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Running title: Performance in a mentally fatigued state

ABSTRACT

Purpose: Mental fatigue is a psychobiological state caused by prolonged periods of demanding cognitive activity and has been observed to decrease time-trial (TT) endurance performance by ~3,5% in normal ambient temperatures. Recently it has been suggested that heat may augment the negative effect of mental fatigue on cognitive performance, raising the question whether it may also amplify the effect of mental fatigue on TT-performance. **Methods:** In 30 °C and 30% relative humidity, ten endurance-trained male athletes (Age: 22 ± 3 y; W_{max}: 332 ± 41 W) completed two experimental conditions: intervention (I; 45-min Stroop task) and control (C; 45-min documentary). Pre and post intervention/control, cognitive performance was followed up with a 5-min Flanker task. Thereafter subjects cycled for 45 min at a fixed pace equal to 60%-W_{max}, immediately followed by a self-paced TT in which they had to produce a fixed amount of work (equal to cycling 15 min at 80%-W_{max}) as fast as possible. **Results:** Self-reported mental fatigue was significantly higher after I compared to C ($P<0.05$). Moreover electroencephalographic measures also indicated the occurrence of mental fatigue during the Stroop ($P<0.05$). TT-time did not differ between conditions (I: 906 ± 30 s, C: 916 ± 29 s). Throughout exercise, physiological (heart rate, blood lactate, core and skin temperature) and perceptual measures (perception of effort and thermal sensation) were not affected by mental fatigue. **Conclusion:** No negative effects of mild mental fatigue were observed on performance and the physiological and perceptual responses to endurance exercise in the heat. Most plausibly mild mental fatigue does not reduce endurance performance when the brain is already stressed by a hot environment.

Key words: exercise, whole-body endurance performance, heat, effort

1. Introduction

Physical performance and more specifically endurance performance is negatively affected by mental fatigue (see Van Cutsem et al. (34) for a systematic review on the topic). Mental fatigue is a psychobiological state caused by prolonged periods of demanding cognitive activity and is characterized by various subjective, physiological and behavioral alterations. It is manifested as an acute increase in subjective ratings of fatigue (4, 16, 17, 33), specific electroencephalography (EEG) alterations (4, 11, 36) and/or an acute decline in cognitive performance (17, 33). Often it is induced by a response inhibition task (e.g. incongruent Stroop task) that requires self-control to inhibit automatic responses to certain stimuli. Prolonged performance on such a task prior to endurance exercise leads to higher perception of effort and impaired endurance performance (34). A higher perception of effort manifests itself as a higher rating of perceived effort (RPE) during fixed workload tests, whilst during time trials the workload is lower relative to RPE. Physiological parameters like respiratory, cardiovascular, metabolic or neuromuscular function during and after endurance exercise (17, 23, 33) do not seem to be influenced by mental fatigue.

Heat stress is also known to impair endurance performance (26, 35). The impairment in performance has frequently been associated with a rise in cardiovascular strain (26) during endurance exercise, a decrease in maximal aerobic capacity, a higher internal body temperature (T_{core}) , higher skin temperature (T_{skin}) (31), hypohydration (31), neuromuscular changes within the central nervous system (19) and an altered metabolic profile in the activity-dependent muscle groups (25). Apart from all these physiological alterations during exercise in the heat, perceptual responses [thermal sensation (T_{sens}), thermal comfort and perception of effort] are also affected. Traditionally the effects of heat on these perceptual responses have been explained as a consequence of the increased physiological strain (31). However, we still do not know why perception of effort is higher during exercise in the heat. This could be due to the increased physiological strain, but direct effects of heat on the brain could also be the cause (20).

Similarly, we do not know why perception of effort during exercise is higher in mentally fatigued individuals (34). However, we can exclude physiological strain (17) and neuromuscular fatigue (24) as there have been no differences observed in these parameters due to mental fatigue. This underlines that the mechanisms causing the higher perception of effort during exercise in conditions of mental fatigue and heat are currently largely unknown. If the mechanisms between the two stressors differ, then one mechanism could add to the other and cause perception of effort to raise further and consequently deteriorate endurance performance even more.

Qian et al. (29) were one of the first to combine both mental fatigue and heat. After thermal exposure [normothermic (25 °C, 1 h) and hyperthermic condition (50 °C, 1 h)], twenty participants performed a twenty-minute psychomotor vigilance test while task-related cerebral blood flow was being registered in a scanner. This revealed that prior heat stress has a potential fatigue-aggravating effect while performing a task demanding continuous attention. They observed a decreased resting-state cerebral blood flow in the fronto-parietal cortex after the heat exposure compared to a thermoneutral situation. This was associated with subsequent slower reaction times, consequently indicating heat may accelerate the occurrence of mental fatigue. The fronto-partietal cortex is an area in the brain that encompasses multiple regions (e.g. dorsolateral prefrontal cortex (DLPFC), anterior prefrontal cortex (APFC) and somatosensory association cortex (SAC)). Specific changes in brain-activity in this part of the cortex have frequently been associated with an impaired cognitive performance and mental fatigue (2, 11, 14, 36).

From the above it is clear that mental fatigue impairs endurance performance and that heat stress may accelerate the occurrence of mental fatigue. Heat stress might augment the effect of mental fatigue on endurance performance by aggravating the mental fatigue induced by a given prolonged demanding cognitive task and/or by affecting perception of effort through a different and additive mechanism. Therefore the main aim of this study was to examine whether mental fatigue decreases subsequent endurance performance in the heat as measured by a time trial (TT). Such TT protocols have been shown to have a high reproducibility (13). Before the self-paced TT we included a fixed-workload period to accentuate fatigue in the heat and better quantify the effects of mental fatigue on physiological and perceptual responses to endurance exercise. From a more applied point of view, this endurance task simulates many cycling races in which a peloton covers the first three quarters of a race at a slower pace than the last quarter. Additionally it would be useful to know whether mental fatigue exerts the same negative influence on endurance performance in warm conditions as has been observed in normal ambient temperatures (5% (22) and 2% (16) decrements). Therefore this study provides useful insights for athletes competing in major sport events like the 2022 (in Qatar) FIFA World Cup and the 2020 Olympic Games in Japan that take place in such a warm climate.

We hypothesized mental fatigue would be induced in the heat, characterized by subjective (higher ratings of mental fatigue (4, 17, 33)), neurophysiological (lower P3b-amplitude (11, 14) and higher fronto-parietal theta (θ)- and alpha (α)-activity (2, 36) due to mental fatigue) and behavioral measures (decreased accuracy and increased reaction time (RT) in time due to mental fatigue (17, 33)). Mental fatigue would subsequently negatively affect performance on the endurance task in the heat. More specifically we expect a higher perception of effort during the 45-min fixed workload part, whilst the time trial (TT) would take longer to complete due to mental fatigue (4, 16, 17). We also expected a bigger decrease in TT-performance due to mental fatigue in heat than the impairments observed in normal ambient temperatures (~3.5%) (16, 22).

2. Methods

2.1 Subjects and ethical approval

Ten trained male cyclist or triathletes (mean ± SD; age: 22 ± 3 y, height: 184 ± 4 cm, weight: 74 ± 7 kg, W_{max}: 332 ± 42 W) volunteered to participate in this study. None of the subjects had any known mental or somatic disorder. Our subjects can be included in the performance level 3 in the classification of subject groups in sport science research (6). 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. All subjects were given written instructions describing all procedures related to the study but were naive of its aims and hypotheses.

2.2 Experimental protocol

On the first visit to the lab subjects underwent a medical examination by a physician. 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. Subjects then completed a maximal cycle ergometer test to determine the maximal wattage (W_{max}). This maximal exercise test was conducted progressively on a cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) in order to determine W_{max} as accurately as possible: the test started at 80 W for 3 min, thereafter the resistance was increased by 40 W each 3 min until exhaustion. The W_{max} was calculated with the formula: $W_{max} = W_{out} + (t/180) \times 40$ [W_{out}: workload of the last completed stage; t: time (seconds) in the final stage]. Before the incremental test the position on the cycle ergometer was adjusted for each subject, and settings were recorded and reproduced at each subsequent visit. Subjects were also given standard instructions for overall RPE using the 15-point scale (6-20) developed by Borg (3). During the incremental exercise test, the scale low and high anchor points were established. In order to acquaint participants with the feelings of exertion that should be rated 7, they were asked to cycle unloaded at 50 rpm for 3 min before the start of the incremental exercise test. To establish the high anchor point participants were asked to assign a rating of 19 to the conscious sensations of how hard, heavy, and strenuous exercise felt at the end of the incremental exercise test.

The subjects were asked to return to the lab for 3 consecutive trials, which were all conducted in the morning and were separated by at least 5 days to ensure full recovery. The first trial was a familiarization trial (to get to know the routine, the equipment and to avoid learning effects), followed by an intervention trial and a control trial in a randomized and counterbalanced order (www.randomization.com). All trials were conducted in 30 °C and in a relative air humidity of 30 %. In both the intervention and the control trial subjects performed a 45-min cognitive task, either involving response inhibition (Stroop task) or a control task (see Cognitive tasks section). In the familiarization trial subjects completed only 15 min of the 45-min Stroop task. Preceding the beginning of the 45-min cognitive task, a urine sample was taken, the subjects' body mass was measured and all physiological measuring instruments were applied (see Physiological and psychological measurements section). After the 45-min cognitive task, subjects performed 45 min of moderate intensity cycling exercise at a fixed workload immediately followed by a self-paced time trial (TT; see Endurance task section). Heart rate (HR) and thermoregulatory measures were followed up at 5-min intervals throughout the entire protocol and RPE at 5-min intervals during the endurance task (see Physiological and psychological measurements section). Cognitive performance was tested before and after the 45-min cognitive task using a Flanker task (see Cognitive tasks section). Brain activity was measured during the 45-min cognitive task (see EEG recordings section). Self-reported mental fatigue was assessed with a visual analog scale (M-VAS) before and after the Flanker tasks, during the 45-min cognitive task and during the endurance task (see Physiological and psychological measurements section). Subjective workload was assessed after the 45-min cognitive task and after the endurance task (see

Physiological and psychological measurements section). Blood glucose levels and salivary concentration of cortisol were assessed before and after the Flanker tasks and salivary cortisol concentrations were measured again after the endurance task (see Physiological and psychological measurements section). An overview of the experimental protocol performed during the intervention and the control trial is presented in Fig. 1.

The subjects were given instructions to sleep for at least 7 hours, refrain from the consumption of caffeine alcohol and not to practice vigorous physical activity 24 hours before each visit. In addition subjects were asked to have the same meal the night before and the morning of each trial and 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 soap) the evening before the experiment.

To ensure high motivation during the Stroop task and the TTs, a reward was given to the best mean performance in the Stroop task (€50) and in the TTs (€50).

2.3 Cognitive tasks

The 45-min tasks used as experimental manipulation in the present study are similar to those used by Smith et al. (32). A 50%-incongruent Stroop task and a documentary were used respectively for the mentally fatiguing task and the control task (32). A brief description of these cognitive tasks can be found below.

2.3.1 Stroop task

The Stroop task requires response inhibition which is a form of inhibitory control. In this task, coloured words ("red", "blue", "green" and "yellow") were presented one at a time on a computer screen and participants were required to indicate the colour of the word, ignoring the meaning of the word itself. The trials were arranged in pseudo-random sequence with 50% of trials being congruent (matched word and colour), while 50% were incongruent, with all incongruent word-colour combinations being equally common. Participants were required to press the button on the keyboard that corresponded to the colour of the word displayed on screen. Each word was presented on screen in fontsize 34 for 1000 ms followed by a blank screen for 1500 ms before the next word was displayed. Therefore a new word was presented every 2500 ms providing a total of 1080 stimuli over the 45-min task. Each 15 min there was a 30-s break in the task to assess M-VAS and T_{sens}. Subjects were instructed to respond as quickly and accurately as possible and were aware that points would be awarded on both performance measures for the €50-prize.

2.3.2 Control task

The control task involved watching a 45-min documentary on the same computer screen as the one used for the Stroop task. The documentary used in this study was "When We Left Earth: The NASA Missions – Episode 6: A Home in Space" (Discovery Channel, USA). The content of this documentary has shown in a previous study (32) to be engaging, yet capable of maintaining a neutral mood and not to induce mental fatigue. In order to prevent sound-artefacts occurring in the EEG recordings (see EEG recordings), subjects watched the documentary without sound. Every 15 min, subjects refrained 30 s from watching the documentary while M-VAS and T_{sens} were assessed.

2.3.3 Flanker task

To assess the influence of the 45-min tasks on cognitive performance independently from time-on-task a modified Flanker task, identical to the one used by Weng et al. (38), was used. This task was chosen because, similar to the Stroop task, it requires inhibitory control (38). The congruency of the flanking items to the target arrows was manipulated in the modified Flanker task, resulting in three conditions: congruent (e.g., > > > > >), incongruent (e.g., < < > < <) and neutral (e.g., - - > - -). Each array of arrows was focally presented in white text (fontsize 34) for 200ms on a black background with a variable inter-stimulus interval of 1000, 1200, 1400, or 1600 ms. For each of the task conditions, 40 trials were presented randomly with right and left target arrows occurring with equal probability, yielding a total of 120 trials. Total Flanker task duration was approximately 5 min. To assess performance on the Flanker task accuracy and RT were collected and 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.

2.5 Endurance task

Subjects had to perform 45-min cycling at 60% W_{max}, immediately followed by a TT that requires the subjects to complete a predetermined amount of work equal to 15 min at 80% W_{max} as quickly as possible. Throughout the endurance task subjects were not verbally encouraged by the experimenter to ensure no bias occurred in motivating subjects. Subjects performed the task on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) and had ad libitum access to plain non pre-cooled water.

2.5.1 45-min fixed workload part

During the 45-min fixed workload part, the cycle ergometer was set in the hyperbolic mode so that the workload (60% W_{max}) was independent of pedaling rate (RPM). Cadence was freely chosen between 60 and 120 RPM. Feedback on elapsed time, RPM, power output and HR was not available to the subject.

2.5.2 Self-paced time trial part

One to two min (to program the TT-protocol) after the 45-min fixed workload part of the endurance task, the self-paced TT began. Similar to the initial part, the cycle ergometer was set in hyperbolic mode. As stated above, the TT required the subjects to complete a predetermined amount of work equal to 15 min at 80% W_{max} as quickly as possible. Subjects began the TT at a workload corresponding to 80% W_{max}, but were free to in- or decrease their power output as desired from the outset. If subjects indicated (orally) they wanted to in- or decrease their power output, the experimenter respectively increased or decreased the workload by a standardized amount of 5 W. Again cadence was freely chosen between 60 and 120 RPM and subjects only received feedback regarding RPM if they dropped below or above the given interval. Furthermore no feedback was provided regarding power output or HR. However they did get feedback regarding the amount of work produced in relation to their goal (equal work to 15 min at 80% W_{max}). Therefore a graph was displayed where the amount of work was depicted on the y-axis and on the x-axis the amount of time elapsed. Subjects were instructed to produce the predetermined amount of work as quickly as possible and were aware that mean performance on the TT was scored for the €50-prize.

2.6 Physiological and psychological measurements

2.6.1 Heart rate

Heart rate was recorded continuously (followed up at 5-min intervals) throughout the entire protocol using a HR monitor (Polar RS400, Polar Electro Oy, Kempele, Finland).

2.6.2 Hydration status and body mass

A urine sample was taken and analyzed for specific gravity (pocket refractometer; Atago, Japan) preceding the start of the protocol and at the end of the protocol. If a hydration status higher than 1.020 was observed, subjects were instructed to drink ~20cl of water to prevent them from starting the protocol in a too dehydrated state. Body mass was also measured before and after the protocol to observe weight loss or gain. As the subjects had ad libitum access to plain water during the protocol the amount of water drunk was also measured to take this into account.

2.6.3 Thermoregulatory measures

During the entire protocol, thermoregulatory measurements were recorded every 5 min. To measure T_{core}, subjects inserted a rectal thermistor 10 cm beyond the anal sphincter (Gram Corporation LT-8A, Saitama, Japan). Skin temperature probes (Gram Corporation LT-8A, Saitama, Japan) were attached to four sites (chest, upper arm, thigh and calf). Subsequently mean weighted skin temperature (T_{skin}) was measured according to the method described by Ramanathan (30). T_{sens}, the subjective feeling of heat, was assessed using a 21-point scale ranging from unbearable cold to unbearable heat.

2.6.4 Blood lactate and glucose

Capillary blood was collected at the ear lobe for the determination of blood lactate (Bla) (determined enzymatically; EKF; BIOSEN 5030, Magdeburg, Germany). Bla levels were measured before and after the 45-min task, during the endurance task (at 5-min intervals) and after the endurance task.

2.6.5 Salivary cortisol

Saliva was used to test for cortisol responses due to its ease of compliance, low invasiveness, and ability to track the biologically active “free” hormone . Saliva (2ml) was collected by passive drool into sterile containers, and these were stored at −80 °C until assay. A saliva sample was taken before the 45-min task, after the 45-min task and after the endurance task. The saliva samples were analysed in duplicate using the “Cortisol II” test of Roche on the Cobas e601 analyser.

2.6.6 Self-reported mental fatigue

Self-reported mental fatigue was measured using M VAS before, during (every 15 min) and after the 45-min task, during (every 15 min) and after the endurance task. Participants were asked to indicate their perceived level of mental fatigue (from not all to completely exhausted) by placing a mark on a 10-cm line.

2.6.7 Perception of effort

During the endurance task, perception of effort was measured at the beginning and each five minutes thereafter using the 15-points RPE scale (3) anchored during the incremental exercise test.

2.6.8 Subjective workload

The National Aeronautics and Space Administration Task Load Index (NASA-TLX;) was used to assess subjective workload. Participants completed the NASA-TLX after the 45-min task and after the endurance task in accordance with a study of Pageaux et al. (24).

2.7 Electroencephalographic recordings and analysis

During the 45-min task preceding the endurance task, brain activity was continuously measured. 32 active Ag/AgCl electrodes were attached on the subjects' head (Acticap, Brain Products, Munich, Germany), according to the "10–20 International System" . 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 and subjects were seated in a dim lit room. During EEG recordings, subjects were seated, 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.

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 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, middle and last 15 min of the Stroop task) artifacts were semi-automatically removed. Then the different stimuli (congruent=S3; incongruent S5) 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 [inferior/orbitofrontal cortex (F7), broca's area (mean of electrodes FC6 and F8), dorsolateral prefrontal cortex (mean of electrodes F3, Fz and F4), anterior prefrontal cortex (mean of electrodes FP1 and FP2), premotor cortex (mean of electrodes FC1 and FC2)] and P3b [somatosensory association cortex (SAC; Pz), angular gyrus (AG; mean of electrodes P3 and P4), fusiform gyrus (FFG; mean of electrodes P7, P8, PO9 and PO10)] components in their specific region of interest (ROI). The P2 is known to be frontally distributed (9) and was therefore analyzed in the frontal ROI, it has been related to attentive stimulus evaluation or the recall of task rules (10). The P2 was defined as the largest positive-going peak occurring within the time window between 150 and 250 ms. The N2 is usually interpreted as an index of conflict monitoring (8) and emerges fronto-centrally after the P2 (9), thus also for the N2 the frontal ROI were analyzed. The N2 was defined as the largest negative-going peak occurring within the time window between 250 and 400 ms. The P3b is linked to salience processing and appears to occur when subsequent attentional resource activations promote memory operations in temporal-parietal areas (27), therefore the FFG, the AG and the 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 200 and 450 ms. Thereafter, the data from Brain Vision Analyzer was exported to SPSS (version 22.0; SPSS, Chicago, IL) for further analysis.

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 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 during both 45-min tasks (first, middle and last 5 min)] 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 (36). 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. 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 mentioned in the ERP analysis with the addition of the primary motor cortex (mean of electrodes C3, Cz and C4).

2.7 Statistical analysis

All data are presented as means ± standard deviation (SD) unless stated otherwise. The one-sample Kolmogorov-Smirnov 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. Paired t-tests were used to assess the effect of condition (intervention vs. control) on mean HR during both 45-min tasks, and on NASA-TLX scores after the 45-min task, and after the endurance task. The effects of condition and time on salivary cortisol were analysed with two-way repeated measure (2 x 3) ANOVAs. Two-way repeated measure (3 x 2) ANOVAs were used to test the effect of time (first, middle and last 15 min) and stimuli (congruent and incongruent) on response accuracy and RT during the Stroop task. Two-way repeated measure (2 x 4) ANOVAs were used to test the effects of condition and time on M-VAS during the 45-min task and during the fixed workload part of the endurance task, during the self-paced part a paired t-test was employed to test the effect of condition. For the EEG-data (θ, α1, α2, β1, β2) three-way repeated measure (2 x 3 x 9) ANOVAs were employed with condition, time and ROI as factors. The different ERP-components (P2, N2 and P3b) were also analyzed with a three-way repeated measure ANOVA with factors time, stimulus-type and ROI. Three-way repeated measure (2 x 2 x 3) ANOVAs were used to test the effects of condition, time and stimuli on mean accuracy and RT during each Flanker task. Two-way repeated measure ANOVAs were used to test the effects of condition and time on HR, T_{core}, T_{skin}, RPE, T_{sens} and Bla during the fixed workload (2 x 10) and the self-paced part (2 x 3; time: 5 min, 10 min and end-point) of the endurance task. If significant interaction effects in the three-way or two-way repeated measure ANOVAs were observed, respectively two-way repeated measure ANOVAs or paired t-tests were performed in order to interpret the effect of condition (intervention vs. control) in each time interval. If no significant interaction effects were observed in the three-way or two-way repeated measure ANOVAs, main effects were immediately observed and further interpreted through pairwise comparisons with Bonferroni correction. Significance was set at 0.05 for all analyses, which were conducted using the Statistical Package for the Social Sciences, version 22 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Markers of mental fatigue

Various physiological, subjective and behavioural markers

Mean HR did not differ during the Stroop task (69 ± 8 beats/min) compared to the control task (67 ± 6 beats/min). The data of the NASA-TLX were not normally distributed and therefore Wilcoxon Signed Ranks Tests were employed. This revealed that 5 out of 6 subscales were perceived as higher/more demanding after the Stroop task compared to the control task. Mental demand (p=0.005), temporal demand (p=0.007), performance (p=0.05), effort (p=0.005) and frustration (p=0.008) were perceived as higher, or worse in the case of performance, in the Stroop task. This subjective higher perceived demand of the Stroop task is confirmed by the cortisol data. Cortisol values were normalized to the value of the saliva sample taken at the beginning of each trial (=100%). An interaction between the condition- and the time-effect was displayed for the normalized cortisol data (F(2, 14)=7.5; p=0.006). Significantly higher cortisol levels were found after the Stroop task compared to after the control task (p=0.033; Fig. 2). Subjectively a higher self-reported mental fatigue was observed after 30 (p=0.006) and 45 min (p=0.002) in the Stroop task compared to in the control task. The accuracy and RT during the Stroop were normalized to the performance in the first 15 min of the task (=100%), however no decrements were observed (for absolute values, see Table 1).

Spectral power analysis

The spectral power data of the first, middle and last 15 min of the Stroop task were normalized to the eyes open-condition before the beginning of the Stroop task (=0%) for each specific frequency band (θ, α1, α2, β1, β2). *θ*. No interactions were observed for θ-activity, it increased significantly in time (F(3, 27)=10.3; p<0.001) and was significantly higher (F(1, 9)=5.2; p=0.048) in the Stroop task (28 ± 5%) compared to in the control task (14 ± 5%). *α1*. The lower alpha band showed a significant main effect of time (F(3, 27)=4.1; p=0.017) and an interaction effect of condition with ROI (F(8, 72)=2.3; p=0.030). In the APFC a subsequent interaction of condition with time was observed (F(3, 27)=5.2; p=0.006). The follow-up paired t-tests showed that only in the middle and last 5 min α1-activity was higher in the Stroop task compared to the control task (p≤0.006; Fig. 3). In the other eight ROI no interaction of condition with time or main effect of condition was observed. *α2*. For the upper alpha band a significant interaction effect of condition with time was observed (F(3, 27)=4.6; p=0.010). A subsequent two-way repeated measure ANOVA (Cond x ROI) in each time interval revealed that in the middle and last 5 min of the cognitive task α2-activity was significantly higher in the Stroop task compared to the control task (F(1, 9)≥5.9; p≤0.037) independently from ROI. *β1*. The lower beta band-activity showed a significant increase in time (F(3, 27)=8.0; p=0.001), however no effect of condition or ROI was observed. *β2*. Similar for the upper beta band-activity only a significant increase in time was observed (F(3, 27)=5.7; p=0.004), condition or ROI again had no effect.

ERP analysis

P2. No interaction effects for P2-amplitude or –latency and also no main effect of time or stimulus-type was observed. N2. No interaction or main effects were found for the N2-amplitude or -latency. P3b. The three-way ANOVA showed that the ROI- and time-effect on P3b-amplitude interacted with each other (F(1.9, 17.3)=3.9; p=0.041). To further unravel the time-effect, a two-way ANOVA (time x stimulus-type) was employed in each ROI. This revealed that amplitude only decreased over time in FFG (F(2, 18)=4.4; p=0.027; Fig. 4) from 3.9 ± 0.7 µV in the first 15 min to 3.2 ± 0.6 µV in the middle 15 min and 2.9 ± 0.5 µV in the last 15 min. In case of the P3b-latency no interactions between the different factors were found, a main effect of time was however present (F(2, 18)=8.1; p=0.003). There was an increase (p=0.002) in latency from the first (311.2 ± 10.9 ms) to the middle 15 min (327.8 ± 11.1 ms), where after it plateaued and even slightly decreased from the middle to the last 15 min (321.2 ± 12.8 ms).

Flanker task

An interaction of condition with time was observed for M-VAS before and after each flanker task ($F(1.8, 16.3)=13.4$; $p<0.001$). In the intervention M-VAS was higher during the post-Flanker task (pre-Flanker: p=0.002; post-Flanker: p=0.001). This higher self-reported mental fatigue was however not associated with a deteriorated cognitive performance. The data for RT and accuracy were normalized to the baseline (i.e. the performance on the first Flanker task (=100%)) to account for day to day variability (for absolute values, see Table 1). In terms of RT, a main effect of time was observed ($F(1, 9)=13.4$; $p=0.005$). Subjects performed faster in the second (96 ± 1%) compared to the first Flanker task (100%) independent of stimulus-type or condition. For the accuracy-data to be normally distributed, the factor 'stimuli-type' was not accounted for and the mean of the three stimuli-types was used, subsequently the effect of condition and time was observed in a two-way ANOVA. Accuracy was found to decline in time ($F(1, 9)=24.5$; $p=0.001$) independently from condition. It dropped from the first (100%) to the second Flanker task (98.9 ± 0.2%; p=0.001). No interaction or main effect of condition was observed.

3.2. Physiological and psychological responses during the fixed workload part of the endurance task

Physiological responses

HR was not significantly altered in both conditions during the fixed workload part, in the intervention trial mean HR was 152 ± 2 bpm and in the control trial this was 152 ± 3 bpm. Non-parametric tests showed that there were no differences in Bla between conditions in any time interval throughout the fixed workload part. In both conditions Bla increased in the first 5 min ($Z\geq-2.5$, $p\leq0.013$) and reached a plateau afterwards (intervention: 2.0 ± 1.0 mmol/L, control: 2.1 ± 0.8 mmol/L). T_{core} ($F(1.8, 14.6)=430.6$; $p<0.001$) and T_{skin} ($F(2.4, 21.4)=86.6$; $p<0.001$) rose throughout the fixed workload cycling part until 38.6 ± 0.3 °C and 36.3 ± 0.4 °C respectively. There was however no difference in T_{core} and T_{skin} between both conditions. T_{core} data of only 9 subjects were used in this analysis.

Perceptual responses

An interaction between condition and time was observed for M-VAS during the fixed part of the cycling task (F(3, 27)=12.3; p<0.001; Fig. 5). Self-reported mental fatigue was higher at the start and after 15 min in the fixed workload part of the cycling task in the intervention compared to control (p≤0.012; Fig. 5). RPE and T_{sens} data were not normally distributed, Wilcoxon tests pointed out that RPE and T_{sens} did not differ significantly in any time interval between both conditions.

3.3. Performance, and physiological and psychological responses during the self-paced time trial part of the endurance task

Endurance performance

The TT was completed in 906 ± 30 s in intervention and in 916 ± 29 s in control. These performances did not differ significantly from each other. The selected power output was, as the TT-time already indicated, similar in both conditions and decreased in time (F(2.0, 17.6)=4.3; p=0.030) independently from condition.

Physiological responses

HR (F(1.2, 10.8)=46.8; p<0.001), Bla (F(2, 18)=24.1; p<0.001) and T_{core} (F(1.1, 8.9)=239.4; p<0.001) increased significantly during the TT. At the end of the TT participants reached a mean HR of 186 ± 3 bpm, mean Bla of 7.1 ± 0.9 mmol/L and a mean T_{core} of 39.1 ± 0.1 °C. No interaction or main effect of condition was observed. Similarly, also for cortisol no effect of condition was observed before and after the endurance task.

Perceptual responses

M-VAS increased both in intervention (p=0.045) and in control (p=0.011) and neither at the start or at the end of the TT a significant difference in M-VAS between conditions was observed. The data of the NASA-TLX was not normally distributed, none of the subscales in relation to the endurance task were perceived differently between conditions. The RPE data had also to be tested non-parametrically, RPE increased significantly in both conditions during the TT ($Chi^2 \geq 14.1$; $p \leq 0.001$) and eventually reached a mean value of 19 ± 1 over all trials. No effect of condition was observed. T_{sens} increased significantly during the TT ($F(2, 18)=23.1$; $p<0.001$) up to 8.4 ± 0.4, no interaction or condition effect was however observed.

4. Discussion

This is the first study that looked at the effect of mental fatigue on endurance performance and cognitive performance in the heat (30°C) in performance level 3 (6) trained athletes.

Markers of mental fatigue

The importance of monitoring subjective, behavioral, and physiological markers of mental fatigue and the interactions between all three manifestation areas to conclude whether mental fatigue was induced or not has been highlighted in the review of Van Cutsem et al. (34). In the present study we strived towards such a quantification of mental fatigue. Subjectively participants rated the Stroop task as more mentally demanding on the NASA-TLX. Physiologically only the Stroop task interfered with cortisols' circadian rhythm. Salivary cortisol levels were higher post-Stroop compared to post-control task, indicating that the Stroop task was more stressful than the documentary. The higher mental demand and stress during the Stroop task eventually resulted in the occurrence of mild mental fatigue. This was indicated subjectively by the higher M-VAS score and was further substantiated physiologically by the neurophysiological indices. Higher θ- and α2-activity was observed in the intervention compared to control throughout all the ROI in the middle and the last 5 min. α1-activity was specifically higher in the APFC during the middle and the last 5 min in intervention compared to control. A recent study of Wascher et al. (36), in which participants had to perform a spatial stimulus-response-compatibility task for an overall duration of 4 h, reported mental fatigue is specifically associated with an increase in frontal theta- (θ) and frontal and occipital alpha- (α) activity. These specific changes in brain-activity indicate a reduced level of arousal and subsequent attention deficits (2). Also the ERP measures indicated mental fatigue was successfully induced. The P300 is a component of an ERP that appears around 300 ms after the onset of a stimulus and its amplitude is suggested to serve as an electrophysiological marker of attentional resource allocation, while its latency reflects the speed of stimulus evaluation (38). Within the P300 a distinction can be made between the P3a that is linked to novelty detection and appears when non-target distractor stimuli are processed and the P3b that appears to occur when subsequent attentional resource activations promote memory operations in temporal-parietal areas (27). In the FFG, a brain area known for object recognition and reading (37), the P3b-amplitude decreased in time, while the P3b-latency increased in time during the Stroop task in the present study. Käthner et al. (14) and Hopstaken et al. (11) both studied the P3b on the Pz-electrode (=an electrode in the parietal region) during a mentally fatiguing task and also found a decrease in P3b amplitude with increasing self-reported mental fatigue and time-on-task. Polich suggested (27) that the P3b is related to temporal–parietal activity, an area where dense norepinephrine inputs are found (27). In addition he also associated P3b-amplitude with dopaminergic activity (28). The associations Polich (27, 28) makes indicate that the altered P3b-amplitude and -latency observed in the present study suggest that altered neurotransmission (i.e. decreased norepinephrine- and dopamine-activity) has a role in the state of mental fatigue. Only behavioral measures did not substantiate that a state of mental fatigue was successfully induced. Contrary to other studies in the field (17, 33), no effect of time was observed in terms of accuracy or RT during the Stroop task. Despite not observing the typical decrease in accuracy and RT associated with mental fatigue, there are arguments to state mild mental fatigue was successfully induced and to expect a decrease in subsequent endurance performance similar to previous studies (16, 17, 22, 33). Studies of Macmahon et al. (16) and Pageaux et al. (22) also did not observe a decrease in accuracy nor an increase in RT with prolonged performance on the mentally fatiguing task and still detected significant reductions in a subsequent endurance task due to mental fatigue.

The Flanker task was included in the study, as proposed in the review of Van Cutsem et al. (34), to be able to quantify cognitive performance independently from time-on-task effects during the Stroop task. In terms of performance on the Flanker task our data only partly confirmed this hypotheses. Accuracy during the Flanker task indeed decreased pre to post the Stroop task, but this was also the case pre to post the control task. The RT-data even contrasted our hypotheses. Instead of increasing, RT during the Flanker task decreased pre to post both 45-min tasks. A trade-off effect between RT and accuracy could possibly explain these results. Meaning that participants adapted their strategy within a trial and performed faster in the post-Flanker task while sacrificing accuracy. Besides a trade-off effect, switching between tasks could also have had a motivational effect that possibly masked a negative effect of mental fatigue on the Flanker task (12). Another explanation could be that participants, despite a 30-min adaptation period to the environmental conditions, did not reach a steady baseline level when performing the pre-Flanker task. Subsequently the faster RT in the post-Flanker task can be explained as an adaptation-effect to the heat stress. This adaptation could also clarify why no higher RT during the Flanker task was observed with after the 45-min Stroop task. However this is rather speculative and because of the fact no effect of condition was found in RT or accuracy during the Flanker task, it was concluded to define the mental fatigue induced in this study as ‘mild’.

Effects of mild mental fatigue on endurance performance in the heat

The endurance task consisted of two parts, a fixed workload part and a subsequent TT. During the fixed workload part, the effects of mild mental fatigue on physiological and perceptual measures could be more accurately assessed, while during the subsequent TT the effect of mild mental fatigue on endurance performance was evaluated. In order to monitor the state of mental fatigue during the fixed workload part of the endurance task, M-VAS was taken each 15 min. According to this measure, mental fatigue was higher during the intervention trial compared to control only in the first 15 min of the endurance task, and decreased thereafter. Contrary to our hypotheses the perceptual measures (i.e. perception of effort and T_{sens}) were not affected by this state of mild mental fatigue. The physiological data did confirm our hypotheses. HR and Bla were unaffected by the mild mental fatigue, confirming the findings of previous studies (16, 17, 33). Moreover thermoregulatory measures, T_{core} and T_{skin}, during exercise in the heat were also unaffected by mild mental fatigue. This adds to the mounting evidence that mild mental fatigue indeed does not influence the traditional physiological responses thought to limit endurance performance. In the subsequent TT, pacing and performance time were the main variables of interest. Contrarily to our hypotheses, both performance time and pacing during the TT were unaffected by mild mental fatigue. Likewise the physiological and perceptual responses during the TT were unaffected. Multiple explanations for these diverging results compared to previous research (4, 16, 17, 22, 33) are possible. First, performing the Stroop task for 45 min might have been insufficient to induce mental fatigue in an already stressful environment (i.e. in the heat). However multiple findings are presented (see markers of mental fatigue) in this study to support that mild mental fatigue was present, and to a similar extent, compared to previous studies (16, 17, 22). A mentally demanding cognitive task as short as 30 min has been shown to negatively affect subsequent endurance performance (22). The mentally fatiguing task in the present study was longer compared to the study of Pageaux et al. (22). Second, the fixed workload part of 45 min could have washed out the effect of mild mental fatigue on performance. Potentially the mild mental fatigue induced in the present study could affect endurance performance in longer duration or open-loop tests. Another possibility could be that, as suggested by the study of Martin et al. (18), endurance trained athletes are more resistant to the negative effects of mild mental fatigue on subsequent endurance performance. The population tested in the present study was slightly better trained [i.e. performance level 3 according to De Pauw et al. (6)] compared to the populations (i.e. performance level 2) used in other studies that did find a negative effect of mental fatigue on endurance performance (17, 22). Therefore the better training status of our participants may explain, in part, the lack of an effect of mild mental fatigue on endurance performance (18). A last and possibly the most reasonable explanation is that mild mental fatigue does not further reduce endurance performance when the brain is already stressed by a hot environment. Consequently we speculate that a floor effect was observed in the present study. Meaning that if one stresses the brain (e.g. heat stress, mental fatigue, …) endurance performance will decrease, at some point however further stressing the brain (e.g. combining heat stress and mental fatigue) will not result in a further reduction of performance and a floor effect is observed. This emphasizes the importance of the brain in endurance performance and might indicate that it is irrelevant which stressor (heat and the increased physiological strain or mental fatigue) leads towards a higher perception of effort; relevant is whether or not the stressor increases perception of effort (see the psychobiological model; (21)(21)). The higher perception of effort experienced during endurance exercise in a hot environment or when mentally fatigued may share a common psychobiological mechanisms: negative valence. Exercising in the heat is associated with thermal discomfort whilst mental fatigue is known to induce a more negative mood. The valence of emotional stimuli have been shown to affect perception of effort (1) and the activity of the cingulate cortex (7), prefrontal and premotor cortical areas (5) related to perception of effort (39, 40). So it is plausible that a similar psychobiological mechanism may explain the effects of heat stress and mental fatigue on perception of effort and why the effects of the two stressors do not summate. In other words, in conditions of thermal discomfort, the negative effects of mild mental fatigue on mood may not lead to further increase in negative valence and perception of effort. EEG data support this hypothesis; Nybo & Nielsen (20) observed that perception of effort during prolonged exercise in hot environments is associated with changes in cerebral electrical activity rather than changes in the electromyogram of the exercising muscles. They reported a higher α/β-activity ratio in the heat, mainly due to a ~50% lower β-activity in the heat (20). Mental fatigue has been repeatedly associated with elevated frontal θ and frontal, central and parietal α-power (15, 36), an association that also is supported by the results in the present study. Consequently mental fatigue might also increase perception of effort via the same mechanism proposed by Nybo & Nielsen (20), raising α/β-activity ratio during an endurance task. This makes a floor effect neurobiologically plausible.

Conclusion

The subjective workload scale and the higher salivary cortisol levels after the Stroop task substantiated perceptually and biologically that the Stroop task was more mentally demanding and stressful than the control task. The demanding nature of the Stroop task eventually caused increases in θ-, α1-, α2-activity and P3b-latency and a decrease in P3b-amplitude. These results and the higher M-VAS support that at least a 'mild form' of mental fatigue was induced. The mild mental fatigue did however not influence participants' psychological or physiological responses during the endurance task, nor their performance. Possible explanations are: 1) the mild form of mental fatigue was insufficient to alter performance on a subsequent endurance task or 2) endurance trained athletes are resistant to the negative effects of mild mental fatigue on subsequent endurance performance or 3) mild mental fatigue does not reduce endurance performance when the brain is already stressed by a relatively hot environment (30 °C).

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The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Conflict of interest

The results of the present study do not constitute endorsement by ACSM. No conflict of interest is declared by the authors.

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Fig. 1 Overview of the protocol depicted on a timeline (min).

Fig. 2 Saliva cortisol levels before (pre-CT; pre 45-min task), after the cognitive task (post-CT; post 45-min task) and after the physical task (post-PT; post endurance task) in the intervention (INT) and control (CON). ^ denotes a significant difference compared to the previous time-point ($p<0.05$), * denotes a significant difference between conditions ($p<0.05$). Data are presented as means ± SE.

Fig. 3 α1-power spectral density during EO, first, middle and last 5 min of the 45-min task in intervention (INT) and control (CON). * Denotes a significant difference between conditions ($p<0.05$). Data are presented as means ± SE.

Fig. 4 Grand-average ERPs at P8 elicited by all (congruent and incongruent) stimuli in the first 15 min, middle 15 min and last 15 min during the Stroop task in intervention. ◊ Denotes a significant main effect of time ($p<0.05$).

Fig. 5 Self-reported mental fatigue during the fixed workload part of the endurance task, at the begin (P0), after 15min (P15), after 30min (P30) and at the end (P45). ^ Denotes a significant difference compared to the previous time-point ($p<0,05$). * Denotes a significant difference between conditions ($p<0,05$). Data are presented as means ± SE.

Figure 1

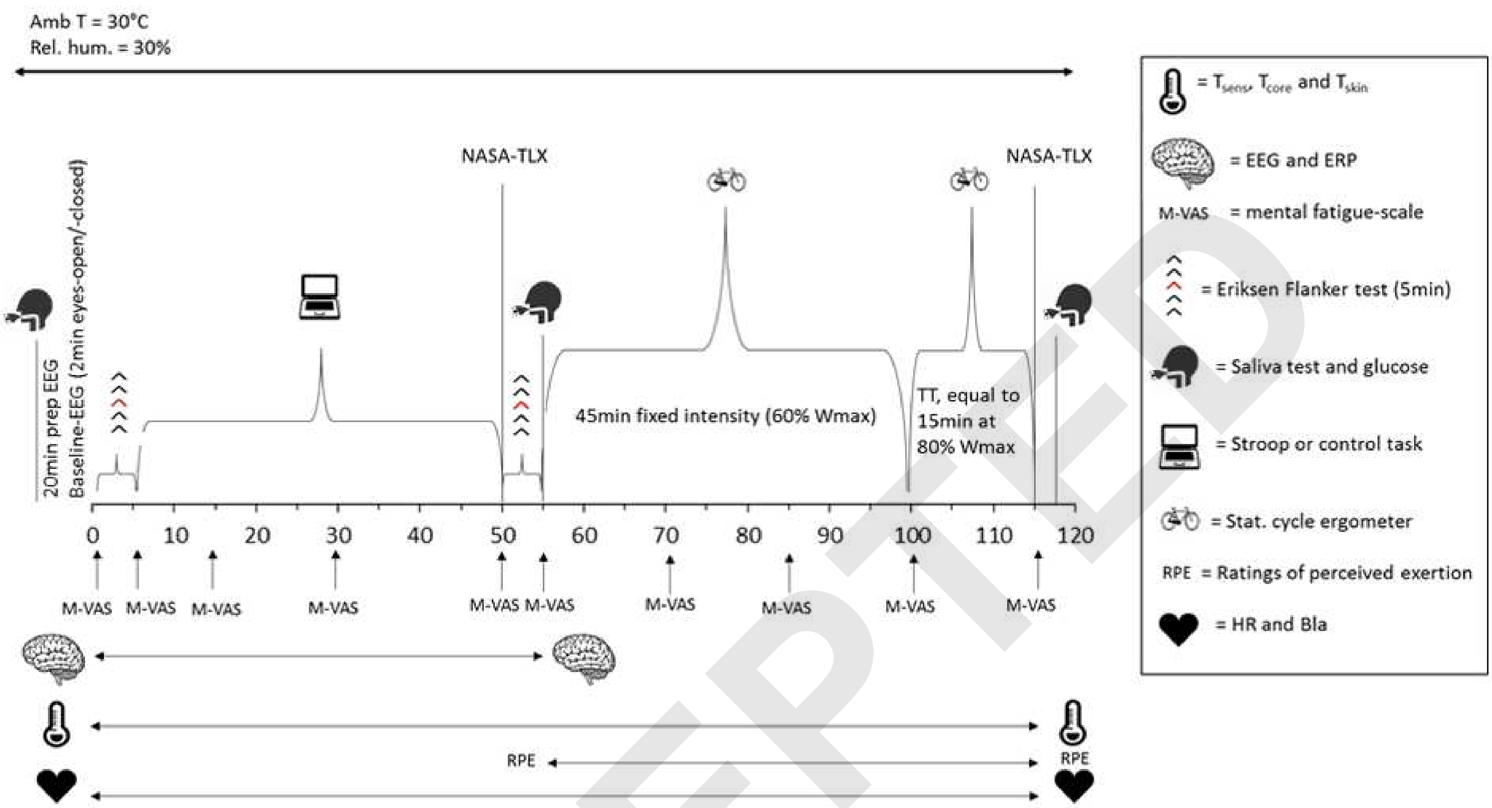
Amb T = 30°C
Rel. hum. = 30%
NASA-TLX
NASA-TLX
20min prep EEG
Baseline-EEG (2min eyes-open/-closed)
45min fixed intensity (60% Wmax)
TT, equal to 15min at 80% Wmax
0 10 20 30 40 50 60 70 80 90 100 110 120
M-VAS
RPE
RPE
= T_sens, T_core and T_skin
= EEG and ERP
M-VAS = mental fatigue-scale
= Eriksen Flanker test (5min)
= Saliva test and glucose
= Stroop or control task
= Stat. cycle ergometer
RPE = Ratings of perceived exertion
= HR and Bla

Figure 2

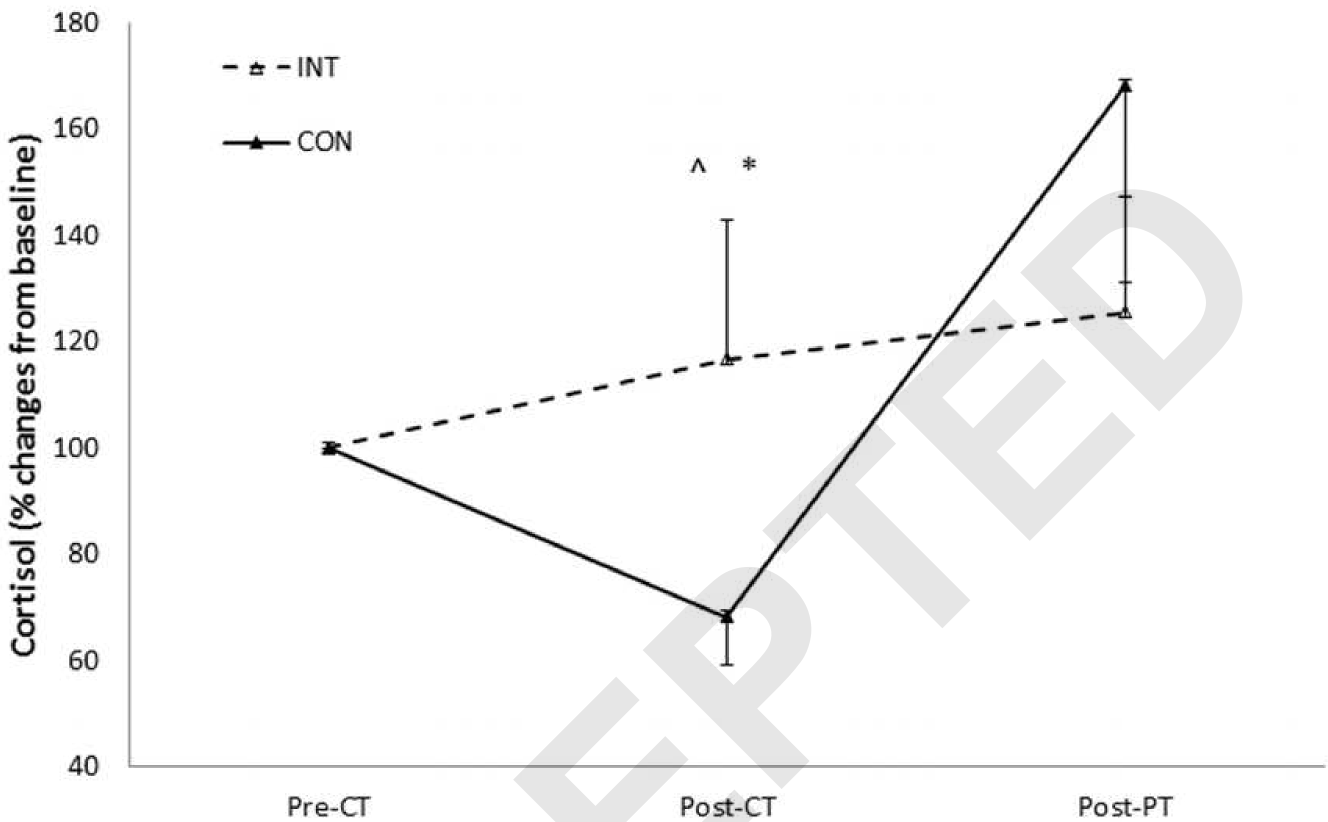
180
160
140
120
100
80
60
40
Cortisol (% changes from baseline)
INT
CON
^
*
Pre-CT
Post-CT
Post-PT

Figure 3

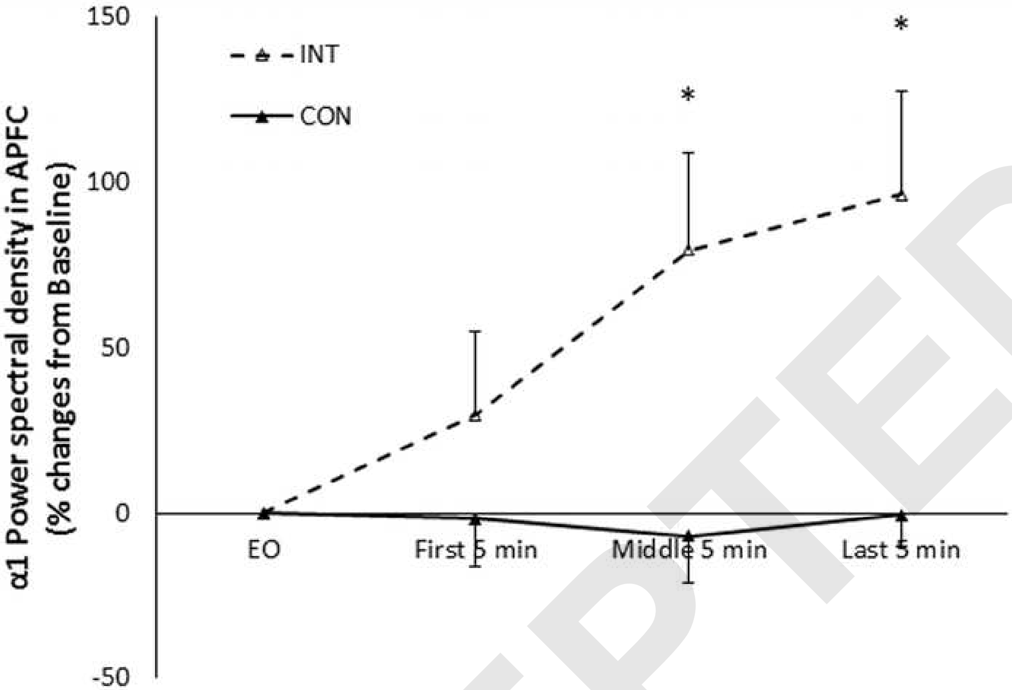
150
100
50
0
-50
INT
CON
*
*
α1 Power spectral density in APFC
(% changes from Baseline)
EO
First 5 min
Middle 5 min
Last 5 min

Figure 4

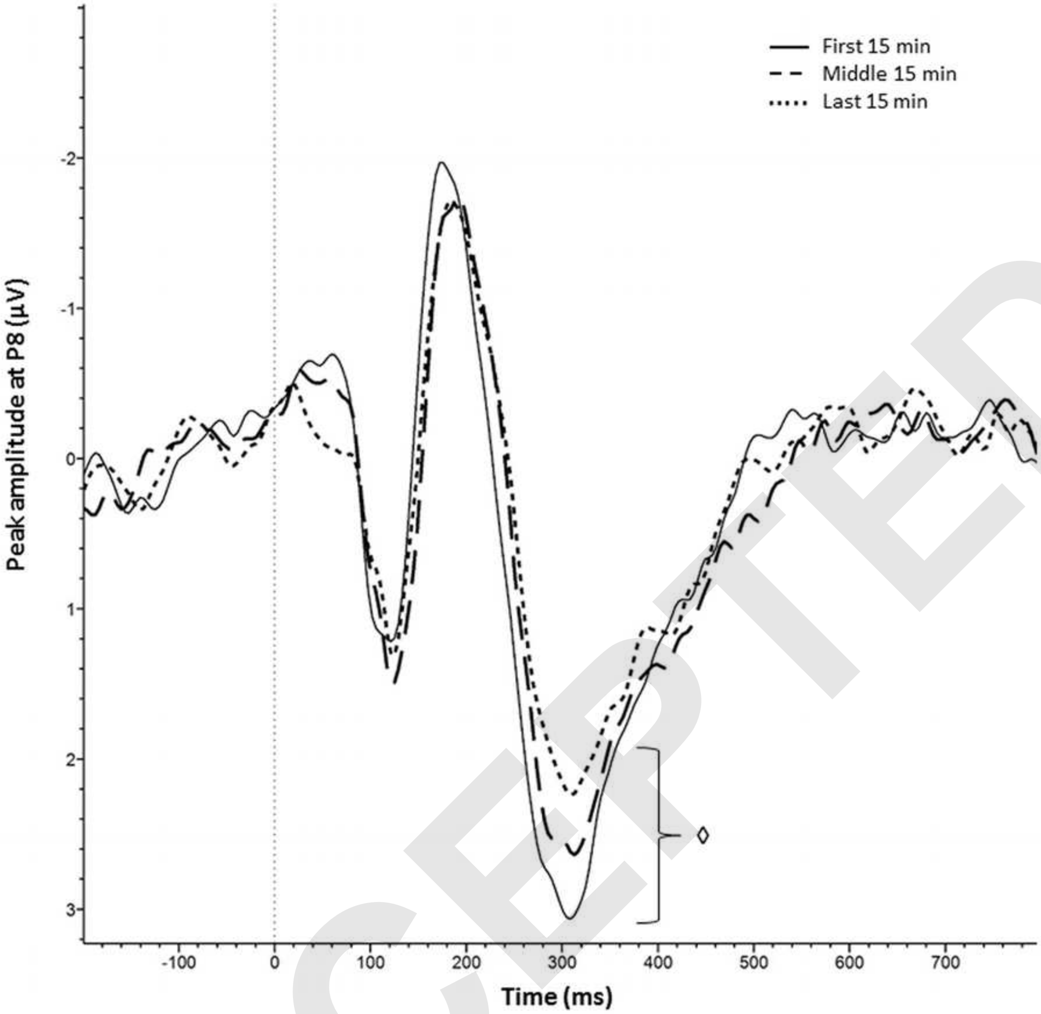
First 15 min
Middle 15 min
Last 15 min
Peak amplitude at P8 (μV)
-2
-1
0
1
2
3
-100
0
100
200
300
400
500
600
700
Time (ms)
◊

Figure 5

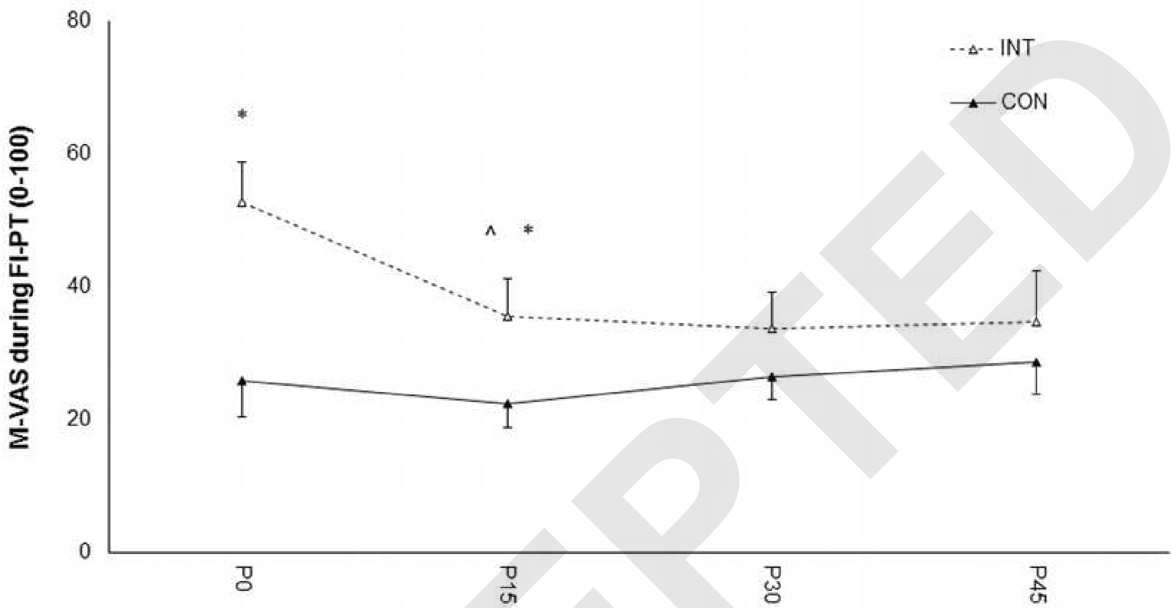

Table 1. RT and accuracy during pre Flanker, first, middle and last 15 min of Stroop and post Flanker, independent of stimulus-type

	INT	CON	INT	CON
Block	**RT ± SD (ms)**	**RT ± SD (ms)**	**Accuracy ± SD**	**Accuracy ± SD**
Pre Flanker	413 ± 13	398 ± 10	0.97 ± 0.1	0.97 ± 0.1
Stroop: First 15 min	633 ± 20	//////////	0.85 ± 0.3	//////////
Stroop: Middle 15 min	632 ± 21	//////////	0.85 ± 0.4	//////////
Stroop: Last 15 min	625 ± 20	//////////	0.82 ± 0.4	//////////
Post Flanker	399 ± 9	379 ± 8	0.96 ± 0.1	0.96 ± 0.1