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Acute effect of energy boost dietary supplement on P3 waveform: double blind, placebo controlled study

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Human cognition may be enhanced by energy drinks containing caffeine and/or other stimulants, which are thought to improve attentional as well as motor performance, and reduce reaction times. Due to the fact that literature shows that even low doses of caffeine may improve cognitive performance, we investigated an acute effect of a single dose of a caffeinated energy dietary supplement, on attention and motor responses by means of event related potentials. Healthy volunteers were examined in double blind, placebo controlled study. EEG recordings from 32 channels were performed in three sessions: before the supplementation (session 1), 30 min after the supplementation (session 2) and 90 min after the supplementation (session 3) in three tasks: visual P3, auditory P3, and motor task. Repeated measures ANOVA analysis showed reduced P3 amplitude increase after energy dietary supplementation (compared to placebo group) throughout all sessions (up to 90 min after consumption) in the visual task, and speeding the classification process observed as a decrease of P3 midpoint latency, but only 30 min after supplementation. The latter effect was present in both, but more pronounced in the visual task. Nonparametric cluster based permutation analysis showed one significant cluster in the placebo group from visual P3 task (approximately between 400 and 520 ms) over the centro-parietal area, which was absent in the study group. Our results suggest that caffeinated energy dietary supplement containing only 55 mg of caffeine may enhance some attentional processes observed by changes in P3 features, but not in motor performance.

Key words: P3, event related potentials, energy drink, dietary supplement, caffeine

INTRODUCTION

Human cognition may be enhanced by energy drinks containing caffeine and/or other stimulants (such as taurine, vitamin B, guarana, yerba mate, acai, ginseng, maltodextrin, inositol, carnitine, creatine, glucuronolactone, and ginkgo biloba), which are thought to improve cognitive performance, alertness, mood, attentional performance, increase wakefulness, help maintain attentional focus, concentration, endurance information processing and vigilance, decrease fatigue, and reduce reaction times (Seidl et al., 2000; Kennedy et al., 2004; Deslandes et al., 2005; Smith et al., 2005; Heckman et al., 2010; Alsunni, 2011; Gurley et al., 2015; Boolani et al., 2017; Socci et al., 2017; Karabay et al., 2018).

The effects of caffeine are known to be dose related, where 200 mg is generally the optimal dose (Smith, 2011). The amount of caffeine in coffee and other beverages differs due to serving size, but it usually ranges from 40 to 300 mg in coffee, from 9 to 50 mg in tea, from 36 to 71 mg in Cola beverages, and from 70 to 200 mg in energy drinks (Higgins et al., 2010; Wesnes et al., 2013).

What's interesting, literature shows that even low doses of caffeine may improve cognitive performance (Smit and Rogers, 2000). Moreover, previous studies showed stimulatory effects after supplementation with a level of caffeine, which are generally considered to be too low to be functionally active. These studies indicated that the evinced effects are not attributable to caffeine content alone. Adequate levels of vitamins and minerals are also essential for the optimal performance

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of many physiological processes (Huskisson et al., 2007; Kennedy et al., 2008; White et al., 2017).

However, some reports indicate these benefits of energy drinks may be limited to improvements in subjective state only, not extended to objective performance (Gurley, 2015) or the doses used in the studies are above normal. Therefore, the aim of this study was to examine the acute effect of energy boost dietary supplement in the form of an effervescent tablet dissolved in water, containing 55 mg of caffeine, guarana, yerba matte, cocoa powder and vitamin B on brain electrophysiological activity. We investigated whether a single dose of such a supplement shows an effect on an attention and motor responses by means of double blind, placebo controlled event related potentials (ERP) studies.

METHODS

Participants

The experiment was conducted on 47 young, healthy people (27 women) at the age of 26.1±4.6 years. They were students or university alumni, right handed, had normal color perception and normal or corrected to normal visual acuity, as well as normal blood pressure and body temperature at the day of the study. All the participants were healthy, physically active, non-smokers, moderate caffeine users and had no neurological medical history. Information about their health condition and life style was gathered in a questionnaire. None of the participants had consumed alcohol, coffee, intoxicant, energizing beverages or other such substances within 12 h prior to the study. They were also asked to get rest, not to participate in a party or other tiring events and not to consume large amounts of alcohol within 24 h before the examination. The experiment was conducted with the understanding and written consent of each subject, according to the Code of Ethics of the World Medical Association. The studies have been approved by the Committee of Ethics of University of Silesia on scientific studies conducted on humans (number 1/2018).

Study design

Double blind, placebo controlled study was used, where neither the participants, nor data collectors knew which group the particular participant belonged to.

Half of the participants (study group) received the active substance. They drank one cup of water with dissolved one effervescent tablet of energy boost dietary supplement. The tablet contained a total amount of 55 mg of caffeine, including caffeine extract, guarana, yerba matte, cocoa powder, vitamin B and other vitamins and minerals.

The other half (placebo group) receives a placebo designed to appear, as much as possible, like the active substance. They drank one cup of water with dissolved one effervescent tablet containing vitamin C in the similar dose as was in the dietary supplement. The drinks in both groups had the same taste, smell and color.

Individuals in both groups didn't know whether they are getting the active substance or placebo. Furthermore, the researchers conducting the measurements were also kept in the dark about which group is receiving which treatment. The treatment (active substance or placebo) was assigned at random by the researcher who did not conduct the measurements.

EEG recordings were performed in three sessions: before the supplementation (session 1), 30 min after the supplementation (session 2) and 90 min after the supplementation (session 3). The study design is presented in Fig. 1.

In each session, there were 7 tasks performed, among which there were three tasks which are analyzed in this paper: visual P3 task, auditory P3 task and motor task. Each task lasted around 5 min. There were 1-2 minute breaks between the tasks and longer breaks between sessions (around 30 min between session 1 and 2 and around 20 min between session 2 and 3). The experiment started at the same time of the day (2 pm ± 2 h), due to the fact that circadian rhythm may influence human cognition (Polich and Kok, 1995).

Stimuli and task

The participants were seated in a comfortable chair in front of the computer screen in a distance of 1 m, in a dimmed room. The P3 tasks were oddball paradigms to stimulate participants with the visual stimuli presented on the 19 inch LCD monitor and with the auditory stimuli presented in headphones. The scenarios were created using Eevoke software (ANT Neuro).

In the visual task, participants were instructed to gaze on the center of the black screen during interstimulus interval and to observe letters and numbers presented in the center of the screen (yellow font on a black background). Letters were standard stimuli and numbers were target stimuli. The parameters of the stimuli were: 200 ms duration and 1000±200 ms interstimulus interval.

In the auditory task, participants were instructed to listen to two tones presented in the headphones, where 1000 Hz tone was a standard stimulus and 2000 Hz was

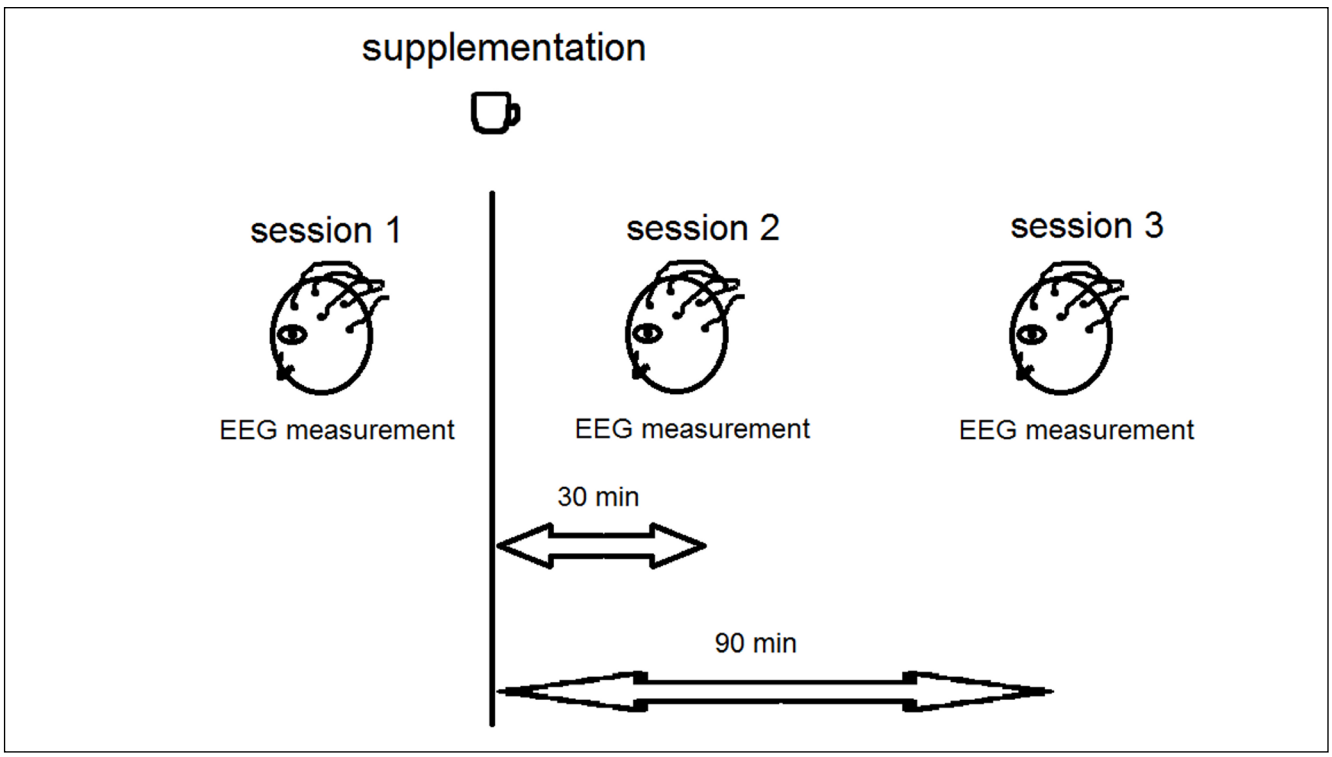

Fig. 1. Study design. EEG recordings were performed in three sessions: before the supplementation (session 1), 30 min after the supplementation (session 2) and 90 min after the supplementation (session 3).

a target stimulus. The parameters of the stimuli were: 100 ms duration and 1000±200 ms interstimulus interval.

In both tasks the stimuli were presented in random order in each session, for each participant. Their probabilities were: 20% for target and 80% for standard stimuli. The overall number of stimuli were 250 (200 standard and 50 target stimuli) and participants were instructed to push a pad button after each target stimulus. In the motor task, a small white cross in the center of a black background was presented in random interstimulus intervals. The participants were instructed to push a pad button as quickly as possible after each presentation.

EEG recording and analysis

Before putting the electrodes on, Everi (Spes Medica s.r.l.) abrasive and conductive paste was used to clean the skin on the forehead. Continuous EEG was recorded from 32 Ag/AgCl electrodes embedded in an elastic Waveguard™ EEG cap (using extended 10/20 EEG montage system) with AFz electrode as the ground electrode. OneStep Clear Gel (ANT Neuro) was inserted into electrodes in order to provide contact between skin and electrodes. The impedances at each electrode site were kept below 5 kΩ.

The signals were recorded using common average reference and then re-referenced offline to the average of the left and right mastoid electrodes. The signal was collected by means of ANT Neuro amplifier (AMP-TR-F40AB model) in DC with 20000 amplification gain and 256 Hz sampling rate. Advanced Source Analysis system ASA-Lab (ANT Neuro) with ASA v.4.8 software was used for acquisition. No high-pass filter was applied during data acquisition.

Offline data processing was performed using the EEGLAB (Delorme and Makeig, 2004) and ERPLAB Toolboxes (Lopez-Calderon and Luck, 2014), which are open-source Matlab packages for EEG-ERP analysis. Recorded EEG signals were filtered using high-pass non-causal Butterworth filter (with 0.1 Hz half-amplitude cutoff and 12 dB/octave slope). 30 Hz low-pass filter was used for figures preparation only. The fragments of signal with clear, distinct artifacts were rejected manually. In order to correct for eye blinks, independent component analysis (ICA) was conducted using EEGLAB's runica algorithm. The criterion for rejecting the components was: the anterior scalp distribution, the location in ERP image and the spectral histogram of independent components (ICs). One or two components for each participant were identified.

Continuous signal was then epoched starting 200 ms before the stimulus (baseline) and ending at 800 ms af-

ter stimulus presentation. Trials with incorrect behavioral responses or eye blinks occurring during stimulus presentation (i.e. from -200 ms before to 200 ms after the stimuli) were excluded from averages. Artifact rejection was applied using ERPLAB's moving window peak-to-peak threshold tool. Trials which peak-to-peak voltage exceeded 100 μV in 200 ms time windows (with 50 ms window step) were detected and excluded from the analysis.

In the final step of the analysis, the individual ERP waveforms were grand averaged in order to obtain mean ERPs for each experimental condition. Only subjects whose minimal number of trials after rejection was 30 were included in the analysis. This resulted in excluding 7 participants from further analyses. Mean number of standard and target stimuli after data processing were: 173±26 and 48±3 for visual P3 task, and 179±24 and 46±4 for auditory P3 task, respectively. Mean number of excluded standard and target error trials were: 27±26 and 2±3 for visual P3 task, and 21±23 and 4±4 for auditory P3 task, respectively.

The P3 measurements were taken from target-minus-standard difference waveforms. The amplitudes were measured as the mean voltage in a given window. In order to avoid biasing the results by using the difference between conditions to define time window, we collapsed the ERPs across conditions and then defined the time window for P3 amplitude as 350-700 ms for visual task and 250-700 ms for auditory task. In addition, we also used a narrower time window: 350-550 ms for visual and 250-450 ms for auditory task. For the P3 latency measurements, 50% area latency was used as the midpoint latency (the time point that divided the area under the curve into two equal halves) within time window 300-500 ms for visual task and 200-400 ms for auditory task, using only positive values (negative values were zeroed). This approach is similar to measuring peak latency, but has several advantages, including greater statistical power (Luck and Kappenman, 2009).

Statistical analysis

In the statistical analysis, following dependent variables were compared: mean P3 amplitude within the defined time windows taken from the difference ERP waveforms as the measure of P3 amplitude, and midpoint latency as the measure of the P3 latency.

Repeated measures ANOVA included between-subject factor: GROUP (placebo and study) and within-subject factors: SESSION (session 1, session 2 and session 3), AREA (frontal, central and parietal) and HEMISPHERE (left, midline, right). The Greenhouse-Geisser correction for nonsphericity and post-hoc comparisons with Bonferroni correction were used. In addition, when the assumption of the sphericity of the data was violated, multivariate Wilk's lambda test was used as an additional confirmation of the obtained corrected univariate results. For data which didn't fulfill the normal distribution and variance homogeneity assumptions, nonparametric Friedman's test was used. In all the analyses, $P<0.05$ was regarded as statistically significant.

In order to take a deeper insight into the changes of ERPs throughout the sessions, we compared ERPs between sessions, time point by time point, using nonparametric cluster based permutation analysis (Maris and Oostenveld, 2007). Monte Carlo correction for MCP (multiple comparison problem) was used by means of FieldTrip software, which is an open source Matlab toolbox for MEG, EEG, iEEG and NIRS analysis (Oostenveld et al., 2011). This method allowed us to compare ERP trials between sessions for both groups (placebo and study) and both tasks (visual and auditory) for each (channel,time) pair and to identify statistically meaningful spatiotemporal clusters. Cluster alpha $P=0.05$ and 0-700 ms time window were used. Since there were three experimental conditions in our study ('session 1', 'session 2' and 'session 1'), they were compared for every (channel, time) pair, by means of F-statistic (alpha 0.05), using ft_statfun_depsamplesFmultivariate function. Cluster-level statistics were calculated by taking the sum of the F-values within every cluster and the maximum of the cluster-level statistics was taken. 2000 draws from the permutation distribution were used. Channel neighbors for spatial clustering were found based on the triangulation method.

The analysis consisted of a comparison of behavioral data and ERPs throughout sessions, between placebo and study groups, taking into account location of the electrodes (area and hemisphere factors). First, behavioral results were presented followed by ERPs results. Within the ERPs analyses, the P3 amplitudes were first analyzed for the visual and auditory tasks. Then, midpoint latencies were analyzed for the same tasks. At the end, nonparametric cluster-based permutation results were presented.

Biological and environmental determinants of P3 waveform

P3 waveform indexes brain activities underlying attention, which can be influenced by many natural and environmental factors, such as: circadian rhythm, body temperature, heart rate, food intake, activity time, age, fatigue, nicotine and alcohol intake, arousal, and anxiety (Polich and Kok, 1995; Guerra et al., 2016;

Vazquez-Marrufo, 2017). Moreover, other factors may also influence reliability of the results: subject characteristics (e.g. caffeine usage levels), proper dosage, abstinence period before examination, and expectancy effect (Barry et al., 2007). We therefore took care to control for the aforementioned factors. In order to control for the circadian rhythm differences, we conducted the experiments at the same time of a day for each participant (2 pm ± 2 h). Body temperature and heart rate, as the physical measures of arousal, were measured before and after each session.

All the subjects were moderate caffeine consumers, non-smokers, and had restrained from energy beverages and alcohol long enough before the examination to get rid of previous contamination. They were also asked not to consume heavy meals at the day of the study.

Fatigue and stress level were assessed by participants in the questionnaire. They determined fatigue level prior to the examination on a 1-4 scale. They also determined their overall anxiety (how stressful they are in social and everyday life). The latter factor was assessed with the use of 10 questions, which the participants answered on a 1-4 scale: 'almost never', 'sometimes', 'often' and 'almost always'. The questions were based on Spielberger's State-Trait Anxiety Inventory test and concerned stressful situations in everyday life or assessment of general anxiety, e.g. 'I feel nervous or restless' or 'I feel stressed about what other people think about me' (Spielberger, 1983; Dionne, 2015).

Neither age, nor any of the initial values of the above mentioned factors: body temperature, heart rate, fatigue, and anxiety level measured prior to the experiment, differed between placebo and study groups (Table I).

In order to investigate potential effects declared by the manufacturer (energy boost, decreased fatigue and improved concentration and vigilance) for the realistic dose of the supplement, we conducted the measurements after single dosage, in two time points (30 min and 90 min after consumption) and compared the measures in addition to pre-task condition. Moreover, in order to notice potential stimulatory effects, which according to the literature manifest mainly in fatigue state, we conducted the experiment in the afternoon, so that people were not fully rested after work or studies.

RESULTS

Hypothesis-driven analysis

Behavioral data

The reaction times (RT) and correct response rates (CR) were calculated for both: P3 visual and P3 auditory task, as well as for the motor response task. In each of the three tasks, there was no significant change in RT between three sessions, neither for the main effect SESSION, nor for the interaction effect SESSIONxGROUP. The statistical results are gathered in Table II. Correct response rate for both P3 tasks did not change between sessions either.

Electrophysiological data

There were 21 subjects in placebo group and 19 subjects in the study group included in the analyses for each P3 task. Fig. 2 presents ERP difference waveforms from all three sessions, for both: visual and auditory P3 tasks, for placebo and study groups, measured from frontal, central and parietal electrodes.

A clear P3 peak is present, arising after 300 ms in visual and auditory P3 tasks. The P3 amplitude increased from frontal through central to parietal areas. There are also N1, P2 and N2 peaks visible. In the upper part of the figure (Fig. 2A), an increase of P3 amplitude between sessions in placebo group in the visual task can be seen over central and parietal areas, whereas P3 amplitudes in the study group are much more similar. In the lower part of the figure (Fig. 2B) showing ERPs from auditory task, differences in P3 amplitude between sessions are smaller. Further statistical analysis will show whether these observations are significant.

Table I. Statistical results of biological measures comparisons between placebo and study groups.

	Mean placebo	Mean study	*t* value	*p*
Age	26.6	25.5	0.64	0.52
Fatigue level	2.9	2.8	0.49	0.63
Body temperature	36.5	36.4	0.65	0.52
Heart rate	71.5	73.4	-0.66	0.52
Anxiety level	1.9	2.0	-0.35	0.73

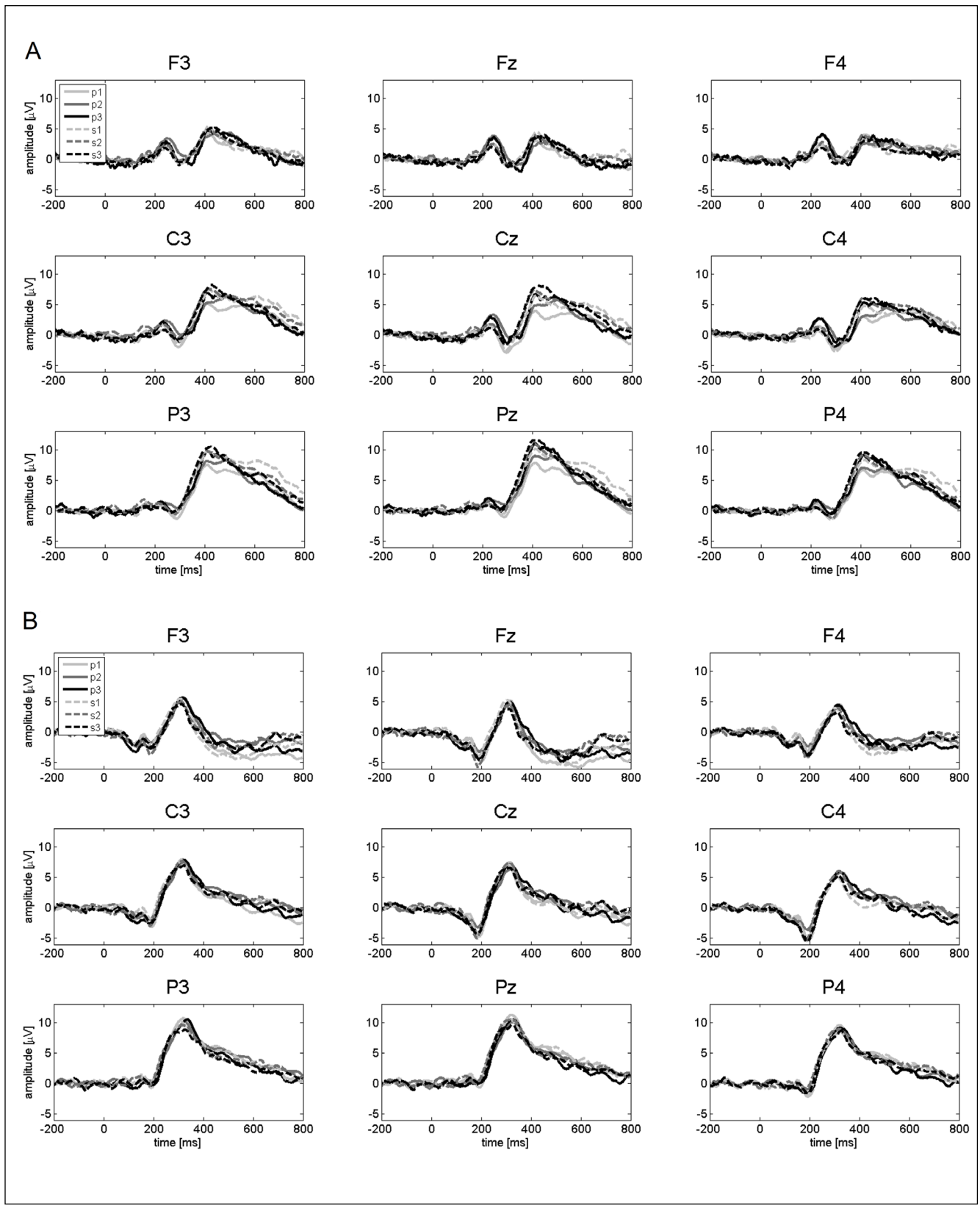

Fig. 2. Grand averaged difference ERPs from visual (A) and auditory (B) P3 tasks, measured at: F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 electrode sites, for: session 1 from placebo group (p1), session 2 from placebo group (p2), session 3 from placebo group (p3), session 1 from study group (s1), session 2 from study group (s2), and session 3 from study group (s3).

Table II. Statistical results of RT comparisons between sessions in P3 visual, P3 auditory and motor tasks.

Task	Effect	Statistical results	
P3 visual	SESSION	F_2=1.3	*pG-G*=0.27
	SESSIONxGROUP	$F_{2,76}$=0.4	*pG-G*=0.62
P3 auditory	SESSION	F_2=2.5	*pG-G*=0.09
	SESSIONxGROUP	$F_{2,76}$=1.2	*pG-G*=0.30
Motor	SESSION	F_2=1.9	*pG-G*=0.16
	SESSIONxGROUP	$F_{2,86}$=0.5	*pG-G*=0.61

P3 amplitude analysis

Visual task

Fig. 3 presents mean P3 amplitudes from the visual task, for all three sessions, within 350-550 ms and 350-700 ms time windows. First row shows the SESSION effect, second row shows the SESSIONxGROUP effect, and third row shows the SESSIONxGROUPxAREA effect.

There were no significant differences in the mean P3 amplitude within 350-700 ms time window between sessions, however a tendency of its increase in placebo (but not in the study) group over central and parietal areas was observed (Fig. 3D-F).

When narrower time window was applied (350-550 ms), statistical analysis revealed main effect SESSION, which showed that mean P3 amplitude significantly increased between sessions (Fig. 3A). More importantly, interaction effects SESSIONxGROUP (with post-hoc significant differences observed only in placebo group) as well as SESSIONxAREA (with post-hoc significant differences observed over central and parietal areas) were also observed. Mean P3 amplitude increased significantly throughout sessions only in placebo group (Fig. 3B). Though the SESSIONxGROUPxAREA effect was not significant, mean P3 amplitude tendency to increase over central and parietal areas may be observed, with a steeper increase in the placebo than in the study group (Fig. 3C). Differences in the P3 amplitude between session 1 and session 3 were: 1.8±3.0 μV over central area and 1.9±2.6 over parietal area in the placebo group and 1.1±3.0 μV over central area and 0.6±2.5 μV over parietal area in the study group. The statistical results of this analysis are presented in Table III.

An additional comparison of P3 amplitude differences between session 3 and session 1 ($P3_{diff}$=$P3_{session3}$ – $P3_{session1}$) revealed main effect GROUP ($F_{1,342}$=6.5, p=0.01) and AREA ($F_{2,342}$=6.0, p=0.0026) showing that the P3 amplitude increase is significantly smaller in the study group than in the placebo group. Moreover, the differences in $P3_{diff}$ between the placebo and the study group became bigger from frontal to parietal area.

Table III. Statistical results of the P3 amplitude analysis in the 350-550 ms time window in the visual task.

Effect	Statistical results		
	ANOVA	Wilks' lambda test	Post hoc differences
SESSION	$F_{2,684}$=20.1, *pG-G*=0.0000	$F_{2,341}$=23.6, p=0.0000	$p_{session1\text{-}session2}$=0.001, $p_{session2\text{-}session3}$=0.013 $p_{session1\text{-}session3}$=0.0000
SESSIONxGROUP	$F_{2,684}$=2.9, *pG-G*=0.06	$F_{2,341}$=3.6, p=0.029	Placebo group: $p_{session1\text{-}session2}$=0.02, $p_{session2\text{-}session3}$=0.03 $p_{session1\text{-}session3}$=0.0000
SESSIONxAREA	$F_{4,684}$=2.8, *pG-G* =0.03	$F_{4,684}$=3.1, p=0.014	Central area: $p_{session1\text{-}session2}$=0.02, $p_{session2\text{-}session3}$=1.0 $p_{session1\text{-}session3}$=0.000003 Parietal area: $p_{session1\text{-}session2}$=1.0, $p_{session2\text{-}session3}$=0.44 $p_{session1\text{-}session3}$=0.0001

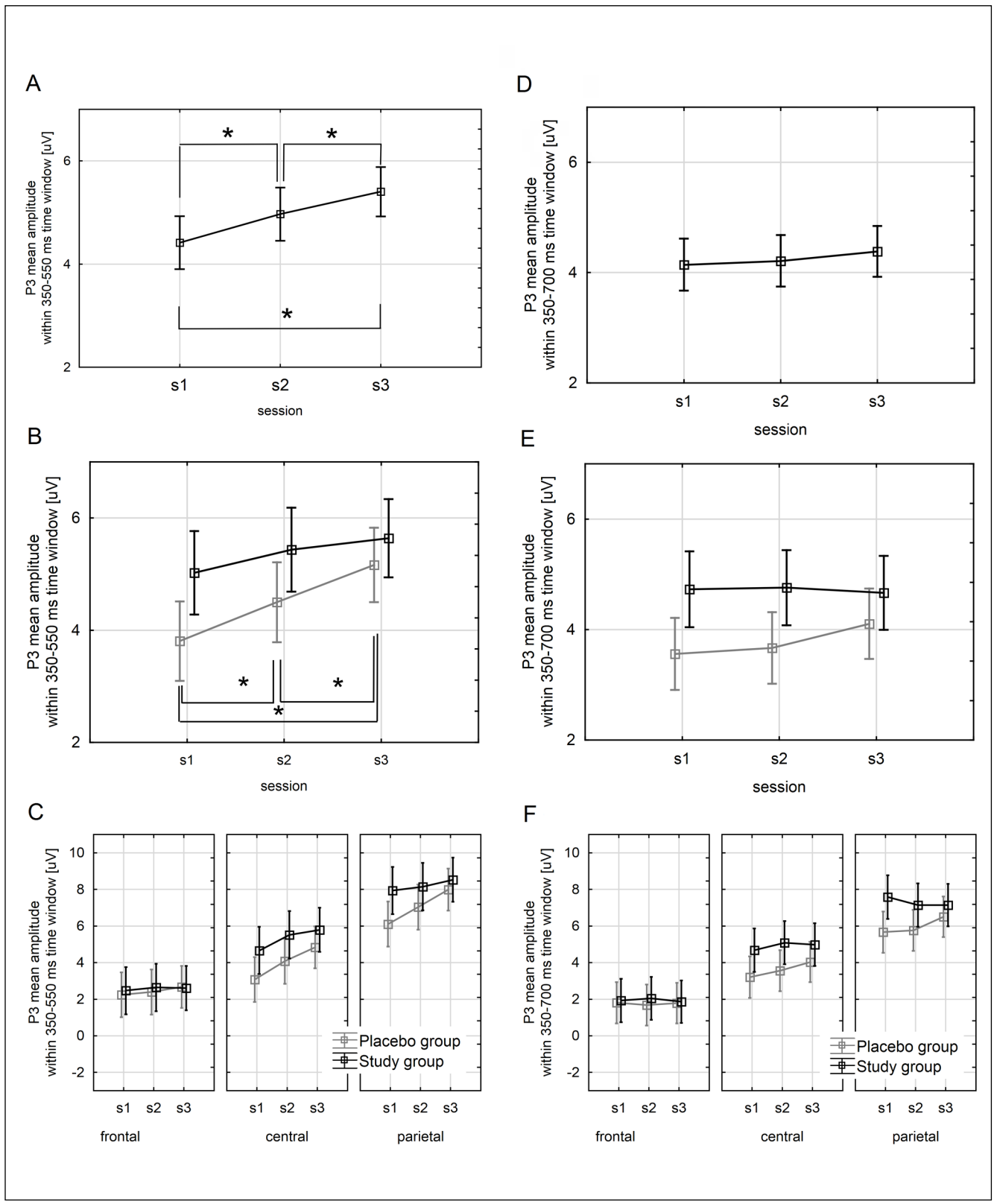

Fig. 3. Mean P3 amplitudes from the visual task, in session 1 (s1), session 2 (s2) and session 3 (s3), from placebo (gray) and study (black) groups, measured within 350-550 ms (left panel) and 350-700 ms time window (right panel). First row (A and D) shows the SESSION effect, second row (B and E) shows the SESSIONxGROUP effect, and third row (C and F) shows the SESSIONxGROUPxAREA effect. Bars: 0.95 confidence level. Significant differences with $p<0.05$ are marked with an asterisk.

Auditory task

In the auditory task, the P3 amplitude changes throughout sessions were similar in the placebo and the study groups, for both time windows (Fig. 4).

For 250-700 ms time window, there was only main effect SESSION (Fig. 4D) and interaction effect SESSIONxAREA (with post-hoc significant differences observed only over frontal area) observed. For 250-450 ms time window, there was neither significant main effect SESSION, nor any interaction effect seen (Fig. 4A-C). The statistical results are presented in Table IV.

P3 latency analysis

Since P3 latencies didn't fulfill variance homogeneity criterion, all the comparisons were performed using non-parametric ANOVA Friedman's tests. Due to the fact that calculating latencies is not trivial and it may be biased by the noise in data (Luck, 2014), this analysis was performed only on the data with clear P3 peak. Therefore, only 23 subjects from each (visual and auditory) task were included in this part of the analysis. Fig. 5 presents mean P3 midpoint latency changes throughout sessions from the visual (Fig. 5A-B) and auditory (Fig. 5C-D) tasks, in the placebo and study groups, for SESSIONxGROUP effect (upper row), and SESSIONxGROUPxAREA effect (lower row).

Visual task

In the P3 visual task, midpoint latency changed differently in the experimental groups: in the placebo group it was the highest in session 2, X^2 (2, N=78)=14, p=0.00093, whereas in the study group it was the lowest in session 2, X^2 (2, N=84)=10.3, p=0.0059 (Fig. 5A). Moreover, these differences were seen on the area level as well, where a difference in the placebo group was significant only over central area, X^2 (2, N=27)=8.4, p=0.015, and differences in the study group were significant over central, X^2 (2, N=30)=7.1, p=0.028 and parietal areas, X^2 (2, N=33)=7.3, p=0.025 (Fig. 5B).

Auditory task

In the P3 auditory task, the midpoint latency didn't change between sessions in the placebo group, X^2 (2, N=108)=2.6, p=0.27, but in the study group it decreased in session 2 and then returned in session 3 to the same level as in session 1, X^2 (2, N=75)=9.1, p=0.01 (Fig. 5C). However, no significant changes were observed at the area level (Fig. 5D).

Nonparametric cluster-based permutation analysis

Due to the fact that ERPs have advantage of high temporal precision, we performed nonparametric cluster-based permutation tests (Maris and Oostenveld, 2007) to compare ERPs between sessions, time point by time point, using Monte Carlo correction for MCP. The MCP arises from the fact that the effect of interest (i.e. a difference between experimental conditions) is evaluated at a large number of (channel, time)-pairs. Using multivariate test for within-subject EEG study, a null hypothesis about the probability distributions of the session-specific averages was tested. This hypothesis involved that trial specific data structures from all three sessions (i.e. trials from 'session 1', 'session 2', and 'session 3') were drawn from the same probability distribution, regardless of the experimental condition. The analysis combines neighboring values that are likely to be correlated (e.g., neighboring time points and spatial locations) to reduce the MCP. Using cluster alpha p=0.05, we found a significant difference between ERPs from 'session 1', 'session 2' and 'session 3' trials only in placebo group from the visual P3 task. This difference resulted in one significant cluster (p=0.003), which extended from approximately 400 to 520 ms over the centro-parietal area. There were no other significant results. A topomap showing cluster based permutation test results with highlighted significant cluster for P3 visual task from placebo group is presented in Fig. 6.

Table IV. Statistical results of the P3 amplitude analysis in the 250-700 ms time window in the auditory task.

Effect	Statistical results		
	ANOVA	Wilks' lambda test	*Post hoc* differences
SESSION	$F_{2,684}$=4.3, $pG\text{-}G$=0.015	$F_{2,341}$=5.2, p=0.006	$p_{session1\text{-}session2}$=0.016, $p_{session2\text{-}session3}$=0.077 $p_{session1\text{-}session3}$=1.0
SESSIONxAREA	$F_{4,684}$=4.5, $pG\text{-}G$=0.002	$F_{4,684}$=3.5, p=0.008	Frontal area: $p_{session1\text{-}session2}$=0.006, $p_{session2session3}$=1.0, $p_{session1\text{-}session3}$=0.44

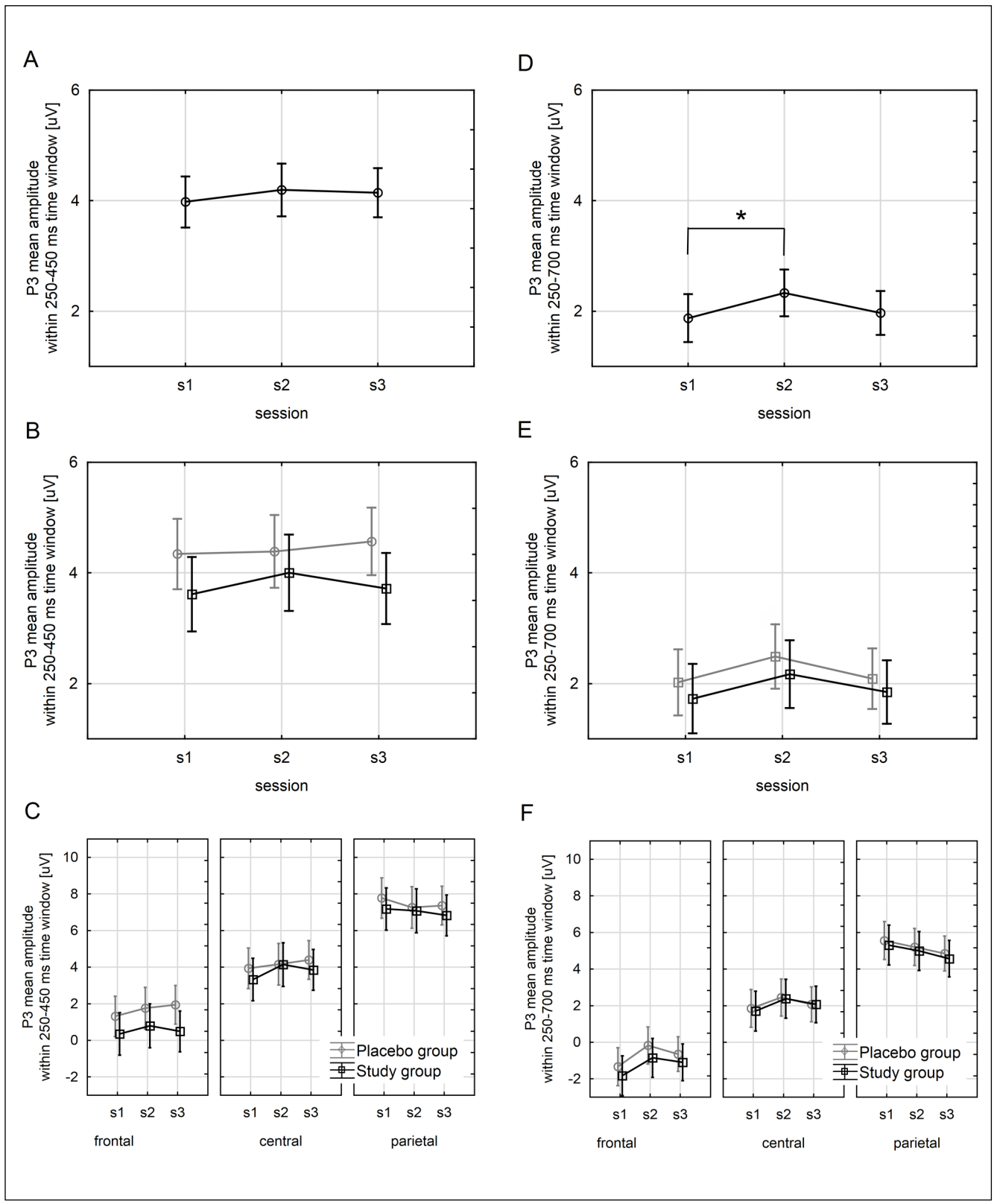

Fig. 4. Mean P3 amplitudes from the auditory task, in session 1 (s1), session 2 (s2) and session 3 (s3), from placebo (gray) and study (black) groups, measured within 250-450 ms (left panel) and 250-700 ms time window (right panel). First row (A and D) shows the SESSION effect, second row (B and E) shows the SESSIONxGROUP effect, and third row (C and F) shows the SESSIONxGROUPxAREA effect. Bars: 0.95 confidence level. Significant differences with $p<0.05$ are marked with an asterisk.

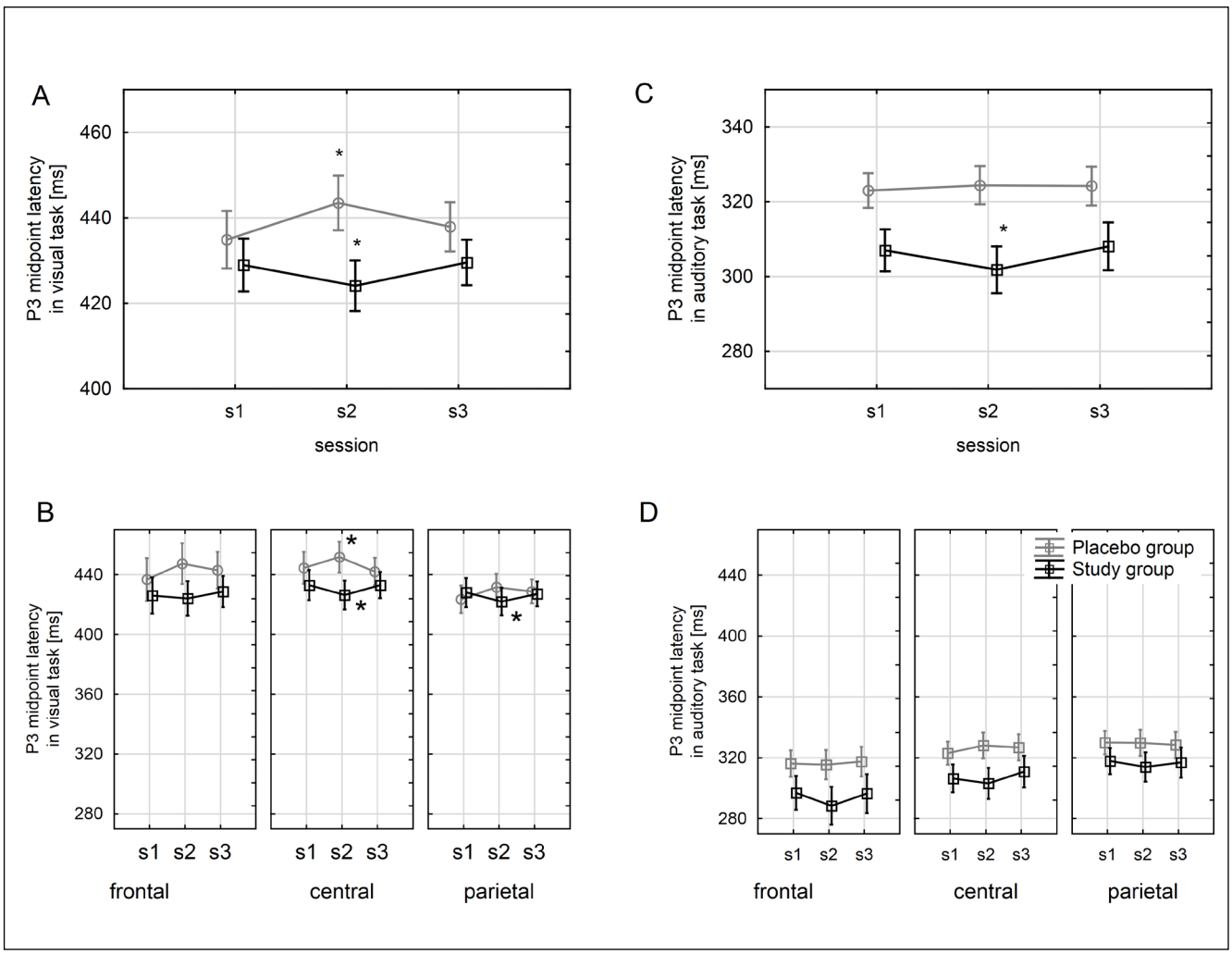

Fig. 5. P3 midpoint latencies measured in session 1 (s1), session 2 (s2) and session 3 (s3), from placebo (gray) and study (black) groups, in visual (left panel) and auditory (right panel) tasks. First row (A and C) shows the SESSIONxGROUP effect, and second row (B and D) shows the SESSIONxGROUPxAREA effect. Bars: 0.95 confidence level. Significant differences with $p<0.05$ are marked with an asterisk.

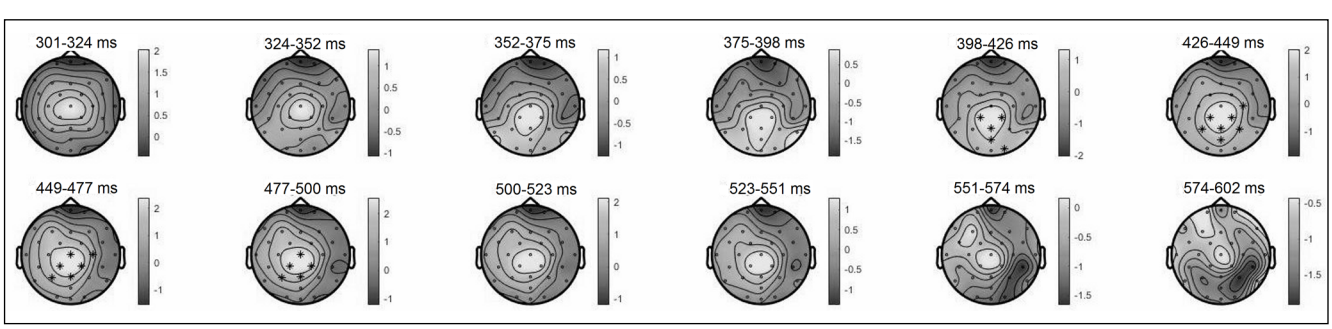

Fig. 6. Topographic distributions of the differences between condition-averaged ERPs: 'session 1', 'session 2' and 'session 3' trials for P3 visual task from placebo group, at $p<0.05$, cluster corrected. Electrode clusters on the basis of which the null hypothesis was rejected are highlighted with asterisks. For each panel all time points and all 30 electrodes were included in the permutation test. Bars show F-values.

DISCUSSION

The amplitude and latency of P3 waveform give two different sources of information about the classification process that is engaged in attention during performing the task. P3 amplitude is larger when more effort is devoted to the task, what has been described as resource allocation (Polich, 2007).

In our study, P3 amplitude in visual task in the placebo group tended to increase throughout sessions within 350-700 ms time (Fig. 3E) and significantly increased (Fig. 3B) when narrower (350-550 ms) time window was used. This increase, though not significant at the area level, was observed over central and parietal areas (Fig. 3C and 3F). Moreover, nonparametric cluster-based permutation analysis showed a significant cluster between approximately 400 and 520 ms over centro-parietal area in this group (Fig. 6). According to Luck (2014), P3 amplitude depends on: uncertainty, probability, and resource allocation. Since in our experiment neither uncertainty nor probability changed between sessions, we considered this increase of P3 amplitude between sessions in the placebo group as an effect of increased amount of resources allocated to perform the task. We regarded this increase as a result of increasing mental fatigue caused by having to complete many tasks during a long experimental time.

No significant increase of the mean P3 amplitude was observed in the study group within 350-700 or 350-550 ms time windows (Fig. 3B-F). The cluster-based analysis didn't reveal any significant clusters in the study group, either. This means that the increase of P3 amplitude throughout sessions was reduced after energy dietary supplementation.

Interestingly, in auditory task no such behavior was observed – the mean amplitude changed between sessions in the same way in the placebo and the study groups (Fig. 4).

One of the possible explanations of the obtained results is that caffeinated energy dietary supplement decreased fatigue level in the visual task, which caused fewer sources needed to be allocated to perform the task.

On the other hand, P3 latency is thought to index classification speed, which is proportional to the time required to detect and process a target item (Kutas et al., 1977; Magliero et al., 1984).

Midpoint latency in the visual task in the placebo group was the highest in session 2 compared to session 1 and 3 (Fig. 5A), with significant difference over central area (Fig. 5B). On the contrary, in the study group, there was a drop in midpoint latency in session 2 (FIG. 5A), which was significant in both: central and parietal areas (Fig. 5B). In the auditory task, midpoint latency didn't change throughout sessions in placebo group, but it dropped in study group in session 2 (though only SESSIONxGROUP effect was significant, Fig. 5C-D). This decrease in P3 midpoint latency in the study group means that the energy dietary supplement speeded up the classification process in both tasks, but more visible in the visual task. However, this effect was observed only 30 min after supplementation. In the session 3 (90 min after supplementation) midpoint latency was on the similar lever to the pre-supplementation level.

Caffeine is rapidly absorbed from the digestive tract and distributed to all tissues, including the brain. The peak plasma concentration is achieved 30 to 120 min after oral intake and the plasma half-life has been reported to vary from 3 to 10 h depending upon the individual (Babu et al., 2008; Wesnes et al., 2013). Literature indicates that the energy drinks help maintain attentional focus, concentration, information processing and vigilance over the 6 h period. Such a short time of action of the energy dietary supplement in our study may be connected to a lower dose of caffeine present in the drink.

The fact that different behavior of the changes of P3 features was observed in visual and auditory task is interesting. Though P3 is an event related potential, which doesn't rely on physical parameters of the stimuli, differences in its features between modalities have been observed (Dreo et al., 2016). However, there might have been task difficulty confound, since there were only two kinds of stimuli: 1000 and 2000 Hz tones in auditory task, while in the visual task, participants had to classify many letters and numbers.

What's important, no changes were observed in the motor task. These results suggest that the aforementioned effects relate to classification process and not to response preparation. Moreover, the lack of effect of energy dietary supplementation on changes in RT and CR between sessions (Table II) suggests that electrophysiological features are more sensitive to lower doses of cognitive stimulants than behavioral measures.

In conclusion, the energy dietary supplementation lowered a P3 mean amplitude increase throughout the whole experiment (3 sessions) in the visual task, compared to the placebo group. This result was considered as a reduction of mental fatigue. The energy dietary supplementation also speeded classification process in both visual and auditory tasks through lowering the P3 midpoint latency, but only 30 min after supplementation. This result was present in both, but more pronounced in the visual task.

CONCLUSIONS

A single dose of an energy dietary supplement containing 55 mg of caffeine (in the form of caffeine ex-

tract, guarana, yerba matte, cocoa powder, vitamin B and other vitamins and minerals), reduced P3 amplitude increase (compared to placebo group) throughout all sessions (up to 90 min after consumption) in the visual task. Nonparametric cluster-based permutation analysis of ERP waveforms from a visual task confirmed this result showing a significant cluster, which corresponded to the P3 time window (approximately 400-520 ms) over centro-parietal area only in placebo group. Since the P3 amplitude is recognized as the measure of resource allocation, this reduction of P3 amplitude increase may be explained by the fact that stimuli categorization in sessions following energy dietary supplementation was less effortful than in the placebo group. Moreover, the energy boost supplementation caused speeding of the classification process observed as a decrease of P3 midpoint latency, but only 30 min after supplementation. This result was present in both, but more pronounced in the visual task. ERP from motor task didn't change significantly between sessions. Performance measured by response time and accuracy was maintained at the same level throughout sessions in both: placebo and study groups, in all three tasks.

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