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Research article

The complexity of caffeine's effects on regular coffee consumers

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ABSTRACT

Why does coffee wake us up? Is it because it contains caffeine, or because we are used to it waking us up after drinking it? To answer this question, we recruited twenty habitual coffee drinkers who received either caffeinated or decaffeinated coffee (placebo) in a double-blind, randomized fashion. The two substances were identical except for the presence of caffeine. We measured cognitive performance, cardiovascular responses, and whole-head EEG during rest and during an auditory-oddball task. The same measurements were done before and after ingestion. We expected to find significant differences between caffeine and placebo groups across the outcome measures. However, except for the resting-state alpha power, changes due to ingestion in physiological responses and in cognitive functioning were not significantly different between the two groups. Actually, only one of the three cognitive measures was found to be significantly altered by the ingestion. These findings suggest that regular coffee consumers respond to coffee-like beverages independently of the presence of caffeine.

1. Introduction

Coffee is arguably the most widespread beverage in history. Social rituals of drinking coffee have roots in every corner of the globe. More than two billion cups of coffee are being consumed every day [1]. By drinking coffee, we ingest caffeine, a favorite drug of many. Effects of caffeine on the human body are intensely researched [2–5], though pros and cons of caffeine are still debated [6,7]. Meanwhile, caffeine is being used to treat headache, migraine [8,9], and various liver conditions [10,11]. Therapeutic use of caffeine in preterm neonates shows promise [12–14].

Physiological and psychological effects of caffeine are largely understood [3,15,16]. After being ingested, caffeine activates the sympathetic nervous system via two pathways. The first is the inhibition of adenosine, which temporarily reduces the feeling of

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sleepiness and tiredness. This is why we feel that our brain functions better after a cup of coffee. Indeed, studies have confirmed caffeine's potential to accelerate information processing, boost alertness, and improve problem-solving [2,5,17,18,6,19,3,20–22]. The up-regulation of the A_{2A} receptors after frequent caffeine use increases the permeability of the blood-brain barrier during withdrawal from caffeine, which facilitates influence on the brain [23]. The second pathway is tied to the cardiovascular system. It differs for habitual and non-habitual caffeine consumers: The former respond by lowering the heart rate and increasing the blood pressure [24,25], whereas in the latter both heart rate and blood pressure increase [6].

In this study, we focused on habitual coffee consumers because they form much of the adult European population [26,27]. For habitual drinkers, coffee is an integral part of everyday routine, meaning they have been using caffeine for many years. Drinking coffee as a habit involves several primary regulatory psychological functions: regulation of emotions, performance goal states, and social connection. Thus, potential mechanisms of conditioned responses such as habituation and anticipation may be composed of different neurophysiological backgrounds, from social responses to various sensory inputs (smell, taste, etc). Expectations related to coffee consumption are not known to influence neurocognitive functions. Caffeine may influence attention-related P3 waves and NoGo-P3 waves, but there is (to our best knowledge) no evidence that caffeine-related expectations affect other electrophysiological indices. Actually, they are known to produce smaller effects than caffeine [28]. However, caffeine anticipation is known to improve accuracy and the ability to filter distracting stimuli.

Studies that used placebo showed that anticipation plays a significant role, where participants expecting caffeine often experience similar cognitive and performance improvements regardless of whether they consume caffeine or a placebo [29]. This placebo effect is particularly relevant in heavy coffee drinkers, who may experience caffeine withdrawal symptoms after a period of abstinence. In such cases, both caffeine and placebo can alleviate these symptoms, leading to similar improvements in performance [30]. This suggests that observed effects may reflect a reduction in withdrawal symptoms rather than direct action of caffeine. Authors of [31] emphasized the importance of craving and withdrawal on cognition. Participants arriving in a withdrawal state may experience improvements due to expectancy alone, complicating the interpretation of the measurements [32]. Moreover, open-label decaf (i.e. placebo) can reduce caffeine withdrawal symptoms, even when people are not consciously expecting it [33].

We asked what exactly triggers behavioral and physiological responses to coffee for habitual consumers? Is it the ingestion of caffeine itself or the ritual of drinking coffee? To tease this out, we need to separate the effect of caffeine from the effects of other ingredients of coffee [34,35]. We chose decaffeinated coffee ("decaf" henceforth) as the placebo: It contains most compounds of coffee, but it is (almost) entirely absent of caffeine. Its taste is virtually indistinguishable from regular coffee, so drinkers are easy to convince that they are drinking the usual coffee. This ensures that any psychological effects of habituation are equally expressed, regardless of the presence or absence of caffeine [35–39].

We recruited a group of habitual coffee consumers that were administered decaf with or without a large dose of caffeine ("caffeine" vs. "placebo"). We examined their cardiovascular response and cognitive performance (see Protocol in the next section). We departed from the assumption that caffeine has an effect and that coffee is not just a ritual. We hence expected that the ingestion of caffeine will make heart rate decrease and blood pressure increase. In an auditory oddball paradigm, we expected a reduction in reaction times and improved performance in a mental arithmetic test, contrary to placebo. Throughout these assessments, we also monitored the brain activity via whole-head electroencephalography (EEG) and hypothesized that the EEG activity will be very different in subjects on caffeine as opposed to placebo. In the analysis of event-related potentials during auditory odd-ball, we expected to see reduced amplitudes of the P3 component. In view of earlier findings by Jung and co-workers [40], we expected to find frequency-specific effects distinguishing caffeine and placebo.

2. Methods

2.1. Procedures

Participants We recruited 20 healthy students from different Slovenian universities who received course credits for participation (10 female, 23.8±1.4 yrs, and 10 male, 25.3±2.9; mean ± SD). The participants were recruited according to the snowball method, all who were willing to participate and fit inclusion criteria during the data collection period were included. They were right-handed [41] and in good general health, with body-mass indices ranging from 20 to 30 and normal hearing and vision. All of them were habitual caffeine consumers (1-3 cups of coffee per day). None of them had a history of neurological, metabolic, or heart conditions, mental disorders, wounds/ulcers in the stomach or intestines and none of them reported using medicines that could influence motor responses, concentration, or cognitive abilities. None of the female subjects was breastfeeding or knowingly pregnant. Participants were instructed to sleep at least seven hours the night before participation in our experiment. Prior to measurements, they abstained from drinking coffee for a minimum of 8 and a maximum of 11, and consumed no food for 2 hours. All these criteria were observed to minimize the chance of adverse side effects of caffeine. The study was approved by the Committee for Research Ethics of the Faculty of Information Studies in Novo mesto, Slovenia (April 2, 2019), application nr. 1/16-20-2. Subjects were familiarized with the purpose of the experiment and provided a signed informed consent. The study complies with the guidelines of good clinical practice in biomedical sciences (ICH-GCP) and with the Declaration of Helsinki.

Protocol All data were collected in a research laboratory at the Faculty of Medicine, of the University of Ljubljana. The participants were welcomed into the lab and sat on a comfortable chair with adjustable armrest. They sat in front of a computer screen approximately 70 cm away from their face. We regularly checked their well-being by asking about their current state. We collected the age, weight, and height, and calculated the body-mass-index. We assessed handedness, measured blood pressure and heart rate. After

mounting the EEG-cap, every participant underwent the first mental arithmetic test [42–44]. Then, the initial EEG recordings were conducted during rest for 3 minutes with eyes open, followed by 3 minutes with eyes closed. Subsequently, participants performed an auditory oddball task that lasted for about 5 minutes. After that, we assigned each participant into caffeine or placebo group. Participants were then offered a cup of coffee. Those belonging to the placebo group received decaf. Those in the caffeine group also received decaf, but we added caffeine powder to it. Specifically, we added 6 mg of caffeine (Kemika, Croatia; $C_8H_{10}N_4O_2$ with a molecular weight of 194.19) per kg of body mass with a maximum of 550 mg. We considered this amount sufficient for a large effect [37]. All participants were convinced they had regular (caffeinated) coffee and reported no suspicion. Participants rested for 20 minutes after caffeine ingestion. Before continuing with EEG measurements, we double checked all electrodes and measured the impedances, which took additional 10 minutes. We then repeated the eyes opened - eyes closed EEG resting state recordings. Participant then performed the second auditory oddball task in the same way as prior to ingestion. Finally, the participants performed a second mental arithmetic test. All data were collected between April and August 2019.

Study design A mixed between-within subjects, randomized double-blind experimental design was used. This means that the participants were randomly assigned to either the decaf or caffeine groups by a researcher, who then did not participate in any further parts of the experimental protocol, via the envelope method. The other researchers were present with the participant (subject) but were kept blind to the assigned condition. The participant was first exposed to the protocol before the ingestion. The measurements were then carried on after the absorption period. The data was analysed in the same way – before and after ingestion, caffeine vs. placebo group, and the interaction between the group and the condition.

Cardiovascular parameters Heart rate, diastolic, and systolic blood pressure were measured at rest with a BM44 upper arm blood pressure monitor (Beurer GmbH, Ulm, Germany). Two measurements separated by 40 minutes were conducted, one before and one after ingestion.

Mental arithmetic test To test for possible changes in cognitive abilities due to ingestion, we carried out a 'simple' arithmetic test [42]: The participant was asked to verbally subtract 7 by 7 starting from 1000 (1000, 993, 986, etc.) before ingestion; after ingestion we used 9 instead of 7. Subjects continued subtracting for 1 minute. We counted the correct and wrong answers, as well as the final number of subtractions.

Auditory oddball task We used a sequence of 400 beeps with low and high pitches (pitch 200 Hz and 470 Hz with duration 115 ms and 90 ms, respectively). Two consecutive beeps were separated by a pause of 700 ms. The pitch of each beep was selected at random, with 75% chance for low and 25% chance for high pitch. The volume on the computer speakers was kept constant so that all participants could hear the beeps equally well. Participants were instructed to push a button with their right index finger each time they heard a high pitch (oddball) beep. Low pitch beeps did not require any action. The experiment was carried out with eyes open.

EEG acquisition We used a 32-channel EEG (g.GAMMAcap2 with Ag/AgCl electrodes in 10–20 system design, g.GAMMAsys, electroconductive gel g.GAMMAgel; Guger Technologies g.tec, Schiedlberg, Austria). An isolated ground was placed at the location of the AFz electrode, and a monoauricular reference was defined on the right earlobe. Impedance was kept below 5 kΩ throughout all recordings. Signals were amplified using g.USBamp amplifiers (g.tec, Schiedlberg, Austria) and sampled at 600 Hz; recordings were controlled via g.Recorder software (v5.14.00; g.tec, Schiedlberg, Austria). We used customized software to generate beeps in the oddball task. We then used a trigger box (g.trigbox; g.tec, Schiedlberg, Austria) to synchronize beeps to both EEG and button pressing. We also applied a 48-52 Hz online notch filter [45] for removal of line noise artifacts.

2.2. Preprocessing of EEG data

All EEG-analyses were conducted using MatLab (ver. 2023a; The MathWorks, Natick, MA, USA) including the open-source toolbox EEGlab ver. 2023.0 [46].

We first examined the data visually for artifacts of excessive signal drifts due to skin conductivity changes (sweating etc.), head movement, or EEG malfunction. We set (rather low) criteria for passing this visual inspection: The data loss due to all above artifacts is not to exceed 15%. Data not meeting this basic criterion were entirely rejected. Ultimately, we rejected none of the resting state datasets and only one of the ERP datasets.

Next, the signals were band-pass filtered. For the resting state signals we employed a windowed sinc FIR filter between 1 and 50 Hz (Hamming window, pass-band ripple 0.0022, stop band attenuation -53 dB; high-pass order of 990 and 2 Hz transition bandwidth, low-pass order of 198 and 10 Hz transition band). For the ERP data we used a filter between 0.1 and 30 Hz (Hamming window, pass-band ripple 0.0022, stop-band attenuation of -53 dB; high-pass order of 19800 and transition band width 0.1 Hz, low-pass order of 198 and transition band width 10 Hz).

Subsequently, we removed all channels that were flat for more than 20 s, had less than 0.8 correlation with nearby channels, more than 5 SDs of high frequency deviation, or more than 7 SDs of root-mean-square voltage. We then conducted a second visual inspection and removal of any large, one-off/rare artifacts.

This was followed by epoching of the ERP data. We defined the onset of each oddball beep as the reference event, used for delimiting successive epochs. 100 ms before each reference event were used as the baseline. 700 ms after the reference event were examined for the effects of caffeine vs. placebo.

As the final step of data cleaning, we employed independent component analysis (ICA; infomax [47] with natural gradient feature [48] and extended-ICA algorithm [49]) on the epoched ERP data and whole resting-state data. ICA components that clearly contained eye-blink artifacts, lateral eye movement, or pronounced muscle artifacts were removed. We also removed the epochs during which the signal exceeded ± 75 μV at any point. We retained, on average, 89.5% of epochs.

The missing channels (due to removal or whatever other reason) were interpolated using spherical splines [50] to guarantee the same topological coverage throughout analysis. Finally, we re-referenced the ERP data to average, and transformed the resting-state data to current source densities (CSD) via parameters $\lambda = 0.00001$, m = 4, head radius = 10 cm.

2.3. Measurements

We used heart rate, systolic and diastolic blood pressure to measure the cardiovascular status of participants, including possible changes due to ingestion.

Cognitive performance was first assessed via mental arithmetic test by counting the total number (rather than the relative number) of answers and errors. Cognitive performance was next assessed in oddball task by measuring the time between a high pitch (oddball) beep and a subject pressing the button (reaction times). Naturally, cognitive performance improves when subjects react faster to oddball beeps, give more answers within 1 minute, and make less errors.

As mentioned above, the studied EEG data consists of two parts: resting-state eyes-open/eyes-closed EEG data (recorded before the oddball task), and Event-related potential (ERP) EEG data (recorded during the oddball task). Resting state data were analyzed in the frequency domain. We calculated the Welch's periodogram for every 3-minute interval (Hann window of width of 1 s, 50% overlap). We used thus obtained periodograms as our outcome measure of resting-state EEG data. In contrast, we analyzed the ERP data in the time domain. We used the raw amplitude of the EEG signal as our measure. Both EEG data were averaged twice: First over the trials (instances of oddball), and then over the participants, which were separated by group and condition.

2.4. Statistical analyses

All cardiovascular and cognitive parameters complied with the assumptions of equivalence of the covariance matrices (Box's test), non-sphericity (Mauchly's test), and the homogeneity of variance (Levene's test). This enabled us to analyze them using a two-way mixed-design ANOVA, *condition* (pre- and post-ingestion; paired) × *group* (caffeine and placebo). We set the statistical significance threshold to $\alpha = 0.05$.

On the other hand, some of EEG measures did not comply with aforementioned requirements for parametric statistics. However, all of them did fulfill the assumption of exchangeability [51], which allowed for switching to permutation statistics. We used 10,000 permutations.[3] We used Statistical Parametric Mapping in 1 Dimension (SPM1D) for a more principled analysis and comparison of continuous-valued datasets, hence avoiding non-interpretable point to point differences.

For the resting state EEG data we used a three-way mixed-design ANOVA, *condition* (pre- and post-ingestion; paired) × *eye state* (open and closed; paired) × *group* (caffeine and placebo). For the oddball ERP data, we decided for two-way ANOVA, *condition* (pre- and post-ingestion; paired) × *group* (caffeine and placebo). Here we applied Holm-Bonferroni correction of the $\alpha = 0.05$ significance threshold. For the 32 ANOVA tests, this yielded a corrected $\alpha = 0.0016$. Whenever ANOVA revealed a significant main effect or interaction, we conducted post-hoc testing, for which we also applied Holm-Bonferroni correction.

We implemented Holm-Bonferroni correction also for the analysis of resting-state EEG data. This was done by first Holm-Bonferroni correcting the p-trajectory of each electrode, then ranking the electrodes in two ways – by the lowest post-correction p-value (lowest-highest) and by the number of post-correction significant p-values (highest-lowest). The ranks were then summed for each electrode and the electrodes ranked again by their sum of ranks. The highest ranked electrode was then (as per the Holm-Bonferroni method) assigned the strictest correction threshold (0.05/32), the second highest a slightly less strict threshold (0.05/31), the third 0.05/30, the fourth 0.05/29, etc. The correction was calculated and implemented for each effect of the ANOVA separately.

For all parametric tests, we used SPSS (ver. 25; IBM, Armonk, NY, USA). The permutation testing was conducted in MatLab (v. 2023a), using the open-source SPM1D package [52].

3. Results

We were able to obtain all measurements for all 20 participants, with one exception: One ERP dataset had to be excluded due to EEG malfunction. Reaction times for this ERP dataset had to be excluded too. In what follows we present our results in four points: Cardiovascular parameters, Cognitive performance, Analysis of ERP datasets, Analysis of resting state datasets.

3.1. Cardiovascular parameters

For all parameters, we found a significant main effect of the condition (pre-/post-ingestion). However, caffeine and placebo groups did not differ significantly in any of the cardiovascular parameters. Also, we could not identify any significant interaction effect. This

[3] We utilized non-parametric permutation methods, since they are known to be at least as powerful as parametric ones when operating on (relatively) small sample size, such as in our case. Also, in contrast to parametric testing, these methods require no special characteristics of data such as normality of distribution, homoscedasticity etc.

Table 1
Statistical results of all 2 × 2 ANOVAs of cardiovascular parameters (pre-/post-ingestion × caffeine/placebo). Numbers in bold face indicate statistical significance ($p < 0.05$).

	condition			group			interaction		
	F-statistic	p	η^2	F-statistic	p	η^2	F-statistic	p	η^2
diastolic	$F_{(1,18)} = \mathbf{6.78}$	**0.018**	**0.27**	$F_{(1,18)} = 4.16$	0.056	0.19	$F_{(1,18)} = 2.73$	0.116	0.13
systolic	$F_{(1,18)} = \mathbf{4.64}$	**0.045**	**0.21**	$F_{(1,18)} = 2.38$	0.141	0.12	$F_{(1,18)} = 2.23$	0.152	0.11
hrt. rate	$F_{(1,18)} = \mathbf{19.53}$	**0.000**	**0.52**	$F_{(1,18)} = 0.04$	0.848	0.00	$F_{(1,18)} = 0.13$	0.721	0.01

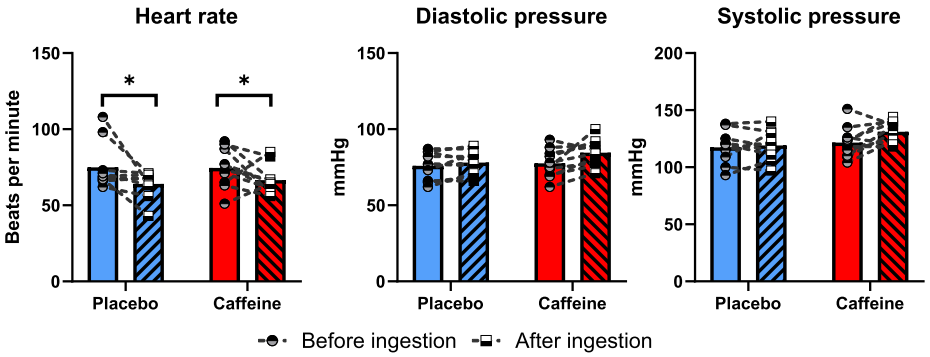

Fig. 1. Post-hoc results for systolic and diastolic blood pressure and for the heart rate. Before ingestion (normal histogram bars) vs. after ingestion (histogram bars dashed). Placebo and caffeine groups are marked by blue and by red, respectively. Trend lines show the direction and the magnitude of change from before to after ingestion (for each participant). Please note, that in some cases multiple subjects had the same outcome values (which could – misleadingly – make it look as if there were less than N subject involved). See text for other details.

Table 2
Results of the two-way mixed-design ANOVAs of the cognitive parameters. Numbers in bold face indicate statistical significance ($p < 0.05$).

	condition			group			interaction		
	F-statistic	p	η^2	F-statistic	p	η^2	F-statistic	p	η^2
answers	$F_{(1,18)} = 2.93$	0.107	0.15	$F_{(1,18)} = 1.59$	0.225	0.09	$F_{(1,18)} = 0.14$	0.247	0.08
errors	$F_{(1,18)} = 0.02$	0.894	0.01	$F_{(1,18)} = 2.91$	0.107	0.15	$F_{(1,18)} = 0.17$	0.690	0.01
reaction	$F_{(1,17)} = \mathbf{9.26}$	**0.008**	**0.37**	$F_{(1,17)} = 0.11$	0.743	0.01	$F_{(1,17)} = 0.007$	0.934	0.00

suggests that the act of ingestion has an effect on cardiovascular activity, but in a way independent of the presence of caffeine. Statistical results are reported in the Table 1.

Results of the corresponding post-hoc t-tests are summarized in the Fig. 1. Heart rate was significantly reduced after the ingestion of both caffeine and placebo ($t_{(9)} = 3.22, p = 0.042$ and $t_{(9)} = 3.23, p = 0.042$, respectively). Both groups also exhibited a significant increase in blood pressure. In other words, blood pressure increased after ingestion of both regular coffee and decaf. To sum up, we found that all cardiovascular parameters changed due to ingestion, regardless of whether this ingestion involved caffeine or not.

3.2. Cognitive performance

Cognitive performance was first assessed via mental arithmetic test, where we counted the number of answers given (i.e., the number of subtractions) and the number of errors (incorrect subtractions) that a subject made. This performance is associated with various aspects of cognition including working and long-term memory, processing speed, and attention. We did not find any significant main effect for neither the number of answers or the number of errors, not for the condition (before/after ingestion), and not for the group (caffeine/placebo). All statistical results are reported in the Table 2.

Cognitive performance was next assessed via auditory oddball task, where we measured the reaction time – the time it took for a subject to press the button once an oddball beep was played (reaction time is not a direct measure of cognition, but it is strongly correlated with several cognitive processes such as decision-making and sustained attention). We found a significant decrease in the reaction times after ingestion of caffeine (see Table 2). In fact, the descriptive statistics shown in Fig. 2 (left panel) reveal a reduction of average reaction time after ingestion for both groups: From 329 ms to 307 ms for placebo, and from 324 ms to 303 ms for caffeine group. This decrease of the average reaction time was found to be statistically significant only in case of the caffeine group.

Middle and right panel in Fig. 2 show the descriptive statistics for the mental arithmetic test, i.e., the number of answers given and the number of errors made. As mentioned above, we found no statistically significant effects here for either of the groups or conditions.

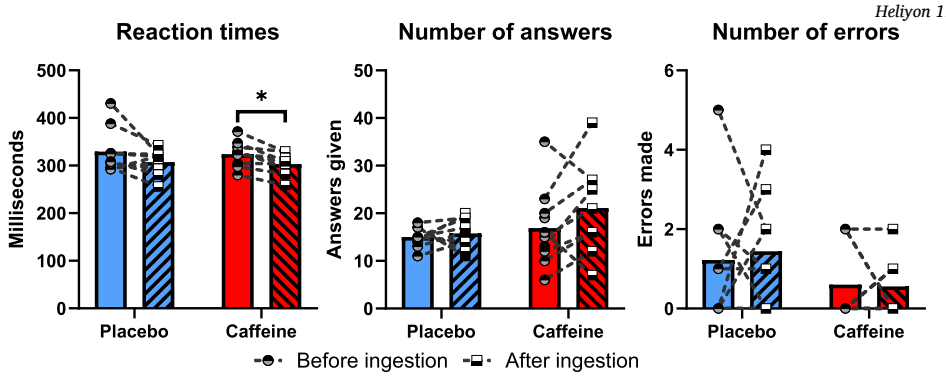

Fig. 2. Descriptive statistics (histograms of measured values) for the reaction times in oddball task (left panel), and number of answers (middle panel) and number of errors (right panel) in the mental arithmetic test. Before ingestion (normal histogram bars) vs. after ingestion (histogram bars dashed). Placebo and caffeine groups are marked by blue and by red, respectively. Trend lines show the direction and the magnitude of change from before to after ingestion (for each participant). See text for other details and note that in some cases multiple subjects had the same outcome values (which could – misleadingly – make it look as if there were less than N subject involved).

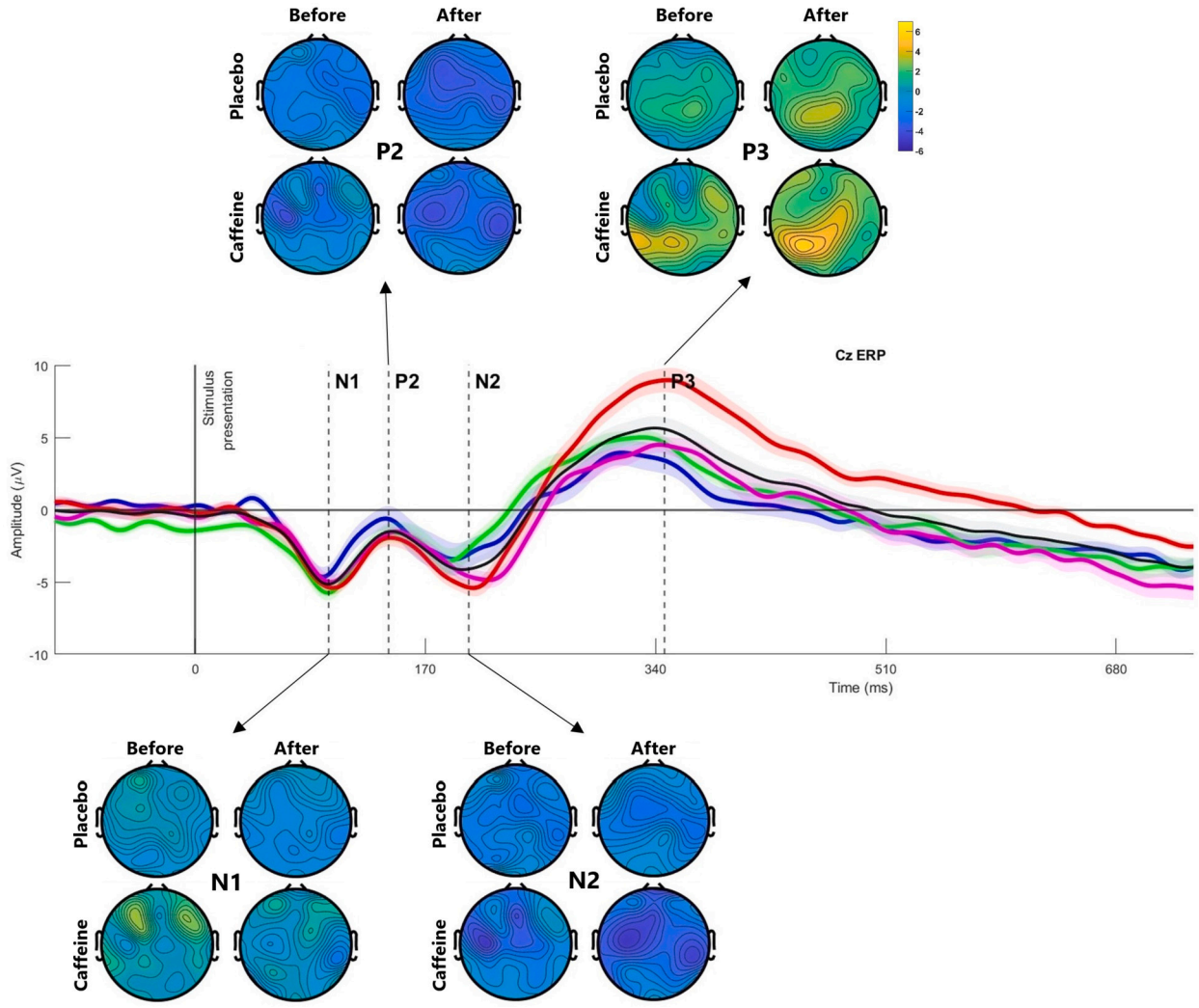

Fig. 3. An example of an ERP waveform at Cz electrode. Groups and conditions are colored as follows: Caffeine before and after ingestion in magenta and red, respectively; placebo before and after ingestion in green and blue, respectively. Mean ERP for all groups and conditions is shown in black. Shaded areas represent standard errors of the mean. Individual ERP components (see text) are marked with dotted vertical lines. For each ERP component, we show the topographies separated by group and condition. All topographies are plotted at a uniform color-scale (in μV).

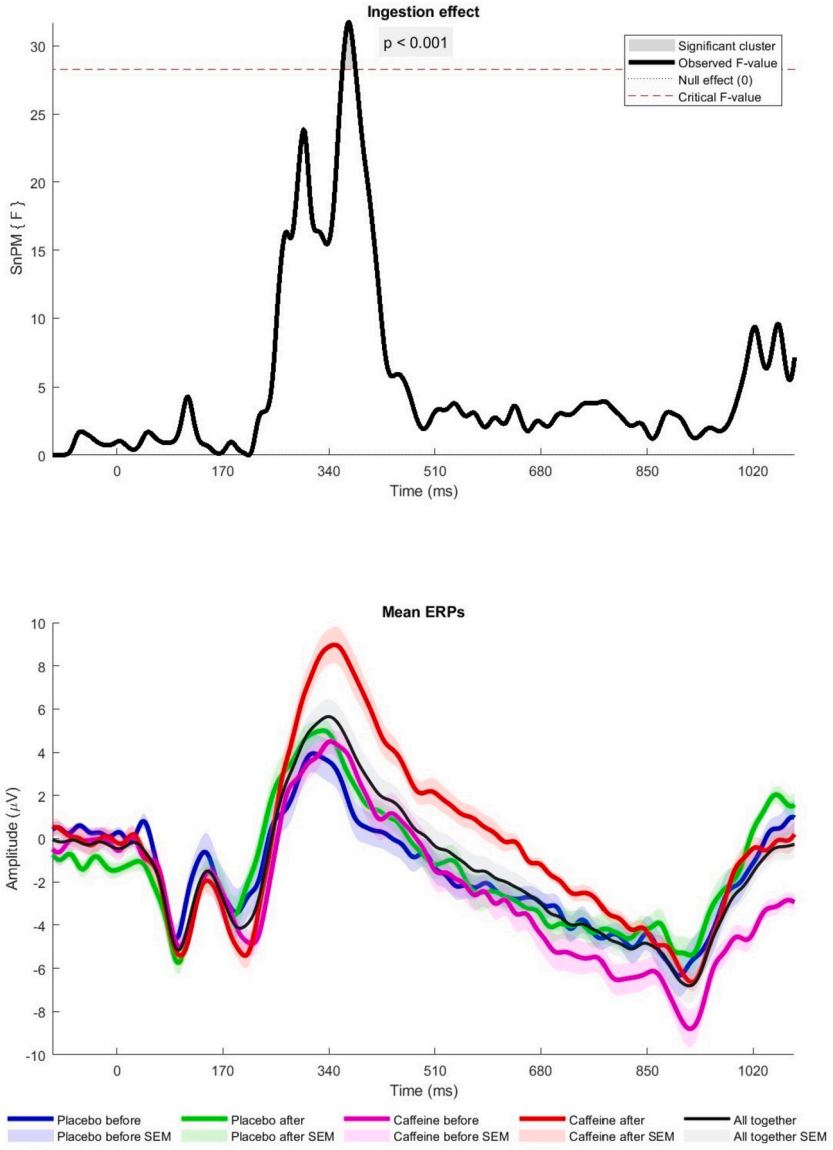

Fig. 4. The significant effect of ingestion at Cz electrode. Omnibus SnPM (statistical non-parametric mapping) testing result with the two-way ANOVA with one repeated measure design. Top panel shows F-values for each data point from -100 ms (pre stimulus) to 1000 ms (post stimulus) for the effect of ingestion (regardless of group). The significant interval is colored light gray, the null effect line ($F = 0$) coincides with the x-axis, and the line of critical F-value (over which the effect is significant) is colored in red. The bottom panel shows the ERP waveforms by group and condition, with the shaded areas representing standard errors of the mean.

3.3. Analysis of ERP datasets

Recall that our ERP data is studied in the time domain and we divided it in epochs, one epoch for each instance of oddball beep. The data was then averaged twice: first over the epochs themselves and then over the subjects (separated by group and condition). 3800 epochs came out from this initially, but we had to remove 398 of them for varying reasons. Of 1216 individual electrode recordings (38 recordings of 32 electrodes), we removed and then interpolated 42. Below we study the remaining ERP datasets.

We start by showing and example of an ERP in the Fig. 3, which was recorded on Cz electrode.

We observe four expected ERP components: N1, P2, N2, and P3. They are considered to reflect the brain's reaction to an (auditory) oddball beep, including (P3) the decision-making processes associated with pressing the button. Their neurological interpretation is available in the literature (see e.g. [52]). In the same figure, we also show the topographical distribution of voltages for these four ERP components, separated by group and condition. They too reflect what is expected in an auditory oddball experiment.

We performed the statistical analysis as described in the Methods section. ANOVA revealed one significant effect of condition (ingestion). It was found on the Cz electrode, between 363 ms and 386 ms after stimulus presentation. We show it in Fig. 4. The time localization of this effect should be interpreted with caution since the SPM method [52] is not very sensitive to localization in time.

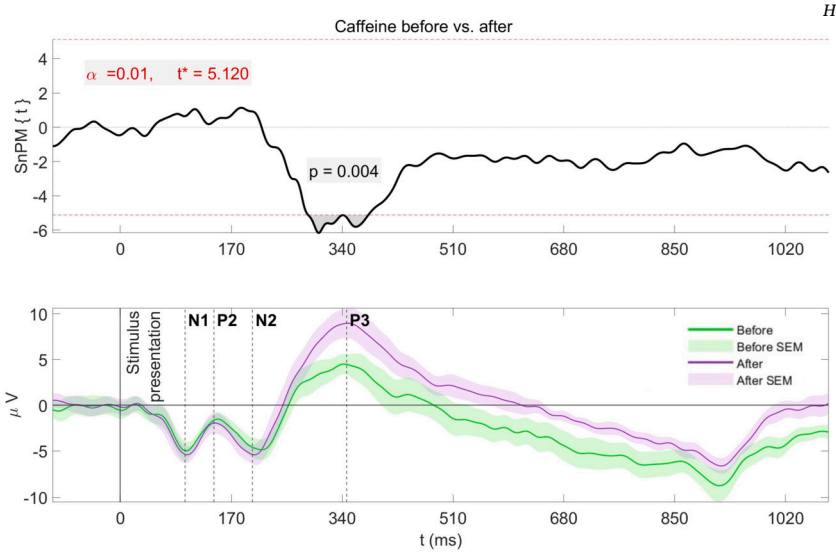

Fig. 5. Top panel: A significant post-hoc effect for the Cz electrode ERP for the caffeine group, cf. top panel in Fig. 4. Bottom panel: Post-ingestion ERP in magenta and pre-ingestion ERP in green for caffeine group.

The observed effect means that there is less than 5% probability (after the correction) that a set of smooth random 1D continua would produce values above the critical F-value threshold as wide (or wider) than observed in our data [52]. This confirms that after the subjects had their beverage – be it coffee or placebo – the activity at Cz electrode changed significantly in that interval. This again suggests that the act of drinking coffee is what makes the change, not the caffeine.

To explain the effect of ingestion seen in Fig. 4, we explored pairwise comparisons in four post-hoc tests. In them, we first compared before/after ingestion ERPs in caffeine and placebo groups separately. Then we compared caffeine and groups' ERPs before and after ingestion. We found one significant effect, this time referring only to the caffeine group. It was found on the Cz electrode in the interval between 243 ms and 381 ms post stimulus. During this interval, post-ingestion ERP exhibited significantly higher values than pre-ingestion ERP. More accurately, we found that there is less than 5% probability (after correction) that a set of smooth random 1D continua would produce values above the critical t-value threshold as wide (or wider) than observed in our data. Fig. 5 shows this effect more closely.

This finding shows that caffeine does have at least some impact on the EEG-revealed brain activity, which is not present in the placebo group. However, note that we found no significant effects on any of the other electrodes.

3.4. Analysis of resting state datasets

Resting state EEG data were taken before the oddball task, both in eyes open and eyes closed state (see Methods). We analyzed it in the frequency domain (power bands).

In Fig. 6 we show the PSDs (power-spectrum densities) for both ingestion conditions, both eye-openness states, and both groups. We also show mean topographies for theta, alpha, and beta frequency bands.

Those bands are of special interest to us, since the power in them has been associated with wakefulness and mental engagement level. They hence provide us with additional avenues for explaining cognitive and cortical effects of ingestion. We found significant effects of the eye openness state on a great majority of electrodes. This is expected, so we will not spend more time on it. We also observed that the power in the beta band was greater in some electrodes during the eyes closed state than during the eyes open state, regardless of group and ingestion. This is interesting, but not unprecedented. Other than this, both PSDs and topographies look as one would expect in this kind of measurement.

We next tested for significant effects of both ingestion condition (before/after) and group (caffeine/placebo). We found a significant interaction effects of group and ingestion condition on several electrodes shown in Fig. 7. They were, respectively, found in the frequency intervals 15–15.7 Hz, 18.1–18.8 Hz, 9.9–10.5 Hz, 9.7 – 9.9 Hz, 10 – 10.4 Hz, and 10 – 10.4 Hz.

For all six electrodes we found less than 5% probability (after correction) that a set of smooth random 1D continua would produce values above the critical F-value threshold as wide (or wider) than the F-trajectory observed in our data. This is a significant effect of belonging to a group vs. before/after ingestion. Therefore, changes of the PSDs for placebo group after ingestion were significantly different from the changes of the PSDs for the caffeine group. This suggests that in this case, both caffeine and the act of drinking played a role.

Finally, we carried out post-hoc tests and found only one significant difference. It was on the FC2 electrode, for PSD before/after ingestion, in the caffeine group. After the ingestion of caffeine, PSD was significantly lower. It reached above the threshold in the frequency intervals 8.95–9.21 Hz and 17.6–18.18 Hz. This result is shown in Fig. 8.

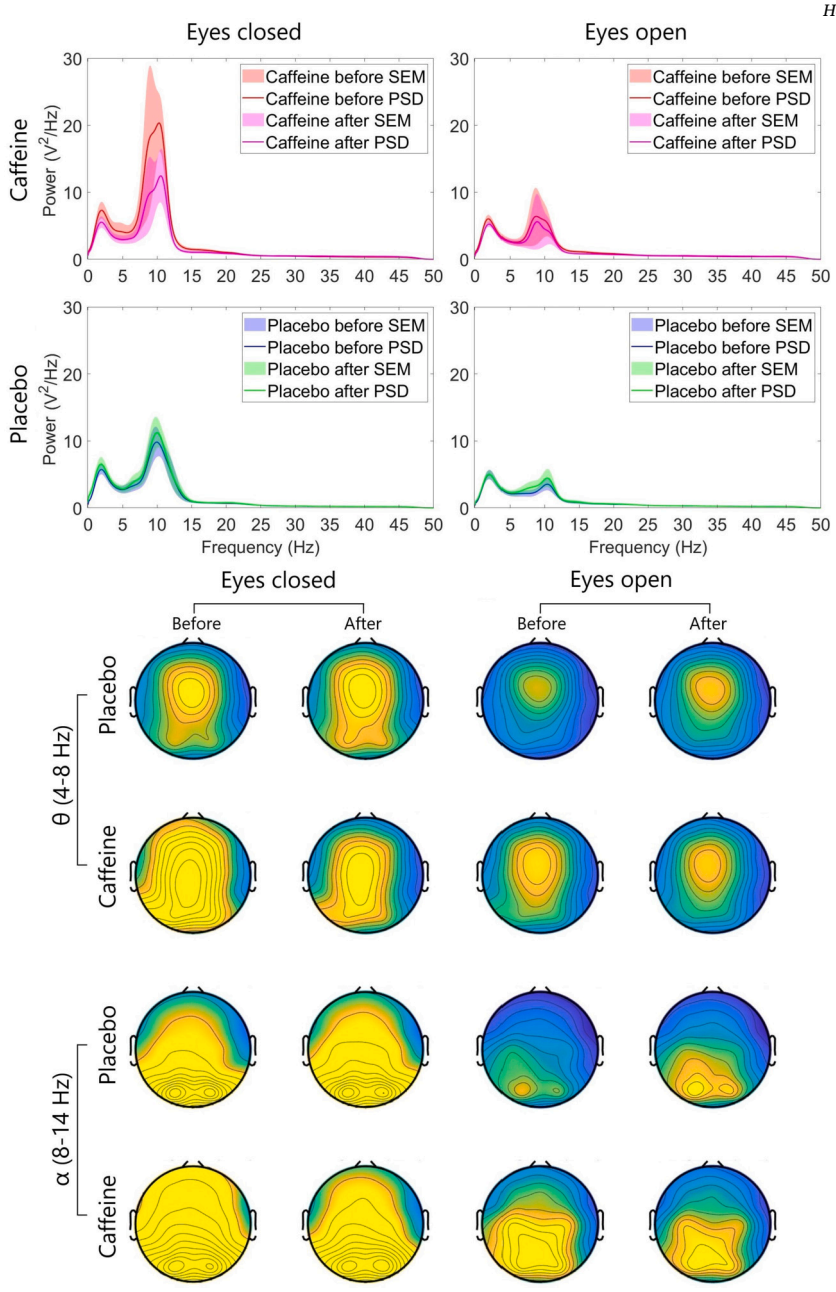

Fig. 6. Top panel: Mean plots of power-spectrum density (PSD), by group, eye openness state, and ingestion condition. Red and magenta are the caffeine group PSDs, green and blue are placebo PSDs (SEM stands for standard error of the mean). Topographies (bottom panel): theta band (4-7 Hz) in the first two rows, alpha band (8-14 Hz) in the last two rows.

We found less than 5% probability that a set of smooth 1D continua could produce supra or sub-threshold values as large (or larger than) the ones observed in the t-trajectory of our data. This shows that in the caffeine group, activity in alpha and beta bands at FC2 electrode were reduced after the ingestion compared to before the ingestion. The same effect was not found in the placebo group.

4. Discussion

We explored the impact of caffeine on cardiovascular parameters, cognitive performance, ERPs, and resting state brain activity. In our $N = 20$ group, the ingestion of both caffeine and placebo significantly increased blood pressure and decreased heart rate. The act of ingestion did not alter the performance in the mental arithmetic test, but it led to a significant shortening of reaction times. In this, no difference between the placebo and caffeine groups reached significance. As for the ERPs, we found that caffeine ingestion

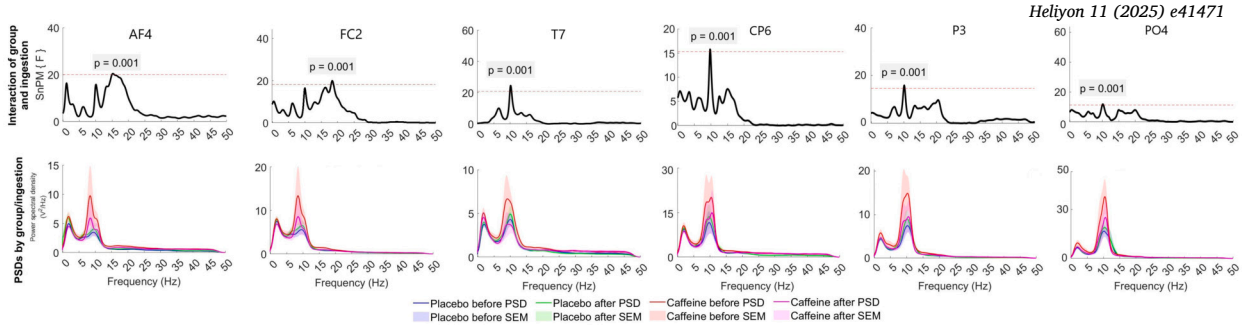

Fig. 7. Significant group × ingestion interaction effects, detected via omnibus SnPM testing, for the electrodes AF4, FC2, T7, CP6, P3, PO4, cf. top panel in Fig. 4. Top row: F-value trajectories, bottom row: PSDs separated by group and ingestion condition.

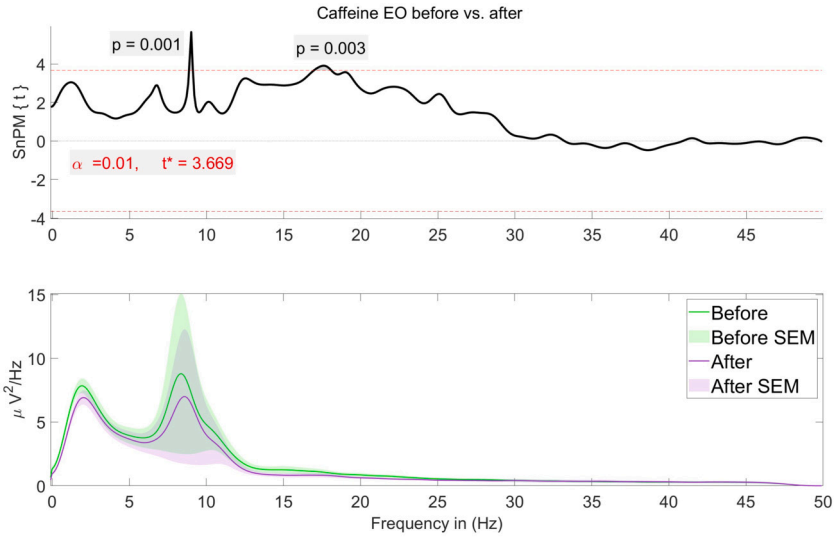

Fig. 8. A significant effect for PSD on the FC2 electrode. Post-ingestion and pre-ingestion caffeine PSDs are colored in magenta and green, respectively, cf. top panel in Fig. 4. This was the only significant post-hoc effect detected.

led to a significant increase of the P3 component at the Cz electrode, while the same increase was not found after ingestion of decaf. Similarly, looking at the resting-state cortical activity, ingesting caffeine or decaf led to significant differential changes in the alpha and beta power bands at electrodes AF4, FC2, T7, CP6, P3, and PO4. Post-hoc testing revealed a decrease in the alpha band power after ingestion of caffeine (but no significant result for the placebo group) for the FC2 electrode only. All other post-hoc tests were not significant. Altogether, our results show that caffeine changes brain activity, albeit to a very small extent, but that change has no significant impact on cardiovascular parameters or cognitive performance. Below we discuss the results and limitations of this work in a few points.

Cardiovascular parameters In our sample of habitual coffee drinkers, we found the heart rate to decrease and blood pressure to increase after ingestion of both caffeine and placebo. In general, this agrees with earlier findings, although there is a debate on some of the issues. Studies on non-habitual coffee consumers showed that ingestion can alter heart rate and blood pressure in different directions [5,24,6,53]. Yoshihara et al. [25] found a decrease of blood pressure for decaf accompanied by a reduction of heart rate. Zimmermann et al. [36] suggested that decaf suppresses vagal activity in habitual coffee drinkers but not in non-habitual ones. This may be related to our findings and explain the lack of differences in cardiovascular responses between the groups. On the other hand, changes we found in blood pressure and heart rate can be also attributed to the habituation of caffeine intake [35] and subsequent autonomic nervous system adjustments. This habituation may compensate for the lack of caffeine. Of course, other active ingredients of coffee might also be at play, but evidence for this is still inconclusive [54].

Cognitive performance We found a reduction in reaction times by 21±5 ms (mean ± SE) after ingestion of caffeine. This agrees with previously found values that range from 10 to 42 ms [5,17,4]. We must stress that comparing these values is challenging, since different studies measure reaction times in different ways and rely on different research designs. For example, while [5] and [17] examined non-habitual coffee drinkers, [4] report no data on habituation of subjects. On the other hand, we also observed a reduction of reaction times by 22±13 ms (mean ± SE) after ingestion of placebo. To our best knowledge, this has not been reported before. This could be due to an anticipation effect that comes from the habituation to coffee. In fact, habitual coffee drinkers have been shown

to exhibit a reduction in reaction times when presented with the smell of coffee. The magnitude of this reduction is similar to what is expected after actual ingestion of caffeine [35]. Our subjects that had placebo were, without exception, convinced they had real coffee. Based on this, we hypothesize that the same phenomenon could be at play.

ERP datasets Statistically significant effects of ingestion were found, in particular, on the P3 component at the Cz electrode, but no significant interaction or group effects. This shows that both groups exhibited a change (an increase) in their ERP after drinking either caffeinated or decaffeinated coffee, but which was not significantly different. However, the post-hoc tests comparing the ERPs before and after ingestion for each group separately showed significant differences for the caffeine group only. One could say that caffeine, being the main active ingredient of coffee, nevertheless produces somewhat stronger effects than the decaf placebo, yet these changes are not large enough to be detected by tests on a sample of our size. The changes in the ERPs of the placebo group, which approach the significance threshold, further this reasoning. Together, the significant group effect, but the absence of other effects in the ANOVA show that the ERPs of both groups changed, with some bias toward a greater increase in the caffeine group, but which was not disparate enough to conclude that caffeine elicited more of a response than placebo.

An increase of the P3 component, as in the present study, has been reported before [5,4,55], but not universally so. The mechanism and replicability of this effect are a matter of ongoing debate. One of the proposed mechanisms involves enhanced excitability of the prefrontal cortex, potentially mediated by adenosine A_{2A} receptor activation. Therefore, while caffeine's enhancement of alertness involves dopaminergic pathways, its effects on the PFC also include direct modulation of glutamatergic synapses via A_{2A} antagonism. This activation increases neuronal excitability and alertness and suggests that A_{2A} controls glutamatergic synaptic plasticity in the PFC independently of dopaminergic modulation [56]. Another mechanism relies on heightened attention resource mobilization that is attributed to caffeine's psycho-stimulative effects. Furthermore, the upregulation of A_{1A} and A_{2A}, alongside purinergic P2X4, P2X7, $P2Y_1$, and $P2Y_4$ receptors after chronic caffeine use can alter the permeability of the blood-brain barrier during caffeine withdrawal [23]. In turn, this can elevate stress levels and diminish cognitive and motor performance [57]. The above mechanisms, in combination with habituation, can explain how caffeine reduces blood-brain barrier permeability and boosts cognitive performance. The same effect, albeit smaller, could be ascribed to stress reduction and activation of habituated neural pathways, when a placebo is ingested.

Resting state datasets The key result of PSD analysis is an interaction between the group belonging and the ingestion condition. We found it on the AF4, FC2, T7, CP6, P3 and PO4 electrodes, in the alpha and beta bands. An explanation in line with our previous results could be that ingestion had an opposite impact on the two groups, causing the power in alpha and beta bands to decrease in caffeine group, and increase in the placebo group. However, we did not confirm these observations by post-hoc testing, which instead showed that the only significant effect was the decrease in the alpha band in the caffeine group (eyes open) post-ingestion, electrode FC2. Similar findings have been reported before, but the location (electrodes) where the significant effects were found and the bands in which they were found vary. Authors of [40] found a reduction in delta and theta bands and an increase in alpha band in the caffeine group. The opposite was seen for their placebo group. Our results are somewhat different from these, but so is the methodology. Namely, Jung et al. grouped and averaged powers in the bands, whereas we used the continuous SPM approach. The study [58] had an interesting placebo strategy involving Coca-Cola variants with/without caffeine and/or sugar. The authors reported a reduction in alpha and beta bands after caffeine ingestion on C3 and C4 electrodes. Note that the C4 electrode is directly adjacent to the FC2 electrode, where we found a significant effect. To tie this to changes in reaction times, it has been suggested that alpha frequency oscillations signify the cortex's readiness to process stimuli [59,60], with strong activity indicating idling, in particular for occipital alpha activity [61]. Therefore, a decreased power in alpha band after caffeine may reflect greater readiness to process information. Such enhanced readiness could speed up the reactions to auditory stimuli. In contrast, the reasons for the reduction in reaction times in the placebo group cannot be gleaned from these analyses.

Confrontation with other studies The recent paper by Pico-Perez et al. [22] contains results similar to our own, but obtained via MRI, rather than EEG. The found decreased DMN connectivity aligns with our EEG findings showing modulation of posterior electrodes (P3, PO4). The frontal changes we observed (FC2, AF4) complement findings of altered frontoparietal connectivity obtained in another similar study [62]. The convergence across methods (EEG frequency changes, fMRI connectivity, behavioral measures) provides robust evidence for caffeine's multi-level effects on brain function, from local oscillations and network reorganization to behavioral outcomes. Notably, our post-hoc finding of decreased alpha power at FC2 after caffeine ingestion may reflect increased impulsivity, as documented in [63] where a high caffeine intake was found to lead to increased risk-taking behavior. Alpha oscillations typically indicate inhibitory control, with decreased power suggesting reduced inhibition. This aligns with the interaction effects we observed in the alpha band (9.7 – 10.5 Hz) across multiple electrodes (T7, CP6, P3, PO4), potentially reflecting a broader modulation of inhibitory control networks. The finding that high caffeine consumption reduced self-control while improving cognitive performance [63] provides a behavioral framework for interpreting these neurophysiological changes. This indicates that caffeine may enhance performance partly through reduced inhibition and increased impulsivity. Effects on impulsivity would explain why reaction times were reduced, but no effect was seen on the mental arithmetic test. Faster responses would be explained by the greater readiness to act, while the setup of the mental arithmetic, forcing the subjects to calculate before giving an answer, could mitigate the increased impulsiveness and thus mask this effect. Even effects of habituation, stimulating the reward pathways of the brain, could mimic these effects and thus reduce reaction times [63]. In animal studies, there is new evidence that regular caffeine consumption has only limited behavioral effects in healthy, rested and non-stressed animals [64], but is contrasted with the ability of caffeine to prevent brain dysfunction in different animal models of brain disease [65]. On the other hand, one must be aware that human coffee consumption involves complex psychological and social dimensions that cannot be adequately captured in animals.

Placebo selection The diversity of reactions to placebo observed in our and in previous studies highlights the importance of which placebo we use [35]. In fact, researchers have used a variety of substances as placebo, including capsules filled with cornflour [5] or starch [53], water [17], and decaffeinated coffee [24,36] as in our case. We propose that the role of placebo choice in studies like these should be studied in more detail. We argue that decaf is the most natural choice, as it includes compounds that amplify the placebo effect via smell, taste, and appearance. This enables a more accurate study of the effects of isolated caffeine, but also of the "ritual" associated with enjoying coffee. On the other hand, regular coffee contains compounds other than caffeine, including polyphenols like hydroxycinnamic acids and flavonoids [38,66,67,54]. Their role can not be entirely excluded. Finally, decaffeinated coffee may retain traces of caffeine during its production. The average caffeine content of decaf is known to be about 1.6 g/kg [38].

Limitations of this work The main limitation of the present study is the sample size of only 20 participants. We did our best to compensate for this by relying on principled statistical analysis. Nevertheless, this could have induced some type II errors (see below). For example, in Fig. 2 one can spot a trend indicating a possible interaction effect pre vs. post ingestion. Unfortunately, due to small sample size, we could not confirm it as statistically significant. In other words, person to person differences in our sample turned out to be large, which means that averaging over subjects might have made some effects less pronounced. This could be remedied by re-designing the study in a way that each subject acts as its own control. We hope that a more nuanced picture of the phenomena will emerge in future studies with larger (and possibly more diverse) sample. Another limitation regards the choice of subtraction from 1000 as a way to measure the cognitive performance. We assumed that subtracting 7 is equally hard as subtracting 9, but this might not be true for every subject. Also, as we noted above, placebo choice might impact results, but it is difficult to know how. Still, some placebo must be used, and a choice must be made. Finally, we stress that our study involved only habitual coffee drinkers. The effect of habituation should thus be verified by comparing reactions of habitual and non-habitual coffee drinkers. Another concern could be raised about the possible presence of effects related to caffeine withdrawal. However, since all participants were regular and not heavy coffee users, we do not expect any withdrawal effects as observed in other studies [32].

Probability of type 2 errors Sensitivity analysis determined that we can only detect very large effects with our sample size of 20 participants, effects being $f = 0.67$, at $\alpha = 0.05$ and $1 - \beta = 0.8$. This makes the probability of a type II error likely in our study. Looking at the results of uncorrected ANOVA tests for EEG analysis gives a perspective to the type II error likelihood. The electrodes showing significant effects in uncorrected analysis were the following:

- ERP analysis: No group effects are detected. Ingestion effects: F7, Fz, FC5, Cz, CP6, P3, PO4. Interaction effects: P7.
- Resting-state analysis: Eye effect: All 32 electrodes exhibit significant effects. Ingestion effect: Fp2, P4. Group-ingestion interaction effect: All electrodes exhibit significant effects, except for T8, O1, Oz, and O2. All other effects exhibit no significance, even in uncorrected analysis.

These electrodes can therefore be more closely investigated in further studies to determine whether these effects are repeatable and also emerge with greater sample sizes.

Conclusions We found clear effects of ingestion of both caffeine and placebo in all streams of analysis, except for the mental arithmetic test that did not reach significance. The effects observed in the placebo group are not significantly different from those found in the caffeine group, except for the spectral distribution of resting-state EEG (alpha power). This highlights the "ritual" dimension of enjoying coffee: Stimuli that closely mimic coffee can produce cognitive and physiological responses markedly similar to those of real coffee, while resting-state power analyses nevertheless show expected readiness-increasing alterations in brain activity. We argue for further study of habituation and conditioning effects in response to caffeine vis-á-vis placebo, investigating how the choice of placebo affects the responses of habitual coffee consumers.

CRediT authorship contribution statement

Mateja Lesar: Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis. **Jakob Sajovic:** Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dušanka Novaković:** Writing – original draft, Methodology, Data curation, Conceptualization. **Maša Primožič:** Investigation, Data curation. **Eva Vetrih:** Project administration, Investigation, Data curation. **Martin Sajovic:** Software. **Anja Žnidaršič:** Writing – review & editing, Validation, Supervision. **Peter Rogelj:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. **Andreas Daffertshofer:** Writing – review & editing, Validation, Supervision. **Zoran Levnajić:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Formal analysis. **Gorazd Drevenšek:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data used in the analyses of this article is available at https://doi.org/10.6084/m9.figshare.25592466.v1.

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