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Cognitive effects of anodal transcranial direct current stimulation and high-dose cocoa flavonoids on an acutely sleep-deprived, highly educated population: a proposed single-center, phase II, randomized, placebo-controlled, double-blind, factorial study - The Cocoaelectric Trial

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Abstract:

Background: *Highly educated students and professionals in cognitively demanding careers are often at risk of acute sleep deprivation. In the past few decades, the trend toward increasing psychostimulant abuse has elicited the need for safer alternatives to cognitive enhancement. Transcranial direct cranial stimulation (tDCS) and high dose cocoa flavonoids (HDCF) have been recently studied as promising alternatives. However, these studies had methodological differences, sometimes conflicting results, and none to date have assessed their combined effects.*

Objective: *To determine if anodal tDCS and HDCF will improve working memory (WM) scores in acutely sleepdeprived highly educated healthy participants. Methods: We propose a single-center, randomized, placebo-controlled (double-dummy), double-blind, early phase II study, in which 164 acutely sleep-deprived 21-40 year-old students or professionals in cognitively demanding fields will be randomized in a 2x2 factorial fashion to one of the following groups: anodal tDCS + HDCF, anodal tDCS + placebo CF, sham tDCS + HDCF, and sham tDCS + placebo CF. The primary outcome is a composite score of nback and dual n-back tests following the intervention. Secondary outcomes include exploratory subgroup analyses for gender, age and cognitive score adjusted for time and task, psychomotor vigilance task, mental fatigue visual analogue scale, quantitative electroencephalogram, and tDCS adverse events questionnaire.*

Potential impact of the study: *This study will allow us to assess the effects of each intervention alone on WM as well as (for the first time) identify any potential synergistic effects resulting from the combined interventions. This, in turn, may generate hypotheses for future studies on cognitive impairment due to both acute/chronic sleep deprivation and pathologic disorders.*

Key-words: *transcranial direct current stimulation, flavonoids, sleep deprivation, working memory, cognition, randomized controlled trials.*

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INTRODUCTION

Students and professionals in cognitively demanding careers often experience acute sleep deprivation, a common cause of cognitive impairment among healthy populations and those with cognitive disorders. The high demand for cognitive enhancement measures often leads to non-therapeutic and potentially harmful habits, such as

worldwide stimulant use or abuse among college students, medical students, and MDs, who are more likely to prescribe stimulants inappropriately for their patients (Amelia M. Arria, Ph.D., Kimberly M. Caldeira, M.S., Kevin E. O'Grady, Ph.D., Kathryn B. Vincent, M.A., Erin P. Johnson, B.A., and Eric D. Wish, 2008; Emanuel et al., 2013; Fond et al., 2016).

Safer, healthier cognitive enhancement alternatives, recently studied, include neuromodulation techniques and certain nutrients and food constituents. Neuromodulation by transcranial direct current stimulation (tDCS) is a safe procedure when safety guidelines are followed (Poreisz, Boros, Antal, & Paulus, 2007), and has positive effects on multiple cognitive subdomains in healthy and neuropsychiatric populations (Hill, Fitzgerald, & Hoy, 2016; Kalu, Sexton, Loo, & Ebmeier, 2012; Summers, Kang, & Cauraugh, 2016). Cocoa bean is a rich source of flavonoids, particularly the flavonol subclass, which epidemiologic studies suggest may be associated with improved cognitive abilities, less cognitive impairment, improved dosedependent cognitive functions with normal aging, and decreased risk of cognitive decline and dementia (Commenges et al., 2000; Crichton, Elias, & Alkerwi, 2016; Letenneur, Proust-Lima, Le Gouge, Dartigues, & Barberger-Gateau, 2007; Moreira, Diógenes, de Mendonça, Lunet, & Barros, 2016; Neshatdoust et al., 2016).

Neuromodulation techniques such as tDCS follow the basic principles of Hebbian neuroplasticity: "neurons that fire together, wire together". In tDCS, a small amount of the electric current alters transmembrane potentials in neurons, polarizing brain tissue without inducing action potentials. Typically, anodal stimulation increases underlying brain tissue excitability, whereas cathodal stimulation has the opposite effect. Stimulation effects are maximal in the area under the electrodes, but they also extend to distant neural networks (Boros, Poreisz, Münchau, Paulus, & Nitsche, 2008). Anodal left dorsolateral prefrontal cortex (LDLPFC) tDCS has been shown to improve cognition as well as cerebral blood flow (CBF) and perfusion (Stagg et al., 2013), potentially countering the negative effects of sleep deprivation on these parameters. tDCS appears to work better in combination with other pharmaceutical or behavioral interventions. Co-administered drugs can lead to the prolongation, blockage or even reversal of tDCS aftereffects (Nitsche et al., 2003).

MATERIALS AND METHODS

We propose a randomized, double-blind, placebo-controlled (double-dummy), exploratory early phase II trial. Study participants will be randomized in a 2x2 factorial design to one of each of the following groups: anodal tDCS + HDCF; anodal tDCS + placebo cocoa flavonoids (CF); sham tDCS + HDCF; sham tDCS + placebo CF. This single-center study will take place in the Spaulding Neuromodulation Center, Boston, Massachusetts.

Eligibility Criteria

Inclusion criteria:

- Healthy males and females;
- 21 to 40 years old;
- Highly educated students or professionals in demanding fields (e.g. science, technology, engineering, mathematics, law, etc.);
- History of normal sleep patterns.

Exclusion criteria:

- Insomnia, sleep apnea or chronic sleep deprivation;
- Smokers;
- History of drug or alcohol abuse over previous year (including stimulants);
- History of drinking more than 4 cups of coffee (or their equivalent) a week over the previous 2 weeks;
- Neuropsychiatric disorders (e.g. depression, dementia);
- Contraindications to tDCS (e.g. intracranial implants, mass brain lesions, unstable medical conditions, risk of seizures) or to CF intake (e.g. intolerance to CF, risk of aspiration);
- Medications, especially drugs affecting the interventions or measurements (e.g. carbamazepine, neurostimulants);
- Pregnancy or lactation;
- Refusal to consent to participate in the study.

Recruitment and Adherence

We will use a broad-based strategy, advertising online, in print media, and by placing flyers at various locations. Subjects will be asked to keep a sleep and diet diary and an activity tracker during the whole study period. They will be required to sign documents attesting adherence to the study protocol, especially to being completely sleepdeprived for 24 hours before the intervention and free of caffeine, alcohol, drugs and stimulants for 48 hours prior to both study visits.

Randomization procedure

Immediately before receiving the intervention in their second visit, subjects will be randomly assigned by a computer-generated random sequence to one of the four groups in a 1:1:1:1 allocation ratio. We will use random blocks of 4 and 6 subjects. An independent investigator will generate the randomization sequence and place each allocation into sequentially numbered, opaque, sealed

envelopes. Subjects will be randomized in the order of receiving the intervention, i.e. the first one about to receive the intervention will be the first one randomized, etc. Subjects and investigators providing any of the other study procedures will not be informed about group allocation.

Blinding

All subjects and assessors will be blinded. Only investigators providing the intervention will be unblinded. The setup and electrode placement will be identical for anodal and sham tDCS. However, the sham groups will receive tDCS for only 30 seconds at the beginning and at the end of the session, when the current is ramped up and down. Thus, sham tDCS subjects will experience cutaneous sensations similar to those of the active groups, but they will not receive any current for the rest of the stimulation period. This method of blinding is reliable, and less than 3 minutes of tDCS does not lead to lasting effects on cortical excitability (Gandiga, Hummel, & Cohen, 2006; Nitsche & Paulus, 2000). Cocoa drinks made by an external manufacturer will be decaffeinated and matched for taste, appearance, calories, macronutrients, micronutrients, and theobromine content. The HDCF sachets will contain 903 mg of CF, compared to 0 mg in the placebo sachets, as described previously (Scholey et al., 2010; Socci, Tempesta, Desideri, De Gennaro, & Ferrara, 2017). Emergency unblinding will only be indicated if knowing the treatment allocation is required to clinically manage the patient, such as in case of a serious adverse event.

Study timeline

Visit 1 - V1: Screening of subjects, signing the informed consent form, baseline well-slept state measurements, one hour break, repeat measurements.

Visit 2 – V2 (within 7-14 days, preceded by acute overnight sleep-deprivation): Sleep-deprived measurements (same measurements as at baseline), followed by randomization, intervention, and repeat measurements (Figure 1).

Interventions

Prior to consent, all subjects will complete a prescreen questionnaire confirming eligibility and adherence to the checklist of sleep and dietary requirements. At the end of V1, they will receive a sleep and dietary adherence checklist and diary as well as an activity tracker. Upon

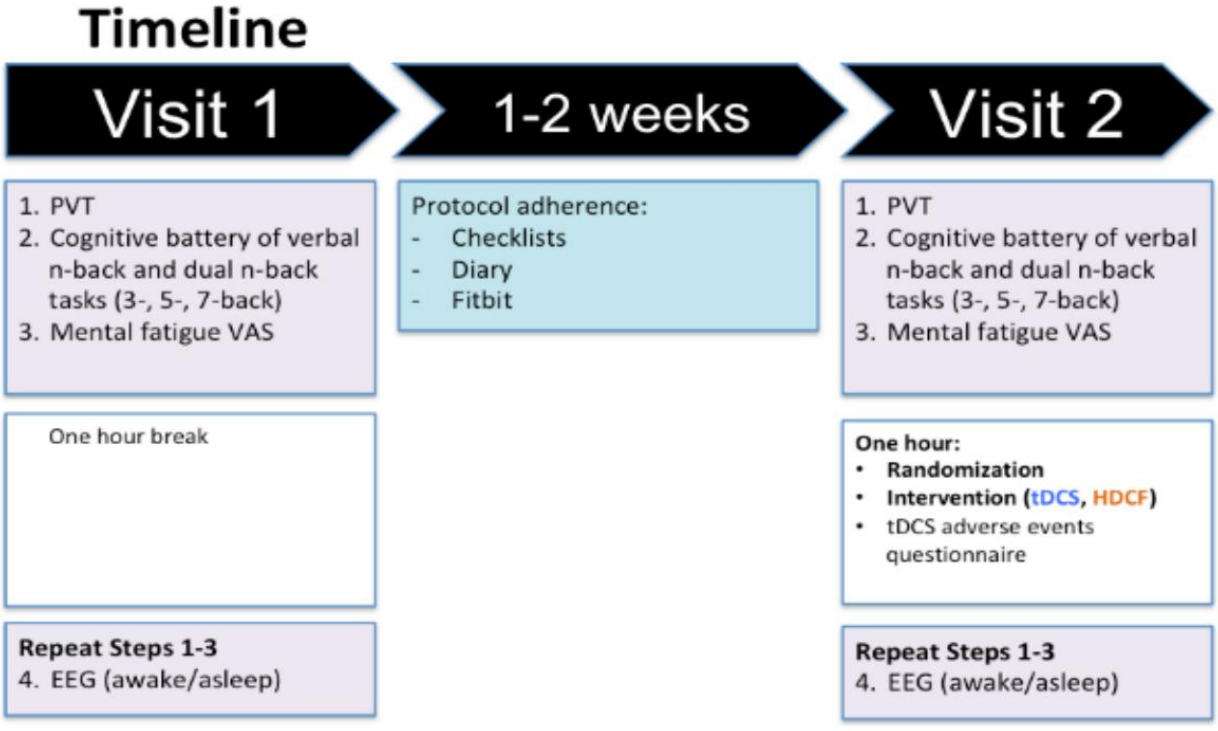

Fig. 1. Study timeline: On Visit 1 the well-slept subjects go through steps 1-3, followed by a one-hour break; they then repeat steps 1-3, followed by a 20-minute EEG in the awake and sleep states. They then receive the protocol adherence checklists, diary and fitbit for use in the 1-2 weeks between Visits 1 and 2. On Visit 2 the acutely sleep-deprived (for 24 hours) subjects go through steps 1-3, after which they are randomized into one of the 4 groups: anodal tDCS + HDCF, anodal tDCS + placebo CF, sham tDCS + HDCF, or sham tDCS + placebo CF; the double-dummy intervention will be followed by the tDCS adverse events questionnaire; subjects then repeat steps 1-3, followed by a 20-minute EEG in the awake and sleep states.

arrival for V2 7-14 days later, all subjects will be asked to submit the diary and activity tracker to ensure eligibility (subjects must have slept an average of 7-8 hours/night for at least 4 weeks before V1 and V2, but must have had 24 hours' sleep deprivation the night before V2, implied by using the activity tracker and by sending investigators an email/voicemail message over each of the following 3 time periods overnight: 12-2 am, 2-4 am, 4-6 am). All computerized tests will be administered using a specific updated Windows software package, as mentioned previously by Scholey et al. (Scholey et al., 2010).

The visits will proceed as shown in Figure 1 and detailed below:

Visit 1 (V1; first baseline; well-slept):

(1) Psychomotor vigilance test (PVT), as described by Basner et al. (Basner et al., 2011a; Basner et al., 2011b).

(2) Cognitive battery of "n-back tasks" and "dual nback tasks", at three task-load levels each (3-back, 5- back, and 7-back) using "Brain Workshop" (an opensource software, access through: http://brainworkshop. sourceforge.net/). We selected these tasks (testing both reaction time and accuracy scores) to assess WM based on the areas we aim to modulate by tDCS and HDCF. They will provide a high WM load to show the differences between the intervention groups within an expected performance period of 45-60 minutes (Kane et a., 2007; Owen et al. ,2005; Schmiedek et al., 2014).

(3) Mental fatigue visual analogue scale (VAS): Subjects will mark their current "mental fatigue" state on a 100 mm VAS (Scholey et al., 2010). Subjects will then take a one-hour break, after which they will repeat steps 1-3 (PVT, cognitive battery, mental fatigue VAS), followed by:

(4) Electroencephalogram (EEG) (awake and asleep): an Electrical Geodesics, Inc. device will be used to perform the EEG (access through: https://www.egi.com/); subjects will be asked to sit on a chair for 10 minutes, then asked to lay down on a bed, covered in a sheet, and try to sleep for 10 minutes with the lights off. Wakefulness and sleep will be identified by visual EEG inspection prior to analysis.

Visit 2 (V2; 24-hour sleep-deprived state; after subject shows compliance to the protocol adherence diary):

(1) PVT;

(2) Cognitive battery (identical to that in V1);

(3) Mental fatigue VAS (steps 1-3 form a second baseline in the sleep-deprived state), followed by:

(4) Randomization;

(5) Intervention per allocated group (allocated drink and stimulation) over one hour;

(6) tDCS Adverse Events (AEs) questionnaire (Brunoni et al., 2011);

(7) PVT;

(8) Cognitive battery (identical to that in V1);

(9) Mental fatigue VAS;

(10) EEG as described above.

An unblinded and otherwise uninvolved investigator with access to the randomization codes will be the only one to provide the drink, perform the tDCS session, and take the AEs questionnaire. Once the assessor informs this unblinded investigator that the subject is ready to be randomized, he or she will obtain the randomization sequence, open a sachet with the correct code for HDCF or placebo CF, and mix the entirety of its contents with 200ml of hot water. All subjects will then drink their allocated HDCF or placebo CF drink over 5 minutes, starting about 30 minutes prior to the tDCS session. The tDCS device by Soterix Medical Inc. will be used (access through: https://soterixmedical.com/). The anode will be placed over the F3 position according to the International 10-20 EEG System (American Clinical Neurophysiology Society, 2006), corresponding approximately to the LDLPFC, and the cathode will be placed over the right supraorbital region. We will use standard sponge electrodes of 5x5 cm each, for a surface area of 25 cm2, allowing for more focal stimulation and a higher current density (0.8 A/m2) in the active tDCS groups, compared to the often-used surface area of 35 cm2 (0.6 A/m2) (Dedoncker et al., 2016). Subjects in the active anodal tDCS condition will receive 2 mA of current for 20 minutes, whereas those receiving the sham procedure will have the current ramped up and down at the beginning and the end of the session, but will receive no current in between (see Blinding section). Following the 20- minute tDCS session, 10 minutes will be allowed for participants to answer the AEs questionnaire and to have their electrodes removed. All investigators will be properly trained and certified as needed, including tDCS certification for investigators providing stimulation.

Primary and Secondary Outcomes

As a primary outcome we will use a combined score of n-back and dual n-back tasks (including accuracy and mean reaction times) as measures of WM after the intervention (Owen et al., 2005). We will use an auditory stimulus with 3-, 5- and 7-back attempts for the n-back tasks and an auditoryspatial stimulus with 3-, 5- and 7-back attempts for the dual tasks. The secondary outcomes will be: 1) Exploratory subgroup analyses for gender, age (21-30 years vs. 31-40 years), and cognitive score adjusted for time and task, including scores for well-slept state in V1 and sleep-deprived state in V2 (pre- and postintervention) so as to contrast the effects during each time point and task to assess whether they are more likely to deteriorate over time or with task complexity; 2) PVT;

3) Mental fatigue VAS (on a scale from 0- 100); 4) Quantitative EEG (QEEG; alpha, beta, theta, delta, and gamma power and frequency ranges, as well as sleep spindles will be used for exploratory purposes to assess potential changes relevant to cognitive performance); 5) tDCS AEs questionnaire (Brunoni et al., 2011).

Study Modification/Discontinuation

tDCS is a safe procedure provided safety guidelines are followed, as mentioned previously. Typical AEs include tingling, itching, burning sensation, local erythema, headache, and general discomfort, but they are usually mild, well-tolerated and last no more than a few minutes after stimulation (Brunoni et al., 2011). More severe AEs, such as burnlike lesions and contact dermatitis, are sometimes observed (Matsumoto and Ugawa, 2017). The dose used in the HDCF group (903 mg) may lead to mild acute side-effects, such as headache, nausea, paresthesias, and may exacerbate pre-existing stressors, such as anxiety, insomnia, or depression for a short period of time (usually 24-48 hours) after intake (Skibola and Smith, 2000). In case of any described or observed intolerance to tDCS and/or to HDCF, the study will be interrupted, and medical support within the research facility will be provided. Emergency unblinding will be applied when necessary for patient management. All AEs will be reported to the Principal Investigator (PI) and the Institutional Review Board (IRB). However, we do not anticipate any severe AEs in this study.

Data Management

We will safeguard the privacy and confidentiality of all information collected during this clinical trial by storing it on paper or in electronic format in accordance with applicable state and federal laws and regulations. Subjects will be given the contact information of the PI and assured that their health care will not be affected if they file a complaint

Data Monitoring and Interim Analysis

Neither an Independent Data Monitoring Committee (IDMC) nor an interim analysis will be necessary, given the short-term, single-center, and lowrisk nature of this trial.

Sample Size Calculation

We based the sample size calculation for this study on a tDCS meta-analysis showing a pooled estimated effect size of 0.65 (Summers et al., 2016). Most of the available studies used a lower current density and an older population (Summers et al., 2016), so we anticipate an effect size at least as large as, or even larger than, this estimate in our study; this may be particularly true for the combination of tDCS with HDCF (there are no reported effect sizes for HDCF) if the potential synergistic effects are confirmed. Considering an alpha level of 0.05 and a statistical power of 80%, we estimate a sample of 39 subjects per group. Adjusting for a 5% dropout rate, the total sample size will be 164 subjects (i.e., 41 per group).

Statistical Analysis Plan

STATA version 14.2 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC) will be used to compile and analyze all the data. The primary outcome analysis will be linear regression of tDCS, HDCF and the interaction term (tDCS x HDCF) on the post-intervention cognitive scores. If the data are not normally distributed, we will log-transform them. For the secondary outcomes, a subgroup analysis will be performed for gender, age (21-30 years vs. 31-40 years) and cognitive scores in V1 and in V2 (pre- and post-intervention), adjusting for task and time point. Pre-post PVT and VAS score differences will be analyzed using two-way ANOVA; if a nonparametric analysis is required, Kruskal-Wallis, followed by Student-Newman-Keuls tests will be used. The QEEG exploratory findings will be descriptive, and the AEs questionnaire results will be analyzed using the Chi-square test. Results will be considered significant if $p < 0.05$. The primary analysis will be an intentionto-treat (ITT) analysis, and will include all randomized subjects. We will perform an additional exploratory per-protocol (PP) analysis and compare it to the ITT group. Secondary analyses will also comprise ITT and PP analyses.

Missing Data

A low dropout rate is expected, as this highly educated study population is likely to be adherent to simple interventions performed at only two visits. We do not anticipate dropouts after randomization unless subjects are unable or refuse to complete any of the study tasks. In the event of data missing-at-random (MAR), we will use the multiple imputation method for missing observations

DISCUSSION

Acute sleep deprivation is a prevalent cause of cognitive impairment in educated populations with a high cognitive demand. Sleep is important for neuronal plasticity, which is induced by thalamocortical activity during both sleep and waking states. A meta-analysis on functional magnetic resonance imaging (fMRI) blood-oxygen-level dependent (BOLD) signals showed that

acute sleep deprivation led to decreased metabolic activity and CBF with dysfunction in the fronto-parietal attention network (Ma, Dinges, Basner, & Rao, 2015). They suggested the brain might try to compensate for an increased cognitive load by activating other regions (such as the PFC and salience network) and/or by increasing CBF in the basal forebrain and anterior cingulate, as well as by maintaining CBF frontoparietally. Our protocol of anodal LDLPFC stimulation (with right supraorbital cathode) and HDCF is designed to improve the neuroplasticity and CBF in these regions in an effort to improve WM.

A number of systematic reviews and metaanalyses collectively showed the following for healthy subjects (Dedoncker et al., 2016a; Dedoncker, Brunoni, Baeken, & Vanderhasselt, 2016b; Elmasry, Loo, & Martin, 2015; Hill et al., 2016; Horvath, Forte, & Carter, 2015; Krause, Márquez-Ruiz, & Cohen Kadosh, 2013; Summers et al., 2016): single-session anodal DLPFC tDCS significantly improved cognitive function (including response times and accuracy); healthy subjects responded faster but without increased accuracy; increasing the charge and/or current density may increase accuracy, particularly in females; and there is a small but significant improvement in reaction time, as well as a trend toward improvement of WM accuracy in offline tasks (after stimulation) but not in online tasks (during stimulation).

A recent paper reviewed 6 studies on cognitive effects of acute CF administration, and all but one showed some improvement in cognitive tasks and/or biomarkers (Socci et al., 2017). Overall it appears that CF doses of 520 to 994 mg might help cognition at certain timepoints, with higher doses being effective in more studies. As with tDCS, young subjects do better than older ones, and females may be more responsive. A high cognitive load might help uncover differences between groups. WM seems more likely to improve while other subdomains of executive function have mixed findings.

As to potential long-term risks, an evidence-based update on tDCS safety showed no serious adverse effects or irreversible injuries in 33,200 sessions, including 1000 subjects with multiple sessions (Bikson et al., 2016). Meanwhile, an invited review concluded that chronic overconsumption of cocoa may lead to weight gain, but that moderate consumption benefits probably exceed the risks (Katz, Doughty, & Ali, 2011).

Thus, tDCS and HDCF may be safe and efficacious options to improve cognition in populations at risk of sleep deprivation and stimulant abuse. This early-phase II, placebo-control RCT will help us better characterize the effects of anodal tDCS and HDCF on WM, and assess whether these interventions have synergistic effects in healthy subjects.

Our study has 3 main potential limitations: 1) it may be underpowered for detecting the interaction effect between the active tDCS and the HDCF groups; 2) dietary and sleep adherence are based primarily on self-report, and any significant lack of adherence among the study participants might produce noise and bias the results (however, we expect adherence to be high, as mentioned above, and the activity tracker may help corroborate sleep activity, albeit imprecisely); 3) the target population might typically drink more than 4 cups of coffee a week (however, this criterion is necessary to reduce confounding; also, the target population has a high prevalence in Massachussetts, and subjects can be instructed to reduce their intake for 2 weeks before enrollment, so this should not be a major limitation to recruitment).

Strengths of the study include its short length and the fact that the population is fairly homogenous and easy-to-recruit, thus limiting baseline confounders and feasibility concerns. Moreover, the findings from our study will elicit hypothesis-generating data on how this population performs on different tasks at different timepoints in both the well-slept and sleep-deprived states, and following single or combined interventions; they will also help clarify the neurophysiologic effects of each intervention. Our exploratory analyses in this factorial trial might uncover cancellation effects caused by pathways activated by both interventions, but not by each intervention alone. This may help inform future cognitive study designs for healthy populations as well as those with cognitive disorders.

CONCLUSION

In view of the encouraging results observed with each of tDCS and HDCF on cognitive enhancement in previous studies, this phase II, randomized, placebo-controlled, factorial trial on healthy highly educated students and professionals may show the effects of these two safe (and potentially synergistic) interventions on cognition at different sleep states and cognitive loads. This may help advance the search for safe alternatives to cognitive improvement in highly demanding professions.

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Conflict of interest and financial disclosure

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REFERENCES

Amelia M. Arria, Ph.D., Kimberly M. Caldeira, M.S., Kevin E. O'Grady, Ph.D., Kathryn B. Vincent, M.A., Erin P. Johnson, B.A., and Eric D. Wish, P. D. (2008). Nonmedical Use of Prescription Stimulants among College Students: Associations with ADHD and Polydrug Use. Pharmacotherapy, 28(2), 156–169. http://doi.org/10.1592/phco.28.2.156. Nonmedical

American Clinical Neurophysiology Society. (2006). Guideline 5: Guidelines for Standard Electrode Position Nomenclature. Clinical Neurophysiology,6,1–3. http://doi.org/10.1080/1086508X.2006.11079580

Basner, M., & Dinges, D. F. (2011). Maximizing sensitivity of the psychomotor vigilance test (PVT) to sleep loss. Sleep, 34(5), 581–91. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21532951

Basner, M., Mollicone, D., & Dinges, D. F. (2011). Validity and Sensitivity of a Brief Psychomotor Vigilance Test (PVTB) to Total and Partial Sleep Deprivation. Acta Astronautica, 69(11–12), 949–959. http://doi.org/10.1016/j.actaastro.2011.07.015

Bikson, M., Grossman, P., Thomas, C., Zannou, A. L., Jiang, J., Adnan, T., … Woods, A. J. (2016). Safety of Transcranial Direct Current Stimulation: Evidence Based Update 2016. Brain Stimulation, 9(5), 641–61. http://doi.org/10.1016/j.brs.2016.06.004

Boros, K., Poreisz, C., Münchau, A., Paulus, W., & Nitsche, M. A. (2008). Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. The European Journal of Neuroscience, 27(5), 1292–300. http://doi.org/10.1111/j.1460- 9568.2008.06090.x

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., & Fregni, F. (2011a). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. International Journal of Neuropsychopharmacology, 14(8), 1133– 1145. http://doi.org/10.1017/S1461145710001690

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., & Fregni, F. (2011b). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP), 14(8), 1133–45. http://doi.org/10.1017/S1461145710001690

Commenges, D., Scotet, V., Renaud, S., Jacqmin-Gadda, H., Barberger-Gateau, P., & Dartigues, J. F. (2000). Intake of flavonoids and risk of dementia. European Journal of Epidemiology, 16(4), 357–63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10959944

Crichton, G. E., Elias, M. F., & Alkerwi, A. (2016). Chocolate intake is associated with better cognitive function: The Maine-Syracuse Longitudinal Study. Appetite, 100, 126– 32. http://doi.org/10.1016/j.appet.2016.02.010

Datla, K. P., Christidou, M., Widmer, W. W., Rooprai, H. K., & Dexter, D. T. (2001). Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a rat model of Parkinson's disease. Neuroreport, 12(17), 3871–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11726811

Dedoncker, J., Brunoni, A. R., Baeken, C., & Vanderhasselt, M.- A. (2016a). A Systematic Review and Meta-Analysis of the Effects of Transcranial Direct Current Stimulation (tDCS) Over the Dorsolateral Prefrontal Cortex in Healthy and Neuropsychiatric Samples: Influence of Stimulation Parameters. Brain Stimulation, 9(4), 501– 517. http://doi.org/10.1016/j.brs.2016.04.006

Dedoncker, J., Brunoni, A. R., Baeken, C., & Vanderhasselt, M.- A. (2016b). The effect of the interval-between-sessions on prefrontal transcranial direct current stimulation (tDCS) on cognitive outcomes: a systematic review and metaanalysis. Journal of Neural Transmission, 123(10), 1159– 1172. http://doi.org/10.1007/s00702-016-1558-x

Elmasry, J., Loo, C., & Martin, D. (2015). A systematic review of transcranial electrical stimulation combined with cognitive training. Restorative Neurology and Neuroscience, 33(3), 263–278. http://doi.org/10.3233/RNN-140473

Emanuel, R. M., Frellsen, S. L., Kashima, K. J., Sanguino, S. M., Sierles, F. S., & Lazarus, C. J. (2013). Cognitive enhancement drug use among future physicians: Findings from a multi-institutional census of medical students. Journal of General Internal Medicine, 28(8), 1028–1034. http://doi.org/10.1007/s11606-012-2249-4

Fond, G., Gavaret, M., Vidal, C., Brunel, L., Riveline, J.-P., Micoulaud-Franchi, J.- A., & Domenech, P. (2016). (Mis)use of Prescribed Stimulants in the Medical Student Community: Motives and Behaviors: A PopulationBased Cross-Sectional Study. Medicine, 95(16), e3366. http://doi.org/10.1097/MD.0000000000003366

Gandiga, P. C., Hummel, F. C., & Cohen, L. G. (2006). Transcranial DC stimulation (tDCS): a tool for doubleblind sham-controlled clinical studies in brain stimulation. Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology, 117(4), 845–50. http://doi.org/10.1016/j.clinph.2005.12.003

Hill, A. T., Fitzgerald, P. B., & Hoy, K. E. (2016). Effects of Anodal Transcranial Direct Current Stimulation on Working Memory: A Systematic Review and MetaAnalysis of Findings From Healthy and Neuropsychiatric

Populations. Brain Stimulation, 9(2), 197–208. http://doi.org/10.1016/j.brs.2015.10.006

Horvath, J. C., Forte, J. D., & Carter, O. (2015). Quantitative Review Finds No Evidence of Cognitive Effects in Healthy Populations From Single-session Transcranial Direct Current Stimulation (tDCS). Brain Stimulation, 8(3), 535–50. http://doi.org/10.1016/j.brs.2015.01.400

Jaeggi, S. M., Buschkuehl, M., Jonides, J., & Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. Proceedings of the National Academy of Sciences of the United States of America, 105(19), 6829– 33. http://doi.org/10.1073/pnas.0801268105

Kalu, U. G., Sexton, C. E., Loo, C. K., & Ebmeier, K. P. (2012). Transcranial direct current stimulation in the treatment of major depression: a meta-analysis. Psychological Medicine, 42(9), 1791–800. http://doi.org/10.1017/S0033291711003059

Kane, M. J., Conway, A. R. A., Miura, T. K., & Colflesh, G. J. H. (2007). Working memory, attention control, and the Nback task: a question of construct validity. Journal of Experimental Psychology. Learning, Memory, and Cognition, 33(3), 615–22. http://doi.org/10.1037/0278- 7393.33.3.615

Katz, D. L., Doughty, K., & Ali, A. (2011). Cocoa and Chocolate in Human Health and Disease. Antioxidants & Redox Signaling, 15(10), 2779–2811. http://doi.org/10.1089/ars.2010.3697

Kirchner,W. K. (1958). Age differences in short-term retention of rapidly changing information. Journal of Experimental Psychology, 55(4), 352–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13539317

Krause, B., Márquez-Ruiz, J., & Cohen Kadosh, R. (2013). The effect of transcranial direct current stimulation: a role for cortical excitation/inhibition balance? Frontiers in Human Neuroscience, 7, 602. http://doi.org/10.3389/fnhum.2013.00602

Letenneur, L., Proust-Lima, C., Le Gouge, A., Dartigues, J. F., & Barberger-Gateau, P. (2007). Flavonoid intake and cognitive decline over a 10-year period. American Journal of Epidemiology, 165(12), 1364–71. http://doi.org/10.1093/aje/kwm036

Ma, N., Dinges, D. F., Basner, M., & Rao, H. (2015). How Acute Total Sleep Loss Affects the Attending Brain: A MetaAnalysis of Neuroimaging Studies. Sleep, 38(2), 233–240. http://doi.org/10.5665/sleep.4404

Matsumoto, H., & Ugawa, Y. (2017). Adverse events of tDCS and tACS: A review. Clinical Neurophysiology Practice, 2, 19–25. http://doi.org/10.1016/J.CNP.2016.12.003

Milbury, P. E., & Kalt, W. (2010). Xenobiotic metabolism and berry flavonoid transport across the blood-brain barrier. Journal of Agricultural and Food Chemistry, 58(7), 3950– 6. http://doi.org/10.1021/jf903529m

Moreira, A., Diógenes, M. J., de Mendonça, A., Lunet, N., & Barros, H. (2016). Chocolate Consumption is Associated with a Lower Risk of Cognitive Decline. Journal of Alzheimer's Disease: JAD, 53(1), 85–93. http://doi.org/10.3233/JAD-160142

Neshatdoust, S., Saunders, C., Castle, S. M., Vauzour, D., Williams, C., Butler, L., … Spencer, J. P. E. (2016). Highflavonoid intake induces cognitive improvements linked to changes in serum brain-derived neurotrophic factor: Two randomised, controlled trials. Nutrition and Healthy Aging, 4(1), 81–93. http://doi.org/10.3233/NHA-1615

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., … Paulus, W. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. The Journal of Physiology, 553(Pt 1), 293–301. http://doi.org/10.1113/jphysiol.2003.049916

Nitsche, M. A., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. The Journal of Physiology, 527 Pt 3, 633–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10990547

Owen, A. M., McMillan, K. M., Laird, A. R., & Bullmore, E. (2005). N-back working memory paradigm: a metaanalysis of normative functional neuroimaging studies. Human Brain Mapping, 25(1), 46–59. http://doi.org/10.1002/hbm.20131

Passamonti, S., Vrhovsek, U., Vanzo, A., & Mattivi, F. (2005). Fast access of some grape pigments to the brain. Journal of Agricultural and Food Chemistry, 53(18), 7029–34. http://doi.org/10.1021/jf050565k

Poreisz, C., Boros, K., Antal, A., & Paulus, W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. Brain Research Bulletin, 72(4–6), 208–14. http://doi.org/10.1016/j.brainresbull.2007.01.004

Schmiedek, F., Lövdén, M., & Lindenberger, U. (2014). A task is a task is a task: putting complex span, n-back, and other working memory indicators in psychometric context. Frontiers in Psychology, 5, 1475. http://doi.org/10.3389/fpsyg.2014.01475

Scholey, A. B., French, S. J., Morris, P. J., Kennedy, D. O., Milne, A. L., & Haskell, C. F. (2010). Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort. Journal of Psychopharmacology, 24(10), 1505–1514. http://doi.org/10.1177/0269881109106923

Skibola, C. F., & Smith, M. T. (2000). Potential health impacts of excessive flavonoid intake. Free Radical Biology & Medicine, 29(3–4), 375–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11035267

Socci, V., Tempesta, D., Desideri, G., De Gennaro, L., & Ferrara, M. (2017). Enhancing Human Cognition with Cocoa Flavonoids. Frontiers in Nutrition, 4. http://doi.org/10.3389/fnut.2017.00019

Stagg, C. J., Lin, R. L., Mezue, M., Segerdahl, A., Kong, Y., Xie, J., & Tracey, I. (2013). Widespread Modulation of Cerebral Perfusion Induced during and after Transcranial Direct Current Stimulation Applied to the Left Dorsolateral Prefrontal Cortex. Journal of Neuroscience, 33(28), 11425–11431. http://doi.org/10.1523/JNEUROSCI.3887-12.2013

Summers, J. J., Kang, N., & Cauraugh, J. H. (2016). Does transcranial direct current stimulation enhance cognitive and motor functions in the ageing brain? A systematic review and meta- analysis. Ageing Research Reviews, 25, 42–54. http://doi.org/10.1016/j.arr.2015.11.004