NeuroRegulation

The Acute Electrocortical and Blood Pressure Effects of Chocolate

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Abstract

Objective: The present study investigated the effects of consuming chocolate on electroencephalograph (EEG) frequencies and localization and on blood pressure. **Method:** Across six conditions, 122 participants consumed either higher (60%) cacao chocolate, low (0%) cacao chocolate, higher cacao chocolate + L-theanine, high sugar water, low sugar water, or water. EEGs, blood pressure, and mood were measured before and after a 60-min digestion period. **Results:** Analyses indicated a decrease in frontal, parietal, and temporal theta and an increase in occipital beta EEG following the consumption of a 60% cacao confection compared with control conditions. Diastolic blood pressure increased with the consumption of higher cacao chocolate when compared to water alone and to higher cacao chocolate + L-theanine. Diastolic and systolic blood pressure decreased following consumption of higher cacao + L-theanine chocolate, averaging 4–8 mmHg. No condition-specific mood changes or gender differences were found. **Conclusions:** This study suggests an acute stimulating effect of cacao on the human brain and vasoconstrictive effects on peripheral vasculature, the latter of which appear to be offset by an L-theanine additive. **Significance:** This is the first known study to investigate acute EEG effects of consuming chocolate and suggests a potential attention-enhancing effect.

Keywords: chocolate, cocoa, cacao, L-theanine, EEG, blood pressure

Citation: Montopoli, M., Stevens, L., Smith, C. J., Montopoli, G., Passino, S., Brown, S., … Wu, J. (2015). The Acute Electrocortical and Blood Pressure Effects of Chocolate. *NeuroRegulation*, *2*(1), 3–28. doi.org/10.15540/nr.2.1.3

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Introduction

Few food products have garnered such attention or have reached such cultural and mythological significance as chocolate. Indeed, chocolate is frequently heralded as an aphrodisiac, a broad-spectrum medicinal agent, a mood-altering substance, a nutritional supplement, an antihypertensive, a stimulant, and as the most frequently craved food in the Western world (Bruinsma & Taren, 1999; di Tomaso, Beltramo, & Piomelli, 1996; Dillinger et al., 2000; Hill & Heaton-Brown, 1994; Rozin, Levine, & Stoess, 1991; Weingarten & Elston, 1991). Research over the past decades has generally been supportive of these effects, if only of minimal magnitude in some cases. Chocolate is made from the cocoa bean—which is actually the seed of the fruit of the *Theobroma cacao* tree—and was originally cultivated by the Olmec, Mayan, and Aztec aristocracy in Mesoamerica and by the Inca in South America. Ancient codices from Mesoamerica indicate that foods made from the raw, fermented, roasted, shelled, and ground cocoa bean (called cacao) were used for a variety of medicinal purposes such as relieving cough, gastrointestinal aids, improving angina and heart palpitations, and even as a sexual stimulant (Dillinger et al., 2000). Modern research has linked the ingestion of flavanols, polyphenolic compounds highly concentrated in the cocoa bean, to stimulation of nitric oxide synthase production (Fisher, Hughes,

Gerhard-Herman, & Hollenberg, 2003). Nitric oxide synthase increases levels of nitric oxide in arterial endothelial cells producing peripheral vasodilation. Cocoa (processed cacao with the cocoa butter removed) and chocolate (processed cacao with cocoa butter not removed) also contain trace amounts of compounds thought to potentially alter brain activity, such as anandamide, a naturally occurring neuromodulator which can bind to cannabinoid-receptors and very mildly mimic the psychoactive properties of plant-derived cannabinoid drugs (di Tomaso et al., 1996).

By far the most heavily researched and most convincing effect of cacao and chocolate ingestion is the effect on blood pressure and vasodilation. The evidence is now highly suggestive that the flavanols in cacao products have vasodilation effects through increases in nitric oxide (NO), which may result in small decreases in blood pressure (Engler et al., 2004; Fraga et al., 2005; Grassi, Lippi, Necozione, Desideri, & Ferri, 2005; Grassi, Necozione, et al., 2005; Taubert, Roesen, Lehmann, Jung, & Schomig, 2007). Taubert, Roesen, and Schomig (2007) conducted a meta-analysis of randomized controlled studies of the effects of cocoa use over a median period of 2 weeks on blood pressure and reported a mean decrease in systolic blood pressure (SBP) of 4.7 mmHg and in diastolic blood pressure (DBP) of 2.8 mmHg. These results translate into a 20%, 10%, and 8% risk reduction in stroke, coronary heart disease, and all-cause mortality, respectively. And, in a cross-sectional study of 470 elderly men, those who consumed the equivalent of 2.3 g of cocoa powder per day over a 5-year period had a significantly lower SBP (-3.7 mmHg) and DBP (-2.1 mmHg) relative to men with low cocoa intake, translating prospectively into a 45–50% decrease in cardiovascular and all-cause risk (Buijsse, Feskens, Kok, & Kromhout, 2006). Such decreases in blood pressure following prolonged consumption of cacao products have been strongly linked in acute effect studies to peripheral vasodilation resulting from endothelium-dependent relaxation of vascular smooth muscles induced by nitric oxide, which is thought to be increased primarily by the flavanol monomer, epicatechin, in cacao products (Fisher et al., 2003; Heiss et al., 2006; Karim, McCormick, & Kappagoda, 2000; Schroeter et al., 2006). This vasodilation has been generally observed to peak at 1–2 hr following consumption of a flavanol-rich chocolate product (Schroeter et al., 2006; Taubert, Rosen, Lehmann et al., 2007). These supportive outcomes notwithstanding, Egan, Laken, Donovan, and Woolson (2010) have pointed to important inconsistencies in outcomes, designs, type, and chemical constituents of cacao confections, dose and time-dependent effects, subject blood pressure (BP) variability, and BP measurement devices, which leave the cardiovascular effects of cacao uncertain at the present time.

The endothelium-dependent relaxation (EDR) effects of cacao are somewhat paradoxical, as cacao also contains a number of sympathomimetics, which have vasoconstrictive and generally stimulatory effects, most notably the biogenic amines, tyramine and phenylethylamine (PEA), and the methylxanthines, caffeine and theobromine (Bruinsma & Taren, 1999; Hurst, Martin, & Zoumas, 1982). Although these substances occur in different amounts in various confections, PEA and theobromine generally are found in larger quantities and tend to potentiate the release of catecholamines, thus causing vasoconstriction and elevations in blood pressure. It is not clear at the present time whether these vasoconstrictive effects are offset by the more prominent EDR vasodilatation effects reported above, or whether the time course of these phenomena are different, resulting in more immediate stimulation followed by a delayed vasodilatation effect. Furthermore, these stimulant effects may well explain the general increase in arousal often reported by chocolate consumers (Bruinsma & Taren, 1999; Dillinger et al., 2000).

If chocolate indeed has an immediate arousing effect, one might expect to see increased arousal in the brain. However, our review of the chocolate literature over the past decades reveals no studies of the effects of chocolate consumption on central nervous system (CNS) arousal. Martin (1998) investigated the electroencephalographic (EEG) effects of olfactory stimulation with the aroma of chocolate, but did not examine the consumption of chocolate in his research. Small, Zatorre, Dagher, Evans, and Jones-Gotman (2001), in a positron emission tomography (PET) study of brain changes following eating chocolate, were interested only in the immediate (within 10 s) reward characteristics of consuming chocolate and did not allow a sufficient time course for cacao constituents to have an effect on cerebral blood flow following digestion. Francis, Head, Morris, and Macdonald (2006) conducted functional magnetic resonance imaging (fMRI) on 16 healthy young women following 5 days of consumption of a flavanol-rich cocoa beverage compared to a low flavanol beverage and examined blood flow changes 1.5 hr after consumption of the beverage during the performance of a cognitive switching task. Although they found no flavanol-specific effects on reaction times, error rates, or

heart rate, they did observe increased fMRI blood oxygenation level-dependent (BOLD) cerebral blood flow (CBF) during the cognitive task following the flavanol-rich cocoa regimen relative to the low flavanol regimen. In a separate pilot study with 4 participants of the time course of CBF changes following an acute dose of flavanol-rich cocoa, Francis et al. reported a peak blood flow response at approximately 2 hr post-ingestion with return to baseline after approximately 6 hr. Reported fMRI images were specific to the cognitive task and not to flavanol ingestion. Although these researchers suggest that the observed effects on CBF may be due to NO-induced EDR, they also point out that the fMRI BOLD response is a neurovascular phenomenon and may result from changes in vascular tone as well as neural activity influenced by stimulants, such as caffeine, in the cocoa product. Nonetheless, these results suggest a flavanol-induced increase in cerebral blood flow consistent with the vasodilation reported in other studies. And, more recently, Camfield et al. (2012), in a study of 61 middle-aged adults who consumed a daily chocolate beverage containing 250 mg or 500 mg of cocoa flavanols compared with a low cocoa flavanol beverage over a 30-day period, found no changes in behavioral measures of accuracy or reaction time on a spatial working memory task but did observe condition-specific amplitude and latency differences on EEG visual evoked potentials (VEP) during the same task. These authors interpreted the observed VEP changes as reflective of increased neural efficiency following the chronic ingestion of cocoa flavanols.

A recent comprehensive review of the neurobiological effects of cocoa flavanols on cognition and behavior indicates rather strong support for neuroprotective effects of long-term consumption of flavanols on age-related and disease-related cognitive decline but less support for the more immediate effects of cocoa consumption on specific brain mechanisms involved in neurogenesis and neuronal function and connectivity, particularly in humans (Sokolov, Pavlova, Klosterhalfen, & Enck, 2013). These authors encourage and offer a template for future research into effects of cacao on human cognition, mood, and behavior.

Given the long-term neuroprotective and neuromodulatory effects of cocoa consumption, the suggested stimulant characteristics of cacao, and the glaring absence of published acute CNS arousal studies, we elected to conduct a controlled EEG study of the comparative effects of consuming a higher cacao-content chocolate (with a high flavanol content) with a low-cacao content chocolate (with no flavanol content) and with balanced sugar and water controls. Sugar controls were included in the present study to control for reported general arousal effects of glucose (Hoffman & Polich, 1998; Hoffman, Friedmann, Saltman, & Polich, 1999). Additionally, as a partial test of the hypothesized acute sympathomimetic effects of cacao, we included a third chocolate condition by the addition of L-theanine to the same higher cacao-content chocolate formulation. L-theanine, an extract of green tea, has been shown in numerous animal and human studies to counteract the stimulating effects of caffeine and stressors, apparently by its ability to bind to the glutamate receptor and to block binding of L-glutamic acid in cortical neurons (Kimura, Ozeki, Juneja, & Ohira, 2007; Mason, 2004;). L-theanine has been found to reduce blood pressure (Yokogoshi et al., 1995; Yokogoshi & Kobayashi, 1998), to elevate posterior EEG alpha activity (Kobayashi et al., 1998), to reduce the psychological and physiological response to a mental stressor (Kimura et al., 2007), and to improve learning in animal models (Juneja, Chu, Okubo, Nagato, & Yokogoshi, 1999). We hypothesized that consumption of the higher cacao-content condition, relative to the low cacao-content, sugar, and water controls, in human volunteers would result in increased activation of the neocortex and increased blood pressure within 1 hr after ingestion and that these effects would be reversed in the higher cocoa-content plus L-theanine condition.

Methods

Participants

A power analysis was conducted to determine the optimal sample size required to detect the hypothesized effect of chocolate on EEG and blood pressure (Howell, 2002). A complex multivariate design was modeled employing 11 dependent variables (9 EEG frequency and 2 blood pressure variables) studied across two repeated measures (pre- and post-ingestion) for each of six treatment conditions. For an alpha level of .05 and a power of .80, sample sizes in each of the 6 treatment cells of 20 allowed a hypothesized medium effect size to be detected.

Consequently, 125 participants (10 males, 10 females in each of the six treatment cells, plus 5 extra participants to allow for possible attrition and outliers) were recruited from the Psychology Department undergraduate voluntary research pool. After exclusion of outliers (3 participants had

clinically-elevated blood pressure readings and were referred to the health center), 122 participants completed the study and were analyzed. Participants were between the ages of 18 and 25 years and were excluded if they used illicit drugs, stimulant or depressant medications, or nicotine, if they had diabetes mellitus, or if they were allergic to chocolate or nuts. Women were not tested during their premenstrual or menstrual phase due to the potentially confounding effect of chocolate cravings during this time and because hormone imbalances during these menstrual phases have been shown to affect the EEG (Dusser de Barenne & Gibbs, 1942; Solis-Ortiz, Ramos, Arce, Guevara, & Corsi-Cabrera, 1994). All participants abstained from caffeine and chocolate intake 24 hr prior to the EEG study. The present study was approved by the NAU Institutional Review Board for the Protection of Human Subjects in Research.

Materials and Equipment

A standard weight scale was used to weigh each participant 1 week prior to study in order to determine the amount of chocolate to administer. Participants were weighed by a same-sex research assistant, and female participants at weigh-in were given a menstrual calendar on which to plot their predicted menstrual cycle. At the initial weigh-in, the participant was briefed as to the nature of the study and the requisite informed consent documents were completed. Blood pressure readings for the study were taken by a HoMedics Automated Blood Pressure Monitor with participants seated and left arm resting at heart level.

The three chocolate treatments were prepared by The Hershey Company, individually wrapped in 40 g squares of identical appearance and coded by contents. The *higher cacao-content chocolate* contained 60% cacao with 15 mg/g of total polyphenols, and 0.37 g/g of sugar; the *low cacao-content chocolate* was a white chocolate (colored with 5% Hansen Brown) that contained 0.4 mg/g of total polyphenols, and 0.56 g/g of sugar; the *higher cacao-content + L-theanine chocolate* contained the identical components as higher cacao-content chocolate above plus 128 mg (3.2 mg/g) of L-theanine (L-theanine has a Generally Recognized as Safe [GRAS] designation by the FDA and, with recommended dosages of 50–200 mg/serving, the amounts used in this study were well within the recommended dosages). Table 1 presents total ingredients of the three chocolate treatments. Three control treatments were also prepared, comprised of a *high sugar beverage treatment* containing an equivalent amount of sugar as the low cacao-content chocolate (23 g/40 g bar or 0.57 g/kg body weight) dissolved in 350 ml (1.5 cups) of water; a *low sugar beverage treatment* containing an equivalent amount of sugar as the higher cacao chocolate (14 g/40 g bar or 0.35 g/kg body weight) dissolved in 350 ml of water; and a 350 ml *water treatment*. For the chocolate and sugar conditions, each participant received 1 g of chocolate for each kg of body weight, with an equivalent amount of sugar for each kg of body weight for the sugar conditions. For example in standard units, a 150 lb participant would receive 2.4 ounces of either of the three chocolate treatments, approximately equivalent to a standard size chocolate bar, 1.38 ounces of sugar in 1.5 cups water, 0.84 ounces of sugar in 1.5 cups water, or 1.5 cups water.

The Positive and Negative Affect Scale (PANAS) was used as a brief measure of emotional changes following chocolate consumption (Watson, Clark, & Tellegen, 1988). The PANAS was administered at the beginning of the study prior to treatment before the EEG was attached, immediately following administration of each condition, and again 1 hr 10 min later after a digestion period and second EEG.

A Mitsar 201 24-channel EEG acquisition system was used to measure each participant's EEG frequencies (Mitsar Co. LTD, 1996). The Mitsar 201 DC amplifiers have a 500 Hz digital sampling rate and input impedance not less than 200 MΩ. EEG data were recorded and preprocessed using WinEEG software (Mitsar Co. LTD, 1996), double visually artifacted by two independent artifactors, and power spectral FFT analyzed utilizing NovaTech EEG Eureka and MHyT software (Nova Tech EEG, Inc., 2006). FFT analysis employed Hamming time domain tapering, Blackman frequency domain smoothing, an overlapping FFT windows advancement factor of 8, and a moving average smoothing filter of 3. The International 10–20 placement system was used to attach 19 Ag/AgCl monopolar electrodes on the scalp with mathematically linked-ear references utilizing the Electro-Cap System (Electro-Cap International, Inc., 2006). Electrode impedances were adjusted to < 5 kohms and to within 1 kohm of each other. All data were recorded in a sound attenuated research suite, appointed with the requisite EEG and blood pressure monitoring equipment. Participants were seated comfortably in a recliner and were able to read magazines during the digestion phase and to sit quietly during the EEG recording phases.

Table 1
Ingredients of the three chocolate confections used in the present study

	High Cacao-Content Chocolate	Low Cacao-Content Chocolate	High Cacao-Content + L-theanine Chocolate
Component	per g	per g	per g
Calories	4.65	4.88	4.65
Fat, g (calculated)	0.339	0.311	0.339
Sat fat, g	0.209	0.173	0.209
Trans fat, g	0.001	0.0016	0.001
Cholesterol, mg	0.078	0.1456	0.078
Sodium, mg	0.153	1.6	0.153
Carbohydrates, g	0.548	0.556	0.548
Dietary fiber, g	0.1099	0	0.1099
Sugar, g	0.37	0.555	0.37
Protein, g	0.0803	0.084	0.0803
Vitamin A, IU	0.9297	6.27	0.9297
Vitamin C, mg	0	0.0117	0
Calcium, mg	0.576	3.13	0.576
Iron, mg	0.107	0	0.107
Magnesium, mg	2.00	0.28	2.00
Potassium, mg	5.27	2.27	5.27
Caffeine, mg	0.63	0.05	0.63
Theobromine, mg	7.14	0.07	6.5
Fat, % (analyzed)	40.1	36.7	38.8
Total polyphenols, mg	15	0.4	22
ORAC, micromoles TE	360	19	250
Catechin, mg	0.16	0.01	0.17
Epicatechin, mg	0.88	0.03	0.95
DMAC, mg	10.10	0.03	12.6
PAC-10, mg/whole product	4.60	< 0.001	5.69
PACs 1 mers	0.83	< 0.001	0.99
PACs 2 mers	0.61	< 0.001	0.78
PACs 3 mers	0.56	< 0.001	0.62
PACs 4 mers	0.56	< 0.001	0.73
PACs 5 mers	0.49	< 0.001	0.51
PACs 6 mers	0.42	< 0.001	1.1
PACs 7 mers	0.36	< 0.001	0.35
PACs 8 mers	0.32	< 0.001	0.22
PACs 9 mers	0.23	< 0.001	0.26
PACs 10 mers	0.24	< 0.001	0.12

Note: Nutrition information calculated using Genesis® R&D *SQL* nutritional analysis and labeling system (ESHA Research, Salem, OR 2007). DMAC = 4-dimethylaminocinnamaldehyde total flavanol content; ORAC = Oxygen Radical Absorbance Capacity general antioxidant activity; PACs = proanthocyanidin flavanol polymers.

Catalogs and magazines were available for participants to read following the ingestion of the treatments. These materials were reviewed in order to remove any overly emotional or stimulating articles that could have potentially affected arousal. Reading materials included clothing, climbing, and hiking catalogs and computer, health-related, and men's and women's magazines.

Procedure

Participants were randomly assigned to 1 of 6 conditions: higher cacao-content, low cacao-content, higher cacao-content + L-theanine chocolates, high sugar water, low sugar water, and water. To preclude blood sugar spikes from an overnight fast from affecting the EEG but to allow more direct effects of each of the conditions without the interference of recent food intake, participants were required to eat breakfast or a meal and then to fast for 4 hr before the experiment. Although cortisol levels were not measured in this study, in order to keep them at their nadir, all participants were run between the hours of 1 and 6 p.m. (Dmitrieva, Almeida, Dmitrieva, Loken, & Pieper, 2013; Karlamangla, Friedman, Seeman, Stawksi, & Almeida, 2013). The type of chocolate condition for each participant was known only to the primary author, who, prior to each test session, carefully weighed out, packaged, and secretly labeled the substance. This package was then passed to a blind research assistant (RA) who conducted the actual test. The sugar water and water conditions were prepared in a similar fashion and the RA was blind as to the nature of the liquid in the cup, with the exception that the RA could visually differentiate the liquid water/sugar water controls from the solid chocolate conditions. Participants were similarly partially blind as to the exact nature of the substance they were consuming, either an unknown chocolate substance or sugar water.

When the participant arrived at the laboratory, they were seated in the recliner and were given the first PANAS to complete; blood pressure was then recorded and the Electro-Cap and EEG equipment were attached, during which time the participant completed a brief questionnaire to substantiate lack of drug use over the past 2 days and lack of caffeine and food intake for the past 24 and 4 hr, respectively. A 10-min, eyes-closed resting baseline EEG was then recorded. Afterwards, each participant was administered their respective chocolate, sugar water, or water treatment, was given 5 min to ingest the substance, and was administered the PANAS again. Sixty min were then allowed for digestion and absorption of the chocolate or water treatments; participants were also visually monitored for alertness. Following the 60-min period, a second 10-min, eyes-closed resting EEG was recorded, then blood pressure was taken, and a final PANAS was administered. The Electro-Cap was then removed and the participant was debriefed. Figure 1 presents the timeline for the study.

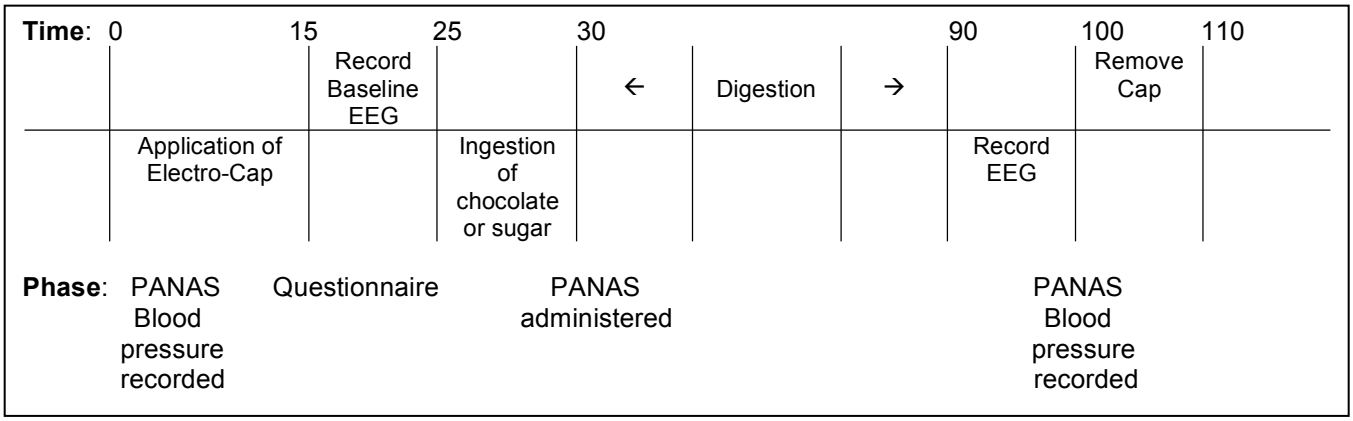

Figure 1. Timeline for the study in minutes.

Design and Analysis

Nine frequency bins were identified for analysis: delta = 0–3.99 Hz, theta = 4–7.99 Hz, low theta = 4–5.99 Hz, high theta = 6–7.99 Hz, alpha = 8–11.99 Hz, low alpha = 8–9.99 Hz, high alpha = 10–11.99 Hz, beta = 12–29.99 Hz, and gamma = 30–60 Hz. Following FFT power spectral analyses, absolute power values for each frequency and for each of the

19 electrode sites were identified and post-treatment minus pre-treatment difference scores for each frequency and site were tabulated, in order to determine the direction and magnitude of change from baseline in absolute EEG power for each condition. Absolute power differences for each electrode were grouped into traditional functional clusters of frontal (Fp1, Fp2, F7, F3, Fz, F4, F8), central (C3, Cz, C4), parietal (P3, Pz, P4), occipital (O1, O2), and temporal (T3, T4, T5, T6) regions. A factorial Analysis of Variance (ANOVA) was then conducted on each functional cluster for each frequency across each of the treatment conditions. In order to investigate the secondary effect of gender, an additional independent variable was included in each analysis, making separate 2 (between-groups: gender) x 6 (between-groups: treatment condition) analyses of post-treatment minus pre-treatment EEG differences for each functional cluster. As this was an investigational study and the percent cacao in the maximal treatment condition was relatively low (60%) thus lowering the magnitude of effect, simple effects analyses of EEGs were conducted utilizing LSD post-hoc *t*-tests. Blood pressure and PANAS effects were assessed utilizing ANOVA with simple effects comparisons made by Tukey HSD tests.

Since surface EEG recordings are aggregates of far-field potentials generated across a 3-dimensional, quasi-spherical cortical space, we wondered what deeper cortical structures might be most impacted by the biochemical constituents of chocolate following our neutral reading task. Low-resolution brain electromagnetic tomography (LORETA) is a neuroimaging software companion to contemporary EEG analyses which allows the triangulation of these surface scalp potentials to their cortical source generators (Pascual-Marqui, 1999; Pascual-Marqui, Esslen, Kochi, & Lehmann, 2002; Pascual-Marqui, Michel, & Lehman, 1994). LORETA algorithms compute a 3-dimensional inverse solution space of cortical gray matter and hippocampi mapped onto a probabilistic Talairach atlas partitioned into 2394 7 mm^3 volumetric units, or voxels. Brodmann anatomical labels may be reported for relevant regions of interest utilizing the Montreal Neurological Institute (MNI) realistic head model (The KEY Institute for Brain-Mind Research, 1995). For the present study, LORETA mapping was utilized in a purely descriptive fashion *post hoc* to identify cortical regions of interest involved in obtained effects. LORETA Current Source Density (CSD) maps were generated from between-groups comparisons of the natural log transformation of FFT power spectral output for each statistically significant frequency and functional cluster.

Results

Participant Characteristics

Our sample of 122 participants was a young, healthy group of undergraduate student volunteers. Mean baseline blood pressures were 73.75 (7.75) mmHg DBP and 117.12 (9.98) mmHg SBP. Males had significantly higher SBP at baseline, 120.32 (8.50) mmHg SBP, than females, 114.03 (10.39) mmHg SBP; $t(120) = 3.65$, $p < .0001$; but these values were within normal limits and there was no significant gender by condition interaction at baseline, $F(1, 5) = 1.37$, $p = .24$.

Analysis of Mood Changes

PANAS scores before, immediately after, and 70-min after consumption of each treatment and for males and females were analyzed by a 2 (gender) x 6 (conditions) repeated measures ANOVA separately for positive and negative moods. Neither analysis revealed significant main effects for treatment, Positive Mood: $F(5, 110) = 1.42$, $p = 0.22$; Negative Mood: $F(5, 110) = 1.56$, $p = 0.18$; or gender, Positive Mood: $F(1, 110) = 0.22$, $p = 0.64$; Negative Mood: $F(1, 110) = 3.06$, $p = 0.08$. There was a trend for males to show slightly higher negative moods across all conditions.

Analysis of EEG Changes

EEG Absolute Power values for each of five primary frequencies for each electrode across each condition at Baseline, at Post-Treatment, and for Difference Scores are presented in Tables 2 through 6 in the Appendix. Values in each table are Absolute Power values for that frequency with decimals removed for ease of presentation (i.e., 608 = .0608, or .0608 x 10^4). In the presentation of these results, a negative difference score indicates that the specified EEG power decreased following treatment (post-treatment – pre-treatment).

For the functional cluster analyses (i.e., frontal, central, parietal, occipital, and temporal), EEG regional cluster scores for individual participants which exceeded 3.29 standard deviation units from the mean for that cluster were identified as outliers and were replaced by the next lower score for that cluster (Tabachnick & Fidell, 2013). Subsequent tests for departures from normality and homogeneity of variance revealed no significant departures for the tested independent variables, with the exception of gamma parietal, which showed a significant departure for the homogeneity of variance

assumption. This latter variable was Log10 transformed, subsequently tested for normality and homogeneity of variance, and was found to meet requirements for analysis. Regional cluster scores for each frequency and condition were then entered into separate 2 x 6 ANOVAs. These results are presented in Table 7.

These ANOVA analyses revealed significant main effects for Condition for frontal theta, $F(5, 110) = 3.12$, $p = .011$, $\eta^2 = .124$; parietal theta, $F(5, 110) = 2.38$, $p = .043$, $\eta^2 = .097$; and temporal theta, $F(5, 110) = 2.72$, $p = .024$, $\eta^2 = .110$; with a trend for central theta as well, $F(5, 110) = 2.00$, $p = .085$, $\eta^2 = .083$. A significant main effect for Gender was also found for frontal theta, $F(1, 110) = 5.94$, $p = .016$, $\eta^2 = .051$, with males showing significantly greater decreases in frontal theta than females across all conditions. No interactions were found to be statistically significant. Planned comparisons revealed frontal theta decreases from Baseline to Post-Ingestion to be significantly greater for the higher cacao-content chocolate condition relative to the water ($p = .006$), high sugar ($p = .001$), low sugar ($p = .005$), and low cacao-content chocolate ($p = .006$) conditions, with these latter conditions actually showing increases in frontal theta. Figure 2 presents these effects graphically for frontal theta. (For Figures 2 through 6, the ordinate scale is set to be equivalent across all figures for ease of magnitude comparisons.)

Similar effects were found for parietal theta and temporal theta with the higher cacao-content chocolate confection showing significant decreases with consumption relative to water ($p = .021$, $.026$), high sugar ($p = .009$, $.002$), and low cacao-content chocolate ($p = .005$, $.008$), which each showed increases across the conditions. Additionally, the higher cacao-content chocolate + L-theanine condition showed significantly smaller increases ($p = .042$) in temporal theta compared to the high sugar condition across treatment. Figures 3 and 4 present these outcomes graphically. These results indicate significant decreases in frontal, parietal, and temporal theta EEG frequencies following the consumption of a 60% cacao confection relative to increases across these cortical regions following consumption of water, high sugar, an approximately 0% cacao-content confection, and, for frontal theta, a low sugar condition.

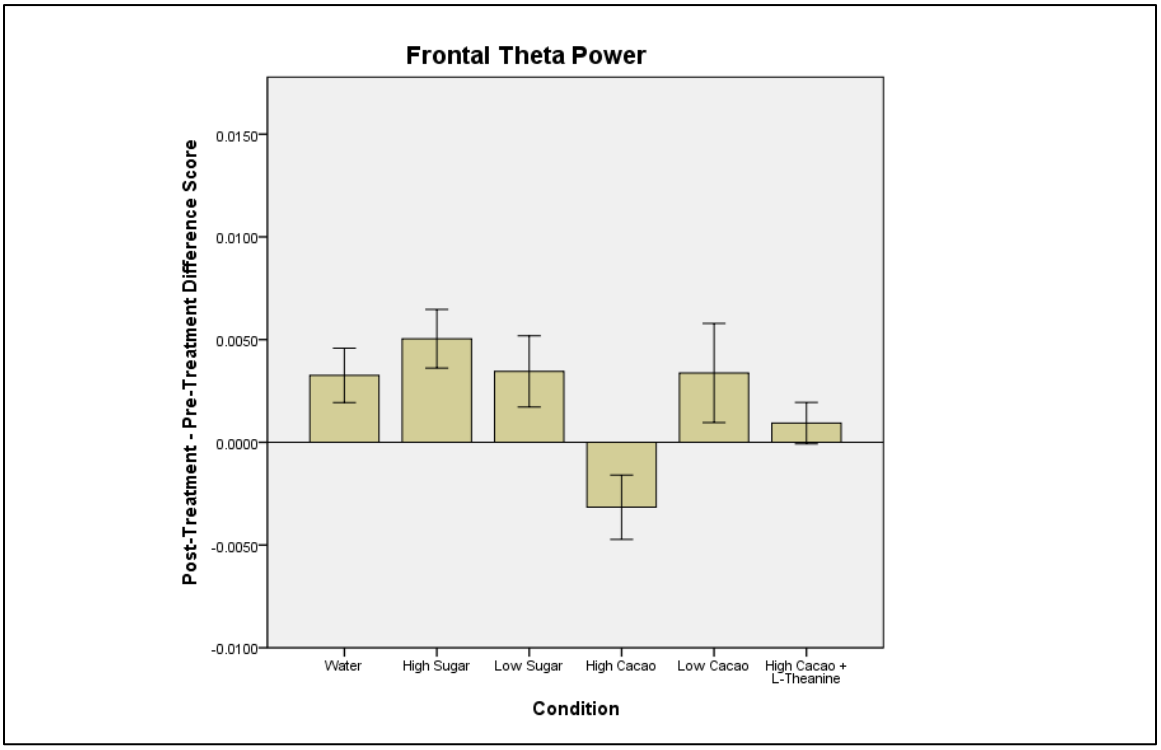

Figure 2. Frontal theta EEG absolute power changes post-treatment minus pre-treatment for each condition, showing significant decreases for higher cacao relative to water, high sugar, low sugar, and low cacao. SE bars = +/- 1 SE.

Table 7
EEG Results for Each Frequency and Regional Electrode Cluster by Condition

	Chocolate Condition		Gender		Interaction	
Cluster	**F (*p*) (η^2)**	**Effect**	**F (*p*) (η^2)**	**Effect**	**F (*p*) (η^2)**	**Effect**
Delta Frontal	.54 (.74) (.02)		.16 (.69) (.001)		.68 (.64) (.03)	
Delta Central	1.01 (.42) (.04)		.16 (.69) (.001)		.46 (.81) (.02)	
Delta Parietal	.70 (.63) (.03)		.17 (.68) (.002)		.58 (.72) (.03)	
Delta Occipital	.44 (.82) (.02)		.08 (.78) (.001)		1.16 (.33) (.05)	
Delta Temporal	.75 (.59) (.03)		.01 (.93) (.000)		.56 (.73) (.03)	
Theta Frontal	**3.12 (.01) (.12)**	d < a,b,c,e	**5.94 (.02) (.05)**	m < f	.52 (.76) (.02)	
Theta Central	2.00 (.09) (.08)		2.61 (.11) (.02)		.16 (.98) (.01)	
Theta Parietal	**2.38 (.04) (.10)**	d < a,b,e	1.67 (.20) (.02)		.41 (.84) (.02)	
Theta Occipital	1.57 (.17) (.07)		.11 (.74) (.001)		.48 (.79) (.02)	
Theta Temporal	**2.72 (.02) (.11)**	d < a,b,e; f < b	2.92 (.09) (.03)		.70 (.63) (.03)	
Low Theta Frontal	**2.46 (.04) (.10)**	d < a,b,c,e	**6.31 (.01) (.05)**	m < f	.32 (.90) (.01)	
Low Theta Central	1.95 (.09) (.08)		2.79 (.10) (.03)		.12 (.99) (.005)	
Low Theta Parietal	**2.75 (.02) (.11)**	d < a,b,c,e	3.45 (.07) (.03)		.20 (.96) (.01)	
Low Theta Occipital	1.99 (.09) (.08)		.05 (.83) (.000)		.06 (.998) (.003)	
Low Theta Temporal	**3.24 (.01) (.13)**	d < a,b,c,e; f < e	3.80 (.054) (.03)		.34 (.89) (.02)	
High Theta Frontal	**3.11 (.01) (.12)**	d < a,b,c,e	**4.88 (.03) (.04)**	m < f	1.13 (.35) (.05)	
High Theta Central	1.98 (.09) (.08)		1.62 (.21) (.02)		.36 (.87) (.02)	
High Theta Parietal	1.79 (.12) (.08)		.92 (.34) (.008)		.77 (.58) (.03)	
High Theta Occipital	1.64 (.15) (.07)		.32 (.57) (.003)		.52 (.76) (.02)	
High Theta Temporal	**2.31 (.049) (.10)**	c,d < b	1.46 (.23) (.01)		.95 (.46) (.04)	
Alpha Frontal	**2.93 (.02) (.12)**	c < a,b,e; d < b,e	1.09 (.30) (.01)		.44 (.82) (.02)	
Alpha Central	1.63 (.16) (.07)		1.31 (.26) (.01)		.67 (.65) (.03)	
Alpha Parietal	1.21 (.31) (.05)		.04 (.84) (.00)		.76 (.58) (.03)	
Alpha Occipital	.74 (.59) (.03)		.21 (.65) (.00)		.57 (.73) (.03)	
Alpha Temporal	1.10 (.37) (.05)		.04 (.84) (.00)		.57 (.72) (.03)	
Low Alpha Frontal	2.38 (.04) (.10)		.51 (.48) (.01)		.78 (.57) (.03)	
Low Alpha Central	1.93 (.10) (.08)		.91 (.34) (.01)		.71 (.62) (.03)	
Low Alpha Parietal	1.61 (.16) (.07)		.13 (.72) (.00)		.72 (.61) (.03)	
Low Alpha Occipital	.91 (.48) (.04)		.00 (.99) (.00)		1.15 (.34) (.05)	
Low Alpha Temporal	1.41 (.23) (.06)		.05 (.82) (.00)		.83 (.53) (.04)	
High Alpha Frontal	1.70 (.14) (.07)		.81 (.37) (.01)		.20 (.96) (.01)	
High Alpha Central	.77 (.57) (.03)		1.64 (.20) (.02)		.51 (.77) (.02)	
High Alpha Parietal	.47 (.80) (.02)		.00 (.95) (.00)		.39 (.86) (.02)	
High Alpha Occipital	.70 (.63) (.03)		.87 (.35) (.01)		.16 (.98) (.01)	
High Alpha Temporal	.45 (.81) (.02)		.30 (.58) (.00)		.19 (.97) (.01)	
Beta Frontal	**2.80 (.02) (.11)**	a,c,d,f < b	.25 (.62) (.00)		1.22 (.30) (.05)	
Beta Central	**3.59 (.005) (.14)**	a,c,d,f < b,e	1.78 (.19) (.02)		.86 (.51) (.04)	
Beta Parietal	1.71 (.14) (.07)		.46 (.50) (.004)		.99 (.43) (.04)	
Beta Occipital	**2.45 (.04) (.10)**	a,c < b; a < d	.13 (.72) (.001)		1.08 (.38) (.05)	
Beta Temporal	.74 (.60) (.03)		.22 (.64) (.002)		1.23 (.30) (.05)	
Gamma Frontal	.67 (.64) (.03)		.61 (.44) (.005)		.78 (.57) (.03)	
Gamma Central	.85 (.51) (.04)		2.66 (.11) (.02)		1.59 (.17) (.07)	
Gamma Parietal	1.25 (.29) (.06)		1.39 (.24) (.01)		1.57 (.18) (.07)	
Gamma Occipital	.52 (.76) (.02)		2.48 (.12) (.02)		2.17 (.06) (.09)	
Gamma Temporal	.33 (.89) (.02)		.43 (.51) (.004)		1.77 (.13) (.07)	

Note: For all analyses, Condition df = 5; Gender df = 1; Condition x Gender df = 5; Error df = 110. All Effects are Post-Condition – Pre-Condition for a = water, b = high sugar, c = low sugar, d = higher cacao-content, e = low cacao-content, f = higher cacao-content + L-theanine; m = male, f = female.

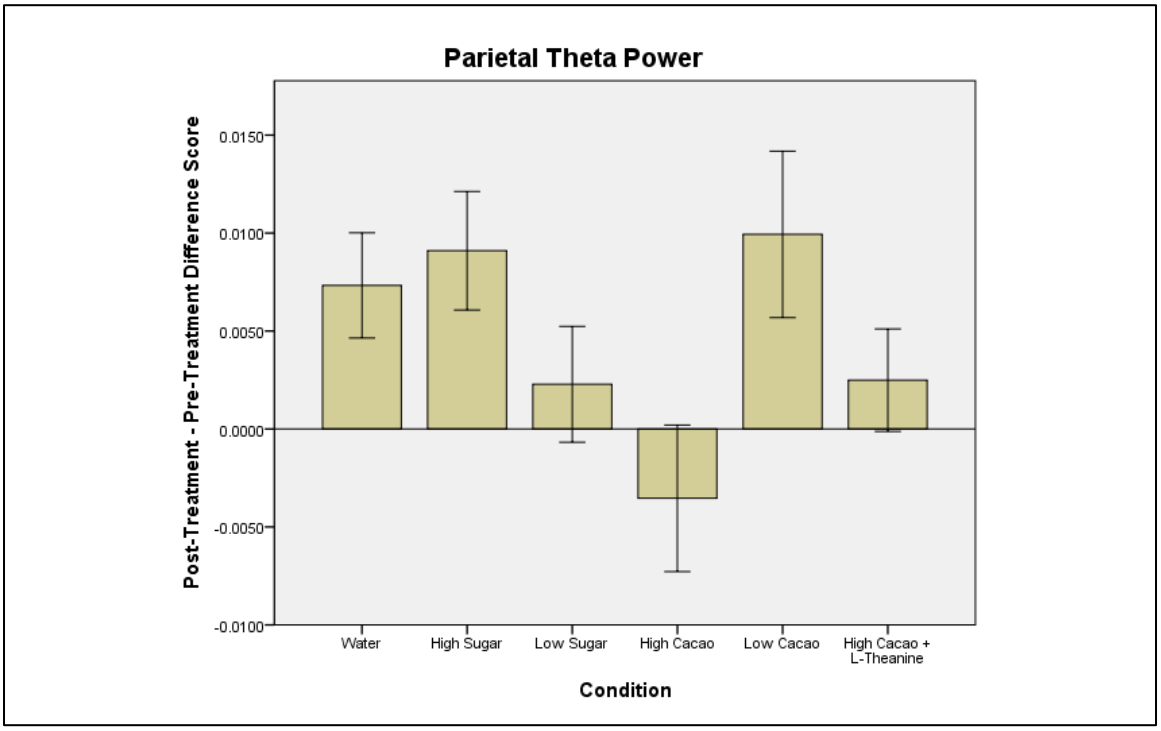

Figure 3. Parietal theta EEG absolute power changes post-treatment minus pre-treatment for each condition, showing significant decreases for higher cacao relative to water, high sugar, and low cacao. SE bars = +/- 1 SE.

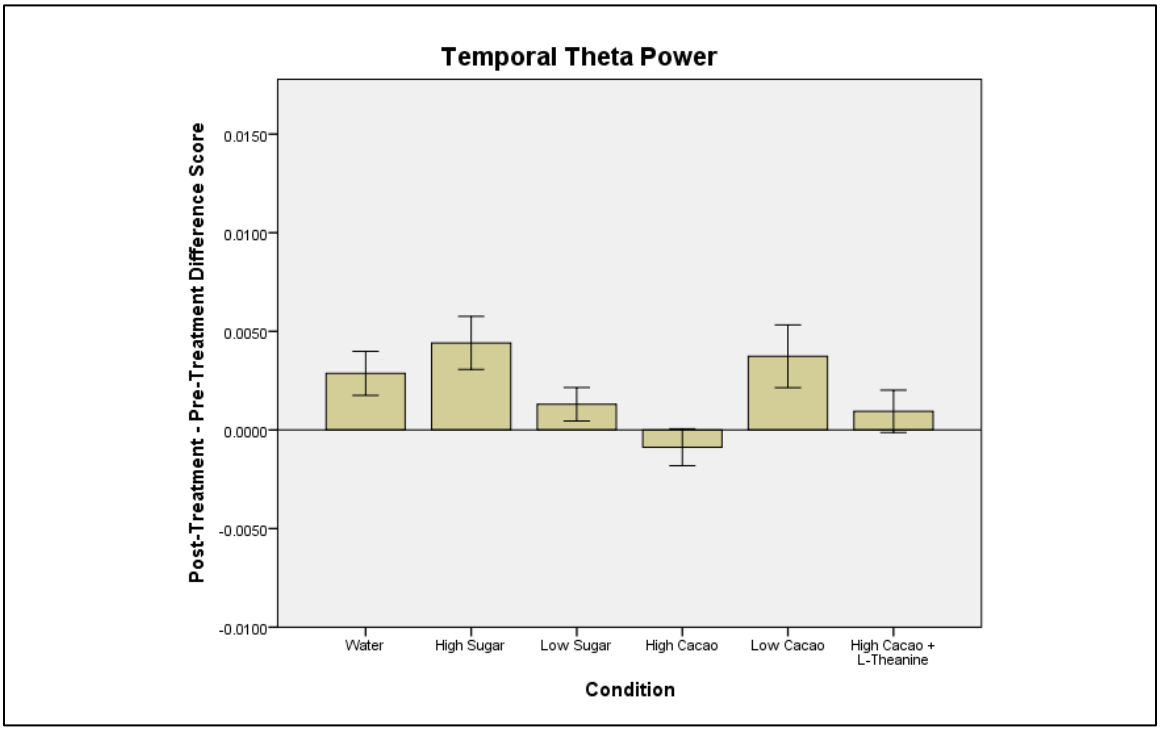

Figure 4. Temporal theta EEG absolute power changes post-treatment minus pre-treatment for each condition, showing significant decreases for higher cacao relative to water, high sugar, and low cacao. SE bars = +/- 1 SE.

Examining narrower frequency bins within the theta band revealed corresponding changes to those reported above for the higher cacao-content chocolate in frontal, parietal, and temporal low theta (4–5.99 Hz) relative to water (p = .030, .010, .011), high sugar (p = .014, .010, .004), low sugar (p = .005, .010, .007), and low cacao-content chocolate (p = .008, .003, .002). Within the high theta (6–7.99 Hz) bin, only the frontal region reached statistical significance for higher cacao-content chocolate relative to water (p = .009), high sugar (p < .0001), low sugar (p = .028), and low cacao-content chocolate (p = .009). These consistent changes in high and low theta frequency bins, primarily in frontal regions, suggest a suppressant effect of cacao on these frequencies. High theta frequency in the temporal region showed very small increases with consumption of low sugar (p = .008) and higher cacao-content chocolate (p = .009) relative to larger high sugar condition increases, an apparent enhancing effect of sugar on the high theta frequency in this region since the higher cacao-content chocolate contained the same amount of sugar as the low sugar condition.

Significant main effects were also obtained for frontal alpha across treatment conditions, $F(5, 110) = 2.93$, p = .016, η^2 = .117. There were no gender or interaction effects for any clusters or alpha frequencies, nor did analysis of any narrow alpha frequency bins result in significant effects. Examination of simple effects for frontal alpha revealed decreases across treatment for the low sugar and higher cacao-content chocolate conditions relative to increases for high sugar (p = .003, .025) and low cacao-content chocolate (p = .006, .047) conditions and for low sugar relative to water (p = .018). Given that low sugar and higher cacao-content chocolate conditions were identical for low sugar levels and that high sugar and low cacao conditions were identical for higher sugar levels, these results suggest an effect of sugar on increasing alpha frequencies in frontal regions. These frontal alpha effects are presented graphically in Figure 5.

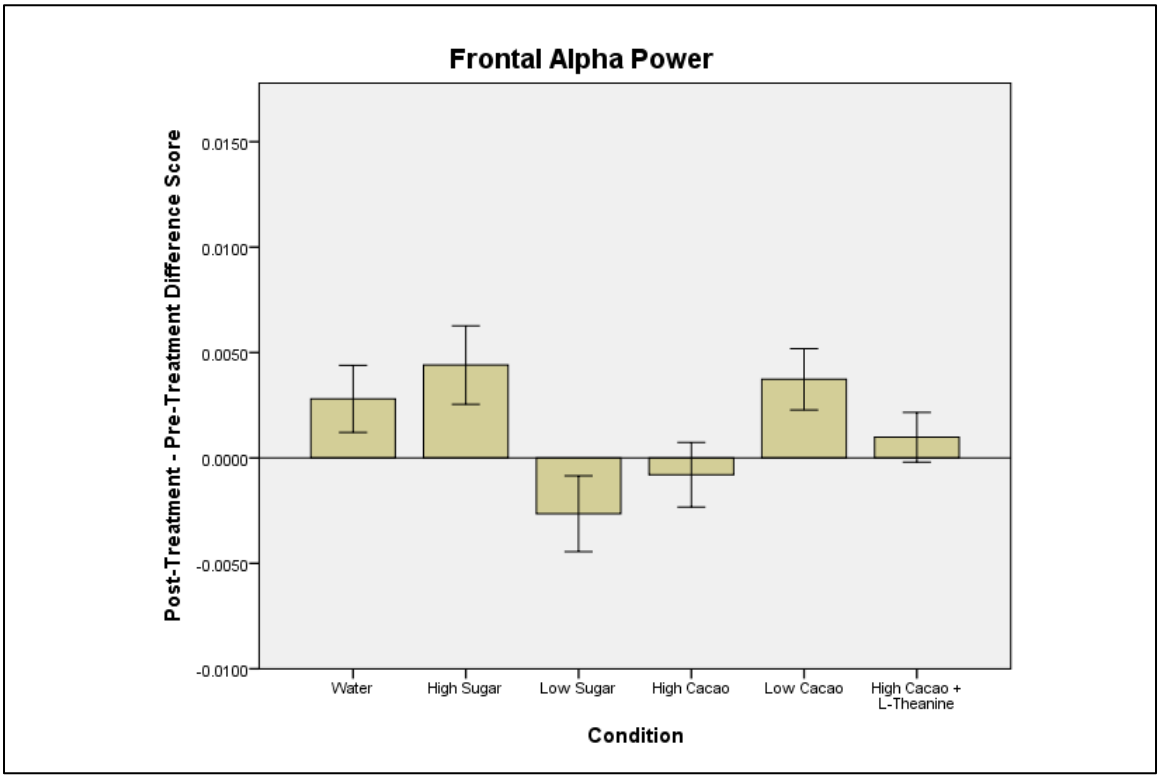

Figure 5. Frontal alpha EEG absolute power changes post-treatment minus pre-treatment for each condition, showing significant decreases for low sugar and higher cacao relative to increases for high sugar and low cacao and for low sugar relative to water. SE bars = +/- 1 SE.

Significant main effects were obtained for frontal beta, $F(5, 110) = 2.80$, $p = .02$, $\eta^2 = .113$; central beta, $F(5, 110) = 3.59$, $p = .005$, $\eta^2 = .14$; and occipital beta EEGs, $F(5, 110) = 2.45$, $p = .038$, $\eta^2 = .10$. No significant gender or interaction effects were obtained. Planned and post-hoc comparisons revealed that for frontal and central regions high sugar was associated with beta increases relative to decreases for water ($p = .003, .006$), low sugar ($p = .019, .016$), higher cacao-content chocolate ($p = .002, .004$), and higher cacao-content chocolate + L-Theanine ($p = .049, .022$), and that for central regions low cacao-content chocolate was associated with beta increases relative to decreases for water ($p = .009$), low sugar ($p = .025$), higher cacao-content chocolate ($p = .007$), and higher cacao-content chocolate + L-Theanine ($p = .035$). In occipital regions, high sugar was also associated with increases in beta relative to decreases for water ($p = .002$) and low sugar ($p = .016$). Again, these beta increases following the consumption of high sugar water in frontal, central, and occipital regions and in central regions following the consumption of a low cacao-content chocolate confection containing comparable high sugar levels, suggest a beta EEG enhancement effect of sugar in these cortical regions. However, an additional, marginally significant ($p = .05$) increase in beta EEG for the higher cacao-content chocolate condition relative to a decrease for water in occipital regions, in the absence of a corresponding increase for the low sugar condition, suggests a potential specific beta enhancement effect for the higher cacao confection. This beta enhancement effect is graphically presented in Figure 6.

No significant main or interaction effects were obtained for delta or gamma EEG frequencies. The absence of these outcomes suggests no statistically significant effect of any of the six conditions on delta and gamma EEG frequencies within the cortical regions studied.

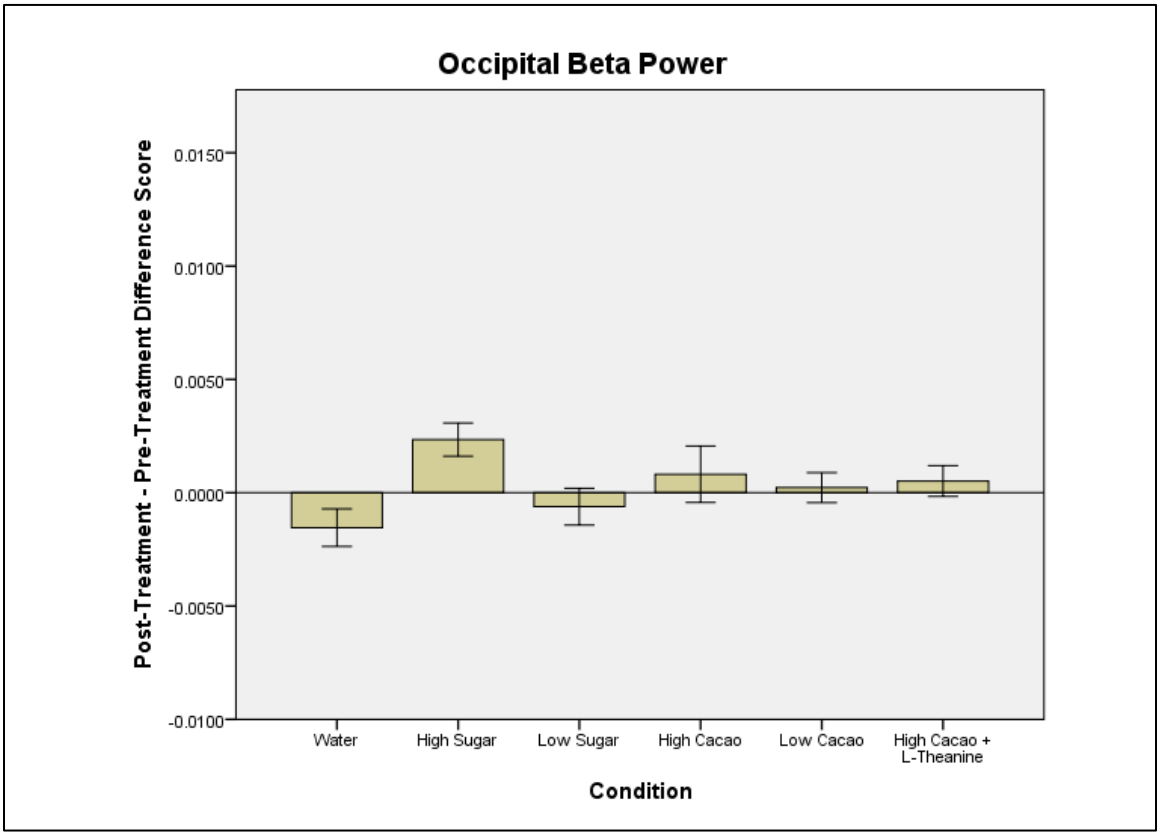

Figure 6. Occipital beta EEG absolute power changes post-treatment minus pre-treatment for each condition, showing significant increases for higher cacao relative to decreases for water. SE bars = +/- 1 SE.

LORETA Source Localization Effects

In order to separate the effects of cacao from sugar in our study, we compared the higher cacao-content condition with the low sugar condition and examined Current Source Density for the higher cacao-content constituents free of sugar effects. Figures 7 and 8 show cortical generators for the low theta and beta frequencies respectively, with relevant neuroimaging parameters reported. The parahippocampal gyrus and sub-gyral hippocampus in the right posterior limbic lobe reflect areas of maximal difference between conditions for the obtained low theta suppressant effect. Maximal differences for the obtained beta enhancement effect involved posterior portions of the medial frontal gyrus and the paracentral lobule in the frontal lobe and the anterior cingulate gyrus in the limbic lobe. Implications of these CSD localization findings are discussed below.

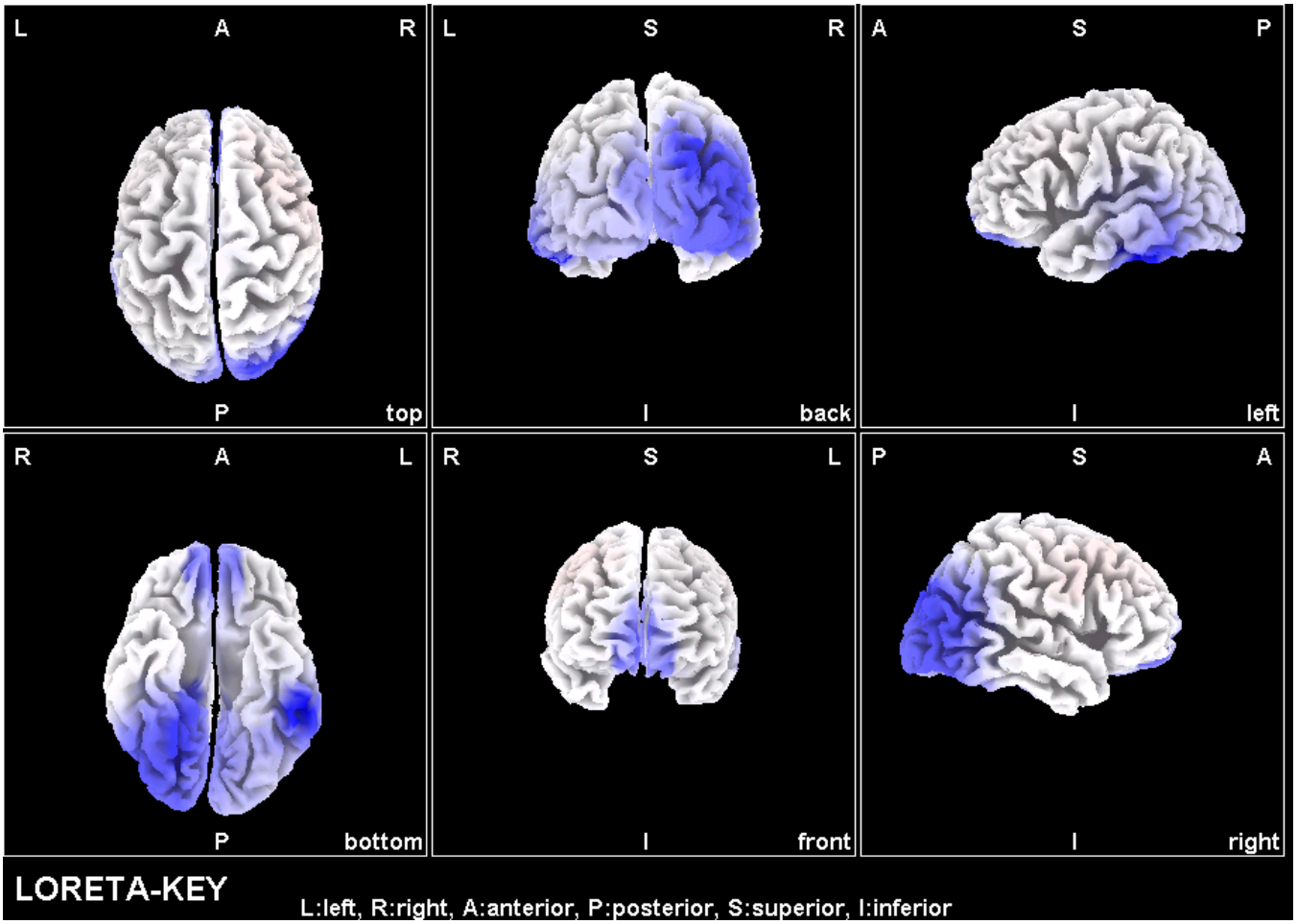

Figure 7. LORETA low theta maximal CSD for the higher cacao-content condition minus the low sugar condition. (Blue color indicates diminished activity.) Mean t = -0.393; SD = 0.420; Maxim t = 4.00; Scale range = +/- 1.36.

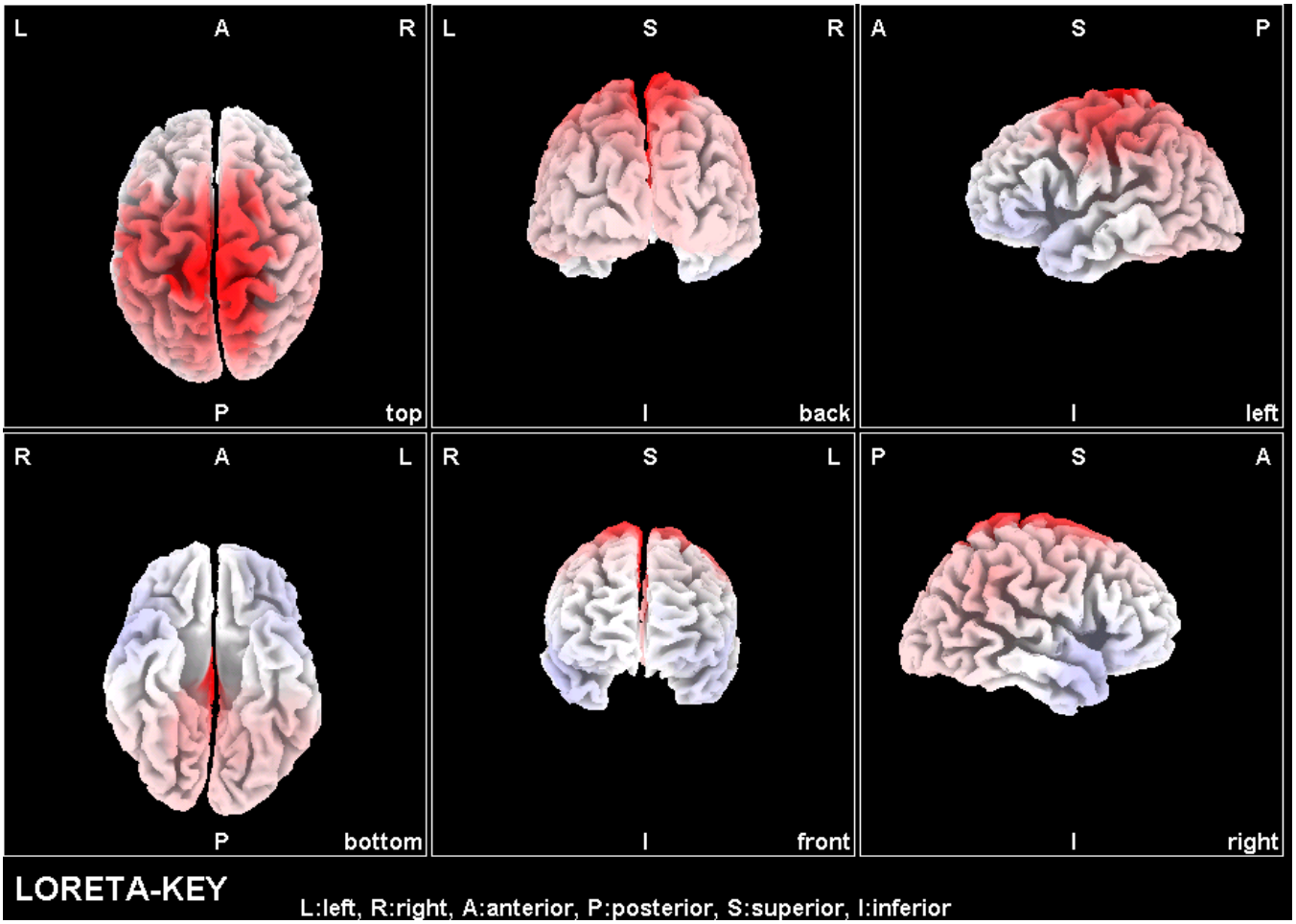

Figure 8. LORETA beta maximal CSD for the higher cacao-content condition minus the low sugar condition. (Red color indicates increased activity.) Mean t = 0.52; SD = 0.76; Maxim t = 4.00; Scale range = +/- 1.92.

Analysis of Blood Pressure Changes

Diastolic and systolic blood pressure changes from baseline to 70-min after ingestion for each treatment and for each gender were analyzed by separate 2 (genders) x 6 (treatments) ANOVAs. For DBP, a significant treatment main effect was found, $F(5, 110) = 6.57$, $p < .0001$, $\eta^2 = .23$, but no significant gender effect, $F(1, 110) = 0.002$, $p = .96$, $\eta^2 < .0001$, and no significant interaction effect, $F(5, 110) = 0.74$, $p = .59$, $\eta^2 = .03$, were obtained. Planned comparisons across treatments for DBP revealed higher cacao-content chocolate DBP to be significantly greater than the higher cacao-content chocolate + L-theanine and water conditions, and higher cacao-content chocolate + L-theanine DBP to be significantly lower than the higher cacao-content chocolate, low cacao-content chocolate, low sugar, and high sugar conditions ($p < .05$). These effects are presented in Table 8 and Figure 9.

For SBP, a significant condition main effect was found, $F(5, 110) = 4.02$, $p = .002$, $\eta^2 = .16$, but no significant gender effect, $F(1, 110) = 0.14$, $p = .71$, $\eta^2 = .001$, and no significant interaction effect, $F(5, 110) = 1.32$, $p = .26$, $\eta^2 = .06$, were obtained. Planned comparisons across conditions for SBP revealed higher cacao-content chocolate + L-theanine SBP to be significantly lower than the higher cacao-content chocolate, low cacao-content chocolate, low sugar, and high sugar conditions ($p < .05$). These effects are presented in Table 8 and Figure 10.

Table 8
Systolic and Diastolic Blood Pressure Changes (Post-Ingestion – Pre-Ingestion)

	Condition Mean (S.E.) BP Change (+ Value = BP Increase)					
	A	B	C	D	E	F
Blood Pressure	Water	High Sugar	Low Sugar	High Cacao	Low Cacao	High Cacao + L-theanine
Systolic	-0.52 (0.72)	+2.55 (1.57)	+1.25 (1.20)	+1.70 (1.65)	+0.57 (1.25)	-5.15 (1.65)** F < B, D, C, E
Diastolic	-0.43 (0.63)	+1.65 (0.71)	+0.85 (0.89)	+4.70 (1.42)* D > F, A	+1.71 (1.06)	-3.65 (1.39)* F < D, E, B, C
N	21	20	20	20	21	20

* = Statistically significant at $p < .05$; ** = Statistically significant at $p < .0001$. All Effects are Post-Condition – Pre-Condition effects for A = water, B = high sugar, C = low sugar, D = higher cacao-content, E = low cacao-content, F = higher cacao-content + L-theanine.

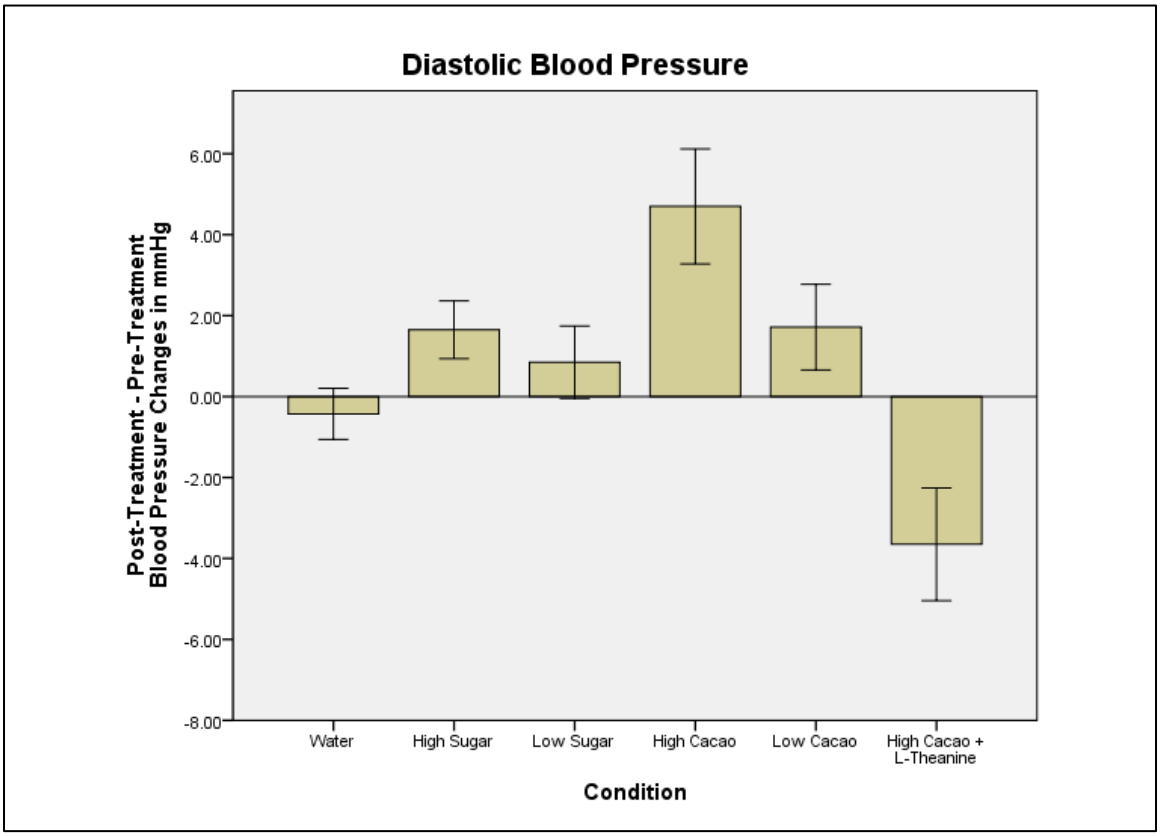

Figure 9. Diastolic blood pressure changes post-treatment minus pre-treatment for each condition, with higher cacao significantly greater than higher cacao + L-theanine and water, and higher cacao + L-theanine significantly lower than higher cacao, low cacao, low sugar, and high sugar. SE bars = +/- 1 SE.

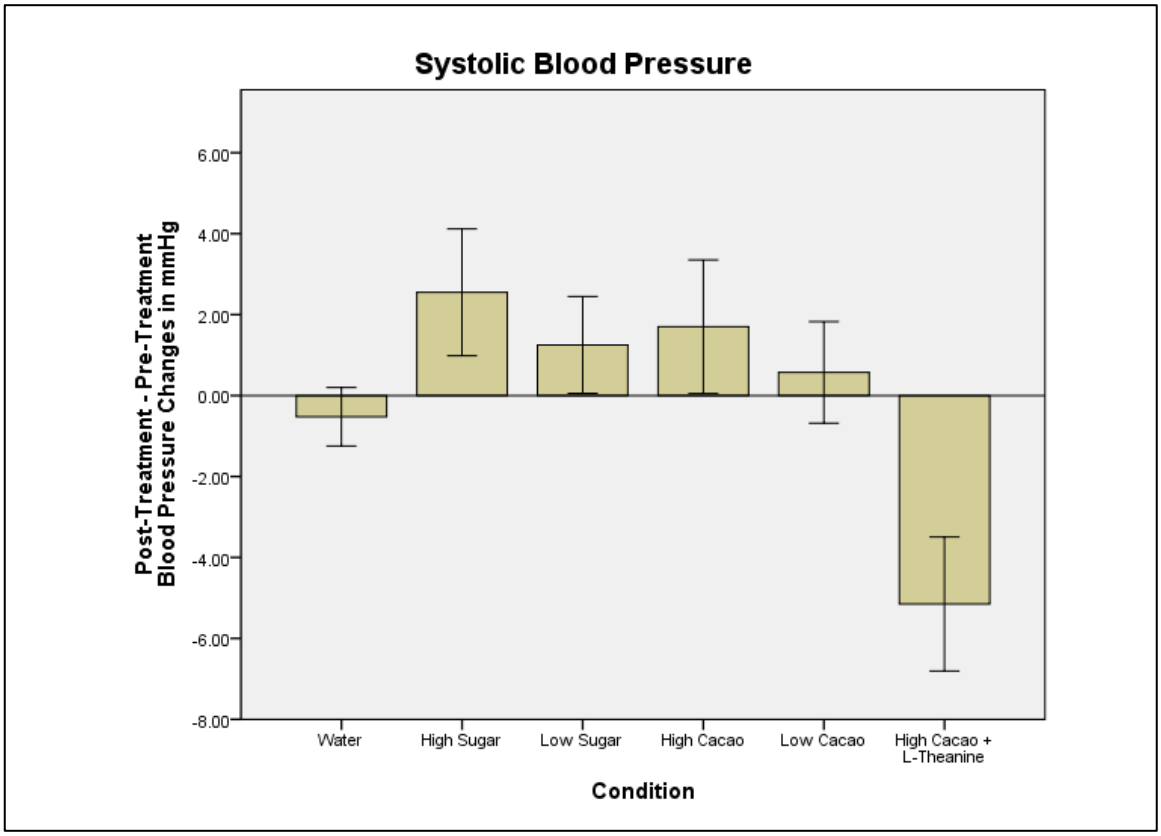

Figure 10. Systolic blood pressure changes post-treatment minus pre-treatment for each condition, with higher cacao + L-theanine significantly lower than higher cacao, low cacao, low sugar, and high sugar. SE bars = +/- 1 SE.

Discussion

General Findings

Consistent with our hypotheses, it appears that chocolate does have an acute stimulatory effect on components of both the central (CNS) and peripheral nervous systems (PNS). In order to understand the nature and implications of these outcomes, it is important to examine the effects on CNS and PNS arousal of specific treatment conditions. The act of sitting quietly and reading neutral magazines during the water condition for our sample of healthy college students tended to diminish CNS arousal, as attested to by general decreases in posterior beta and increases in anterior theta EEG frequencies. The combination of decreased beta and increased theta is commonly seen in attentional disorders and often reflects inattention and "spacing out" (Lubar, Swartwood, Swartwood, & O'Donnell, 1995; Mann, Lubar, Zimmerman, Miller, & Muenchen, 1992), not to be unexpected among college students sitting quietly and reading uninteresting magazines. This diminution of the CNS arousal was inhibited by the consumption of a high sugar drink (increased diffuse beta) and by the consumption of a higher cacao-content chocolate (decreased anterior theta and increased posterior beta). Interestingly, although the high sugar drink was very effective in increasing cortical beta in general, it was not effective in decreasing theta across the cortex, whereas the higher cacao-content chocolate condition was statistically equivalent to high sugar in increasing posterior beta but far more effective in decreasing more diffuse theta activity, even though the higher cacao-content condition contained less sugar (0.35 g/kg body weight) than the high sugar condition (0.57 g/kg body weight). This effect is further supported by a significantly diminished beta enhancement by the low sugar condition relative to high sugar, the former containing an equivalent amount of sugar to the higher cacao-content conditions but none of the cacao bioactive components.

Furthermore, the possibility of these effects being also due to changes in mu rhythms during page turning and lambdoid waves during saccadic eye movements while reading seems unlikely in that both the pre-ingestion and post-ingestion EEGs were recorded eyes closed before and after, respectively, the reading of magazines, with approximately 5 minutes intervening after stopping reading and before post-ingestion EEG recording while the Electro-Cap was repositioned, impedances were checked, and good clean EEG traces were obtained. These observations suggest a supplemental and differential stimulating effect of the bioactive compounds in cacao (such as biogenic amines and/or methylxanthines) over that of sugar, with sugar increasing cortical beta and cacao decreasing cortical theta.

Potential Biogenic Amines Underlying EEG Cacao Effects

Changes in biogenic amine neurotransmission are associated with distinctive patterns of EEG change. In Fischer rats for example, small declines in EEG slow wave activity have been associated with administration of dopamine agonists (Dimpfel, 2005). These declines were followed, 90 min later, by increases in theta, delta, and alpha 2 activity in the hippocampus and frontal cortex. The administration of the highest L-Dopa dose replicated the biphasic low dose finding but predominately in the frontal cortex. It is noteworthy that increases in theta spectral power have been associated with reports of increased tiredness and sedation in humans (Dimpfel, 2008; Dimpfel & Schober, 2001; Vyazovskiy & Tobler, 2005) and also with increased attentional demands during mental work (Schober, Schellenberg, & Dimpfel, 1995; Schwarz-Ottersbach & Goldberg, 1986). Also, administration of direct D2 agonists was found to decrease alpha 2 power in the frontal cortex, hippocampus, and striatum but not in the reticular formation. Predictably the administration of a DA2 antagonist dramatically increased alpha 2 power in the frontal cortex 3 hr after administration of the highest dose. Our data, collected on humans within an hour after the administration of varied cacao doses (with or without theanine) from frontal, temporal, and parietal sites, are consistent with these acute theta wave declines reported by Dimpfel (2008).

Given the known relationship between dopamine (D) regulation and acetylcholine (ACh) neurotransmission, these low dose agonist effects are thought to be associated with the activation of heterosynaptic presynaptic D2 receptors located on cholinergic neurons involving the cAMP inhibition of activity in the b-arrestin pathway (Dimpfel, 2008). Data presented by Zhang, Zhou, and Dani (2004) demonstrating increased dopamine release following administration of an ACh esterase inhibitor supports this conclusion. Conversely, declines in alpha 2 activity have been associated with the administration of an ACh M1 antagonist. Clearly, the availability of different receptor subtypes and their pharmacological selectivity in different neuroanatomical circuits (cortical and subcortical) help regulate both DA and ACh neurotransmission and EEG frequency pattern alterations. However, the precise subcortical neuroanatomical circuitry responsible for these changes has yet to be elucidated and behavioral data collected concurrently are limited. Moreover, these effects are dose-dependent and area specific, as well as time-, drug-, and task-dependent. To complicate matters further, norepinephrine reuptake inhibitors have been demonstrated to increase theta wave activity in the septo-hippocampal area (Hajós, Hoffman, Robinson, Yu, & Hajós-Korcsok, 2003) and administration of the antihypertensive, anxiolytic, and alpha 1 adrenergic receptor agonist clonidine has also been demonstrated to increase theta EEG activity (Dimpfel & Schober, 2001). With regard to the biogenic amines, alterations in EEG outcomes not only appear to involve differing signal activity in different cortical and subcortical neuroanatomical pathways, but also the integration of differing subcellular neuronal processes involved in complex neuronal patterns and behavior associated with cacao-induced electrocortical changes.

For example, cacao flavanols are known to cross the blood brain barrier, to increase blood circulation in brain, to exert antioxidative effects, to increase nitric oxide production, and to trigger protein-receptor synthesis via mitogen-activated protein, phosphoinositide 3-kinase, and extra-signal regulated subcellular cascades, all of which are associated with the neuromodulation of long-term potentiation integral to the formation of memories and neurocognitive function (Sokolov et al., 2013). The neurological impacts of cacao flavanols have also been reported to exert neuroprotective and neuromodulatory effects that promote synaptic connectivity, alter cognition and behavior and promote endothelium-dependent vasodilation (Sokolov et al., 2013). Flavanoids have also been demonstrated to promote neurogenesis and memory formation and to protect against neuronal cell death by increasing the expression of brain derived neurotrophic factor in the hippocampus. And, with regard to acute and chronic consumption effects, the

short-term oral exposure to 100 mg/100 g body weight of cacao exerted an anxiolytic effect on elevated T-maze behavior in rats, whereas exposure to cacao for 2 weeks increased brain serotonin concentration and turnover rate but failed to alter elevated T-maze behavior (Yamada, Yamada, Okano, Terashima, & Yokogoshi, 2009). Nonetheless, in humans, the effects of cacao-derived flavanols on cognitive function and mood have not been clearly elucidated and the effects of acute vs. long-term exposure to flavanols on arousal and EEG changes relating to these processes have not been adequately investigated.

L-theanine EEG Effects

L-theanine has a documented EEG alpha enhancement effect in the research literature (Juneja et al., 1999; Kimura et al., 2007; Kobayashi et al., 1998). While we did not find such an effect with our higher cacao-content + L-theanine confection, we theorize this to be due to the sympathomimetic ingredients in chocolate suppressing slow wave (alpha and theta) and enhancing fast wave (beta) activity. The absence of a significant alpha suppression effect with the higher cacao-content condition is noteworthy. EEG alpha has been historically associated with quiet rest and relaxation. Activation of the brain with increased beta and decreased alpha could actually be perceived by participants as an increase in anxiety and agitation. The finding of increased beta, decreased theta, and a stable alpha frequency with a moderate cacao-content confection suggests that our participants were neurologically activated but without the agitation that might have been perceived had alpha actually been suppressed as well. These CNS arousal without anxiety effects are supported by the absence of significant changes in PANAS mood scores, particularly those related to anxiety/agitation. As noted above, our results suggest that a high sugar beverage can actually increase alpha over our 60% cacao confection, perhaps having implications for a combination of these two substances.

Cortical Source Generators

LORETA cortical source localizations of the surface potentials generated by cacao consumption, independent of sugar effects, suggest some intriguing implications for the impact of cacao on the human brain. Although theta rhythms have been associated with visual imagery, problem solving, perceptual processing, attentive performance in cognitive tasks, creativity, and dissociative states (Stevens et al., 2004), low theta during quiet waking predicts the subsequent development of sleep slow-wave activity and an increase in sleep propensity (Makeig, Jung, & Sejnowski, 2000; Vyazovskiy & Tobler, 2005). Therefore, the suppression of low theta following the consumption of cacao in the present study indicates a counteracting of natural drowsiness induced by an hour of quiet reading of neutral magazines. Since the structures activated are primarily involved with the encoding and recognition of scenes such as landscapes, cityscapes, etc., the content of many of the magazines available during the digestion phase, and with episodic memory (Orrison, 2008), these results suggest an activation of task-related processes in the brain following the consumption of the higher cacao-content confection independent of sugar. Similarly, the enhancement of posterior frontal and anterior cingulate beta frequencies indicates an activation of such executive functions as the recognition of similarities and differences, retention of long term memories, learning, problem-solving, and mental conflict resolution (Orrison, 2008). Taken together, the localization of cortical source generators of the observed surface potentials suggests an enhancement of task-related activities following consumption of cacao in our study. Furthermore, the nature of these brainwave changes directly counteracts those specific frequencies seen during diminished attention.

Acute Blood Pressure Effects

Peripherally, the acute effects (1 hr after consumption) of higher cacao-content and of higher cacao-content + L-theanine ingestion on blood pressure were rather remarkable, with BP changes on the order of 3–5 mmHg. It is noteworthy that while the higher cacao-content condition significantly increased DBP relative to the water condition, it did not significantly do so when compared with the low cacao-content condition. These higher and lower cacao conditions differed considerably in the presence of the psychoactive biogenic amines, tyramine and, particularly, PEA, and the methylxanthines, caffeine and, particularly, theobromine, and would be expected to have a stronger differential effect on blood pressure. Although not statistically significant, these differences were in the predicted direction and were on the order of 3 mmHg different. As mentioned below, it is likely that the only moderate levels of cacao used in this study contributed to this small effect. It is also possible that more prominent vasoconstrictive effects of these sympathomimetics were counteracted by beginning vasodilatation effects of the epicatechin polyphenols in the higher cacao-content product, thus diminishing treatment differences. One can only speculate at this point

what these differences would have been for a higher cacao-content chocolate containing more of these psychoactive compounds.

While the more immediate effect of consuming a higher cacao-content confection was an increase in diastolic BP of 4.7 mmHg on average, the higher cacao-content + L-theanine confection actually counteracted this effect by lowering diastolic BP 3.65 mmHg on average and systolic BP 5.15 mmHg. The potential antihypertensive effect of lowering diastolic blood pressure from the 4.7 mmHg increase seen with higher cacao-content to the 3.65 mmHg decrease seen with higher cacao-content + L-theanine represents an 8.4 mmHg decrease in diastolic blood pressure. These blood pressure lowering outcomes following a single recommended dose of the L-theanine additive represent approximately one-third to one-half the effects of sustained use of standard antihypertensive medications, and without documented side effects (Mason, 2004; Wu et al., 2005). Given the apparent ability of L-theanine to inhibit the more immediate sympathomimetic effects of cacao and to acutely lower blood pressure, combined with the documented longer term antihypertensive effects of polyphenols in cacao, there is clearly the possibility of an application of this combination of L-theanine and cacao in the treatment of hypertension. This exciting possibility is certainly speculative at the present time and awaits further directive research into the longer term consequences of cacao + L-theanine use, particularly for higher doses of both constituents.

Limitations

Overall, these CNS arousal effects suggest that the constituents in cacao (polyphenols, biogenic amines and/or methylxanthines) can inhibit naturally occurring deactivation of the brain during mundane and less interesting tasks. The relative enhancement of beta and suppression of theta frequencies found in this study indicate that higher cacao-content chocolate may have an impact upon electrocortical processes implicated in diminished attention, a common complaint among college students attending lectures and reading academic material. A limitation of this study was that we did not directly measure attentional behavior. Given our findings, it would be of interest in future studies of the effects of chocolate to do so. It is also important to note that this same combination of suppressed beta and enhanced theta has been reported in diagnosed Attention Deficit Disorder (ADD; Lubar, et al., 1995; Mann, et al., 1992). While our participants were not ADD patients, it would be interesting to replicate this study and to observe not only EEG changes but also measures of attentional performance with such a clinical sample.

For reasons of palatability and availability, our study utilized a dark chocolate confection containing only moderate amounts (60%) of cacao. This choice of chocolate confections was a major limitation of this study and quite likely resulted in the small effect sizes (< .25; See Cohen, 1988) found in our analyses, even though the results reported were statistically significant. There are quite palatable chocolate preparations publicly available containing up to 90% cacao. Certainly this study should be replicated with a palatable chocolate confection containing higher percentages of cacao or increased concentrations of cacao bioactives to increase the magnitude of effect and to better understand which cacao constituents are predominantly responsible for these effects. Also of interest for future research would be to examine these enhanced effects for more individualized frequency bins, as suggested by Klimesch (1999).

Acknowledgements

Parts of the present study were conducted as a Master of Arts Thesis project by Michelle Montopoli under the direction of Dr. Larry Stevens.

Chocolate products for this study were provided by a generous grant in supplies from The Hershey Company, P.O. Box 810, 100 Crystal A Drive, Hershey, PA 17033. Grateful appreciation is expressed to Dr. Debra Miller and to the staff at The Hershey Company for their guidance and support throughout this project and for their careful review of this manuscript prior to submission.

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Received: February 5, 2015
Accepted: March 11, 2015
Published: April 14, 2015

Appendix

Table 2

*Absolute **Delta** Power for each electrode site for each condition at Baseline, after Treatment, and Differences (Post Treatment – Baseline)*

Electrode Position	Mean Values	Water Control	High Sugar Control	Low Sugar Control	High Cacao	Low Cacao	Theanine + High Cacao
Fp1	Baseline	608	679	696	669	731	382
	Post-Rx	537	793	651	823	790	449
	Difference	-071	114	-045	153	058	067
Fp2	Baseline	605	657	717	714	702	467
	Post-Rx	538	786	668	850	796	468
	Difference	-066	129	-049	137	095	001
F7	Baseline	481	557	565	693	632	304
	Post-Rx	411	665	621	647	649	317
	Difference	-069	108	055	-046	018	012
F3	Baseline	494	573	582	593	649	356
	Post-Rx	502	651	644	593	719	380
	Difference	008	078	062	-001	070	024
Fz	Baseline	537	571	592	620	669	387
	Post-Rx	555	677	693	589	756	408
	Difference	018	106	101	-030	087	022
F4	Baseline	487	520	551	677	626	350
	Post-Rx	480	610	624	547	724	366
	Difference	-007	090	074	-130	098	018
F8	Baseline	449	454	504	591	544	244
	Post-Rx	374	602	520	531	552	264
	Difference	-075	148	016	-060	007	020
T3	Baseline	247	305	311	307	348	194
	Post-Rx	229	333	344	298	396	203
	Difference	-018	028	034	-008	048	009
C3	Baseline	410	522	514	519	623	320
	Post-Rx	438	591	595	478	692	340
	Difference	027	069	081	-041	069	021
Cz	Baseline	545	654	649	725	760	436
	Post-Rx	606	752	774	623	923	460
	Difference	062	098	125	-102	163	024
C4	Baseline	413	481	486	537	565	341
	Post-Rx	432	549	564	488	742	347
	Difference	019	068	079	-049	177	007
T4	Baseline	208	240	263	265	283	259
	Post-Rx	197	289	324	254	325	169
	Difference	-012	049	060	010	042	-090
T5	Baseline	309	416	372	372	426	246
	Post-Rx	319	458	420	376	503	252
	Difference	011	042	048	004	077	007
P3	Baseline	413	550	525	541	633	334
	Post-Rx	431	618	581	523	701	346
	Difference	018	068	055	-018	069	012
Pz	Baseline	502	628	596	655	712	393
	Post-Rx	518	709	667	620	828	407
	Difference	016	081	071	-035	115	014
P4	Baseline	416	549	500	549	601	335
	Post-Rx	431	611	559	523	692	340
	Difference	015	062	059	-026	091	004
T6	Baseline	301	406	356	459	447	294
	Post-Rx	318	447	402	358	496	259
	Difference	017	041	047	-101	048	-035
O1	Baseline	431	582	511	532	569	362
	Post-Rx	483	658	546	550	647	371
	Difference	053	077	035	001	077	009
O2	Baseline	421	586	516	533	510	333
	Post-Rx	476	671	551	535	608	364
	Difference	054	085	035	002	098	031

NOTE: Values in the table are Absolute EEG Power uV^2 x 10^4 for ease of presentation.

Table 3

*Absolute **Theta** Power for each electrode site for each condition at Baseline, after Treatment, and Differences (Post Treatment – Baseline)*

Electrode Position	Mean Values	Water Control	High Sugar Control	Low Sugar Control	High Cacao	Low Cacao	Theanine + High Cacao
Fp1	Baseline	241	301	302	322	378	268
	Post-Rx	263	342	324	302	419	275
	Difference	022	041	023	-020	041	007
Fp2	Baseline	242	290	298	317	359	271
	Post-Rx	260	331	319	304	402	269
	Difference	019	041	021	-013	044	-001
F7	Baseline	195	255	239	280	314	221
	Post-Rx	206	287	266	252	346	221
	Difference	012	032	026	-028	033	000
F3	Baseline	307	450	432	445	562	347
	Post-Rx	359	518	480	392	645	362
	Difference	052	068	049	-053	083	015
Fz	Baseline	352	498	472	504	604	392
	Post-Rx	420	577	533	441	711	405
	Difference	069	079	061	-064	107	013
F4	Baseline	297	417	340	449	491	330
	Post-Rx	348	482	447	384	580	349
	Difference	051	065	048	-064	089	019
F8	Baseline	171	203	200	239	230	165
	Post-Rx	175	230	216	214	256	178
	Difference	004	027	016	-025	026	013
T3	Baseline	154	224	199	216	254	176
	Post-Rx	160	251	218	205	287	185
	Difference	006	026	018	-011	034	009
C3	Baseline	304	485	448	454	518	341
	Post-Rx	353	545	481	399	618	360
	Difference	049	060	033	-055	100	019
Cz	Baseline	423	618	578	657	698	446
	Post-Rx	509	700	651	533	843	466
	Difference	086	082	073	-124	145	020
C4	Baseline	300	475	440	466	507	351
	Post-Rx	354	523	453	399	611	364
	Difference	054	049	013	-067	104	013
T4	Baseline	115	167	162	172	170	148
	Post-Rx	123	182	168	158	196	141
	Difference	008	015	006	-014	026	-007
T5	Baseline	226	460	334	368	360	265
	Post-Rx	276	531	354	379	468	283
	Difference	050	071	020	010	108	018
P3	Baseline	336	633	508	516	597	385
	Post-Rx	400	732	528	494	695	408
	Difference	064	099	019	-022	098	022
Pz	Baseline	437	737	585	607	690	447
	Post-Rx	522	841	613	563	838	475
	Difference	084	104	027	-044	015	028
P4	Baseline	336	682	466	481	535	370
	Post-Rx	407	752	487	441	641	395
	Difference	072	070	022	-040	106	025
T6	Baseline	225	436	299	361	379	261
	Post-Rx	276	500	307	341	451	279
	Difference	051	064	008	-020	072	018
O1	Baseline	321	625	470	443	488	405
	Post-Rx	417	744	498	554	605	441
	Difference	096	119	028	111	117	035
O2	Baseline	313	722	446	460	420	365
	Post-Rx	400	850	492	437	525	395
	Difference	087	128	047	-023	105	031

NOTE: Values in the table are Absolute EEG Power uV^2 x 10^4 for ease of presentation.

Table 4

*Absolute **Alpha** Power for each electrode site for each condition at Baseline, after Treatment, and Differences (Post Treatment – Baseline)*

Electrode Position	Mean Values	Water Control	High Sugar Control	Low Sugar Control	High Cacao	Low Cacao	Theanine + High Cacao
Fp1	Baseline	282	384	305	291	296	270
	Post-Rx	308	425	274	282	321	279
	Difference	025	040	-031	-009	025	009
Fp2	Baseline	277	373	296	281	284	267
	Post-Rx	298	413	267	273	310	273
	Difference	021	040	-029	-008	026	006
F7	Baseline	220	320	240	237	260	209
	Post-Rx	242	347	221	229	284	219
	Difference	022	027	-019	-008	025	011
F3	Baseline	368	560	460	400	425	374
	Post-Rx	405	614	417	391	475	387
	Difference	037	054	-043	-009	051	013
Fz	Baseline	406	607	496	434	448	421
	Post-Rx	451	675	453	427	505	429
	Difference	045	068	-043	-007	058	008
F4	Baseline	350	526	427	383	386	362
	Post-Rx	385	581	390	374	440	372
	Difference	035	054	-037	-008	054	010
F8	Baseline	188	266	196	191	196	175
	Post-Rx	199	291	185	184	219	186
	Difference	011	025	-011	-007	023	012
T3	Baseline	196	336	249	219	257	193
	Post-Rx	214	361	227	231	288	206
	Difference	019	025	-021	012	031	013
C3	Baseline	435	804	630	478	553	452
	Post-Rx	465	817	559	484	633	469
	Difference	030	013	-071	006	080	017
Cz	Baseline	551	875	693	592	644	531
	Post-Rx	597	945	620	578	736	547
	Difference	047	070	-073	-014	091	016
C4	Baseline	447	820	649	477	548	484
	Post-Rx	483	852	540	455	627	490
	Difference	036	032	-109	-022	079	006
T4	Baseline	162	271	183	176	191	188
	Post-Rx	173	279	162	168	214	193
	Difference	011	007	-021	-008	023	005
T5	Baseline	641	1149	652	690	676	556
	Post-Rx	746	1200	573	686	858	599
	Difference	105	051	-079	-004	181	044
P3	Baseline	885	1474	1014	954	1072	754
	Post-Rx	998	1521	870	915	1181	797
	Difference	113	047	-144	-039	108	043
Pz	Baseline	1063	1716	1234	1140	1171	869
	Post-Rx	1151	1763	1067	1083	1369	925
	Difference	088	047	-166	-057	198	056
P4	Baseline	937	1623	1036	976	1019	828
	Post-Rx	1046	1643	892	933	1123	898
	Difference	109	020	-143	-043	105	070
T6	Baseline	758	1147	682	698	985	651
	Post-Rx	854	1209	584	791	1000	769
	Difference	096	062	-099	092	016	118
O1	Baseline	1689	2328	1513	1327	1550	1505
	Post-Rx	1839	2505	1325	1399	1717	1620
	Difference	151	177	-188	072	166	115
O2	Baseline	1663	2651	1538	1639	1608	1370
	Post-Rx	1710	2826	1430	1696	1864	1543
	Difference	047	175	-108	058	256	173

NOTE: Values in the table are Absolute EEG Power uV^2 x 10^4 for ease of presentation.

Table 5

*Absolute **Beta** Power for each electrode site for each condition at Baseline, after Treatment, and Differences (Post Treatment – Baseline)*

Electrode Position	Mean Values	Water Control	High Sugar Control	Low Sugar Control	High Cacao	Low Cacao	Theanine + High Cacao
Fp1	Baseline	063	049	050	051	046	045
	Post-Rx	055	060	049	050	049	048
	Difference	-009	011	-001	-001	004	003
Fp2	Baseline	075	049	046	047	051	044
	Post-Rx	058	060	047	046	047	045
	Difference	-017	011	001	-001	-004	002
F7	Baseline	043	054	040	043	043	037
	Post-Rx	040	057	039	041	046	040
	Difference	-004	004	-001	-002	002	004
F3	Baseline	060	065	059	059	058	052
	Post-Rx	056	075	060	056	064	053
	Difference	-004	010	001	-003	005	000
Fz	Baseline	062	064	060	060	059	055
	Post-Rx	057	076	060	059	064	054
	Difference	-005	012	000	-001	006	-001
F4	Baseline	067	061	056	057	055	051
	Post-Rx	058	071	057	055	061	051
	Difference	-008	011	001	-002	005	000
F8	Baseline	036	042	032	036	033	030
	Post-Rx	035	044	032	033	038	032
	Difference	-001	002	000	-003	005	002
T3	Baseline	063	067	042	050	051	040
	Post-Rx	053	068	038	045	050	045
	Difference	-009	001	-003	-005	-001	005
C3	Baseline	065	077	064	064	065	054
	Post-Rx	058	083	061	062	074	054
	Difference	-007	006	-004	-002	009	000
Cz	Baseline	071	076	069	075	071	062
	Post-Rx	064	087	067	074	078	060
	Difference	-006	011	-001	-001	007	-002
C4	Baseline	064	075	064	065	062	056
	Post-Rx	056	082	059	064	068	054
	Difference	-008	007	-005	-001	006	-003
T4	Baseline	040	077	054	039	059	041
	Post-Rx	036	067	046	034	047	033
	Difference	-003	-009	-008	-005	-012	-008
T5	Baseline	071	086	066	072	071	060
	Post-Rx	068	096	065	090	081	067
	Difference	-003	010	-000	018	010	007
P3	Baseline	086	091	078	094	086	071
	Post-Rx	079	101	074	110	087	072
	Difference	-007	010	-004	017	002	001
Pz	Baseline	095	094	087	106	090	077
	Post-Rx	086	106	081	123	093	077
	Difference	-009	013	-006	017	002	-000
P4	Baseline	087	092	078	096	084	072
	Post-Rx	079	102	074	107	084	071
	Difference	-007	010	-005	011	-000	-002
T6	Baseline	072	075	061	073	078	054
	Post-Rx	065	084	057	081	074	061
	Difference	-007	009	-004	008	-003	007
O1	Baseline	145	142	120	156	123	129
	Post-Rx	128	166	113	181	123	129
	Difference	-017	024	-007	025	-001	000
O2	Baseline	138	141	116	170	119	117
	Post-Rx	124	164	111	187	124	127
	Difference	-014	023	-005	017	006	010

NOTE: Values in the table are Absolute EEG Power uV^2 x 10^4 for ease of presentation.

Table 6

*Absolute **Gamma** Power for each electrode site for each condition at Baseline, after Treatment, and Differences (Post Treatment – Baseline)*

Electrode Position	Mean Values	Water Control	High Sugar Control	Low Sugar Control	High Cacao	Low Cacao	Theanine + High Cacao
Fp1	Baseline	020	013	014	013	012	012
	Post-Rx	016	014	014	012	013	014
	Difference	-003	001	-000	-001	001	001
Fp2	Baseline	027	014	012	012	017	011
	Post-Rx	019	016	012	011	012	012
	Difference	-008	003	000	-001	-005	001
F7	Baseline	012	024	009	011	013	008
	Post-Rx	010	021	008	009	012	011
	Difference	-002	-003	-001	-002	-001	003
F3	Baseline	009	011	008	008	007	006
	Post-Rx	008	010	007	007	008	007
	Difference	-001	-000	-000	-001	001	001
Fz	Baseline	007	007	006	006	006	005
	Post-Rx	006	007	005	006	006	005
	Difference	-001	000	-000	-001	-000	000
F4	Baseline	013	009	007	007	008	006
	Post-Rx	009	009	007	006	009	006
	Difference	-003	-000	-000	-001	001	001
F8	Baseline	009	016	006	009	009	006
	Post-Rx	009	014	006	007	012	008
	Difference	000	-003	-001	-002	003	002
T3	Baseline	022	032	010	014	015	010
	Post-Rx	017	026	008	010	012	012
	Difference	-005	-005	-003	-004	-003	001
C3	Baseline	007	013	007	008	007	005
	Post-Rx	006	011	006	005	010	005
	Difference	-001	-002	-001	-003	003	001
Cz	Baseline	006	007	006	006	006	005
	Post-Rx	006	007	006	006	006	005
	Difference	-001	000	-000	-000	000	000
C4	Baseline	007	012	006	007	007	005
	Post-Rx	006	011	005	006	009	005
	Difference	-001	-001	-001	-001	003	001
T4	Baseline	013	035	014	010	021	013
	Post-Rx	009	027	012	007	017	009
	Difference	-003	-008	-003	-003	-004	-004
T5	Baseline	007	009	007	009	007	008
	Post-Rx	006	009	007	006	008	011
	Difference	-001	001	-001	-003	000	004
P3	Baseline	006	007	006	007	006	005
	Post-Rx	006	007	006	006	007	005
	Difference	-001	000	-001	-001	000	000
Pz	Baseline	007	007	006	007	007	005
	Post-Rx	006	007	006	006	006	005
	Difference	-001	000	-000	-000	-000	-000
P4	Baseline	007	007	007	006	007	005
	Post-Rx	006	008	006	006	006	005
	Difference	-000	000	-001	-000	-000	000
T6	Baseline	009	009	007	007	007	007
	Post-Rx	007	011	007	007	007	007
	Difference	-002	001	-000	000	000	001
O1	Baseline	011	010	016	015	011	014
	Post-Rx	009	012	013	010	011	010
	Difference	-002	002	-002	-005	-000	-003
O2	Baseline	011	012	015	012	013	012
	Post-Rx	012	014	014	015	012	013
	Difference	001	002	-001	003	-001	001

NOTE: Values in the table are Absolute EEG Power uV^2 x 10^4 for ease of presentation.