Research article

Influence of phosphatidylserine on cognitive performance and cortical activity after induced stress

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The aim of this study was to investigate the effect of phosphatidylserine (PS) on cognition and cortical activity after mental stress. After familiarization, 16 healthy subjects completed cognitive tasks after induced stress in a test–re-test design (T1 and T2). Directly after T1, subjects were assigned double-blind to either PS or placebo groups followed by T2 after 42 days. At T1 and T2, cortical activity was measured at baseline and immediately after stress with cognitive tasks using electro-encephalography (EEG). EEG was recorded at 17 electrode positions and fast Fourier transforms (FFT) determined power at Theta, Alpha-1, Alpha-2, Beta-1 and Beta-2. Statistics were calculated using ANOVA (group x trial x time). The main finding of the study was that chronic supplementation of phosphatidylserine significantly decreases Beta-1 power in right hemispheric frontal brain regions (F8; $P < 0.05$) before and after induced stress. The results for Beta-1 power in the PS group were connected to a more relaxed state compared to the controls.

Keywords: phosphatidylserine, EEG, cortical activity, stress, cognitive tasks

Introduction

Phosphatidylserine (PS) is an essential component of all biological membranes and has important regulatory functions within mammalian cells. In humans, PS is most concentrated in the brain where it represents 15% of the total phospholipid pool. PS has been used as a nutritional supplement for many years. Historically, it was obtained from the bovine cortex but, due to potential infectious diseases, soy-derived phosphatidylserine has become a safe alternative.[1] Exogenous PS supplementation has shown benefits in physical exercise[2,3] and mental performance, where it is known for improving brain functions including long-term memory and recognition, especially in elderly people. Cenacchi *et al*.[4] and Amaducci *et al*.[5] both reported an improvement in cognitive performance in elderly people with cognitive decline as a result of Alzheimer's disease after a PS-supplementation. Benton *et al*.[6] demonstrated, for the first time, an effect of PS on young adults. They showed that subjects who received PS supplementation felt less stressed and had a better mood during a cognitive task. In another study, there is a discussion about using PS in attention deficit/hyperactivity disorder (ADHD) children with benefits in attention and learning.[7]

Therefore, PS appears to have a positive affect on cognitive functions including attention, concentration and recognition,[8] but nothing is known about the influence of PS after induced stress. Only Kingsley *et al*.[3] investigated the influence of PS supplementation on oxidative stress during recovery following physical exercise/stress and found no significant effects.

To investigate this idea further, we have used a double-blind, placebo-controlled paradigm where

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stress was induced before the subjects have to perform two cognitive tasks. In a test–re-test design, this was conducted before and after a chronic supplementation period (PS/placebo). To examine the underlying cortical mechanisms, we measured EEG cortical activity before and after the induced stress. In association with attention and brain functions, EEG spectral power analysis is a widely used method to evaluate changes in cortical activity during and after cognitive[9–11] and sensorimotor tasks.[12–14] In this context, the Beta-1 spectral power plays a role in mental[15,16] and physical stress[17,18] whereas the frontal Theta and global Alpha-1 components are closely related to task-specific and unspecific attention.[9,19]

It was the purpose of the current study to examine the effect of a chronic PS supplementation on: (i) cognitive performance; and (ii) cortical activity after induced stress. We hypothesized that PS supplementation would result in better cognitive performance and changes in cortical activity compared to a control group.

Subjects and methods

Subjects

A total of 16 male, right-handed volunteers (age, 25 ± 3 years; weight, 78.3 ± 6.9 kg; height, 184 ± 4 cm) participated in the study. The subjects were divided randomly into a PS (n = 8; age, 24 ± 3 years; weight, 77.0 ± 8.3 kg; height, 183 ± 4 cm) and a placebo group (n = 8; age, 26 ± 2 years; weight, 80.0 ± 5.6 kg; height, 185 ± 4 cm). All subjects were recruited from the University of Paderborn. They all were non-smokers and had no history of neurological, cardiovascular or other major disorders, no current use of medications or drugs and no physical or psychological exposure (*e.g.* intensive training sessions, examinations) 24 h prior to the trials. All subjects had normal or corrected-to-normal vision at the time of the experiment. The participants were instructed to avoid changes in their every-day behaviour (especially their diet). The study was done in accordance with the rules and regulations established by the Institutional Review Board for ethical treatment of human subjects. All subjects signed an informed consent after the explanation of the testing procedure. All data were treated with confidentiality.

Experimental design

All subjects had one preliminary visit to the laboratory to undergo a familiarization trial in order to avoid learning effects in the cognitive tasks before conducting the two main trials.

All participants then performed the two main trials, which were separated by exactly 42 days (6 weeks). The subjects were assigned (in a randomized, double-blind design) to receive either one nutritional bar (IQ PLUS brain bar; Giventis, Germany) per day containing 200 mg soy-based PS (PS group; n = 8) or a corresponding placebo bar (control group; n = 8). Each bar had a weight of 35 g, providing 149 kcal, 4.8 g protein, 20 g carbohydrate, 5.5 g fat and vitamins (1.4 mg vitamin B_1, 1.4 mg vitamin B_6, 42 mg vitamin C, 4.6 mg vitamin E, 2.8 mg niacin, and 4.2 mg pantothenic acid). The supplementation was started immediately after the first trial and was continued until the day before the second trial. During the supplementation period, the subjects were weighed and their food intake recorded 3 days a week to ensure the same diet over the supplementation phase.

Main trial procedures

On both test days (trial 1 and trial 2), all subjects received a standardized breakfast and were not allowed caffeine or any other stimulants. On arriving in the laboratory, the EEG electro cap and the HR belt (Polar, Sporttester, Germany) were attached to the subjects. They were asked to lie supine and relax for a 10-min period. After relaxing, the participants were comfortably seated in a chair in front of a computer monitor with keyboard and mouse. The EEG baseline measurement (M1) was conducted at rest with eyes open for 2 min. To induce stress, delayed auditory feedback (DAF) was performed. In this task, the normal auditory feedback of speech is drowned and disturbed by a timed, delayed feedback (Novel SVG 3, NEG, Germany). The subject has to read sections from Immanuel Kant's *Kritik der reinen Vernunft*; because of its complexity, it is unlikely be memorized and is, therefore, suitable for repeated use. Subjects hear everything they have spoken after an adjustable delay. In this case, the delay was set at 175 ms. Afterwards, the cognitive tasks were performed in the order stoop-colour word test and D2 test, only interrupted by the EEG measurement (M2–M4; 2 min eyes open) immediately after each task. Finally, there was a 10-min relaxing period followed by an EEG measurement with eyes closed (M5).

Cognitive tasks

The subjects had to perform two different cognitive tasks.

Stroop colour–word interference test[20]

The Stroop task was selected as a measure of inhibition, a key executive function. The task requires

a suppression of response to a dominant stimulus pattern (printed words) while attending and responding to a secondary stimulus characteristic (ink colour). In the experimental session, two words are presented on the monitor, the upper one of which is always written in a certain colour (red, blue, green, yellow), while the lower one is always in white. The test subject must decide whether the meaning of the lower word reflects the characteristic (ink colour) of the upper word. For a 'correct' decision, the subject has to push the left button of a computer mouse; for an 'incorrect' decision, the right button must be pressed. In the analysis, three conditions appear: congruent (below and above colours co-incide), incongruent (below colour does not co-incide with the colour above), and neutral (there is no connection between the semantic category and the characteristic to be named – often meaningless sequences of letters). In total, 216 reaction times (72 in each condition) will be analyzed per test. The duration of the test was 20 min.

D2 concentration test[21]

This task was used to document objectively alertness, capacity to deal with stress and capacity of concentration. Within a preset time, the subject must cross out specific letters that are arranged in rows: the lower case 'd' with two dots above must be crossed out. Two types of mistakes can occur: omission (correct letters were not crossed out) and confusion (incorrect letters were crossed out). Total numbers of letters worked on was calculated as concentration performance (CP), omissions (O) and false answers (F).

Heart rate measures

Heart rate was continuously recorded at each 5-s interval using short-range telemetry (Polar Sporttester; Polar Electro, Finland). Heart rates were averaged (M1–M5).

Cortical (EEG) measures

The EEG was recorded by a stretchable electro cap (ElectroCap Inc., USA) in accordance with standards of the international 10:20 System. At baseline, directly after the induced stress, the cognitive performance of each task (Stroop, D2) and after 10-min recovery (M1–M5), the EEG was recorded continuously for 2 min from 17 scalp locations (Fz, F3, F4, F7, F8, Cz, C3, C4, Pz, P3, P4, T3, T4, T5, T6, O1, O2) using the central electrode (Cz) as physical reference. All EEG data were recorded and stored using the CATEEM system (MediSyst, Linden, Germany). The signals were sampled at 512 Hz/12 bit and amplified (DC = 20 MΩ). Before each measurement, an impedance test ensured a sufficient signal-to-noise ratio. The physiological signals were high-pass filtered at 0.86 Hz. An automatic artefact detection (depending on amplitude level and signal slope) was followed by a visual inspection. Only artefact-free segments were used for analysis.

In the EEG signals, fast Fourier transforms (FFT) were calculated on 50% overlapped, 512-sample Hanning windows at each electrode for all artefact-free segments with the CATEEM system (MediSyst). The power spectra were divided into different frequencies: Theta (4.75–6.75 Hz), Alpha 1 (7.0–9.5 Hz), Alpha 2 (9.75–12.5 Hz), Beta 1 (12.75–18.5 Hz) and Beta 2 (18.75–35.0 Hz). For the statistical analyses, a logarithmic transformation of the power values was necessary to stabilize the variances.[22] Average log-transformed power spectra were computed across all time samples (M1–M5) in both trials for each subject.

Statistical analysis

For statistical analysis, SPSS v.12.0G software was used. All results are given as mean values and standard deviation. The Kolmogorov–Smirnov test was used to determine if variables fit the Gaussian distribution. Subject characteristics were compared under supplementation groups using independent *t*-tests. To examine differences in peripheral and cortical parameters, a mixed-model, repeated-measures ANOVA (within-subject factors – trial and time; between-subjects factor – supplementation groups) was conducted. Compound symmetry, or sphericity, was verified by the Mauchley test. When the assumption of sphericity was not met, the significance of F-ratios was adjusted according to the Greenhouse–Geisser procedure. If a significant interaction was identified for group x trial x time, a PS-supplementation effect was accepted. If a significant main effect of time appeared, multiple Bonferroni-corrected paired *t*-tests were made. The outcome of statistical calculations were declared significant if $p \leq 0.05$. The partial η^2 statistic provided estimates of the effect sizes in significant effects.

Results

Cognitive task performance

The results for cognitive testing are shown in Table 1. In the tasks (Stroop colour-word test, D2 attention test), the subjects in both groups increased their performance significantly from trial 1 to trial 2 (trial effect; see Table 1). There were no significant between-groups differences (DAF [RW]: $F_{1,14} = 0.527$, $P = 0.480$; Stroop [neutral]: $F_{1,14} = 0.464$, $P = 0.507$, [congruent]: $F_{1,14} = 4.262$, $P = 0.058$, [incongruent]:

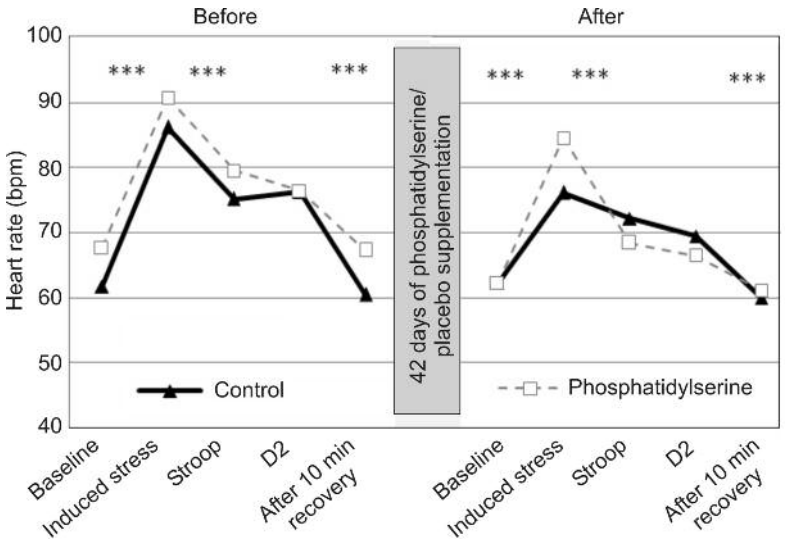

Figure 1 Heart rate of both groups during baseline (M1), induced stress (M2), Stroop (M3), D2 (M4) and after 10-min recovery (M5) before and after chronic supplementation period. Post hoc significance level in both groups: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$)

$F_{1,14}$ = 3.868, P = 0.078; D2 [CP]: $F_{1,14}$ = 0.823, P = 0.380, [F]: $F_{1,14}$ = 0.590, P = 0.455, [O]: $F_{1,14}$ = 0.256, P = 0.621). There was no interaction effect of group x trial x time existent in any task parameter.

Heart rate measures

The mean heart rate changed significantly within-subjects (within-factor time: $F_{4,56}$ = 61.570, $P < 0.001$, partial η^2 = 0.815; Fig. 1). No significant between-subject effect is existent (between-factor group $F_{1,14}$ = 0.280, P = 0.605) and PS-supplementation has no effect on heart rate values.

Cortical activity (EEG) measures

There was a main within-subject change over time (within-factor time) in different frequencies. In the Theta frequency, significant power changes appear in frontal (Fz, F3, F4, F7, F8: $F_{4,56} \geq 8.657$, $P \leq 0.001$, partial $\eta^2 \geq 0.382$; Fig. 2; *post hoc* Fz, F3, F4, F7 [$P \leq 0.05$]: M1–M5, M2–M5, M3–M5) and temporal (T3, T4, T5, T6: $F_{4,56} \geq 4.365$, $P \leq 0.004$, partial $\eta^2 \geq 0.238$; *post hoc* T3, T6 [$P \leq 0.05$]: M2–M5, M3–M5, M4–M5; *post hoc* T4 [$P \leq 0.05$]: M3–M5, M4–M5; *post hoc* T5 [$P \leq 0.05$]: M1–M5, M2–M5, M3–M5, M4–M5) regions. The Alpha-1 power values demonstrate significantly increased spectral power values over time in frontal (Fz, F3, F4, F7, F8: $F_{4,56} \geq 9.319$, $P < 0.001$, partial $\eta^2 \geq 0.400$; *post hoc* Fz, F3, F4, F7, F8 [$P \leq 0.05$]: M1–M5, M2–M5, M3–M5, M1–M4, M2–M4, M3–M4), central (Cz, C3, C4: $F_{4,56} \geq 9.709$, $P < 0.001$, partial $\eta^2 \geq 0.410$; *post hoc* Cz, C3, C4 [$P \leq 0.05$]: M1–M5, M2–M5, M3–M5, M1–M4, M2–M4), parietal (Pz, P3: $F_{4,56} \geq 10.095$, $P < 0.001$, partial $\eta^2 \geq 0.419$; *post hoc* Pz, P3 [$P \leq 0.05$]: M1–M5, M2–M5, M3–M5, M1–M4, M2–M4), and temporal (T3, T4, T5, T6: $F_{4,56} \geq 9.364$, $P < 0.001$, partial $\eta^2 \geq 0.400$; *post hoc* T3, T4, T5, T6 [$P \leq 0.05$]: M1–M4, M1–M5, M2–M5) electrode positions. Beta-1

Table 1 Performance in cognitive tasks before and after chronic supplementation period

Test	Parameter	Trial	Control (ms)	PS (ms)	Main time effect F	P	Partial η^2
Stroop	Congruent	1	807.8 ± 76.6	758.9 ± 77.9	13.327	0.003	0.488
		2	747.6 ± 58.2	680.5 ± 55.5			
	Neutral	1	793.6 ± 63.7	771.7 ± 54.9	36.836	< 0.001	0.725
		2	705.4 ± 54.2	691.0 ± 66.3			
	Incongruent	1	958.1 ± 64.8	850.6 ± 87.5	38.316	< 0.001	0.732
		2	813.8 ± 84.2	762.1 ± 81.9			
D2	CP	1	620 ± 67	593 ± 41	27.852	< 0.001	0.666
		2	667 ± 55	642 ± 68			
	O	1	6.88 ± 3.5	7.50 ± 4.1	10.978	0.005	0.440
		2	3.50 ± 2.2	4.75 ± 5.8			
	F	1	7.25 ± 3.7	8.63 ± 4.1	10.427	0.006	0.428
		2	3.63 ± 2.2	5.13 ± 6.2			

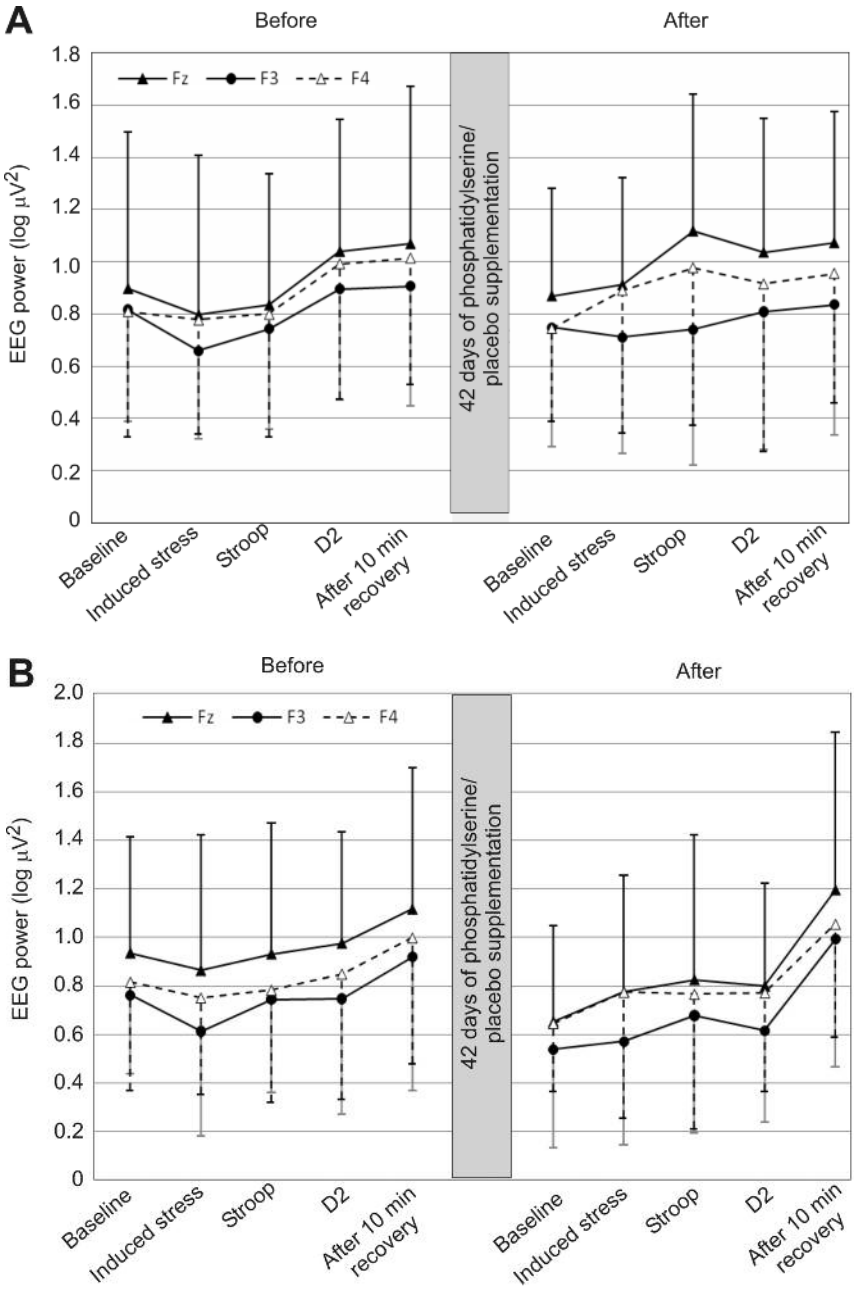

Figure 2 Overview of frontal (Fz, F3, F4) Theta power values of PS (A) and control (B) group immediately after rest (M1), induced stress (M2), Stroop (M3), D2 (M4) and 10-min recovery (M5) before and after chronic supplementation period. See text for post hoc significance level

power values increased over time at frontal (Fz: $F_{4,56}$ = 12.310, $P < 0.001$, partial $\eta^2 = 0.468$; *post hoc* [$P \leq 0.05$]: M1–M2, M1–M4, M1–M5, M2–M4; F3: $F_{4,56} = 9.127$, $P < 0.001$, partial $\eta^2 = 0.395$; *post hoc* [$P \leq 0.05$]: M1–M4, M1–M5, M2–M5) and parietal (Pz: $F_{4,56}$ = 12.157, $P < 0.001$, partial $\eta^2 = 0.465$; P3: $F_{4,56} = 8.520$, $P < 0.001$, partial $\eta^2 = 0.387$; *post hoc* Pz, P3 [$P \leq 0.05$]: M1–M4, M1–M5, M2–M4, M2–M5, M3–M4, M3–M5) sites. Alpha-2 and Beta-2 show no significance in within-subject differences.

Significant main group effects between the PS-group and the placebo group could not be detected in any frequency band.

The supplementation of PS has a significant effect (supplementation group x trial x time) in the Beta-1 right-frontal sites F4 ($F_{4,56} = 3.324$, $P = 0.016$, partial $\eta^2 = 0.192$; *post hoc* inner-subject factor trial [$P \leq 0.05$]: control M1 before – M1 after, Fig. 3) and F8 ($F_{4,56} = 3.101$, $P = 0.022$, partial $\eta^2 = 0.181$; *post hoc* inner-subject factor trial [$P \leq 0.05$]: control M1 before – M1 after, between-subject factor group [$P \leq 0.05$]: M1 after, M3 after, Fig. 4).

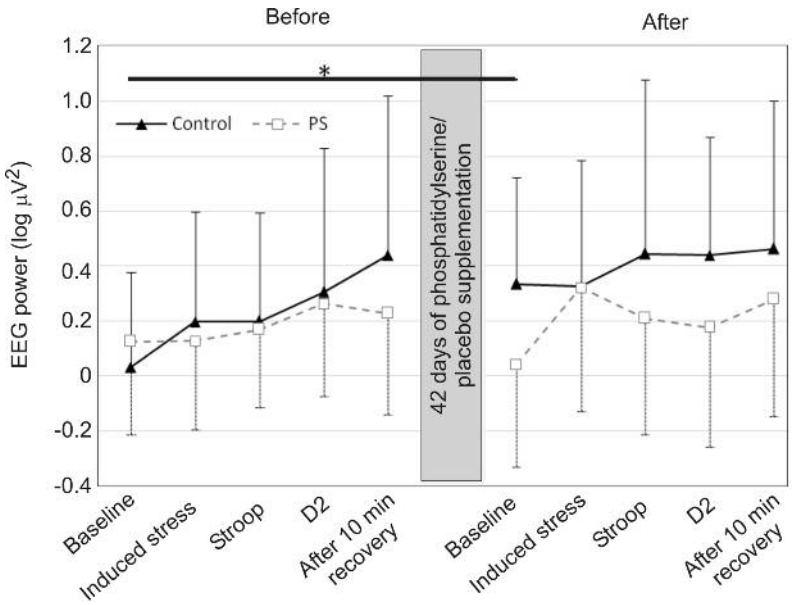

Figure 3 Right-frontal (F4) Beta-1 power values of both groups immediately after rest (M1), induced stress (M2), Stroop (M3) and D2 (M4) and 10-min recovery (M5) before and after chronic supplementation period. Post hoc significance level: $*P \leq 0.05$

Discussion

The main finding of the study was that supplementation of phosphatidylserine administered for 42 days significantly decreases Beta-1 power in right hemispheric frontal brain regions before and after induced stress.

Increased Beta-1 spectral power is described as an indicator of activation associated with cognitive task demands,[23,24] higher and neurophysiological function.[25] Adey[15] described an increased frontal Beta-1 power in astronauts in association with an increased visual information overload, simulating hazardous flight conditions, as an 'information overflow'. Diego *et al.*[16] investigated three types of relaxation (massage and vibrator effects) and found a significant decrease in frontal Beta-1 activity. They interpreted this finding in connection with the participant's response to relaxation during the stimulation. Taken together, it can be

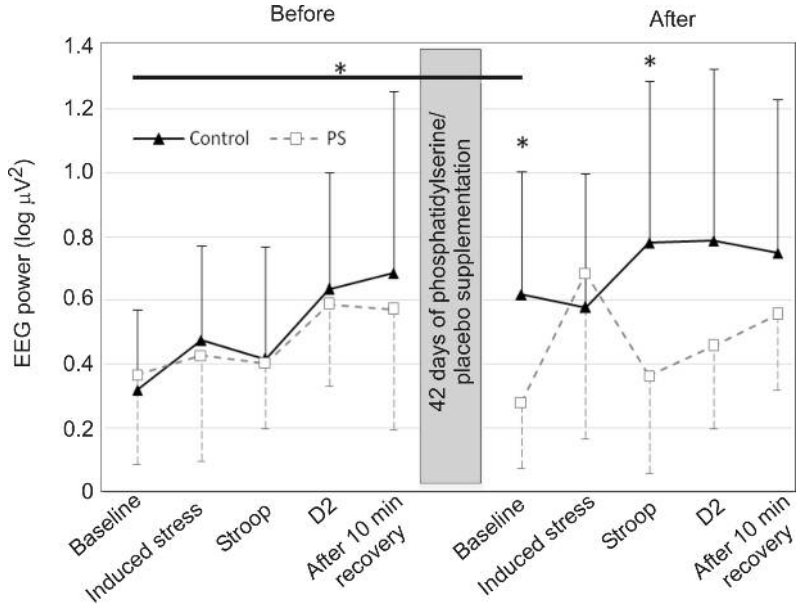

Figure 4 Right-frontal (F8) Beta-1 power values of both groups immediately after rest (M1), induced stress (M2), Stroop (M3) and D2 (M4) and 10-min recovery (M5) before and after chronic supplementation period. Post hoc significance level: $*P \leq 0.05$

concluded that a higher frontal neural activity is associated with increased Beta-1 power and, conversely, a decrease in frontal Beta-1 power values demonstrated a form of relaxation.[19] In this study, the PS supplementation lead to significant decreases in the right hemispheric frontal brain area before and after induced stress whereas the Beta-1 power was higher in the control group indicating a higher activation state. Therefore, the PS group demonstrated a higher state of relaxation after, and even before, the induced stress which could not be detected before the supplementation period.

Frontal and prefrontal regions of the brain mediate executive processes (*i.e.* inhibition, selection, planning, attention, co-ordination, concentration).[26,27] Given the higher activation (expressed by higher Beta-1 activity) in frontal brain regions immediately after the cognitive tasks, it can be speculated that more resources of executive functions were used in the control group due to the given mental stress by the delayed auditory feedback. It seems that PS induced a more relaxed state which resulted in a suppressed frontal Beta-1 activity. In conjunction with an 'information overflow',[15] it seems that the control group without any supplementation is not able to relax and is probably still engaged in processing information, still focusing on the performed task and activating frontal resources for executive functions.

The cognitive performance in the Stroop colour-word test and D2-test improved after the supplementation period in the PS group as well as in the control subjects. This may be due to a familiarization effect. The participants knew the situation and tasks during the second trial and, therefore, may have attained better results. The supplementation of PS has a long history in age-related diseases to improve cognitive performance in memory and learning. Crook *et al.*[28] divided patients with age-related memory impairment into a PS supplementation group and controls (placebo). After a 12-week intervention, the PS group performed better in memory and learning tasks relative to the controls. Although clinical trials demonstrated some improvements in memory performance in Alzheimer's disease patients,[5] only Benton *et al.*[6] proved an effect of PS in young healthy subjects during arithmetic tasks. There were no differences in task performance but the PS-supplemented participants achieved the same results and felt less stressed compared to a control group. To conclude, the PS group was able to perform as well as the control group in a more relaxed state indicated by the frontal Beta-1 spectral power values. To elucidate this effect, more research is needed to investigate the underlying mechanism.

Both groups were able to perform better in all given tasks after the supplementation period. Given the highest activation immediately after the delayed auditory feedback in both groups, one can assume that stress was really induced. This is supported by the significant increases before and decreases after the DAF in heart rate during the tasks.

Independent of supplementation with PS, the study regimen demonstrated significant increases after the induced stress in different frequency bands (Theta and Alpha-1). The frontal Theta power tended to result in increased values after the DAF over time in both groups. Recent data have indicated a generation of frontal Theta power in the anterior cingulate cortex.[9] Neuro-imaging and brain-lesion studies have shown that this anatomical region is an important component of the human attentional system.[11,29] Different authors have found that frontal Theta power increases when task performance and attention must be sustained over time.[9,12,30,31] Therefore, the attentional resources used at the end (M5) of the test seemed to be increased compared to those at the beginning, independently of PS-supplementation. Furthermore, the frontal Theta power increases after the stress task indicating a higher attention level.

In addition, the results show an increase over time in the slow Alpha-1 frequency band at all electrode positions in all regions oft the brain. Alpha is the dominant frequency in the human scalp of EEG of adults. Alpha activity is often described as a form of cortical idling with its amplitude inversely related to the number of neuronal populations activated during cognitive and motor processes.[19,32] The slow alpha band component (Alpha-1) is attenuated over broad regions of the cortex and is a non-specific parameter of attentional and expectancy processes. An increase in the Alpha-1 frequency band over time supports the idea of a decrease of non-specific attention over time but PS-supplementation plays no role in this during the trials.

Conclusions

It can be concluded that 42 days of chronic PS supplementation influenced the frontal Beta-1 frequency before and after induced stress. Both groups were able to perform better in cognitive tasks after the supplementation period with a decreased activation in frontal brain regions immediately after the tasks in the PS group compared to the controls. To our knowledge, this is the first study to show these effects of PS indicating a more relaxed state after induced stress. Further research is required to confirm this findings.

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