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The impact of blackcurrant juice on attention, mood and brain wave spectral activity in young healthy volunteers

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There is a growing body of evidence from randomized controlled trials which indicates that consumption of berries has a positive effect upon the cognitive function of healthy adults. It has been recommended that studies combining cognitive and physiological measures be undertaken in order to strengthen the evidence base for the putative effects of flavonoid consumption on cognitive outcomes. This pilot study utilized a randomized, double-blind and placebo controlled crossover design to assess the influence of the acute administration of anthocyanin-rich blackcurrant juice, standardized at 500 mg of polyphenols, on mood and attention. Additionally, this trial used electroencephalography (EEG) to assess if any changes in cognitive performance are associated with changes in localized prefrontal cortex neuronal activity in nine healthy young adults. Outcomes from the pilot EEG data highlight an anxiolytic effect of the consumption of a single serve blackcurrant juice, as indexed by a suppression of α spectral power, and an increase in the slow wave δ and θ spectral powers. There was also an indication of greater alertness and lower fatigue, as indexed by an increase in β power and suppression of α spectral power. Outcomes from the CogTrack™ system indicated a small acute increase in reaction times during the digit vigilance task.

Keywords: Blackcurrants, Attention, Computerized cognitive testing, CogTrack, EEG

Introduction

Epidemiological data suggest a strong association between the chronic consumption of flavonoid-rich foods and reductions in cognitive decline.[1–7] Research using animal models has shown that supplementation of Berry-derived flavonoids, as well as dried fruit or fruit juices can positively impact several aspects of memory. These include spatial and short-term working memory,[8–10] reversal learning and rapid memory acquisition,[11] long-term reference memory,[12] slow memory acquisition,[13] and memory retrieval.[14] Furthermore, the consumption of polyphenol-rich extracts has been shown to protect rodent cognitive function from a range of neuronal insults and natural ageing,[15] as well as to delay the onset of symptoms of Alzheimer's disease.[16] Evidence from controlled human trials also demonstrates a link between acute flavonoid intake and improvements in cognitive function in healthy individuals[3,4] with effects ranging from improvements in mood and working memory[17] to improvements in simple reaction time.[18] However, there is an ever-growing body of literature from randomized controlled trials which indicates positive effects of berries upon healthy aged[19,20] and young adults.[21] Bell *et al.*,[3] recommended studies combining cognitive and physiological measures in order to strengthen the evidence base for the putative positive effects of flavonoid consumption on cognitive outcomes. Electroencephalographs (EEG) provide a physiological basis to detect and record brain wave activity and to interprete such recordings in terms of alertness, attention, relaxation, and concentration. However, there is a paucity of data combining EEG outputs with cognitive outcomes following flavoniod supplementation studies. Okello *et al.*,[22] showed that θ, α, and β brain wave activities increased in healthy young volunteers 1 hour after black and green tea consumption. Scholey *et al.*,[23] showed that the acute

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supplementation of the tea flavonoid Epigallocatechin gallate (300 mg) was associated with a significant increase in θ, α and β activity in healthy young volunteers. Both studies support the putative positive effects of tea flavonoid consumption on relaxed, alert, attentive and concentration states.

Blackcurrants, *Ribes nigrum* L., (Family: Grossulariaceae) are a berry fruit native to Europe and Northern Asia which are rich in polyphenols with the major constituents being anthocyanins.[24] Data from randomized controlled trials highlight the potential of blackcurrants to modulate physiological parameters which could influence human behaviour. These include changes to blood flow,[25] modulations of peripheral dopaminergic tone[21] and modulations in post prandial blood glucose profiles.[21,26] Watson *et al.*,[21] showed that acute consumption of a blackcurrant juice standardized at 500 mg per 60 kg body weight improved sustained attention and psychomotor speed in healthy young adults as indicated by improvements in reaction times during a digit vigilance task. These effects upon sustained attention could indicate a potential modulation of neuronal activity within the frontal cortex.[27]

The study aimed to collect pilot data assessing the influence of the acute administration of anthocyanin-rich blackcurrant juice on localized prefrontal cortex neuronal activity during cognitive activity as measured using EEG.

Methods

Design

The intervention study followed a double-blind; placebo controlled, and cross-over design. Volunteers were randomly allocated into treatment orders, of either the Blackcurrant or placebo treatment, by means of a Williams Latin Square. Ethical approval was granted by the Faculty of Science, Agriculture and Engineering Ethics committee, Newcastle University.

Volunteers

Nine healthy adults (three male and six female) with a mean age of 23 years were recruited using opportunity sampling at Newcastle University. Prior to enrolment, volunteers attended a screening session during which signed consent to take part in the trial was received. Volunteers were screened for any contraindications to the trial with the use of an exclusion questionnaire. The volunteer exclusion criteria included: a diagnosis of any physical or mental illness; the use of recreational, over-the-counter or prescribed drugs; use of dietary supplements and the use of tobacco products (including vaping). Volunteers were also excluded if they had a body mass index (BMI) <18 >30 kg/m^2. The average volunteer demographics are shown in Table 1.

Table 1. Volunteer ($n = 9$) mean demographics.

Sex	*n*	Age (years)	Weight (kg)	BMI
M	3	24	78.5	25
F	6	22	56.6	22
Group	9	23	67.5	23.5

Treatments

Volunteers consumed two study drinks, with at least 1-week washout between study visits. These drinks were either a cold-pressed blackcurrant juice, standardized to 500 mg of polyphenols per drink or a sugar, vitamin C and flavour matched placebo.

Treatment drinks

Twelve kilogram of frozen blackcurrants (Ben Hope cultivar) were defrosted overnight at room temperature and pressed. The juice was collected in a single vat and then separated into 50 ml aliquots to be stored at −20° C until used (approximately 4 months). When a volunteer was scheduled to attend, aliquots were thawed overnight in a refrigerator and prepared into treatment drinks the following day.

Polyphenols/anthocyanins

The total polyphenol content of black currant juice was estimated using the Folin-C method as described by Deighton *et al.*[28] Values are expressed as mg of Gallic acid equivalents, the standard compound used for quantification. Total anthocyanin content was estimated using the colorimetric method outlined by Deighton *et al.*,[28] and amounts expressed as mg cyanidin-glucoside equivalents. Polyphenolic composition of the blackcurrant juice was assessed by liquid-chromatography mass spectrometry (LC–MS) analysis as described by Boath *et al.*[29] The LC–MS analysis blackcurrant juice (Fig. 1) is characteristic of blackcurrant with high levels of anthocyanins dominating the polyphenolic profile. Trace 'A' shows the absorbance profile at 280 nm. Trace B shows absorbance profile at 520 nm, where the red-coloured anthocyanins absorb best. The predominance of anthocyanins is illustrated as trace 'B' makes up the majority of the profile. The level of anthocyanins is similar to that reported for other blackcurrant extracts and juices[30,31] as is the Vitamin C content.[30,32]

Vitamin C

Vitamin C content was measured by the HPLC method outlined previously[32] and quantified after suitable dilution against standard curves of vitamin C.

Sugars

Sucrose, fructose, and glucose contents of the blackcurrant juice were estimated using a method based on Dionex high-performance anion exchange chromatography against standard curves of the three sugars.[33]

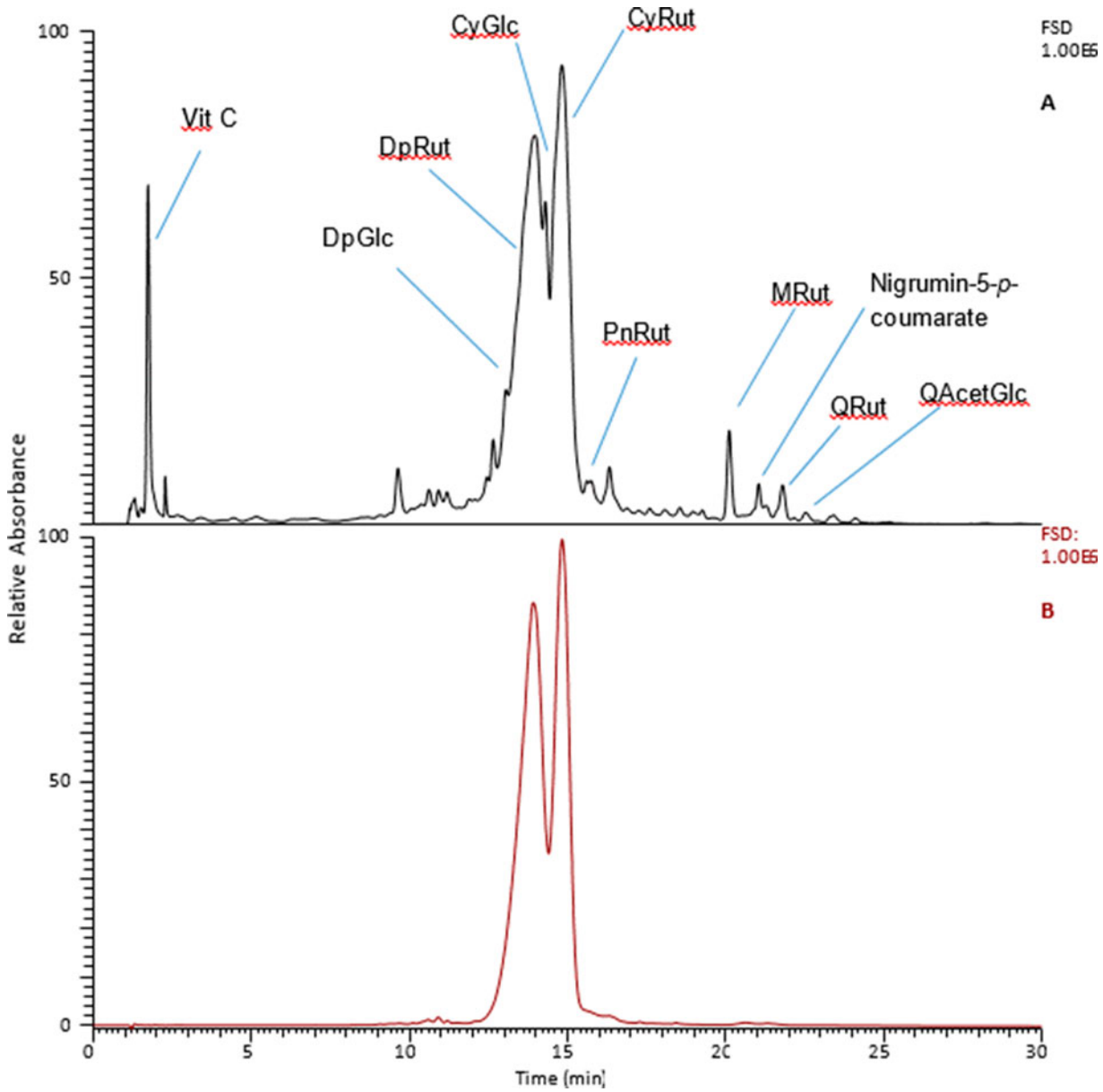

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Figure 1. Liquid chromatography mass spectrometry graph to show the phytochemical profile of the blackcurrant treatment. AQ8

The sugar measurements were found to be within normal levels for blackcurrant juice.[34]

The quantities of the naturally occurring constituents in the blackcurrant Juice are shown in Table 2.

Preparation of treatment drinks

Active treatment drinks were standardized to contain 500 mg of polyphenols in the form of blackcurrant juice. It was, therefore, calculated that 96.96 ml of Ben Hope blackcurrant juice was needed to ensure 500 mg of polyphenols were present in the blackcurrant treatment drink. Active and control drinks contained 152.90 mg of Vitamin C, 2.47 g of Glucose, 3.91 g of Fructose and 0.60 g of Sucrose. 150 ml of Blackcurrant Cordial (Schweppes blackcurrant cordial) and 10 g of a sucralose-based sweetener (Splenda) was added to each treatment drink to improve palatability. To make up for the differences in volume, 96.96 ml of water was also added to the placebo drink. The breakdown of the drink constituents is shown in Table 3.

Table 2. Compounds in the blackcurrant juice.

Constituents	Blackcurrant Juice (100 ml)
Vitamin C (mg)	157.7
Total Polyphenols (mg)	515.7
- Anthocyanins (mg)	118.7
Glucose (g)	2.55
Fructose (g)	4.03
Sucrose (g)	0.62

Cognitive assessments

CogTrack™ system

Cognitive tasks were presented using the CogTrack™ System[35,36] an online set of cognitive tests (www.wesnes.com). Prior to the start of each test, all of the

Table 3. Ingredient breakdown of each 250 ml treatment drink prepared.

Ingredients per single serve drink (approx. 250 ml)	Treatment drink	
	Blackcurrant	Placebo
Blackcurrant Juice (ml)	96.96	0.00
- Total Polyphenols (mg)	500.00	0.00
- Anthocyanins (mg)	115.09	0.00
- Vitamin C (mg)	152.90	152.90
- Glucose (g)	2.47	2.47
- Fructose (g)	3.91	3.91
- Sucrose (g)	0.60	0.60
- Water (ml)	0.00	96.96
Splenda Sweetener (g)	10.00	10.00
Blackcurrant Cordial, Schweppes© (ml)	150.00	150.00
- Sucrose (g)	18.6 g	18.6 g

required task resources were downloaded within the browser, which ensures that the rates of stimuli presentation and the assessments of the speed and accuracy of each response are managed locally. It is not until the end of each task that the data are returned to the server, which prevents poor internet connections from influencing the conduct of each test. The instructions for each task were presented on the computer screen, and remained there until the volunteer initiated the test by pressing the right arrow keyboard key. The stimuli for all tasks were presented on screen with the in-task responses made using the computer keyboard.

In this study, three cycles of the 7-minute battery involving three attention tests (Simple Reaction Time, Digit Vigilance, Choice Reaction Time) from the CogTrack™ system were used. The three attention tasks are described below in the order in which they were completed.

Simple reaction time: The volunteers were instructed to press the right arrow key on the keyboard as quickly as possible every time a stimulus was presented in the centre of the screen (the inter-stimulus interval varied randomly between 1 and 3.5 seconds). The volunteers were informed that fifty stimuli would be presented one at a time and that the stimuli would remain there until a response was made. The speed of each response was recorded.

Digit vigilance: A target digit from 1 to 9 was randomly selected and constantly displayed to the right-hand side of the screen. Digits were then presented one at a time in the centre of the screen at the rate of 150 per minute. The volunteers were asked to press the right arrow key as quickly as possible every time a digit matched the target digit. Correct detections, the speed of the detections and responses made in error (false alarms) were recorded. The duration of the task was 3 minutes, and the target density was 15 per minute.

Choice reaction time: The two possible stimuli in this task are either the right facing arrow with the word 'YES' in the middle, or a left facing arrow, with the word 'NO' in the middle. On each of 50 successive trials, one of these two stimuli were selected randomly (but with equal probability) and presented in the centre of the screen, remaining there until a response was made. Volunteers were asked to respond as quickly and accurately as possible. The accuracy and speed of each response was recorded.

Mood assessments

Visual analogue scales

Paper-based visual analogue scales (VAS) were given to volunteer's post-completion of each cycle of the battery in order to assess volunteers' subjective physical and mental states. These were presented to volunteers at baseline and after each repetition of the CogTrack™ attention tests. A 100 mm sale was anchored at either and with the adjectives extremely and not at all. Volunteers were required to use two separate scales to subjectively state how 'mental fatigued' and how 'physically fatigued' they felt at that exact moment in time.

Electroencephalography (EEG)

A simplified EEG was purchased from Alpha-Active Ltd (UK) and set up for data acquisition as described by Okello *et al.*[22] This is a non-invasive technique used to measure and record qualitative electrical activity of the brain. Depending on their frequency, brainwaves can be classified into four main groups according to frequency: delta (δ) – 0.5–4 Hz, theta (θ) – 4–8 Hz, alpha (α) – 8–12 Hz, and beta (β) – 12–30 Hz and each is related to different levels of awareness, focus, and excitation.[22] Briefly; the apparatus has five individually labelled EEG electrodes: two were placed behind the left and right ear (L– , R–) near the mastoid, two electrodes placed on the right and left side of the forehead (L+, R+) which refer to the frontal and temporal lobes of the brains left and right sides, respectively and a control electrode was placed in the middle of the forehead.

EEG recordings commenced with each CogTrack™ test. Volunteers were also instructed to remain as motionless as possible to minimize the impact of movement as an EEG artefact.

Experimental procedure

Training visit

Volunteers were required to attend a 2-hour training visit 7 days before their first study session. During this visit, volunteers were familiarized with the trial procedures and completed three sets of the cognitive battery to eliminate practice effects.[37,38]

Study visits

Volunteers were required to abstain from caffeine for 12 hours prior to each study visit and abstain from

alcohol and the consumption of dark coloured fruits for 24 hours prior to each session. Volunteers arrived for each study day in a 2-hour fasted state. The study took place in the NU-Food Research Facility (Newcastle University) in specially designed rooms where volunteers are isolated from any external stimulus. The total study period for each volunteer was approximately 4 hours, split over two separate study days, with at least 5 days' washout between each study day.

On arrival at the facility, volunteers confirmed that pre-trial procedures had been followed. Cleansing wipes were provided to cleanse the electrode attachment site of any makeup or oil residues- ensuring the EEG electrodes were fitted to uncontaminated skin. Volunteers rested for 10 minutes to ensure they became accustomed to the study environment and sensation of the EEG apparatus.

Volunteers were then instructed to complete one cycle of the 7-minute cognitive assessment, to provide baseline measurements of EEG and cognitive performance. Volunteers were then provided with a treatment drink, either placebo or active, and instructed to consume the full amount within 5 minutes. The drinks were served in undisguisable bottles so that neither the volunteer nor researcher could identify which drink was which. Whilst viewing a non-mentally stimulating documentary (Grand Designs, Channel 4), volunteers were left to rest for a further 45-minute period to allow for absorption of treatment drinks. Matsumoto *et al.*,[25] found that anthocyanins reached peak plasma concentration at 1 hour after ingestion. This would suggest that a 45-minute absorption period would put peak plasma concentration 15 minutes into the 21-minute test period. After this, volunteers were instructed to begin a further three cycles of the CogTrack™ attention battery. At the end of the cognitive assessments EEG recording ceased and volunteers were free to leave. A basic outline of the overall procedure is given in Fig. 2.

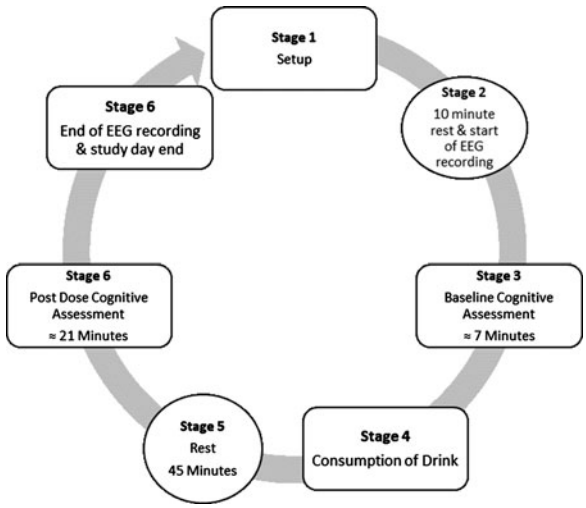

Figure 2. A basic outline of the experimental procedure. Completed once on each volunteers first study day, and repeated on each volunteers second study day.

Statistics

Mood and cognition

Baseline differences were calculated for all measures using a one-way (treatment) repeated measures ANOVA. All CogTrack™ and mood results were computed into change from baseline scores, calculated by subtracting the post-treatment scores from the pre-treatment scores. Further, the accuracy scores from the three attention tests were combined to create the Sustained Attention Index (Wesnes *et al.* 2016), calculated at baseline and for each repetition. Treatment effects were analysed with linear mixed models including the terms treatment, assessment, treatment × assessment as fixed effects and treatment order as cofactor.

Pairwise comparisons (LSD) were conducted on all outcomes with a P value <0.05 from the initial mixed model analysis to ascertain any differences between treatments for the whole session and at specific epochs. All data were tabulated using Microsoft Excel 2013 and analyses were conducted with IBM SPSS Statistics 22.

EEG

For each volunteer, study time-points (task and task repetition) were identified as a separate epoch. HEADCOACH© recorded the brain spectral activity from the left and right hemispheres as distinct and separate channels; which were averaged to give an overall mean value of prefrontal cortical wave spectral activity. EEG data were then processed to remove artefacts (extracerebral factors[39]) by subtraction of the standard deviation from each time point. EEGLAB (Swartz Centre for Computational Neuroscience, CA, USA) was then used to generate a mean value for each study epoch (combination of task and repetition) and wavelength (Delta (δ), Theta (θ), Beta (β) and Alpha (α)). All values were then processed to give change from baseline values, calculated by subtracting the post-treatment scores from the pre-treatment scores (baseline). Repeated measures ANOVA was conducted on both placebo and Blackcurrant treatment baseline values to test for significance of task variance between the two treatments. Repeated measures ANOVA was conducted on placebo treatment and Blackcurrant treatment data to determine the overall effect of treatment, of task repetition, and for the combined effect of treatment and task repetition. On all treatment-related effects, *post hoc* pairwise comparisons identified where relationships were found. All results were reported at a significance level of $P < 0.05$.

Table 4. Mean change from baseline scores and standard deviations (SD) and main ANOVA outcomes for subjective ratings of mental fatigue, physical fatigue and task difficulty, for each task repetition (1–3), following supplementation with either the Blackcurrant or placebo treatment drinks.

Outcome	Treatment	Repetition 1		Repetition 2		Repetition 3		Effect of treatment	Treatment*repetition interaction
		Mean	SD	Mean	SD	Mean	SD		
Mental fatigue (mm)	Blackcurrant	−10.89	37.07	−9	29.77	−15.22	27.87	$F = 0.695$	$F = 0.162$ $P = 0.833$
	Placebo	−5.44	22.72	1.44	22.28	−3	29.87	$P = 0.429$	
Physical fatigue (mm)	Blackcurrant	3.33	23.52	2.67	29.96	2.22	26.19	$F = 0.000$	$F = 0.270$ $P = 0.767$
	Placebo	2.33	23.25	−0.22	27.46	5.56	22.13	$P = 0.987$	
Task difficulty (mm)	Blackcurrant	8.22	13.41	3.89	13.82	2.11	22.83	$F = 0.890$	$F = 0.939$ $P = 0.386$
	Placebo	−6.11	15.82	−2.11	19.48	−6.67	22.82	$P = 0.373$	

Results

No significant difference was found between treatments at baseline for any outcome.

Mood scales

Raw change from baseline post dose data and mixed model outcomes for all VAS are shown in Table 4. No interpretable effects of treatment were found in the mixed model analysis for any of the outcomes.

Cognitive assessment

Mean post dose change from baseline scores, standard deviations and mixed model outcomes are shown in Table 5.

Choice reaction time

A significant treatment effect was found for the speed of responses during the choice reaction time task [$F(1,8) = 5.40$, $P = 0.028$] with the blackcurrant treatment increasing reaction time by 19.8 ms when compared with placebo. No treatment * Repetition interactions were found [$F(2,16) = 1.67$, $P = 0.20$]. This change in reaction time did not influence task accuracy [$F(1,8) = 1.31$, $P = 0.28$] (Table 5; Fig. 3).

EEG

Mean post dose change from baseline scores, standard deviations and ANOVA outcomes can be found in Table 6. All interpretable effects of treatment are summarized below.

Simple reaction time

Alpha

No effect of treatment alone was found for the α waveband [$F = (1,8) = 2.385$, $P = 0.161$]. However, a significant treatment*repetition effect was found [$F = (2,16) = 43.682$, $P = 0.000$].

Pairwise comparisons revealed that the strength of the α brainwaves became significantly lower after supplementation with the Blackcurrant juice, at the third repetition of the SRT test ($P = 0.000$) when compared with the placebo (Figure 4A).

Beta

No significant effect of treatment was found for the β brainwave [$F = (1,8) = 1.586$, $P = 0.243$]. However, a significant treatment*repetition interaction was found [$F(2,16) = 24.193$, $P = 0.000$].

Table 5. Mean change from baseline scores, standard deviations (SD) and main ANOVA outcomes for simple reaction time, digit vigilance and choice reaction time, for each task repetition (1–3), following supplementation with either the Blackcurrant or placebo treatment drinks.

Task	Task measure	Treatment	Repetition 1		Repetition 2		Repetition 3		Effect of treatment	Treatment*repetition interaction
			Mean	SD	Mean	SD	Mean	SD		
Simple reaction time	Reaction time median (Msec)	Blackcurrant	13.10	22.20	28.73	24.27	34.58	26.87	$F = 2.12$ $P = 0.15$	$F = 0.10$ $P = 0.71$
		Placebo	25.20	24.67	45.64	28.51	47.08	32.62		
Digit vigilance	Accuracy (%)	Blackcurrant	−1.73	4.94	−0.98	3.70	−1.97	5.38	$F = 0.16$ $P = 0.69$	$F = 0.54$ $P = 0.21$
		Placebo	0.98	2.96	−0.98	6.20	−1.72	9.54		
	Reaction time (msec)	Blackcurrant	3.90	36.18	7.78	30.36	32.47	30.90	$F = 0.26$ $P = 0.60$	$F = 2.02$ $P = 0.12$
		Placebo	12.54	27.61	24.16	37.08	17.75	44.6		
	False alarms (#)	Blackcurrant	0	1.22	0.11	1.61	−0.55	1.87	$F = 1.00$ $P = 0.24$	$F = 1.65$ $P = 0.20$
		Placebo	−0.44	1.01	−0.08	0.92	0.55	1.13		
Choice reaction time	Reaction time (msec)	Blackcurrant	18.93	25.57	15.01	17.72	28.47	28.47	$F = 5.40$ $P = 0.028$	$F = 1.67$ $P = 0.20$
		Placebo	−8.88	20.36	7.90	22.58	1.73	41.64		
	Accuracy (%)	Blackcurrant	−1.15	3.84	0.88	3.01	0.88	3.48	$F = 0.03$ $P = 0.85$	$F = 1.31$ $P = 0.28$
		Placebo	−0.44	1.94	−0.22	1.855	0	3.31		
Sustained attention index		Blackcurrant	−1.72	6.33	−1.23	6.72	0.37	7.39	$F = 0.32$ $P = 0.57$	$F = 0.09$ $P = 0.75$
		Placebo	0.49	2.49	0.37	2.94	−0.41	5.34		

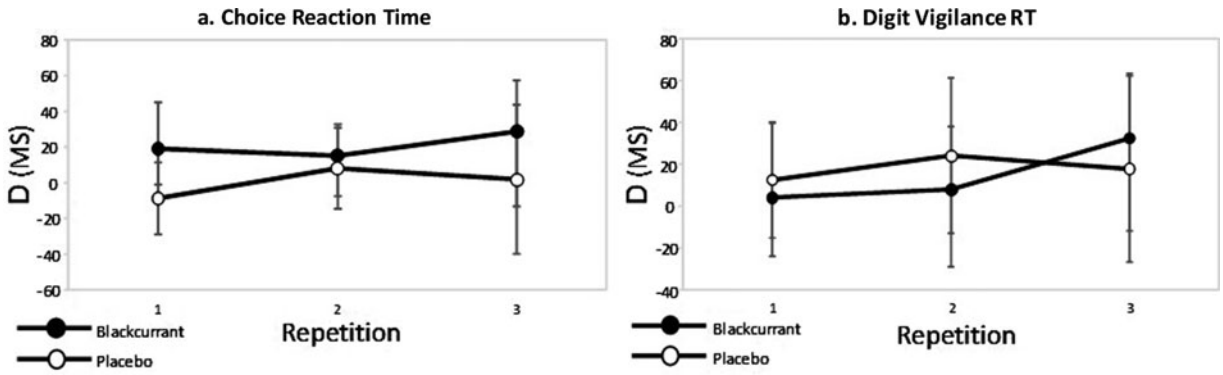

Figure 3. (A) Mean change from baseline scores for each repetition for the choice reaction time where the mixed model showed an overall treatment effect ($P = 0.02$) and (B) mean change from baseline scores for each repetition for the digit vigilance reaction time where the mixed model showed a trend treatment*repetition interaction ($P = 0.12$).

Pairwise comparisons revealed that, after supplementation with the Blackcurrant juice, repetition 1 gave significantly lower strength Beta brainwaves ($P = 0.003$) than the placebo (Fig. 4B)

Digit vigilance

Baseline

No significant differences were found between baseline $\delta[F = (1,8) = 0.516,\ P = 0.493]$, $\theta[F = (1,8) = 0.499,\ P = 0.500]$, $\alpha[F = (1,8) = 0.565,\ P = 0.474]$ or β $[F = (1,8) = 0.675,\ P = 0.435]$ wavelength spectral power.

Alpha

No significant effect of treatment was found $[F = (1,8) = 0.000,\ P = 0.986]$. There was however a significant treatment*repetition interaction $[F = (2,16) = 30.943,\ P = 0.000]$.

Pairwise comparisons showed that, after supplementation with the Blackcurrant juice, the strength of the brainwaves were significantly higher in repetition 1 ($P = 0.002$) but significantly lower in repetition 3 ($P = 0.001$) of the DV test, when compared with placebo (Figure 4D).

Beta

No significant effect of treatment was found $[F = (1,8) = 0.898,\ P = 0.371]$, There was however a significant treatment*repetition interaction $[F = (2,16) = 9.039,\ P = 0.002]$.

Pairwise comparisons showed that, after supplementation with the Blackcurrant juice, repetition

Table 6. Mean change from baseline scores, standard deviations (SD) and main ANOVA outcomes for Simple Reaction Time, Digit Vigilance and Choice Reaction Time, for each task repetition (1–3), following supplementation with either the Blackcurrant or Placebo treatment drinks.

			Repetition 1		Repetition 2		Repetition 3			
Task	Task Measure	Treatment	Mean	SD	Mean	SD	Mean	SD	Effect of treatment	Treatment*repetition interaction
SRT	Delta	Blackcurrant	−0.08	3.41	−0.99	3.23	−0.14	3.74	$F = 1.374$	$F = 0.416$ $P = 0.593$
		Placebo	1.55	4.28	−0.31	2.64	2.23	4.09	$P = 0.275$	
	Theta	Blackcurrant	−0.40	3.37	−1.01	3.23	−0.45	3.46	$F = 1.505$	$F = 0.246$ $P = 0.738$
		Placebo	0.89	3.18	0.57	2.42	1.53	2.48	$P = 0.255$	
	Alpha	Blackcurrant	−0.45	3.43	−4.58	2.09	−4.43	2.55	$F = 2.385$	$F = 43.682$ $P = 0.000$
		Placebo	−2.87	3.03	−2.89	2.47	1.39	2.23	$P = 0.161$	
	Beta	Blackcurrant	−0.20	2.59	2.68	3.27	3.04	3.40	$F = 1.586$	$F = 24.193$ $P = 0.000$
		Placebo	4.19	2.25	3.89	1.91	1.06	1.78	$P = 0.243$	
DV	Delta	Blackcurrant	0.36	2.20	1.06	3.55	1.16	2.38	$F = 0.883$	$F = 1.042$ $P = 0.376$
		Placebo	−1.31	1.32	0.82	1.26	0.61	1.48	$P = 0.375$	
	Theta	Blackcurrant	0.14	2.06	1.09	3.59	1.14	2.37	$F = 1.000$	$F = 0.419$ $P = 0.655$
		Placebo	−1.13	1.48	0.67	1.45	0.56	1.30	$P = 0.347$	
	Alpha	Blackcurrant	−0.41	1.99	−2.24	2.83	−2.40	2.29	$F = 0.000$	$F = 30.943$ P= 0.000
		Placebo	−3.47	1.82	−2.09	1.80	0.54	1.32	$P = 0.986$	
	Beta	Blackcurrant	0.41	2.82	3.74	3.57	3.61	2.69	$F = 0.898$	$F = 9.039$ P= 0.002
		Placebo	1.71	1.76	3.34	1.95	0.38	1.44	$P = 0.371$	
CRT	Delta	Blackcurrant	1.32	2.19	0.79	2.55	0.52	2.52	$F = 8.469$	$F = 2.629$ $P = 0.103$
		Placebo	−1.22	1.51	−0.44	1.47	−0.09	2.03	$P = 0.020$	
	Theta	Blackcurrant	1.40	2.11	0.85	2.29	0.56	2.51	$F = 4.841$	$F = 2.842$ $P = 0.088$
		Placebo	−0.96	1.26	−0.23	1.54	0.18	1.97	$P = 0.059$	
	Alpha	Blackcurrant	−2.87	2.26	−3.22	2.36	−0.38	3.55	$F = 0.458$	$F = 1.925$ $P = 0.184$
		Placebo	−4.26	1.28	−3.62	1.50	0.28	1.99	$P = 0.518$	
	Beta	Blackcurrant	4.58	1.55	4.01	2.12	0.27	2.02	$F = 2.286$	$F = 3.368$ $P = 0.060$
		Placebo	2.69	1.64	3.39	2.12	0.20	1.91	$P = 0.169$	

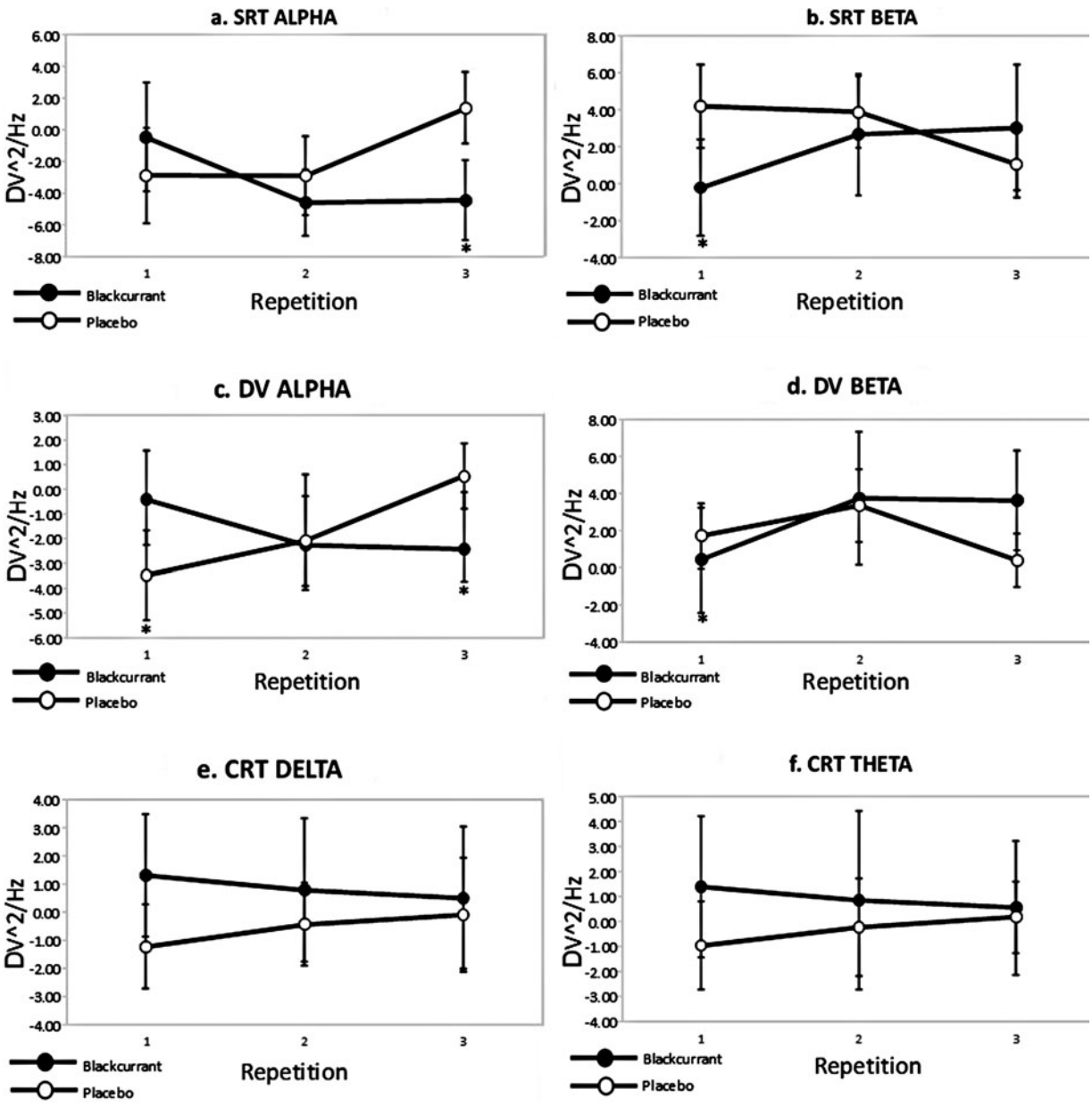

Figure 4. Brain spectral activity at each epoch for outcomes which elicited a significant effect of treatment or treatment × repetition interaction. (*$P < 0.05$). SRT = single reaction time, DV = Digit Vigilance CRT = choice reaction time.

1 gave significantly lower strength β spectral power ($P = 0.003$) compared to the placebo (Fig. 4C)

Choice reaction time

Baseline

No significant differences were found between baseline δ[$F = (1,8) = 0.049$, $P = 0.830$], θ[$F = (1,8) = 0.028$, $P = 0.871$], α [$F = (1,8) = 0.127$, $P = 0.731$] or β [$F = (1,8) = 0.161$, $P = 0.698$] spectral power.

Delta

A significant effect was found between treatments [$F = (1,8) = 8.469$, $P = 0.020$] and further analysis with pairwise comparisons revealed that supplementation with the Blackcurrant juice gave significantly higher strength δ spectral power ($P = 0.020$) when compared with placebo, irrespective of repetition. No significant treatment*repetition interaction was found [$F = (2,16) = 2.629$, $P = 0.103$] (Fig. 4F).

Theta

There was a non-significant trend effect of treatment [$F = (1,8) = 4.841$, $P = 0.059$]. Pairwise comparisons revealed that supplementation with the Blackcurrant juice gave higher strength θ spectral power ($P = 0.059$) when compared with placebo, irrespective of repetition. No significant treatment*repetition interaction was found [$F = (2,16) = 2.842$, $P = 0.088$] (Fig. 4E).

Discussion

Results from the current trial provide evidence of a modulation of pre frontal cortex brain wave spectral activity in small sample of healthy young adults after the acute supplementation of a single serve cold-

pressed blackcurrant juice drink standardized to contain 500 mg of polyphenols. Outcomes from the CogTrack™ system indicate a small increase in reaction times during the digit vigilance task, with no interpretable impact upon accuracy.

The observation of an increase in choice reaction time after supplementation with blackcurrant juice compared with placebo was unexpected but could be explained by the EEG spectral power activity data. The significant increase in the slow wave δ and θ spectral powers is indicative of enhanced or deep relaxation perhaps caused by the anxiolytic effects of the anthocyanin-rich blackcurrant juice[40,41] a potential effect of blackcurrants monoamine oxidase inhibitory properties,[21] which may have impacted on speed of reaction although interestingly not accuracy. Oscillations in the alpha-band are important in a number of critical selective attentional processes and as such, natural compounds that have clear effects on this rhythm are of significant interest.[42] Alpha suppression, or attenuation, is associated with heightened attention,[42–45] furthermore increases in Alpha power are associated with fatigue.[46] Suppression of alpha power has previously been shown after supplementation with a polyphenol beverage containing 58% anthocyanins,[47] suggesting that anthocyanins may have been responsible for this effect in this study.

SRT is a much 'simpler' task compared to CRT. The observation that α wave spectral power decreased with repetition is indicative of increased attention. Alpha waves, the normal alert but relaxed state waves, are known to decrease with attention while β waves which are associated with attention and fast activities increase as demonstrated by the significant increase in their spectral power over task repetitions. Likewise DV requires increase in attention and concentration, and like the STR this is demonstrated by a decrease in α and an increase in β spectral powers. The concurrent increases in beta brainwaves observed are also associated with heightened attention[45] and alertness[48,49]; and decreases associated with fatigue.[46] Brain wave spectral activity acquired from the EEG in the current study, therefore, suggests that after supplementation with the Blackcurrant treatment, volunteers had higher attention levels, and lower fatigue compared to placebo. However, this did not translate into improved psychomotor outcomes.

It may be interpreted that higher beta power/lower alpha power is indicative of higher mental exertion needed to perform. This view is shared by Kiroy *et al.*,[50] who found that after a 6-hour performance on cognitive tasks, a significant increase in Beta power occurred. Kiroy *et al.*,[50] stated that fatigued individuals, who must nevertheless perform the task, show evidence of high physiological arousal and beta activity increase,[50] a conclusion that is supported by a number of previous studies.[51,52] It could therefore be suggested, that after supplementation with the Blackcurrant treatment, fatigued volunteers were more focussed and eager to perform the tasks correctly, causing an increase in Beta power. However, no impact was seen upon cognitive outcomes.

A previous acute intervention assessing the effect of blackcurrants upon cognitive function[21] found a significant attenuation of mental fatigue, and a significantly higher self-reported rating of alertness, during a cognitive demand battery, after supplementation with a blackcurrant drink standardized at 500 mg of polyphenols per 60 kg/bw. However, it is important to note that the study used many more repetitions (seven in total) and changes only became significantly different on the final repetition of the tasks. Results from the two trials indicate that volunteers need to be under considerable cognitive loads for a prolonged period for the anti-fatigue effects of blackcurrant juice to be seen.

Bell[3] stated that the timings of cognitive effects are likely to be related to the digestion, absorption, and metabolism of flavonoids. The significant effects observed at 150 minutes in the study by Watson *et al.*,[21] coincided with a significant increase in plasma anthocyanins. In the present study, volunteers only participated in tasks up until 70 minutes after supplementation which may not have been long enough to yield a significant effect of anthocyanins on cognitive performance. Research suggests that peak concentrations of anthocyanins post berry consumption appear between 1 and 4 hours' post dose.[25,53] The actual times to peak plasma concentrations are most likely altered by the matrix of the bolus, a factor on which there is no data in the literature. Known pharmacokinetics of anthocyanins would suggest that, in the present study, volunteers may have experienced maximal anthocyanin levels in the blood during at the third repetition of the CogTrack™ battery. As a limitation of the present study, no plasma anthocyanin measurements were made, and therefore the levels of plasma anthocyanins, and corresponding time points they were highest in plasma remain unknown. The relationship between the levels of phytochemicals in plasma and cognitive performance is an area which should be investigated. Additionally, the small sample size of this study could have contributed to the absence of any cognitive findings which previous work has identified and as such, future work should power studies appropriately using the data produced by this pilot study.

Evidence from the pilot EEG data obtained in the current trial highlight an anxiolytic effect of the consumption of a single serve blackcurrant juice, and an indication of greater alertness and lower fatigue in healthy young adults. However, these changes did

not improve cognitive performance and slowed responses in the choice reaction time task. Berries, such as blackcurrants are an affordable and readily accessible food to many people; more research in this area will help to define the optimal dosage, preparation and potential cognitive benefits via acute or chronic consumption.

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