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ORIGINAL INVESTIGATION

Electro-physiological changes in the brain induced by caffeine or glucose nasal spray

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Abstract
Objective A direct link between the mouth cavity and the brain for glucose (GLUC) and caffeine (CAF) has been established. The aim of this study is to determine whether a direct link for both substrates also exist between the nasal cavity and the brain.
Methods Ten healthy male subjects (age 22 ± 1 years) performed three experimental trials, separated by at least 2 days. Each trial included a 20-s nasal spray (NAS) period in which solutions placebo (PLAC), GLUC, or CAF were provided in a double-blind, randomized order. During each trial, four cognitive Stroop tasks were performed: two familiarization trials and one pre- and one post-NAS trial. Reaction times and accuracy for different stimuli (neutral, NEUTR; congruent, CON; incongruent INCON) were determined. Electroencephalography was continuously measured throughout the trials. During the Stroop tasks pre- and post-NAS, the P300 was assessed and during NAS, source localization was performed using standardized low-resolution brain electromagnetic tomography (sLORETA).
Results and discussion NAS activated the anterior cingulate cortex (ACC). CAF-NAS also increased θ and β activity in frontal cortices. Furthermore, GLUC-NAS increased the β activity within the insula. GLUC-NAS also increased the P300 amplitude with INCON ($P = 0.046$) and reduced P300 amplitude at F3-F4 and P300 latency at CP1-CP2-Cz with NEUTR ($P = 0.001$ and $P = 0.016$, respectively). The existence of nasal bitter and sweet taste receptors possibly induce these brain responses.
Conclusion Greater cognitive efficiency was observed with GLUC-NAS. CAF-NAS activated cingulate, insular, and sensorymotor cortices, whereas GLUC-NAS activated sensory, cingulate, and insular cortices. However, no effect on the Stroop task was found.

Keywords EEG · Stroop · Attention · ERP P300 · Source localization · sLORETA

Introduction

The ingestion of substrates, such as glucose (GLUC) and caffeine (CAF), results in a peripheral and a central action. Rinsing a solution within the mouth cavity rules out the metabolic action of the exogenous substrate and enables researchers to examine underlying brain responses. Brain imaging techniques revealed that oral GLUC sensing activates the frontal cortex (Benton et al. 1994; De Pauw et al. 2015; Diukova et al. 2012; Gagnon et al. 2012), a brain area involved in several cognitive processes, reward, and motor control (Chambers et al. 2009). CAF mouth rinsing was also shown to influence the activity within the dorsolateral prefrontal (DLPFC) and orbitofrontal cortex (De Pauw et al. 2015). Taste receptors within the mouth cavity are possibly responsible for the link to the brain, because they might activate dopamine pathways in the brain reward centers (de Araujo et al. 2010; Jeukendrup et al. 2013).

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Mouth rinsing is an administration route, which enables investigation of the central mechanisms of substrates. The direct link between the mouth cavity and the brain has been well investigated using exercise performance and brain measures (Burke and Maughan 2015; Jeukendrup et al. 2013). Another administration route also allows us to determine brain responses to substrates, i.e., the nasal cavity. The high permeability of the nasal epithelium allows molecules with a mass cutoff at approximately 1000 Da to enter the brain (Jogani et al. 2008). This signaling pathway avoids the hepatic first-pass effect, reduces substrate delivery to non-targeted sites, and facilitates the administration of lower doses (Jogani et al. 2008). The region situated in the roof of the nasal cavity, i.e., the olfactory region, is densely covered with blood vessels and offers a direct access to the central nervous system via the fibers of the olfactory nerves (Pires et al. 2009; Stevens and Lowe 1997). Therefore, nasal delivery of substances and vaccines are already in use (Jogani et al. 2008).

Since GLUC and CAF have a molecular mass of 180 and 194 Da, respectively, it is assumed that these substrates will be transported across the nasal epithelium. Shah et al. (2009) already found that sensory bitter taste receptors in the motile cilia of the nasal cavity sensed bitter compounds and increased the intracellular Ca^{2+} concentration. Additionally, evidence is available that GLUC is permeable from the serosal to the mucosal side of the porcine mucosa (comparable to human nasal mucosa) and vice versa (Wadell et al. 1999).

A direct link between the nasal cavity and the brain might be evidenced with cognitive measures. GLUC and CAF ingestion already demonstrated the potential to alter cognitive performance. CAF ingestion improves attention, increases alertness and psychomotor speed, and reduces simple and complex reaction times (Diukova et al. 2012; Giles et al. 2012; Hogervorst et al. 2008; Smith et al. 2005). GLUC beneficially alters specific aspects of attention, like selective and divided attention (Benton et al. 1994; Sünram-Lea et al. 2002) and memory functioning (Brown and Riby 2013; Messier 2004; Smith et al. 2009). A facilitating effect of GLUC ingestion was observed with reaction times (RT) on more complex stimuli (Brandt et al. 2013). Furthermore, positive effects were observed during a recognition memory task (Smith et al. 2009) and the inhibition condition of the Stroop task (Gagnon et al. 2010).

Responses to stimuli are an end product of many different cognitive operations and thus do not provide direct information about the effects of substrates on brain functioning. To investigate the specific effects of CAF and GLUC on brain responses during task performance, additional event-related potential (ERP) analysis is recommended (Lorist and Tops 2003). The ERP component P300 is of importance for stimulus evaluation, selective attention, and conscious discrimination (Patel and Azzam 2005). The amplitude of P300 is proportional to the amount of attentional resources devoted to task stimuli (Wickens et al. 1983). The P300 latency is a sensitive measure of stimulus-evaluation time (Coles et al. 1995). The visual three-stimulus oddball task has been shown to moderate the magnitude and latency of the P300 at medial-temporal lobes and the prefrontal cortex with glucose ingestion (Riby et al. 2008). On the other hand, Geisler and Polich (1994) and Knott et al. (2001) did not observe glucose modulation of the P300 during a visual oddball task and a visual memory task, respectively. More consistent findings exist regarding CAF ingestion and reduced P300 latency at the frontal cortex during cognitive performance tasks (Deslandes et al. 2004, 2005; Diukova et al. 2012; Reeves et al. 1999). Martin and Garfield (2006) also showed increased P300 amplitude in the choice RT task with CAF ingestion.

The question arises whether nasal administration of CAF or GLUC induces brain responses. Thus, the aim of the current experiment is to examine the influence of nasal sprays (NAS) containing GLUC, CAF, or placebo (PLAC) on brain activity using the cognitive Stroop task and electroencephalography (EEG) (standardized low-resolution brain electromagnetic tomography or sLORETA and the ERP component P300). In line with the existing mouth rinse research, it is hypothesized that both GLUC and CAF-NAS alter the activity within the frontal cortex and beneficially influence cognitive performance.

Method

Subjects

Ten healthy, non-smoking male subjects (PL2 according to De Pauw et al. 2013) participated in this experiment (age 22 ± 1 years, height 1.82 ± 0.07 m, weight 78 ± 13 kg). The daily CAF intake of the subjects was very low: 0.5 ± 0.6 (<50 mg/day) caffeine-containing beverages. The subjects were asked to abstain from beverages containing CAF, other psychoactive substances, or medication for at least 24 h before each experimental trial. All experimental trials were planned in the morning (06:00–09:30), and the subjects were only allowed to drink water before the experimental trials. Preceding each experimental trial, the participants were asked about information regarding nasal irritation and sinusitis. The experiment was approved by the institutional medical ethical committee of UZ Brussel and Vrije Universiteit Brussel (Belgium) (B.U.N. 143201421380). The subjects were provided written and oral information about the experimental procedures and potential risks before giving informed consent to participate in this study. After receiving a general description of the experiment, the

subjects were prepared (placement of EEG electrodes) for the first trial.

Protocol

The subjects reported three times to a sound-insulated laboratory separated by at least 2 days. During the whole experiment, the subjects were seated in a comfortable chair, wore earplugs, and kept the same body posture. Three NAS solutions were given to the subjects in a double-blind, placebo-controlled, randomized manner. To determine the effect of the NAS solutions on brain activity, electrodes were placed on the subjects' head before the start of each experimental trial. The EEG was continuously measured during the whole experimental trial (approximately 45 min). Cognitive tasks (Stroop tasks) were performed: two familiarization trials to minimize learning effects and one baseline (Stroop pre) and one post the 20-s NAS period (Stroop post) (Fig. 1).

Stroop task

The Stroop task was programmed and performed on E-prime 2.0 software (Psychology Software Tools, Inc., Pittsburg, PA). This multiple-choice RT test comprised two parts: the first part included 50 neutral stimuli (NEUTR; Stimulus X, colored in red, green, yellow, and blue), and the second part randomly presented 100 color words [stimulus, red, green, yellow, and blue; responses, keyboard buttons V, H, F, and B (AZERTY), respectively] written in congruent (CON, color-word matching; 50 %) and incongruent (INCON, color-word non-matching; 50 %) color. The interval response-stimulus-onset was set at 500 ms and the distance to the screen was approximately 40 cm. During the second familiarization trial, the lights were turned off. For each series, the response time (in ms) and accuracy of the responses (% of correct responses) were determined. NEUTR, CON, and INCON were marked during continuous EEG measurements as S1 (response marker, S2), S3 (response marker, S4), and S5 (response marker, S6), respectively. An express card enabled the connection between the two separate computers (computer 1, E-prime; computer 2, Brain Vision Recorder).

Nasal spray solutions

Different solutions were nasally sprayed, i.e., CAF, GLUC, or PLAC, in a randomized, double-blind, placebo-controlled manner. NAS were prepared by Qualenica (Malle, Belgium). The basic solution was prepared according to the Therapeutic Magistral Formulary, which is a reference work for pharmaceutical compounding, and consisted of distilled water up to 400 ml with benzalkoniumchloride (40 mg) to preserve the NAS and natrium chloride. Additionally, in order to modify particle morphology and flowability, the following two excipients were used: hydroxypropylmethylcellulose (2 g) and mannitol (2.57 g) (Sacchetti et al. 2002). The final [CAF] was 15 mg/ml and the ratio CAF/mannitol/HPMC 65.1:27.9:2 (Sacchetti et al. 2002). In most mouth rinse researches, %1.2 *w/v* CAF (Beaven et al. 2013) and %6.4 *w/v* maltodextrin (Sinclair et al. 2014) are used. Thus, the amount of GLUC in the mouth rinse solutions was 5.333 times higher compared to the concentration of CAF. Therefore, GLUC-NAS contained a final [GLUC] of 80 mg/ml.

Preceding each experimental trial, the nose was cleared. Each time the subjects sprayed the solution within the nasal cavity, they alternately sprayed twice in the right and twice in the left nostril in order to optimally disperse the solution within the nasal cavity.

Electrophysiological measurements

Continuous EEG data were derived from 32 active Ag/AgCl electrodes attached on the subjects' head (Acticap, Brain Products, Munich, Germany) according to the "10–20 International System" (Jasper 1958). The sampling rate was set at 500 Hz (Brain Vision Recorder, Brain Products, Munich, Germany). Electrode impedance was kept <5 kΩ throughout the experiment. During the cognitive tasks and NAS period, the subjects were instructed to relax and maintain the same posture. To reduce distraction of the subjects and to minimize sound artifacts, the lights were turned off and the subjects wore earplugs. After the second familiarization trial, the subjects had to remain seated with eyes closed for 1 min (baseline measurement). The latency and amplitude of the stimulus-locked ERP P300 were analyzed during the Stroop tasks before and after the NAS period. During the 20-s NAS period, we applied the source localization technique sLORETA to determine electrocortical brain alterations in response to the three NAS solutions (compared to baseline).

Event-related potential analysis

The program Brain Vision Analyzer (version 2.0.4) was used to preprocess and process the datasets. The ERP component P300 was analyzed. Raw data were down-sampled to 256 Hz,

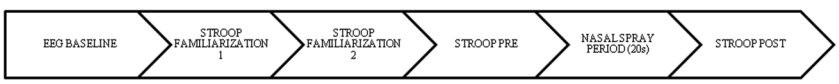

Fig. 1 Timeline of one experimental trial (Stroop pre: Stroop task at baseline and Stroop post: Stroop test after NAS period)

filtered (high pass: 0.1 Hz, low pass 45 Hz and Notch; Slope 48 dB/oct) with a Butterworth filter design, and re-referenced to an average reference of all electrodes. For each dataset of interest (i.e., ERP during the cognitive task at baseline, EEG during baseline and the NAS period, and ERP during the post-NAS period), artifacts (signal shifts and distortions across all electrodes) were manually removed. Then, the different stimuli (S1, S3, and S5) were extracted from the EEG datasets. For stimulus-locked ERP analysis, a data window was set at −200 to 800 ms relative to the stimulus onset. For each ERP epoch, independent component analysis (ICA) and inverse ICA further reduced periodic recurrent artifacts, such as eye blink artifacts. Furthermore, a baseline correction was induced (period, −200 to 0 ms). Epochs were then averaged and the visually evoked ERP component was assessed. Peak amplitude and onset latency were measured, which was defined as the largest positive-going (P300) peaks occurring within the time window between 240 and 470 ms. Thereafter, the data from the Brain Vision Analyzer were exported to SPSS (v 22.0; Chicago, IL) for further analysis. Electrodes were clustered into several regions of interest (ROI) according to the location of the electrodes and, consequently, brain functions. The ROIs (n = 7) were FP1-FP2 (anterior prefrontal cortex), F3-F4 (Brodmann area 8, involved in planning complex movements), FC1-FC2 (premotor cortex and supplementary motor areas), C3-C4 (primary somatosensory and motor cortex), F7-F8 (orbitofrontal cortex), CP1-CP2-Cz (Brodmann area 5, somatosensory processing and association), and CP5-CP6 (supramarginal gyrus).

Standardized low-resolution brain electromagnetic tomography

The ICA and inverse ICA were also applied on EEG datasets during the NAS period to remove eye and muscle artifacts. Segments of each dataset were averaged to a 4-s window (data points, 1024; frequency resolution, 0.25 Hz). These artifact-free datasets were exported and inserted in the program sLORETA. The latter program is a source localization method that attempts to solve the inverse problem by assuming related orientations and strengths of neighboring neuronal sources (Pandey et al. 2012; Pascual-Marqui et al. 2002). First, electrode names (.txt files) were converted to an electrode coordinate file (.sxyz file) and a transformation matrix (.spinv file). The classical frequency bands delta (δ; 1.5–6 Hz), theta (θ; 6.5–8 Hz), alpha 1 (α1; 8.5–10 Hz), alpha 2 (α2; 10.5–12 Hz), beta 1 (β1; 12.5–18 Hz), beta 2 (β2; 18.5–21 Hz), and beta 3 (β3; 21.5–30 Hz) were selected. Second, EEG datasets (.dat files) were converted to cross spectra files (.crss files) and then the program sLORETA computed the corresponding 3D distribution of the electric neuronal generators (.slor files). The latter files were computed for each subject and dataset for each aforementioned frequency band.

Statistical analysis

Statistics were computed using SPSS 22.0 (Chicago, IL). All data (RT, accuracy, P300 peak amplitudes, and latencies) were normally distributed.

For each type of stimulus of the Stroop task, RT and accuracy data were processed with the repeated-measures general linear models [factors time (2) and interventions (3)]. The amplitude and latency of the P300 were analyzed per stimulus of the Stroop task (NEUTR, CON, and INCON) using a three-way repeated-measures general linear models [factors ROI (7) ∗ intervention (3) ∗ time (2)]. When the assumption of sphericity was not met (epsilon <0.75 or nothing is known about sphericity), the Greenhouse-Geisser correction was applied. Significant interaction effects were further analyzed using two-way repeated measures ANOVA (with factors ROI ∗ intervention − ROI ∗ time or intervention ∗ time) and post hoc tests with Bonferroni correction. The significance level was set at $P < 0.05$.

sLORETA statistical analyses are performed at voxel level, involving the formation and assessment of a statistical non-parametric map, which shows the highest possible statistical power (Nichols and Holmes 2002). Furthermore, multiple tests are performed at all voxels simultaneously, since no “a priori” hypotheses exist (Nichols and Holmes 2002). To correct for these multiple comparisons, the statistical program of sLORETA is based on Fisher’s permutation test (Fisher 1935) and relies on a bootstrap method with 5000 randomizations. An important outcome measure of sLORETA statistics is the classical critical t value ($t_{critical}$). Voxels with statistical values exceeding the $t_{critical}$ have their null hypotheses rejected. The omnibus null hypothesis (combined voxel hypotheses) states that there was no activation anywhere in the brain, and, if rejected (at $P < 0.01$), a significant difference in a specific frequency band existed at these voxels between two conditions. The statistical non-parametric map method provided voxel information, i.e., Montreal Neurological Institute/Talairach coordinates, Brodmann area (BA), lobe, and structure. Thereafter, Brodmann areas were clustered according to brain functions in the following brain regions: primary somatosensory cortex (Brodmann areas 1, 2, and 3), the secondary association cortex (SAC, Brodmann areas 5 and 7), motor cortices (Brodmann areas 4, 6, and 8), the DLPFC (Brodmann areas 9, 10, 44, 45, and 46), cingulate cortices (Brodmann areas 23, 24, 29, 30, 31, 32, and 33), as well as the insula (Brodmann area 13); the supramarginal gyrus (Brodmann area 40);and Brodmann areas 27, 39, and 43. The sum of the significantly activated voxels within these brain areas was calculated.

Results

Stroop task

Repeated measures ANOVA did not reveal any significant difference for both the RT and accuracy of the Stroop task (Table 1).

For the amplitude of P300, three-way ANOVA revealed significant interaction effects for INCON and NEUTR. An interaction effect intervention ∗ time was observed for the INCON [F (2,18) = 4.851, P = 0.021]. Further two-way ANOVAs revealed a significant time effect for GLUC independent of ROI [F (1,9) = 5.339, P = 0.046; P300 amplitude pre 1.7 ± 1.1 μV, P300 amplitude post 2.4 ± 0.9 μV). Furthermore, the three-way ANOVA revealed a significant interaction effect ROI ∗ intervention ∗ time for NEUTR [F(3.574, 108) = 3.050, P = 0.035]. Further two-way ANOVAs revealed significant interaction effects intervention ∗ time for different ROIs. At F3-F4, an interaction effect (factors, intervention ∗ time) was observed [F (2,18) = 6.067; P = 0.01]. Post hoc comparisons with Bonferroni correction (P < 0.017) showed for GLUC a significantly reduced P300 amplitude at F3-F4 when NEUTR were presented (P = 0.001; P300 amplitude pre 2.5 ± 0.9 μV, P300 amplitude post 1.4 ± 0.7 μV). We also observed at F7-F8 an interaction effect (intervention ∗ time) [F (2,18) = 3.654, P = 0.047]. A one-way ANOVA with Bonferroni correction showed no significant difference for P300 amplitude at F7-F8 for CAF-NAS compared to PLAC-NAS in the post-NAS period when NEUTR were visualized [F (2,18) = 3.078, P = 0.071; pairwise comparisons P = 0.083; CAF, 2.6 ± 1.5 μV; PLAC, 1.4 ± 1.4 μV].

For the latency of P300, a significant interaction effect ROI ∗ intervention was observed for NEUTR [F(4.741, 108) = 2.821, P = 0.029]. Further two-way ANOVAs revealed a significant effect at CP1-CP2-Cz [F(Deslandes et al. 2005) = 3.926; P = 0.038]. A significant lower latency of the P300 at CP1-CP2-Cz was observed when NEUTR were displayed for GLUC (P300 latency pre 373 ± 60 ms, P300 latency post 358 ± 31 ms; P = 0,016).

Nasal spray period (standardized low-resolution brain electromagnetic tomography)

The $t_{critical}$ values for CAF, PLAC, and GLUC-NAS were 6.249, 3.298, and 5.947, respectively. When comparing baseline measures with NAS periods per NAS solution, sLORETA clearly showed that CAF-NAS activated most voxels within the somatosensory cortices, motor cortices, DLPFC, and cingulate cortices (Figs. 2 and 3).

With PLAC-NAS, increased activity within different frequency ranges were observed in the ACC (δ: n = 29, 3.49; θ: n = 5, 3.75; α1: n = 20, 4.00; β1: n = 7, 3.46; β2: n = 17, 3.58). Additionally, PLAC-NAS increased the δ and θ activity of the DLPFC (δ: n = 44, 3.43; θ: n = 48, 3.63) and orbitofrontal cortex (δ: n = 18, 3.43; θ: n = 1, 3.31). PLAC-NAS also increased α1 activity of the DLPFC (n = 38, 3.70) and the insula (n = 8, 3.44).

Whereas PLAC-NAS did not show any significant difference in any voxel of the somatosensory cortices and motor cortices, CAF-NAS altered the activity of the primary somatosensory cortex (δ n = 35, 8.80; θ : n = 13, 7.58; α2 : n = 5, 7.11; β: see Table 2), SAC (β2: n = 29, 6.82; β3: n = 312, 10.41), and motor cortices (δ: n = 73, 9.17; θ: n = 120, 9.74; α1: n = 95, 9.74; α2: n = 100, 9.91; β1: n = 142, 10.35, β2: n = 45, 9.66, and β3: n = 110, 7.05). GLUC-NAS also increased activity in the primary somatosensory cortex (δ: n = 9, 6.49; α2: n = 1, 6.17), SAC (β1: n = 60, 8.62; β2: n = 6, 6.60), and motor cortices (δ: n = 22, 7.20; α2: n = 16, 7.57).

In the ACC, all NAS solutions significantly altered the activity of voxels in all frequency ranges (see Table 2, representing data for the β frequency ranges) with the highest amount of voxels in the CAF-NAS (δ: n = 10, 7.89; θ: n = 8, 6.98; α1: n = 28, 7.20; α2: n = 71, 10.55; β1: n = 61, 9.33; β2: n = 30, 8.18; β3: n = 19, 7.21) and GLUC-NAS groups (α2: n = 1, 6.02; β1: n = 1, 6.11). Furthermore, CAF and GLUC-NAS increased the activity of the posterior cingulate cortex (CAF-NAS: β3: n = 76, 7.93; GLUC-NAS: β1: n = 24, 7.53; β2: n = 11, 6.64).

Within the prefrontal cortex, CAF-NAS clearly showed the highest amount of voxels showing a t value above the $t_{critical}$ in the DLPFC (δ: n = 82, 9.93; θ: n = 192, 11.08; α1: n = 61, 8.86; α2: n = 126, 9.78; β1: n = 153, 10.08; β2: n = 1, 6.76) and orbitofrontal cortex (θ: n = 108; 7.73; α2: n = 96, 8.81; β1: n = 18, 7.61). GLUC-NAS increased the activity of the

Table 1 Reaction times (mean ± SD) on congruent (CON), incongruent (INCON), and neutral stimuli (NEUTR) for the three NAS conditions

	GLUC-NAS		CAF-NAS		PLAC-NAS	
	Pre	Post	Pre	Post	Pre	Post
CON (ms)	560 ± 42	561 ± 84	564 ± 60	568 ± 83	624 ± 87	600 ± 86
INCON (ms)	624 ± 79	628 ± 103	635 ± 118	661 ± 130	665 ± 77	674 ± 82
NEUTR (ms)	566 ± 95	585 ± 94	582 ± 94	581 ± 106	585 ± 70	613 ± 130

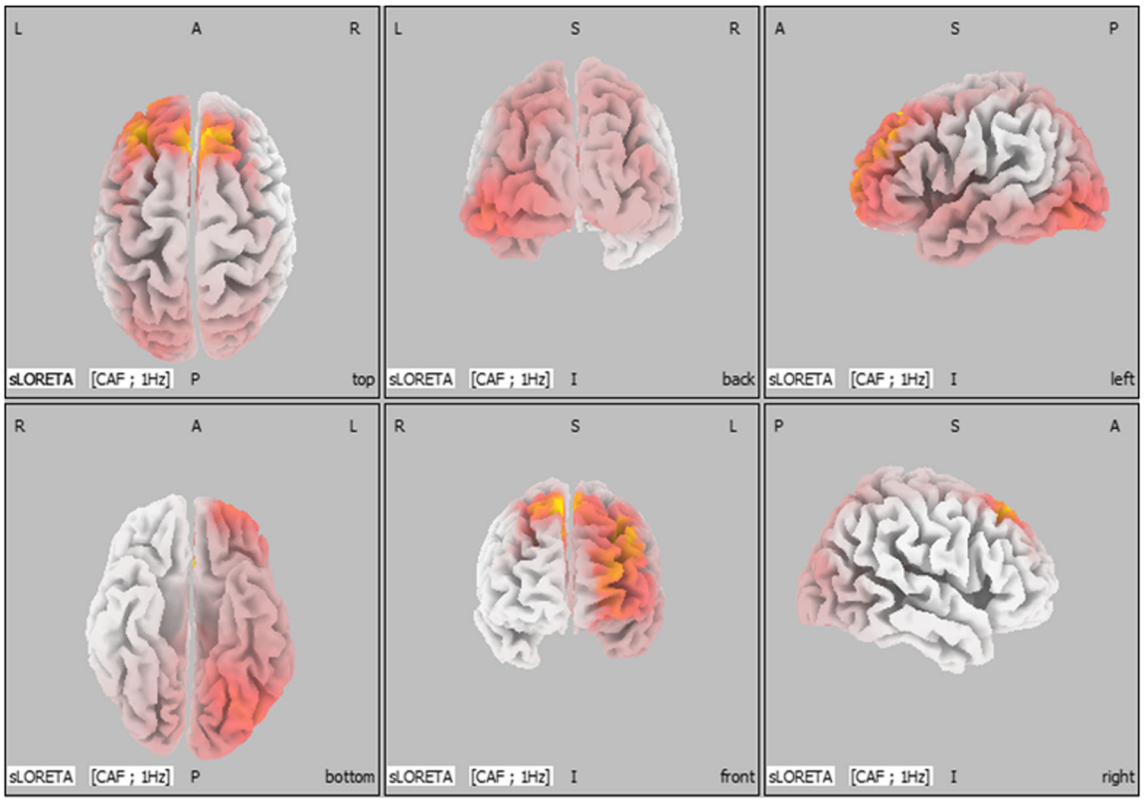

Fig. 2 Brain areas with altered β1 activity (marked in *yellow* and *red*) during CAF-NAS (*A* anterior, *P* posterior, *L* left, *R* right, *S* superior, *I* inferior)

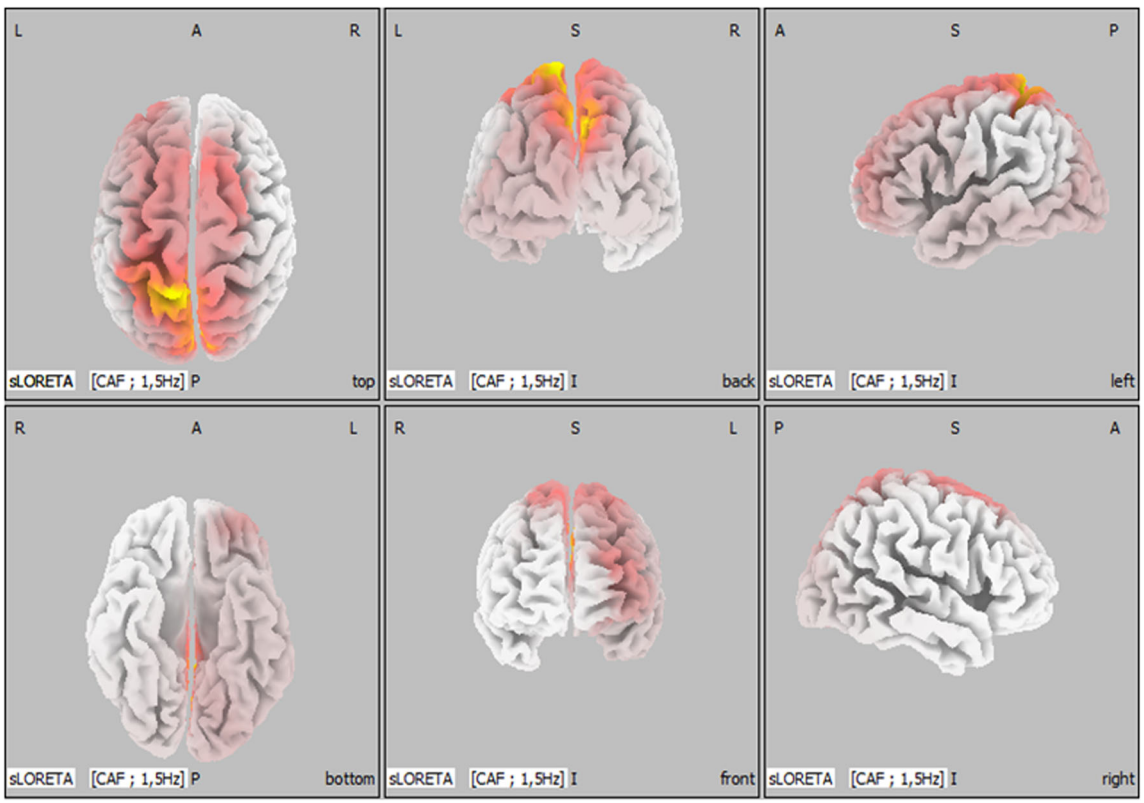

Fig. 3 Brain areas with altered β3 activity (marked in *yellow* and *red*) during CAF-NAS (*A* anterior, *P* posterior, *L* left, *R* right, *S* superior, *I* inferior)

Table 2 Amount of significantly activated voxels per brain region and NAS solution for β1, β2, and β3 frequency ranges

Brain regions	β1			β2		β3	
	PLAC	CAF	GLUC	CAF	GLUC	PLAC	CAF
Primary somatosensory cortex	–	–	–	–	–	–	39
SAC	–	–	60	29	6	–	312
Motor cortices	–	142	–	45	–	–	110
DLPFC	–	153	–	1	–	–	–
Orbitofrontal cortex	–	18	–	–	–	–	–
ACC	7	61	1	30	–	17	19

SAC secondary association cortex, *DLPFC* dorsolateral prefrontal cortex, *ACC* anterior cingulate cortex

DLPFC (δ: n = 1, 6.10; α1: n = 2, 6.22; α2: n = 72, 7.32), orbitofrontal cortex (α2: n = 2, 6.41), and Brodmann area 4 (precentral gyrus: θ: n = 1; 5.95).

CAF and GLUC-NAS also increased the activity of the insula (CAF-NAS, δ: n = 33, 8.87; θ: n = 34, 8.25; GLUC-NAS: β2: n = 3, 6.18) and supramarginal gyrus (CAF-NAS, δ: n = 46, 8.22; θ: n = 19, 10.47; β3: n = 10, 8.82; GLUC-NAS: δ: n = 47, 7.87; β1: n = 4, 6.04).

Discussion

The current study investigated brain responses when CAF or GLUC were nasally administered. One of the main findings is that CAF-NAS activated the prefrontal brain areas and the cingulate, insular, somatosensory, and motor cortices during the NAS period compared to baseline. GLUC-NAS resulted in greater cognitive efficiency when complex stimuli were displayed and faster stimulus evaluation times at central electrodes when NEUTR were presented. GLUC-NAS also showed to increased (β) activity of the SAC, cingulate cortex, and insular cortex. PLAC-NAS also showed increased (β) activity within the ACC.

The current study showed that CAF-NAS increased the θ activity in many voxels of the ACC and frontal brain areas. Previous research described that the frontal θ power is generated in the ACC (Gevins et al. 1997), which has neuronal projections to frontal brain areas (Barbas 1995) and is functionally connected with the frontal lobe (Koski and Paus 2000). Increased θ activity of the frontal lobe and the ACC was also shown when a herbal drop was nasally administered (Chan et al. 2011). In the present study, CAF-NAS also increased β activity in the ACC (as well as the posterior cingulate cortex) and prefrontal brain areas. The underlying mechanism is possibly the activation of extraoral bitter taste receptors, i.e., receptors within the nasal cavity (Devillier et al. 2015; Lee and Cohen 2014; Tizzano and Finger 2013), which recognize many compounds, such as caffeine. This in turn triggers the activation of the primary taste cortex, located in the anterior insula and frontal operculum, and the putative secondary taste cortex in the orbitofrontal cortex (Jeukendrup et al. 2013; Small et al. 2007), which have projections to the DLPFC and ACC. The latter brain area might provide the link between this sensory pathway and the appropriate emotional, cognitive, and behavioral response (Kringelbach 2004). Previous research already demonstrated that sensory bitter taste receptors in the motile cilia of the nasal cavity sensed bitter compounds and increased the intracellular Ca^{2+} concentration (Shah et al. 2009). This underlying mechanism is in line with the beneficial effects of mouth rinsing on exercise performance due to the activation of taste receptors (Jeukendrup et al. 2013).

Sweet taste receptors have also been identified in the nasal cavity (Lee et al. 2014). However, the function and role of the sweet taste receptor in the nasal chemosensory cells remain unknown (Lee et al. 2014). In the current study, GLUC-NAS increased the β activity within the anterior insula and cingulate cortex, but no increased β (or θ) activity was observed in the frontal brain areas. The increased activity of the insular cortex is in accordance to the findings of Turner et al. (2014), who observed that maltodextrin mouth rinsing activated the insular cortex. Thus, a possible mechanism inducing brain responses through the nasal administration of substrates is the presence of the sweet and bitter taste receptors. Bitter taste receptors are expressed in solitary chemosensory cells in the human nose (Barham et al. 2013; Braun et al. 2011). These chemosensory cells also express subunits comprising the human sweet taste receptor (Tizzano et al. 2011). Another possible pathway that triggers brain responses via the nasal administration of substrates is the olfactory pathway. The activation of olfactory sensory neurons might initiate a signal transduction cascade, which activates brain areas involved in olfaction. De Araujo et al. (2003) also outlined that olfactory inputs in the human brain converge in the far anterior insular cortex, which plays a role in higher-order cognitive processes and motor control (hand-eye movement) and is reported to be active during conflict

processing in the Stroop literature (Grandjean et al. 2012). The current findings implicate that the localization of electrocortical alterations or brain responses of substrates can be investigated via nasal administration. In line with previous research on nutrition intake or mouth rinsing, the nasal administration of substrates activates brain areas involved in executive functions, sensory information processing, motor control, and planning and thus might also beneficially influence endurance and/or sprint performance via central mechanisms.

It should be mentioned that the PLAC-NAS condition increased the β activity of the ACC, as well as the θ frequency within the ACC, DLPFC, and the orbitofrontal cortex. From an anatomical point of view, the olfactory nerves connect the nasal cavity with the ACC (de Araujo et al. 2003). This means that any solution sprayed within the nasal cavity activates the olfactory bulb and, consequently, the ACC.

Alpha activity is probably generated in several areas of the cortex due to cortico-cortical and thalamo-cortical interactions (Niedermeyer and Lopes da Silva 2005). In the current study, GLUC and CAF-NAS increased the fast α frequency activity (10.5–12 Hz), which indicates a synchronization of the signal within this frequency range and a lower number of neuronal populations activated during the NAS period. The increased α activity has also been observed when a glucose beverage (Knott et al. 2001) or a carbohydrate supplement (Wang et al. 2004) was orally administered, but the functional significance remains inconclusive.

In the current study, the cognitive Stroop test was implemented, because it has been consistently associated with a large fronto-parietal network, typically involving the ACC, DLPFC, inferior frontal gyrus, inferior and superior parietal cortex, and insula (Nee et al. 2007; Roberts and Hall 2008). Furthermore, GLUC and CAF ingestion and mouth rinsing are known to activate the frontal cortex and reward circuitry. Although the ACC, sensory cortices, and the insula were activated, no significant differences in Stroop measures were found for all solutions. Several studies did not find an effect of CAF ingestion on the Stroop test (Bottoms et al. 2013; Edwards et al. 1996; Hameleers et al. 2000). Additionally, De Pauw et al. (2015) did not find a significant effect of GLUC mouth rinsing on Stroop measures. The involvement of several brain areas for a successful Stroop task might explain the unchanged attentional measures for the NAS conditions (Laird et al. 2005; Nee et al. 2007; Roberts and Hall 2008). In line with this reasoning, Deslandes et al. (2005) observed alterations within the ERP components, whereas no significant changes were found with the Stroop task when CAF was ingested.

The ERP component important to stimulus evaluation, selective attention, and conscious discrimination is the P300 (Patel and Azzam 2005). Several alterations of the amplitude and latency of the P300 with GLUC were observed. ERP components reflect the reception and processing of sensory information and higher-level processing. Research about the effect of CAF on ERP components is scarce. CAF ingestion has been shown to increase the P300 amplitude (Lorist et al. 1994; Ruijter et al. 1999) and reduce the P300 latency, but not in a complex task (Lorist et al. 1996). The current study showed no signficant differences for ERP components with the CAF-NAS intervention. Since the sample size is rather small, it is worth mentioning that CAF-NAS elicited a higher (but not significant) P300 amplitude at frontal electrodes compared to PLAC-NAS when NEUTR were displayed. The frontal electrodes F3-F4 measure the electrocortical activity of Brodmann area 8, which is involved in the planning of complex movements. Deslandes et al. (2004) investigated the influence of CAF ingestion on P300 at Fz, Cz, and Pz. They observed a greater cognitive efficiency via the reduced latency at the frontal midline electrode.

The P300 latency varies as a function of factors governing stimulus evaluation time (Hoffman 1990; Patel and Azzam 2005). GLUC-NAS reduced the P300 latency at central electrodes, which is located above Brodmann area 5, a brain area involved in somatosensory processing and association. Furthermore, GLUC-NAS increased the P300 amplitude for INCON at all ROIs. The P300 amplitude is proportional to cortical arousal (Ruijter et al. 2000) and related to resource demands available in the information processing system (Donchin et al. 1986). Previous findings are associated with greater cognitive efficiency, but the current study also revealed a reduced P300 amplitude at frontal electrodes when NEUTR appeared. Shorter P300 latencies were also detected with a more complex cognitive task, i.e., the visual three-stimulus oddball task, as well as a reduced P300 amplitude (Riby et al. 2008), which is typically associated with memory storage operations (Polich and Criado 2006).

One of the limitations of the study is the low sample size, which reduced the likelihood of finding a statistically significant difference. Furthermore, several electrode sites were clustered into ROIs, which influences the incidence of a type I error. Future research should examine the effect of the combination of GLUC and CAF on brain responses. Since the nasal administration of substrates activates brain regions involved in sensorimotor information processing and cognitive functioning, future research should also investigate the effect of nasal sprays with CAF and GLUC on endurance and/or sprint performance.

To conclude, all NAS solutions activated the anterior cingulate cortex. Greater cognitive efficiency was observed with GLUC-NAS. CAF-NAS activated the prefrontal brain areas, cingulate, insular, somatosensory, and motor cortices, whereas GLUC-NAS activated the SAC, cingulate, and insular cortex. Although the direct link between the nasal cavity and the brain was shown, the altered brain responses did not influence the cognitive performance.

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Compliance with ethical standards The experiment was approved by the institutional medical ethical committee of UZ Brussel and Vrije Universiteit Brussel (Belgium) (B.U.N. 143201421380). The subjects were provided written and oral information about the experimental procedures and potential risks before giving informed consent to participate in this study.

Conflict of interest The authors declare that they have no conflict of interest.

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