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Artificial food coloring affects EEG power and ADHD symptoms in college students with ADHD: a pilot study

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ABSTRACT

Objectives: Removing artificial food coloring (AFC) is a common dietary intervention for children with Attention-Deficit/Hyperactivity Disorder (ADHD), but has not been tested in young adults. This pilot study examined the effects of AFC on ADHD symptoms and electroencephalography (EEG) in college students with and without ADHD.

Methods: At baseline, control and ADHD participants completed the Adult ADHD Self-Report Scale (ASRS), simple and complex attention measures, and resting-state EEG recordings. ADHD participants ($n = 18$) and a subset of controls (extended control group or EC, $n = 11$) avoided AFC in their diet for 2 weeks and then were randomized to a double-blind, placebo-controlled crossover challenge. Subjects received either 225 mg AFC disguised in chocolate cookies or placebo chocolate cookies for 3 days each week, with testing on the third day each week. Baseline comparisons were made using Student's t-test or Wilcoxon rank sum tests and challenge period analyses were run using General Linear Modeling.

Results: The ADHD group had significantly greater scores on the ASRS ($p < 0.001$), confirming a symptom differential between groups; however, there were no differences in attentional measures or EEG at baseline. The AFC challenge resulted in an increase in posterior mean gamma power ($p = 0.05$), a decrease in posterior relative alpha power ($p = 0.04$), and a marginal increase in inattentive symptoms ($p = 0.08$) in the ADHD group. There were no effects of AFC in the EC group.

Discussion: This study indicates that AFC exposure may affect brainwave activity and ADHD symptoms in college students with ADHD. Larger studies are needed to confirm these findings.

KEYWORDS
Attention Deficit Hyperactivity disorder (ADHD); food additives; electroencephalography (EEG); artificial food coloring; adults

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder defined by persistent inattention, hyperactivity, and/or impulsivity [1]. Approximately 5–11% of children under the age of 18 are diagnosed with ADHD [2]. Over 50% of individuals carry this diagnosis into adulthood, with an adult ADHD prevalence rate of 4.4% in the United States [3,4]. While the etiology of ADHD is not fully understood, genetic underpinnings, neurobiological dysfunction, and environmental/lifestyle factors have been linked to the disorder [5–7].

Diet is a specific lifestyle factor that has been implicated in the treatment of ADHD. One dietary intervention for ADHD that has been studied since the 1970s is the removal of artificial food coloring (AFC) from the diet. The Kaiser-Permanente (K-P) diet was the first diet to systematically remove AFC, naturally occurring salicylates, and artificial flavors from the diet in children diagnosed as hyperactive or behaviorally deviant. The K-P diet resulted in a reduction of hyperactive symptoms in 11–33% of children both at school and at home [8,9]. Since then, the effects of AFC on children with ADHD has been heavily researched with reports of increased hyperactive behavior, impulsivity, motor responses, sleep disturbances, and negative mood after being exposed to varying doses (26–150 mg) of AFC [10]. In a meta-analysis of 15 double-blind placebo-controlled trials, parental reporting of hyperactive behaviors was significantly different during challenge with AFC as compared to placebo [11]. Another more recent meta-analysis concluded that approximately 8% of children with ADHD may benefit from removing AFC from their diet [12].

While AFC research has been conducted for decades, there are two major factors that remain under

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researched: investigating the effects of AFC in young adults with ADHD and the use of objective outcome measures. First, to our knowledge, all dietary research within the ADHD population has been conducted in children and adolescents. While ADHD is the most prevalent neurodevelopmental disorder, and therefore highly diagnosed in children, individuals can carry the diagnosis into adulthood or be diagnosed as an adult [1]. Second, the potential connection between AFC and symptoms of ADHD has relied mostly on subjective reporting of symptoms from parents, teachers, and clinicians, with only a handful of studies employing quantifiable learning or behavioral tasks [9]. Using objective measures for dietary research is vital to understand how dietary components, like AFC, influence ADHD.

Resting-state electroencephalography (EEG) has been used in ADHD research as an objective measure of brain activity. EEG, compared to other imaging techniques such as functional neuroimaging, is useful when studying ADHD because there is a large extant literature, it can be completed in a short time frame, and the data is visualized during recording which can reduce artifacts. Resting-state EEG has been conducted in adults with ADHD, with theta power being consistently increased, and other power bands having mixed findings (Table 1). An increased theta/beta ratio (TBR) was once hoped to be a biomarker of ADHD, but a meta-analysis found that the initial specificity and sensitivity rates (>85%) have not held across replication [13]. Importantly, only three studies have focused on young adults [14–16] and no studies have investigated dietary treatments for adults.

To address these gaps in the literature, this pilot study aimed to examine the effects of AFC on college students with and without ADHD using a double-blind placebo-controlled crossover challenge. EEG is used as an objective outcome measure, in addition to computerized cognitive tests of attention and a self-report measure of adult ADHD symptoms. It was hypothesized that: (1) baseline theta and TBR would be increased in the ADHD population, (2) consuming high doses of AFC would change EEG power, compared to placebo, across all frequency bands, and (3) ADHD symptoms would be increased while attentional scores decreased during AFC exposure as compared to placebo.

Methods

Participants

Eighteen participants with ADHD and 41 controls were recruited from a mid-Atlantic University through on-campus advertisements. Inclusion criteria included being enrolled at the university, 18–24 years old, with stable medications for >3 months. The ADHD group had to also provide documentation of diagnosis and be willing to suspend ADHD medication for 24–48 h before testing. Exclusion criteria were presence of a seizure disorder, past hospitalization for asthma, or comorbid disorders requiring anti-psychotic medication. One ADHD participant dropped out of the study before the challenge period due to scheduling conflicts.

Procedure

The study was approved by the university's IRB and written informed consent was obtained. Baseline assessments for participants included collection of demographic/anthropometric data, completion of cognitive testing using CNS Vital Signs® (CNSVS) software, the Adult

Table 1. Differences in absolute and relative EEG spectral power between Adults with ADHD and Controls.

Author	Year	EC/EO	Mean age (yrs)	Delta	Theta	Alpha	Beta	Gamma	Theta/Beta
Bresnahan	1999	EO	30	↑↑	↑↑	↓	↓		↑
Bresnahan	2002	EO	31	↑	↑↑	↑	↑		↑
Hermens	2004	EC	41	–	↑	–	↓		
Bresnahan	2006	EO	32	↓↑	↑↑	–	↓		↑
Clarke	2008	EC	22	↓	↑	–	↓↓	–	
Hale	2009	EC/EO	45			↑			
Koehler	2009	EC	33	–	↑	↑	–		
van Dongen-Boomsma	2010	EC/EO	34		–	–	–		↑
Woltering	2012	EC/EO	26		↑↑	↓↓	↓↓		
Liechti	2013	EC/EO	43	–	–	–	–		
Buyck	2014	EC	26		–		–		–
Kitsune	2015	EO	19	↑	↑	–	↑		
Skirrow	2015	EO	29	–	–	–	–		–
Rommel	2017	EO	19	–	–	–	–		
Li	2017	EC	26	–	↑	↓	↑		
Markovska-Simoska	2017	EO	36	–	–	–	–		–

Arrows up indicate an increase in power, arrows down indicate a decrease in power, and straight bars indicate no difference in power. Blue arrows: absolute power. Red arrows: relative power. EC: Eyes-Closed; EO: Eyes-Open.

ADHD Self-Report Scale (ASRS) [17], and 4 minutes of eyes-closed resting-state EEG. Four minutes of eyes-closed data was recorded to ensure enough usable segments of EEG data, and was based on the average length of EEG recordings in adult ADHD research. To avoid medication effects, ADHD medication use was suspended 24–48 h before testing, as appropriate for the half-life of the medication.

After baseline testing, ADHD participants and a subset of control participants (extended controls or EC, n = 11) completed in-person dietary training on how to identify and avoid AFC in the diet, including reviewing the AFC names, identifying such names on food labels, reviewing common foods containing AFC, and talking through their average diet to identify foods containing AFC. Participants also received a paper and PDF copy of the dietary training.

Participants avoided AFC in the diet for the remaining 4 weeks of the study. Participants followed the diet for 2 weeks (to washout AFC consumption and standardize diets) before beginning the 2-week crossover challenge. After 2 weeks on the diet, participants completed an AFC specific Food Frequency Questionnaire (FFQ) to measure dietary compliance and then were randomized to a double-blind placebo-controlled crossover challenge for 3 days each, of 2 consecutive weeks. The challenge materials were either 225 mg of mixed powder AFC (i.e. Red No. 40, Red No. 3, Yellow No. 5, Yellow No. 6, Blue No. 1, Blue No. 2) disguised in chocolate cookies, or placebo chocolate cookies with no AFC. Chocolate cookies work well for blinding since these colors look brown when mixed together. The mixture of AFC was chosen to incorporate the colors most often consumed in the diet, and the amount of each AFC used was half of the Estimated Daily Intake (EDI) of a high-consumer, as determined by the FDA [18]. A previous study showed that a washout period of 2 days did not result in carry-over effects, [19] however the study only used one AFC, so the washout period was doubled (4 days) to account for the combined AFC challenge. The cognitive testing, ASRS, and EEG recordings were repeated at the end of each 3-day exposure (Figure 1).

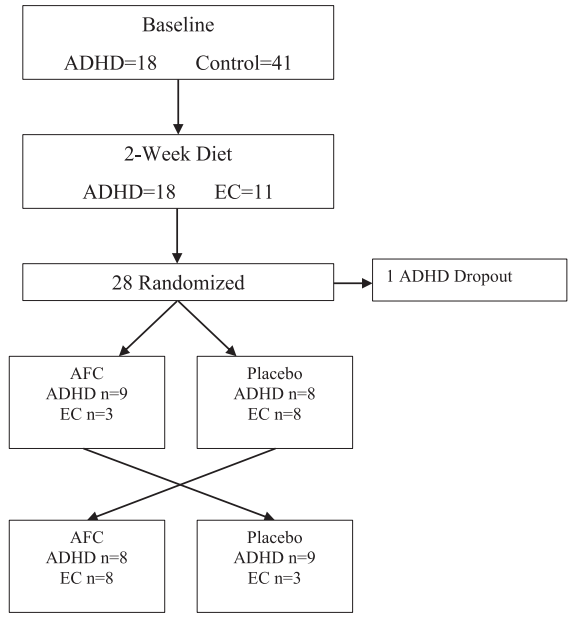

Figure 1. Study design. A double-blind placebo-controlled crossover design with an AFC challenge was implemented. One ADHD participant dropped out prior to randomization.

Adult ADHD Self-Report Scale (ASRS)

The ASRS-v1.1 is a reliable and valid scale used for assessing ADHD symptoms based on the DSM-IV-TR criteria in the adult population [17]. The ASRS includes 6 questions in Part A (considered most predictive of adult ADHD) and 12 questions in Part B (specific symptom profile questions). Scoring is based on the frequency of symptoms, ranging from 0 for 'never' to 4 for 'very often', with a higher score indicating a higher likelihood of ADHD. The ASRS asks about symptoms over the last 6 months, however participants were asked to answer questions based on the week leading up to the testing to capture potential short-term effects of AFC. Additionally, ASRS questions were broken up into 'inattentive' or 'hyperactive' sub-classifications based on CNSVS protocol for adult ADHD testing [20], resulting in nine inattentive and nine hyperactive questions, and then were scored. Thus, each participant received an inattentive, hyperactive, and total ASRS score. The ASRS was administered at the end of the CNSVS adult ADHD testing battery.

CNSVS cognitive testing for attention

CNS Vital Signs® (CNSVS) software was used to test simple and complex attention. Simple attention is measured using a Continuous Performance Test (CPT) to analyze the correct response rate and whether the correct responses are more frequent than commission errors. Complex attention is a composite score from performance on a Stroop task, CPT, and Shifting Attention Task. One control participant had invalid test scores for both simple and complex attention (indicating that they did not understand the instructions), therefore their data were excluded from these analyses.

Quantitative electroencephalography (EEG)

Four minutes of eyes-closed resting-state data was collected in a sound attenuated room at baseline and after

each challenge period. Participants were instructed to remain still while being relaxed, but awake.

EEG recording was completed using g.tec equipment (http://www.gtec.at). Twenty-five Ag/AgCL g.SCARABEO Z electrodes (16 × 10 × 5 mm, 125 cm lead) were used according to the International 10–20 System with the reference on the right earlobe and the ground electrode at Fpz. Impedance remained below 5 kOhm for all electrodes. A sampling rate of 256 Hz, input signal filter of 0.5–60 Hz, and a 60 Hz notch filter were applied online.

EEG recordings were analyzed in EEGLAB [21] and The Batch Electroencephalography Automated Processing Platform (BEAPP) [22]. In EEGLAB, the first 30 s and last 10 s of recording were trimmed and channels F5 and P8 were interpolated on baseline data for 24 participants after they are incorrectly placed in the array (but within the correct region of analysis). In BEAPP, a bandpass filter was applied (1–50 Hz). To remove artifacts, Independent Component Analysis (ICA) with multiple artifact rejection algorithm (MARA) was used to automatically reject multiple types of artifacts [23]. The data were re-referenced to the average and segmented into 2-second epochs, with time points rejected if more than 0.01% of the epoch was >150 μV. Power spectral density was calculated using the Fast Fourier Transform with a Hanning window. Mean power and relative power were calculated for delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), and gamma (30–50 Hz) frequency bands. Mean power is the sum of the power values of each frequency band divided by the frequency bandwidth, a measure similar to absolute power. Relative power is the absolute power of a frequency band divided by the total power of all bands. The theta-beta ratio (TBR) was also calculated. Each band and ratio was then averaged over frontal electrodes (Fp1, Fp2, F3, F4, F5, F6, F7, F8, Fz, AF3, AF4), central electrodes (Cz, C3, C4), and posterior/temporal electrodes (Pz, P3, P4, P7, P8, P9, P10, T7, T8, O1, O2).

Data analysis

All data were analyzed in SPSS® V25.0. Baseline comparisons between ADHD and controls for demographic and anthropometric data were run using Wilcoxon rank sum test for non-normal continuous variables, Student's t-test for normal continuous variables, or Chi-Square test for categorical variables. Baseline comparisons of mean and relative EEG power between groups were analyzed using Wilcoxon rank sum test. Challenge analyses for the ADHD and EC group were run using General Linear Modeling (GLM) with the within-subjects variable being the challenge week data (AFC/placebo) and the between-subjects variable being the order of challenge materials. GLM analyses were run separately for each group to test for effect modification. GLMs were run for ASRS scores (inattentive, hyperactive, and total), simple and complex attention measures, each frequency band, and TBR in the frontal, central, and posterior regions. At least 75% of the EEG data segments had to be useable after preprocessing to be included in the statistical analyses, resulting in the elimination of 6 controls at baseline, and 1 ADHD and 2 EC participants during the challenge analyses. Spearman correlations were run between significant challenge period EEG findings and simple attention, complex attention, and all three ASRS measures.

Since this is the first study on this topic, a power analysis for sample size was not able to be performed. Therefore, uncorrected p-values are reported to the *a priori* significance level of $p = 0.05$ to note possible effects of AFC across all measures. Hedge's g was used to determine effect sizes of findings (small effect = 0.2, medium effect = 0.5, large effect = 0.8).

Results

Baseline comparisons

The only significant difference between the ADHD and control group for demographic or anthropometric data at baseline was for age, with the ADHD group being one year younger on average ($t(57) = 2.04$, ($p = 0.05$) (Table 2)). Most ADHD participants were diagnosed by a psychiatrist (50.0%) or psychologist (38.9%), with only two participants being diagnosed by a primary care doctor (11.1%). The majority of ADHD participants (78%) were currently taking ADHD medication, with stimulant medication being prescribed most often (61.2%). The ADHD group was also more likely to be taking other types of medication when compared to healthy controls ($\chi^2(1) = 4.02$, $p = 0.05$), including antidepressants, anti-anxiety, birth control, and allergy medications. The ADHD group had significantly higher inattentive ($t(57) = -6.24$), hyperactive ($t(57) = -6.28$), and total ASRS scores ($t(57) = -7.14$) than the control group ($p < 0.001$), confirming clinical symptomology differences between groups. There were no baseline differences in simple or complex attention scores, or in EEG power between the ADHD and control group.

There were no significant differences between the ADHD and EC group for demographic or anthropometric data. As would be expected, the ADHD group had significantly higher inattentive, hyperactive, and total ASRS scores than the EC group across all testing points (Table 3). The ADHD (mean = 7.69, SD = 10.68) and the EC groups (mean = 4.18, SD = 4.38) had similar dietary compliance ($p > 0.05$).

Table 2. Demographics of ADHD and control groups.

	ADHD ($N = 18$)	Controls ($N = 41$)	P-value*
	Median (IQR)	Median (IQR)	
Age (yrs)	19.0 (2)	20.0 (2)	0.05
GPA	3.45 (0.67)	3.60 (0.46)	0.16
BMI	22.26 (6.82)	24.10 (4.93)	0.24
Simple Attention	92.0 (27)	98.0 (13)	0.61
Complex Attention	100.0 (27)	108.0 (16)	0.84
	Mean (SD)	Mean (SD)	P-value**
Inattentive ASRS	23.73 (5.99)	14.41 (4.94)	<0.001
Hyperactive ASRS	19.39 (6.09)	10.01 (4.85)	<0.001
Total ASRS	43.44 (11.30)	24.49 (8.46)	<0.001
	N (%)	N (%)	P-value***
Sex (N(%) Female)	13 (72.2%)	27 (65.9%)	0.63
Race			0.31
White	13 (72.2%)	24 (58.5%)	
African American	1 (5.6%)	1 (2.4%)	
Asian	1 (5.6%)	7 (17.1%)	
Hispanic	2 (11.1%)	5 (12.2%)	
Middle Eastern	1 (5.6%)	0 (0.0%)	
Mixed	0 (0.0%)	4 (9.8%)	
Right-Handed	17 (94.4%)	36 (87.8%)	0.44
Year in School			0.10
Freshman	11 (61.1%)	12 (29.3%)	
Sophomore	3 (16.7%)	7 (17.1%)	
Junior	3 (16.7%)	14 (34.1%)	
Senior	1 (5.6%)	8 (19.5%)	
Head Injury	7 (38.9%)	11 (26.8%)	0.35
Concussion	7 (38.9%)	7 (17.1%)	0.07
Diagnoses	6 (75%)	13 (31.7%)	0.90
Anxiety	4 (22.2%)	2 (4.8%)	
Depression	3 (16.7%)	1 (2.4%)	
Asthma	1 (5.6%)	3 (7.3%)	
Other	4 (22.2%)	9 (22.0%)	
Medication Use	13 (72.2%)	18 (43.9%)	0.05
Anti-depressant	5 (27.8%)	1 (2.4%)	
Anti-anxiety	3 (16.7%)	1 (2.4%)	
Birth Control	4 (22.2%)	12 (29.3%)	
Allergy	3 (16.7%)	1 (2.4%)	
Other	9 (50.0%)	7 (17.1%)	
Substance Use			
Alcohol	18 (100%)	36 (87.8%)	0.12
Nicotine	6 (33.3%)	6 (14.6%)	0.10
Marijuana	11 (61.1%)	17 (41.5%)	0.16
Other	0 (0%)	0 (0%)	

Wilcoxon signed rank test*, Student's T-test**, Chi-Square Test***.

Challenge period results

ASRS scores and attentional measures

There was a marginal increase in inattentive scores during the AFC challenge relative to the placebo in the ADHD group ($F(1,15) = 3.65$, $p = 0.08$; Hedge's $g = 1.38$), with no differences observed in the EC group. There were no significant differences between the challenge periods for total or hyperactive ASRS or attentional measures in either group.

Table 3. ASRS score comparison between ADHD and EC groups across conditions.

	ADHD Mean (SD)	EC Mean (SD)	T-stat (df = 26)	P-value
Baseline				
Total ASRS	43.44 (11.30)	26.27 (8.62)	−4.32	<0.001
Hyperactive	19.39 (6.09)	10.73 (4.25)	−4.13	<0.001
Inattentive	23.72 (5.99)	15.55 (4.93)	−3.80	<0.001
AFC				
Total ASRS	42.06 (13.37)	24.63 (9.87)	−3.71	0.001
Hyperactive	18.29 (6.85)	10.18 (4.47)	−3.47	0.002
Inattentive	23.76 (7.14)	14.45 (6.06)	−3.56	0.001
Placebo				
Total ASRS	41.00 (13.03)	23.81 (11.00)	−3.61	0.001
Hyperactive	18.24 (6.49)	9.55 (5.15)	−3.74	0.001
Inattentive	22.71 (6.98)	14.27 (6.18)	−3.26	0.003

Eyes-closed EEG mean and relative power

There were differences in mean and relative power across challenge periods, only in the ADHD group (Table 4). Patterns emerged for several frequency bands in the posterior region. Band power during AFC challenge resembled baseline, while band power after placebo was decreased when compared to baseline or the AFC period, except for relative alpha power which was increased (Figure 2).

In the ADHD group only, mean posterior beta power was marginally increased ($F(1,14) = 3.93$, $p = 0.07$; Hedge's $g = 0.12$) and gamma power was significantly increased ($F(1,14) = 4.55$, $p = 0.05$; Hedge's $g = 0.11$) in the AFC challenge relative to placebo (Figure 3). Mean posterior gamma band power had a significant order effect ($F(1,14) = 4.88$; $p = 0.04$)), indicating a potential carryover when AFC was administered before placebo.

Relative posterior alpha power was significantly decreased ($F(1,14) = 4.88$, $p = 0.04$; Hedge's $g = 0.13$) and posterior gamma power was marginally increased ($F(1,14) = 4.36$, $p = 0.06$; Hedge's $g = 0.20$) in the AFC challenge relative to the placebo (Figure 4).

Correlations between EEG, attentional measures, and ASRS scores

Relative posterior alpha power was negatively correlated with total ASRS ($r = -0.49$, $p = 0.05$) and hyperactive ASRS score ($r = -0.62$, $p = 0.01$) during the AFC challenge. Higher ASRS scores indicate a worsening of symptoms; therefore, as relative alpha power decreased, overall and hyperactive ADHD symptoms worsened. There were no significant correlations between simple or complex attention measures or ASRS scores with other EEG frequency bands that varied across challenge periods.

Discussion

This pilot study aimed to examine the possible effects of AFC on young adults with and without ADHD using EEG, ASRS, and attentional outcomes. Challenge with AFC resulted in increased posterior mean gamma power and decreased relative alpha power, relative to placebo. Additionally, inattentive ASRS scores were marginally increased in the AFC challenge relative to the placebo. These results were only observed in those with ADHD and not in controls, however the EC group was

Table 4. ADHD eyes-closed resting-state EEG in posterior region mean (SD) (baseline $N = 18$, AFC/placebo $N = 16$).

	Delta	Theta	Alpha	Beta	Gamma	TBR
Mean power (μV^2/Hz)						
Baseline	5.53 (6.85)	1.77 (1.42)	3.30 (2.53)	0.33 (0.22)	0.10 (0.10)	5.51 (3.04)
Placebo	3.09 (3.25)	1.60 (1.69)	3.17 (2.75)	0.25 (0.13)	0.07 (0.04)	5.38 (3.40)
AFC	3.19 (1.93)	1.96 (2.40)	3.27 (3.19)	0.32 (0.23)$^{\Delta}$	0.10 (0.08)*	5.38 (2.57)
Relative power (%)						
Baseline	0.29 (0.14)	0.16 (0.04)	0.40 (0.13)	0.16 (0.05)	0.05 (0.02)	
Placebo	0.25 (0.08)	0.18 (0.05)	0.42 (0.11)	0.16 (0.05)	0.05 (0.02)	
AFC	0.26 (0.07)	0.19 (0.06)	0.40 (0.11)*	0.16 (0.05)	0.06 (0.03)$^{\Delta}$	

*$p \leq 0.05$, $^{\Delta}p < 0.08$ uncorrected from GLM.

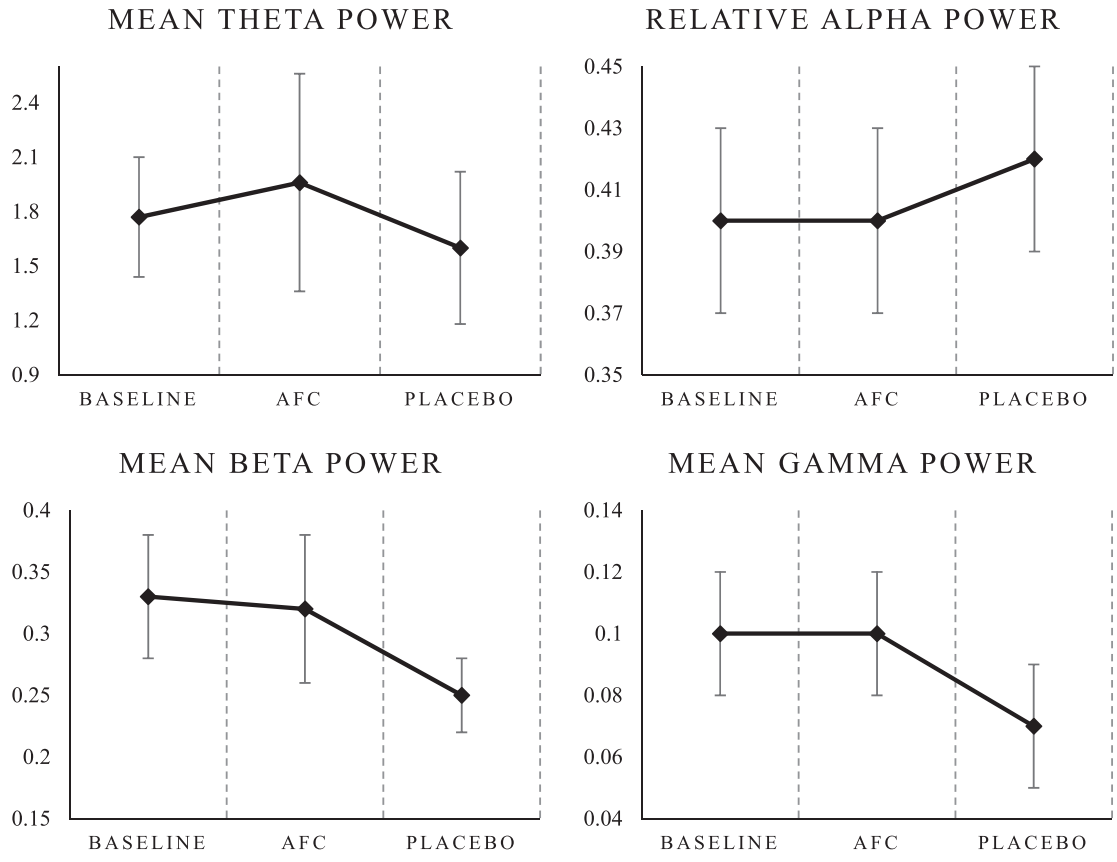

Figure 2. Posterior frequency band mean (SE) patterns in ADHD subjects across testing days. Several frequency bands showed distinct patterns when comparing baseline, AFC, and placebo testing days. Only relative alpha power (AFC < placebo) and mean gamma power (AFC > placebo) were significantly different across challenge weeks.

very small, which may be contributing to this differential effect.

Several studies using EEG have been conducted in adults with ADHD as compared to controls, with varying results (Table 1). Few studies have focused on young adults with ADHD and they show theta power as consistently elevated when compared to controls, with other bands being inconsistent [15,16,24]. However, other studies within the young adult population have reported no differences between those with and without ADHD [14,25]. The current study is consistent with such results as no differences were found in EEG between young adults with and without ADHD, going against the hypothesis that baseline theta and TBR would be increased in the ADHD group. Based on recent reviews and meta-analyses of TBR research, this finding fits the current theory that TBR is not a consistent biomarker of ADHD [13,26].

Only two studies have examined the effects of AFC using EEG in children with ADHD. However, both studies had methodological flaws. The first study recruited only 4 children with ADHD and 4 age-matched controls and tested an undisclosed about of Red No.40 on alpha band power, resulting in a difference in power in the ADHD group during both the AFC and placebo conditions when compared to controls [27]. The

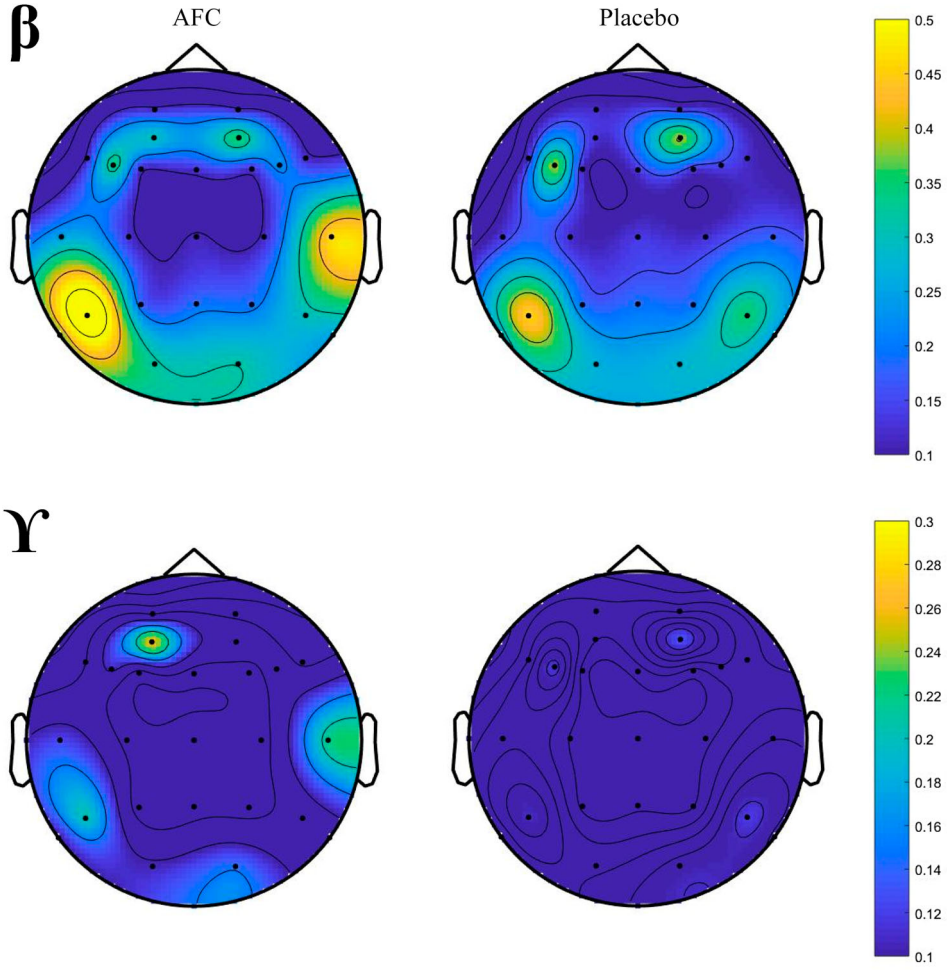

Figure 3. Topographic maps of mean beta and gamma power in ADHD group. The left panel shows power during the AFC challenge and the right panel shows power during the placebo challenge. AFC > placebo in the posterior region for beta (top) and gamma (bottom).

second study investigated the exposure to an array of 'provoking' foods (i.e. AFC, beet sugar, wheat, cow milk, bananas, eggs, citrus, cacao, beef, pork, and oats) in children with food-induced ADHD using a cross-over challenge [28]. When the children consumed provoking foods, there was an increase in relative beta and theta power in the frontal region, with the opposite during avoidance [28]. Neither study included baseline EEG profiles and the results were difficult to interpret based on sample size and study design. Despite the methodological issues, they provide evidence that certain food items, including AFC, may contribute to changes in brainwave activity in children with ADHD.

Our study demonstrated significant differences in both mean gamma power and relative alpha power in the posterior region with eyes-closed EEG. Mean posterior gamma was increased during the AFC condition relative to placebo. Gamma power is not often reported in the ADHD literature, with only one study in adults reporting null findings as compared to controls [29]. Posterior gamma power is modulated by low-frequency oscillations (<14 Hz) and is related to sustained attention, visual processing, and cognitive control from the anterior attentional systems [30]. Since the eyes-closed task eliminated visual processing, the increase in posterior gamma power during the AFC condition may be due to increased attentional demands, leading to the activation of anterior attentional systems. The gamma power band did not have a significant correlation with the simple and complex attentional measures used, however more nuanced attentional measures may be better suited to understand the relation of posterior gamma power and attention, in young adults with ADHD.

Potential changes in the anterior attentional networks by AFC are further supported by the changes in alpha power. The current study observed a decrease in alpha power in the posterior region during AFC challenge. Alpha power may be an important factor in adult ADHD as it is connected to attentional self-control and active inhibition of external stimuli [24,31].

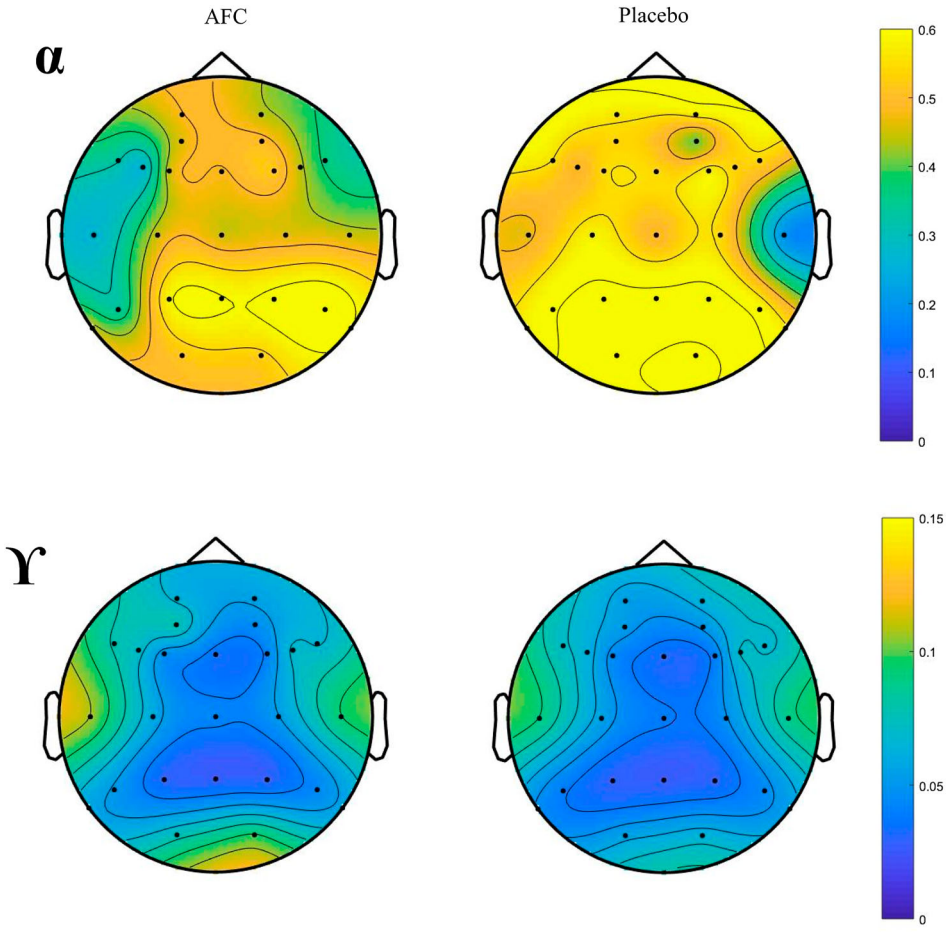

Figure 4. Topographic maps of relative alpha and gamma power in ADHD group. The left panel shows power during the AFC challenge and the right panel shows power during the placebo challenge. AFC < placebo in the posterior region for alpha (above) and AFC > placebo in the posterior region for gamma (below).

Interestingly, posterior alpha showed a significant negative correlation with hyperactive ASRS scores and overall ADHD symptoms, suggesting that posterior alpha may be associated with symptoms of ADHD. This is consistent with a published report of lower posterior alpha in college students with ADHD as compared to controls [24]. It is hypothesized that a decrease in posterior alpha power may result in more attention paid to external stimuli during eyes-closed resting state, a condition where external stimuli interference should be highly restricted. This orienting towards external stimuli rather than internal stimuli may result in a worsening of ADHD symptoms [24].

When examining results across the study, a pattern emerged within the ADHD group for theta, alpha, beta, and gamma bands (Figure 2). Power bands observed after AFC challenge resembled the baseline power values, while the placebo challenge power bands were decreased (or increased for relative alpha). This is a pattern of interest, especially within the theta and alpha bands as they are often implicated in ADHD as being increased or decreased, respectively, when compared with controls (Table 1). However, without baseline AFC FFQ data, the comparison between baseline and AFC challenge is simply exploratory. A larger sample size and data on baseline AFC consumption is needed to better understand the implications of these patterns.

There were no differences at baseline in attentional measures between college students with and without ADHD. Importantly, the participants were all students at a selective university. Cognitive research suggests that individuals with ADHD who attend college have distinct cognitive skills as compared to their peers who do not attend college, resulting in similar cognitive performance to their neuro-typical peers [32]; this may account for the lack of differences in simple/complex attention measures in the current study.

There are four main strengths of this research. This is the first study, to our knowledge, to test the effects of AFC in young adults. Second, this study uses the largest dose (225 mg) of combined AFC to date, an amount that

is still physiologically appropriate and able to be consumed by an adult while eating normal food products. Past studies have been criticized for not using large enough doses of AFC during the challenge period [33]. As children tend to consume more AFC than adults per body weight, this amount would also be appropriate for children. Third, using powdered AFC removed potential confounding effects from other chemicals used in liquid AFC (sodium benzoate and propylene glycol). Fourth, using the objective measures of EEG and cognitive testing for attentional measures limited the reliance on subjective self-report measures.

There are also some limitations to the current study. Since this is a pilot study, the goals of this research were to (1) assess AFC effects on EEG or ADHD symptoms in young adults, and (2) provide estimates of effect size so that other researchers can use these for power analyses for future studies. However, the small sample size limits the inferences that can be made. Future work can now use these estimates to accurately calculate optimal sample size. While the EC group did not seem to be affected by AFC on any measure, randomization of the EC group to challenge weeks was imperfect and the sample was even smaller than the ADHD group. Having a larger and equal size sample of young adults with and without ADHD will better determine if these results are specific to ADHD. Another limitation was that the AFC FFQ was only administered once, after avoiding AFC in the diet for 2 weeks and before randomization to challenge weeks, which limited comparisons to baseline diet. Future studies should assess AFC intake at every time point. Additionally, mean posterior gamma had a significant order effect, indicating carry-over effects when AFC was administered before placebo; suggesting future research should use a washout period longer than 4 days. Lastly, during the challenge weeks participants were asked to report ADHD symptoms over the past week, however the reliability and validity of ASRS for durations <6 months has not yet been tested.

Future research should focus on addressing the above limitations, as well as examining other food additives (e.g. monosodium glutamate or artificial sweeteners) in relation to ADHD [34]. Additionally, gamma band power is rarely explored in the ADHD population, yet we found differences during AFC consumption. We would recommend including the gamma frequency band in future EEG studies to better understand how high-frequency variations may be contributing to ADHD. Lastly, expanding the population to include young adults who are not in college may provide insight into any potential attentional effects of AFC that were not seen in this sample.

Conclusion

In conclusion, this pilot study is the first to investigate the effects of AFC on young adults with and without ADHD, using EEG, a measure of adult ADHD, and attentional tests. AFC was shown to have differential effects on mid and high-frequency bands in the posterior brain regions of young adults with ADHD. Specifically, mean gamma power was increased and relative alpha power was decreased during AFC exposure, relative to placebo. Inattentive ADHD symptoms were also marginally increased upon exposure to AFC relative to placebo. A future study should be undertaken to attempt to replicate these results in a larger sample.

The authors declare that there are no conflicts of interest.

The data that support the findings of this study are available from the corresponding author, K.F.H., upon reasonable request.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Anna E. Kirkland, Mackenzie T. Langan and Kathleen F. Holton are part of the Nutritional Neuroscience Lab at American University, which studies the negative effects of food additives on glutamatergic neurotransmission, as well as the protective effects of certain micronutrients against excitotoxicity, with the aim of optimizing neurological function through dietary treatment.

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