Brain-heart interactions are optimized across the respiratory cycle via interoceptive attention

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Abstract

Respiration and heartbeat continuously interact within the living organism at many different levels, representing two of the main oscillatory rhythms of the body and providing major sources of interoceptive information to the brain. Despite the modulatory effect of respiration on exteroception and cognition has been recently established in humans, its role in shaping interoceptive perception has been scarcely investigated so far.

In two independent studies, we investigated the effect of spontaneous breathing on cardiac interoception by assessing the Heartbeat Evoked Potential (HEP) in healthy humans. In Study 1, we compared HEP activity for heartbeats occurred during inhalation and exhalation in 40 volunteers during resting-state. We found higher HEP amplitude during exhalations, compared to inhalations, over fronto-centro-parietal areas. This suggests increased brain-heart interactions and improved cortical processing of the heartbeats during exhalations. In Study 2, we tested the respiratory-phase dependent modulation of HEP activity in 20 volunteers during Exteroceptive and Interoceptive conditions of the Heartbeat Detection (HBD) task. In these conditions, participants were requested to tap at each heartbeat, either recorded or felt, respectively. Results showed higher HEP activity and higher detection accuracy at exhalation than inhalation in the Interoceptive condition only. These effects were positively correlated, suggesting that both cortical processing of cardiac signals and perception of heartbeats are optimized across the respiratory cycle. Direct comparisons of Interoceptive and Exteroceptive conditions confirmed stronger respiratory-phase dependent modulation of HEP and accuracy when attention was directed towards the interoceptive stimuli.

We provide data showing that respiration shapes cardiac interoception at the neurophysiological and behavioural levels. Specifically, exhalation may allow attentional shift towards the internal bodily states.

Keywords: heartbeat evoked potential, respiration, respiratory phase, heartbeat detection task, interoception, interoceptive accuracy, predictive coding, electroencephalogram, electrocardiogram

Introduction

Respiration and heartbeat are inextricably interconnected. Together, they produce two of the dominant oscillatory rhythms of the organism and represent major sources of interoceptive information (Chen et al., 2021; Khalsa et al., 2009; Weng et al., 2021). Interoception has been commonly defined as the process by which the brain receives, elaborates and interprets signals originating from the peripheral organs, continuously updating the conscious and (mostly) unconscious representations of the physiological condition of the body (Berntson and Khalsa, 2021; Craig, 2003; Critchley et al., 2004). However, within the field of interoception research, respiratory and cardiac signals processing have been studied mainly separately so far. In addition, there is no question that cardiac interoception has attracted most of the attention in the field. Since the Schandry's first proposal (Schandry, 1981), various behavioural tasks have been suggested to assess the individual interoceptive accuracy, as the ability to voluntarily focus on one's own heart and correctly report its beating. Importantly, research has shown that higher sensitivity to cardiac signals supports the capacity to regulate emotions and behaviour (Dunn et al., 2007; Herbert et al., 2011, 2012; Herbert and Pollatos, 2014; Wiens, 2005), while it is negatively associated to the susceptibility to mental health problems (de la Fuente et al., 2019; Lutz et al., 2019; Schulz et al., 2015; Yoris et al., 2017). However, heartbeat sensations remain for the most part outside of the field of awareness, and methodological limitations of cardiac interoceptive tasks have emerged (Brener and Ring, 2016).

More recently, researchers have increasingly focused on a more objective, electrophysiological index of the cortical processing of single heartbeats, the so-called Heartbeat Evoked Potential (HEP). The HEP is an Electroencephalographic (EEG) event-related potential, time-locked to participants' Electrocardiogram (ECG) R-peak or T-peak (Pollatos and Schandry, 2004). Physiological pathways underlying the HEP are currently under study, but they mainly involve signals originating from baroreceptor activity. Baroreceptors are stretch receptors located near the aortic arch and the carotid arteries, whose discharge activity is time-locked to cardiac systole and is driven by rhythmic changes in arterial blood pressure (Park and Blanke, 2019b). Baroreceptor-mediated information is sent upstream via the vagus nerve to the nucleus of the solitary tract in the brainstem and to the ventromedial posterior nucleus of the thalamus. Cardiac interoceptive information is finally elaborated in the brain at the level of the insula, amygdala, anterior cingulate, and somatosensory cortices, which represent the main cerebral sources of the HEP activity (Babo-Rebelo et al., 2016; Canales-Johnson et al., 2015; Kern et al., 2013; Park and Tallon-Baudry, 2014). Importantly, HEP activity increases when individuals voluntarily orient their attention to the heart (García-Cordero et al., 2017; Mai et al., 2018; Petzschner et al., 2019; Salamone et al., 2018; Villena-González et al., 2017), and is also positively associated to their accuracy in detecting heartbeats during cardiac interoceptive tasks (Canales-Johnson et al., 2015; Marshall et al., 2017; Pollatos et al., 2005).

In line with the inextricable interconnection between heartbeat and respiration, separate studies have independently shown that both HEP and cardiac interoceptive accuracy are modulated by respiration. For instance, one study from Baumert et al. (2015) assessed respiratory phase-dependent (inhale vs. exhale) HEP peak amplitude in children with sleep disordered breathing, as compared to healthy controls, during REM sleep. The authors found decreased peak HEP amplitude during exhalation in the sleep disordered breathing group. Moreover, MacKinnon and colleagues (2013) observed increased peak HEP amplitude over central EEG electrodes during "resonant breathing" (i.e., breathing at a rate of 6 breaths per minute), compared to spontaneous breathing. Finally, two recent studies (Smith et al., 2020, 2021) consistently showed that breath-holding can improve participants' cardiac interoceptive accuracy, as assessed with a modified version of the Heartbeat Detection (HBD) task. However, all these studies investigated the effect of respiration on

HEP changes and interoceptive accuracy separately and in a "perturbated" physiological setting, that is during systemic alteration of respiratory activity (REM sleep, slow breathing, and breath-hold). A systematic and multilevel investigation of the complex neuro-cardio-respiratory interactions (Corcoran et al., 2018) in an "ecological" setting is still lacking. This would allow the simultaneous characterization of the effects of spontaneous breathing on both HEP and interoceptive accuracy, as well as their possible relationship.

Spontaneous breathing occurs mostly outside of the field of awareness, and comprises a phase of active inspiration, that involves the contraction of the diaphragm and the external intercostals muscles, and a phase of passive expiration due to their subsequent relaxation. However, unlike the heartbeat, breathing can be easily accessed consciously and voluntarily controlled in its depth and frequency (Del Negro et al., 2018; Feldman et al., 2013). Respiration represents one of the most salient conscious forms of interoception. Despite this, it has received relatively low scientific interest until very recently, when increasing evidence has been accumulating about respiratory phase-dependent changes of perception and cognitive-emotional functions. For example, Zelano et al. (2016) found increased emotional recognition and episodic memory encoding and retrieval during inhalation, as compared to exhalation. Accordingly, phase-locking of stimulus onset to inhalation increased participant's near-threshold somatosensory perception (Grund et al., 2021) visuospatial recognition abilities (Kluger et al., 2021; Perl et al., 2019), and memory performance (Huijbers et al., 2014). Differently, self-initiated motor actions (Park et al., 2020), and trace eyeblink conditioning learning (Waselius et al., 2019) were more frequent during exhalation, while the exhalation-to-inhalation phase transition improved recognition memory performance (Nakamura et al., 2018). Based on this and other evidence, within the framework of predictive coding theories of brain functioning (Clark, 2013; Friston, 2010), respiration has been recently interpreted as a form of "active sensing" (Allen et al., 2021; Boyadzhieva and Kayhan, 2021;

Corcoran et al., 2018): that is, spontaneous inhalation may adaptively modulate cortical excitability to facilitate reception and elaboration of incoming sensory information from the environment, namely, exteroceptive information (Tort et al., 2018). Hence, the question arises of whether spontaneous respiration plays a role in shaping the processing of interoceptive information generated from inside the body as well.

In particular, the role of the respiratory phases assumes a special psychophysiological interest in the context of cardiac interoception for the following reasons. First, heartbeat and respiration are deeply linked at the body level, given the coupling between respiratory cycle and baroreceptor activity. This interaction is commonly observed during respiratory phase-dependent heart rate changes known as Respiratory Sinus Arrhythmia (RSA, Brecher and Hubay, 1955). Second, heartbeat and respiration share similar interoceptive pathways, both reaching the central nervous system through the vagus nerve at the level of the anterior and posterior insula, hippocampus, precuneus, somatosensory, and cingulate cortices (Farb et al., 2013; Wang et al., 2019), suggesting the possibility that respiratory and cardiac interoceptive signals interact also at the brain level, with possible effects on the individual interoceptive accuracy.

Therefore, the aim of the present work is twofold: i) to investigate and characterize respiratory phase-dependent modulations of neural responses to single heartbeats, as measured via HEP activity at rest; and ii) to relate respiratory phase-dependent HEP modulations to cardiac interoceptive attention. To fulfil these objectives, we performed two independent studies, simultaneously recording participant's EEG, ECG, and respiratory activity. In Study 1, we tested 40 healthy volunteers during a resting-state condition, while in Study 2 we tested 20 healthy volunteers during the performance of the HBD task, which requires to focus attention on the heart and tap a button in synchrony with each heartbeat.

Materials and methods

Ethics Statement

The studies were approved by the Institutional Review Board of Psychology, Department of Psychological, Health and Territorial Sciences, “G. d'Annunzio” University of Chieti-Pescara (Protocol Number 44_26_07_2021_21016), in compliance with the Italian Association of Psychology and the Declaration of Helsinki guidelines and its later amendments. All subjects signed a written informed consent.

Study 1 – Resting-state condition

Participants

Forty healthy volunteers with normal or corrected-to-normal vision (29 females; two left-handed; age: 26.67 ± 4.56 years [mean ± SD]) took part in the study. We estimated the sample size through the G*Power 3 software (v3.1.9.7; Faul et al., 2007) based on the results of a recent meta-analysis on HEP activity (Coll et al., 2021). We estimated a medium effect size of Cohen’s d = 0.5, set the significance level to alpha = 0.05, and the desired power at 0.80 (estimated sample size = 34). One participant was excluded from EEG analysis because of excessive movement-related artifacts. The inclusion of each volunteer was based on the following criteria: i) no personal or family history of neurological, psychiatric, or somatic disorders; and ii) not having taken any drug acting on the central nervous system in the previous week.

Experimental procedure

Participants were asked to rest for 10 minutes with eyes open, letting their mind wander while watching at a fixation cross at the centre of a computer screen (Raichle et al., 2001). Mind-wandering implies a shift in attention from the external to the internal environment, characterized by self-related mentation, memories and feelings (Smallwood and Schooler, 2006). No specific instruction on breathing was given, and participants were blind to the experimental goals. EEG, ECG, and respiratory signals were simultaneously recorded throughout the session.

Electrophysiological recordings

EEG signal was recorded from 64 scalp electrodes using a BrainAmp EEG acquisition system (BrainCap MR, BrainVision, LLC), according to the international 10-20 system. The midfrontal electrode (FCz) was used as the reference, the inion electrode (Iz) as the ground. Electrode impedance was kept lower than 10 kΩ for all channels using an electrode paste. ECG data were obtained from three ECG electrodes, two placed over the left and right clavicles, and the ground located on the right costal margin (BIOPAC Systems, Inc). Another ECG electrode was connected to the EEG net and placed on the left breast, serving as a back-up. Respiratory activity was recorded via a respiratory belt positioned around the chest (respiratory transducer TSD201, BIOPAC Systems, Inc). All signals were recorded with a sampling rate of 2 kHz; band-pass filtering from 0.016 to 250 Hz was applied, along with 50 Hz notch filtering.

Electrophysiological data pre-processing

ECG and respiratory signals were high-pass filtered (0.1 Hz) to remove baseline fluctuations. A low-pass filter at half the resampling frequency (i.e., 128 Hz) was applied to the data to avoid aliasing effects, then signals were down-sampled to 256 Hz. R-peaks were extracted from the ECG using the Pan-Tompkins algorithm (Pan and Tompkins, 1985; Sedghamiz, 2014), and mis-detected peaks were corrected using a point process model (Citi et al., 2012). The obtained RR-interval data set (tachogram) was processed using Kubios Oy free software (v3.4.3, Tarvainen et al., 2014) and a set of HRV feature of interest were extracted. Time-domain parameters (Heart Rate - HR), and frequency domain parameters: power in High-Frequency band (HF - 0.15-0.4 Hz)-log value (Malik et al., 1996), Heart Rate Variability (HRV) total power, and Low Frequency/High Frequency (LF/HF) ratio (Supplementary Material 1, SM1). Respiratory signals were processed using BreathMetrics (Noto et al., 2018) toolbox algorithms, to extract inhale and exhale onsets and offsets, as well as the set of respiratory features of interest (breathing rate, average inhale duration, average exhale duration, Inhalation/Exhalation (I/E) ratio; SM1). Inhale and exhale onsets and offsets were then visually inspected, and noisy breathing cycles were manually rejected. A respiratory cycle was defined starting from the beginning of inhalation and ending at the beginning of the following inhalation. Before starting the session, participants had to verbally confirm that they could not feel their heartbeat through the respiratory belt.

EEG data were pre-processed offline using EEGLAB (v2021.1; Delorme and Makeig, 2004) toolbox algorithms running on a MATLAB environment (R2021a, MathWorks Inc.). After all individual blocks were concatenated, signals were down-sampled to 256 Hz. Before resampling, a low-pass filter at half the resampling frequency (i.e., 128 Hz) was applied to the data to avoid aliasing effects. Datasets were then filtered using a Hamming windowed FIR filter (0.5-40 Hz). Signals were visually inspected for the removal of artefacts and detection of noisy channels, and bad segments were manually rejected. Noisy EEG channels were then removed and interpolated using their neighbouring channels (Al et al., 2021; Junghöfer et al., 2000). Rejected channels were generally few (~5%, depending on the EEG recording). Retained epochs were submitted to Independent Component Analysis to visualize and manually remove sources of heartbeat, ocular and muscle artifacts (FastICA algorithm; Hyvärinen, 1999). Particular attention was given to cardiac field artifact (CFA), by visually selecting the components whose activities followed the time course of R-peak and/or T-peak of the ECG (Al et al., 2020, 2021). Signals were finally re-referenced to the average signal.

HEP analysis

HEP was analysed using ERPLAB toolbox algorithms (v8.30; Lopez-Calderon and Luck, 2014). HEP was computed on EEG signals time-locked to the T-peak of the ECG (Babo-Rebelo et al., 2019; Babo-Rebelo, Richter, et al., 2016; Park et al., 2014). ECG T-peak positions were identified using the HEPLAB toolbox (Perakakis, 2019). Automatic detection of T-peaks was then followed by visual inspection and manual correction (Al et al., 2021). EEG data were epoched and baseline-corrected between -100 and 0 ms, using the T-peak event as temporal reference (epoch length: -100 to 350 ms after T-peak) (Park et al., 2014). The time window of interest for the statistical analysis was set to 80-350 ms, coincident with cardiac relaxation, when the CFA is minimum (Babo-Rebelo et al., 2019; Babo-Rebelo, Richter, et al., 2016; Dirlich et al., 1997). Additionally, we rejected all epochs in which the signal recorded at any channel exceeded a threshold of 100 µV (Blankenship et al., 2018; Villena-González et al., 2016), using a moving window peak-to-peak threshold function implemented in ERPLAB (moving window size: 200 ms; step size: 100 ms) (Lopez-Calderon and Luck, 2014). The number of rejected epochs was very low (less than 1%). Artifact-free epochs were assigned to “inhale” if the respective T-peak fell within the inhale onset/inhale offset time-range, and to “exhale” if the T-peak fell within the exhale onset/exhale offset time-range. HEP corresponding to inhale and exhale were computed by averaging EEG epochs assigned to the respective respiratory phase for each phase. We analysed HEP epochs occurring exclusively during inhalation or exhalation, that is, excluding those occurring across both respiratory phases, to avoid uncontrolled effects due to inhalation or exhalation onset (e.g., respiratory-related evoked potentials; Davenport et al., 2007; Webster and Colrain, 2000). Finally, we excluded epochs including T-peaks followed by an R-peak by less than 350 ms, to avoid overlap between the HEP activity and the following R-peak residual CFA (Babo-Rebelo et al., 2019).

HEP differences between phases were statistically assessed in the EEG artifact-free time window 80–350 ms after the T-peak, using a cluster-based permutation t-test implemented in the FieldTrip toolbox (Oostenveld et al., 2011), which corrects for multiple comparisons in time and space. First, paired t-values were computed between the two respiratory phases. Then, all adjacent spatiotemporal data points with a p-value below 0.05 formed a cluster, and the cluster-level t-values were calculated by taking the sum of the t-values within each cluster. Statistical significance was calculated by comparing the experimentally observed cluster-level statistics with a randomized null distribution of cluster-level statistics, which was obtained by randomly shuffling “inhale” and “exhale” phase labels 10000 times. For each randomization, the largest randomly obtained cluster-level statistic was entered into the null distribution. Finally, experimentally observed cluster-level statistics were compared against the null distribution. Clusters with a p-value below 0.05 were considered significant (two-tails). We did not use any a priori spatial or temporal region of interest for the comparison between inhale and exhale HEP, hence including the entire EEG sensor space and epoch time window (80-350 ms) (Petzschner et al., 2019).

Source analysis

The neural sources of the EEG signal were reconstructed with the BrainStorm toolbox (v3.210416; Tadel et al., 2011), using a standard structural T1-weighted MRI template (ICBM152) (Fonov et al., 2009), segmented with Freesurfer 5.3 into 15000 vertices of the cortex surface. A 3-shell Boundary Element Model was constructed with OpenMEEG (Gramfort et al., 2010; Kybic et al., 2005) to compute the lead field matrix. Sources were estimated with the Minimum Norm Estimation method using sLORETA normalization (Pascual-Marqui, 2002), by keeping constrained current dipole orientations (i.e., normally oriented to the cortical surface). For the statistical test of the neural sources of differential HEP amplitudes among different phases (inhale vs. exhale), we used cluster-based statistics in the source space (1000 randomizations) from the FieldTrip toolbox (Oostenveld et al., 2011).

Additional cardiorespiratory analyses during resting-state

To investigate the possible explanatory role of cardiac and respiratory physiology on HEP activity among respiratory phases, we performed the following additional analyses. First, to exclude confounding influence of the CFA, we tested for differences in the ECG signal amplitude between the inhale and exhale phases (Petzschner et al., 2019). We submitted the averaged ECG signal time-locked to the T-peak (epoch length: -100 to 350 ms) from the inhale and exhale phases to a repeated-measures, two-tailed t-test for all time points within the time window of observed significant HEP differences (Groppe et al., 2011), corrected with the Benjamini and Yekutieli (2001) procedure for control of the False Discovery Rate (FDR). In addition, we calculated ΔHEP for each participant by subtracting the mean HEP value during inhale from the mean HEP value during exhale in the significant time-window, averaged within the cluster of significant electrodes. Next, we computed ΔECG by subtracting the mean ECG value during inhale from the mean ECG value during exhale in the significant time-window. Finally, we tested for any relationship between cardiorespiratory activity and HEP changes across participants using Pearson’s correlation analyses relating mean HEP changes among respiratory phases (ΔHEP) to both changes in the ECG (ΔECG) and participants’ cardiac and respiratory features of interest (SM1).

Study 2 - Heartbeat Detection Task

Participants

Twenty healthy volunteers with normal or corrected-to-normal vision took part in the study (14 females; two left-handed; mean age: 25.21 ± 2.64 years [mean ± SD]). Sample size was estimated through G*Power 3 (v3.1.9.7; Faul et al., 2007) based on the results of a previous study investigating HBD task accuracy (Fittipaldi et al., 2020). We estimated a medium/high effect size of Cohen's d = 0.8, set the significance level to 0.05, and the power at 0.80 (estimated sample size = 15). We checked for the same inclusion criteria of Study 1. One participant was excluded from EEG analysis because of excessive movement-related artifact.

Experimental procedure

Following a brief training, participants were asked to perform the two conditions of a validated HBD task (Fittipaldi et al., 2020; García-Cordero et al., 2017; Yoris et al., 2017). In the first condition, named Exteroceptive Condition (EC), participants were presented with digitally constructed heartbeat sounds and instructed to press a button in synchrony with it with their dominant hand. They were given the following instructions: "You will hear the beating of a heart. Tap the button with your dominant hand as soon as you hear each heartbeat. Please avoid anticipated responses by guessing the recorded heart rhythm". The EC consisted of 4 blocks, each lasting 2.5 minutes, for a total of 10 minutes. In two blocks the heartbeats were presented at a regular frequency (60 bpm), while in the rest of the blocks the heartbeats were presented with irregular heartbeat intervals. In the second condition, named Interoceptive Condition (IC), participants were asked to focus their attention on their heart and tap a button in synchrony with their own heartbeats, in the absence of any external cues. Instructions were as follows: "Now, you must follow the beating of your own heart by tapping a button with your dominant hand for every beat you feel. You should not guide your responses by checking your arterial pulse in your wrists or neck. If you are unable to feel these sensations, you should appeal to your intuition trying to respond whenever you think your heart is beating" (Fittipaldi et al., 2020). The IC consisted of 4 blocks lasting 2.5 min each, for a total of 10 minutes. As in Study 1, during both conditions, participants kept their eyes open and watched a fixation cross at the centre of the monitor. Also, no specific instruction on breathing was given. EEG, ECG, and respiratory signals were simultaneously recorded throughout IC and EC.

Electrophysiological recordings, signals pre-processing, and HEP analysis

We applied the same analysis pipeline of Study 1 to the recording of the EEG and cardiorespiratory signals, as well as to the electrophysiological data pre-processing and HEP analysis.

Task accuracy assessment and comparison

Participants' interoceptive accuracy was assessed by calculating the interoceptive accuracy index, based on previously reported standard procedures (Fittipaldi et al., 2020; García-Cordero et al., 2017; Yoris et al., 2017). Correct answers were estimated by time-locking participant's tapped responses with a corresponding time window around each R-peaks registered by the ECG. To control for HR differences between participants in the IC, we considered three different time windows depending on the participant's HR. Unlike canonical procedure, which calculates the time windows of accurate response depending on mean HR, we relied on the participants' instantaneous HR, calculated for each R-peak based on the R-R interval from the following complex. This allowed us to control for ongoing HR changes during the task, in particular, for those coupled with the respiratory cycle (i.e., RSA). We thus considered 750 ms after the R-peak for an instantaneous HR less than 69.76 bpm; 600 ms after the R-peak for an instantaneous HR between 69.75 and 94.25 bpm; and 400 ms after the R-peak for an instantaneous HR higher than 94.25 bpm (Fittipaldi et al., 2020; García-Cordero et al., 2017; Yoris et al., 2017). A response was considered accurate if it fell within these temporal windows. The interoceptive accuracy index was calculated with the formula:

$$1 - (\text{Recorded heartbeats} - \sum \text{Correct Answers}) / \text{Recorded heartbeats}$$

The interoceptive accuracy score range falls between 0 and 1, and higher scores indicate better interoceptive performance.

In the EC, we calculated the exteroceptive accuracy index by time-locking each tapped response with the corresponding time window for each presented heartbeat sound. A tapped response was considered correct if it fell between 0 to 750 ms from the recorded heartbeat (Fittipaldi et al., 2020; García-Cordero et al., 2017; Yoris et al., 2017). As for the interoceptive score, the exteroceptive accuracy index was calculated with:

$$1 - (\text{Heartbeats sounds} - \sum \text{Correct Answers}) / \text{Heartbeat sounds}$$

Mean latencies were also calculated as the average time between every correct tapped response and its corresponding recorded heartbeat (IC) and heartbeat sound (EC). Finally, both IC and EC tapped responses were assigned to “inhale” if the respective R-peak fell within the inhale onset/inhale offset time range and to “exhale” if the R-peak fell within the exhale onset/exhale offset time range. Using the same procedures described above, we calculated interoceptive and exteroceptive accuracy scores and mean latencies corresponding to the inhale and exhale phase of respiration (e.g., “interoceptive accuracy-inhale”, “interoceptive accuracy-exhale”, “exteroceptive accuracy-inhale”, “exteroceptive accuracy-exhale”, etc.). An overview of the experimental procedure and data analysis is presented in Figure 1. We tested for changes in task accuracy and mean latencies among respiratory phases (inhale vs. exhale) with a bootstrapped paired t-test (2000 permutations) within both the EC and the IC of the HBD task. Then, to investigate the interaction effect of task * respiratory phase on task accuracy, we performed a two-way repeated-measures ANOVA with task accuracy as dependent variable, and task condition (exteroceptive vs.

interoceptive) and respiratory phase (inhale vs. exhale) as within-participants factors. The ANOVA for mean latencies scores was not performed due to non-significant differences in neither IC and EC latencies scores among respiratory phases.

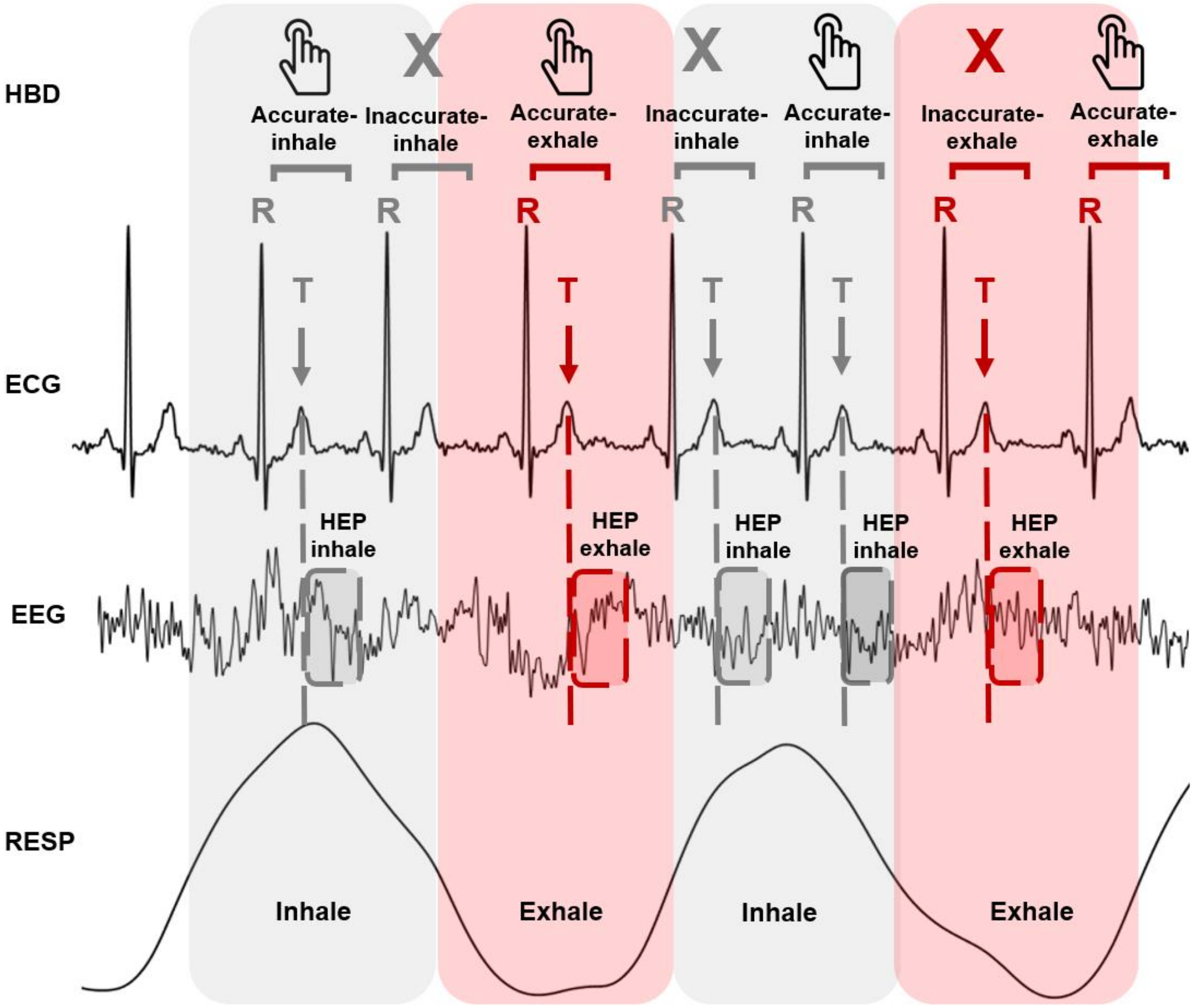

Figure 1. Schematic representation of data analysis. Grey areas represent exhale phases, pink areas represent inhale phases. The HBD row refers to the performance at the Heartbeat Detection task. “Hand” icons represent correct tapped responses. “X” icons represent inaccurate responses. The ECG row represents an exemplary trace of the electrocardiogram. Correct tapped responses were defined as the responses occurring within a given time-window time-locked to the previous R-peak of the ECG signal. The width of the

time window was individualized, based on the heart rate of each participant. The EEG (electroencephalogram) row represents an exemplary trace of one EEG channel. EEG data were epoched and time-locked to the T-peak (epoch length: -100 to 350 ms). Grey and red squares superimposed on the EEG trace represent the time-window where HEP were analysed. We analysed HEP epochs occurring either during inhalation or exhalation, excluding those across respiratory phases. RESP row represents an exemplary trace of the respiratory activity.

HEP amplitude statistical analysis

HEP differences between respiratory phases were statistically assessed as in Study 1. To investigate the interaction effect of task * respiratory phase on mean HEP amplitude, we performed a two-way repeated-measures ANOVA with mean HEP amplitude as dependent variable, and task condition (exteroceptive vs. interoceptive) and respiratory phase (inhale vs. exhale) as within-participants factors. In addition, to relate inter-individual differences in the effect of respiratory phase on the HEP to changes in interoceptive accuracy, we calculated the participants' mean HEP difference (ΔHEP) between respiratory phases, by subtracting the mean HEP during inhale from the mean HEP during exhale in the significant time-window, averaged within the cluster of significant electrodes, as done in Study 1. We next calculated participants' interoceptive accuracy difference (Δaccuracy) between respiratory phases by subtracting the accuracy scores of the inhale phase ("interoceptive accuracy-inhale") from the accuracy scores of the exhale phase ("interoceptive accuracy-exhale"). Then, based on previous evidence (Katkin et al., 1991; Mai et al., 2018; Montoya et al., 1993; Pollatos and Schandry, 2004; Schandry and Montoya, 1996), we tested the linear positive relationship between participant's Δaccuracy and ΔHEP using Pearson's correlation analysis. The same analysis was not performed for the EC, due to non-significant differences in both HEP activities and task accuracy scores among respiratory phases.

Additional cardiorespiratory analyses during the Heartbeat Detection Task

To investigate the role of cardiac and respiratory physiology on HEP activity and task accuracy, we performed the following additional analyses (Petzschner et al., 2019). Differences in the ECG amplitude between the IC and EC of the HBD task were first tested to exclude confounding cardiac effects. We compared the averaged ECG signal time-locked to the T-peak (epoch length: -100 to 350 ms) between the EC and IC using a t-test with FDR correction for all time points within the time window of observed significant HEP differences (Groppe et al., 2011). Then, differences in cardiorespiratory features among the EC and IC were assessed using bootstrapped paired t-test (2000 permutations). Within the IC, we also tested if the cardiac activity was different between respiratory phases (inhale vs. exhale), as done in Study 1 (Groppe et al., 2011). Within the EC, this test was not performed due to non-significant changes in HEP activity among respiratory phases. Finally, we tested for any relationships between mean HEP differences (ΔHEP), interoceptive accuracy changes (Δaccuracy), and cardiorespiratory features of interest (SM1) across participants with a series of Pearson's correlation analyses. Again, Pearson's correlation analyses for the EC were not carried out due to non-significant differences in both HEP activity and task accuracy scores among respiratory phases.

Throughout the manuscript, p-values (one for each feature) were adjusted for multiple testing using Benjamini and Yekutieli procedure (FDR, Benjamini and Yekutieli, 2001). FDR threshold was set at $p = 0.05$. All statistical analyses were performed in jamovi (v2.2.2; The jamovi project, 2021).

Results

Study 1 – Resting-state condition

Overview

We characterized HEP activity dependent from the respiratory phase by testing for changes in the HEP between inhalation and exhalation during a 10-minutes resting-state condition, using a comprehensive approach that included the entire EEG sensor space and epoch time window (Petzschner et al., 2019). The homogeneity of the inhale and the exhale phases in terms of number of registered heartbeats and analysed epochs was first verified by performing between-phases paired t-test comparisons (SM2).

Heartbeat-evoked potentials activity among respiratory phases at rest

We determined whether a HEP occurred during inhale and exhale and performed a cluster-based permutation t-test comparing the HEP during the two respiratory phases between 80 and 350 ms following the T-peak over the whole scalp. In a time window ranging from 180 to 350 ms after the T-peak, HEP showed increased positivity, during exhalation, in a wide cluster of frontal, central, and parietal electrodes (FC1, FC2, FC3, Cz, C1, C2, C3, C4, CPz, CP1, CP2, CP3, CP4, Pz, P1, P3, P4, POz), peaking on CPz (cluster-based permutation t-test, 10000 permutations, $t(38) = 2.0248$, $p_{corrected} = 0.0074$) (Figure 2A-B). Source reconstruction analysis with sLORETA showed that HEP amplitude was significantly different between the two respiratory phases in two postero-central cortical areas, one on the left hemisphere (cluster-based permutation *t*-test, 1000 permutations, $p_{corrected} = 0.002$, cluster statistic (maxsum) = 13494, cluster size = 3904) and the other on the right hemisphere (cluster-based permutation *t*-test, 1000 permutations, $p_{corrected} = 0.004$, cluster statistic (maxsum) = 12089, cluster size = 3662), overlapping areas of the Sensorimotor Network (bilateral post-central, paracentral, and pre-central gyrus) and the Default Mode Network (left inferior parietal lobule, and bilateral precuneus, cuneus, intraparietal sulcus, parieto-occipital cortex, and parietal superior lobule) (Figure 2C).

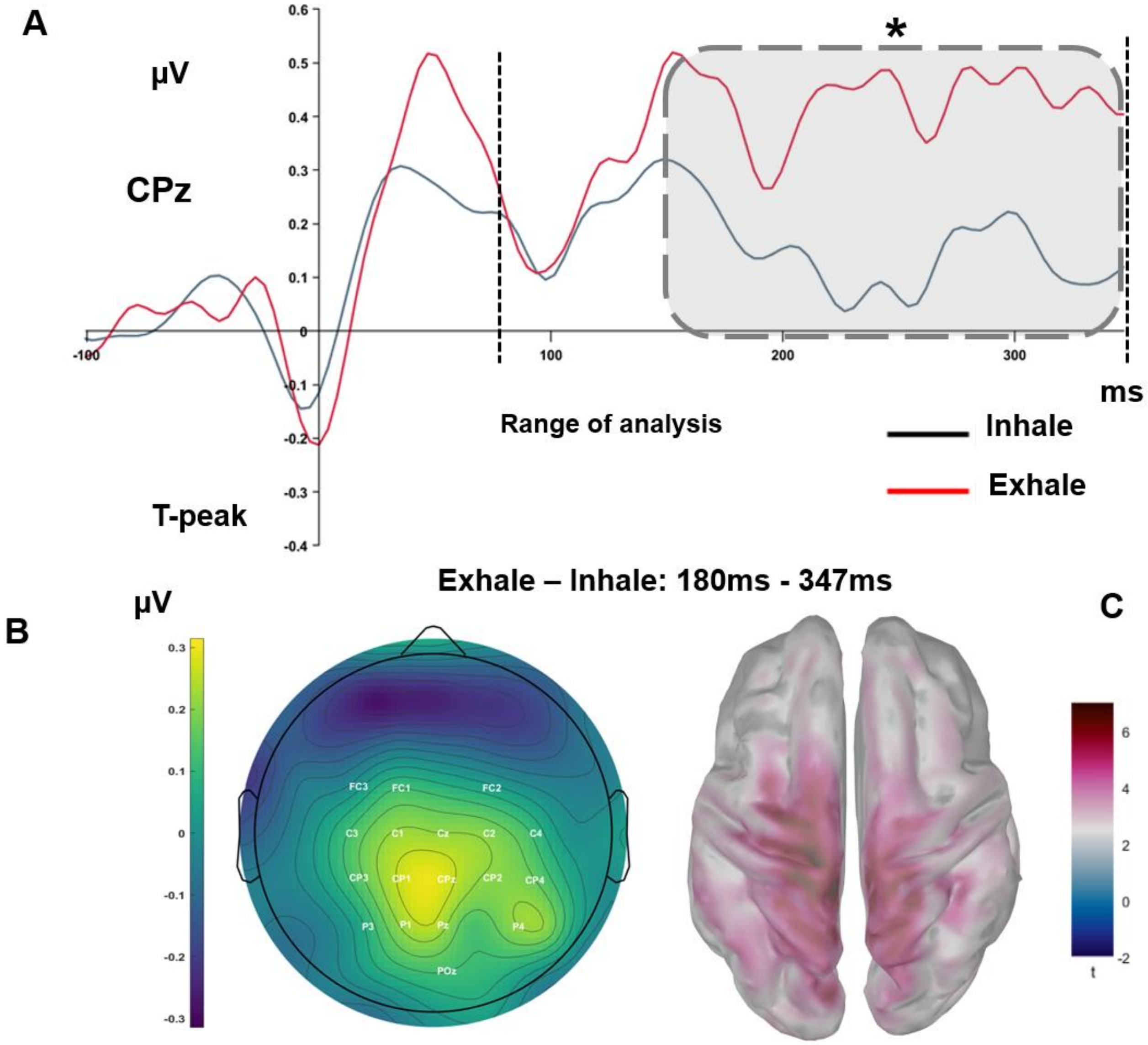

Figure 2. HEP activity changes among respiratory phases at rest. (A) Grand-average HEP waveforms at CPz. HEP waveform occurring at inhale is indicated in black, HEP waveform occurring at exhale is indicated in red. The dotted lines represent the temporal window of interest used for the statistical analysis (80-350 ms after the T-peak) of the HEP components. The grey rectangle marks the time window of significant differences (180–347 ms after the T-peak) (cluster-based permutation test). (B) Topographical scalp distribution showing

the contrast between significant mean HEP differences (180–347 ms after the T-peak) at exhale and at inhale. (C) sLORETA source-reconstruction of significant HEP amplitude changes between the exhale and inhale phases in the Sensorimotor Network and the Default Mode Network (cluster-based permutation test).

Additional cardiorespiratory analyses during resting-state

Heart physiology significantly differs between inhalation and exhalation (Draghici and Taylor, 2016; Riganello et al., 2018; Shaffer et al., 2014; Shaffer and Venner, 2013), and our experimental design makes the possible confounding influence of CFA a serious issue. Therefore, we performed additional analyses to support the assumption that observed differences in HEP amplitude between inhalation and exhalation were not driven by CFA modifications between phases, but they reflected differences in heartbeat cortical processing (Petzschner et al., 2019). First, we tested for differences in ECG amplitude across the two phases with a repeated-measures two-tailed t-test at all time points within the time window of significant HEP differences, followed by FDR correction for multiple comparisons. We found statistically significant differences between inhale and exhale in ECG amplitude, characterized by increased ECG negativity in the exhale phase (paired t-test, $t(38) = -4.674$, $p_{FDR} < 0.001$) (Supplementary Figure 1A, SF1A). This result may be an index that the heartbeat itself was different during exhalations. We, therefore, tested whether the effect of respiratory phase on HEP correlated with individual differences in ECG signal among respiratory phases (ΔECG) with a bootstrapped Pearson's correlation (2000 permutations). There was no significant correlation between subject's HEP changes (ΔHEP) and changes in ECG signal amplitude (Pearson's correlation ΔECG, $r = 0.222$, uncorrected $p = 0.175$ (SF1B), excluding a direct role of heartbeat physiology in explaining respiratory phase-related HEP modulations. We then estimated participants' cardiorespiratory features of interest during the resting-state (Supplementary Table 1, ST1): that is, cardiac (HR, HF power, HRV total power, LF/HF ratio) and respiratory (breathing rate, inhale duration, exhale duration, I/E ratio) features. Then, we performed a correlation analysis testing for relationship between the observed mean HEP differences among respiratory phases (ΔHEP) and cardiorespiratory features of interest, as well as with the number of registered heartbeats and analysed epochs, all of which resulted in null findings (SM3).

Study 2 – Heartbeat Detection Task

Overview

We tested HEP activity changes across the respiratory cycle in participants performing the IC and the EC of the HBD task. During EC, participants heard a digital heartbeat sound and they had to tap a button with their dominant hand in synchrony with it. During IC, they were asked to focus their attention on their own heart and tap a button at each heartbeat. As in Study 1, we confirmed the homogeneity of the inhale and the exhale phases in terms of number of registered heartbeats and analysed epochs by performing between-phases paired t-test comparisons, both within the EC and the IC of the HBD task (SM4).

Heartbeat-evoked potentials change across the respiratory cycle during IC

We first tested our hypothesis that during IC HEP activity changes among respiratory phases. As for the resting-state condition, we determined when a HEP occurred during inhale and exhale, and performed a cluster-based permutation t-test comparing HEP during the two respiratory phases between 80 and 350 ms after the T-peak over the whole scalp. The results showed that during a time window ranging from 164 to 350 ms following the T-peak, HEP increased in positivity in a cluster of central and frontal electrodes (F2, F4, FC2, FC4, FC6, Cz, C1, C2, C4, C6, CPz, CP2, FT8), peaking on CPz and CP2, confirming increased exhalation-related cortical processing of the heartbeat during the IC (cluster-based permutation *t*-test, 10000 permutations, t(18) = 2.1018, $p_{corrected} = 0.0136$) (Figure 3A-B, IC).

Second, we tested whether HEP modulations among respiratory phases were present also during the EC: we compared HEP activity during expiratory phases of respiration with inhalation during the execution of the EC. We adopted two different approaches: i) we compared inhale and exhale HEP over the whole scalp and in the whole time window (80-350 ms), as done for IC; and ii) we focused on space regions of the main respiratory effects previously detected within the IC (i.e., F2, F4, FC2, FC4, FC6, Cz, C1, C2, C4, C6, CPz, CP2, FT8) in the significant time-window (164-350 ms). In both cases, cluster-based permutation t-tests revealed no differences in any cortical cluster (cluster-based permutation *t*-test, 10000 permutations, $p_{corrected} > 0.05$) (Figure 3A-B, EC). This null effect during EC suggests that identified respiratory phase-dependent HEP changes in the IC are related to the different direction of top-down attention between the two conditions.

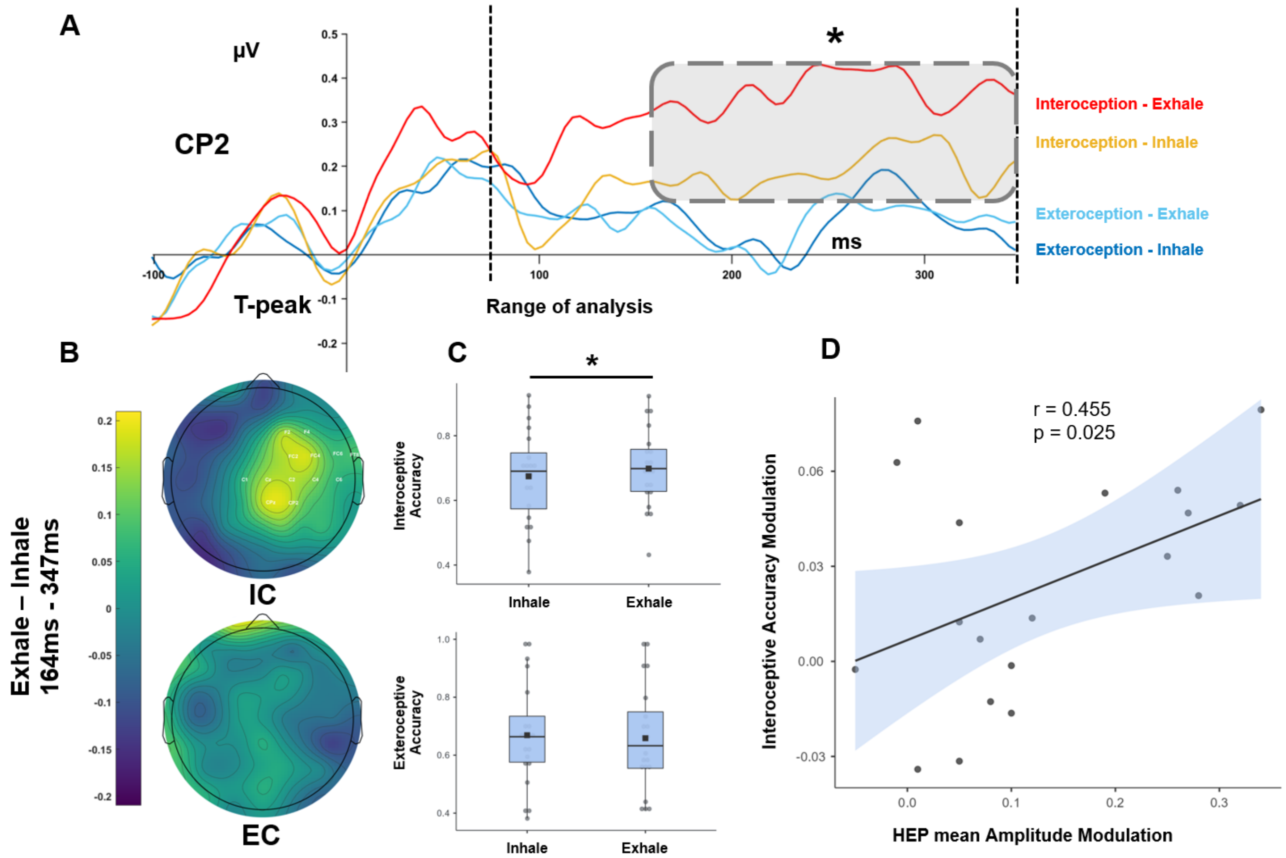

Figure 3. HEP activity and interoceptive accuracy changes across the respiratory cycle during the HBD task. (A) Grand average HEP waveform at CP2. The time courses of the HEP are shown for "interoception-exhale" (red), "interoception-inhale" (orange), "exteroception-exhale" (light blue), and "exteroception-inhale" (dark blue). The dotted lines represent the temporal window of interest used for the statistical analysis (80-350 ms after the T-peak) of the HEP components. The grey rectangle marks the time window of significant differences (180–347 ms after the T-peak) (cluster-based permutation test). (B) Topographical scalp distribution (164–347 ms after the T-peak) showing the difference between the exhale and inhale phases for both the Interoceptive Condition (up) and the Exteroceptive Condition (down) of the HBD task. (C) Interoceptive (up) and Exteroceptive (down) accuracy scores of the HBD task for tapped responses plotted against the respiratory phases. * represents significant differences between interoceptive accuracy scores at inhale and exhale. (D) Scatter plot of the linear relationship (Pearson's correlation, one tail) between the mean HEP amplitude changes (ΔHEP) and interoceptive accuracy changes (Δaccuracy) among respiratory phases. Mean HEP amplitude changes (ΔHEP) were computed by aggregating the following channels: F2, F4, FC2, FC4, FC6, Cz, C1, C2, C4, C6, CPz, CP2, FT8.

To investigate the interaction effect of task * respiratory phase on mean HEP amplitude, we performed a two-way repeated-measures ANOVA with mean HEP amplitude as dependent variable, and task condition (exteroceptive vs. interoceptive) and respiratory phase (inhale vs. exhale) as within-participants factors. The ANOVA did not show a main effect of task (repeated-measures ANOVA task F(1, 18) = 0.017, p = 0.899), but showed a main effect of respiratory phase (repeated-measures ANOVA respiratory phase F(1, 18) = 5.153, p = 0.036), and an interaction effect of task * respiratory phase on mean HEP amplitude (repeated-measures ANOVA task * respiratory phase F(1, 18) = 7.517, p = 0.013), indicating that respiratory phase-related HEP changes are significantly stronger when top-down attention is directed towards the heart than towards the environmental sounds.

Interoceptive accuracy changes across the respiratory cycle

We hypothesized that, along with HEP activity, interoceptive accuracy is modulated by respiratory phases. Therefore, we tested if accuracy of tapped responses given during IC in the exhale phase of respiration was different from the inhale phase. After the calculation of participants' interoceptive and exteroceptive accuracy and mean latency (SM5), we specifically considered interoceptive and exteroceptive accuracy scores and mean latencies corresponding to the inhale and exhale phases of respiration. Mean interoceptive accuracy was 69.84 ± 12.56 % [mean ± SD] for the exhale phase, and 67.45 ± 14.71 % [mean ± SD] for the inhale phase. In line with HEP findings, a bootstrapped paired t-test (2000 permutations) revealed significant changes in interoceptive accuracy among respiratory phases, indicating increased detection of the heartbeat sensations during exhalations (paired *t*-test, t(19) = 3.14, p = 0.005) (Figure 3C - IC). Mean latency for accurate responses was the same among respiratory phases, being 335.11 ± 36.77ms [mean ± SD] for the inhale phase, and 336.68 ± 41.20ms [mean ± SD] for the exhale phase (paired *t*-test, t(19) = 0.223, p = 0.86).

We then hypothesized that differences in accuracy among respiratory phases were specific to interoception. Therefore, as for IC, we calculated exteroceptive accuracy in the EC during exhale and compared it to the inhale phase of respiration. Mean exteroceptive accuracy was 64.10 ± 16.98 % [mean ± SD] for the exhale phase, and 65.23 ± 16.94 % [mean ± SD] for the inhale phase. We did not find any changes in exteroceptive accuracy due to respiratory phases during EC, showing that respiration did not affect the exteroceptive counterpart of the HBD task (paired *t*-test, t(19) = 1.35, p = 0.193) (Figure 3C - EC). Again, mean latency for accurate responses did not change among respiratory phases, being 194.81 ± 66.32ms [mean ± SD] for the inhale phase, and 200.64 ± 74.27ms [mean ± SD] for the exhale phase (paired t-test, t(19) = -0.682, p = 0.504).

Next, we performed a two-way repeated-measures ANOVA with task accuracy as dependent variable, and task condition (exteroceptive vs. interoceptive) and respiratory phase (inhale vs. exhale) as within-participants factors. The ANOVA showed no main effect of respiratory phase (repeated-measures ANOVA respiratory phase F(1, 19) = 2,263, p = 0.150), and of task condition (repeated-measures ANOVA respiratory phase F(1, 19) = 0.583, p = 0.455), but found an interaction effect of task * respiratory phase on accuracy (repeated-measures ANOVA task * respiratory phase F(1, 19) = 6.464, p = 0.020), confirming that respiratory phase-related modulations of accuracy are specific to the interoceptive task.

Finally, we interestingly found that inter-individual respiratory phase-related HEP amplitude modulations were directly linked to interoceptive accuracy, by running a Pearson's correlation analysis relating participants' interoceptive accuracy changes (Δaccuracy) to participants' changes of HEP mean amplitude (ΔHEP). We found a positive relationship showing that higher HEP activity increases positively correlated with higher interoceptive accuracy increases (Pearson's correlation r = 0.455, p = 0.025, one tail) among respiratory phases (Figure 3D).

Additional cardiorespiratory analyses during the Heartbeat Detection Task

In order to control if our results were determined by differences in heart activity between IC and EC, we first tested for differences in ECG amplitude across conditions with a repeated-measures, two-tailed t-tests at all time points within the time window of significant HEP differences (80-350 ms) followed by FDR correction for multiple comparisons. We did not find differences between IC and EC in the ECG signals (paired *t*-test, $p_{FDR} = 0.318$) (SF1D). Then, within the IC, we tested if the CFA equally impacted the HEP among respiratory phases (inhale vs. exhale) in the time window of significant HEP differences (IC: 164 - 350 ms). Unlike Study 1, we observed no differences in cardiac activity between inhale and exhale phases (paired *t*-test, $p_{FDR} = 0.2805$) (SF1C). We then estimated the participants' cardiorespiratory features of interest during the EC and the IC of the HBD task (ST2), and compared the two conditions with a bootstrapped paired t-test (2000 permutations). Participants had lower respiratory rate during the IC, compared to EC (paired *t*-test breathing rate, t(19) = -3.927, uncorrected p = 0.001, $p_{FDR} = 0.0217$; paired *t*-test inhale duration, t(19) = 3.511, uncorrected p = 0.002, $p_{FDR} = 0.0217$; paired *t*-test exhale duration, t(19) = 3.033, uncorrected p = 0.006, $p_{FDR} = 0.0435$), probably due to the different arousing nature of the two conditions. However, differences in breathing rate across IC and EC were not related to observed HEP modulations during IC (SM6). The other cardiorespiratory features did not significantly differ between IC and EC (ST2). Finally, during IC, we performed a series of correlation analyses testing for relationships between: i) mean HEP differences (ΔHEP) and cardiorespiratory features (SM7); and ii) interoceptive accuracy changes (Δaccuracy) and cardiorespiratory features (SM8). All the above-mentioned analyses did not yield statistically significant results, suggesting that observed differences in HEP activity and interoceptive accuracy among respiratory phases during IC are not significantly attributable to cardiac and respiratory physiology alone.

Discussion

The fundamental influence of respiration on brain activity and cognitive functions in humans has been increasingly recognized in the last few years (Heck et al., 2017; Kluger and Gross, 2021; Varga and Heck, 2017). However, its potential role in fine-tuning brain-heart interactions has gone mostly unstudied within the interoception research field so far. In the present work, we performed two studies with the general aim of investigating the role of spontaneous respiration in shaping the cortical processing of cardiac-related information. In both studies, we focused on HEP activity modulations, an EEG event-related potential time-locked to the ECG signal, commonly regarded as an objective electrophysiological index of brain-heart interactions (Coll et al., 2021; Park and Blanke, 2019b). Our first specific aim was to perform an investigation of the interplay between the cardiac, respiratory and brain activity in a resting-state condition, that is while participants were let mind-wander and spontaneously breath. Hence, in Study 1, we recruited healthy volunteers and computed HEP levels separately for heartbeats occurred during the inhalation and the exhalation phase of the respiratory cycle. We found that during exhalation, the HEP showed higher amplitude compared to inhalation, indicating increased brain-heart interactions and improved cortical processing of the heartbeats. HEP levels significantly increased in a time-window ranging from 180 to 350 ms after the T-peak over frontal, central, and parietal electrodes, and peaked on CPz. Source-level respiratory effects on HEP were localized in cortical regions overlapping the Sensorimotor Network and the Default Mode Network: cortical areas included the left inferior parietal lobule, the bilateral post-central, paracentral, and pre-central gyri, the precuneus, cuneus, intraparietal sulcus, superior parietal lobule, and parieto-occipital cortex. These results are consistent with evidence from Babo-Rebelo and colleagues (2016), who observed higher HEP activity over medial posterior areas associated to self-related processes during mind-wandering.

A first, low-level interpretation of present results may refer to the strong coupling between the respiratory cycle and the baroreflex. This well-known interaction is commonly referred to as RSA, a form of cardiorespiratory synchronization resulting in cyclic HR increases during inhalations and subsequent decreases during exhalations (Brecher and Hubay, 1955; Eckberg et al., 1985). RSA is mechanically induced by inhalation-dependent decrease of pleural pressure driven by chest expansion, which facilitates venous return to the right heart. This causes a reduction of the left ventricular stroke volume, decreasing aortic blood pressure, which in turn is sensed by aortic baroreceptors. Lower baroreceptor stimulation during inhalation then triggers the baroreflex: to stabilize cardiac output over the short term, the HR temporarily increases (Bainbridge, 1915; Magder, 2018). However, this also means that when HR increases, heartbeats are inherently weaker (Draghici and Taylor, 2016; Riganello et al., 2018; Shaffer et al., 2014; Shaffer and Venner, 2013). Regarding the present study, it is possible that being inhalations associated to increases in HR, they are also inextricably linked to weaker (or less salient) heartbeats, eventually resulting in the observed reduction of the HEP during inhalations compared to exhalations. Accordingly, a recent behavioural study found that the performance at a modified Heartbeat Tracking task was modulated by fluctuations in the frequency of heartbeats: participants' interoceptive accuracy was higher when HR was lower (Larsson et al., 2021). To further explore this hypothesis, we performed additional analyses on both the ECG signal and cardiorespiratory physiology. On the one hand, supporting the interpretation of increased HEP levels resulting from stronger heartbeats during exhalations, we found that the ECG signal time-locked to the T-peak had increased amplitude compared to inhalations. On the other hand, differences in the ECG signal did not correlate with mean HEP changes among respiratory phases, likely suggesting that the two phenomena are likely independent from each other. Additionally, respiratory phase-related HEP changes did not significantly correlate with any cardiorespiratory features of interest, such as the HR, HRV total power, and HF power (i.e., an indicator of RSA; Lewis et al., 2012), indicating a minor role of heart physiology in explaining HEP modulations.

Another possible interpretation is that bottom-up interoceptive information travelling from the heart to the cerebral cortex could be modulated by simultaneous sensorimotor signals generated by pulmonary afferents as well as by the active movement of the chest (see Baumert et al., 2015 for a similar interpretation). Indeed, heartbeat and respiratory interoceptive signals follow similar bottom-up pathways, as both aortic baroreceptors and rapidly-adapting pulmonary stretch receptors send information about the state of the cardiac and respiratory systems through vagal afferents and the nucleus of the solitary tract (Park and Blanke, 2019b). It is worth highlighting here that respiratory sensorimotor and interoceptive information is sent upstream primarily during inhalations (Noble and Hochman, 2019; Streeter et al., 2012). Therefore, in this specific respiratory phase, weaker cardiac baroreceptor-mediated information may compete with, and likely lose against, stronger respiratory-mediated information, increasing bottom-up sensorimotor interference between the two signals, and reducing the likelihood that the former could be entirely transferred from the periphery to the brain. In addition, at the central level, cardiac and respiratory interoceptive information is then processed within the same brain regions, which include the insula and somatosensory cortices (Farb et al., 2013; Park and Blanke, 2019b; Wang et al., 2019). Consequently, from a purely computational standpoint, interoceptive heartbeat-mediated information will be less processed within those areas that are already involved in the processing of respiratory-related signals. Supporting this hypothesis, we found that source-level HEP reduced activity during inhalations, compared to exhalations, was localized over brain regions that have been classically associated to respiratory phase-dependent fMRI modulations, that is the Sensorimotor Network (Bijsterbosch et al., 2017; Birn et al., 2006; Glasser et al., 2018; Power et al., 2020). This possibly reflects an interference between respiratory and heartbeat-related information processing.

A third intriguing explanation of our results relies on the predictive coding model of interoceptive perception (Barrett and Simmons, 2015; Seth, 2013; Seth and Friston, 2016). In brief, recent models (Allen et al., 2019; Allen et al., 2021) posit that cardiorespiratory interoception is able to shape the neural gain (i.e., the balance of neural excitation vs. inhibition) across several brain regions by modulating the computational precision (i.e., the inverse of noise) of perceptual, cognitive, and emotional processes. More in detail, periodic physiological changes related to rhythmic cardiorespiratory oscillations are computed by the brain as stable predictions, and are centrally suppressed via a sensory attenuation process in order to minimize their interference. Accordingly, recent studies (Al et al., 2020, 2021; Grund et al., 2021) on heart functions found that the systolic phase of the cardiac cycle (i.e., when baroreceptor activity is at a maximum) was related to simultaneous attenuation of somatosensory perception. This effect was explained by the functional cortical overlapping between somatosensory perception and cardiac interoception, at the level of the primary somatosensory cortices: the same sensory attenuation process that minimized systole-related oscillations in those areas also reduced somatosensory processing within those same regions (Al et al., 2020, 2021). Similarly, we propose that the brain receives recursive and predictable interoceptive signals during inhalations forming stable predictions about each respiratory cycle, and consequently suppressing the physiological signals associated to it within the Sensorimotor Network (Birn, 2012). Since also heartbeat-related information processing involves these areas (Park and Blanke, 2019b), cardiac-related sensations of heartbeats occurring during inhalations could be suppressed together with those related to respiration, hence explaining the observed inhalation-related HEP activity decrease within this network.

Our second specific aim was to explore if the above-described neuro-cardio-respiratory interactions are further modulated by endogenous attention towards interoceptive vs. exteroceptive signals, and if this modulation relates to individual interoceptive vs. exteroceptive accuracy at behavioural level. Hence, in Study 2 we simultaneously recorded EEG, ECG, and respiratory activity in healthy volunteers during the IC and the EC of the HBD task. We observed significant respiratory-phase dependent modulation of HEP activity exclusively during IC. As in Study 1, HEP activity increased during exhalations in a time window ranging from 164 to 350 ms after the T-peak. This effect was found over a cluster of central and frontal electrodes, peaking on CPz and CP2. During EC, we observed no effects of the respiratory phase on HEP activity, suggesting that when attention is focused on the external environment, heart-brain interactions are no longer mediated by respiratory activity. When considering task accuracy measures, we found that the exhalation phase of respiration was beneficial exclusively for interoceptive accuracy, and not for exteroceptive accuracy, suggesting that respiratory phase-related modulations of accuracy are specific to the IC. By directly comparing the two conditions (i.e., interoceptive vs. exteroceptive) we found more complex neuro-cardio-respiratory interactions when attention is directed towards the heart, with higher ΔHEP and higher Δaccuracy during the IC, compared to the EC. Finally, we found that HEP activity changes among respiratory phases (ΔHEP) positively correlated with interoceptive accuracy changes (Δaccuracy). As in Study 1, additional analyses on both the ECG signal and cardiorespiratory features of interest revealed no significant relationships with observed respiratory phase-related changes in HEP activity and interoceptive accuracy, indicating a negligible confounding influence of the CFA, as well as a minor explanatory role of cardiorespiratory physiology alone on present results. To summarize, Study 2 showed that both cortical processing of cardiac signals and perception of heartbeats during an IC are optimized at exhalation. Importantly, this suggests a crucial role of interoceptive attention in shaping neuro-cardio-respiratory interactions, which are instead strongly reduced when attention is directed towards the external environment (see Herrero et al., 2018 for similar results on interoception and EEG-breath coherence).

To better understand our results, we can interpret the role of attention towards the heartbeat and towards the environment during the performance of the IC and the EC within the framework of the predictive coding model. According to this model, top-down attention increases the precision of what is relevant for the organism in a specific moment, by modulating neuronal gain that represents the target objects at the expense of others (Feldman and Friston, 2010; Smout et al., 2019; Boyadzhieva and Kayhan, 2021). Then, during IC, the brain may assign the highest priority to heartbeat signals; on the opposite, during EC, the brain may assign the highest priority to the heard sounds. Notably, however, during both IC and EC the brain also receives recursive, predictable and strong respiratory-related noise during inhalations. Then why did we observe different respiratory-phase dependent modulations of HEP activity between the IC (higher ΔHEP) and the EC (lower ΔHEP)? According to the above-described model (Allen et al., 2019; Allen et al., 2021), interoceptive prediction is not an all-or-none phenomenon, but a highly context-sensitive process determining the precision of incoming sensations based on the ongoing task-oriented cognition. Therefore, during the IC, to be able to correctly perceive and process heartbeat sensations across the respiratory cycle, the brain may adaptively increase the precision of interoceptive cardiac input specifically during exhalations, that is, when inhalation-related physiological noise is absent. This is not necessary during the EC, because auditory signal processing at the level of auditory areas is much less interfered by cardiac and respiratory signal processing. Hence, comparing the two conditions, an interoceptive optimization process of the heartbeat signal among respiratory phases may occur during the IC, reflected by higher HEP increases during exhalations (higher ΔHEP), but not during the EC (lower ΔHEP). Notably, this interpretation is further supported by the linear correlation between respiratory phase-induced modulation of both HEP and interoceptive accuracy, with higher ΔHEP (i.e., interoceptive optimization) associated to higher increase of cardiac interoceptive accuracy (Δaccuracy). This clearly suggests that the ΔHEP index herein reported for the first time reflects a degree of optimization of interoceptive processing that is also relevant for interoceptive perception.

Overall, the present findings reveal a so far unnoticed influence of respiration on cardiac interoception: when contextualized within a breathing organism, cardiac interoception is highly interconnected with the respiratory cycle, in addition to task-oriented cognition. Accordingly, we propose that the respiratory phase-dependent HEP modulation (ΔHEP) could represent an objective physiological index of cardiac interoceptive optimization, at neurophysiological and behavioural levels, above and beyond HEP activity alone. In general, we underline the importance of investigating the synergic interplay between different visceral signals, which have been classically studied in isolation within the field of interoception research (Garfinkel et al., 2016). In particular, building on the present research, future studies should investigate neuro-cardio-respiratory interactions during different interoceptive and exteroceptive tasks. In fact, a limitation of the present study is that the occurrence of motor tapped responses during the HBD task made the comparison between ΔHEP during task vs. rest inappropriate, due to motor-related confounding activity intrinsic to the HBD task. To overcome this limitation, future studies could, for instance, adopt the Schandry task (Schandry, 1981), a non-motor-based cardiac interoceptive task making direct comparisons of ΔHEP during rest and task possible. It could be also tested if voluntary breath-control, in particular the slowing down of the breathing frequency or the specific increase of the exhalation duration (i.e., the decrease of the I/E ratio), may modulate neurophysiological and behavioural signatures of cardiac interoception (MacKinnon et al., 2013). This assumes a special interest particularly concerning the relationships between interoception and mental health, because breath-control is a highly recommended practice in a whole range of clinical applications and mind-body interventions (Farb et al., 2015; Paulus, 2013; Weng et al., 2021; Zaccaro et al., 2018, 2022). Finally, the link between ΔHEP and interoception could be deeply tested in different clinical or sub-clinical populations with known altered or reduced interoceptive skills, such as generalized anxiety disorder, major depression, and schizophrenia (Ardizzi et al., 2016; Bonaz et al., 2021).

To conclude, in line with the active sensing interpretation of respiratory activity, we speculate on the existence of a breathing-related attentional “switch” between interoceptive and exteroceptive brain modalities. It is already known that inhalation leads to adaptive modulation of neuronal gain in order to facilitate reception and elaboration of sensory information from the external environment (Grund et al., 2021; Huijbers et al., 2014; Kluger et al., 2021; Perl et al., 2019; Zelano et al., 2016). As inhalation itself improves exteroception-related cognitive functions, exhalation may reflect a more general attentional shifts towards the internal bodily states and the self, hence modulating interoceptive perception as well as self-consciousness (Molle and Coste, 2022; Park et al., 2018; Park and Blanke, 2019a).

That is, spontaneous breathing may continuously tune the brain to switch from a working mode of increased external (extrinsic) processing during inhalation, to an internal (intrinsic) processing mode during exhalation (Golland et al., 2007).

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