

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

3
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14
15 **Abstract**

16
17 Evidence for the health benefits of blueberries is well documented. In particular memory and
18 executive function benefits have both been found for children aged 7 – 10 in the 6 hour
19 period following acute blueberry consumption. Previous research has utilised a limited
20 number of tasks when considering these domains. Therefore, in two separate experiments, we
21 employed extended memory and executive function task batteries to further understand the
22 extent of blueberry benefits. Following blueberry intervention, children aged 7 – 10 were
23 tested on a memory battery at 75 minutes and an executive function battery at 3 hours.
24 Shorter memory reaction times were observed on the visuo-spatial grid task and shorter
25 executive function reaction times were observed on the congruent trials of the attention
26 network task. Whilst providing further evidence for the cognitive benefits of blueberry
27 consumption in school age children, these findings contrast with previous research where
28 improved accuracy and reaction time benefits have most commonly been found on more
29 cognitively demanding trials. Further research targeted to consider the areas of the brain
30 related to each cognitive domain and how they coincide with mechanisms of action, such as
31 increases in cerebral blood flow following blueberry intervention, is therefore recommended.

32
33 **Introduction**

34
35 There is evidence for beneficial effects of blueberries on a number of health outcomes, one of
36 which is cognitive function¹. Much of the supporting evidence for this comes from human
37 intervention trials whereby cognitive function is assessed with a battery of tests following a
38 single acute dose of blueberries, or following regular daily consumption over several weeks.
39 A review of this literature² reported eleven blueberry interventions in various populations
40 including children, healthy adults, and adults with mild cognitive impairment (MCI) with
41 benefits being reported for various aspects of cognition including memory, executive function
42 and psychomotor function. It is hypothesised that these effects can be ascribed to the high
43 flavonoid content of blueberries, for which various mechanisms of action have been
44 proposed³. There are some indications that the specific cognitive domains affected by acute
45 flavonoid ingestion vary with the age of participants, i.e. benefits to executive function seem
46 most prevalent in healthy young adults, whilst episodic memory effects are seen in older
47 adults and adults with MCI. These differences may be attributable to the different stages of
48 physiological and neuronal development in the brain across the lifespan, however it should
49 also be noted that benefits are most evident where the cognitive demand of the task is high or
50 the participant is cognitively compromised^{4, 5}. This suggests that failure to find effects across

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

56

57 Previous research in children has shown acute benefits for cognitive function following wild
58 blueberry consumption. For example, Whyte and Williams⁷ demonstrated improved verbal
59 episodic memory 2 hours post consumption of a 30g wild blueberry drink in children aged 8-
60 10⁷. In a subsequent study, further evidence was found for episodic memory benefits at 75
61 minutes and 6 hours with executive function benefits being found at 3 hours. These findings
62 suggested that the beneficial effects of blueberry in children were modulated by the level of
63 demand, or difficulty, associated with the task⁸. Specifically, the 7- to 10-year-old children
64 showed better performance on the more cognitively-demanding incongruent trials (but not the
65 easier congruent trials) on a flanker task assessing executive function. This effect for
66 executive function 3 hours post consumption was replicated in a further study⁵ with a
67 Modified Attention Network Task (MANT), where benefits were again seen for the most
68 demanding aspects of the task. Interestingly, executive function effects have been
69 consistently observed 3 hours post consumption, however, recent research by Barfoot et al.
70 has demonstrated, benefits for both memory and executive function at 2 hours⁹. It should be
71 noted that, at this earlier time point, the executive function benefits found by Barfoot et al.⁹
72 differed slightly from earlier findings⁵ in that benefits were found on the shorter stimulus
73 presentation trials and no effect of congruence was evident (see Whyte et al.⁵ for discussion
74 regarding overall task difficulty). It is plausible that the different time course for effects on
75 executive function and memory are associated with subtle differences in the mechanisms of
76 action by which flavonoids may interact with the relevant brain regions (i.e. the hippocampus
77 for episodic memory, and the frontal cortex for executive functions). However, this is
78 speculative as specific mechanisms of action are not well known.

79

80 Previous studies of blueberries in children have typically only used a single task to measure
81 either memory or executive function. Therefore, the aim of this research was to extend our
82 knowledge of the benefits of blueberries using a range of tests in order to provide a more
83 comprehensive assessment of (i) memory function and (ii) executive function. The length of
84 the task batteries precluded the use of the same participants in the same experiments,
85 therefore two different groups, drawn from the same population (children aged 7- to 10-
86 years) participated. Use of two separate samples also allowed for the targeting of testing
87 points where post-consumption benefits of blueberry intervention have previously been
88 found; 75 minutes for memory function⁸ and 3 hours for executive function^{5, 8}. This approach
89 also avoided interference effects, thus allowing a purer examination of each cognitive
90 domain. To be clear with regards to time course, the aim here was to test each cognitive
91 domain at a single time point where it has previously been shown to be sensitive to blueberry
92 consumption, rather than testing each domain at multiple time points.

93

94 The Auditory Verbal Learning Test (AVLT)¹⁰ used in previous studies⁷⁻⁹ gives measures of
95 both episodic memory and interference effects. In order to provide a more focused measure
96 of each of these areas we introduced a paradigm targeted specifically at assessment of
97 proactive interference (the Brown-Peterson task). The AVLT is also retained in the battery,
98 however, the interference list presentation and recall has been removed making the task a
99 purer measure of episodic memory. Furthermore, previous research with this age group has
100 focused on the auditory modality of episodic memory, therefore, we also include here a test

101 of visual memory (Picture Recognition Task) and visuo-spatial working memory (Visuo-
 102 Spatial Grid Task). The executive function experiment incorporated three tests assessing
 103 aspects of executive function including inhibition (Stop-Go Task), rule switching (Task
 104 Switching) and a response interference task for which previous studies have shown sensitivity
 105 to blueberries in children (Attention Network Task, ANT, see²). In addition, within the ANT
 106 task demand can be manipulated, which is important as previous studies have shown that
 107 blueberries are most effective when cognitive demand is high^{5, 8}. Broadly, we hypothesised
 108 that benefits would be observed following blueberry consumption for episodic memory and
 109 executive function measures. Furthermore, benefits were expected to be particularly evident
 110 for the most cognitively demanding aspects of the tasks such as the incongruent, or initial
 111 switch trials on the executive function tasks, or delayed recall on the memory tasks.

112 **Methods**

114 Design

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

131 Table 1 Analysis results of WBANA freeze dried wild blueberries showing total
 132 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 |

133

134

135 Participants

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

149 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) |

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

152 Cognitive Tests

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

155 Experiment (i) Memory Battery

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

164 (List A) presented auditorily at a rate of 1 word/second. After a 2 minute delay, during which
165 time the participants completed viewing the stimulus for the Picture Recognition Task, there
166 was then a further free recall of List A (Recall 6). This was followed by a fifteen minute
167 delay where participants completed the remaining tasks. A final free recall of List A (Recall
168 7) was then performed. Finally, participants were shown a list of 50 nouns, containing all the
169 words from List A plus an additional 35 filler words to match the number used in Lezak, and
170 asked to circle only the words from List A. The baseline lists and session 2 lists as employed
171 in Whyte et al.⁸ were used for this experiment. Different versions were created for repeated
172 administration, which were counterbalanced across conditions. All words had an age of
173 acquisition (AOA) rating of less than 400 (equivalent to age 7 and below) and were matched
174 for concreteness and familiarity. For each test session the following outcomes as specified in
175 Lezak were calculated: Immediate Word Span (Recall 1); Number of Words Learned (Recall
176 5 minus Recall 1); Final Acquisition level (Recall 5); and Word Recognition expressed as the
177 number of correctly circled words.

178 The Picture Recognition Task examined delayed visual recognition and was designed by the
179 researchers to reflect the AVLT. Participants were shown 15 pictures of different landscapes
180 at a rate of 1 per second in a randomised order. A 15 minute delay followed whilst
181 participants completed other tasks (see above). Participants were then shown the original 15
182 pictures along with 35 novel pictures in a randomised order and were instructed to press a
183 green key ('right arrow' on the keyboard) if they had seen the item previously, or press a red
184 key ('left arrow' on the keyboard) if the item was a novel. The pictures were displayed at a
185 size of 6 x 6 cm and were drawn from the Sun database¹², with memorability ratings between
186 46-54. Matched versions were created and administered in a counterbalanced order across
187 test days. Outcome variables were correct picture recognitions and reaction time and correct
188 novel picture rejections and reaction time.

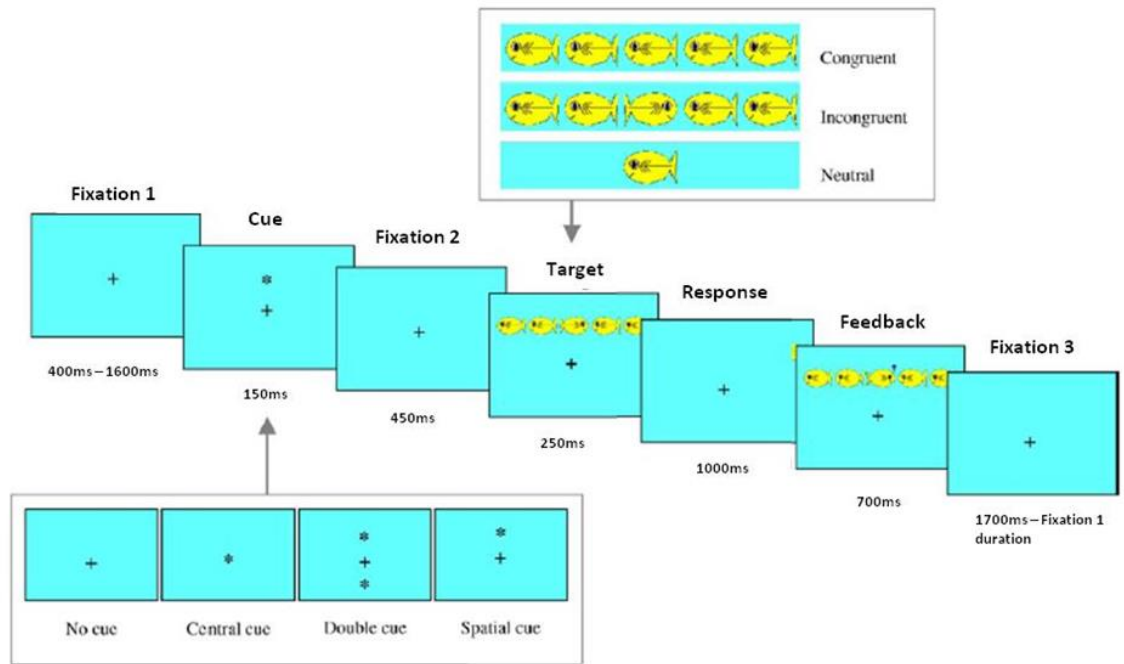
189 The Brown Peterson Task examines proactive interference (PI), and release from proactive
190 interference (RPI). Participants were auditorily presented with 3 letters at a rate of 1/second,
191 excluding vowels and the letter y, with each triplet of letters controlled in such a way as no
192 letters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

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

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

216 Experiment (ii) Executive Function Battery

217 The Attention Network Task (ANT) measures executive attention (response interference)
218 orienting and alerting¹³. Following an initial fixation slide of 400-1600 ms duration, either a
219 centre cue, a double cue, a spatial cue, or no cue were randomly presented for 150 ms. There
220 was then a further short fixation period of 400ms. Stimuli (in the form of yellow cartoon fish
221 on a blue background) were then displayed either above or below the fixation point for
222 250ms and could be congruent, incongruent or neutral depending on whether they matched
223 the direction of the central fish. Stimuli position and congruence type were randomised. A
224 mouse press was required within 1250ms corresponding to the direction the central fish was
225 facing. Feedback was presented in the form of a ‘buzz’ for an incorrect response or the fish
226 reappearing along with a ‘whoohoo’ sound for a correct response. Three blocks with 48 trials
227 in each were presented (see Figure 1 for schematic). A practice block of 24 trials preceded
228 the test phase. If an accuracy of below 60% was recorded for the practice a second practice
229 was performed. The outcome measures were accuracy and response times (RTs) for
230 congruency and cue type.



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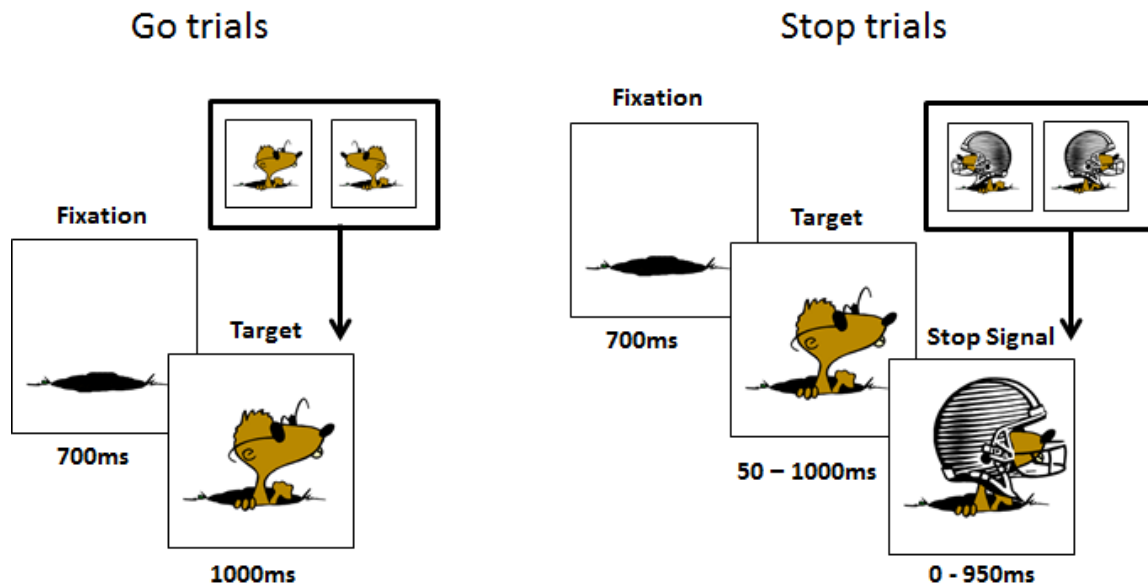
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Figure 1: Schematic of the Attention Network Task (adapted from Rueda et al.¹³).

233

The Stop Go Task (SGT) measures response inhibition¹⁴. 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.

246



247

248

Figure 2: Schematic of the Stop-Go task.

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

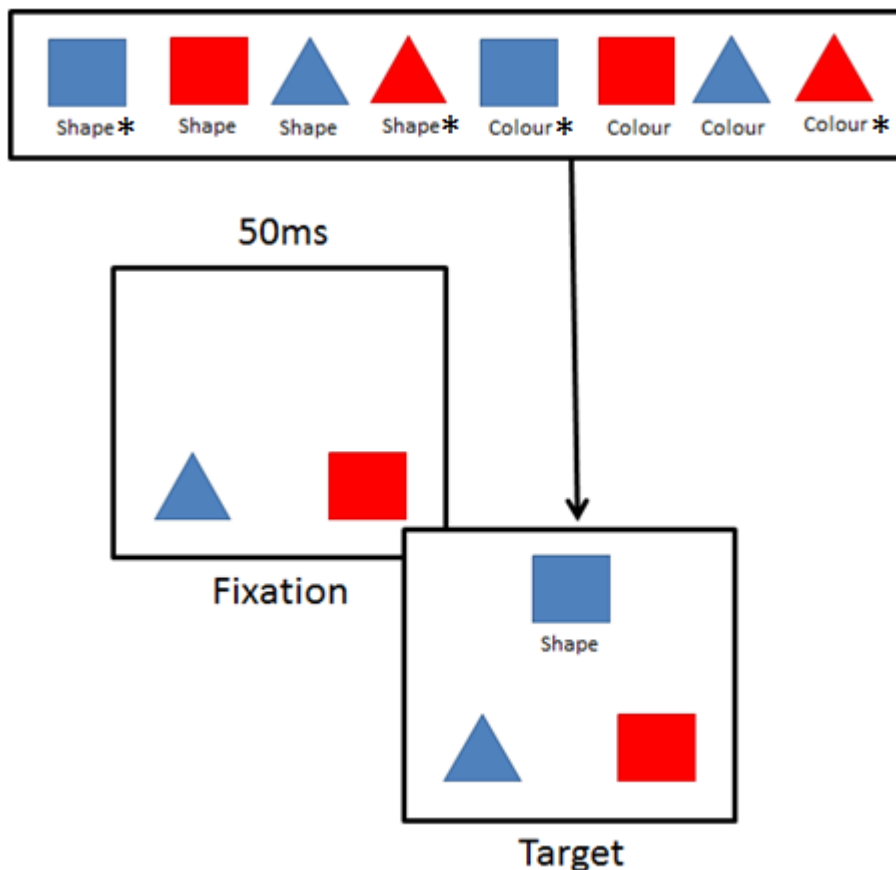


Figure 3: Schematic of the Switching Task. ‘*’ Denotes incongruent targets.

267

268

269

270 Procedure

271 Upon recruitment all participants were invited for a screening session where demographic
 272 information was collected, exclusion inclusion criteria were checked, fluid intelligence was
 273 assessed with Raven’s Colour Progressive Matrices (RPM), and a practice version of the
 274 cognitive battery was administered. Twenty four hours before each test session participants
 275 were instructed to consume a low flavonoid diet avoiding a list of high flavonoid foods. Food
 276 diaries completed by the guardians were collected to ensure compliance. On completion of
 277 the first food diary, guardians also recorded how many portions of fruit and how many of
 278 vegetables the participants consumed on a typical day, with a portion being defined as the
 279 amount the child could comfortably hold in the palm of their hand. On each test day the
 280 participants were requested to consume a low flavonoid lunch consisting of a ham or cheese
 281 sandwich, crisps and a banana. Water consumption was unlimited during each test day. Half
 282 an hour before consumption, a confederate prepared the drinks, which were consumed
 283 through a black straw, thus ensuring doubling blinding. All drinks were consumed at the
 284 participant’s school and all cognitive testing took place at the University of Reading. In order
 285 to coincide with the time points where significant effects on memory were previously
 286 observed⁸, for experiment (i) the drink was consumed at 1445 or 1515 hours and testing took

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

291 Statistical analysis

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

301 Results

302 Memory Function Experiment (i)

303 As shown in Table 3, for the AVL T, Brown Peterson Task and Picture Recognition Task
 304 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 $t^{16} = -.275, p = .787$

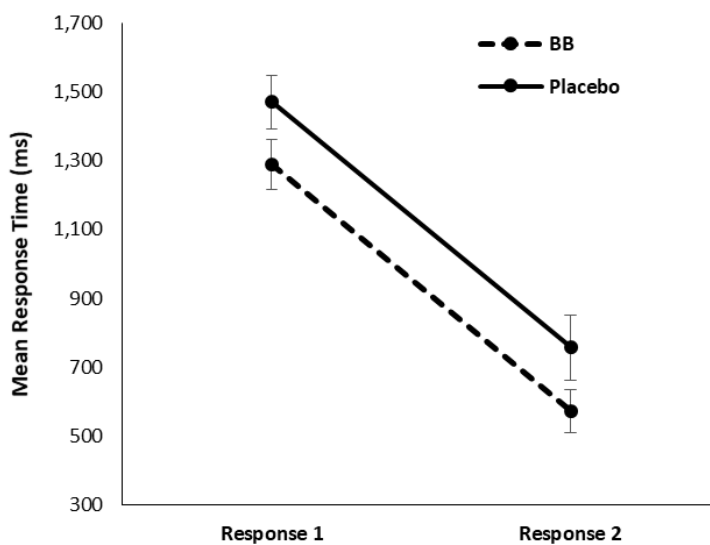
Response x Treatment RT (interaction) $F^{1,16} = .001, p = .972$

Response x Treatment RT (main effect treatment) $F^{1,16} = 4.87, p = .042^*$

305 *Significant at $p < .05$

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

312



313

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

317

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

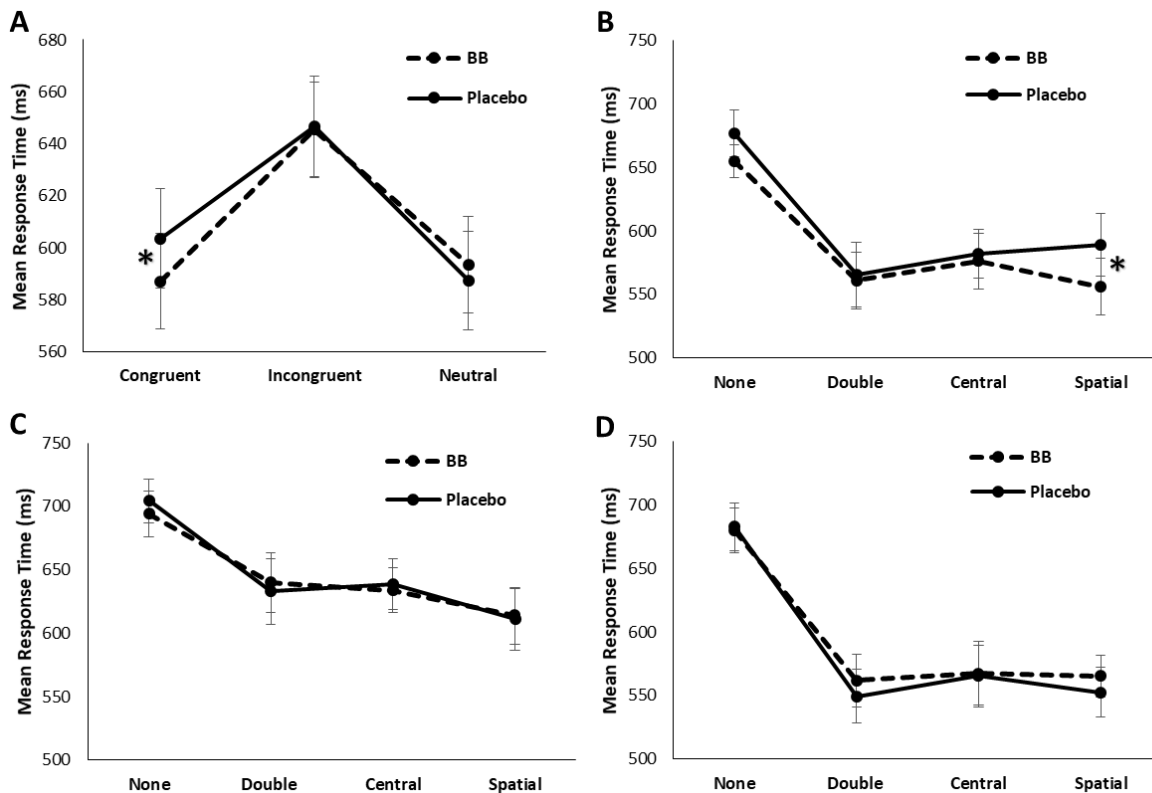
Table 4. Treatment-related results for tasks employed in Experiment (ii)

| 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 | |
| Congruency x Switch Trial x Treatment (3 way interaction) | $F^{3,51}=.123, p=.946$ |
| Congruency x Switch Trial x Treatment (Switch Trail x Treatment) | $F^{1,97,33.5}=.973, p=3.87$ |
| Congruency x Switch Trial x Treatment (Congruency x Treatment) | $F^{1,17}=.136, p=.717$ |
| Congruency x Switch Trial x Treatment (Treatment main effect) | $F^{1,17}=.116, p=.738$ |
| Switching task - Accuracy | |
| Congruency x Switch Trial x Treatment (3 way interaction) | $F^{3,51}=.853, p=.472$ |
| Congruency x Switch Trial x Treatment (Switch Trail x Treatment) | $F^{3,51}=.198, p=.898$ |
| Congruency x Switch Trial x Treatment (Congruency x Treatment) | $F^{1,17}=.374, p=.549$ |
| Congruency 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 Treatment (interaction) | $F^{1,17}=.349, p=.563$ |
| Task x Treatment (Treatment main effect) | $F^{1,17} = .092, p = .765$ |
| Switching task - simple task vs mixed task comparison Accuracy | |
| Task x Treatment (interaction) | $F^{1,17}=.008, p=.929$ |
| Task x Treatment (Treatment main effect) | $F^{1,17}=.062, p=.806$ |

322 *Significant at $p < .05$

323

324 For the ANT a significant Treatment*Congruence interaction was observed [$F^{2,34}=3.3$,
 325 $p=.049$] for reaction time data. As show in Figure 2, this interaction is partially explained by
 326 a trend for faster responses following BB (mean = 587ms) relative to placebo (mean =
 327 604ms) for congruent trials ($p=.062$), particularly for the spatial cues though post-hoc
 328 analysis only revealed a weak trend ($p=.094$) for this measure, however the
 329 Treatment*Congruence*Cue Type interaction was not significant. No other significant effects
 330 of Treatment were observed for the ANT.



331

332 **Figure 5.** Attention Network Task mean response times (\pm SE) showing A) the interaction
 333 between treatment and congruence. For congruent trials there is evidence of more rapid
 334 response times following the blueberry drink compared to placebo (non-significant trend;
 335 $p=.062$), however, this trend is not seen for neutral or incongruent trials. Mean response times
 336 (\pm SE) are also shown as a function of treatment and warning type for B) Congruent, C)
 337 Neutral, and D) Incongruent trials. For congruent trials following a spatial cue, there is
 338 evidence of more rapid response times following blueberry drink compared to placebo, (non-
 339 significant trend; $p=.094$), however, this trend is not seen for any of the other comparisons.
 340 * $p<. 05$

341

342 Discussion

343 The aim of this research was to examine whether episodic memory and executive function
344 were improved at 75 minutes and 3 hours (respectively) after consumption of a wild
345 blueberry beverage in children aged 7- to 10-years, and whether any effects extended to
346 various aspects of these cognitive domains. The results from experiment (i) showed no
347 significant differences between the blueberry and placebo for immediate recall, delayed
348 recall, delayed recognition, or proactive interference. Participants, however, responded
349 significantly faster on aspects of the VSGT at 75 minutes following blueberry, revealing for
350 the first time increases in the speed of visual memory processing following blueberry within
351 this age group. In support, other flavonoid intervention studies which have also shown no
352 accuracy effect in visuo-spatial memory have shown improvement in speed of processing (i.e.
353 Pipingas et al.¹⁵). This was also the case here where there were significantly faster first and
354 second responses following anthocyanin intervention in comparison to the vehicle. A
355 consideration in relation to previous findings for episodic memory is the time of testing.
356 Previously participants were tested in the morning at 1145 hours⁸ whilst in the current
357 experiment they were tested at 1600 hours. Variables such as fatigue and levels of exercise
358 (as part of the school day curriculum) may have contributed to the absence of effects on
359 memory accuracy. However, when children were tested in the afternoon two hours following
360 blueberry consumption, Barfoot et al.⁹ did show that verbal memory accuracy was improved.
361 It is possible that a longer time course is needed (i.e. 120 minutes rather than 75 minutes) to
362 observe effects for episodic memory when testing after lunch, possibly due to variations in
363 speed of digestion which can be influenced by the macronutrient composition of the lunch
364 interfering with digestion of the intervention. Furthermore, the children in experiment (i)
365 showed higher fluid intelligence than the published norms for the RCPM (70th percentile).
366 Fluid intelligence is strongly related to performance on visuo-spatial working memory tasks¹⁶
367 and it is therefore possible that the particular sample of participants in this study had an
368 increased aptitude for the Visuo-Spatial Grid Task which would have elevated their
369 performance regardless of intervention and reduced the scope for the blueberry drink to
370 reveal an accuracy benefit. For example, higher RCPM scores were observed here compared
371 to other studies in children showing benefits of blueberry⁹. The lack of significant delayed
372 memory effects on the AVLTL were unexpected given that this has been a robust effect found
373 in previous blueberry research with this age group⁷⁻⁹. It should be noted that the version of
374 the AVLTL used here did not employ an 'interference' list which is normally presented before
375 the delayed recall element of the task. Given there was no retroactive interference the delayed
376 recall in this version of the task would have been less cognitively demanding than the
377 versions employed in previous studies and it is possible the task was no longer sufficiently
378 sensitive to demonstrate blueberry related cognitive benefits. Going further, it is possible that
379 this indicates that this episodic memory assessment is not sensitive to a blueberry
380 intervention in children under these conditions.

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

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

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

444

445 **Conflicts of interest**

446 There are no conflicts of interest to declare.

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451

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453

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