The cognitive effects of an acute wild blueberry intervention on 7- to 10-year-olds using extended memory and executive function task batteries

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Adrian R. Whyte[1#], Daniel J. Lamport[1#], Graham Schafer[1], & Claire M. Williams[1*]

[1] School of Psychology & Clinical Language Sciences, University of Reading, Earley Gate, Reading, Berkshire, RG6 6AL.

[#] Joint first authors

[*] Corresponding author: Professor Claire Williams, School of Psychology & Clinical Language Sciences, University of Reading, Earley Gate, Reading, Berkshire, RG6 6AL. Tel: 0118 378 7540; Fax: 0118 387 6715; Email: Claire.williams@reading.ac.uk

Abstract

Evidence for the health benefits of blueberries is well documented. In particular memory and executive function benefits have both been found for children aged 7 – 10 in the 6 hour period following acute blueberry consumption. Previous research has utilised a limited number of tasks when considering these domains. Therefore, in two separate experiments, we employed extended memory and executive function task batteries to further understand the extent of blueberry benefits. Following blueberry intervention, children aged 7 – 10 were tested on a memory battery at 75 minutes and an executive function battery at 3 hours. Shorter memory reaction times were observed on the visuo-spatial grid task and shorter executive function reaction times were observed on the congruent trials of the attention network task. Whilst providing further evidence for the cognitive benefits of blueberry consumption in school age children, these findings contrast with previous research where improved accuracy and reaction time benefits have most commonly been found on more cognitively demanding trials. Further research targeted to consider the areas of the brain related to each cognitive domain and how they coincide with mechanisms of action, such as increases in cerebral blood flow following blueberry intervention, is therefore recommended.

Introduction

There is evidence for beneficial effects of blueberries on a number of health outcomes, one of which is cognitive function[1]. Much of the supporting evidence for this comes from human intervention trials whereby cognitive function is assessed with a battery of tests following a single acute dose of blueberries, or following regular daily consumption over several weeks. A review of this literature[2] reported eleven blueberry interventions in various populations including children, healthy adults, and adults with mild cognitive impairment (MCI) with benefits being reported for various aspects of cognition including memory, executive function and psychomotor function. It is hypothesised that these effects can be ascribed to the high flavonoid content of blueberries, for which various mechanisms of action have been proposed[3]. There are some indications that the specific cognitive domains affected by acute flavonoid ingestion vary with the age of participants, i.e. benefits to executive function seem most prevalent in healthy young adults, whilst episodic memory effects are seen in older adults and adults with MCI. These differences may be attributable to the different stages of physiological and neuronal development in the brain across the lifespan, however it should also be noted that benefits are most evident where the cognitive demand of the task is high or the participant is cognitively compromised[4, 5]. This suggests that failure to find effects across

all domains may be a result of tasks not being sufficiently sensitive or optimised for the particular age group being tested. This notwithstanding, children seem sensitive to both executive function and episodic memory tasks (for review see Bell et al.[6]) and are of particular interest as they represent a population who are experiencing rapid neuronal and cognitive development.

Previous research in children has shown acute benefits for cognitive function following wild blueberry consumption. For example, Whyte and Williams[7] demonstrated improved verbal episodic memory 2 hours post consumption of a 30g wild blueberry drink in children aged 8-10[7]. In a subsequent study, further evidence was found for episodic memory benefits at 75 minutes and 6 hours with executive function benefits being found at 3 hours. These findings suggested that the beneficial effects of blueberry in children were modulated by the level of demand, or difficulty, associated with the task[8]. Specifically, the 7- to 10-year-old children showed better performance on the more cognitively-demanding incongruent trials (but not the easier congruent trials) on a flanker task assessing executive function. This effect for executive function 3 hours post consumption was replicated in a further study[5] with a Modified Attention Network Task (MANT), where benefits were again seen for the most demanding aspects of the task. Interestingly, executive function effects have been consistently observed 3 hours post consumption, however, recent research by Barfoot et al. has demonstrated, benefits for both memory and executive function at 2 hours[9]. It should be noted that, at this earlier time point, the executive function benefits found by Barfoot et al.[9] differed slightly from earlier findings[5] in that benefits were found on the shorter stimulus presentation trials and no effect of congruence was evident (see Whyte et al.[5] for discussion regarding overall task difficulty). It is plausible that the different time course for effects on executive function and memory are associated with subtle differences in the mechanisms of action by which flavonoids may interact with the relevant brain regions (i.e. the hippocampus for episodic memory, and the frontal cortex for executive functions). However, this is speculative as specific mechanisms of action are not well known.

Previous studies of blueberries in children have typically only used a single task to measure either memory or executive function. Therefore, the aim of this research was to extend our knowledge of the benefits of blueberries using a range of tests in order to provide a more comprehensive assessment of (i) memory function and (ii) executive function. The length of the task batteries precluded the use of the same participants in the same experiments, therefore two different groups, drawn from the same population (children aged 7- to 10-years) participated. Use of two separate samples also allowed for the targeting of testing points where post-consumption benefits of blueberry intervention have previously been found; 75 minutes for memory function[8] and 3 hours for executive function[5, 8]. This approach also avoided interference effects, thus allowing a purer examination of each cognitive domain. To be clear with regards to time course, the aim here was to test each cognitive domain at a single time point where it has previously been shown to be sensitive to blueberry consumption, rather than testing each domain at multiple time points.

The Auditory Verbal Learning Test (AVLT)[10] used in previous studies[7-9] gives measures of both episodic memory and interference effects. In order to provide a more focused measure of each of these areas we introduced a paradigm targeted specifically at assessment of proactive interference (the Brown-Peterson task). The AVLT is also retained in the battery, however, the interference list presentation and recall has been removed making the task a purer measure of episodic memory. Furthermore, previous research with this age group has focused on the auditory modality of episodic memory, therefore, we also include here a test

of visual memory (Picture Recognition Task) and visuo-spatial working memory (Visuo-Spatial Grid Task). The executive function experiment incorporated three tests assessing aspects of executive function including inhibition (Stop-Go Task), rule switching (Task Switching) and a response interference task for which previous studies have shown sensitivity to blueberries in children (Attention Network Task, ANT, see[2]). In addition, within the ANT task demand can be manipulated, which is important as previous studies have shown that blueberries are most effective when cognitive demand is high[5, 8]. Broadly, we hypothesised that benefits would be observed following blueberry consumption for episodic memory and executive function measures. Furthermore, benefits were expected to be particularly evident for the most cognitively demanding aspects of the tasks such as the incongruent, or initial switch trials on the executive function tasks, or delayed recall on the memory tasks.

Methods

Design

For both experiments participants consumed a wild blueberry drink (BB) or placebo according to a crossover, double-blind design with order of consumption counterbalanced and a seven day washout between test days. Cognitive function was assessed at one time point post consumption (see procedure). A baseline practice day occurred seven days prior to the first test day, for which no drink was consumed. The 200ml BB drink contained 30g freeze dried wild blueberry powder mixed with 170ml water and 30ml vehicle (Rocks Orange Squash). The BB drink contained 253mg anthocyanins, 8.9g fructose, 7.99g glucose, 4mg vitamin C, and 116.4kcal. The placebo was matched with the BB drink for volume, fructose, glucose, vitamin C and kcal, and consisted of 30ml vehicle, 170ml water, and added sugars and vitamin C as described. The vehicle also contained 13.2mg total polyphenols (Narirutin & Hesperidin). The freeze dried blueberries were provided free of charge by the Wild Blueberry Association of North America (WBANA) with the same batch being used for both experiments. Analysis of anthocyanin content was carried out by independent researchers from the University of Reading using the methods described in Rodriguez-Mateos et al.[11], indicating anthocyanin content of 8.43 mg/g which, given a freeze dried to fresh ratio of 7/1, is equivalent to 120.5 mg/100g fresh (see Table 1).

Table 1 Analysis results of WBANA freeze dried wild blueberries showing total anthocyanin content and content broken down by sub class.

	mg/g freeze dried	Stdev	mg/ 100 g fresh BB	stdev
Delphinidin	3.29	0.21	46.94	3.03
Cyanidin	1.17	0.07	16.64	0.95
Petunidin	1.58	0.08	22.50	1.16
Peonidin	0.37	0.02	5.24	0.30
Malvidin	2.04	0.10	29.14	1.50
Total	**8.43**	**0.48**	**120.47**	**6.92**

Participants

For blueberry interventions considering cognitive outcomes, previously published a priori power analysis, using G*Power, with an effect size of 0.45 and alpha level of 0.05 has indicated that 21 participants would be required to achieve a power of 0.85. Exclusion criteria for both experiments were, diagnosis of ADHD (attention deficit hyperactivity disorder) or dyslexia, or a known intolerance to any fruit, whilst an inclusion criterion was English as first spoken language. For experiment (i) twenty children were initially recruited, however, two were excluded for non-compliance (consuming only half of the blueberry drink), and one further child was excluded as an extreme outlier on the Ravens Coloured Progressive Matrices (RCPM). Seventeen children (12 female) aged 7- to 10-years (mean 8.8, s.d. 0.67) were therefore included in the study. For experiment (ii), nineteen children were recruited, though one failed to attend the final test session leaving eighteen (11 females) aged 7-10 (mean age 8.4, s.d. 0.4) in the study. Table 2 shows the characteristics of the samples for both experiments.

Table 2: Participant characteristics; frequencies, means and standard deviations

	Experiment (i) Episodic Memory			**Experiment (ii) Executive Function**		
	All	Females	Males	All	Females	Males
N	17	12	5	18	11	7
Age (yrs)	8.8 (.67)	8.1 (.61)	8.2 (.59)	8.4 (.4)	8.4 (.4)	8.4 (.5)
RCPM	29.1 (3)	28.7 (3.2)	30.2 (2.3)	26.7 (5.9)	26.4 (6.1)	27.1 (6)
RCPM %tile	70.2	64.1	85	66.1	64.1	69.3
Fruit & Veg*	4.5 (1.2)	4.6 (1.3)	4.4 (1.1)	4.6 (1.6)	4.2 (1.9)	5.1 (.6)

* Portions per day as assessed with a questionnaire at screening. RCPM = Ravens Coloured Progressive Matrices

Cognitive Tests

E-Prime V2 (Psychology Software Tools, Inc.) running on a PC with a 15” screen was used to display the stimuli and record participant responses.

Experiment (i) Memory Battery

The cognitive tests were presented in the following order: Auditory Verbal Learning Test (AVLT) Recalls 1-5; Picture Presentation; AVLT trial 6; Brown Peterson; Visuo-Spatial Grid Task; Picture Recognition; AVLT Recall 7; AVLT word recognition. The AVLT followed the same protocol as described in Lezak [10] minus the presentation and recall of interference list B which, as discussed above, was removed to allow for a purer measure of episodic memory. It assesses word learning via free recall and recognition. Verbal responses from the participants were recorded by the experimenter both on paper and using a digital recorder. The AVLT consisted of five consecutive free recalls (Recalls 1 to 5) of the same 15 nouns

(List A) presented auditorily at a rate of 1 word/second. After a 2 minute delay, during which time the participants completed viewing the stimulus for the Picture Recognition Task, there was then a further free recall of List A (Recall 6). This was followed by a fifteen minute delay where participants completed the remaining tasks. A final free recall of List A (Recall 7) was then performed. Finally, participants were shown a list of 50 nouns, containing all the words from List A plus an additional 35 filler words to match the number used in Lezak, and asked to circle only the words from List A. The baseline lists and session 2 lists as employed in Whyte et al.[8] were used for this experiment. Different versions were created for repeated administration, which were counterbalanced across conditions. All words had an age of acquisition (AOA) rating of less than 400 (equivalent to age 7 and below) and were matched for concreteness and familiarity. For each test session the following outcomes as specified in Lezak were calculated: Immediate Word Span (Recall 1); Number of Words Learned (Recall 5 minus Recall 1); Final Acquisition level (Recall 5); and Word Recognition expressed as the number of correctly circled words.

The Picture Recognition Task examined delayed visual recognition and was designed by the researchers to reflect the AVLT. Participants were shown 15 pictures of different landscapes at a rate of 1 per second in a randomised order. A 15 minute delay followed whilst participants completed other tasks (see above). Participants were then shown the original 15 pictures along with 35 novel pictures in a randomised order and were instructed to press a green key ('right arrow' on the keyboard) if they had seen the item previously, or press a red key ('left arrow' on the keyboard) if the item was a novel. The pictures were displayed at a size of 6 x 6 cm and were drawn from the Sun database [12], with memorability ratings between 46-54. Matched versions were created and administered in a counterbalanced order across test days. Outcome variables were correct picture recognitions and reaction time and correct novel picture rejections and reaction time.

The Brown Peterson Task examines proactive interference (PI), and release from proactive interference (RPI). Participants were auditorily presented with 3 letters at a rate of 1/second, excluding vowels and the letter y, with each triplet of letters controlled in such a way as no letters presented were phonetically similar. As a distraction task, 15 colour blocks were presented at a rate of 1/second and the task was to name each colour as it appeared. Participants then recalled the previously presented letters. The process was repeated for a further 3 trials, with a novel set of letters. This concluded the PI section of the task. Three numbers were then presented followed by the same colour block distraction task, followed by recall. As the final numbers trial was from a different semantic category to the letters trials, this final trial was considered to be an RPI measure. For each session a PI measure was calculated by subtracting recall 1 from recall 4 and an RPI measure by subtracting recall 4 from recall 5.

The Visuo-Spatial Grid Task (VSGT) examined visuo-spatial working memory. Participants were shown a 4 x 4 grid on which blue circles would appear within a square of the grid one at a time for 1 second. As each circle appeared the previous one was removed. The main task was preceded by four practice trials. The task was to press the screen in the boxes of the grid where the circles had appeared and in the order that they appeared. Responses started 1

second following the final circle presentation signalled by a beep. Each response left a smaller red circle in the box. After each correct trial the words 'Well done, press space to continue' appeared. For errors, the words 'oops you made a mistake, press space to continue' appeared. If participants failed to complete a minimum of 3 correct responses they were given further coaching to ensure they fully understood the task. The main task followed the same procedure as above, however it commenced with a sequence of 2 circles and an additional circle was added after every two trials. The task was terminated at the point participants were no longer able to correctly recall both trials for a given number of circles. Outcome measures were the maximum number of circle presentations reached without making a mistake and response time for each screen press.

Experiment (ii) Executive Function Battery

The Attention Network Task (ANT) measures executive attention (response interference) orienting and alerting[13]. Following an initial fixation slide of 400-1600 ms duration, either a centre cue, a double cue, a spatial cue, or no cue were randomly presented for 150 ms. There was then a further short fixation period of 400ms. Stimuli (in the form of yellow cartoon fish on a blue background) were then displayed either above or below the fixation point for 250ms and could be congruent, incongruent or neutral depending on whether they matched the direction of the central fish. Stimuli position and congruence type were randomised. A mouse press was required within 1250ms corresponding to the direction the central fish was facing. Feedback was presented in the form of a 'buzz' for an incorrect response or the fish reappearing along with a 'whoohoo' sound for a correct response. Three blocks with 48 trials in each were presented (see Figure 1 for schematic). A practice block of 24 trials preceded the test phase. If an accuracy of below 60% was recorded for the practice a second practice was performed. The outcome measures were accuracy and response times (RTs) for congruency and cue type.

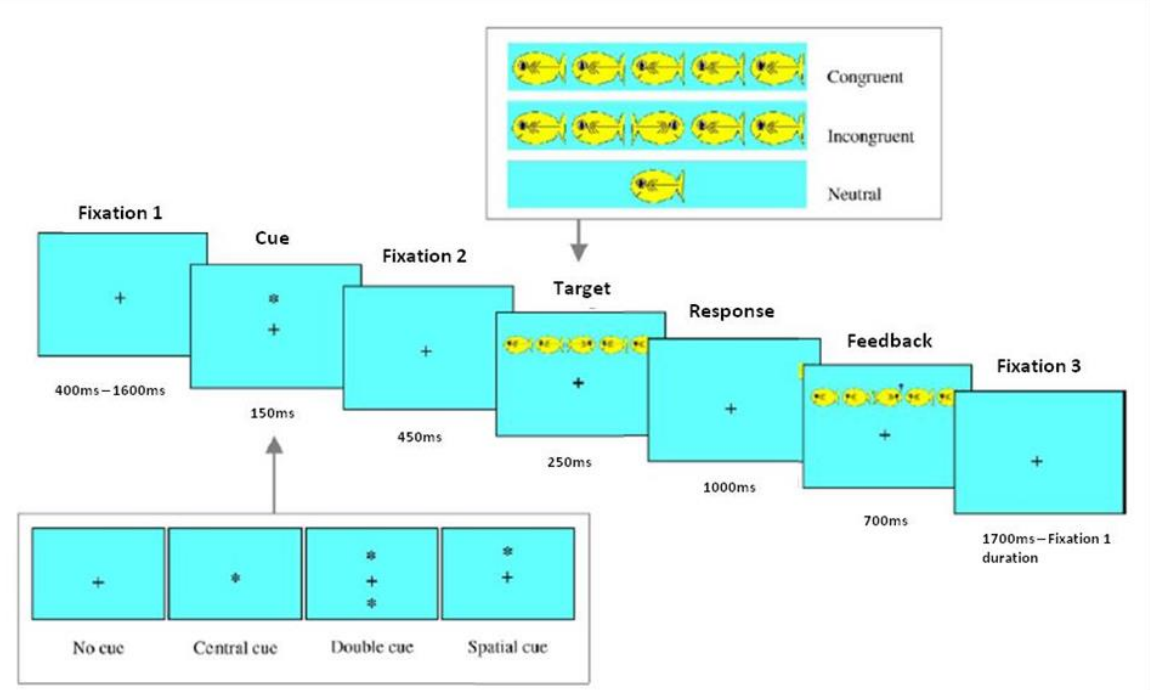

Figure 1: Schematic of the Attention Network Task (adapted from Rueda et al.[13]).

The Stop Go Task (SGT) measures response inhibition[14]. Following a 700ms fixation slide a stimulus slide was presented for 1000ms (a cartoon mole popping out of a hole). A mouse click was required corresponding with the direction the mole was facing (left or right). On 25% of the trials, a stop signal (a helmet on the moles head) was displayed for which participants were instructed to refrain from pressing either mouse button. The initial stop signal was displayed after a 250ms delay with subsequent delays being dynamically 'staircased' so that a correct inhibition added 50 ms, thus making inhibition harder, and failure to inhibit subtracted 50 ms, thus making inhibition easier. This manipulation was performed in order to "handicap" performance so that participants performed at approximately 50% accuracy on stop trials (see Figure 2 for schematic). An initial overall 60% accuracy rate was required from a 48 trial practice prior to commencing the main task. The main task consisted of 200 trials (50 inhibitions). Outcome measures were accuracy for go trials, go-signal reaction times (GSRT), stop-signal delays (SSD), and stop-signal reaction time (SSRT) measured by subtracting SSD from GSRT.

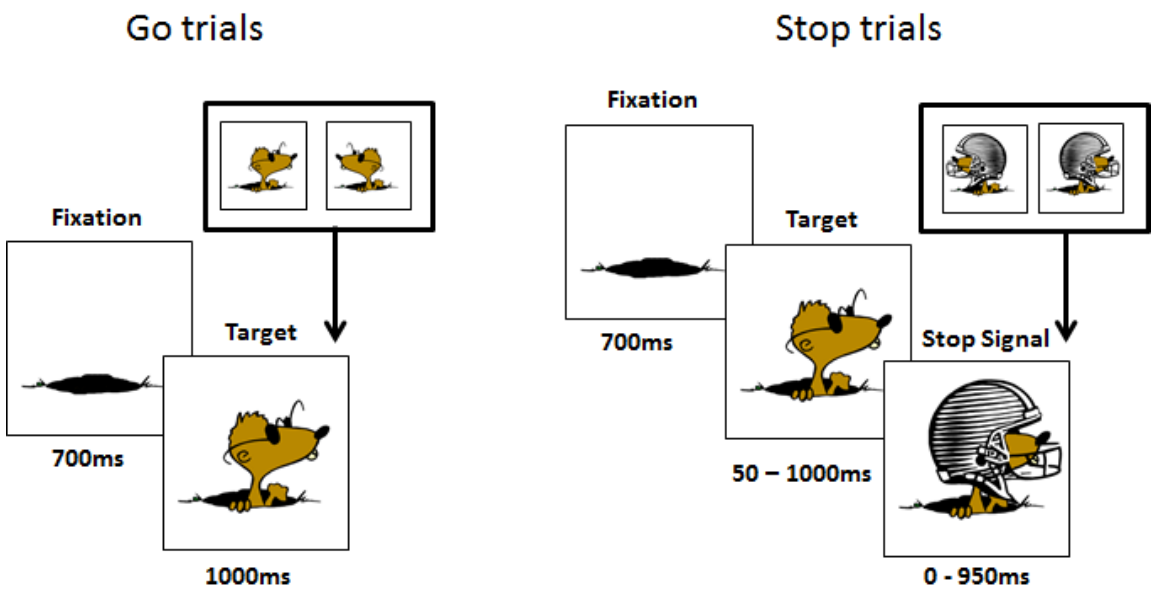

Figure 2: Schematic of the Stop-Go task.

The Switching Task measures cognitive flexibility. A blue triangle in the bottom left corner and a red square in the bottom right were simultaneously presented with a stimulus item shown in the top centre of the screen; either a blue triangle, a blue square, a red triangle, or a red square. Below this stimulus was an instruction word which was either 'shape' or 'colour'. According to the instruction word, participants were required to match the stimulus to the same shaped or same coloured item at the bottom of the screen by pressing a keyboard key on the corresponding side. Therefore, the stimuli were either congruent (same shape / same colour following both instruction words) or incongruent (same shape / different colour following the 'shape' instruction and different shape / same colour following the 'colour' instruction). There was no time limit. A 50ms fixation screen showing only the bottom two items appeared after each response. Three separate blocks were performed; the first 'colour' block consisted of 52 colour-only trials and the second 'shape' block consisted of 52 shape-only trials. The third 'mixing' block was designed to investigate the cost of switching task and therefore consisted of alternating the instruction that was presented every four trials. Each set of four trials contained each of the four stimulus items presented in a random order (see Figure 3 for a schematic). The main task blocks were preceded by a 48 trial mixing block practice. Accuracy of 60% was required to progress from the practice to the main task. The outcome variables were accuracy and reaction time.

Figure 3: Schematic of the Switching Task. '*' Denotes incongruent targets.

Procedure

Upon recruitment all participants were invited for a screening session where demographic information was collected, exclusion inclusion criteria were checked, fluid intelligence was assessed with Raven's Colour Progressive Matrices (RPM), and a practice version of the cognitive battery was administered. Twenty four hours before each test session participants were instructed to consume a low flavonoid diet avoiding a list of high flavonoid foods. Food diaries completed by the guardians were collected to ensure compliance. On completion of the first food diary, guardians also recorded how many portions of fruit and how many of vegetables the participants consumed on a typical day, with a portion being defined as the amount the child could comfortably hold in the palm of their hand. On each test day the participants were requested to consume a low flavonoid lunch consisting of a ham or cheese sandwich, crisps and a banana. Water consumption was unlimited during each test day. Half an hour before consumption, a confederate prepared the drinks, which were consumed through a black straw, thus ensuring doubling blinding. All drinks were consumed at the participant's school and all cognitive testing took place at the University of Reading. In order to coincide with the time points where significant effects on memory were previously observed[8], for experiment (i) the drink was consumed at 1445 or 1515 hours and testing took

place 75 minutes later. Similarly, to coincide with the time points for which effects have been observed for executive function[5, 8], for experiment (ii) the drinks were consumed at 1300 hours and testing took place three hours later. This research was given a favourable opinion for conduct from the University of Reading, School of Psychology Ethics Committee.

Statistical analysis

Data were not collected on practice days, and reaction times <100ms were excluded. The following analyses were performed: 2x7 (Treatment*Recall) ANOVA for AVLT data; 2x5 (Treatment*Recall) for Brown-Peterson data; 2x3x4 (Treatment*Congruence*Cue Type) ANOVA for ANT data; 2x2 (Treatment * Response) for VSGT reaction time data (only the first 2 responses were included in the analysis because not all participants managed to progress beyond this point); 2x2x4 (Treatment*Congruence*Switch Set) for Switching Task data. For all other outcome measures within-subject t-tests were performed. For conciseness, only main effects and interactions which involve Treatment are reported here. Bonferroni corrections were applied to all post hoc analysis of significant interactions.

Results

Memory Function Experiment (i)

As shown in Table 3, for the AVLT, Brown Peterson Task and Picture Recognition Task there were no significant main effects or interactions involving Treatment.

Table 3. Treatment-related results for tasks employed in Experiment (i)

Dependent Variables	Statistics
RAVLT	
Recall x Treatment (interaction)	$F^{6,96}= 1.18$, p = .325
Recall x Treatment (main effect Treatment)	$F^{1,16}= .222$, p = .644
Immediate Recall	$t^{16}= -.436$, p = .668
Final Acquisition	$t^{16}= .746$, p =.466
Amount Learned	$t^{16}= 1.13$, p = .275
Total Acquisition	$t^{16}= -.511$, p = .616
Delayed Recall	$t^{16}= -.313$, p = .748
Delayed Recognition	$t^{16}= .544$, p = .594
Brown Peterson Task	
Recall x Treatment (interaction)	$F^{2.2,35.3}= .199$, p =.841
Recall x Treatment (main effect Treatment)	$F^{1,16}= 2.2$, p = .157
Proactive Interference	$t^{16}= .344$, p = .735
Release from Proactive Interference	$t^{16}= 0$, p = 1
Picture Recognition Task	
Picture Recognition Accuracy	$t^{16}= .771$, p = .452
Novel Picture Rejection Accuracy	$t^{16}= -1.577$, p = .134
Picture Recognition RT	$t^{16}= .536$, p = .599
Novel Picture Rejection Accuracy RT	$t^{16}= -.745$, p= .467

Visuo-Spatial Grid Task	
Maximum circle positions recalled	t^{16}= -.275, p = .787
Response x Treatment RT (interaction)	$F^{1,16}$= .001, p = .972
Response x Treatment RT (main effect treatment)	$F^{1,16}$= 4.87, p = .042*

*Significant at p<.05

For the Visuo-Spatial Grid Task a main effect of Treatment was observed for reaction time [$F^{1,16}$=4.87, p=.042], such that responses were faster following BB relative to placebo (see Figure 1). Importantly, this reaction time benefit was achieved with no cost to accuracy performance with no significant difference being found between the treatments on this measure [t^{16}=.275, p=.787]. No other significant effects of Treatment were observed for the VSGT.

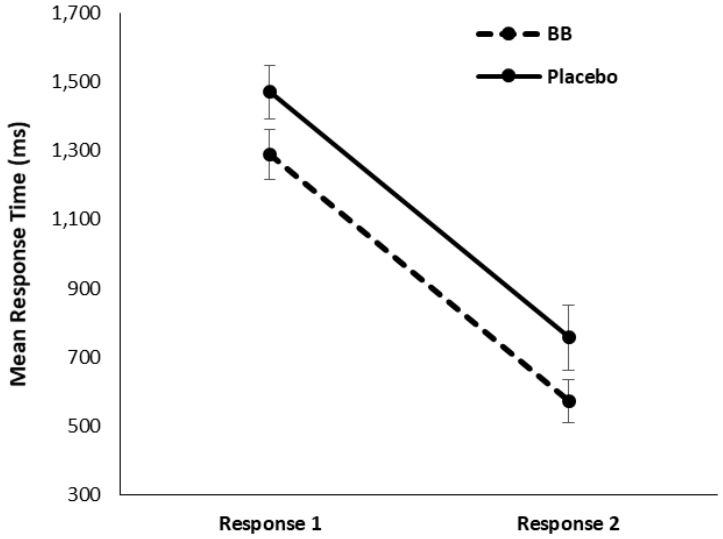

Figure 4: Mean reaction times (± SE) for the first two screen press responses on each trial of the VSGT showing the main effect of faster response times following anthocyanin intervention in comparison to vehicle (p<0.05).

318 Executive Function Experiment (ii)

319 As shown in Table 4, for the Stop-Go Task and the Switching Task there were no significant
320 main effects or interactions of Treatment.

321

Table 4. Treatment-related results for tasks employed in Experiment (ii)

Dependent Variables	Statistics
Attention Network Task - RT	
Congruency x Cue x Treatment (3 way interaction)	$F^{6,102}$=.434, p= .855
Congruency x Cue x Treatment (Cue x Treatment interaction)	$F^{3,51}$=.537, p=.659
Congruency x Cue x Treatment (Congruency x Treatment interaction)	$F^{2,34}$=3.30, p=.049*
Congruency x Cue x Treatment (Treatment main effect)	$F^{1,17}$=.199, p=.662
Attention Network Task - Accuracy	
Congruency x Cue x Treatment (3 way interaction)	$F^{6,102}$=.530, p=.784
Congruency x Cue x Treatment (Cue x Treatment interaction)	$F^{3,51}$=.720, p=.545
Congruency x Cue x Treatment (Congruency x Treatment interaction)	$F^{2,34}$=.759, p=.476
Congruency x Cue x Treatment (Treatment main effect)	$F^{1,17}$=2.28, p=.150
Stop-Go Task	
Go trial accuracy	t^{17} = -.263, p = .795
Go trial RT	t^{17} = -1.08, p = .295
Stop signal delay	t^{17} = -.558, p = .584
Stop signal reaction time	t^{17} = .088, p = .931
Switching Task – RT	
Congrency x Switch Trial x Treatment (3 way interaction)	$F^{3,51}$=.123, p=.946
Congrency x Switch Trial x Treatment (Swtich Trail x Treatment)	$F^{1.97,33.5}$=.973, p=3.87
Congrency x Switch Trial x Treatment (Congruency x Treatment)	$F^{1,17}$=.136, p=.717
Congrency x Switch Trial x Treatment (Treatment main effect)	$F^{1,17}$=.116, p=.738
Switching task - Accuracy	
Congrency x Switch Trial x Treatment (3 way interaction)	$F^{3,51}$=.853, p=.472
Congrency x Switch Trial x Treatment (Swtich Trail x Treatment)	$F^{3,51}$=.198, p=.898
Congrency x Switch Trial x Treatment (Congruency x Treatment)	$F^{1,17}$=.374, p=.549
Congrency x Switch Trial x Treatment (Treatment main effect)	$F^{1,17}$=.171, p=.684
Switching task - simple task vs mixed task comparison RT	
Task x Treament (interaction)	$F^{1,17}$=.349, p=.563
Task x Treament (Treatement main effect)	$F^{1,17}$ = .092, p = .765
Switching task - simple task vs mixed task comparison Accuracy	
Task x Treament (interaction)	$F^{1,17}$=.008, p=.929
Task x Treament (Treatement main effect)	F=^{1,17}.062, p=.806

322 *Significant at p<.05

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For the ANT a significant Treatment*Congruence interaction was observed [$F^{2,34}$=3.3, p=.049] for reaction time data. As show in Figure 2, this interaction is partially explained by a trend for faster responses following BB (mean = 587ms) relative to placebo (mean = 604ms) for congruent trials (p=.062), particularly for the spatial cues though post-hoc analysis only revealed a weak trend (p=.094) for this measure, however the Treatment*Congruence*Cue Type interaction was not significant. No other significant effects of Treatment were observed for the ANT.

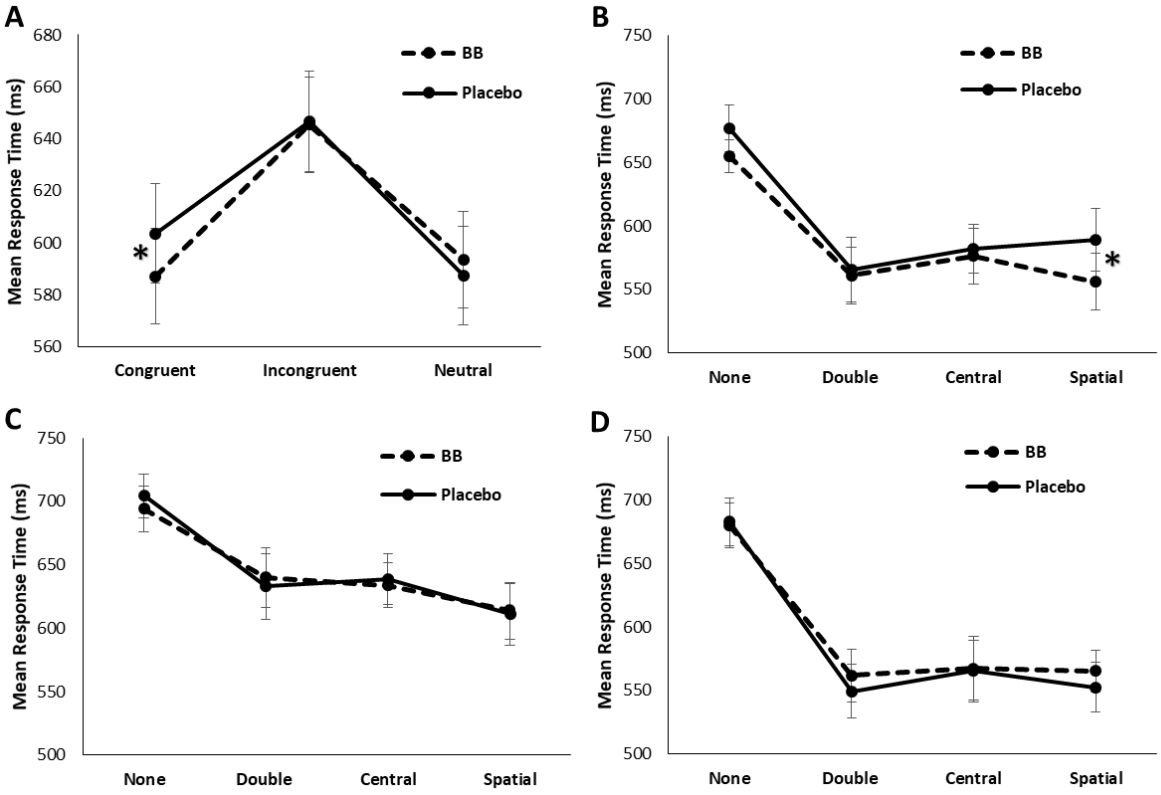

Figure 5. Attention Network Task mean response times (±SE) showing A) the interaction between treatment and congruence. For congruent trials there is evidence of more rapid response times following the blueberry drink compared to placebo (non-significant trend; p=.062), however, this trend is not seen for neutral or incongruent trials. Mean response times (±SE) are also shown as a function of treatment and warning type for B) Congruent, C) Neutral, and D) Incongruent trials. For congruent trials following a spatial cue, there is evidence of more rapid response times following blueberry drink compared to placebo, (non-significant trend; p=.094), however, this trend is not seen for any of the other comparisons. *p<. 05

Discussion

The aim of this research was to examine whether episodic memory and executive function were improved at 75 minutes and 3 hours (respectively) after consumption of a wild blueberry beverage in children aged 7- to 10-years, and whether any effects extended to various aspects of these cognitive domains. The results from experiment (i) showed no significant differences between the blueberry and placebo for immediate recall, delayed recall, delayed recognition, or proactive interference. Participants, however, responded significantly faster on aspects of the VSGT at 75 minutes following blueberry, revealing for the first time increases in the speed of visual memory processing following blueberry within this age group. In support, other flavonoid intervention studies which have also shown no accuracy effect in visuo-spatial memory have shown improvement in speed of processing (i.e. Pipingas et al.[15]). This was also the case here where there were significantly faster first and second responses following anthocyanin intervention in comparison to the vehicle. A consideration in relation to previous findings for episodic memory is the time of testing. Previously participants were tested in the morning at 1145 hours [8] whilst in the current experiment they were tested at 1600 hours. Variables such as fatigue and levels of exercise (as part of the school day curriculum) may have contributed to the absence of effects on memory accuracy. However, when children were tested in the afternoon two hours following blueberry consumption, Barfoot et al. [9] did show that verbal memory accuracy was improved. It is possible that a longer time course is needed (i.e. 120 minutes rather than 75 minutes) to observe effects for episodic memory when testing after lunch, possibly due to variations in speed of digestion which can be influenced by the macronutrient composition of the lunch interfering with digestion of the intervention. Furthermore, the children in experiment (i) showed higher fluid intelligence than the published norms for the RCPM (70th percentile). Fluid intelligence is strongly related to performance on visuo-spatial working memory tasks[16] and it is therefore possible that the particular sample of participants in this study had an increased aptitude for the Visuo-Spatial Grid Task which would have elevated their performance regardless of intervention and reduced the scope for the blueberry drink to reveal an accuracy benefit. For example, higher RCPM scores were observed here compared to other studies in children showing benefits of blueberry [9]. The lack of significant delayed memory effects on the AVLT were unexpected given that this has been a robust effect found in previous blueberry research with this age group[7-9]. It should be noted that the version of the AVLT used here did not employ an 'interference' list which is normally presented before the delayed recall element of the task. Given there was no retroactive interference the delayed recall in this version of the task would have been less cognitively demanding than the versions employed in previous studies and it is possible the task was no longer sufficiently sensitive to demonstrate blueberry related cognitive benefits. Going further, it is possible that this indicates that this episodic memory assessment is not sensitive to a blueberry intervention in children under these conditions.

The results of experiment (ii) revealed a positive effect of wild blueberry for faster response times on congruent trials during the ANT task, which indicates a benefit for blueberries on the attention aspect of the task. However, there was no evidence to benefits for other aspects

of executive function including response inhibition in the Stop Go task, cognitive flexibility in the Switching Task, or on the most cognitively demanding (incongruent) trials of the ANT as evidenced by an absence of significant effects for the outcome measures of these tasks. Interestingly, the benefit for attentional response speed is consistent with others [5, 9] who also report increased speed of response following blueberry with a modified version of the ANT task used here. However, these previous studies report benefits when demand was high, i.e. faster response for the more difficult incongruent rather than congruent trials [5] and trials of shorter duration [9] which the authors argue require greater executive function resources than longer trials. The slight discrepancy between the present findings and others could be accounted for by the nature of the task. The modified ANT included additional elements and stimuli (e.g. noise and load variables), which increase the complexity and demand of the task and therefore, it is possible that the present version, which did not include these variables, was not sufficiently challenging to induce the demand effect. Importantly there was a fixation period between trails in this version of the task which varied between 2100ms and 3300ms whereas previous versions where reaction time benefits have been recorded had no gap between trials[5, 9]. This extended gap between trials may have had the consequence of allowing the participants a period where concentrated attention on stimuli was not required and thus reduced the overall demand of the task. A similar effect may also have been present in the switching task. Here, there was no time constraint on response, with the participants being free to take as long as they wished to respond on each trial. This lack of time pressure may again have lessened the cognitive demand and reduced the sensitivity of the task to any reaction time or accuracy benefits. The absence of effects for the Stop-Go task are consistent with the null effects for a similar Go-No-Go task [8] which could indicate that response inhibition is less sensitive to blueberry flavonoids in children than other aspects of executive function. Direct comparisons between the executive function and episodic memory outcomes in the present study are limited in light of the different, albeit matched samples recruited for each of two experiments. The rationale for this design is outlined in the introduction (i.e. to avoid interference and procedural order effects), however, it would be beneficial to apply this experimental design with a single cohort following a randomised cross-over design to enable investigation of possible differences in performance between executive function and episodic memory tasks. It is also important to acknowledge that, owing to difficulties with recruitment, the anticipated sample size was not achieved leading to a possible loss of power and further research with a larger sample size to address this is recommended. Furthermore, across the two experiments there is a risk that the observed significant effects reflect type 1 error, particularly given the complexity of the analysis models. Having said that, appropriate post hoc corrections were applied and only significant interactions and main effects were explored. The addition of sugar to the vehicle was required in order to match the placebo and blueberry drinks for sugar content, and to ensure that the drink was palatable to the children. In support, this vehicle is similar to other studies in children [5, 8, 17, 18], and whilst it is true that the sugar content may affect performance, we can be confident that differences in performance between the placebo and blueberry drinks are not due to the sugar content given that they are matched on this constituent. Future studies would benefit from a measure of physical activity in the children as it is plausible that health parameters not measured here such as level of fitness, habitual diet, and BMI could affect response to the intervention.

The research was designed to examine whether consumption of a flavonoid-rich wild blueberry drink can improve episodic memory at 75 minutes post consumption and executive function at 3 hours post consumption (respectively) in children aged 7-10. The results offer some support for this hypothesis, with improved response times for some elements of the episodic memory and executive function measures, however there were no apparent blueberry benefits for accuracy outcomes. It was also hypothesised that blueberry consumption would improve performance on the most demanding aspects of the tasks, however there was no clear support for this hypothesis. As discussed, this may reflect that the versions of the task used were not of sufficient demand. In summary, this research adds some support for the evidence base (see [2] for review) that blueberry flavonoids can benefit cognitive function, specifically response speed, in children aged 7-10. Further research is required to understand if the time course of these effects is different depending on the area of the brain and cognitive domain targeted, and how this coincides with mechanisms of action. For example, the time course of the peripheral vascular responses has been reasonably well documented [19, 20] but further work is required to identify the cerebral vascular response, and whether any such changes can directly impact cognitive function.

Conflicts of interest

There are no conflicts of interest to declare.

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