A caffeine-maltodextrin mouth rinse counters mental fatigue.

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Abstract

Introduction: Mental fatigue is a psychobiological state caused by prolonged periods of demanding cognitive activity that has negative implications on many aspects in daily life. Caffeine and carbohydrate ingestion have been shown to be able to reduce these negative effects of mental fatigue. Intake of these substances might however be less desirable in some situations (e.g. restricted caloric intake, Ramadan, …). Rinsing caffeine or glucose within the mouth has already been shown to improve exercise performance. Therefore we sought to evaluate the effect of frequent caffeine-maltodextrin (CAF-MALT) mouth rinsing on mental fatigue induced by a prolonged cognitive task.

Methods: Ten males (age:23±2y, physical activity:7.3±4.3h/week, low CAF-users) performed two trials. Participants first completed a Flanker task (3 min), then performed a 90-min mentally fatiguing task (Stroop task), followed by another Flanker task. Before the start and after each 12.5% of the Stroop task (8 blocks) subjects received a CAF-MALT mouth rinse (MR;0.3g/25ml CAF;1.6g/25ml MALT) or placebo (PLAC;25ml artificial saliva).

Results: Self-reported mental fatigue was lower in MR ($p=0.017$) compared to PLAC. Normalized accuracy (accuracy first block=100%) was higher in the last block of the Stroop in MR ($p=0.032$) compared to PLAC. P2-amplitude in the dorsolateral prefrontal cortex (DLPFC) decreased over time only in PLAC ($p=0.017$).

Conclusion: Frequent mouth rinsing during a prolonged and demanding cognitive task reduces mental fatigue compared to mouth rinsing with artificial saliva.

Keywords: Cognitive fatigue, Mouth Rinse, Electroencephalography, Cognitive performance

1. Introduction

Mental fatigue is a psychobiological state caused by prolonged periods of demanding cognitive activity (Desmond and Hancock 2001; Job and Dalziel 2001) with subjective and objective manifestations [for an extensive definition see Van Cutsem et al. (2017)]. Subjectively, increased feelings of tiredness and lack of energy are reported (Boksem and Tops 2008), as well as a decrease in alertness (van der Linden et al. 2006). Objectively, mental fatigue can also result in a decline in cognitive performance (Marcora et al. 2009) and alterations in brain activity (Brownsberger et al. 2013; Cook et al. 2007). Multiple studies have shown a negative effect of mental fatigue on many aspects of daily life (Chaudhuri and Behan 2004; McCormick et al. 2012; Van Cutsem et al. 2017). In the workplace for example, mental fatigue has been found to predict an increased risk of error of surgeons (McCormick et al. 2012), while during military operations it has been found to impair physical and cognitive ability (Weeks et al. 2010). In daily life, fatigue can ensue after only 60 min of driving and is associated with a difficulty in maintaining skilled driving behavior (Lal and Craig 2002). In addition it is also one of the most common symptoms experienced by individuals with neurological disorders (Chaudhuri and Behan 2004) and recognized as one of the most common and distressing side effects of cancer and its treatment (Bower 2014).

Therefore, an interest in how to reduce mental fatigue has emerged. Caffeine (1,3,7-trimethylxanthine) is the most commonly consumed psychoactive stimulant in the world, and is often found to enhance human vigilance and mental alertness (Lieberman 2001). Consequently the intake of caffeine, with its ability to cross the blood-brain barrier and block the adenosine receptors in the brain (Glade 2010; Lorist and Tops 2003), was the first mental fatigue countermeasure to be tested and found to be successful (Lorist and Tops 2003; McLellan et al. 2016). Furthermore carbohydrate ingestion has also been found to positively affect mental fatigue (Childs 2014). There is even some evidence that, when administered together, interactions between glucose and caffeine counteract mental fatigue even more successful (Childs 2014). A combination of caffeine and carbohydrates (e.g. glucose) is the main ingredient of the energy drinks that have become so popular in recent years to enhance alertness and both physical and cognitive performance (Childs 2014; Wolk et al. 2012).

Caffeine, as well as carbohydrate intake, might however come with some unwanted side-effects. Caffeine-intake for example, can cause tremors, nausea, nervousness, increased levels of anxiety or gastrointestinal distress (McLellan et al. 2016). In addition it may also adversely affect sleep patterns (McLellan et al. 2016). Ingesting carbohydrates might also be forbidden (e.g. during Ramadam) or unwanted for health reasons (e.g. diabetes and obesity), especially in the form of energy drinks (Malik et al. 2010). Therefore, an alternative to caffeine and/or carbohydrate ingestion may be useful to people who want to reduce their mental fatigue without the potential negative effects of caffeine and carbohydrate ingestion.

One of the alternatives to caffeine and/or carbohydrate ingestion is mouth rinsing. Mouth rinsing is a nutritional strategy that involves rinsing of substrates within the mouth for several seconds (5–20 s) without ingesting the solution, and thus avoids the negative side-effects of intake of the substance. The rinsing of a solution containing carbohydrates, caffeine or both, has been shown to reduce fatigue and performance during exercise (Beaven et al. 2013; de Ataide e Silva et al. 2013; Rollo and Williams 2011). The combination of both carbohydrates and caffeine has even been observed to have an additive positive effect compared to the separate use of both substances (Beaven et al. 2013). However, to the best of our knowledge, the use of mouth rinsing with both carbohydrates and caffeine as a mental fatigue countermeasure has never been tested. Therefore, we sought to assess the effects of frequent

mouth rinsing with a caffeine-maltodextrin (CAF-MALT) solution during a prolonged and demanding cognitive task (serial mouth rinsing) on various markers of mental fatigue. We hypothesized that the subjective markers (i.e. self-reported mental fatigue), objective markers (decline in cognitive performance) and brain activity alterations typically associated with mental fatigue (i.e. a decrease in P2- and P3-amplitude and an increase in θ and α activity) would be positively counteracted by the serial CAF-MALT mouth rinsing.

2. Methods

2.1 Subjects and ethical approval

Ten active healthy male students volunteered to participate in this study (mean ± SD; age: 23 ± 2 y, physical activity hours(h)/week: 7.3 ± 4.3 h/week). They were all low caffeine users (caffeine usage/day: 101 ± 97 mg/day) and none had any known mental or somatic disorder. Each subject gave written informed consent prior to the study. Experimental protocol and procedures were approved by the Research Council of the Vrije Universiteit Brussel, Belgium.

2.2 Experimental protocol

Subjects were asked to return to the lab for 3 consecutive trials. The first trial was a familiarization trial (to get to know the routine, the equipment and to avoid learning effects), followed by two experimental trials [i.e. a mouth rinse-trial (MR) and a placebo-trial (PLAC)] , all separated by at least 6 days (mean: 8 days, SD: 3days) to ensure full recovery. All trials were conducted in thermoneutral conditions (20°C, humidity 45%) and took approximately 2 h (Fig. 1). Preceding the beginning of the familiarization trial subjects were asked about their health status, were given written instructions describing all procedures related to the study and got the opportunity to ask questions. Subjects were excluded if they presented with any medical history, family history or medication or drug use that would prevent them from safely completing the experiment.

After an overnight fast, subjects entered a sound-insulated and dim lit laboratory at the same time of day. Subjects were seated in a comfortable chair, wore earplugs, and kept the same body posture during the entire experiment. The MR- and PLAC-trial were completed in a double-blinded, randomized, crossover protocol. To determine the effect of the mouth rinse solutions on brain activity, 32 active Ag/AgCl electrodes were attached on the subjects' head preceding each trial (Acticap, Brain Products, Munich, Germany), according to the "10–20 International System" (Jasper 1958). EEG was continuously measured during all cognitive tasks (see EEG recordings for more information). All trials began with one baseline Flanker task (duration 3 min), followed by a 90-min mentally fatiguing task (in the familiarization trial this task was performed for 30 min) and ended with the same 3-min Flanker task as in the beginning (Fig. 1).

INSERT FIG.1 HERE

Flanker task In the Flanker task all cues were incongruent, meaning the flanking arrows pointed in the opposite direction as the target arrow (e.g., < < > < <), requiring a great level of inhibitory control over the flanker arrows in order to execute an accurate response. Each array of arrows was focally presented in white text for 200 ms on a black background with a variable inter-stimulus interval of 1000, 1200, 1400, or 1600 ms. One hundred and twenty trials were presented randomly with right and left target arrows occurring with equal probability. Total Flanker task duration was approximately 3 min. Participants were instructed to respond as quickly and accurately as possible to the direction of a target arrow while ignoring two flankers on each side. The second Flanker task was performed exactly 20 s after completion of the 90-min mentally fatiguing task to avoid a rest break influencing the effect of the mentally fatiguing task on the Flanker task performance. To assess performance on the Flanker task accuracy and response times (RT) were collected.

Mentally fatiguing task A modified Stroop task (Pageaux et al. 2015; Smith et al. 2016) of 90 min, partitioned in 8 blocks of 252 stimuli, was used as mentally fatiguing task. Between each block a small intersection of 20 stimuli was implemented (participants perceived no stop in the Stroop task) that, without the participants' knowing, was not accounted for in the accuracy-, RT- and EEG-analysis (see MR protocol for the purpose). The Stroop task is a task that requires inhibition and selective attention on controlled processes (MacLeod and MacDonald 2000). In this task, four coloured words ("red", "blue", "green" and "yellow") were presented one at a time on a computer screen. The participants were required to indicate the colour of the word (i.e. 'colour' stimuli), ignoring the meaning of the word itself. If however, the ink colour was red, the button to be pressed was the button linked to the real meaning of the word, not the ink colour (i.e. 'meaning' stimuli). The word presented and its ink colour were randomly selected by the computer (100% incongruent), with all incongruent word-colour combinations being equally common (meaning, in each block 63 words were presented in the colour red, yellow, green and blue). Each word was presented on screen in 34 point font for 1000 ms with a variable inter-stimulus interval of 1100, 1500 or 1900 ms, with each inter-stimulus interval being equally common. Subjects were instructed to respond as quickly and accurately as possible. Performance was assessed similarly to the Flanker task and a €50-reward for the best mean performance on the mentally fatiguing task in both conditions was offered. This should minimize the negative effects of poor motivation and disengagement on Stroop-performance.

MR solutions The MR solutions were bottled (volume 25 ml) and flavored with an amount of sodium salt of saccharin (0.03 g PLAC MR, 0.45 g CAF-MALT MR) by an independent pharmacy to blind the caffeine-taste, and stored in the dark at room temperature. PLAC MR consisted of the main ionic components of saliva, meaning distilled water containing 25 mmol KCl (0.047 g) and 2.5 mmol $NaHCO_3$ (0.005 g) (O'Doherty et al. 2000), which is tasteless and odor-free. This 'artificial saliva' solution was employed, instead of pure water, to minimize the activation of cortical taste areas which are sensitive to water in the mouth (de Araujo et al. 2003). The same solution was used for CAF-MALT MR with the addition of 1.2% w/v CAF powder (0.3 g) and 6.4% w/v MALT powder (1.6 g).

MR protocol Before the start of the Stroop and every 12.5% completion of the 90 min (Chambers et al. 2009) participants had to rinse their mouth with a given solution. A block of 20 stimuli was presented during and immediately after the mouth rinse. At this time the accuracy and RT data were collected, but not included in any analysis. This created the urge to keep on performing and avoided the subjects regaining motivation due to a rest-break The subjects had to rinse the MR solution for 10 s before expectorating it into a waste container. The MR-

solution was provided in a 30 ml cup, the researcher poured the drink in the mouth of the participant so the participant did not have to detach his hands from the keyboard to be able to rinse the solution in the mouth and expectorate it afterwards in another cup. The subjects' subjective rating of the pleasantness of the bitterness/sweetness of the taste stimuli was also assessed after each trial using a rating scale (O'Doherty et al. 2000) (+2= very (pleasant), 0= neutral, and -2= very (unpleasant)).

During the entire protocol subjects were equipped with a heart rate monitor (HR; Polar RS400, Polar Electro Oy, Kempele, Finland). Before and after the entire protocol blood glucose concentration was assessed (Bayer, Contour Next Link, Medtronic, Vienna, Austria) by collecting capillary blood at the ear lobe. Subjective psychological assessment took place before the start of the cognitive tasks with the Situational Motivation Scale (SIMS) to assess participants' motivation towards the upcoming 90-min Stroop task (Guay et al. 2000), during the cognitive tasks with a mental fatigue- and motivation-scale (0-100) and after the cognitive tasks with the National Aeronautics and Space Administration Task Load Index [NASA-TLX; (Hart and Staveland 1988)] and the Profile Of Mood States (POMS). The SIMS was filled in before the start of the first Flanker task and is a 16-item self-report inventory, which is designed to measure intrinsic motivation, identified regulation, external regulation and amotivation. Both a mental fatigue and movitation-scale (0-100) were taken before and after both Flanker tasks and within each timeframe of the 20 unrecorded stimuli during the mentally fatiguing task. These scales assessed respectively how mentally fatigued the subject was feeling (MFS; '0 = not at all' to '100 = completely exhausted') and how motivated the subject was feeling towards the next block in the Stroop task ['0 = not at all' to '100 = extremely motivated'; (Brownsberger et al. 2013)]. For the subjects to be able to keep their hands in place on the keyboard they indicated their level of mental fatigue and motivation vocally by announcing a number between 0 and 100. The NASA-TLX scale was taken after the completion of the second Flanker task and is composed of six subscales assessing subjective workload. The 32-item POMS scale was also assessed after completion of the second Flanker task and consists of five subscales; tension, depression, anger, fatigue and vigor. All items had to be scored from 0 (not at all) until 4 (extremely). The higher the score on a category, the more participants felt this mood state was present. The questionnaire was translated into the native language of the participants [Dutch; (Wald and Mellenbergh 1990)].

The subjects were given instructions to sleep for at least 7 h, refrain from the consumption of caffeine, alcohol and not to practice vigorous physical activity the day before each visit. In addition, to intra-individually standardize calorie- and macronutrient-intake, subjects were asked to have the same meal (in terms of content and quantity) the evening before each trial and not to have any food/drink intake but water after 22:00 the night before each trial. The use of any kind of medicinal products during and between the trials was prohibited. If subjects could not meet these standards they were excluded from the study. To facilitate the contact between the EEG-electrodes and the subjects' head, they were also asked to wash their hair (with neutral shampoo) the evening before the experiment.

2.3. EEG recordings and analysis

During the two Flanker tasks and the modified Stroop task, brain activity was continuously measured. Thirty two active Ag/AgCl electrodes were attached on the subjects' head (Acticap, Brain Products, Munich, Germany), according to the "10–20 International System" (Jasper 1958). The sampling rate was set at 500 Hz (Brain Vision Recorder, Brain Products, Munich, Germany). Electrode impedance was kept <10 kΩ throughout the recording. Baseline measurements were taken 2 min with eyes open, 2 min with eyes closed. During EEG recordings, subjects

were seated in a dim lit room, inserted earplugs and had been instructed to minimize movement of the head and eye blinking, to avoid frowning, to maintain the same posture and not to touch their head with their hands in order to minimize movement, sound and muscle artifacts.

Event-related potential (ERP) analysis The program Brain Vision Analyzer (version 2.1) was used to preprocess and process the data sets. Raw data were down-sampled to 256 Hz, filtered (high pass 0.1 Hz, low pass 45 Hz and Notch, Slope 48 dB/oct) with a Butterworth filter design and re-referenced to an average reference. For each data set of interest [i.e., ERP during the first, fourth and eighth block in the Stroop task] artifacts were semi-automatically removed. Then the different stimuli (Flanker task: incongruent; Stroop task: colour / meaning) were extracted from the EEG data sets. For stimulus locked ERP analysis, a data window was set at -200 to 800 ms relative to stimulus onset. Trials in which performance errors occurred were excluded. For each ERP epoch, independent component analysis (ICA) and inverse ICA further reduced artifacts. Furthermore, a baseline correction was applied (period -200 to 0 ms). Epochs were then averaged and the visually evoked potentials, P2, N2, P3b were assessed. Peak amplitudes and onset latencies were measured for the P2, N2 and P3b components in their specific region of interest (ROI; Table 1). The P2 is known to be frontally distributed (Gajewski and Falkenstein 2015) and was therefore analyzed in the orbitofrontal cortex (OFC) and dorsolateral prefrontal cortex (DLPFC). It has been related to attentive stimulus evaluation or the recall of task rules (Gajewski et al. 2008). The P2 was defined as the largest positive-going peak occurring within the time window between 80 and 260 ms and was visually confirmed. The N2 is usually interpreted as an index of conflict monitoring (Folstein and Van Petten 2008) and emerges fronto-centrally after the P2 (Gajewski and Falkenstein 2015), thus also for the N2 the OFC and DLPFC was analyzed. The N2 was defined as the largest negative-going peak occurring within the time window between 180 and 440 ms and was visually confirmed. The P3b is linked to salience processing and appears to occur when subsequent attentional resource activations promote memory operations in temporal-parietal areas (Polich 2007). Therefore the fusiform gyrus (FFG), the angular gyrus (AG) and the somatosensory association cortex (SAC) were analyzed to observe any effects on the P3b. The P3b was defined as the largest positive-going peak occurring within the time window between 180 and 415 ms and was visually confirmed. Thereafter, we exported the data from Brain Vision Analyzer to SPSS (version 22.0; SPSS, Chicago, IL) for further analysis.

INSERT Table 1 HERE

Spectral power analysis Similar to the ERP analysis, the program Brain Vision Analyzer (version 2.1) was used to preprocess and process the data sets for the analysis of the total power. Raw data were down-sampled to 256 Hz, filtered (high pass 0.1 Hz, low pass 45 Hz and Notch, Slope 48 dB/oct) with a Butterworth filter design and re-referenced to an average reference. For each data set of interest [i.e., Continuous EEG measurements from the fifth to the eighth minute in the first (5-8 min), fourth (38 min 45 sec-41 min 45 sec) and eighth (83 min 45 sec-86 min 45 sec) block in the Stroop task] artifacts were semi-automatically removed. For each continuous EEG data set of interest segments with a length of 4 s and with an overlap of 2 s were extracted (Wascher et al. 2014). Subsequently ICA and inverse ICA further reduced artifacts. The resulting data segments were tapered with a Hanning window with 10% of the total segment length. Fast Fourier transform (FFT) power spectra with a spectral

resolution of 0.25 Hz were calculated for both sides of the spectrum, resulting in FFT segments containing the full spectral information. The resulting FFT segments were averaged to stabilize the spectral content. The power in the FFT was extracted for theta (θ, 3.5–7.5 Hz), alpha (α1, 7.5–10 Hz; α2, 10–12.5 Hz) and beta (β1, 12.5–18 Hz; β2, 18–35 Hz) in each ROI.

2.4. Statistical analysis

All data are presented as means ± standard error (SE) unless stated otherwise. The Shapiro-Wilk test was used to test the normality of the data, sphericity was verified by the Mauchly's test. When the assumption of sphericity was not met, the significance of F-ratios were adjusted with the Greenhouse-Geisser procedure. Subjective data. NASA-TLX-, SIMS- and POMS-data were not normally distributed and therefore Wilcoxon signed ranked tests were used to test the effect of condition (MR vs. PLAC) in each subscale. Paired sample t-tests were employed to assess the effect of condition on the pleasantness-rating of the taste stimuli. Two-way repeated measure ANOVAs were used to test for the effect of condition and time on motivation (2 x 10) and MFS (2 x 11). Behavioral data. Stroop accuracy-data had to be normalized to a baseline performance (i.e. performance on the first block = 100%) within each condition and subject (see Results) in order to be normally distributed. The effect of condition, time (second to eighth block in the Stroop) and stimuli (colour vs. meaning) on normalized Stroop accuracy-data was tested with a three-way repeated measures ANOVA (2 x 7 x 2). The same three-way repeated measures ANOVA with an extra level in the time variable (the first block; 2 x 8 x 2) was used to analyze Stroop RT-data. A two-way repeated measure ANOVA (2 x 2) was used to assess the effect of condition and time on Flanker-accuracy and -RT. (Neuro)Physiological data. The effect of condition and time on blood glucose concentration (2 x 2) and HR (2 x 11) was tested with a two-way repeated measure ANOVA. Four-way repeated measure ANOVAs were used to assess the effect of condition, time (first to eighth block in the Stroop), stimuli (colour vs. meaning) and ROI (see Table 1) on multiple ERP (P2-, N2- and P3b-amplitude and -latency). Square root transformed normalized (spectral power during baseline eyes open condition = 100%) spectral power (α1, α2 and θ) variables associated with mental fatigue were analyzed with a three-way repeated measures ANOVA (condition, time and ROI; 2 x 3 x 9; see Table 1). If significant interaction effects in the repeated measure ANOVAs were observed, subsequent repeated measure ANOVAs or paired sample t-tests (depending on the amount of interacting factors) were performed in order to elucidate the main effect of the interacting factors. If no significant interaction effects were observed, main effects were immediately observed and further interpreted through pairwise comparisons with Bonferroni correction. Within-subjects correlation coefficients (r) were computed for the correlations between MFS/P2-amplitude in DLPFC and Stroop accuracy on 'meaning' stimuli/P2-amplitude in DLPFC using the method described by Bland and Altman (Bland and Altman 1995). This method adjusts for repeated observations within participants by using multiple regression with "participant" treated as a categorical factor using dummy variables. Significance was set at 0.05 for all analyses. All statistical tests were conducted using the Statistical Package for the Social Sciences, version 24 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Subjective data

No significant condition x time interaction was found for self-reported mental fatigue. Self-reported mental fatigue significantly increased in time from 10.1 ± 2.5 before the first Flanker task to 42.7 ± 7.4 after the second Flanker task ($F(1.2, 9.3)=12.7$; $p=0.005$) and was, on average, significantly lower in MR (23.2 ± 4.4) compared to PLAC (30.4 ± 4.6; $F(1, 8)=9.0$; $p=0.017$; Fig. 2). The POMS however showed no difference between conditions in fatigue or any other subscale (depression, tension, anger and vigor). No difference between conditions was observed in terms of general motivation towards the Stroop (assessed with the oral 0-100 scale) or intrinsic motivation, identified regulation, external regulation and amotivation (assessed with the SIMS). General motivation (assessed with the oral 0-100 scale) did decrease in time from 86.5 ± 2.9 at the start of the protocol to 69.9 ± 5.6 at the end of the Stroop task ($F(1.6, 14.8)=9.9$; $p=0.003$). The NASA-TLX data revealed that there was no effect of condition on any subscale (mental demand, physical demand, temporal demand, performance, effort, frustration). Although efforts were made to mask the differing taste between both mouth rinse solutions, participants scored the pleasantness of the bitter/sweet taste in MR (-0.9) significantly lower than in PLAC (0.7; $p=0.008$).

INSERT FIG.2 HERE

3.2. Behavioral data

Stroop performance

A triple condition x time x stimulus-type interaction ($F(6, 54)=2.7$; $p=0.022$) for the normalized Stroop accuracy was found. A post hoc condition x time ANOVA in each stimulus-type revealed a condition x time interaction within the 'meaning' stimuli ($F(6, 54)=3.3$; $p=0.008$) and a main effect of time within the 'colour' stimuli ($F(6, 54)=2.4$; $p=0.039$; see Table 2). Concerning the 'meaning' stimuli, follow-up paired sample-t-tests within each time interval revealed that accuracy was higher in the eighth and last block of the Stroop in MR (100.4 ± 1.6%) compared to PLAC (91.0 ± 3.5%; $p=0.032$; see Table 2). Regarding Stroop RT, a time x stimulus-type interaction was observed in the three-way-ANOVA ($F(7, 63)=8.1$; $p<0.001$). The post-hoc condition x time ANOVA in each stimulus-type revealed that for the 'meaning' stimuli participants reacted faster in time ($F(1.9, 17.1)=4.9$; $p=0.023$; see Table 2) independent of condition. For the 'colour' stimuli no interaction or main effects were found.

INSERT Table 2 HERE

Flanker performance

No interaction effect or time effect was observed for accuracy, participants performed worse in PLAC (0.89 ± 0.02) than in MR (0.91 ± 0.01; F(1, 9)=6.6; p=0.031; see Table 3). Regarding RT no interaction effect or main effect of condition or time was observed (see Table 3).

INSERT Table 3 HERE

3.3. (Neuro)Physiological data

Heart rate and blood glucose

For HR no interaction effect or main effect of condition was observed. HR did however decrease in time in both conditions (F(4.1, 37.0)=5.0; p=0.002) from 71 ± 3 bpm before the pre-Flanker to 63 ± 3 bpm after the post-Flanker. For blood glucose no interaction effect or main effect of time was found. There was however a main effect of condition (F(1, 8)=21.6; p=0.002). Blood glucose was higher in MR (95.9 ± 3.9 mg/dl) than in PLAC (88.4 ± 3.2 mg/dl).

Event related potentials

P2. For P2-amplitude a condition x stimulus-type x ROI (F(1, 9)=5.3; p=0.046) and time x stimulus-type x ROI (F(2, 18)=4.3; p=0.03) interaction effect in the four-way ANOVA led towards the implementation of a post hoc condition x time x ROI ANOVA in each stimulus type. This revealed a condition x ROI interaction (F(1, 9)=5.8; p=0.039) for the 'meaning' stimuli and a condition x time interaction (F(2, 18)=5.0; p=0.018) for the 'colour' stimuli. Subsequently, for the 'meaning' stimuli, follow-up condition x time ANOVAs in both ROIs were performed. For the 'colour' stimuli condition x ROI and time x ROI ANOVAs were performed. Within the 'meaning' stimuli, the P2-amplitude in the OFC was larger in MR (2.6 ± 0.5 µV) than in PLAC (1.9 ± 0.4 µV; F(1, 9)=9.9; p=0.012) independently from time. In the DLPFC a condition x time interaction was present (F(2, 18)=4.2; p=0.032). Amplitude decreased in time in PLAC (F(2, 18)=5.1; p=0.017; see Fig. 3a), whilst in MR time did not have an effect (Fig. 3b). Paired sample-t-tests indicated that P2-amplitude was larger in PLAC in the first time-interval (p=0.049; see Fig. 3a & b), while in the third and last time-interval it was larger in MR (p=0.034; see Fig. 3a & b). Within the 'colour' stimuli, P2-amplitude decreased over time in PLAC (F(2, 18)=11.1; p=0.001) independently from ROI. In MR P2-amplitude did not differ over time (no time x ROI interaction or main effect of time) in the 'colour' stimuli. No interaction effects or main effect of time or condition were observed for latency. N2. For N2-amplitude no interaction effects or main effect of time or condition were observed. For N2-latency a time x stimulus-type interaction was present (F(2, 18)=6.4; p=0.008). N2-latency on the 'meaning' stimuli became longer in time (304.2 ± 12.0 ms → 330.8 ± 19.9 ms) independent of condition (F(2, 18)=4.3; p=0.029). On the 'colour' stimuli, N2-latency did not differ between conditions or in time. P3b. No interaction effects or main effect of time or condition were observed for P3b-amplitude. A time x ROI interaction was present in the four-way ANOVA for P3b-latency. Subsequent follow up condition x time x stimulus-type ANOVAs in all three ROIs

revealed that only in the FFG, P3b-latency became longer in time (261.9 ± 11.8 ms → 293.0 ± 8.5 ms; F(2, 18)=11.6; p=0.001) independent of condition.

INSERT FIG. 3a & 3b HERE

Spectral power

In all spectral power frequencies (θ, α1, α2, β1 and β2) no interaction or main effect of condition and time was observed. Only β1-power was found to decrease in time during the Stroop task (F(2, 18)=5.6; p=0.013).

3.4. Correlations

Within-subjects correlation coefficients were computed for those parameters that, similar to the markers of mental fatigue, were affected by the intervention (i.e. an increase in self-reported mental fatigue and a decrease in accuracy). No significant correlations were however observed between P2-amplitude and MFS or Stroop accuracy on the 'meaning' stimuli.

4. Discussion

This study is the first to assess the ability of serial CAF-MALT mouth rinsing to counteract mental fatigue. It was observed that a serial CAF-MALT mouth rinse-intervention counteracts subjective, behavioral and electrophysiological (i.e. decreased P2-amplitude was counteracted in MR) mental fatigue.

In both conditions, MR as well as PLAC, the 90-min Stroop task elicited mental fatigue. This was measured as an increase in self-reported mental fatigue and a decrease in accuracy. However, small but important differences were observed between both conditions. Despite that self-reported mental fatigue significantly increased in both conditions, it increased significantly less in MR. Participants commenced the protocol in both conditions with a similar degree of self-reported mental fatigue (MR: 11.2 ± 2.7; PLAC: 8.9 ± 3.2), whilst at the end this differed substantially (MR: 34.7 ± 7.8; PLAC: 50.7 ± 8.3). POMS measures however showed no difference in fatigue between conditions. It was suggested by Van Cutsem et al. (2017) that the POMS may be less capable of detecting small but relevant short-term changes in mental fatigue and this seems to be confirmed in the present study.

Behaviorally, performance on the 'meaning' stimuli (which represent the stimuli in which subjects had to respond to the meaning of the word, and not the color), also indicated a difference was present between conditions. This became specifically apparent in the eighth and last block of the Stroop task. Accuracy on the 'meaning' stimuli in this block was higher in MR compared to PLAC. This means that participants were able to keep up their cognitive performance for longer. Furthermore participants reacted faster on the 'meaning' stimuli in time, independent from the condition. This indicates participants adopted the higher risk strategy with increasing time-on-task with more success in MR than in PLAC, as participants were only able to keep up accuracy in MR condition. The lower occurrence rate (25% of all stimuli) of these 'meaning' stimuli brings along a higher mental demand, which could explain why performance on these stimuli was more sensitive to the intervention-effect. In order to be able to

observe the effect of mental fatigue on cognitive performance independently from time-on-task, a Flanker task was completed before and after the mentally fatiguing task. Performance on this task confirmed that cognitive performance was better in MR, mainly due to a higher accuracy on the Flanker task completed after the mentally fatiguing task (see Table 3). Consequently also this measure indicates that participants were more resistant to mental fatigue in MR.

Multiple functional magnetic resonance imaging- (Chambers et al. 2009; Frank et al. 2008; Haase et al. 2009) and EEG-studies (De Pauw et al. 2015) have demonstrated that rinsing a solution containing glucose and/or caffeine activates certain brain regions (e.g. anterior cingulate cortex, orbitofrontal cortex, striatum, …). An objective effect of CAF-MALT mouth rinsing on the brain was confirmed by our data. Specifically, serial CAF-MALT mouth rinsing prevented the decrease in P2-amplitude in time. The P2 is a component of an ERP that appears around 200 ms after the onset of a stimulus (Gajewski and Falkenstein 2015) and its amplitude is suggested to serve as an electrophysiological marker of the process in which cue information is associated to the functional properties of this information (i.e. the recall of task rules) (Gajewski and Falkenstein 2015; Lorist 2008). In other words, serial CAF-MALT mouth rinsing prevented a decrease in the ability to select relevant cue information. Nonetheless, the change in P2-amplitude was not significantly correlated with the other markers of mental fatigue. In contrast, the frequently reported shift of EEG power towards low-frequency bands (δ, θ, α) in mentally fatigued subjects (Aeschbach et al. 1997; Cajochen et al. 1995; Phipps-Nelson et al. 2011; Trejo et al. 2015; Wascher et al. 2014; Zhao et al. 2012) was not observed in the present study. It is possible this shift in spectral distribution was prevented in both MR and PLAC by the brain activating properties of both substances. In the study of Chambers et al. (2009) it is shown that caloric (e.g. the solution used in MR in the present study) as well as non-caloric sweetened (e.g. the solution used in PLAC in the present study) solutions activate multiple brain areas (i.e. right insula, frontal operculum, left dorsolateral prefrontal cortex). The present results suggest that a prevention of this shift in spectral distribution [i.e. a decrease in arousal (Tops and Boksem 2010)] is however not sufficient to postpone the occurrence of mental fatigue, subjectively and behaviorally. Unfortunately we did not include a control trial (i.e. without mouth rinse) to confirm whether the spectral distribution-shift occurred in the first place in the present study. Therefore we can also not further substantiate this suggestion.

A potential mechanism for the serial CAF-MALT mouth rinsing positively affecting mental fatigue might be the absorption through the oral mucosa, especially for a lipophilic agent like caffeine (Shargel and Yu 2016). Caffeine, rapidly taken up in the bloodstream through the buccal mucosa, may arrive in the brain via the systemic circulation and exert its' known effects (i.e. antagonist of adenosine). Doering et al. (2014) investigated however the effects of serial caffeine mouth rinsing on endurance cycling time-trial performance and found no significant increase in plasma caffeine concentration after repeated oral exposure. Similarly, Rollo et al. (2010) assessed whether peripheral blood glucose concentration is affected by serial carbohydrate mouth rinsing (i.e. Lucozade Sport, Brentford, England) and did not observe any increase. This is confirmed by our results, although blood glucose was higher in MR, it was not altered differently in time in both conditions. In addition, the time dynamics of the changes in other parameters like P2-amplitude and accuracy during the Stroop task cannot be explained by the higher blood glucose in MR. Therefore this difference in glucose between MR and PLAC, most probably, does not explain our results. Although both caffeine and maltodextrin were probably not taken up systemically during the serial CAF-MALT mouth rinsing, it could still be that minimal amounts of both substances were absorbed through the oral mucosa and exerted their effect in the brain without showing up in the blood. A placebo effect

(Wehrwein and Carter 2016) could also explain the observed results and could have been triggered by the participants' awareness that they were expected to perform better in one trial compared to the other. Participants were however informed in a way that they were naïve of the study's real aims and hypotheses. This also anticipated the fact that pleasantness of taste was rated lower in MR than in PLAC. A possible role of motivation in the observed results was also accounted for. A monetary incentive was provided in an attempt to prevent task disengagement and to restrict possible alterations in motivation throughout the task performance (Gergelyfi et al. 2015; Van Cutsem et al. 2017). In addition the level of motivation was monitored before the start of the task (SIMS) and during the task (oral 0-100 scale). In none of the two methods an effect of the intervention was found. A final potential mechanism by which the serial CAF-MALT mouth rinsing attenuated mental fatigue is the activation of specific brain regions (i.e. the anterior cingulate cortex and the right caudate, that forms part of the striatum) known to produce dopamine (Chambers et al. 2009; Turner et al. 2014). A mechanism that has already been suggested in the self-control literature, where a carbohydrate-mouth rinse has been shown to successfully counter on self-control impairments (Hagger and Chatzisarantis 2013). Moreover mental fatigue has already been associated multiple times with alterations in the anterior cingulate cortex (Boksem et al. 2005; Boksem and Tops 2008; Marcora et al. 2009; Mostofsky and Simmonds 2008). This way a mouth rinse containing caffeine and maltodextrin might be able to (partly) restore dopaminergic transmission in the striatum and anterior cingulate cortex and postpone mental fatigue by activating sensory neurons in the mouth and initiating a signal transduction cascade towards the brain.

Future research

In the present study caffeine and maltodextrin were combined in one mouth rinse in order to increase the chances to successfully counter the electrophysiological changes associated with mental fatigue. This assumption was based on a study conducted by Beaven et al. (2013), who found that there was an additive effect of combining caffeine and carbohydrates on power production during repeated sprints on a cycle ergometer. The additive positive effect suggested that distinct mechanisms are involved in the performance enhancement (Beaven et al. 2013). This indicates that it would be interesting to look at the separate effects of a maltodextrin mouth rinse and a caffeine mouth rinse on the occurrence of mental fatigue. Looking at the separate effects of both mouth rinses might further increase our knowledge on the electrophysiological measures laying at the base of the occurrence of mental fatigue. The present study was completed with low caffeine users in a fasted state, meaning that it is still to be confirmed whether these effects would still occur in another population at another time of day and when switching between tasks. Another important aspect that needs further investigation is whether serial mouth rinsing is also capable of postponing mental fatigue in a less demanding way. The mouth rinse intervention in the present study was based on a study of Carter et al. (2004), were participants also had to rinse their mouth every 12.5% task completion. A less demanding mouth rinse-protocol, that is still able to postpone mental fatigue, would greatly improve its applicability. Caution is however warranted as a recent study of Kumar et al. (2016) reported that a single carbohydrate mouth rinse preceding a 20-min cognitive task, was unable to prevent a decrease in cognitive performance in time.

5. Conclusion

A 90-min Stroop task induced mental fatigue in both MR and PLAC. However in the MR condition mental fatigue was induced to a lesser extent, indicated by a slower increase in self-reported mental fatigue, the ability to keep up

cognitive performance and by preventing a mental fatigue-associated electrophysiological change (i.e. decreasing P2-amplitude) occurring with increasing time-on-task. Two potential mechanisms to account for the ability of serial CAF-MALT mouth rinsing to counter mental fatigue are: 1) absorption of caffeine and maltodextrin via the brain that does not show up systemically; 2) restored dopaminergic brain transmission via sensory neurons initiating a signal transduction cascade towards the brain. In addition, for individuals that do not want to or cannot ingest caffeine and/or carbohydrates but need to reduce mental fatigue, these findings provide a possible practical solution.

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Fig. 1 Protocol timeline (min). T = ambient temperature; RH = relative humidity; POMS = profile of mood states; SIMS = situational & intrinsic motivation scale; GLUC = blood glucose; MFS = self-reported mental fatigue;  = mouth rinse;  = Electroencephalography;  = heart rate

Fig. 2 Self-reported mental fatigue throughout the protocol; before and after the first Flanker task (Pre F1, S0), after each block during the Stroop task (S1, …, S8) and after the last Flanker task (Post F2). ‡ Significant effect of time ($p<0,05$). * Significant effect of condition ($p<0,05$). Data are presented as means ± SE.

Fig. 3 a. Grand-average ERPs at Fz elicited by the 'meaning' stimuli (i.e. indicate the meaning of the word) in the first block (solid black line), fourth block (large dashed line) and eighth block (small dashed line) during the Stroop task in PLAC. ‡ Significant main effect of time ($p<0.05$). **b.** Grand-average ERPs at Fz elicited by the 'meaning' stimuli in the first block (solid black line), fourth block (large dashed line) and eighth block (small dashed line) during the Stroop task in MR.

Table 1 Regions Of Interest (ROI) defined by Brodmann Areas (BAs) and electrode sites according to the "10–20 International System". V denotes the ROI checked for that particular electroencephalography-measure.

Table 2 Stroop performance; Accuracy and RT in all eight blocks (11min15sec) of the Stroop task. Accuracy-data were normalized to the performance on the first block within each condition (=100%), data are presented as means ± SE; † indicates a trend towards a time-effect; ‡ indicates a significant time-effect; * indicates a significant difference compared to the analogous time interval in PLAC.

Table 3 Flanker performance; Accuracy and RT in pre and post Flanker task. Data are presented as means ± SE; * indicates a significant difference compared to PLAC.

A caffeine-maltodextrin mouth rinse counters mental fatigue.

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Abstract

Introduction: Mental fatigue is a psychobiological state caused by prolonged periods of demanding cognitive activity that has negative implications on many aspects in daily life. Caffeine and carbohydrate ingestion have been shown to be able to reduce these negative effects of mental fatigue. Intake of these substances might however be less desirable in some situations (e.g. restricted caloric intake, Ramadan, …). Rinsing caffeine or glucose within the mouth has already been shown to improve exercise performance. Therefore we sought to evaluate the effect of frequent caffeine-maltodextrin (CAF-MALT) mouth rinsing on mental fatigue induced by a prolonged cognitive task.

Methods: Ten males (age:23±2y, physical activity:7.3±4.3h/week, low CAF-users) performed two trials. Participants first completed a Flanker task (3 min), then performed a 90-min mentally fatiguing task (Stroop task), followed by another Flanker task. Before the start and after each 12.5% of the Stroop task (8 blocks) subjects received a CAF-MALT mouth rinse (MR;0.3g/25ml CAF;1.6g/25ml MALT) or placebo (PLAC;25ml artificial saliva).

Results: Self-reported mental fatigue was lower in MR ($p=0.017$) compared to PLAC. Normalized accuracy (accuracy first block=100%) was higher in the last block of the Stroop in MR ($p=0.032$) compared to PLAC. P2-amplitude in the dorsolateral prefrontal cortex (DLPFC) decreased over time only in PLAC ($p=0.017$).

Conclusion: Frequent mouth rinsing during a prolonged and demanding cognitive task reduces mental fatigue compared to mouth rinsing with artificial saliva.

Keywords: Cognitive fatigue, Mouth Rinse, Electroencephalography, Cognitive performance

1. Introduction

Mental fatigue is a psychobiological state caused by prolonged periods of demanding cognitive activity (Desmond and Hancock 2001; Job and Dalziel 2001) with subjective and objective manifestations [for an extensive definition see Van Cutsem et al. (2017)]. Subjectively, increased feelings of tiredness and lack of energy are reported (Boksem and Tops 2008), as well as a decrease in alertness (van der Linden et al. 2006). Objectively, mental fatigue can also result in a decline in cognitive performance (Marcora et al. 2009) and alterations in brain activity (Brownsberger et al. 2013; Cook et al. 2007). Multiple studies have shown a negative effect of mental fatigue on many aspects of daily life (Chaudhuri and Behan 2004; McCormick et al. 2012; Van Cutsem et al. 2017). In the workplace for example, mental fatigue has been found to predict an increased risk of error of surgeons (McCormick et al. 2012), while during military operations it has been found to impair physical and cognitive ability (Weeks et al. 2010). In daily life, fatigue can ensue after only 60 min of driving and is associated with a difficulty in maintaining skilled driving behavior (Lal and Craig 2002). In addition it is also one of the most common symptoms experienced by individuals with neurological disorders (Chaudhuri and Behan 2004) and recognized as one of the most common and distressing side effects of cancer and its treatment (Bower 2014).

Therefore, an interest in how to reduce mental fatigue has emerged. Caffeine (1,3,7-trimethylxanthine) is the most commonly consumed psychoactive stimulant in the world, and is often found to enhance human vigilance and mental alertness (Lieberman 2001). Consequently the intake of caffeine, with its ability to cross the blood-brain barrier and block the adenosine receptors in the brain (Glade 2010; Lorist and Tops 2003), was the first mental fatigue countermeasure to be tested and found to be successful (Lorist and Tops 2003; McLellan et al. 2016). Furthermore carbohydrate ingestion has also been found to positively affect mental fatigue (Childs 2014). There is even some evidence that, when administered together, interactions between glucose and caffeine counteract mental fatigue even more successful (Childs 2014). A combination of caffeine and carbohydrates (e.g. glucose) is the main ingredient of the energy drinks that have become so popular in recent years to enhance alertness and both physical and cognitive performance (Childs 2014; Wolk et al. 2012).

Caffeine, as well as carbohydrate intake, might however come with some unwanted side-effects. Caffeine-intake for example, can cause tremors, nausea, nervousness, increased levels of anxiety or gastrointestinal distress (McLellan et al. 2016). In addition it may also adversely affect sleep patterns (McLellan et al. 2016). Ingesting carbohydrates might also be forbidden (e.g. during Ramadam) or unwanted for health reasons (e.g. diabetes and obesity), especially in the form of energy drinks (Malik et al. 2010). Therefore, an alternative to caffeine and/or carbohydrate ingestion may be useful to people who want to reduce their mental fatigue without the potential negative effects of caffeine and carbohydrate ingestion.

One of the alternatives to caffeine and/or carbohydrate ingestion is mouth rinsing. Mouth rinsing is a nutritional strategy that involves rinsing of substrates within the mouth for several seconds (5–20 s) without ingesting the solution, and thus avoids the negative side-effects of intake of the substance. The rinsing of a solution containing carbohydrates, caffeine or both, has been shown to reduce fatigue and performance during exercise (Beaven et al. 2013; de Ataide e Silva et al. 2013; Rollo and Williams 2011). The combination of both carbohydrates and caffeine has even been observed to have an additive positive effect compared to the separate use of both substances (Beaven et al. 2013). However, to the best of our knowledge, the use of mouth rinsing with both carbohydrates and caffeine as a mental fatigue countermeasure has never been tested. Therefore, we sought to assess the effects of frequent

mouth rinsing with a caffeine-maltodextrin (CAF-MALT) solution during a prolonged and demanding cognitive task (serial mouth rinsing) on various markers of mental fatigue. We hypothesized that the subjective markers (i.e. self-reported mental fatigue), objective markers (decline in cognitive performance) and brain activity alterations typically associated with mental fatigue (i.e. a decrease in P2- and P3-amplitude and an increase in θ and α activity) would be positively counteracted by the serial CAF-MALT mouth rinsing.

2. Methods

2.1 Subjects and ethical approval

Ten active healthy male students volunteered to participate in this study (mean ± SD; age: 23 ± 2 y, physical activity hours(h)/week: 7.3 ± 4.3 h/week). They were all low caffeine users (caffeine usage/day: 101 ± 97 mg/day) and none had any known mental or somatic disorder. Each subject gave written informed consent prior to the study. Experimental protocol and procedures were approved by the Research Council of the Vrije Universiteit Brussel, Belgium.

2.2 Experimental protocol

Subjects were asked to return to the lab for 3 consecutive trials. The first trial was a familiarization trial (to get to know the routine, the equipment and to avoid learning effects), followed by two experimental trials [i.e. a mouth rinse-trial (MR) and a placebo-trial (PLAC)] , all separated by at least 6 days (mean: 8 days, SD: 3days) to ensure full recovery. All trials were conducted in thermoneutral conditions (20°C, humidity 45%) and took approximately 2 h (Fig. 1). Preceding the beginning of the familiarization trial subjects were asked about their health status, were given written instructions describing all procedures related to the study and got the opportunity to ask questions. Subjects were excluded if they presented with any medical history, family history or medication or drug use that would prevent them from safely completing the experiment.

After an overnight fast, subjects entered a sound-insulated and dim lit laboratory at the same time of day. Subjects were seated in a comfortable chair, wore earplugs, and kept the same body posture during the entire experiment. The MR- and PLAC-trial were completed in a double-blinded, randomized, crossover protocol. To determine the effect of the mouth rinse solutions on brain activity, 32 active Ag/AgCl electrodes were attached on the subjects' head preceding each trial (Acticap, Brain Products, Munich, Germany), according to the "10–20 International System" (Jasper 1958). EEG was continuously measured during all cognitive tasks (see EEG recordings for more information). All trials began with one baseline Flanker task (duration 3 min), followed by a 90-min mentally fatiguing task (in the familiarization trial this task was performed for 30 min) and ended with the same 3-min Flanker task as in the beginning (Fig. 1).

INSERT FIG.1 HERE

Flanker task In the Flanker task all cues were incongruent, meaning the flanking arrows pointed in the opposite direction as the target arrow (e.g., < < > < <), requiring a great level of inhibitory control over the flanker arrows in order to execute an accurate response. Each array of arrows was focally presented in white text for 200 ms on a black background with a variable inter-stimulus interval of 1000, 1200, 1400, or 1600 ms. One hundred and twenty trials were presented randomly with right and left target arrows occurring with equal probability. Total Flanker task duration was approximately 3 min. Participants were instructed to respond as quickly and accurately as possible to the direction of a target arrow while ignoring two flankers on each side. The second Flanker task was performed exactly 20 s after completion of the 90-min mentally fatiguing task to avoid a rest break influencing the effect of the mentally fatiguing task on the Flanker task performance. To assess performance on the Flanker task accuracy and response times (RT) were collected.

Mentally fatiguing task A modified Stroop task (Pageaux et al. 2015; Smith et al. 2016) of 90 min, partitioned in 8 blocks of 252 stimuli, was used as mentally fatiguing task. Between each block a small intersection of 20 stimuli was implemented (participants perceived no stop in the Stroop task) that, without the participants' knowing, was not accounted for in the accuracy-, RT- and EEG-analysis (see MR protocol for the purpose). The Stroop task is a task that requires inhibition and selective attention on controlled processes (MacLeod and MacDonald 2000). In this task, four coloured words ("red", "blue", "green" and "yellow") were presented one at a time on a computer screen. The participants were required to indicate the colour of the word (i.e. 'colour' stimuli), ignoring the meaning of the word itself. If however, the ink colour was red, the button to be pressed was the button linked to the real meaning of the word, not the ink colour (i.e. 'meaning' stimuli). The word presented and its ink colour were randomly selected by the computer (100% incongruent), with all incongruent word-colour combinations being equally common (meaning, in each block 63 words were presented in the colour red, yellow, green and blue). Each word was presented on screen in 34 point font for 1000 ms with a variable inter-stimulus interval of 1100, 1500 or 1900 ms, with each inter-stimulus interval being equally common. Subjects were instructed to respond as quickly and accurately as possible. Performance was assessed similarly to the Flanker task and a €50-reward for the best mean performance on the mentally fatiguing task in both conditions was offered. This should minimize the negative effects of poor motivation and disengagement on Stroop-performance.

MR solutions The MR solutions were bottled (volume 25 ml) and flavored with an amount of sodium salt of saccharin (0.03 g PLAC MR, 0.45 g CAF-MALT MR) by an independent pharmacy to blind the caffeine-taste, and stored in the dark at room temperature. PLAC MR consisted of the main ionic components of saliva, meaning distilled water containing 25 mmol KCl (0.047 g) and 2.5 mmol $NaHCO_3$ (0.005 g) (O'Doherty et al. 2000), which is tasteless and odor-free. This 'artificial saliva' solution was employed, instead of pure water, to minimize the activation of cortical taste areas which are sensitive to water in the mouth (de Araujo et al. 2003). The same solution was used for CAF-MALT MR with the addition of 1.2% w/v CAF powder (0.3 g) and 6.4% w/v MALT powder (1.6 g).

MR protocol Before the start of the Stroop and every 12.5% completion of the 90 min (Chambers et al. 2009) participants had to rinse their mouth with a given solution. A block of 20 stimuli was presented during and immediately after the mouth rinse. At this time the accuracy and RT data were collected, but not included in any analysis. This created the urge to keep on performing and avoided the subjects regaining motivation due to a rest-break The subjects had to rinse the MR solution for 10 s before expectorating it into a waste container. The MR-

solution was provided in a 30 ml cup, the researcher poured the drink in the mouth of the participant so the participant did not have to detach his hands from the keyboard to be able to rinse the solution in the mouth and expectorate it afterwards in another cup. The subjects' subjective rating of the pleasantness of the bitterness/sweetness of the taste stimuli was also assessed after each trial using a rating scale (O'Doherty et al. 2000) (+2= very (pleasant), 0= neutral, and -2= very (unpleasant)).

During the entire protocol subjects were equipped with a heart rate monitor (HR; Polar RS400, Polar Electro Oy, Kempele, Finland). Before and after the entire protocol blood glucose concentration was assessed (Bayer, Contour Next Link, Medtronic, Vienna, Austria) by collecting capillary blood at the ear lobe. Subjective psychological assessment took place before the start of the cognitive tasks with the Situational Motivation Scale (SIMS) to assess participants' motivation towards the upcoming 90-min Stroop task (Guay et al. 2000), during the cognitive tasks with a mental fatigue- and motivation-scale (0-100) and after the cognitive tasks with the National Aeronautics and Space Administration Task Load Index [NASA-TLX; (Hart and Staveland 1988)] and the Profile Of Mood States (POMS). The SIMS was filled in before the start of the first Flanker task and is a 16-item self-report inventory, which is designed to measure intrinsic motivation, identified regulation, external regulation and amotivation. Both a mental fatigue and movitation-scale (0-100) were taken before and after both Flanker tasks and within each timeframe of the 20 unrecorded stimuli during the mentally fatiguing task. These scales assessed respectively how mentally fatigued the subject was feeling (MFS; '0 = not at all' to '100 = completely exhausted') and how motivated the subject was feeling towards the next block in the Stroop task ['0 = not at all' to '100 = extremely motivated'; (Brownsberger et al. 2013)]. For the subjects to be able to keep their hands in place on the keyboard they indicated their level of mental fatigue and motivation vocally by announcing a number between 0 and 100. The NASA-TLX scale was taken after the completion of the second Flanker task and is composed of six subscales assessing subjective workload. The 32-item POMS scale was also assessed after completion of the second Flanker task and consists of five subscales; tension, depression, anger, fatigue and vigor. All items had to be scored from 0 (not at all) until 4 (extremely). The higher the score on a category, the more participants felt this mood state was present. The questionnaire was translated into the native language of the participants [Dutch; (Wald and Mellenbergh 1990)].

The subjects were given instructions to sleep for at least 7 h, refrain from the consumption of caffeine, alcohol and not to practice vigorous physical activity the day before each visit. In addition, to intra-individually standardize calorie- and macronutrient-intake, subjects were asked to have the same meal (in terms of content and quantity) the evening before each trial and not to have any food/drink intake but water after 22:00 the night before each trial. The use of any kind of medicinal products during and between the trials was prohibited. If subjects could not meet these standards they were excluded from the study. To facilitate the contact between the EEG-electrodes and the subjects' head, they were also asked to wash their hair (with neutral shampoo) the evening before the experiment.

2.3. EEG recordings and analysis

During the two Flanker tasks and the modified Stroop task, brain activity was continuously measured. Thirty two active Ag/AgCl electrodes were attached on the subjects' head (Acticap, Brain Products, Munich, Germany), according to the "10–20 International System" (Jasper 1958). The sampling rate was set at 500 Hz (Brain Vision Recorder, Brain Products, Munich, Germany). Electrode impedance was kept <10 kΩ throughout the recording. Baseline measurements were taken 2 min with eyes open, 2 min with eyes closed. During EEG recordings, subjects

were seated in a dim lit room, inserted earplugs and had been instructed to minimize movement of the head and eye blinking, to avoid frowning, to maintain the same posture and not to touch their head with their hands in order to minimize movement, sound and muscle artifacts.

Event-related potential (ERP) analysis The program Brain Vision Analyzer (version 2.1) was used to preprocess and process the data sets. Raw data were down-sampled to 256 Hz, filtered (high pass 0.1 Hz, low pass 45 Hz and Notch, Slope 48 dB/oct) with a Butterworth filter design and re-referenced to an average reference. For each data set of interest [i.e., ERP during the first, fourth and eighth block in the Stroop task] artifacts were semi-automatically removed. Then the different stimuli (Flanker task: incongruent; Stroop task: colour / meaning) were extracted from the EEG data sets. For stimulus locked ERP analysis, a data window was set at -200 to 800 ms relative to stimulus onset. Trials in which performance errors occurred were excluded. For each ERP epoch, independent component analysis (ICA) and inverse ICA further reduced artifacts. Furthermore, a baseline correction was applied (period -200 to 0 ms). Epochs were then averaged and the visually evoked potentials, P2, N2, P3b were assessed. Peak amplitudes and onset latencies were measured for the P2, N2 and P3b components in their specific region of interest (ROI; Table 1). The P2 is known to be frontally distributed (Gajewski and Falkenstein 2015) and was therefore analyzed in the orbitofrontal cortex (OFC) and dorsolateral prefrontal cortex (DLPFC). It has been related to attentive stimulus evaluation or the recall of task rules (Gajewski et al. 2008). The P2 was defined as the largest positive-going peak occurring within the time window between 80 and 260 ms and was visually confirmed. The N2 is usually interpreted as an index of conflict monitoring (Folstein and Van Petten 2008) and emerges fronto-centrally after the P2 (Gajewski and Falkenstein 2015), thus also for the N2 the OFC and DLPFC was analyzed. The N2 was defined as the largest negative-going peak occurring within the time window between 180 and 440 ms and was visually confirmed. The P3b is linked to salience processing and appears to occur when subsequent attentional resource activations promote memory operations in temporal-parietal areas (Polich 2007). Therefore the fusiform gyrus (FFG), the angular gyrus (AG) and the somatosensory association cortex (SAC) were analyzed to observe any effects on the P3b. The P3b was defined as the largest positive-going peak occurring within the time window between 180 and 415 ms and was visually confirmed. Thereafter, we exported the data from Brain Vision Analyzer to SPSS (version 22.0; SPSS, Chicago, IL) for further analysis.

INSERT Table 1 HERE

Spectral power analysis Similar to the ERP analysis, the program Brain Vision Analyzer (version 2.1) was used to preprocess and process the data sets for the analysis of the total power. Raw data were down-sampled to 256 Hz, filtered (high pass 0.1 Hz, low pass 45 Hz and Notch, Slope 48 dB/oct) with a Butterworth filter design and re-referenced to an average reference. For each data set of interest [i.e., Continuous EEG measurements from the fifth to the eighth minute in the first (5-8 min), fourth (38 min 45 sec-41 min 45 sec) and eighth (83 min 45 sec-86 min 45 sec) block in the Stroop task] artifacts were semi-automatically removed. For each continuous EEG data set of interest segments with a length of 4 s and with an overlap of 2 s were extracted (Wascher et al. 2014). Subsequently ICA and inverse ICA further reduced artifacts. The resulting data segments were tapered with a Hanning window with 10% of the total segment length. Fast Fourier transform (FFT) power spectra with a spectral

resolution of 0.25 Hz were calculated for both sides of the spectrum, resulting in FFT segments containing the full spectral information. The resulting FFT segments were averaged to stabilize the spectral content. The power in the FFT was extracted for theta (θ, 3.5–7.5 Hz), alpha (α1, 7.5–10 Hz; α2, 10–12.5 Hz) and beta (β1, 12.5–18 Hz; β2, 18–35 Hz) in each ROI.

2.4. Statistical analysis

All data are presented as means ± standard error (SE) unless stated otherwise. The Shapiro-Wilk test was used to test the normality of the data, sphericity was verified by the Mauchly's test. When the assumption of sphericity was not met, the significance of F-ratios were adjusted with the Greenhouse-Geisser procedure. Subjective data. NASA-TLX-, SIMS- and POMS-data were not normally distributed and therefore Wilcoxon signed ranked tests were used to test the effect of condition (MR vs. PLAC) in each subscale. Paired sample t-tests were employed to assess the effect of condition on the pleasantness-rating of the taste stimuli. Two-way repeated measure ANOVAs were used to test for the effect of condition and time on motivation (2 x 10) and MFS (2 x 11). Behavioral data. Stroop accuracy-data had to be normalized to a baseline performance (i.e. performance on the first block = 100%) within each condition and subject (see Results) in order to be normally distributed. The effect of condition, time (second to eighth block in the Stroop) and stimuli (colour vs. meaning) on normalized Stroop accuracy-data was tested with a three-way repeated measures ANOVA (2 x 7 x 2). The same three-way repeated measures ANOVA with an extra level in the time variable (the first block; 2 x 8 x 2) was used to analyze Stroop RT-data. A two-way repeated measure ANOVA (2 x 2) was used to assess the effect of condition and time on Flanker-accuracy and -RT. (Neuro)Physiological data. The effect of condition and time on blood glucose concentration (2 x 2) and HR (2 x 11) was tested with a two-way repeated measure ANOVA. Four-way repeated measure ANOVAs were used to assess the effect of condition, time (first to eighth block in the Stroop), stimuli (colour vs. meaning) and ROI (see Table 1) on multiple ERP (P2-, N2- and P3b-amplitude and -latency). Square root transformed normalized (spectral power during baseline eyes open condition = 100%) spectral power (α1, α2 and θ) variables associated with mental fatigue were analyzed with a three-way repeated measures ANOVA (condition, time and ROI; 2 x 3 x 9; see Table 1). If significant interaction effects in the repeated measure ANOVAs were observed, subsequent repeated measure ANOVAs or paired sample t-tests (depending on the amount of interacting factors) were performed in order to elucidate the main effect of the interacting factors. If no significant interaction effects were observed, main effects were immediately observed and further interpreted through pairwise comparisons with Bonferroni correction. Within-subjects correlation coefficients (r) were computed for the correlations between MFS/P2-amplitude in DLPFC and Stroop accuracy on 'meaning' stimuli/P2-amplitude in DLPFC using the method described by Bland and Altman (Bland and Altman 1995). This method adjusts for repeated observations within participants by using multiple regression with "participant" treated as a categorical factor using dummy variables. Significance was set at 0.05 for all analyses. All statistical tests were conducted using the Statistical Package for the Social Sciences, version 24 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Subjective data

No significant condition x time interaction was found for self-reported mental fatigue. Self-reported mental fatigue significantly increased in time from 10.1 ± 2.5 before the first Flanker task to 42.7 ± 7.4 after the second Flanker task ($F(1.2, 9.3)=12.7$; $p=0.005$) and was, on average, significantly lower in MR (23.2 ± 4.4) compared to PLAC (30.4 ± 4.6; $F(1, 8)=9.0$; $p=0.017$; Fig. 2). The POMS however showed no difference between conditions in fatigue or any other subscale (depression, tension, anger and vigor). No difference between conditions was observed in terms of general motivation towards the Stroop (assessed with the oral 0-100 scale) or intrinsic motivation, identified regulation, external regulation and amotivation (assessed with the SIMS). General motivation (assessed with the oral 0-100 scale) did decrease in time from 86.5 ± 2.9 at the start of the protocol to 69.9 ± 5.6 at the end of the Stroop task ($F(1.6, 14.8)=9.9$; $p=0.003$). The NASA-TLX data revealed that there was no effect of condition on any subscale (mental demand, physical demand, temporal demand, performance, effort, frustration). Although efforts were made to mask the differing taste between both mouth rinse solutions, participants scored the pleasantness of the bitter/sweet taste in MR (-0.9) significantly lower than in PLAC (0.7; $p=0.008$).

INSERT FIG.2 HERE

3.2. Behavioral data

Stroop performance

A triple condition x time x stimulus-type interaction ($F(6, 54)=2.7$; $p=0.022$) for the normalized Stroop accuracy was found. A post hoc condition x time ANOVA in each stimulus-type revealed a condition x time interaction within the 'meaning' stimuli ($F(6, 54)=3.3$; $p=0.008$) and a main effect of time within the 'colour' stimuli ($F(6, 54)=2.4$; $p=0.039$; see Table 2). Concerning the 'meaning' stimuli, follow-up paired sample-t-tests within each time interval revealed that accuracy was higher in the eighth and last block of the Stroop in MR (100.4 ± 1.6%) compared to PLAC (91.0 ± 3.5%; $p=0.032$; see Table 2). Regarding Stroop RT, a time x stimulus-type interaction was observed in the three-way-ANOVA ($F(7, 63)=8.1$; $p<0.001$). The post-hoc condition x time ANOVA in each stimulus-type revealed that for the 'meaning' stimuli participants reacted faster in time ($F(1.9, 17.1)=4.9$; $p=0.023$; see Table 2) independent of condition. For the 'colour' stimuli no interaction or main effects were found.

INSERT Table 2 HERE

Flanker performance

No interaction effect or time effect was observed for accuracy, participants performed worse in PLAC (0.89 ± 0.02) than in MR (0.91 ± 0.01; F(1, 9)=6.6; p=0.031; see Table 3). Regarding RT no interaction effect or main effect of condition or time was observed (see Table 3).

INSERT Table 3 HERE

3.3. (Neuro)Physiological data

Heart rate and blood glucose

For HR no interaction effect or main effect of condition was observed. HR did however decrease in time in both conditions (F(4.1, 37.0)=5.0; p=0.002) from 71 ± 3 bpm before the pre-Flanker to 63 ± 3 bpm after the post-Flanker. For blood glucose no interaction effect or main effect of time was found. There was however a main effect of condition (F(1, 8)=21.6; p=0.002). Blood glucose was higher in MR (95.9 ± 3.9 mg/dl) than in PLAC (88.4 ± 3.2 mg/dl).

Event related potentials

P2. For P2-amplitude a condition x stimulus-type x ROI (F(1, 9)=5.3; p=0.046) and time x stimulus-type x ROI (F(2, 18)=4.3; p=0.03) interaction effect in the four-way ANOVA led towards the implementation of a post hoc condition x time x ROI ANOVA in each stimulus type. This revealed a condition x ROI interaction (F(1, 9)=5.8; p=0.039) for the 'meaning' stimuli and a condition x time interaction (F(2, 18)=5.0; p=0.018) for the 'colour' stimuli. Subsequently, for the 'meaning' stimuli, follow-up condition x time ANOVAs in both ROIs were performed. For the 'colour' stimuli condition x ROI and time x ROI ANOVAs were performed. Within the 'meaning' stimuli, the P2-amplitude in the OFC was larger in MR (2.6 ± 0.5 µV) than in PLAC (1.9 ± 0.4 µV; F(1, 9)=9.9; p=0.012) independently from time. In the DLPFC a condition x time interaction was present (F(2, 18)=4.2; p=0.032). Amplitude decreased in time in PLAC (F(2, 18)=5.1; p=0.017; see Fig. 3a), whilst in MR time did not have an effect (Fig. 3b). Paired sample-t-tests indicated that P2-amplitude was larger in PLAC in the first time-interval (p=0.049; see Fig. 3a & b), while in the third and last time-interval it was larger in MR (p=0.034; see Fig. 3a & b). Within the 'colour' stimuli, P2-amplitude decreased over time in PLAC (F(2, 18)=11.1; p=0.001) independently from ROI. In MR P2-amplitude did not differ over time (no time x ROI interaction or main effect of time) in the 'colour' stimuli. No interaction effects or main effect of time or condition were observed for latency. N2. For N2-amplitude no interaction effects or main effect of time or condition were observed. For N2-latency a time x stimulus-type interaction was present (F(2, 18)=6.4; p=0.008). N2-latency on the 'meaning' stimuli became longer in time (304.2 ± 12.0 ms → 330.8 ± 19.9 ms) independent of condition (F(2, 18)=4.3; p=0.029). On the 'colour' stimuli, N2-latency did not differ between conditions or in time. P3b. No interaction effects or main effect of time or condition were observed for P3b-amplitude. A time x ROI interaction was present in the four-way ANOVA for P3b-latency. Subsequent follow up condition x time x stimulus-type ANOVAs in all three ROIs

revealed that only in the FFG, P3b-latency became longer in time (261.9 ± 11.8 ms → 293.0 ± 8.5 ms; F(2, 18)=11.6; p=0.001) independent of condition.

INSERT FIG. 3a & 3b HERE

Spectral power

In all spectral power frequencies (θ, α1, α2, β1 and β2) no interaction or main effect of condition and time was observed. Only β1-power was found to decrease in time during the Stroop task (F(2, 18)=5.6; p=0.013).

3.4. Correlations

Within-subjects correlation coefficients were computed for those parameters that, similar to the markers of mental fatigue, were affected by the intervention (i.e. an increase in self-reported mental fatigue and a decrease in accuracy). No significant correlations were however observed between P2-amplitude and MFS or Stroop accuracy on the 'meaning' stimuli.

4. Discussion

This study is the first to assess the ability of serial CAF-MALT mouth rinsing to counteract mental fatigue. It was observed that a serial CAF-MALT mouth rinse-intervention counteracts subjective, behavioral and electrophysiological (i.e. decreased P2-amplitude was counteracted in MR) mental fatigue.

In both conditions, MR as well as PLAC, the 90-min Stroop task elicited mental fatigue. This was measured as an increase in self-reported mental fatigue and a decrease in accuracy. However, small but important differences were observed between both conditions. Despite that self-reported mental fatigue significantly increased in both conditions, it increased significantly less in MR. Participants commenced the protocol in both conditions with a similar degree of self-reported mental fatigue (MR: 11.2 ± 2.7; PLAC: 8.9 ± 3.2), whilst at the end this differed substantially (MR: 34.7 ± 7.8; PLAC: 50.7 ± 8.3). POMS measures however showed no difference in fatigue between conditions. It was suggested by Van Cutsem et al. (2017) that the POMS may be less capable of detecting small but relevant short-term changes in mental fatigue and this seems to be confirmed in the present study.

Behaviorally, performance on the 'meaning' stimuli (which represent the stimuli in which subjects had to respond to the meaning of the word, and not the color), also indicated a difference was present between conditions. This became specifically apparent in the eighth and last block of the Stroop task. Accuracy on the 'meaning' stimuli in this block was higher in MR compared to PLAC. This means that participants were able to keep up their cognitive performance for longer. Furthermore participants reacted faster on the 'meaning' stimuli in time, independent from the condition. This indicates participants adopted the higher risk strategy with increasing time-on-task with more success in MR than in PLAC, as participants were only able to keep up accuracy in MR condition. The lower occurrence rate (25% of all stimuli) of these 'meaning' stimuli brings along a higher mental demand, which could explain why performance on these stimuli was more sensitive to the intervention-effect. In order to be able to

observe the effect of mental fatigue on cognitive performance independently from time-on-task, a Flanker task was completed before and after the mentally fatiguing task. Performance on this task confirmed that cognitive performance was better in MR, mainly due to a higher accuracy on the Flanker task completed after the mentally fatiguing task (see Table 3). Consequently also this measure indicates that participants were more resistant to mental fatigue in MR.

Multiple functional magnetic resonance imaging- (Chambers et al. 2009; Frank et al. 2008; Haase et al. 2009) and EEG-studies (De Pauw et al. 2015) have demonstrated that rinsing a solution containing glucose and/or caffeine activates certain brain regions (e.g. anterior cingulate cortex, orbitofrontal cortex, striatum, …). An objective effect of CAF-MALT mouth rinsing on the brain was confirmed by our data. Specifically, serial CAF-MALT mouth rinsing prevented the decrease in P2-amplitude in time. The P2 is a component of an ERP that appears around 200 ms after the onset of a stimulus (Gajewski and Falkenstein 2015) and its amplitude is suggested to serve as an electrophysiological marker of the process in which cue information is associated to the functional properties of this information (i.e. the recall of task rules) (Gajewski and Falkenstein 2015; Lorist 2008). In other words, serial CAF-MALT mouth rinsing prevented a decrease in the ability to select relevant cue information. Nonetheless, the change in P2-amplitude was not significantly correlated with the other markers of mental fatigue. In contrast, the frequently reported shift of EEG power towards low-frequency bands (δ, θ, α) in mentally fatigued subjects (Aeschbach et al. 1997; Cajochen et al. 1995; Phipps-Nelson et al. 2011; Trejo et al. 2015; Wascher et al. 2014; Zhao et al. 2012) was not observed in the present study. It is possible this shift in spectral distribution was prevented in both MR and PLAC by the brain activating properties of both substances. In the study of Chambers et al. (2009) it is shown that caloric (e.g. the solution used in MR in the present study) as well as non-caloric sweetened (e.g. the solution used in PLAC in the present study) solutions activate multiple brain areas (i.e. right insula, frontal operculum, left dorsolateral prefrontal cortex). The present results suggest that a prevention of this shift in spectral distribution [i.e. a decrease in arousal (Tops and Boksem 2010)] is however not sufficient to postpone the occurrence of mental fatigue, subjectively and behaviorally. Unfortunately we did not include a control trial (i.e. without mouth rinse) to confirm whether the spectral distribution-shift occurred in the first place in the present study. Therefore we can also not further substantiate this suggestion.

A potential mechanism for the serial CAF-MALT mouth rinsing positively affecting mental fatigue might be the absorption through the oral mucosa, especially for a lipophilic agent like caffeine (Shargel and Yu 2016). Caffeine, rapidly taken up in the bloodstream through the buccal mucosa, may arrive in the brain via the systemic circulation and exert its' known effects (i.e. antagonist of adenosine). Doering et al. (2014) investigated however the effects of serial caffeine mouth rinsing on endurance cycling time-trial performance and found no significant increase in plasma caffeine concentration after repeated oral exposure. Similarly, Rollo et al. (2010) assessed whether peripheral blood glucose concentration is affected by serial carbohydrate mouth rinsing (i.e. Lucozade Sport, Brentford, England) and did not observe any increase. This is confirmed by our results, although blood glucose was higher in MR, it was not altered differently in time in both conditions. In addition, the time dynamics of the changes in other parameters like P2-amplitude and accuracy during the Stroop task cannot be explained by the higher blood glucose in MR. Therefore this difference in glucose between MR and PLAC, most probably, does not explain our results. Although both caffeine and maltodextrin were probably not taken up systemically during the serial CAF-MALT mouth rinsing, it could still be that minimal amounts of both substances were absorbed through the oral mucosa and exerted their effect in the brain without showing up in the blood. A placebo effect

(Wehrwein and Carter 2016) could also explain the observed results and could have been triggered by the participants' awareness that they were expected to perform better in one trial compared to the other. Participants were however informed in a way that they were naïve of the study's real aims and hypotheses. This also anticipated the fact that pleasantness of taste was rated lower in MR than in PLAC. A possible role of motivation in the observed results was also accounted for. A monetary incentive was provided in an attempt to prevent task disengagement and to restrict possible alterations in motivation throughout the task performance (Gergelyfi et al. 2015; Van Cutsem et al. 2017). In addition the level of motivation was monitored before the start of the task (SIMS) and during the task (oral 0-100 scale). In none of the two methods an effect of the intervention was found. A final potential mechanism by which the serial CAF-MALT mouth rinsing attenuated mental fatigue is the activation of specific brain regions (i.e. the anterior cingulate cortex and the right caudate, that forms part of the striatum) known to produce dopamine (Chambers et al. 2009; Turner et al. 2014). A mechanism that has already been suggested in the self-control literature, where a carbohydrate-mouth rinse has been shown to successfully counter on self-control impairments (Hagger and Chatzisarantis 2013). Moreover mental fatigue has already been associated multiple times with alterations in the anterior cingulate cortex (Boksem et al. 2005; Boksem and Tops 2008; Marcora et al. 2009; Mostofsky and Simmonds 2008). This way a mouth rinse containing caffeine and maltodextrin might be able to (partly) restore dopaminergic transmission in the striatum and anterior cingulate cortex and postpone mental fatigue by activating sensory neurons in the mouth and initiating a signal transduction cascade towards the brain.

Future research

In the present study caffeine and maltodextrin were combined in one mouth rinse in order to increase the chances to successfully counter the electrophysiological changes associated with mental fatigue. This assumption was based on a study conducted by Beaven et al. (2013), who found that there was an additive effect of combining caffeine and carbohydrates on power production during repeated sprints on a cycle ergometer. The additive positive effect suggested that distinct mechanisms are involved in the performance enhancement (Beaven et al. 2013). This indicates that it would be interesting to look at the separate effects of a maltodextrin mouth rinse and a caffeine mouth rinse on the occurrence of mental fatigue. Looking at the separate effects of both mouth rinses might further increase our knowledge on the electrophysiological measures laying at the base of the occurrence of mental fatigue. The present study was completed with low caffeine users in a fasted state, meaning that it is still to be confirmed whether these effects would still occur in another population at another time of day and when switching between tasks. Another important aspect that needs further investigation is whether serial mouth rinsing is also capable of postponing mental fatigue in a less demanding way. The mouth rinse intervention in the present study was based on a study of Carter et al. (2004), were participants also had to rinse their mouth every 12.5% task completion. A less demanding mouth rinse-protocol, that is still able to postpone mental fatigue, would greatly improve its applicability. Caution is however warranted as a recent study of Kumar et al. (2016) reported that a single carbohydrate mouth rinse preceding a 20-min cognitive task, was unable to prevent a decrease in cognitive performance in time.

5. Conclusion

A 90-min Stroop task induced mental fatigue in both MR and PLAC. However in the MR condition mental fatigue was induced to a lesser extent, indicated by a slower increase in self-reported mental fatigue, the ability to keep up

cognitive performance and by preventing a mental fatigue-associated electrophysiological change (i.e. decreasing P2-amplitude) occurring with increasing time-on-task. Two potential mechanisms to account for the ability of serial CAF-MALT mouth rinsing to counter mental fatigue are: 1) absorption of caffeine and maltodextrin via the brain that does not show up systemically; 2) restored dopaminergic brain transmission via sensory neurons initiating a signal transduction cascade towards the brain. In addition, for individuals that do not want to or cannot ingest caffeine and/or carbohydrates but need to reduce mental fatigue, these findings provide a possible practical solution.

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Fig. 1 Protocol timeline (min). T = ambient temperature; RH = relative humidity; POMS = profile of mood states; SIMS = situational & intrinsic motivation scale; GLUC = blood glucose; MFS = self-reported mental fatigue;  = mouth rinse;  = Electroencephalography;  = heart rate

Fig. 2 Self-reported mental fatigue throughout the protocol; before and after the first Flanker task (Pre F1, S0), after each block during the Stroop task (S1, …, S8) and after the last Flanker task (Post F2). ‡ Significant effect of time ($p<0,05$). * Significant effect of condition ($p<0,05$). Data are presented as means ± SE.

Fig. 3 a. Grand-average ERPs at Fz elicited by the 'meaning' stimuli (i.e. indicate the meaning of the word) in the first block (solid black line), fourth block (large dashed line) and eighth block (small dashed line) during the Stroop task in PLAC. ‡ Significant main effect of time ($p<0.05$). **b.** Grand-average ERPs at Fz elicited by the 'meaning' stimuli in the first block (solid black line), fourth block (large dashed line) and eighth block (small dashed line) during the Stroop task in MR.

Table 1 Regions Of Interest (ROI) defined by Brodmann Areas (BAs) and electrode sites according to the "10–20 International System". V denotes the ROI checked for that particular electroencephalography-measure.

Table 2 Stroop performance; Accuracy and RT in all eight blocks (11min15sec) of the Stroop task. Accuracy-data were normalized to the performance on the first block within each condition (=100%), data are presented as means ± SE; † indicates a trend towards a time-effect; ‡ indicates a significant time-effect; * indicates a significant difference compared to the analogous time interval in PLAC.

Table 3 Flanker performance; Accuracy and RT in pre and post Flanker task. Data are presented as means ± SE; * indicates a significant difference compared to PLAC.

Table. 1

Table 1. Regions Of Interest (ROI)

ROI	Brain region	BAs	Electrode sites	P2 & N2	P3b	EEG
1	Inferior/Orbitofrontal cortex	11, 47	F7	V	-	V
2	Broca's area	44, 45	FC6, F8	-	-	V
3	Dorsolateral prefrontal cortex	8, 9, 46	F3, Fz, F4	V	-	V
4	Anterior prefrontal cortex	10	FP1, FP2	-	-	V
5	Premotor cortex	6	FC1, FC2	-	-	V
6	Primary motor cortex	4	C3, Cz, C4	-	-	V
7	Somatosensory Association Cortex	7	Pz	-	V	V
8	Angular Gyrus	39	P3, P4	-	V	V
9	Fusiform Gyrus	37	P7, P8, PO9, PO10	-	V	V

Table. 2

Table 2. Stroop performance; Accuracy and RT in all eight blocks (11min15sec) of the Stroop task

	Meaning stimuli								**Colour stimuli**							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
MR (accuracy ± SE; %)	100	97.3 ± 1.1	97.5 ± 2.3	97.7 ± 2.2	97.0 ± 1.3	98.9 ± 1.7	97.9 ± 1.8	100.4 ± 1.6*	100	99.0 ± 0.6	98.9 ± 1.0	97.3 ± 0.9	98.1 ± 1.5	97.4 ± 1.2	96.7 ± 1.4	97.3 ± 1.7‡
PLAC (accuracy ± SE; %)	100	98.6 ± 1.9	98.6 ± 1.8	98.1 ± 2.0	95.4 ± 2.6	93.0 ± 3.1	96.2 ± 2.9	91.0 ± 3.5†	100	96.8 ± 1.0	96.7 ± 1.2	95.6 ± 2.4	94.7 ± 3.1	93.9 ± 2.6	93.6 ± 2.5	92.5 ± 3.1‡
MR (RT ± SE; ms)	642 ± 12	609 ± 10	603 ± 9	595 ± 13	601 ± 12	603 ± 15	598 ± 12	604 ± 17‡	557 ± 10	559 ± 10	563 ± 11	551 ± 10	560 ± 14	550 ± 12	559 ± 12	561 ± 13
PLAC (RT ± SE; ms)	638 ± 8	604 ± 4	613 ± 10	606 ± 9	613 ± 12	615 ± 13	603 ± 11	609 ± 11‡	553 ± 8	557 ± 11	566 ± 11	564 ± 11	571 ± 16	564 ± 13	566 ± 15	565 ± 14

Table. 3

Table 3. Flanker performance; Accuracy and RT in pre and post Flanker task

	Pre	**Post**
MR (accuracy ± SE)*	0.91 ± 0.02	0.91 ± 0.01
PLAC (accuracy ± SE)	0.92 ± 0.01	0.86 ± 0.03
MR (RT ± SE; ms)	350 ± 13	342 ± 11
PLAC (RT ± SE; ms)	360 ± 8	365 ± 11

Figure 1

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Fig. 1

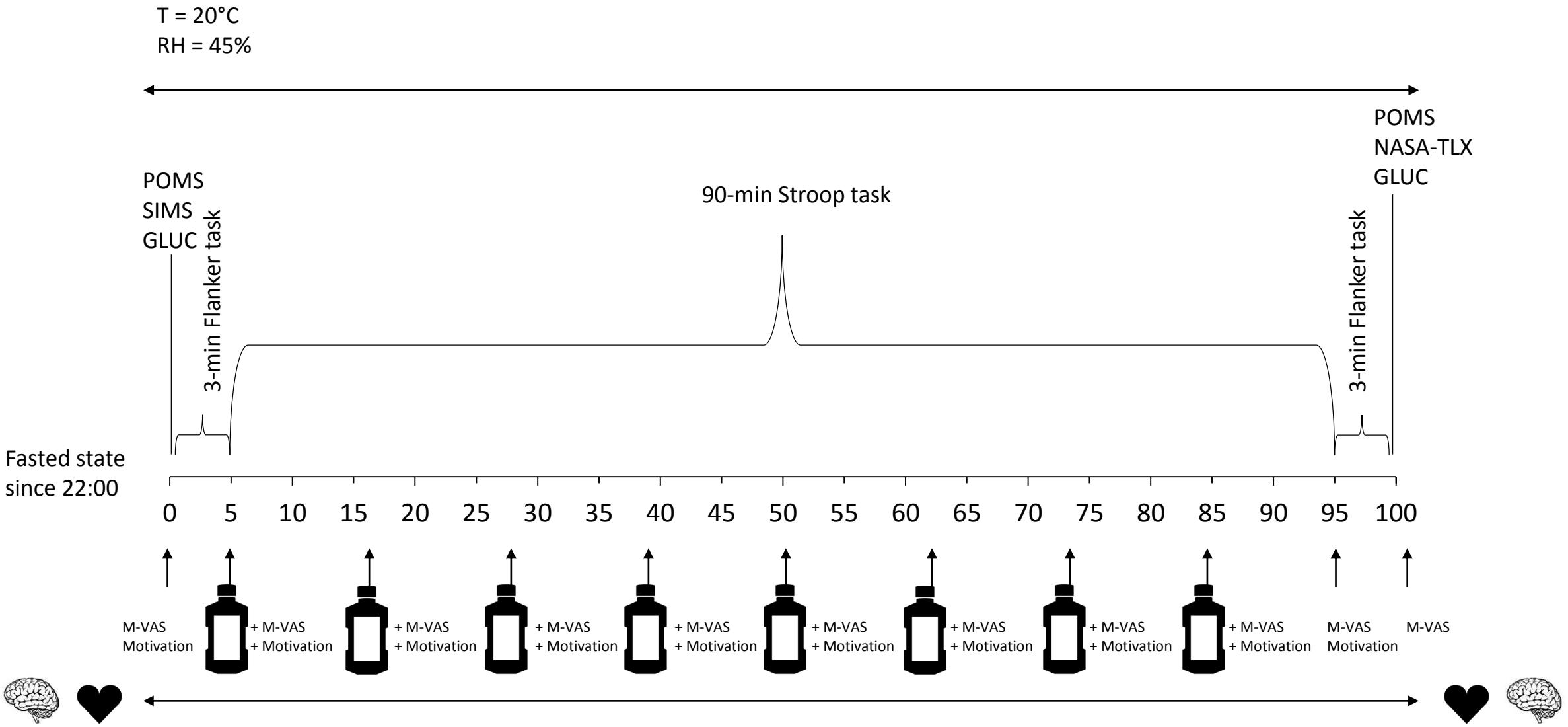

Figure 2

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Fig. 2

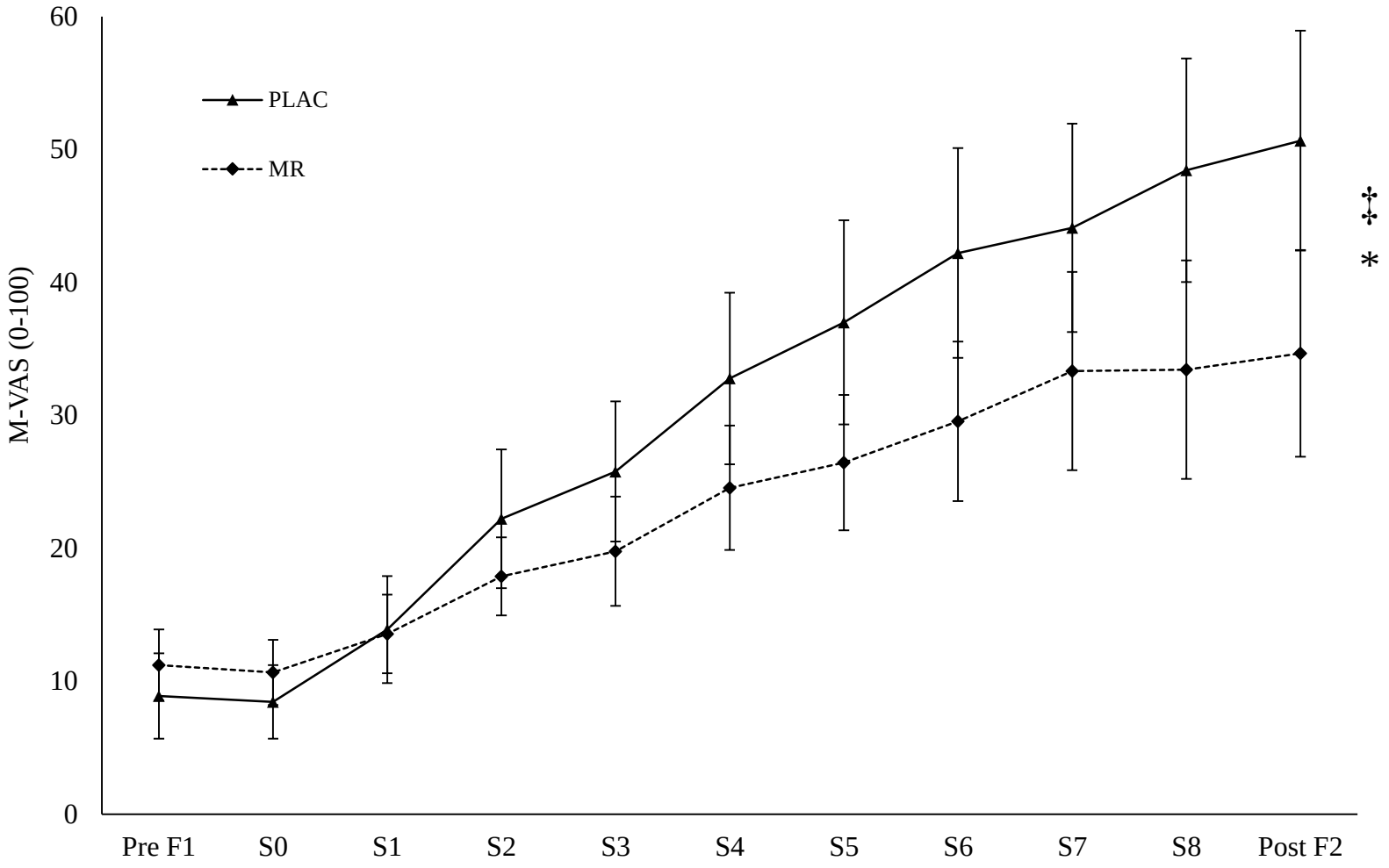

Figure 3

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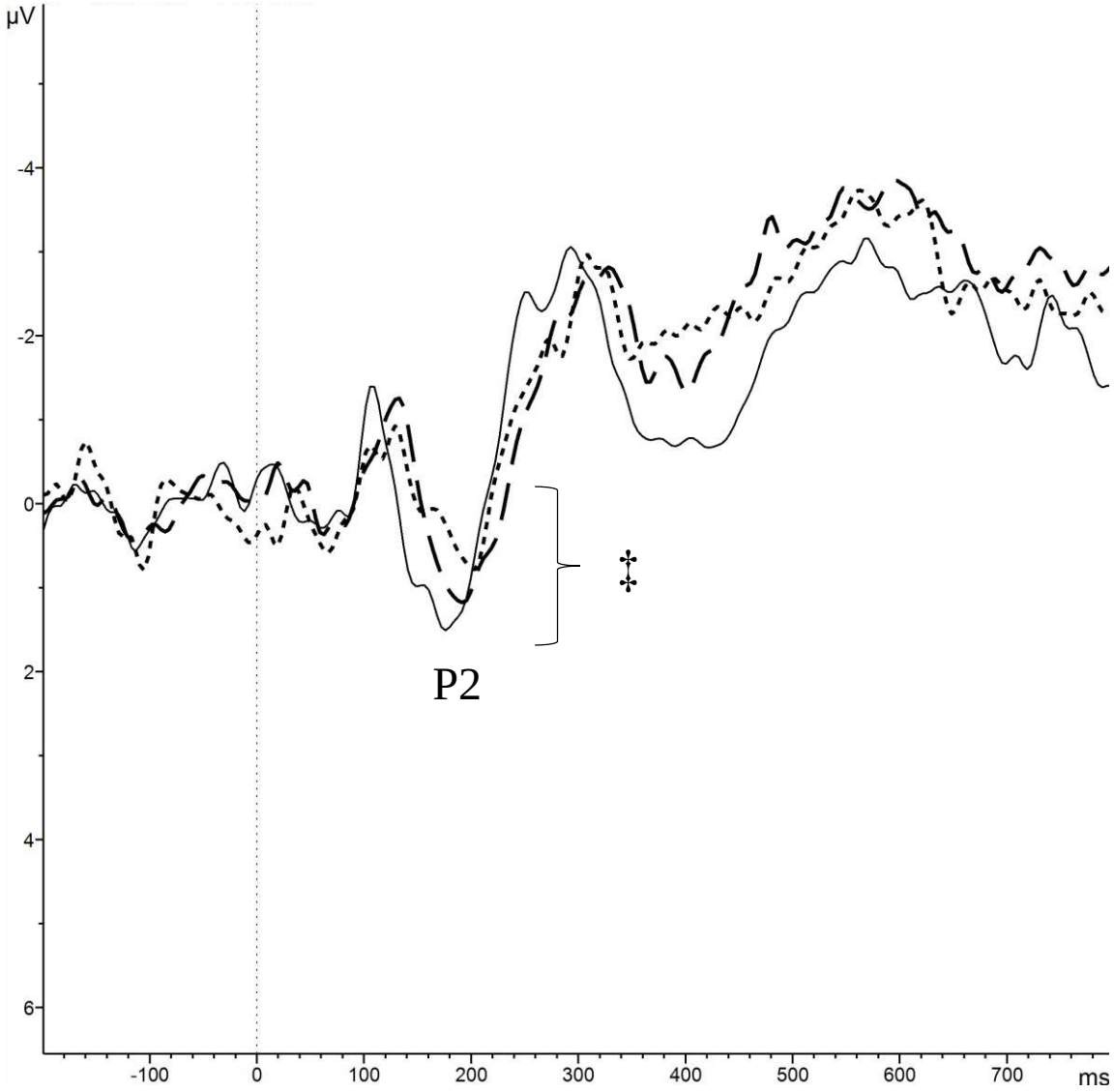
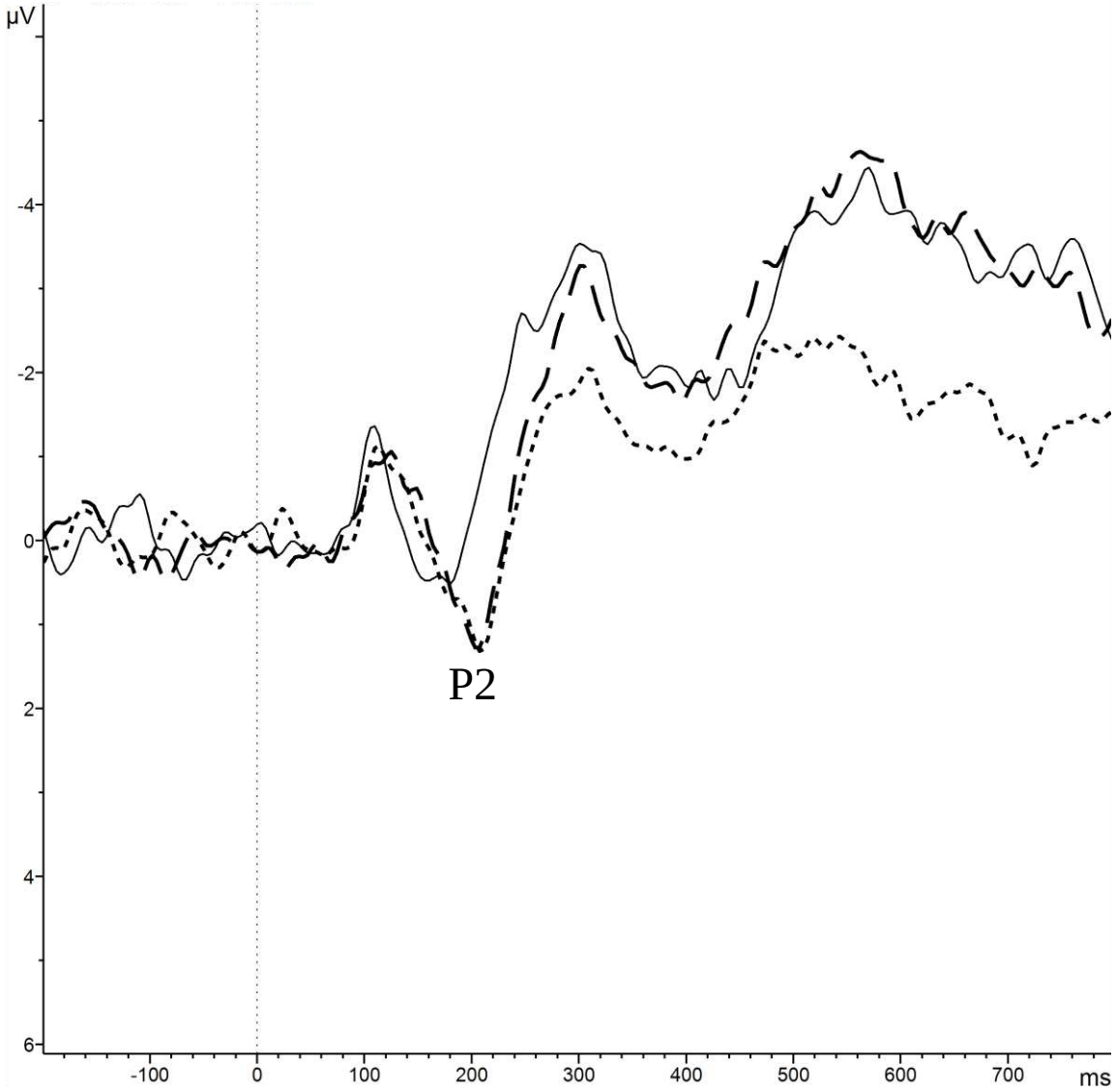
Fig. 3

a.



b.



Reviewer #1: The manuscript provides evidence that a combined caffeine-maltodextrin mouth rinse attenuates the decline in markers of mental fatigue during a 90 min task. The paper is generally well-written with clearly defined aims, comprehensively described methods, and valid conclusions. However, there are a few points I feel require further clarification.

We thank the reviewer for the revision and his/her constructive comments.

1) Ten volunteers completed the study, but the methods section states that were excluded from the study if they did not meet the dietary/lifestyle instructions prior to the study visits. How many volunteers were recruited and excluded? In addition, was an a priori sample size calculation performed to confirm that 10 volunteers would provide sufficient statistical power to detect differences in the primary outcomes?

An a priori sample size calculation was performed based on the effect size mentioned in the paper of De Pauw et al. [1]. This calculation indicated a sample size of 7 would result in sufficient statistical power to detect differences in the cognitive performance measures. To account for a possible drop-out we decided to recruit 10 participants. None of these 10 participants were excluded as all were successful in following up the dietary/lifestyle instructions.

2) The methods state that experimental trials were separated by at least 6 days. What was the mean and range for this?

The mean time between both experimental trials was 8 days, the standard deviation was 3 days. In two participants the two experimental trials were separated by 13 days, this explains the standard deviation of 3 days.

This was added in the paper as follows:

… all separated by at least 6 days (mean: 8 days, SD: 3days) to ensure full recovery. …

3) Can you please specify the definition of 'the same evening meal'? Was this standardised for calories and macronutrients?

Participants were asked to have the same meal the evening before each trial. During the familiarization trial subjects chose a meal that they would have the evening before their next trial and were asked to have this same meal (in terms of content and quantity) the evening before their last trial. This instruction was given to standardize calorie- and macronutrient-intake intra-individually. The adherence to this instruction was checked orally before the commencement of each experimental trial and participants were found to follow up this instruction with success. As each participant was free to choose what kind of meal they wanted to have the evening before each trial, the calories and macronutrients-intake was standardized intra-individually, but not inter-individually. In order to provide some more clarity on this matter, following changes were made in the manuscript:

… In addition, to intra-individually standardize calorie- and macronutrient-intake, subjects were asked to have the same meal (in terms of content and quantity) the evening before each trial and not to have any food/drink intake but water after 22:00 the night before each trial. …

4) The study found a significant main effect of condition for blood glucose, being significantly higher in MR compared to PLAC. Whilst not significant at any particular time, with the significant main overall effect, I don't agree that blood glucose was not different between trials, as the authors' state. Could the results be explained by the 8.5% higher blood glucose levels in the MR trial?

We agree with the reviewer that we cannot state that blood glucose was not different between trials, as we indeed observed there was a main effect of condition. However, from our point of view we never stated this?

We presume the reviewer is referring to the following statement in the discussion: '*This is confirmed by our results, blood glucose was found not to be altered differently in time in both conditions.*'. This statement was made In order to point out that, despite a 8.5% higher blood glucose level in the MR trial, serial CAF-MALT mouth rinsing did not result in an increase in blood glucose in time. We adjusted this statement as follows in the manuscript:

… This is confirmed by our results, although blood glucose was higher in MR, it was not altered differently in time in both conditions. …

Hypothetically our findings might also be explained by the 8.5% difference in the blood glucose level. Yet if this was the case, we would not have expected interaction effects between time and condition in the other measures (i.e. P2-amplitude and accuracy on the meaning stimuli during the Stroop task). The interaction effects that were observed indicate that the effect of condition was dependent on time, and therefore cannot be explained by a general 8.5% difference in blood glucose. These interaction effects do however stroke with a gradual effect in time of serial CAF-MALT mouth rinsing. Apart from whether the higher blood glucose in MR could have played a role or not, we do acknowledge the importance of this discussion and therefore added the following to the discussion:

… This is confirmed by our results, although blood glucose was higher in MR, it was not altered differently in time in both conditions. In addition, the time dynamics of the changes in other parameters like P2-amplitude and accuracy during the Stroop task cannot be explained by the higher blood glucose in MR. Therefore this difference in glucose between MR and PLAC, most probably, does not explain our results. Although both caffeine and maltodextrin were probably not taken up systemically during the serial CAF-MALT mouth rinsing, it could still be that minimal amounts …

By checking the manuscript on the appearance of the word 'glucose', it was noticed that it was not mentioned in the method-section how glucose was measured. Therefore the following was added to the manuscript:

… Before and after the entire protocol blood glucose concentration was assessed (Bayer, Contour Next Link, Medtronic, Vienna, Austria) by collecting capillary blood at the ear lobe. …

1. De Pauw, K., et al., *Effects of caffeine and maltodextrin mouth rinsing on P300, brain imaging, and cognitive performance.* J Appl Physiol (1985), 2015. **118**(6): p. 776-82.