefore, vary across studies.

To clarify some of these factors, the effects of ATD, specifically on word learning, has recently been examined in a mega-analysis of data from a number of studies, including our own, in order to examine the effects of various variables including age and gender (8). The main findings from this analysis are that ATD did impair delayed word recall but that immediate recall was also impaired. This finding was greater in females and was independent of age, although unsurprisingly, cognitive function declined with age. The magnitude of these effects did not correlate with plasma TRP levels, a possible measure of the degree of the challenge to the 5-HT system.

Data from healthy elderly subjects investigated in two separate studies have therefore been presented previously:

- (a) As two separate studies in which the elderly subjects were control groups in studies of Alzheimer's disease (4,9) and recovered depression (5) and in which mood and neuropsychological effects of ATD were compared between the control and patient groups.
- (b) Data from the Rey Auditory Verbal Learning Task (RAVLT) (10) have been analysed in a mega-analysis of the effects of ATD on word learning and mood with data pooled from these and seven other studies of the effects of ATD on cognitive function (8).

The purpose of this analysis was to extend these observations by combining data to make a large group of healthy older people, across an age range of 60–81 years. This has the advantage of examining data from a larger group of elderly subjects than in the two individual studies and across a wider range of cognitive functions than the analysis of Sambeth et al. Factors which can be examined in this analysis are the overall effects of ATD in a larger group and in addition the effects of age and gender. The analysis can also directly compare the effects of two different strength amino acid drinks. We hypothesised the following:

- (a) That in this combined elderly group, ATD would induce deficits in executive function.
- (b) That given the greater power in this study, we would be able to demonstrate impaired memory during ATD.
- (c) That females and, in particular, those receiving the higher strength drink would be more vulnerable to the effects of ATD.

Methods

Participants

Thirty-six healthy subjects took part in the two studies [$n = 17$ in study 1 (4); and $n = 19$ in study 2 (5)]. All were aged between 60 and 81 years of age. No subject reported a personal or family history of depression or was experiencing current depressive illness and all had a Montgomery-Asberg Depression Rating Scale (MADRS) (11) score of ≤ 10 (study 1) or < 8 (study 2) and a Geriatric Depression Scale (12) score of < 12 . No subject had dementia as defined by a Mini-Mental State Examination (13) score of ≤ 27 . There was no history of significant head injury and no subject was on medication known to affect the 5-HT system. A physical and psychiatric examination found no current physical or psychiatric illness. After a full description of the study, all subjects gave written, informed consent. The study was approved by Newcastle and North Tyneside Local Research Ethics Committee.

One female subject withdrew from study 1 during the first visit because of nausea. Two subjects from study 2 declined to return after the first visit. Thirty-three subjects completed both experimental days, of whom 18 were male and 15 were female and results are given for these subjects.

Procedure

Subjects were tested twice, at least 1 week apart, in a double-blind, placebo-controlled, counterbalanced,

Mace et al.

randomised crossover designed study. Subjects attended the research unit at 0830 hrs following an overnight fast and underwent baseline assessments. An amino acid drink was administered at 0900 hours on both occasions, with subjects receiving either the depleting or the placebo composition according to the experimental design. All drinks were mixed in 300 ml of water, flavoured with blackcurrant and sweetened with saccharin.

Drink composition

In study 1, 50 g was given to both males and females. In study 2, males received 100 g and females 80 g. In each study, both depletion and placebo were of identical composition, with the exception of the placebo drink, which in the 100 g strength contained an additional 2.3 g of L-TRP. The composition of the 100 g drink as described by Young (14) was: L-alanine 5.5 g, L-arginine 4.9 g, L-cysteine 2.7 g, L-glycine 3.2 g, L-histidine 3.2 g, L-isoleucine 8.0 g, L-leucine 13.5 g, L-lysine monohydrochloride 11 g, L-methionine 3 g, L-phenylalanine 5.7 g, L-proline 12.2 g, L-serine 6.9 g, L-threonine 6.5 g, L-tyrosine 6.9 g and L-valine 8.9 g. The composition of the smaller drinks was 80% (80 g) and 50% (50 g) of the larger drink. The 100 g (80 g for females) drink will be referred to as 'high dose', while the 50 g drink will be referred to as 'low dose'.

Biochemical assessment

Ten millilitres of venous blood was taken during each experimental session at 0, 4 and 7 h post-drink. The blood was added to anticoagulant and the plasma was immediately separated by centrifugation. A sample for free TRP was further centrifuged using an ultrafiltrate tube. All samples were stored at -20°C until assay. Plasma total and free TRP was determined by high pressure liquid chromatography by the method of Marshall et al. (15).

Mood assessment

Mood was assessed three times on each test day at 0, 4 and 7 h. A higher score reflects a lower mood.

Montgomery-Asberg Depression Rating Scale. The MADRS (11) is a subjective rating of mood with 10 items scored on a six-point scale. Total score is out of 60.

Neuropsychological assessment

Neuropsychological testing was carried out between 4 and 6 h after consumption of the drink. Within each study, tests were performed on both occasions

in the same order, by the same administrator who was blind to whether the drink was active or placebo. Where available, parallel versions of the tests were used on the second test day. Computerised tests were taken from the Cambridge Neuropsychological Test Automated Battery (CANTAB) (16).

In study 1 (4), tests were administered in the following order: Modified Mini-Mental State Examination (3MS) (17), Digit Span Forwards and Backwards (18), Vigil (19), Motor Screening, Spatial Working Memory (SWM), RAVLT (10), Paired Associate Learning (PAL), Simultaneous (SMTS) and Delayed Matching to Sample (DMS), Rey Visual Design Learning Task (RVDLT), Controlled Oral Word Association (COWA) task (20), Verbal Fluency Performance Test (VFPT) (21), Pattern Recognition Memory, Spatial Recognition Memory.

In study 2 (5), tests were administered in the following order: 3MS, Digit Span Forwards, RAVLT (recognition trial omitted), RVDLT (delayed recall and recognition trials omitted), Simultaneous (SMTS) and DMS, RAVLT, Vigil AK, Digit Span Backwards, COWA, VFPT, SWM, Tower of London (TOL).

In study 1, the VIGIL task used response to a single letter K, then in a second task response to a letter K immediately preceded by the letter A. As we argued at the time, this created a go-no-go phenomenon, which we believe makes it incomparable with the AK form used alone in the second study. Different forms of PAL were used in the two studies making it untenable to combine data. Likewise for RAVLT recognition and RVDLT delayed recall/recognition trials.

Therefore tests common to both studies were as given below.

Global cognitive functioning. Modified Mini-Mental State examination. A clinical test of a range of cognitive domains (17).

Attention and Executive function. COWA test. The COWA test (20) assesses verbal fluency and comprises three trials, each lasting 60 s, in which subjects are required to generate as many words as possible beginning with the given letters, in sequential order, 'F', 'A' and 'S'. Subjects are asked to exclude proper nouns, repetitions and grammatical variations of the same word. Performance is assessed as the sum of acceptable words generated across the three trials. In the parallel form, the letters 'C', 'F' and 'L' are used.

Verbal Fluency Performance Test. Excluded letter fluency was assessed by the number of words

subjects can generate in two 60-s trials that do not contain a specified letter. In the first trial, subjects are required to produce as many words as possible not containing 'E', and in the second trial words not containing 'A'. The total number of words produced across the two trials is summed (21).

Digit span. Digit span (22) comprises two trials. In the first trial (Digit Span Forwards), the subject is asked to remember a series of digit spans, increasing in length from three to nine numbers, and repeat them back to the investigator. In the second trial (Digit Span Backwards), the subject is asked to follow the same procedure but is asked to repeat each span of digits in reverse order. The spans increase in length from two to eight. The number of spans correct is tallied for a total score in each component.

CANTAB Spatial working memory. Subjects search through a number of 'boxes' (4, 6 or 8) for a hidden 'token', without returning to a box they have already examined (to avoid 'within search errors') or have already emptied (to avoid 'between search errors') on the same trial. Accuracy and latency are recorded for all levels plus an overall strategy score.

Learning and memory. Rey Auditory Verbal Learning Test. Subjects are read a list (A) of 15 words at a constant rate and asked to repeat these back to the administrator in any order. The list is repeated four times, the subject being asked on each occasion to repeat as many words as possible. A distracter list (B) is read and subjects asked to recall as many words as possible from this. Without repeating list A, the subject is asked again to remember as many words as possible from this list. After 20 activity-filled minutes, the subject is asked to recall the words from list A (10).

Rey Visual Design Learning Test. This is a visual analogue of the RAVLT (10). The procedure is essentially the same except that the subject is shown 15 simple geometric designs and at the end of each presentation asked to recall and draw as many designs as possible. The procedure is repeated five times. Unlike the RAVLT, there is no distracter set of designs or a delayed recall, but there is a recognition trial in which subjects are shown 30 designs and asked to indicate which comes from the set already viewed.

SMTS and DMS. The subject is shown a complex visual pattern (index) and then four patterns, one (the target pattern) of which is identical to the

index pattern plus three novel (distracter) patterns. The subject is asked to touch the pattern that matches the index pattern. After a practice session, there are 40 counterbalanced test trials in which the index, target and distracter patterns are shown either simultaneously or after a delay of 0, 4 or 12 s. Response rate and accuracy are recorded for all levels. For example, DMS Total Correct (simultaneous), reports the number of trials for which the subject selects the correct stimulus in simultaneous trials (when the stimulus is left in view while the target stimulus and three distracters are simultaneously presented).

Statistical analysis

SPSS for Windows Release 13 (SPSS, Chicago, IL, USA) was used. Demographic data between studies were compared using unpaired *t*-tests and in the case of gender chi-squared tests. All variables were analysed by repeated measures analysis of covariance with 'treatment' (placebo or depletion) as a within-subject factor. When a test was administered at different times during the day (e.g. MADRS) 'time' was entered as a further within-subject factor. 'Dose' was classified as high (100/80 g) or low (50 g). This factor and 'gender' were entered as between-subject factors. Order of administration of drinks (placebo first vs. depletion first) was entered as a between-subject factor but subsequently omitted from the analysis if there was no main effect or interaction with 'treatment'. Age was entered as a covariate. Reported *p* values were corrected using the Huynh-Feldt correction factor when the sphericity assumption for the 'time' factor was not met. For clarity, uncorrected degrees of freedom are reported. Data are quoted as estimated marginal means \pm standard errors.

Results

Demographic

The mean age was 70.4 years (SD = 5.61, range = 61–81). Eight males and eight females had the low dose. Ten males and seven females had the high dose.

Comparison of demographic and baseline data between studies is given in Table 1. Groups were well matched between studies except on age, which was significantly younger in the high-dose study ($F_{1,31} = 3.05$; $t = 0.005$).

Missing data

Biochemical data from two subjects were missing: one male having the low dose and one female having the high dose.

Table 1. Demographics of subjects compared between studies

Variable	Low-dose study		High-dose study	
	Mean	SD	Mean	SD
Age	73.13	4.9	67.82	5.1
Gender (male/female)	8/8		10/7	
National Adult Reading Test Score (NART IQ)	107.8	8.4	112.0	10.9
Baseline CAMCOG	99.2	3.5	99.6	3.9
Baseline MADRS	1.94	2.2	1.06	1.9

Biochemical

There was a significant effect of depletion, significant two-way interactions between treatment and dose, and depletion and time and a significant three-way interaction between treatment, dose and gender on free TRP (Fig. 1 and Table 2).

Mood

Treatment had no significant effects on MADRS score and there was no interaction between treatment and time. There was no significant interaction between treatment and other variables.

Neuropsychological measures

Effects of treatment and interactions between treatment, gender and dose are shown in Table 2. There were no main effects of treatment on any neuropsychological variables. There were no independent effects of age, interaction of age with treatment or three-way interactions between age, treatment and dose or gender. There were no significant main effects of order or interactions with treatment. Only statistically significant results for individual assessments are referred to in the text.

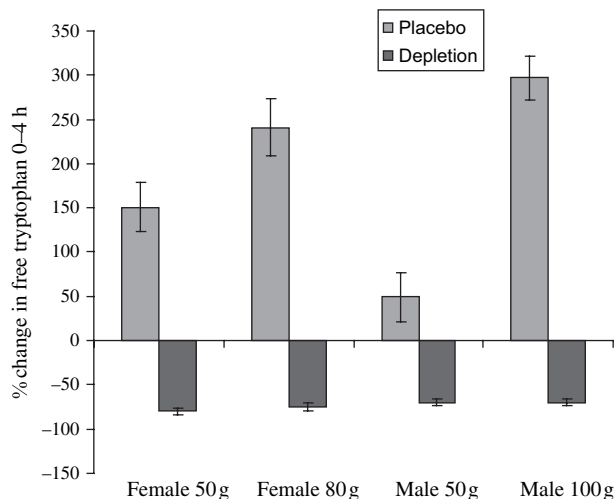


Fig. 1. Percentage change in free TRP levels.

Digit Span Forwards. On Digit Span Forwards (DigitsF), there was a significant interaction between treatment, dose, and gender ($F_{1,24} = 4.20, p = 0.05$). Estimated marginal means are shown in Fig. 2. The greatest reduction in performance during ATD was in the females receiving the high-dose amino acid drink.

RAVLT trials I–V. On RAVLT trials A1 to A5, there was a significant interaction between treatment, dose, and gender ($F_{1,28} = 5.74, p = 0.024$). The greatest reduction in performance during ATD was again in the females receiving the high-dose amino acid drink.

COWA: Letter fluency. There was a significant interaction between treatment, dose, and gender ($F_{1,28} = 4.40, p = 0.045$). Females receiving the high dose had a lower score during ATD but the reduction in performance was greatest in males receiving the low dose.

CANTAB SMTS total correct. There was a significant interaction between treatment, dose and gender ($F_{1,28} = 5.66, p = .024$). While there was a reduction during ATD in females receiving the high dose, there was an increase of greater magnitude in females receiving the low dose.

Discussion

The current analysis is the first to examine the effects of ATD on a range of cognitive functions in a large group of healthy elderly participants. The principal findings of this analysis are as follows:

- There was an interaction between treatment, dose and gender on free TRP levels. Reduction in free TRP was similar in males and females and with both doses. However, the increase in free TRP following placebo was greater following the higher dose and more so in males (see Fig. 1).
- There was a significant interaction between treatment, dose and gender on several neuropsychological measures: Digit Span Forwards, total words recalled on the RAVLT trials 1–5, COWA and SMTS.
- The pattern of this interaction in Digit Span Forwards and RAVLT was the same with the greatest difference between depletion and placebo (in each case scores were lower during depletion) being in females following the higher dose amino acid drink (Fig. 1). In the COWA, there was a reduction in performance in females receiving the higher dose, but not as

Tryptophan depletion and cognition in the elderly

Table 2. Effects of ATD on cognitive measures. Repeated measures analysis of variance was used

Assessment	Variable	Treatment	Treatment × dose	Treatment × gender	Treatment × dose × gender
		F(df) p	F(df) p	F(df) p	F(df) p
Free TRP	% change 0–4 h	0.01(1,26) 0.92	34.72(1,26) 0.00*	1.51(1,26) 0.23	7.68(1,26) 0.01*
Free TRP	% change 0–7 h	1.36(1,26) 0.26	16.05(1,26) 0.00*	0.61(1,26) 0.44	2.47(1,26) 0.13
3MS		0.03(1,28) 0.86	0.30(1,28) 0.59	0.01(1,28) 0.95	3.13(1,28) 0.09
Digit Span	Forwards	0.33(1,24) 0.57	2.20(1,24) 0.15	0.15(1,24) 0.70	4.20(1,24) 0.05*
	Backwards	2.13(1,28) 0.16	0.99(1,28) 0.33	0.28(1,28) 0.60	0.99(1,28) 0.33
	Total	1.79(1,28) 0.19	0.02(1,28) 0.89	0.34(1,28) 0.56	0.20(1,28) 0.66
COWA		0.03(1,28) 0.86	0.11(1,28) 0.74	0.12(1,28) 0.73	4.40(1,28) 0.045*
VFPT		0.12(1,28) 0.73	0.58(1,28) 0.45	0.79(1,28) 0.38	0.03(1,28) 0.86
RAVLT	Trials I–V	0.96(1,28) 0.34	0.85(1,28) 0.36	1.71(1,28) 0.20	5.74(1,28) 0.024*
	Delayed recall	1.88(1,28) 0.18	0.001(1,28) 0.97	0.016(1,28) 0.90	0.65(1,28) 0.43
RVDLT	Trials I–V	0.49(1,26) 0.49	1.85(1,26) 0.19	0.69(1,26) 0.41	0.29(1,26) 0.60
SWM	Strategy	0.98(1,28) 0.33	0.56(1,28) 0.46	4.12(1,28) 0.052	0.88(1,28) 0.36
SWM	Between errors	1.20(1,28) 0.28	1.25(1,28) 0.27	0.39(1,28) 0.54	0.06(1,28) 0.82
SMTS	Accuracy	0.49(1,28) 0.49	0.42(1,28) 0.52	0.002(1,28) 0.97	5.66(1,28) 0.024*
SMTS	Latency	0.72(1,28) 0.40	2.59(1,28) 0.12	0.00(1,28) 0.99	0.45(1,28) 0.51
DMS	Accuracy total	0.21(1,28) 0.65	0.22(1,28) 0.64	1.21(1,28) 0.28	1.34(1,28) 0.26
DMS	Latency total	2.67(1,28) 0.11	2.15(1,28) 0.15	0.05(1,28) 0.83	0.98(1,28) 0.33

* $p < 0.05$.

great as that seen in the males receiving the lower dose drink. In SMTS, the reduction in the females receiving the higher dose was not as great as the increase in the females receiving the lower dose drink.

As noted, there was an interaction between treatment, dose and gender on free TRP levels probably accounted for by a greater increase in free TRP in the males receiving the high-dose placebo drink (Fig. 1). The most likely explanation for this

finding is simply that males in study 2 received a 100-g drink and therefore the largest dose of L-TRP (see Drink composition, Methods section). The finding suggests that the males did not compensate for the greater TRP load in the placebo drink by faster peripheral metabolism or any other factor. The fact that plasma-free TRP was equally reduced in males and females receiving either dose of the TRP-free mixture suggests that in these cases peripheral protein synthesis during the procedure could only increase by a certain amount

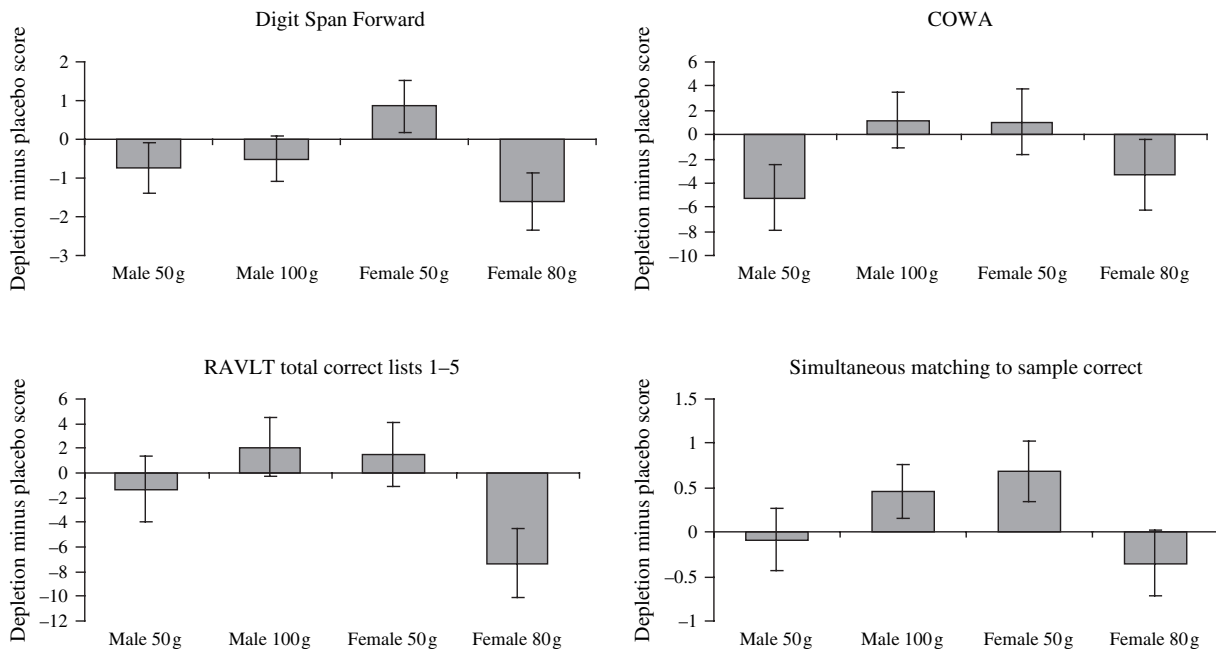


Fig. 2. Neuropsychological tests for male and female subjects following high- or low-dose drink (mean ± standard error).

and reached a ceiling, above which it would not increase regardless of an additional load of amino acids. However, the other mechanism of reduction of central 5-HT synthesis in this technique is by increased competition for transport across the blood–brain barrier by other large neutral amino acids (LNAA). It is likely that this competition is greater in a larger drink and that the effect on central TRP availability and hence 5-HT synthesis was greater. Unfortunately, we did not measure LNAA to allow comparison of the TRP/LNAA ratio between doses.

On neuropsychological measures, a similar pattern of response was observed in the RAVLT immediate word recall and Digit Span Forward tasks (Fig. 2). In both, the greatest effect was an ATD-associated reduction in the scores in females having the higher dose. Our finding of a gender-specific effect is in keeping with previous studies suggesting greater vulnerability to behavioural effects of ATD in females. A ‘mega-analysis’ (including the RAVLT data from this study) showed that the effect of ATD on aspects of word learning was greater in females than males (8). Nishizawa et al. (2), Okazawa et al. (23) and Sakai et al. (24) in positron emission tomography (PET) and magnetic resonance imaging (MRI) studies have also shown a 40–50% higher rate of 5-HT synthesis in males compared with females, and data from cerebrospinal fluid (CSF) studies report greater 5 hydroxyindolacetic acid (5-HIAA) concentration in females (25). These findings suggest that although reduction in free TRP levels did not vary between gender, females may be more vulnerable to the effects of ATD. Furthermore, in earlier work, we have shown that the low-dose amino acid drink has no effect on a range of neuropsychological domains in younger, healthy, male subjects (26).

An effect on Digit Span Forwards and immediate word recall in the absence of an independent effect on delayed word recall suggests an effect on encoding or registration. The findings on the COWA and SMTS are different and difficult to interpret. However, there is consistency at least in the fact that performance was worse during ATD than placebo with the 80-g drink in females. The analysis of Sambeth et al. (8) did find an overall effect on recall of the first repetitions of word lists, and the authors suggest that at least some of this effect is because of impaired encoding and would therefore accord with our current findings. Clearly not all tasks involving a degree of encoding showed the same pattern however. For instance, there were no effects on the DMTS or Digit Span Backwards. It may be that verbal tasks are more

sensitive in this regard and that Digit Span Backwards is complicated by a greater executive load.

Previous studies have been consistent in showing an effect of ATD on delayed recall. No such effect was seen in this analysis. In particular, there was no effect of ATD on delayed recall of words, a variable that has been consistently impaired in previous studies. A possible explanation for our negative finding, compared with studies in younger subjects, is that scores on this measure in elderly subjects are more variable (8) than those in studies in the young, thereby increasing the variance and making statistical significance less likely. The absence of an interaction between age and ATD in this analysis may be explained simply by the relatively narrow age range. As such, the analysis does not answer the question of whether normal ageing affects the vulnerability of the 5-HT system to challenge in this way. The study of Sambeth et al. (8) did not find an interaction between age and ATD on word learning suggesting that ageing does not affect the response to 5-HT depletion.

Potential advantages of this pooled analysis are as follows:

- (a) The larger number of subjects has allowed analysis of factors such as gender with less risk of type II error.
- (b) Compared with that of Sambeth et al. (8), the current analysis covered a broader range of cognitive tasks.
- (c) The studies used two different drink sizes in similar groups in the same setting, thus allowing analysis of the effects of amino acid dose.

There are some important methodological issues and disadvantages.

- (a) The testing schedule was not identical in the two studies and was therefore different between high and low dose. The tests carried out and the order in which this was performed is noted in the Methods section. It is possible that these differing schedules may have introduced a systematic difference between groups because of either differential fatigue or differential interference in performance from previous tests (proactive interference). We believe that differences are more likely to be the result of the effects of the size of the drink especially because they occurred in a consistent fashion in a number of tasks regardless of task placement within the battery. However, the only way to

examine this question definitively would be to randomise subjects to receive either the high- or low-dose drink.

- (b) The high- and low-dose groups were not matched *a priori* on all important variables. We attempted to overcome this in two ways. First, by co-varying for age in each analysis. Secondly, because the analysis was within subjects, differing baseline performance was taken into account. We note that on the screening tool Cambridge Cognitive Examination (CAMCOG), the groups were well matched and that baseline cognitive performance was therefore similar. Moreover, although there was a significant difference in age between the two studies in practical terms, the difference was small (5 years), and, there were no age effects associated with any of the cognitive variables.
- (c) We did not measure LNAA levels and cannot therefore calculate TRP/LNAA ratios. TRP competes with other LNAAs for entry into the brain at a specific carrier protein. The ratio is vital in determining 5-HT synthesis. It is likely that the higher dose reduced central TRP to a greater extent than the low dose because of greater competition by other LNAAs despite similar TRP concentrations. However, we can only infer this from our data because we have no measurement of the ratio.
- (d) Another issue is the neutrality of the placebo drink, which in our study raised the TRP levels more in males having the high-dose drink. In a study using the same technique, in the same centre, TRP/LNAA ratios were calculated in adults suffering from schizophrenia, receiving identical 100-g drinks. TRP/LNAA ratios were not altered by the placebo drink (27). Likewise, in three studies (28–30) using a smaller 75 g dose. This suggests that the balanced amino acid placebo drink is a neutral manipulation. However, because the ratios were determined from different studies in younger medicated subjects with a neuropsychiatric condition, they do not necessarily reflect the situation in the elderly.
- (e) While the analysis was of a large group of elderly subjects and comprised 18 males and 15 females, in the low dose there were only 8 males and 8 females and in the high dose 10 males and 7 females. Therefore, the study did have relatively less power to examine the interaction between dose and gender.
- (f) We have conducted the analysis on a large number of neuropsychological variables and did not use a correction for this. We preferred

to examine neuropsychological results without correction to determine whether a domain-specific pattern emerged. We suggest that the findings on Digit Span Forwards and RAVLT may constitute a pattern that is consistent with the literature. However, the findings on COWA and SMTS may well be due to chance.

Summary

This analysis was able to examine effects of ATD and the interaction of this with dose of depleting drink, age and gender in a large group of healthy elderly subjects. Results are largely negative apart from an interaction between ATD, gender and dose on some neuropsychological variables. We suggest that females receiving high-dose ATD have reduced encoding or registration. This effect is in keeping with previous literature.

Authors' contributions

All authors were involved in analysis and writing of the paper. Richard Porter and John O'Brien produced the protocol. Richard Porter and Peter Gallagher were involved in the conduct of the studies.

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