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Soft shoulder massage amplifies glucose-induced increases in medial prefrontal activity: a pilot study employing a neurovisceral integration perspective

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Glucose intake has a modulating effect on autonomic activity at rest, indicating shared central mechanisms in the regulation of glucose homeostasis and autonomic responses. However, it is still unclear how glucose influences vagal and neuronal activity in response to changing environmental demands. To address this question, we studied the effect of glucose consumption on parasympathetic, and medial prefrontal reactivity in response to regenerative processes. For this, we invited fasted, healthy adult participants (n = 62, age *mean* = 23.0 years, *SD* = 4.05, 69.4% female) to the laboratory. After the consumption of either water or a drink containing glucose, participants were randomly assigned to a soft shoulder massage or a resting control group. Throughout the experiment, we simultaneously monitored cardiac vagal activity, indexed by root mean square of successive differences (RMSSD), and changes in medial prefrontal activation, indexed by changes in O_2Hb concentrations, via continuous electrocardiogram (ECG) and functional near-infrared spectroscopy (fNIRS) recording. In contrast to previous findings, we could not replicate an amplifying effect of glucose consumption on the physiological relaxation response. While we did not find a positive association between changes in medial prefrontal O_2Hb concentration and vagal reactivity to the relaxation intervention, findings from our exploratory analysis suggest that higher blood glucose availability is associated with increases in medial prefrontal oxygenation. We discuss the results in the context of the neurovisceral integration theory.

Keywords: glucose, massage, neurovisceral integration model, heart rate variability, fNIRS

Despite contributing only up to 2% of the entire body mass, the human brain is the largest source of energy consumption and accounts for more than 20% of the overall energy requirement at rest (Watts et al., 2018). Therefore, cerebral metabolism depends on a constant supply of glucose and oxygen. Glucose homeostasis involves the maintenance of blood glucose levels within a range of 90-120 mg/dl, independent from nutrient intake or changing environmental demands (Arble & Sandoval; 2013). To ensure optimal autonomic, endocrine, and behavioral responses to physiological and environmental challenges, the brain integrates afferent metabolic signals from peripheral tissues and coordinates adaptive changes in glucose uptake, utilization, and storage via efferent autonomic activity (Roh et al., 2016). There is a large body of evidence that rapid increases in blood glucose, triggered by acute glucose intake, lead to significant short-term alterations of autonomic nervous system (ANS) activity (Paolisso et al., 2000; Sverker, 1991; Synowski et al., 2012; Uijtdehaage et al., 1994; Weissman et al., 2006). These findings indicate shared central mechanisms in the regulation of autonomic activity and the maintenance of glucose homeostasis. Specifically, previous research has reported elevations of cardiac sympathetic activity following glucose consumption under conditions of rest (Brown et al., 2008; Paolisso et al., 2000; Weissman et al., 2006). However, findings regarding the effect of glucose ingestion on cardiac

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parasympathetic activity are inconsistent: While some studies have linked elevated blood glucose levels after glucose intake with decreases in cardiac parasympathetic activity (Oliveira et al., 2021; Weissman et al., 2006), other studies reported increases in cardiac parasympathetic activity immediately after the intravenous administration of glucose or insulin (Stockhorst et al., 2011) and 40 minutes after the consumption of a drink with glucose (Brown et al., 2008). Expanding on findings that suggested an association between elevated blood glucose levels and cardiac autonomic alterations at rest, Meier et al. (2022) investigated the effect of glucose consumption on physiological reactivity to changing environmental demands. The authors reported that higher euglycemic blood glucose concentrations after the consumption of a sugary drink were related to enhanced parasympathetic reactivity to challenging as well as regenerative tasks. These findings might indicate increased autonomic flexibility in times of high blood glucose availability, which could be adaptive when being confronted with environmental challenges.

The neurovisceral integration theory assumes that higher vagal tone is the result of the ability to establish adequate mappings between sensory input, internal state, and motor output. There is an extensive body of research investigating the pathways by which this neural control is achieved (Thayer et al., 2009). Neuroimaging and pharmacological blockage studies provided evidence that specific brain regions within the central autonomic network (CAN) modulate cardiovascular functions. (Allen et al., 2015; Thayer et al., 2009). In a meta-analysis of human neuroimaging studies of heart rate variability (HRV), Thayer et al. (2012) identified brain regions associated with the neurovisceral integration model that were activated in conjunction with task-evoked increases in high-frequency (HF) HRV, a vagally mediated HRV component. Across all studies and independent of task domain, HF-HRV was associated with functional subdivisions within the medial prefrontal cortex and the amygdala. These results were supported by a second meta-analysis from Beissner et al. (2013), showing that task-induced changes in medial prefrontal activity positively correlated with task- induced changes in HF-HRV. As these findings might not be indicative of neural pathways generally related to resting cardiac activity, Jennings et al. (2015) examined whether brain regions that previously have been related to cardiac vagal reactivity were also associated with cardiac vagal activity at rest by using functional magnetic resonance imaging (fMRI). Their results showed that there was a communality between neural regulation of resting HF-HRV and task-induced reductions in HF-HRV. Moreover, their analysis also revealed that the directionality of the relationships between HF-HRV and neural activation was inverse during the resting state. According to the neurovisceral integration model, higher resting HRV (tonic component) is typically associated with greater task-induced reductions in HRV to challenging and increases to regenerative stimuli (negative correlation), reflecting greater adaption skills. Further, as confirmed by the two meta-analyses described above, the model claims that large reductions in HRV are associated with less neural activation (positive correlation). Consequently, relatively high levels of resting HRV must be associated with relatively low levels of neural activation (negative correlation). To test these assumptions, Condy et al. (2020) simultaneously assessed prefrontal neural activation via functional-near infrared spectroscopy (fNIRS) and root mean square of successive differences (RMSSD), a time-domain measure to estimate vagally mediated changes in heart rate, during a baseline period as well during cognitive tasks. Their results were consistent with the findings from the fMRI study by Jennings et al. (2015), showing an inverse relationship between RMSSD and prefrontal activation during baseline and a positive relationship between task-induced reactivity of RMSSD and neural activation.

Although currently considered as the “gold standard” to non-invasively assess functional brain activity, fMRI imaging has practical constraints limiting the ecological validity of experimental paradigms that can be performed using MRI. For example, as the MRI signal can be contaminated by motion artifacts, there are strict restraints on motion during data acquisition (Scarapicchia et al., 2017). Another significant weakness of fMRI is its limited temporal resolution. Typically, peak hemodynamic responses to brain activation occurs approximately 5-6 seconds after stimulus onset (Glover, 2012). In recent years, fNIRS has emerged as an alternative functional brain imaging method that exploits the principles of near-infrared spectroscopy and cerebral hemodynamics and overcomes several limitations associated with fMRI. Within the near-infrared spectrum (650–1000 nm), light can penetrate biological tissues. While most of the light scatters within the underlying tissue, some light is absorbed by chromophores, such as hemoglobin. The absorption spectrum of hemoglobin depends on the oxygenation level, i.e., oxyhemoglobin (O_2Hb) > 800 nm and deoxyhemoglobin (HHb) < 800 nm. FNIRS makes use of this principle to detect relative changes in O_2Hb and HHb concentration and thereby indirectly assesses changes in neural activation via optical sensors (optodes) placed on the

surface of the head (Kohl et al., 2020). Compared to fMRI, fNIRS signals are less sensitive to motion artifacts and allow for paradigms with greater task flexibility in naturalistic settings. In addition, fNIRS has a superior temporal resolution with peak hemodynamic responses on a one to two seconds time scale (Scarapicchia et al., 2017; Wilcox & Biondi, 2016). Due to these advantages, fNIRS might be superior to fMRI when exploring the relationship between central and autonomic activity in laboratory settings.

Taken together, a considerable body of evidence suggests that acute glucose consumption has a modulating effect on autonomic activity at rest, indicating shared central mechanisms in the regulation of autonomic responses and the maintenance of glucose homeostasis. However, it is still unclear how high blood glucose concentrations influence vagal and neuronal activity in response to changing environmental demands. Consistent with the neurovisceral integration model, findings from neuroimaging studies about neurovisceral regulatory circuits indicate that medial prefrontal cortical activity is associated with resting vagal tone and stimulus- induced changes in cardiac vagal activity. Even though the theory makes specific predictions regarding neural control of tonic and phasic levels of cardiac vagal activity, no study has yet empirically investigated the relationship between neuronal and vagal reactivity to regenerative processes. Therefore, the aim of this study was to investigate whether the intake of a sugary drink would influence cardiac vagal and neuronal medial prefrontal reactivity to psychophysiological relaxation, as induced by a soft shoulder massage. To address this question, we invited fasted, healthy adult participants to the laboratory. After the consumption of either water (condition *water*) or a drink containing glucose (condition *glucose*), participants were randomly assigned to a soft shoulder massage or a resting control group. Directly after that, they performed the d2-R sustained attention test. Throughout the experiment, we repeatedly assessed blood glucose concentration, blood pressure and mood. Further, we simultaneously monitored cardiac vagal activity, indexed by RMSSD, and changes in medial prefrontal activation, indexed by changes in O_2Hb concentrations, via continuous electrocardiogram (ECG) and functional near-infrared spectroscopy (fNIRS) recording.

Hypothesis. We expected that the consumption of glucose augments the physiological relaxation response. Further, we aimed at exploring the relationship between medial prefrontal reactivity and cardiac vagal reactivity in response to the relaxation intervention. As this was the first experiment in which we used fNIRS to monitor hemodynamic changes, the fNIRS data were considered pilot data and examined in the context of an exploratory analysis.

Methods and Material

Participants

Participants were recruited trough the University of Konstanz subject pool management software (SONA Systems), through the distribution of printed flyers (advertising a "relaxation study") at the facilities of the University of Konstanz, the city of Constance as well as via social media advertisements. To control for the influence of variables related to altered central or cardiovascular regulation, participants completed an online eligibility screening using the questionnaire tool Qualtrics (Qualtrics, Provo, UT, USA) prior to being invited to the laboratory. If not otherwise stated, single-item measures were used to assess the respective self-reported criterium. Exclusion criteria of the screening questionnaire were: (1) age < 18 years, (2) lack of German language skills, (3) past or current relevant physical or mental diseases (cardiovascular disease, neurological disease, respiratory disease, metabolic disease, psychiatric disease, somatic disease, allergies) (Quintana et al., 2016), (4) acute and/or regular medication intake affecting the autonomous or central nervous system (e.g., psychopharmaceutic, anti-histaminic or anticoagulant medication) (Carpenter et al., 2011), (6) smoking (> 5 cigarettes per day) (Quintana et al., 2016), (7) feeling unable to fast for 4 hours, (8) clinically relevant, moderate to severe symptoms of depression (indicated by Beck's Depression Inventory II sum score >19) (Beck et al., 1996; Kühner et al., 2007), (9) past or current orthopedic problems (e.g., scoliosis, discopathy, spinal fracture or whiplash) (Meier et al., 2020).

In total, 141 people filled in the online screening to determine eligibility for this study. 62 eligible adults were invited to the laboratory and participated in the study. Due to an irregular heart rhythm or > 10% ectopic beats, as identified during the ECG data pre-processing, (n = 3), n = 59 (age *mean* = 22.71 years, *SD* = 2.76, 67.8% female) participants were included in the final analysis. A flow diagram visualizing the sample of the study is depicted in Appendix *Figure S1*.

Prior to its conductance, the study was approved by the Ethics Committee of the University of Konstanz (IRB statement 12/2017). The experiment was carried out in accordance with the ethical standards of the

Declaration of Helsinki. Prior to participation, all participants gave written informed consent. After completion of the experiment, they received a written debriefing were compensated with either 15€ or 1.5 course credits.

Study Design and Experimental Procedure

Our study comprised a mixed methods design with the between-subject factors *drink condition* (glucose, water) and *relaxation intervention* (massage, rest). The physiological outcome parameters (O_2Hb concentration, RMSSD, HR, blood glucose levels) were assessed continuously/repeatedly over *time* (within-subject factor).

Participants were randomly assigned to the between-subject factors and both participants and the experimenter were blind to of this assignment prior to the experimental session. To control for possible sex effects in the giver/receiver interaction during the massage, participants were tested by an experimenter with the same sexual orientation (the variable "sexual orientation" was assessed via self-report in the screening questionnaire). All experimental sessions were performed in the afternoon at either 3 or 5 p.m. to account for circadian variations in the glucose/insulin metabolism (Kalsbeek et al., 2014). The sampling took place at the Center for Psychiatry Reichenau and lasted for approximately 90 minutes. If eligible, participants were invited to the laboratory session via email. They were asked to refrain from smoking, food and drinks (except for water or unsweetened tea) 4 hours prior to the laboratory session. In addition, participants were instructed to follow a normal sleeping routine in the night before the experiment (Miglis, 2017) and not to engage in heavy physical activity on the day of the experiment (Sammito & Böckelmann., 2015). Due to the ongoing Covid-19 pandemic at the time of testing, the experimenter wore a face mask to minimize the risk of infection. The experiment was conducted in the same room with the same equipment throughout the entire testing period. All procedures were carried out while participants were sitting in a chair in front of a desk. Prior to each laboratory session, the experimenter took note of the temperature and humidity in the test room using a thermo-/hygrometer (Temeo Hygro indicator, Bresser, Rhede, Germany).

Upon entering the laboratory, participants were informed about the procedure of the experimental session and gave written informed. Then, they were introduced to the Affect Grid that was used to assess changes in subjective relaxation along the dimensions of valence and arousal during the experiment (Russell, Weiss & Mendelsohn, 1989). Participants were then outfitted with seven ECG electrodes of a portable electrocardiogram (ECG) and impedance cardiography (ICG) device (Asmuth GmbH Medizintechnik, Minden, Deutschland). ECG (Bio1) and impedance (Z0) signals were visually examined to ensure that the physiological equipment was applied properly. Once this was completed, circumferential head measurements were performed to identify reference points based on the international 10-20 system, followed by the application and adjustment of the fNIRS cap. Before starting the measurement, signal quality was ensured by an automated signal optimization algorithm by the acquisition software Aurora fNIRS 2021.4.0 (NIRx Medical Technologies LLC, Berlin, Germany).

The session started with an acclimatization period, in which participants filled in questionnaires and gave a subjective relaxation rating using Qualtrics' offline survey application (Qualtrics, Provo, UT, USA) on an iPad (Apple Inc., Cupertino, CAL, USA). For a physiological baseline recording, participants were asked to rest and sit in silence for 5 minutes. They were instructed to adopt an upright seating position placing both feet flat on the floor (knees bent at a 90° angle), hands on their thighs and to keep their eyes opened. Directly after, the first blood pressure measurement was performed, participants were asked to give a subjective relaxation rating and blood glucose was measured. Participants were assigned to consume one of two different drinks (*glucose* or *water*). After drink consumption, participants filled in further questionnaires. After 15 minutes, when the drink content was assumed to be digested and absorbed into the bloodstream (Ferrannini et al., 1985; Meier et al., 2022), the second blood pressure measurement, subjective relaxation rating and blood glucose measurement was performed. Directly afterwards, a relaxation intervention (soft shoulder massage or rest) during which participants remained seated and rested their heads in their palms was carried out. The intervention was carried out in silence and lasted 10 minutes. This was followed by another blood pressure measurement, subjective relaxation rating and blood glucose measurement. To raise subjective and physiological arousal levels, the d2-R sustained-attention test was performed for approximately 5 minutes. After successful completion of the test, participants rated their subjective relaxation level for the fourth time. Subsequently, participants were asked to rest and sit in silence for another 5 minutes to record physiological recovery. Afterwards, a final block of questionnaires was completed, and a final subjective relaxation

rating was performed. Lastly, the debriefing and compensation of the participant took place The full study procedure is depicted in *Figure 1.*

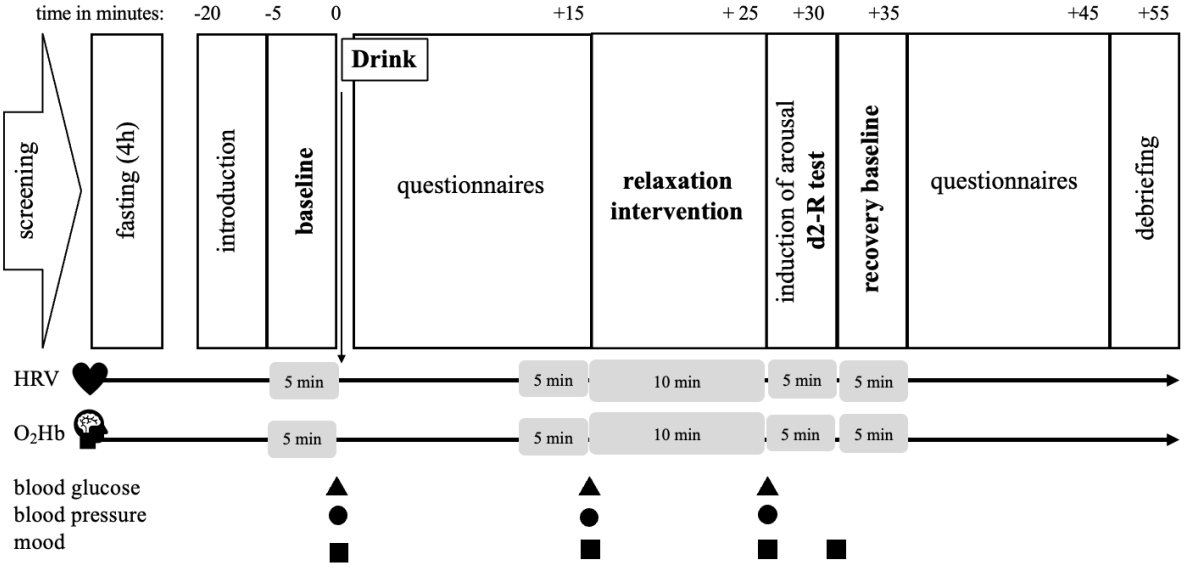

Figure 1. Schematic illustration of the experimental procedure. Experimental manipulation involved drinking 200 ml of water, or 200 ml of water with 75 g dextrose as well as a relaxation intervention (soft shoulder massage or rest). Horizontal grey blocks represent the time intervals of interest from the ECG/ICG and fNIRS recording.

Tasks and Measures

Drinks

Participants were randomly assigned to one of the two *drink conditions* (glucose, water) utilizing a computerized randomization list. The randomization key was controlled by a university staff member not involved in the testing procedure. The key was not revealed until analyses of the primary outcome parameters were finished. Therefore, drinks were administered in a double-blind fashion for both the experimenter and participant. The control group (condition *water)* was implemented to map the bradycardic and cardiac vagal activity increasing effect of drinking 200 ml of water (Routledge et al., 2002) in both groups and to ensure that any physiological effects found were due to differences in drink composition. The basis of all drinks was 200 ml still mineral water. Energy content was manipulated by adding 75 g of dextrose (Müller's Mühle Traubenzucker, Müller's Mühle GmbH, Gelsenkirchen, Germany), reflecting a caloric difference of about 300 kcal between the drink conditions. A comparable amount of polysaccharide has been used in other studies investigating the effect of glucose on physiological parameters (von Dawans et al., 2021; Kirschbaum et al., 1997; Meier et al., 2022). The drink of the experimental group was characterized by a high glycemic index (GI) (~100), ensuring rapid digestion and absorption into the bloodstream. All drinks were prepared by a university staff member not involved in the testing procedure. The drinks were cooled at a temperature of ~8 °C.

Relaxation Intervention

In both conditions (*massage* and *rest*), participants were asked to adopt a comfortable seating position, place their head on their arms onto the table, and close their eyes. The intervention phase was carried out in silence. To avoid a one-sided stimulation, participants in the *massage* group were instructed to align their head as straight as possible. Further, they were instructed to avoid speaking during the 10-minutes intervention, except for indicating potential feelings of psychological or physical discomfort. In this case, the massage was interrupted immediately. The standardized soft shoulder massage intervention consisted of light pressure stroking and softly touching the neck and shoulder area (Meier et al., 2020). Participants in the resting control condition were not exposed to any physical contact and were asked to rest in silence for 10 minutes.

Sustained-Attention Test

To raise physiological and psychological arousal levels, participants performed the d2-R. The d2-R measures sustained attention by means of a paper and pencil cancellation test (Steinborn et al., 2018). The test has a total duration of 4 minutes and 40 seconds. Participants are presented 14 rows of 47 letters containing p's and d's with one to four dashes placed above or below the letters. The test requires the selection of relevant stimuli (d's marked with two dashes above and/or below) under time pressure (20 seconds per row).

Cardiac Activity (ECG)

To assess cardiac activity, an electrocardiogram (ECG) and an impedance cardiogram (ICG) was obtained using a portable MindWare Mobile device (Mindware Technologies, Gahanna, OH) with a sampling rate of 500 Hz (Quintana et al., 2016). Seven electrodes (ECG electrodes ASF50, Asmuth Medizintechnik GmbH, Minden, Germany) were placed on the chest, abdomen and back of the participants. The electrode setup followed the standard Lead II configuration and the standard tetrapolar electrode system (Sherwood et al., 1990). For each participant, the following five time intervals were analyzed (see horizontal grey blocks in *Figure 1*): (1) the resting baseline (5 min), (2) the last five minutes of the questionnaire phase directly after drink consumption (5 min), (3) the relaxation intervention (10 min), (4) the d2-R sustained attention test (5 min), (5) the recovery baseline (5 min).

The analysis of the raw ECG signal was performed using the MindWareTM Heart Rate Variability Analysis Application (version 3.2.3) (MindWare Technologies LTD, Gahanna, OH, USA). For the extraction of HRV, 60 second intervals within the 5-minute time intervals were used. After filtering the raw signal, an algorithm detected the R-peaks. An irregular peak was marked if IBI values differed from preceding and subsequent beats more than a threshold value or if a value was outside of an acceptable physiological range. We manually corrected missed or unidentified R-peaks and replaced them manually, or by linear interpolation, as recommended by the Mindware Technologies guidelines. Finally, participants' heart rate signal was interpolated at a sampling frequency of 4 Hz and the root mean square of successive differences (RMSSD) was extracted as cardiovascular measure of parasympathetic cardiac control. For each time interval of interest, we calculated mean RMSSD per minute. As we use a similar measure of variability to indicate changes in cerebral activation over time, we will refer to the HRV RMSSD as HRV-RMSSD in the following.

Cerebral Activity (fNIRS)

We used a multi-channel, continuous wave fNIRS instrument (NIRSport 2, NIRx Medical Technologies LLC, Berlin, Germany) operating at two wavelengths (760 nm and 850 nm) and to measure hemodynamic changes in relative oxygenation levels (O_2Hb). Our system contained a total of 8 light-sources and 8 detectors placed on textile EEG caps (EASYCAP, Herrsching, Germany), creating 17 channels of interest. Regions of Interest (ROIs) (i.e., the medial prefrontal cortex and the bilateral somatosensory cortex) were identified according to the craniocerebral topography within the international 10-20 system. For the present research project, we defined nine channels of interest in the medial prefrontal region. The optode configuration within the caps was done using NIRSite 2021.4 montage software (NIRx Medical Technologies, LLC, Berlin, Germany). The eight fNIRS sources were positioned at FPz, Fz, AF1, AF2, C5, CP3, CP4, C6 and the eight fNIRS detectors were placed at the AFz, FCz, F1, F2, C3, CP5, C4, CP6 with an interoptode distance of 30 mm (see Appendix *Figure S2*).

Data was collected at a sampling rate of 10.2 Hz using the acquisition software Aurora fNIRS 2021.4.0 (NIRx Medical Technologies LLC, Berlin, Germany). For each participant, the following five time intervals were analyzed (see horizontal black blocks in *Figure 1*): (1) the resting baseline (5 min), (2) the last five minutes of the questionnaire phase directly after drink consumption (5 min), (3) the relaxation intervention (10 min), (4) the d2-R sustained attention test (5 min), (5) the recovery baseline (5 min).

Data pre-processing was completed using Satori (version 1.6.4 for Windows) (Brain Innovation B.V., Maastricht, the Netherlands). First, raw data time course values were converted into optical density values and channels with a scarp coupling index (SCI) < .75 were excluded from further analyses steps. Next, optical density data was converted to hemoglobin concentration using the modified Beer-Lambert law. The modified Beer-Lambert Law allows for the quantification of changes in chromophore concentrations, namely oxyhemoglobin and deoxyhemoglobin, by relating differential changes in light transmission to differential changes in tissue absorption (Baker et al., 2014). Linear trend removal and bandpass filtering algorithms were applied to remove high frequency (≥ .01 Hz; heartbeat, respiratory and instrument noise) and low frequency (< .04 Hz; blood pressure fluctuations) information from the signal (Hocke et al., 2018). To address the issue of spike contamination, we applied an iterative spike removal algorithm with a z-score threshold of 3.5 and a

time lag of 5s. Gaps left from spike removal were interpolated linearly. Motion artifacts were removed by Temporal Derivative Distribution Repair (TDDR) motion correction (Fishburn et al., 2019). As we did not use a block design, the resulting O_2Hb concentrations represent changes in O_2Hb concentration in reference to an initial baseline at the beginning of the recording (seconds prior to the start of the experimental session). The subsequent processing steps were performed in R (version 4.1.2) (R Core Team, 2021) with the user interface RStudio (version 2022.7.1.554) (RStudio Team, 2022).

Event markers from each participants' data file were used to calculate mean O_2Hb concentration per minute during the *relaxation intervention* time interval across the nine channels of interest in the medial prefrontal region (see Appendix *Figure 2*). First, the mean O_2Hb concentration during the first minute of the relaxation intervention was used as a reference value and we computed minute-to-minute change scores for the period of the *relaxation intervention*. Second, we added up all minute-to minute change scores to quantify total O_2Hb reactivity in response to the *relaxation intervention* (ΔO_2Hb). Third, to obtain a measure that considered differences in the variability of changes in O_2Hb concentration per minute, we calculated the root mean square of successive minute-to-minute differences in O_2Hb concentration (O_2Hb-RMSSD).

Blood glucose concentrations

Blood glucose concentrations (mg/dl) in capillary blood of the fingertip from the non-dominant hand were measured at three scheduled time points (see black triangles in *Figure 1*). After disinfection of the middle finger, disposable lancets (Accu-Check®, Roche Diabetes Care GmbH, Mannheim, Germany) were used to prick the fingertip of the middle finger. The first drop of blood was dabbed, and blood glucose concentration (mg/dl) was determined using the second drop of blood with a blood glucometer (GlucoMen®, A. Menarini Diagnostics, Berlin, Germany).

Statistical Analysis

Processing of the data and statistical analyses were conducted using the open-source software R (version 4.1.2) (R Core Team, 2021) with the user interface RStudio (version 2022.7.1.554) (RStudio Team, 2022) and *nlme* (Pinheiro et al., 2022). Graphs were created using *ggplot2* (Wickham, 2016).

Prior to data pre-processing and statistical analyses, we preregistered our hypotheses and analysis plan on the Open Science Framework (see https://osf.io/ap27q/; date of registration: September 15, 2022). Originally, we planned to quantify the relationship between HRV-RMSSD and O_2Hb concentration by using linear mixed effect models with HRV-RMSSD as the predictor and O_2Hb concentration as the outcome for each participant at each channel. However, due to the questionable data quality of our pre-processed fNIRS data, the presented analyses deviated from our preregistered approach. To acknowledge this, we decided to examine the relationship between changes in O_2Hb concentration, indexed by ΔO_2Hb and O_2Hb-RMSSD, across all channels and cardiac vagal activity, indexed by HRV-RMSSD, in the context of an exploratory analysis.

Data Cleaning

Prior to statistical analysis, raw HRV-RMSSD and blood glucose concentration data were investigated for plausibility. If an HRV-RMSSD or blood glucose value exceeded the mean of the experimental group by more than 3 standard deviations (SDs), it was defined as an outlier and replaced by the respective 3 SD value of the corresponding physiological marker. If more than 30% of a person's physiological data was outlying or missing, the participant was excluded from the subsequent analyses. Missing blood glucose level data at the first or last assessment of a given time interval were imputed by the mean of the respective experimental group at this timepoint. If values were missing at other timepoints, they were replaced by the mean of the participant's value prior and after to the missing value. As the HRV-RMSSD data was not normally distributed, we decided to log-transform the data to adjust for unequal variance prior to statistical analyses (Laborde et al., 2017). In the following, we will refer to ln(HRV-RMSSD) as HRV-RMSSD.

Preliminary Analysis

First, we examined whether potential person-related covariates (variables that were not equally distributed across the experimental conditions) might have an influence on our analysis results. A one-way Analysis of Variance (ANOVA) with *experimental condition* (four levels: *glucose-massage, glucose-rest, water-massage, water-rest)* and *age, BDI-Score* (Beck's Depression Inventory II sum score), *blood glucose baseline, HRV-RMSSD baseline* and *heart rate baseline* as dependent variables was conducted to detect potential differences between the four experimental groups. Then, to assess whether *session start* (3 p.m. / 5 p.m.) and *sex* (male / female) were equally distributed across the experimental groups, we calculated Pearson's Chi-squared tests. Variables that differed significantly between the experimental groups were treated as potentially

confounding variables. Their impact was evaluated by adding their main effect to the statistical models.

Statistical Approach

The level of significance was set to alpha = .05. To test for normality, Shapiro-Wilk tests were used, and homoscedasticity was tested using Levene's test. Moreover, model assumptions were visually checked by evaluating residual plots (homoscedasticity), histograms (normality) and QQ-plots (normality and linearity of residuals). To detect autocorrelation in residuals of linear regression models, we calculated Durbin-Watson tests. Finally, to identify potential outliers, we computed Cook's distance. If not otherwise reported, all assumptions were met, and no influential outliers were detected.

Whenever we analyzed changes in physiological outcome variables (blood glucose concentration and HRV-RMSSD) repeatedly measured over *time,* we computed multilevel growth curve models that allow for accounting for individual differences in baseline levels (random intercepts) and differences in trajectories over time (random slopes) (Curran et al., 2010). In all models, we built the growth curves in a hierarchical approach: First, we defined a fixed intercept model (blood glucose concentration/HRV-RMSSD predicted by intercept), then introduced a random intercept on participant level, followed by fixed time effects for linear, quadratic, and cubic time trends as well as random effects of the best-fitting time trend. To account for autocorrelation in the repeated measures data, we further added an autoregressive order 1 correlation structure (CAR1). Finally, we added the predictors *relaxation intervention* and *drink condition,* potential covariates identified in the preliminary analyses, as well as their interaction with *time* to the model. To identify the best-fitting model, we compared the log-likelihood ratios of the nested models using analysis of variances (ANOVA). Then, using the R package *performance* (Lüdecke et al., 2021), we computed marginal R^2 to quantify the variance explained only by the fixed effects and conditional R^2 to quantify the variance explained by both fixed effects and random effects. Finally, significant main or interaction effects were further examined by Bonferroni-corrected post-hoc t-tests.

Further, as a measure of reactivity to the experimental paradigms, we computed two HRV-RMSSD change scores. The first change score indicated HRV-RMSSD reactivity in response to the relaxation intervention (*ΔHRV-RMSSD-RELAX)* and was calculated by subtracting mean HRV-RMSSD values during the drink time interval from the values during the relaxation intervention time interval. The second change score represented HRV-RMSSD reactivity in response to the d2-R test (*ΔHRV-RMSSD-D2)* and was calculated by subtracting mean HRV-RMSSD values during the d2-R test from the values during the relaxation intervention interval.

Manipulation Checks

We computed growth curves to examine whether blood glucose changes over time depended on *drink condition.* This would be indicated by a significant *time x drink condition* interaction effect in the growth curve approach. Further, to test whether the *relaxation intervention* led to a significant increase in *HRV-RMSSD* compared to the *questionnaire phase following drink consumption* across experimental groups, we conducted a paired samples t-test.

Confirmatory Analysis

To test whether the consumption of glucose augmented the physiological relaxation response to the soft shoulder massage compared with the consumption of water, we examined whether *drink condition* (glucose vs. water) was a significant predictor of changes in HRV-RMSSD over *time* (indicated by a significant *time x drink condition* interaction effect).

Exploratory Analysis

Being exploratory, we computed correlation coefficients across groups and per experimental condition to examine whether changes in O_2Hb concentration in response to the relaxation intervention (ΔO_2Hb) were related to changes in RMSSD (*ΔHRV-RMSSD)* to the relaxation intervention. Further, to test whether mean RMSSD during the relaxation intervention was linked to O_2Hb-RMSSD, we calculated correlation coefficients between mean HRV-RMSSD (*HRV-RMSSD_mean)* and mean O_2Hb-RMSSD (*O_2Hb-RMSSD_mean)* across groups and per experimental condition. Finally, to obtain a cumulative index of O_2Hb reactivity to the relaxation intervention (Pruessner et al., 2003), we computed an area under the curve with respect to the increase (AUCi) for each group and conducted a one-way ANOVA to examine potential differences between the four experimental groups.

Results

Preliminary Analyses

Descriptive statistics and participants characteristics of the four experimental conditions (*glucose-massage, glucose-rest, water-massage, water-rest*) can be found in Appendix *Table S1*. We found no significant difference between the groups in *age, sex* (male / female), *blood glucose baseline, BDI-Score* (Beck's

Depression Inventory II sum score), *O_2Hb baseline, RMSSD baseline*, *heart rate baseline* and *session start* (3 p.m. / 5 p.m.).

Changes in blood glucose concentration over time

Growth curve analyses (177 blood glucose observations nested in 59 participants) of the three blood glucose assessments revealed that the best model for blood glucose trajectories over time included random effects for intercepts and slopes and a linear and quadratic trend of *time*. Using an ANOVA, the evaluation of the final model (conditional R^2 = .94, marginal R^2 = .79) indicated a significant main effect of *time*, $F(2, 114) = 56.1$, $p < .001$, partial Cohen's $f = .99$, no significant main effect of *drink condition* $F(2, 114) = 2.94$, $p = .092$, partial Cohen's $f = .23$, and a significant *drink condition* by *time* interaction effect, $F(2, 114) = 46.0$, $p < .001$, partial Cohen's $f = 0.90$.

Bonferroni corrected post-hoc t-tests showed that blood glucose levels significantly increased in the group consuming glucose (all $p < .001$), whereas there was no significant change in blood glucose levels over the course of the experiment for participants consuming water (all $p > .05$). Overall, these results confirm the successful manipulation of blood glucose concentrations in participants consuming glucose.

Effect of Relaxation Intervention on HRV-RMSSD

Across all experimental groups, RMSSD was significantly higher during the *relaxation intervention* (*mean* = 3.89, *SD* = 0.50) as compared with the *questionnaire phase following drink consumption* (*mean* = 3.74, *SD* = 0.53), $t(58) = 3.81$, $p < .001$, $d = 0.31$. This confirms the successful manipulation of RMSSD through the *relaxation intervention.*

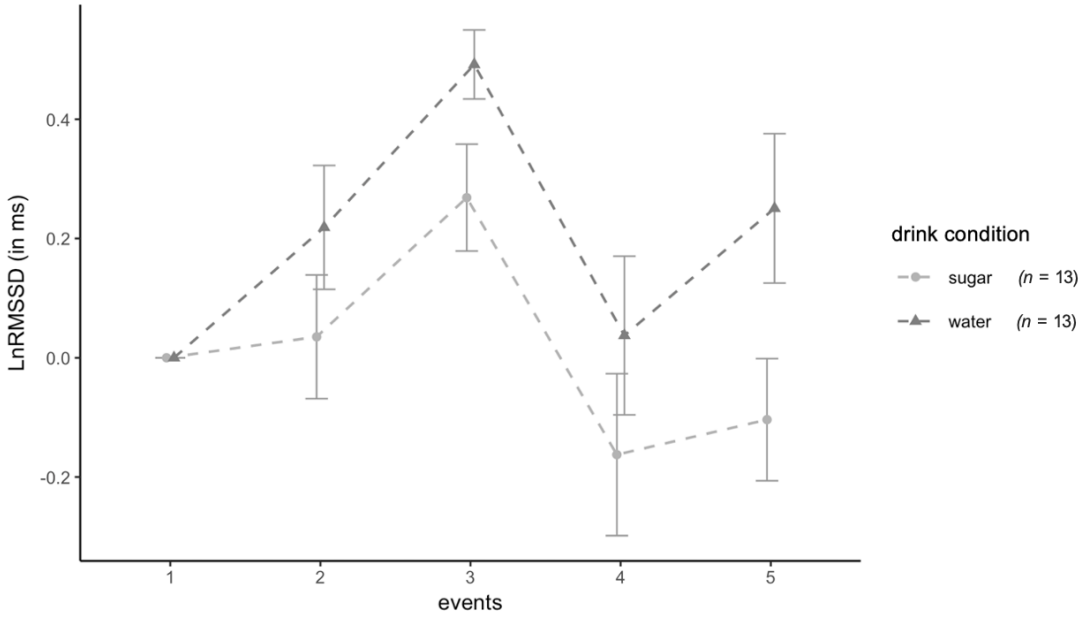

Figure 2. Changes in baseline-controlled ln(HRV-RMSSD) over time (in ln(ms)) per *drink condition* in the *massage* groups. Values are depicted as *mean* ± *SD.* Description of events: 1 = baseline, 2 = questionnaire period after drink consumption, 3 = relaxation intervention, 4 = d2-R test, 5 = recovery baseline.

Confirmatory Analysis

Effect of drink condition on HRV-RMSSD trajectories in the massage group

The model fit of the growth curve predicting HRV-RMSSD trajectories in the *massage* group over time (130 HRV-RMSSD observations nested in 26 participants) increased significantly upon the inclusion of random intercepts, a linear, quadratic, and cubic trend of time as well as an autoregressive covariance structure. The evaluation of the final model (conditional R^2 = .84, marginal R^2 = .17) indicated a significant main effect of *time*, $F(3, 98) = 26.0$, $p < .001$, partial Cohen's $f = .89$, but neither a significant main effect of *drink condition*, $F(1, 24) = 1.95$, $p = .175$, partial Cohen's $f = .29$, nor a significant *drink condition* by *time* interaction effect, $F(3, 98) = 1.47$, $p = .228$, partial Cohen's $f = .21$. Across both groups, HRV-RMSSD baseline (*mean* = 3.62, *SD* = 0.45) was not significantly lower than HRV-

RMMSD in the questionnaire period following drink consumption (*mean* = 3.74, *SD* = 0.56), $t(25) = 1.71$, $p = .100$, $d = 0.34$. HRV-RMSSD significantly increased in response to the relaxation intervention (*mean* = 4.00, *SD* = 0.47), $t(25) = 5.13$, $p < .001$, $d = 1.01$, significantly decreased in response to the d2-R test (*mean* = 3.56, *SD* = 0.59), $t(25) = -6.27$, $p < .001$, $d = 1.23$ and significantly increased in the recovery baseline period (*mean* = 3.69, *SD* = 0.61), $t(25) = 2.50$, $p = .019$, $d = 0.49$. Compared to baseline, HRV-RMSSD was significantly higher during the relaxation intervention, $t(25) = 6.69$, $p < .001$, $d = 1.31$. While HRV-RMSSD did not significantly differ between *drink conditions* during baseline, the questionnaire phase following drink consumption, and the d2-R test, the *water* group showed significantly higher HRV-RMSSD levels during the massage intervention (*mean* = 4.10, *SD* = 0.50), than the *glucose* group (*mean* = 3.89, *SD* = 0.42), $t(24) = 2.13$, $p = .044$, $d = 0.83$. Similarly, HRV-RMSSD was significantly higher in the *water* group (*mean* = 3.89, *SD* = 0.55), than the *glucose* group (*mean* = 3.46, *SD* = 0.62), during the recovery baseline period $t(24) = 2.22$, $p = .036$, $d = 0.87$. In contrast to our hypothesis and in line with the non-significant *drink condition* by *time* interaction effect, ΔHRV-*RMSSD-RELAX* did not differ significantly between groups, $t(24) = -0.40$, $p = .700$, $d = 0.16$. Similarly, we found no significant difference in ΔHRV-*RMSSD-D2* between the *water* and *glucose* group, $t(24) = -0.16$, $p = .870$, $d = 0.064$. HRV-RMSSD trajectories per drink condition in the massage groups are depicted in *Figure 2.*

Table 1. Correlation between O_2Hb and HRV-RMSSD reactivity in response to the relaxation intervention.

variables	experimental group	*r*	*p*
ΔO_2Hb & ΔHRV-RMSSD	all participants	-.13	.340
ΔO_2Hb & ΔHRV-RMSSD	sugar-massage	-.15	.670
ΔO_2Hb & ΔHRV-RMSSD	sugar-rest	-.17	.533
ΔO_2Hb & ΔHRV-RMSSD	water-massage	-.060	.845
ΔO_2Hb & ΔHRV-RMSSD	water-rest	-.23	.384

Note. Summary of the correlation analysis parameters across all participants and per experimental group.

Exploratory Analyses

As summarized in *Table 1*, correlational analyses revealed that changes in O_2Hb concentration in response to the relaxation intervention (*ΔO_2Hb)* were neither significantly correlated with changes in HRV-RMSSD (*ΔHRV-RMSSD)* in response to the relaxation intervention across groups, nor per experimental condition.

As summarized in *Table 2*, heart rate variability during the relaxation intervention (*HRV RMSSD_mean)* was not significantly correlated with mean variability in minute-to-minute changes in O_2Hb concentration (O_2Hb-*RMSSD_mean).*

Table 2. Correlation between mean O_2Hb-RMSSD and mean HRV-RMSSD during the relaxation intervention.

variables	experimental group	*r*	*p*
O_2Hb-RMSSD_mean & HRV-RMSSD_mean	all participants	-.04	.778
O_2Hb-RMSSD_mean & HRV-RMSSD_mean	sugar-massage	-.24	.480
O_2Hb-RMSSD_mean & HRV-RMSSD_mean	sugar-rest	.18	.513
O_2Hb-RMSSD_mean & HRV-RMSSD_mean	water-massage	-.30	.313
O_2Hb-RMSSD_mean & HRV-RMSSD_mean	water-rest	-.25	.328

Note. Summary of the correlation analysis parameters across all participants and per experimental group.

Effect of experimental condition on changes in O2Hb concentration

Evaluation of the one-way ANOVA showed that there was a significant difference in AUC_{iO2Hb} between the four experimental groups, $F(3, 53) = 3.39$, $p = .024$, $\eta_p^2 = .16$. While on average, AUC_{iO2Hb} was positive in the groups that consumed a drink with *glucose (sugar-massage*: *mean* = 363, *SD* = 645*; sugar-rest*: *mean* = 16.9, *SD* = 329), the groups that consumed *water* had negative AUC_{iO2Hb} values *(water-massage*: *mean* = -104*, SD* = 426; *water-rest*: *mean* = -187, *SD* = 462). Post-hoc t-tests revealed that only the difference between the *sugar-massage* and *water-control group* was statistically significant, $t(17) = 2.45$, $p = .026$, $d = 1.02$. *Figure 3* depicts O_2Hb trajectories during the relaxation intervention per experimental group.

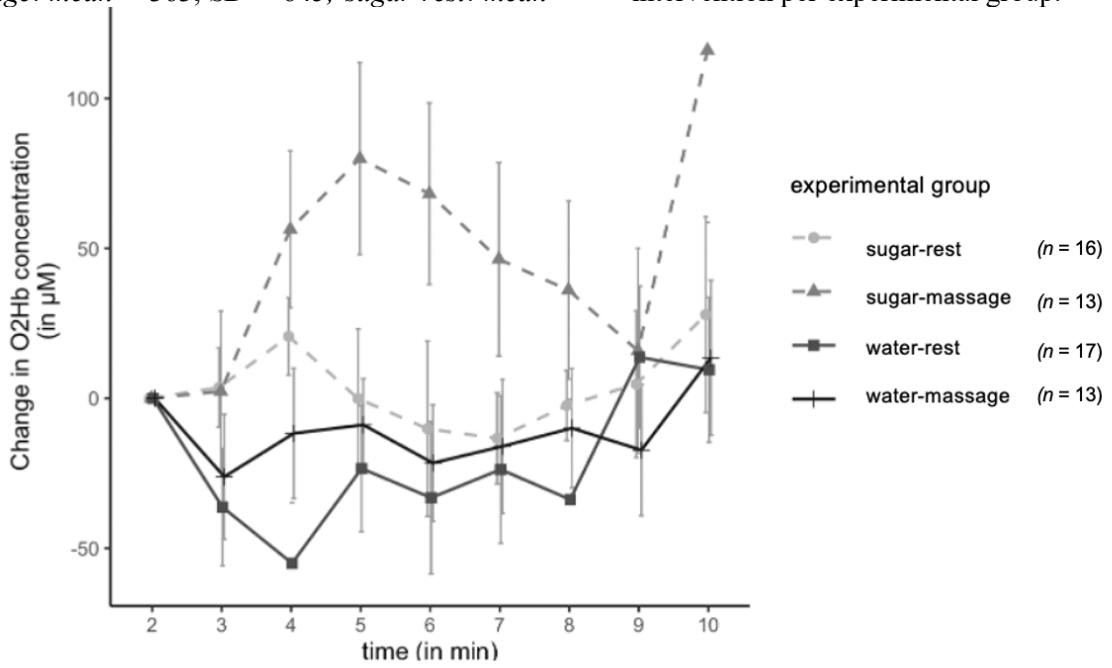

Figure 4. Changes in baseline-controlled O_2Hb concentration during the relaxation intervention per minute per experimental group. The change in O_2Hb concentration from minute 1 to minute 2 was defined as the reference value for the subsequent changes. Values are depicted as *mean* ± *SD.*

Discussion

The primary aim of this work was to investigate the impact of a drink with glucose compared to water on cardiac vagal and neuronal medial prefrontal activity in response to physiological relaxation, as induced by a standardized massage intervention. Further, we examined the relationship between changes in medial prefrontal oxygenation, resting cardiac vagal tone and changes in cardiac vagal reactivity to regenerative processes. In contrast to previous research (Meier et al, 2021), our data did not support the hypothesis that glucose had an amplifying effect on the physiological relaxation response. Moreover, we neither found an association between changes in medial prefrontal O_2Hb concentration and cardiac vagal reactivity in response to the relaxation intervention, nor between changes in medial prefrontal O_2Hb concentration and mean cardiac vagal activity during the relaxation intervention. Findings from our exploratory analysis suggest that higher blood glucose availability is associated with increases in medial prefrontal oxygenation.

In contrast to our main hypothesis, our results did neither show a significant effect of *drink condition* on HRV-RMSSD trajectories, nor on HRV-RMSSD reactivity to the massage or the d2-R test. These contrasting findings might at least partly be explained by the impact of blood glucose concentrations on parasympathetic (re-)activity. Meier et al. (2022) found a positive relationship between blood glucose concentrations and cardiac vagal reactivity to the d2-R and the slow-paced breathing task, indicating that higher blood glucose concentrations augment parasympathetic reactivity to challenging tasks as well as regenerative processes. However, it is possible that enhanced parasympathetic modulations following glucose ingestion only occur at higher euglycemic and hyperglycemic blood glucose concentrations. Findings from oral glucose tolerance tests indicate that in healthy individuals, blood glucose concentrations usually peak 30-60 minutes after the ingestion of 75 g glucose (Esposito & Giugliano, 2006; Takahashi et

al., 2018; Weissman et al., 2006). While in the study by Meier et al. (2022), the relaxation intervention was performed approximately 30 minutes after drink consumption, in our study, the massage intervention took place directly after the questionnaire period following drink consumption, approximately 15 minutes after glucose intake. Therefore, compared to our study, participant's blood glucose levels in the *glucose* group have likely been higher in the study by Meier et al. (2022).

Moreover, it is feasible that the augmenting effect of glucose on cardiac vagal reactivity depends on the extent of vagal activity prior to stimulus induction. In the study by Meier et al. (2022), participants completed the d2-R test prior to the relaxation intervention, whereas participants in our study filled in questionnaires. Therefore, vagal activity prior to the relaxation intervention was significantly lower in participants of the study by Meier et al. (2022). The boosting effect of glucose might only come into effect when changing situational demands require pronounced changes in autonomic activity. However, at this point, these considerations are only speculative and should be targeted by future research.

The non-significant correlation between changes in HRV-RMSSD and O_2Hb concentration in response to the relaxation conflict with previous meta-analyses, reporting that task-induced changes in medial prefrontal activity positively correlated with task- induced changes in HF-HRV (Beissner et al. 2013; Thayer et al., 2012). Moreover, we did not find a correlation between mean HRV-RMSSD and mean O_2Hb-RMSSD. These contradictory findings might be explained by the utilization of different experimental designs for the HRV-fNIRS signal collection. All studies included in the meta-analyses used block designs to examine prefrontal hemodynamic responses to a specific stimulus. A typical block design consists of alternating presentations of a single stimulus type for an extended time interval (task condition) and an inter-stimulus-interval (control condition) of sufficient duration for the stimulus-evoked hemodynamic response to build up and return to baseline level (Luke et al., 2020). In contrast, we did not implement a resting period directly prior to and after the relaxation intervention. Moreover, we calculated the change in HRV-RMSSD and O_2Hb concentration over a time interval of 10 minutes, whereas task durations in block designs traditionally are in the range of seconds (Chee et al., 2003). However, when analyzing O_2Hb trajectories during the relaxation intervention per experimental group, we found that cumulative reactivity was significantly higher in the *sugar- massage* than in the *water-rest* group. Interestingly, average AUC_{iO2Hb} values were negative for the groups that consumed *water* and positive for the groups that consumed a drink with *glucose* prior to the relaxation intervention. This implies that on average, after the consumption of glucose, medial prefrontal oxygenation increased, whereas oxygenation in the mPFC decreased after the consumption of water. One possible explanation for the diverging activation pattern might be provided by an increase in overall cerebral metabolism following glucose ingestion. To assess the relationship between neuronal glucose uptake and subsequent changes in brain activity, Lundgaard et al. (2015) administered a near-infrared glucose analogue into the peri-arterial space surrounding large penetrating cortical arteries of awake mice. Using in vivo-two photon imaging, they demonstrated that direct neuronal glucose uptake increased neuronal activity in brain regions related to the control of energy homeostasis, including the hypothalamus and the mPFC. In summary, these findings imply that higher blood glucose concentrations might indirectly induce increases in cerebral metabolism, reflected in increased functional activity.

The present study has several limitations. First, given the homogenous, predominantly female sample composition, the generalizability of the results is limited to young, mentally, and physical healthy individuals. Related to this, since we did not account for menstrual cycle phase or oral contraceptive use in our analyses, we cannot exclude the possibility of hormonal impacts on our results. Similarly, we did neither consider nor control for potential confounding variables on PNS (re-)activity such as obesity (Body Mass Index > 30) (Karason et al., 1999), temperature, humidity (Meier et al., 2022) and chronic stress levels (Kim et al., 2018) in our analyses. Another major limitation is the relatively small sample size, which raises the possibility of power issues. For these reasons, our findings need to be confirmed in replication studies with a more diverse sample and a larger sample size. Although fNIRS data were considered pilot data and therefore examined in the context of an exploratory analysis, we would like to discuss several limitations associated with our study design. First, our experimental design diverged significantly from other studies that used fNIRS to monitor cerebral oxygenation. Traditionally, fNIRS research relies on block design, event-related design, or mixed-design paradigms to assess stimulus-related changes in hemodynamic activity compared to a pre-defined baseline value (Yücel et al., 2021). Our analysis approach to calculate mean changes in O_2Hb concentration per minute for each time interval of interest did not fully utilize the temporal information of fNIRS O_2Hb signals. Therefore, the resulting values need to be interpreted with caution. Alternatively, a block design and

subsequent general linear model (GLM) based statistical approach might overcome these limitations and significantly enhance the signal-to-noise ratio (von Lühmann et al., 2020). Second, we did not place a retaining overcap over the fNIRS cap. FNIRS is highly susceptible to signal artifacts caused by ambient light and relative motion between the optodes and the scalp (Cooper et al., 2012). Therefore, a retaining overcap can optimize data quality by blocking external light luminance and ensuring continuous optode stability and contact with the scalp (Kassab et al., 2015).

Finally, our probe configuration was based on single-distanced long-channels with source-detector distance of 30 mm. The distance between source and detector determines the sensitivity to changes in chromophore concentrations in a tissue layer, with longer channels being more sensitive to neuronal activity in deeper tissue layers. Increased sensitivity to deeper tissues, however, comes at the expense of a reduced signal-to-noise ratio. However, it has been demonstrated that the parallel usage long- and short-separation channels significantly improves the accuracy and reliability of fNIRS measurements (Brigadoi & Cooper, 2015). Specifically, the short-separation channel signal might be used to regress superficial components from the standard long-separation channel fNIRS signal, isolating functional cortical responses. Therefore, implementing a probe configuration with multi-distance channels might be a good compromise between reasonable depth sensitivity and an acceptable signal-to noise ratio.

Apart from these limitations, our approach to examine neurovisceral integration by the simultaneous collection of HRV and neuroimaging data via continuous ECG and fNIRS recording represents a promising research method to investigate the relationship between central and autonomic activity at rest and in response to a variety of experimental paradigms. However, given the questionable quality of our fNIRS data, the validity of our findings is limited, and future studies following best practice guidelines for fNIRS research should follow up upon the presented pilot data of our study.

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Author Contributions

LH: conceptualization, methodology, investigation, data curation, project administration, formal analysis, visualization, writing—original draft. MM: conceptualization, methodology, data curation, project administration, supervision, writing—review and editing. JCP: conceptualization, methodology, resources, funding acquisition, supervision, writing—review and editing. All authors approved the final version.

Preregistration

An Open Science Framework preregistration of this project is available online at https://osf.io/ap27q/ (date of preregistration: September 15, 2022).

Code Availability

Code is available online at https://osf.io/ap27q/ (Open Science Framework Project AP27Q).

Appendix

Supplementary information is available at the end of this article.

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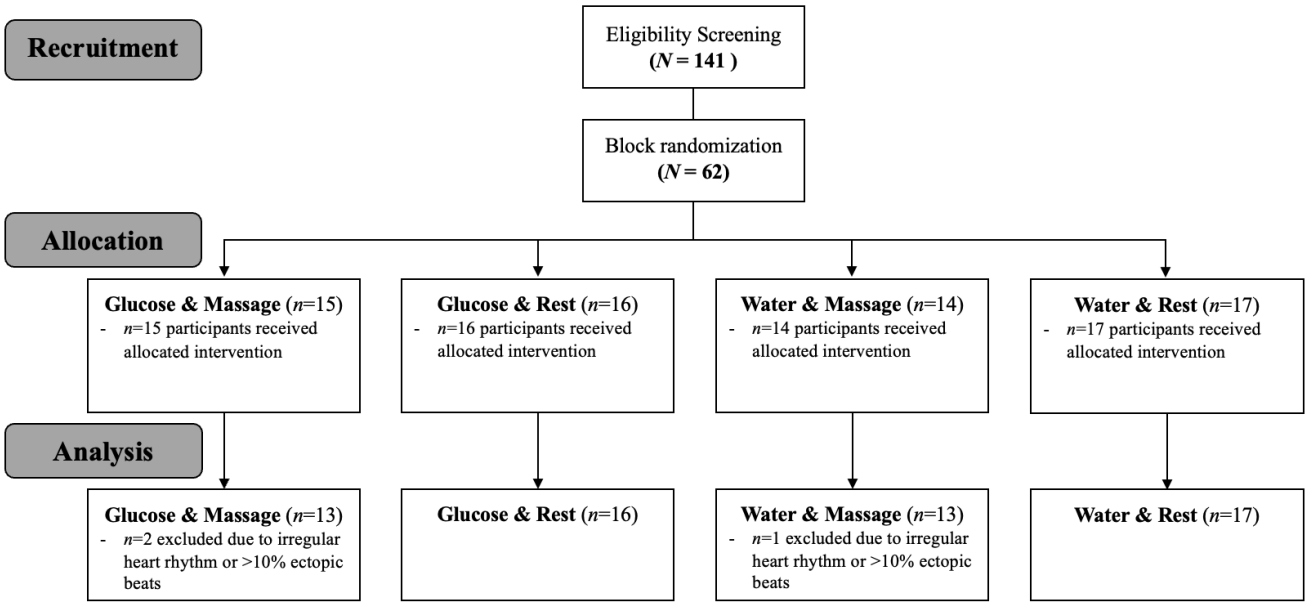
Appendix

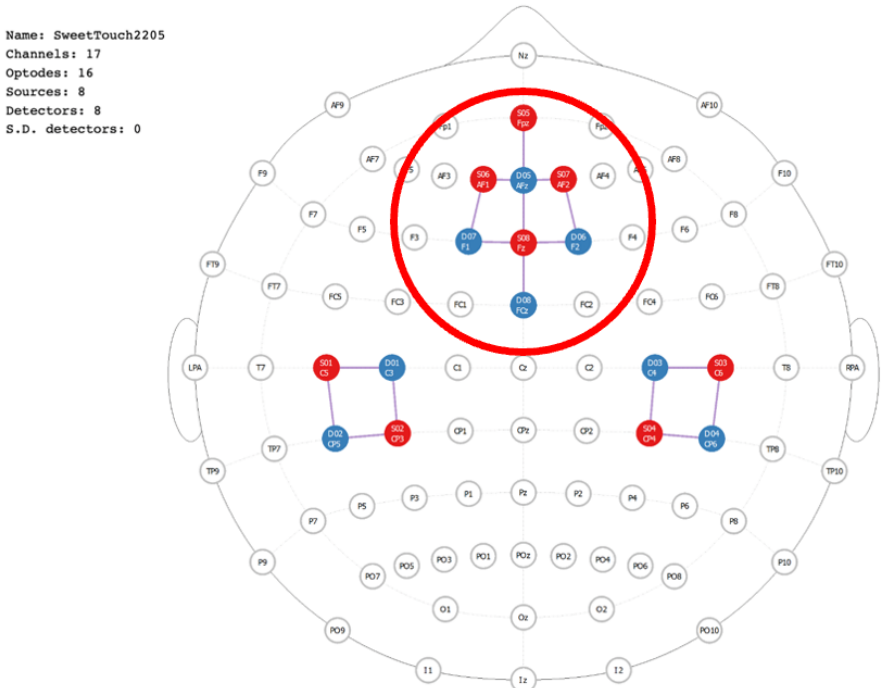
Appendix Table S1
Descriptive statistics of the experimental groups

variable	water-rest *(n=17)*	water-massage *(n=13)*	glucose-rest *(n=16)*	glucose-massage *(n=13)*	*p*
age (years)	22.3 ± 2.66	23.1 ± 2.69	22.7 ± 2.65	22.92 ± 3.30	.881
sex: male/female[a]	6/11	3/10	5/11	5/8	.847
blood glucose baseline (mg/dl)	94.2 ± 13.0	94.5 ± 17.4	91.6 ± 10.4	89.2 ± 11.6	.678
BDI-Score	2.53 ± 2.76	3.31 ± 3.12	3.38 ± 3.12	3.38 ± 4.07	.853
RMSSD baseline (ms)	38.2 ± 18.9	44.2 ± 20.2	50.3 ± 31.5	38.0 ± 16.1	.389
HR baseline (bpm)	75.9 ± 9.8	73.5 ± 11.9	71.7 ± 9.39	74.0 ± 11.8	.721
session start: 3/ 5 p.m.	8/9	3/10	8/8	4/9	.386

Note. We calculated multiple one-way Analysis of Variances by *experimental condition* to examine whether the groups differed with respect to *age, blood glucose baseline, BDI-Score, RMSSD baseline* and HR baseline. In these cases, data is expressed as *mean* ± *SD*. To test whether the experimental groups differed with respect to *sex* and *session start,* we conducted Pearson's Chi-squared tests. There were no statistically significant differences between the groups. BDI = Beck's Depression Inventory. RMSSD = Root Mean Square of Successive Differences. HR = Heart Rate.
[a] The variable "sex" was assessed in self-report as assigned sex at birth.



Appendix Figure S1. Flow diagram visualizing the sample of the study



Appendix Figure S2. Configuration of fNIRS optodes and channels. Red circles denote sources, blue circles denote detectors. fNIRS channels are defined by the blue lines. Labels within the circles denote the locations of the international 10-20 EEG placement. The interoptode distance was considered 3 cm. The red circle in the prefrontal region labels the nine channels of interest for the present research project.