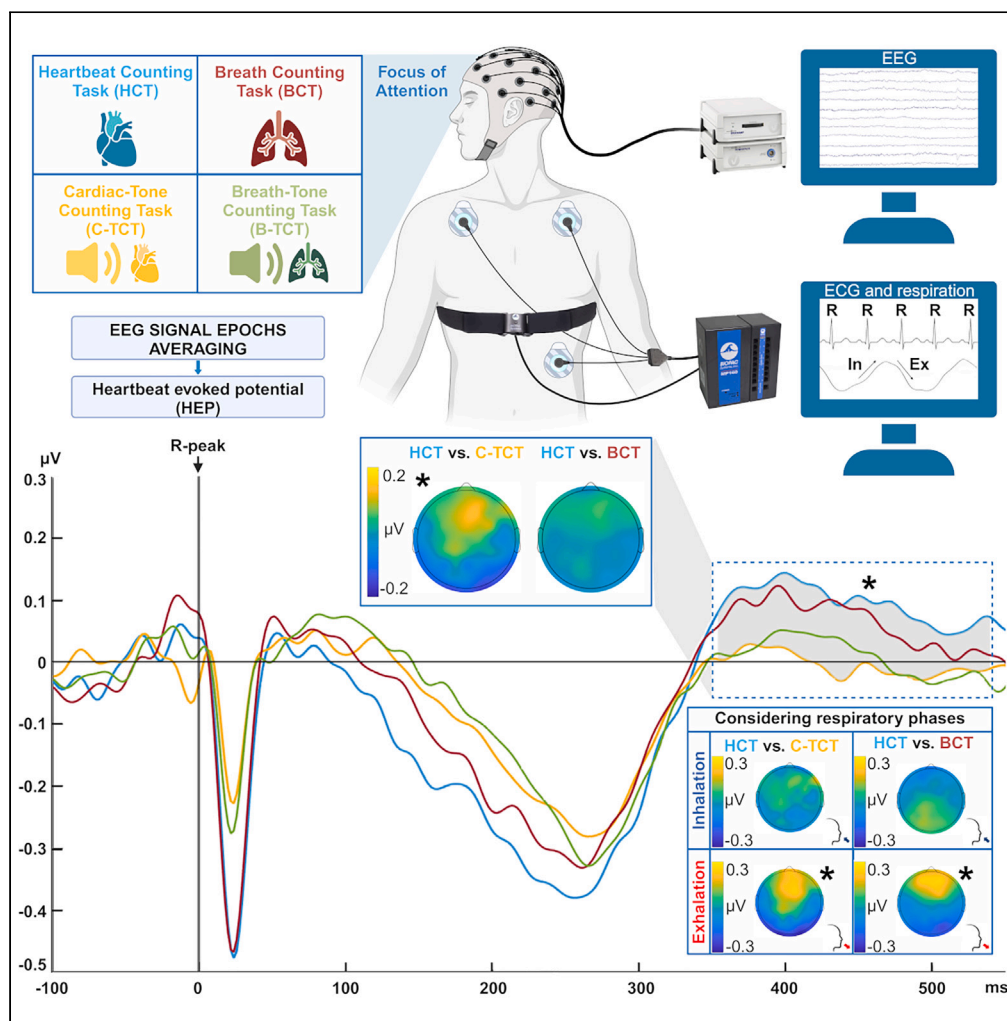**Article**

Attention to cardiac sensations enhances the heartbeat-evoked potential during exhalation

Andrea Zaccaro, Francesca della Penna, Elena Mussini, Eleonora Parrotta, Mauro Gianni Perrucci, Marcello Costantini, Francesca Ferri

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Highlights

Late HEP positivity increases during interoceptive tasks

Early HEP negativity increases non-specifically during exhalation

Late HEP increases specifically in the cardiac interoceptive task at exhalation

HEPs recorded at exhalation are linked to mindfulness and interoceptive sensibility

Article

Attention to cardiac sensations enhances the heartbeat-evoked potential during exhalation

Andrea Zaccaro,[1,4,*] Francesca della Penna,[2] Elena Mussini,[2] Eleonora Parrotta,[2] Mauro Gianni Perrucci,[2,3] Marcello Costantini,[1,3] and Francesca Ferri[2,3]

SUMMARY

Respiration and cardiac activity intricately interact through complex physiological mechanisms. The heartbeat-evoked potential (HEP) is an EEG fluctuation reflecting the cortical processing of cardiac signals. We recently found higher HEP amplitude during exhalation than inhalation during a task involving attention to cardiac sensations. This may have been due to reduced cardiac perception during inhalation and heightened perception during exhalation through attentional mechanisms. To investigate relationships between HEP, attention, and respiration, we introduced an experimental setup that included tasks related to cardiac and respiratory interoceptive and exteroceptive attention. Results revealed HEP amplitude increases during the interoceptive tasks over fronto-central electrodes. When respiratory phases were taken into account, HEP increases were primarily driven by heartbeats recorded during exhalation, specifically during the cardiac interoceptive task, while inhalation had minimal impact. These findings emphasize the role of respiration in cardiac interoceptive attention and could have implications for respiratory interventions to fine-tune cardiac interoception.

INTRODUCTION

The pulsation of the heart and the flow of respiration are intricately interconnected, together constituting two prominent oscillatory rhythms within the organism and representing major sources of interoceptive information. Cardiac interoceptive information is conveyed via the vagus and glossopharyngeal nerves to the nucleus of the solitary tract and the ventromedial posterior thalamic nucleus.[1–6] Subsequently, it reaches various brain regions including the amygdala, insula, cingulate, and somatosensory cortices. The modulation of EEG activity associated with the heartbeat and typically observed over fronto-central areas reflects this interoceptive information.[7–9] This phenomenon is specifically referred to as the heartbeat-evoked potential (HEP). The HEP is an event-related potential time-locked to the cardiac cycle derived by averaging EEG epochs around simultaneously recorded ECG R-peaks. Originally described by Schandry and colleagues,[10] it has gained interest in the interoception research field,[11,12] where it is considered an electrophysiological signature of the cortical processing of the heartbeat. The physiological pathways underlying HEP generation are still under study, but they are likely associated with mechanoreceptive signals from baroreceptors located in the aortic arch, carotid sinus, and carotid arteries.[13–16]

Cardiac activity is widely intertwined with respiration, another fundamental interoceptive rhythm in the human body.[12,17,18] Given the strong interactions between cardiac function and respiration at the physiological level,[19] we recently investigated the effects of respiratory phases on cardiac interoception in healthy participants. We observed an increase in late HEP amplitude and cardiac interoceptive accuracy during exhalation compared to inhalation while performing the interoceptive condition of the Heartbeat Detection Task (HBD).[20] This task involves focusing attention on the heart and pressing a button in synchrony with it.[21,22] Our interpretation was grounded in the continuous reception of both cardiac and respiratory sensations by the brain through vagal pathways, with the latter predominantly conveyed during inhalation.[23,24] Consequently, heartbeat sensations during inhalation may compete with concurrent respiratory sensations. This competition may decrease during exhalation, resulting in a reduction of respiratory interference. Therefore, during HBD, when cardiac sensations are heightened by attention, increased HEP and interoceptive accuracy may manifest during exhalation.[20]

Despite these findings, some ambiguities remained regarding the relationships between HEP, attention, and respiratory phases. Firstly, while previous studies have compared HEP amplitude between cardiac interoceptive tasks and exteroceptive tasks,[16] there is a lack of research comparing HEP amplitude between cardiac interoceptive tasks and *non-cardiac* interoceptive tasks, which involve focusing on interoceptive sensations unrelated to the heart. As such, HEP augmentation has been associated not only with attention focused toward the heart,

but also on the bodily self or the self in a broader sense.[25–29] Secondly, in our previous study,[20] confounding factors such as motor activity involved in HBD posed challenges for direct comparison of respiratory phase-related HEP effects between the interoceptive and exteroceptive condition.

Here, we have developed an experimental approach to distinguish the impact of respiratory phases on HEP during different types of interoceptive and exteroceptive attention. We used the Heartbeat Counting Task (HCT) to elicit cardiac interoception while avoiding potential movement-related issues present in HBD. In the first analysis, we compared HEP amplitude between tasks focused on cardiac interoception and exteroception, as well as tasks targeting *non-cardiac* interoception and exteroception, specifically focusing on respiration. Given the established links between HEP and cardiac interoceptive attention,[16] we expected an increase in HEP amplitude during the cardiac interoceptive task (HCT) compared to the exteroceptive task, with no HEP changes during the respiratory interoceptive task. In the second analysis, we explored how attention influenced HEP responses in interaction with respiration, aiming to determine whether putative changes in HEP amplitude during HCT were specific to a given respiratory phase. Given the connections between interoceptive sensibility, trait mindfulness, and HEP activity,[30–32] we also investigated whether they were associated with HEPs recorded during exhalation and inhalation. Based on our previous findings that showed higher late HEPs during exhalation,[20] we predicted a stronger contribution of exhalation in driving the expected HEP amplitude increase during HCT and its associations with interoceptive sensibility and mindfulness. If confirmed, these findings would highlight a previously overlooked role of respiratory phases in modulating cardiac interoception. In addition, recognizing the role of each specific respiratory phase in shaping interoception may provide valuable insights for clinical populations that show altered interoceptive functions, such as major depression or anxiety disorders.[33] For example, specific impairments of cardiac signal processing during exhalation may benefit from the development of respiratory-based interventions to fine-tune cardiac interoception. These findings could potentially refine therapeutic applications, such as mindfulness and slow breathing practices.

RESULTS

Overview

After a baseline resting state with eyes open, participants underwent four tasks involving interoceptive and exteroceptive attention (attention focus factor) focused on the cardiac and the respiratory systems (system factor). Tasks included HCT[10,13] and the Breath Counting Task (BCT)[34] as interoceptive tasks, and the Cardiac-Tone Counting Task (C-TCT)[35] and the Breath-Tone Counting Task (B-TCT; adapted from[34]) as exteroceptive tasks.

Heartbeat-evoked potential activity is related to interoceptive attention

To test whether HEP activity was modulated by attention, we performed a cluster-based permutation test over the whole scalp, with HEP amplitude as the dependent variable and System (cardiac vs. respiratory) and Attention Focus (interoceptive vs. exteroceptive) as within-participant factors (see STAR Methods for details on the interaction and main effects calculation). Included epochs in each task are shown in Table S1. Based on previous research,[4,20,30,36–42] we tested HEP amplitude at each electrode and time point separately in an early (100–350 msec) and a late time window (350–600 msec) after the R-peak.

In the early time window, there was no difference in HEP amplitude between interoceptive and exteroceptive attention (Attention Focus: $t_{31} = -1.312$, $p_{clust} = 0.115$, $d = 0.232$) nor between the cardiac and respiratory system (System: $t_{31} = 0.892$, $p_{clust} = 0.234$, $d = 0.158$). However, there was a significant System by Attention Focus interaction from 242 to 313 msec after the R-peak over the following electrodes: Fp1, AF7, AF3, F3, F1, Fpz, AFz, Fz, FCz, Fp2, AF8, AF4, and F2 ($t_{31} = -4.034$, $p_{clust} = 0.018$, $d = 0.713$, Figure S1A). However, despite the interaction, none of the planned t-tests were significant, showing no HEP difference when comparing the tasks (HCT vs. C-TCT: $t_{31} = -2.376$, $p_{clust} = 0.069$, $d = 0.42$; HCT vs. BCT: $t_{31} = 2.631$, $p_{clust} = 0.067$, $d = 0.465$; BCT vs. B-TCT: $t_{31} = -0.114$, $p_{clust} = 0.434$, $d = 0.02$; and C-TCT vs. B-TCT: $t_{31} = 2.724$, $p_{clust} = 0.312$, $d = 0.485$).

In the late time window, a higher HEP positivity was observed for interoceptive attention over F3, F1, FC1, C3, C1, CP1, CP3, Fpz, AFz, Fz, FCz, Cz, AF4, F6, F2, FC4, and FC2 from 352 to 508 msec after the R-peak (Attention Focus: $t_{31} = 5.039$, $p_{clust} = 0.017$, $d = 0.891$, Figures 1A and S1B). Neither the main effect of the system ($t_{31} = 0.338$, $p_{clust} = 0.738$, $d = 0.06$) nor the System by Attention Focus interaction ($t_{31} = 1.28$, $p_{clust} = 0.872$, $d = 0.227$) were significant. These findings suggest that the impact of attention on late HEP amplitude did not specifically depend on the system being monitored (cardiac vs. respiratory) but was associated with attention directed toward the body. Despite the non-significant interaction, planned t-tests conducted based on previous studies[16] revealed increases in HEP activity only during HCT compared to C-TCT from 352 to 539 msec after the R-peak over F1, FC3, FC1, C1, AFz, Fz, FCz, AF4, F6, F4, F2, FC4, and FC2 ($t_{31} = 4.13$, $p_{clust} = 0.013$, $p_{FDR} = 0.039$, $d = 0.73$, Figures 1B and S1C), but not compared to BCT ($t_{31} = 1.34$, $p_{clust} = 0.19$, $d = 0.273$, Figure 1B). No differences were observed between BCT vs. B-TCT ($t_{31} = 0.627$, $p_{clust} = 0.231$, $d = 0.111$, Figure 1B), nor between C-TCT vs. B-TCT ($t_{31} = -0.683$, $p_{clust} = 0.5$, $d = 0.121$, Figure 1B).

Heartbeat-evoked potential activity is related to the respiratory phase

To test whether HEP activity was modulated by the respiratory phase, we differentiated HEPs occurring during inhalation and exhalation. We found more epochs during inhalation than exhalation, likely due to higher Heart Rate (HR) during inhalation, resulting in the detection of more R-peaks (Table S2). We first ran a cluster-based permutation t-test comparing HEP amplitude between inhalation and exhalation during the baseline. In the early time window, the analysis showed a positive posterior cluster of higher HEP amplitude during exhalation compared to inhalation from 195 to 328 msec after the R-peak (CP1, CP3, CP5, P1, P3, P5, PO3, CPz, Pz, POz, C4, CP2, CP4, CP6, P2, P4, P6, and PO4)
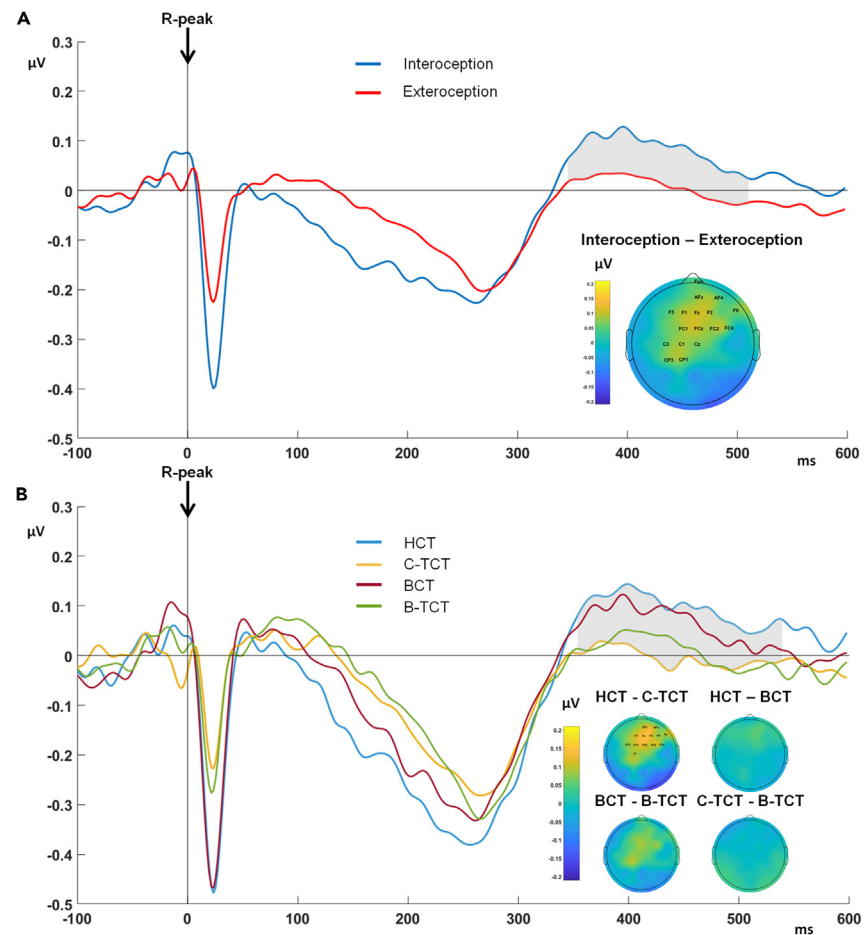
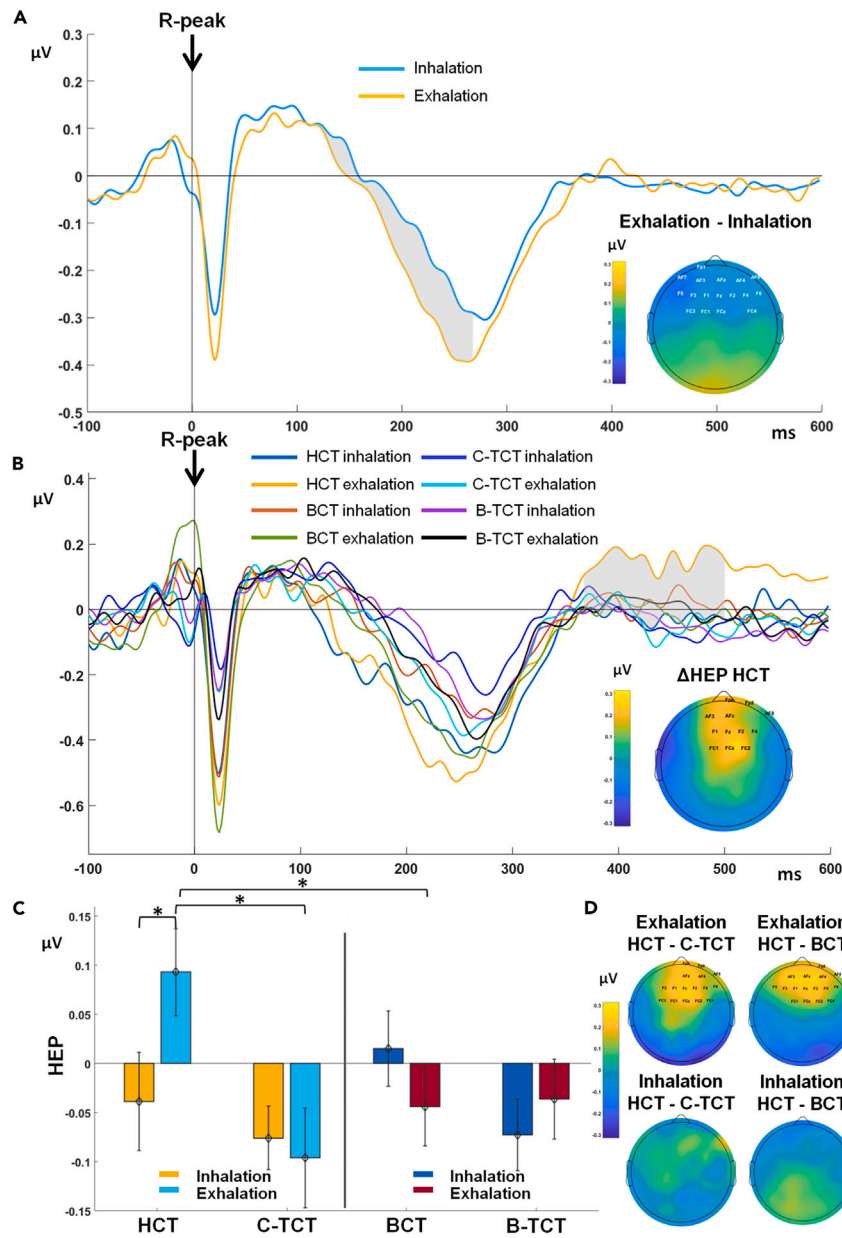**Figure 1. HEP activity is related to interoceptive attention**

(A) Grand-average HEP pooled for significant electrodes illustrating the main effect of Attention Focus. The shaded area marks significant differences between Interoception (blue) and Exteroception (red). The topographical distribution represents HEP differences between Interoception vs. Exteroception. Labels represent significant electrodes (cluster-based t-test: $t_{31} = 5.039$, $p_{clust} = 0.017$, $d = 0.891$). The scale bar unit of measurement is μV.

(B) Grand-average HEP pooled for significant electrodes during HCT (light blue), C-TCT (orange), BCT (purple), and B-TCT (green). The shaded area marks significant differences between HCT and C-TCT. The topographical distributions represent HEP differences between HCT vs. C-TCT, HCT vs. BCT, BCT vs. B-TCT, and C-TCT vs. B-TCT. Labels represent significant electrodes (cluster-based t-test: $t_{31} = 4.13$, $p_{clust} = 0.013$, $p_{FDR} = 0.039$, $d = 0.73$). The scale bar unit of measurement is μV. Abbreviations: HCT-Heartbeat Counting Task, C-TCT-Cardiac-Tone Counting Task, BCT-Breath Counting Task, B-TCT-Breath-Tone Counting Task.

($t_{27} = 3.9$, $p_{clust} = 0.012$, $d = 0.738$, Figure S2) and a negative frontal cluster from 195 to 336 msec (Fp1, AF7, AF3, F5, F3, F1, FC5, FC3, C3, C5, Fpz, AFz, Fz, FCz, Fp2, AF8, AF4, F6, F4, F2, FC6, FC4, FC2, and C6) ($t_{27} = 3.18$, $p_{clust} = 0.012$, $d = 0.601$, Figure S2). We found no significant differences in the late time window ($t_{27} = 2.7$, $p_{clust} = 0.303$, $d = 0.489$).

We then conducted two cluster-based permutation tests: one for the early and one for the late time window, both focused on the significant fronto-central cluster observed in the previous analysis that did not consider the respiratory phase. In these analyses, HEP amplitude served as the dependent variable, with System (cardiac vs. respiratory), Attention Focus (interoceptive vs. exteroceptive), and respiratory Phase (inhalation vs. exhalation) as within-participant factors. In the early time window, we found a main effect of Phase from 117 to 266 msec after the R-peak over Fp1, AF7, AF3, F5, F3, F1, FC3, FC1, AFz, Fz, FCz, AF8, AF4, F6, F4, F2, and FC4 ($t_{27} = -3.32$, $p_{clust} = 0.007$, $d = 0.627$, Figures 2A and S3A), indicating that HEP negativity was consistently higher during exhalation compared to inhalation across all tasks. There was no main effect of System ($t_{27} = -1.65$, $p_{clust} = 0.054$, $d = 0.311$) or Attention Focus ($t_{27} = -2.71$, $p_{clust} = 0.472$, $d = 0.512$). Moreover, no interactions were significant (System by Attention Focus by Phase: $t_{27} = 0.544$, $p_{clust} = 0.089$, $d = 0.103$; System by Attention Focus: $t_{27} = 1.68$, $p_{clust} = 0.698$, $d = 0.318$; Attention Focus by Phase: $t_{27} = -0.674$, $p_{clust} = 0.55$, $d = 0.127$; System by Phase: $t_{27} = 1.40$, $p_{clust} = 0.098$, $d = 0.265$).

In the late time window, we found no main effect of System ($t_{27} = 0.2$, $p_{clust} = 0.319$, $d = 0.038$), Attention Focus ($t_{27} = 2.057$, $p_{clust} = 0.857$, $d = 0.389$), or Phase ($t_{27} = 0.777$, $p_{clust} = 0.174$, $d = 0.147$) on HEP. Similarly, there were no significant System by Attention Focus ($t_{27} = -1.705$, $p_{clust} = 0.974$, $d = 0.322$), System by Phase ($t_{27} = -0.45$, $p_{clust} = 0.318$, $d = 0.085$), or Attention Focus by Phase interactions ($t_{27} = 1.058$,

Figure 2. HEP activity is related to the respiratory phase

(A) Grand-average HEP pooled for significant electrodes illustrating the main effect of Phase. The shaded area marks significant differences between Exhalation (orange) and Inhalation (light blue). The topographical distribution represents HEP differences between Exhalation vs. Inhalation. Labels represent significant electrodes (cluster-based t-test: $t_{27} = -3.32$, $p_{clust} = 0.007$, $d = 0.627$). The scale bar unit of measurement is μV.

(B) Grand-average HEP pooled for significant electrodes during different respiratory phases: HCT-inhalation (dark turquoise), HCT-exhalation (orange), BCT-inhalation (brown), BCT-exhalation (green), C-TCT-inhalation (blue), C-TCT-exhalation (light blue), B-TCT-inhalation (purple), B-TCT-exhalation (black). The shaded area marks significant differences between exhalation and inhalation (ΔHEP) during HCT. The topographical distribution represents HEP differences between Exhalation vs. Inhalation during HCT. Labels represent significant electrodes (cluster-based t-test: $t_{27} = 3.8$, $p_{clust} = 0.005$, $p_{FDR} = 0.02$, $d = 0.719$). The scale bar unit of measurement is μV.

(C) Mean HEP amplitude over significant electrodes across tasks and respiratory phases. Data are presented as mean +/− standard error. Significant planned t-tests are indicated by asterisks (*p < 0.05).

(D) Topographical distributions represent HEP differences during exhalation between HCT vs. C-TCT and HCT vs. BCT, and during inhalation between HCT vs. C-TCT and HCT vs. BCT. Labels represent significant electrodes (Exhalation HCT vs. C-TCT cluster-based t-test: $t_{27} = 3.668$, $p_{clust} = 0.003$, $p_{FDR} = 0.02$, $d = 0.693$; Exhalation HCT vs. BCT cluster-based t-test: $t_{27} = 3.572$, $p_{clust} = 0.018$, $p_{FDR} = 0.048$, $d = 0.675$). The scale bar unit of measurement is μV. Abbreviations: HCT-Heartbeat Counting Task, C-TCT-Cardiac-Tone Counting Task, BCT-Breath Counting Task, B-TCT-Breath-Tone Counting Task.

p_{clust} = 0.676, d = 0.199). Crucially, however, the three-way System by Attention Focus by Phase interaction was significant from 430 to 523 msec over Fp1, AF3, F5, F3, F1, FC1, Fpz, AFz, Fz, Fp2, AF8, AF4, F4, F2, FC4, and FC2 (t_{27} = 3.98, p_{clust} = 0.031, d = 0.583, Figure S3B). This interaction was explained by the following planned t-tests, which showed that HEP positivity increased during exhalation compared to inhalation specifically in HCT, from 367 to 500 msec after the R-peak, over AF3, F1, FC1, Fpz, AFz, Fz, FCz, Fp2, AF8, F4, F2, and FC2 (t_{27} = 3.8, p_{clust} = 0.005, p_{FDR} = 0.02, d = 0.719, Figures 2B, 2C, and S3C). In contrast, there were no changes in HEP activity between exhalation and inhalation in BCT (t_{27} = −0.357, p_{clust} = 0.129, d = 0.067), C-TCT (t_{27} = 0.116, p_{clust} = 0.667, d = 0.022), or B-TCT (t_{27} = 1.01, p_{clust} = 0.392, d = 0.192). The ΔHEPs waveforms, defined as the difference between HEPs during exhalation and HEPs during inhalation, are represented for each task in Figure S4. Interestingly, HEPs recorded during exhalation in HCT were higher than those recorded during exhalation in C-TCT from 383 to 508 msec after the R-peak over F3, F1, FC3, FC1, Fpz, AFz, Fz, FCz, Fp2, AF8, AF4, F6, F4, F2, FC4, and FC2 (t_{27} = 3.668, p_{clust} = 0.003, p_{FDR} = 0.02, d = 0.693, Figures 2C, 2D, and S3D), and during exhalation in BCT from 445 to 500 msec over AF3, F5, F3, F1, FC1, AFz, Fz, FCz, Fp2, AF8, AF4, F6, F4, F2, FC4, and FC2 (t_{27} = 3.572, p_{clust} = 0.018, p_{FDR} = 0.048, d = 0.675, Figures 2C, 2D, and S3E). In contrast, there were no changes between HEPs recorded during inhalation in HCT and those recorded during inhalation in C-TCT (t_{27} = 0.428, p_{clust} = 0.654, d = 0.081, Figure 2D) nor BCT (t_{27} = −1.061, p_{clust} = 0.895, d = 0.2, Figure 2D). These results showed higher late HEP positivity during exhalation compared to inhalation specifically for HCT. Importantly, they highlight that the observed increases in HEP amplitude during HCT primarily stem from HEPs recorded during exhalation, with minimal contribution from HEPs recorded during inhalation.

Heartbeat-evoked potential and cardio-respiratory physiology

We performed control analyses at both the participant and trial levels to assess whether observed changes in HEP amplitude were related to cardio-respiratory physiology[8,43–47] (STAR Methods). Cardio-respiratory features of interest are described in the STAR Methods. We found negligible effects of cardio-respiratory physiology on HEP changes across tasks and respiratory phases (Figures S5–S10; Tables S3–S5).

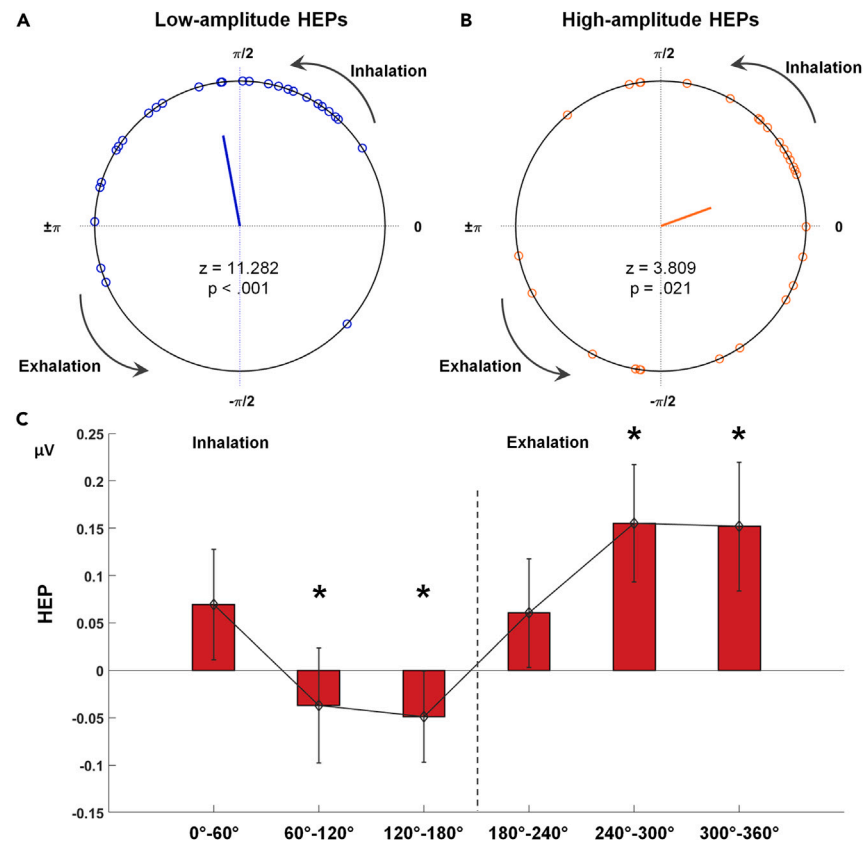### Respiratory cycle analyses during the Heartbeat Counting Task

To capture HEP changes across the respiratory cycle during HCT, we employed circular statistics and divided the respiratory cycle into phase bins. We used the interquartile range to categorize HEPs into low and high-amplitude HEPs. The Rayleigh test rejected the uniform distribution for both low (z = 11.282, p < 0.001) and high-amplitude HEPs (z = 3.809, p = 0.021). Low-amplitude HEPs were clustered around the middle phase of inhalation (first and second quadrants; mean angle = 100.45°, Figure 3A), while high-amplitude HEPs were clustered around the early phase of inhalation (first quadrant) and extended to the exhalation phase (third and fourth quadrant), with the mean direction occurring near the transition from exhalation to inhalation (19.99°, Figure 3B). The Watson-Williams test revealed significant differences between low and high-amplitude HEPs ($F_{1,54}$ = 15.665, p < 0.001).

We assessed HEP amplitude changes in HCT across the respiratory cycle using six equally sized bins covering the 0°–360° interval. A one-way repeated measures ANOVA revealed significant HEP amplitude changes across phase bins ($F_{5,135}$ = 3.23, p = 0.009, ηp2 = 0.107). Planned t-tests showed that HEPs detected in the 60°–120° range (middle inhalation) had lower amplitude than those detected in the 240°–300° range (middle exhalation: t_{27} = −2.995, p = 0.006, p_{FDR} = 0.027, d = 0.566) and the 300°–360° range (late exhalation: t_{27} = −2.43, p = 0.022, p_{FRD} = 0.049, d = 0.459). Furthermore, HEPs detected in the 120°–180° range (late inhalation) had lower amplitude than those detected in the 240°–300° range (middle exhalation: t_{27} = −3.063, p = 0.005, p_{FRD} = 0.027, d = 0.578) and the 300°–360° range (late exhalation: t_{27} = −2.731, p = 0.011, p_{FRD} = 0.033, d = 0.516) (Figure 3C). All the other comparisons did not show significant differences.

Correlation between the heartbeat evoked potential, behavioral and psychometric data

Task accuracy changed across tasks (Table S6). A 2 × 2 repeated measures ANOVA revealed a main effect of System ($F_{1,31}$ = 73.1, p < 0.001, ηp2 = 0.702) and Attention Focus ($F_{1,31}$ = 108.5, p < 0.001, ηp2 = 0.778), with lower accuracy in the cardiac than respiratory condition and in the interoceptive than exteroceptive condition. We also found a significant system by attention focus interaction ($F_{1,31}$ = 110.7, p < 0.001, ηp2 = 0.781). Planned t-tests revealed lower task accuracy in HCT compared to C-TCT (t_{31} = −10.78, p < 0.001, d = 1.91) and BCT (t_{31} = −9.66, p < 0.001, d = 1.71), and lower task accuracy in B-TCT compared to C-TCT (t_{31} = −2.6, p = 0.048, d = 0.365). No differences were observed between BCT and B-TCT (t_{31} = −1.81, p = 0.08, d = 0.32). Correlations with HEP amplitude showed no relationship with HCT accuracy (r = 0.117, p = 0.524) or confidence (r = 0.108, p = 0.558). Similarly, HEPs amplitude during inhalation or exhalation did not correlate with HCT accuracy (inhalation: r = 0.074, p = 0.708; exhalation: r = −0.047, p = 0.812) or confidence (inhalation: r = 0.183, p = 0.352; exhalation: r = −0.086, p = 0.663). The absence of correlation might arise from the inherent limitations of HCT in assessing accuracy.[48–50] In addition, while interoceptive accuracy is related to the conscious perception of heartbeats, HEP changes may also be attributed to unconscious processes.[51]

Given previous associations of HEP activity with specific self-reported interoceptive sensibility and trait mindfulness,[30–32] we examined the correlations between scores obtained on the Trusting and Not-Worrying scales of the Multidimensional Assessment of Interoceptive Awareness (MAIA) and the total score of the Five Facet Mindfulness Questionnaire (FFMQ) with HEP amplitude recorded during inhalation and exhalation during HCT. HEPs recorded during inhalation did not correlate with any of the scales (FFMQ Total Score: r = −0.01, p = 0.963, Figure 4A; MAIA Trusting: r = 0.163, p = 0.437; Figure 4B; MAIA Not-Worrying: r = −0.348, p = 0.088; Figure 4C). In contrast, HEPs recorded during exhalation exhibited a positive correlation with FFMQ total score (r = 0.407, p = 0.044, Figure 4D) and MAIA Trusting scale (r = 0.399, p = 0.048, Figure 4E), but not with MAIA Not-Worrying scale (r = 0.139, p = 0.506, Figure 4F). None of the other MAIA scales correlated with HEP values. These findings suggest that the relationship between HEP amplitude, interoceptive sensibility, and trait mindfulness is related to HEPs recorded during exhalation.

Figure 3. Respiratory cycle analyses during HCT

Circular distribution of mean angles (HEPs onsets relative to the respiratory cycle) for (A) low-amplitude HEPs (blue) and (B) high-amplitude HEPs (orange). Zero degree corresponds to inhalation onset. Each dot indicates the mean angle of one participant. Line direction indicates the mean angle across the participants. Arrow length represents the mean resultant length.

(C) The mean amplitude of HEPs as a function of six equally sized bins of the respiratory phase (from 0° to 360°). Data are presented as mean +/− standard error. Significant paired t-tests comparing inhalation and exhalation phase bins are indicated by asterisks (*p < 0.05): 60°–120° vs. 240°–300° (t_{27} = −2.995, p = 0.006, p_{FDR} = 0.027, d = 0.566); 60°–120° vs. 300°–360° (t_{27} = −2.43, p = 0.022, p_{FRD} = 0.049, d = 0.459); 120°–180° vs. 240°–300° (t_{27} = −3.063, p = 0.005, p_{FRD} = 0.027, d = 0.578); 120°–180° vs. 300°–360° (t_{27} = −2.731, p = 0.011, p_{FRD} = 0.033, d = 0.516).

DISCUSSION

We assessed HEP activity in healthy volunteers during tasks prompting either interoceptive or exteroceptive attention. Our aim was to investigate HEP and ΔHEP (respiratory phase-related HEP modulations) changes across different interoceptive and exteroceptive attention tasks. The results revealed late HEP amplitude increases during the interoceptive tasks at fronto-central electrodes, while only partially supporting the idea that HEP increases are specifically induced by cardiac interoceptive attention. Additionally, the results suggest that, despite some limitations that may influence its validity in assessing accuracy,[48–50] HCT is a suitable task to elicit cardiac interoceptive attention. Crucially, when respiratory phases were taken into account, a specific modulation of HEP amplitude induced by cardiac interoceptive attention clearly emerged. When comparing the respiratory phase-induced modulations of HEPs between the different tasks (HCT, BCT, C-TCT, and B-TCT), results revealed increased HEP positivity during exhalation compared to inhalation only during the cardiac interoceptive task (HCT). In contrast, no changes in HEP activity were observed between exhalation and inhalation during the other tasks. Circular statistics showed that in HCT low-amplitude HEPs were clustered around the middle part of inhalation and high-amplitude HEPs were concentrated around exhalation and the transition from exhalation to inhalation. We also observed a gradual increase in HEP amplitude throughout the respiratory cycle, peaking at the end of exhalation (240°–360° respiratory phase bins). Importantly, the experimental design allowed a direct comparison of HEP amplitude between inhalation and exhalation among tasks. We found that HEPs recorded during exhalation in HCT showed higher amplitude than HEPs recorded during exhalation in C-TCT and BCT. Notably, no differences were found when comparing HEPs recorded during inhalation in HCT with HEPs recorded during inhalation in C-TCT or BCT. These results extend our previous findings regarding changes in HEP amplitude between respiratory phases.[20] Firstly, they corroborate previous results using HCT in addition to HBD. Secondly, they establish that late HEP modulations related to the respiratory phase are specific to the *cardiac* interoceptive task and do not extend to *non-cardiac* interoceptive tasks (BCT). This also suggests that ΔHEP modulation is a more specific index for cardiac interoceptive attention
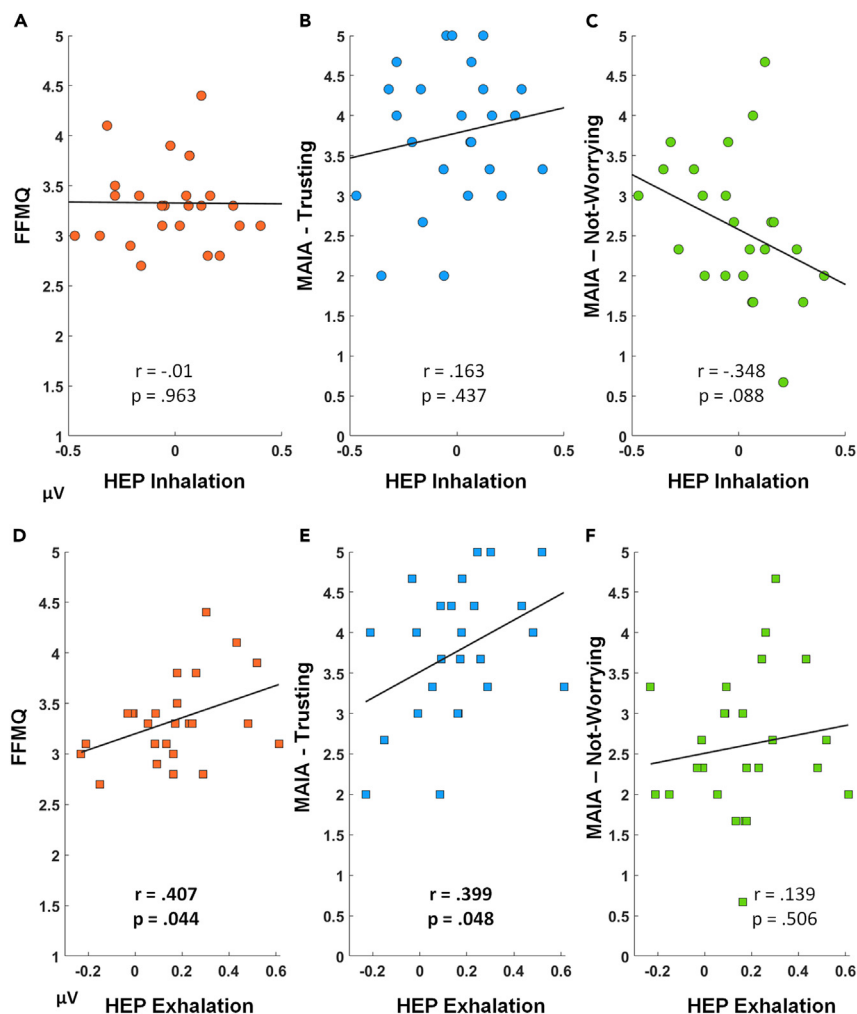
Figure 4. Correlation between questionnaires and HEPs recorded at inhalation and exhalation
Scatterplots of the linear relationships (Pearson's correlation) between the FFMQ total score, MAIA Trusting scale, and MAIA Not-Worrying scale and mean HEP amplitude recorded during inhalation (A, B, C) and exhalation (D, E, F).

than HEP amplitude alone, when respiratory phases are not taken into account. Lastly, the results indicate that, during HCT, the brain does not enhance heartbeat sensations during inhalation, but enhances heartbeat sensations exclusively during exhalation.

An additional finding is the association between HEP activity and interoceptive sensibility and trait mindfulness. During HCT, HEP amplitude recorded during exhalation was related to the trusting and not-worrying scales of MAIA and FFMQ total score. In contrast, no association was found between HEP amplitude during inhalation and these indices. This suggests that only HEPs assessed during exhalation are linked to interoceptive sensibility and mindfulness, which are important indicators of well-being and mental health.[33,52–56] These results could explain non-significant findings regarding cardiac interoceptive accuracy changes in mindfulness practitioners (see[57,58] for meta-analyses) that might be attributed to the failure to consider respiratory phases. It is possible that cardiac interoception in experienced meditators is specifically improved during exhalation. These findings may also provide an explanation for previously reported null associations between trait mindfulness and HEP,[32] a link that may be evident only during exhalation. Future studies may shed light on this topic.

We conclude that the increases in HEP amplitude during HCT are driven primarily by HEPs recorded during exhalation, with minimal contribution from HEPs during inhalation. This strengthens our previous interpretation of changes in interoception across the respiratory cycle during HBD[20] and introduces a new perspective on HEP activity that emphasizes the role of exhalation. Our results show that late HEP amplitude increases only when attention is focused on the heart *and* during exhalation. In other words, exhalation may not only serve as a respiratory phase that enhances attention to cardiac interoception compared to inhalation; it may also be a necessary condition for directing attention to heartbeat sensations during cardiac interoceptive tasks. If the first scenario were true, we would also expect to find higher HEP amplitude during inhalation in HCT compared to the other tasks (C-TCT and BCT), which was not observed. Several factors could explain

this phenomenon. A higher-level explanation is in line with a recent hypothesis regarding the role of respiration in interoceptive perception.[59] According to this hypothesis, the brain is constantly engaged in a trade-off between directing attention toward interoceptive or exteroceptive stimuli. Inhalation can hence give priority to external perception by redirecting attention to the environment, while exhalation can optimize interoception by allocating attentional resources to bodily sensations. Imbalance in the allocation of attentional resources toward exteroception during inhalation (e.g., visual,[60] somatosensory;[61] and auditory processing[62]) can disrupt the processing of interoceptive signals, particularly in the case of cardiac sensations, which are notoriously very ambiguous. This is consistent with the observed positive effects of exhalation on *non-exteroceptive* brain functions, such as associative conditioned learning[63,64] and the initiations of voluntary actions[65] and mental imagery of voluntary actions.[66] Alternatively, a peripheral explanation may lie in the connection between respiration and the baroreflex, known as respiratory sinus arrhythmia (RSA).[19] RSA causes faster but weaker heartbeats during inhalation compared to exhalation,[67] potentially resulting in reduced HEP amplitude during inhalation. The non-specific increase observed in the early time window across all tasks, irrespective of attentional focus, could be precisely attributed to this bottom-up mechanism. However, control analyses of late HEP amplitude and cardio-respiratory physiology seem to rule out the substantial contribution of these bottom-up components. Nevertheless, changes in cardiac physiology between respiratory phases do not exclude a concomitant top-down modulation of attention: heartbeats strength reduction during inhalation may enhance exteroceptive attention also due to reduced interference of cardiac signals, while exhalation may facilitate interoceptive attention also due to already increased cardiac sensations.

Regardless of the specific mechanism involved, future studies should differentiate between HEPs recorded between respiratory phases, as exhalation has demonstrated stronger associations with interoceptive attention and sensibility than inhalation. Additionally, the influence of the respiratory phase on cardiac interoception in mental and somatic disorders should be studied. Numerous studies have linked HEP amplitude to clinical conditions such as social anxiety,[68] anorexia nervosa,[69] depression,[70] post-traumatic stress disorder,[71] depersonalization,[72] borderline personality,[73] and chronic pain.[74] However, it is unclear whether these findings reflect an overall change in HEP activity or a change limited to a particular respiratory phase. An interesting exception is the study by Baumert and colleagues,[75] who reported a decrease in HEP amplitude during exhalation compared to inhalation in children with sleep-disordered breathing. Notably, after therapy (adenotonsillectomy), they observed an increase in HEP amplitude during exhalation and no changes during inhalation. This finding is in line with ours, suggesting that the effects of an intervention on cardiac interoception are more easily observed during exhalation. Consequently, the possibility that cardiac interoception in various disorders may be specifically impaired during exhalation may lead to the development of tailored interoceptive interventions to fine-tune cardiac interoception. Mindfulness-based interventions[18,76–78] or slow breathing practices[79,80] could be designed to specifically target this respiratory phase.

In conclusion, our data suggest that respiratory phases play a crucial role in influencing cardiac interoception. It is crucial for future studies to investigate the impact of respiratory phases on other aspects of interoception beyond cardiac sensations. These domains include gastric interoception,[81–83] interoceptive touch,[84–86] and pain perception.[87–90] Recognizing the role of respiration in shaping other interoceptive processes will be critical for developing a comprehensive understanding of reciprocal interactions between the body and the brain.[91–96]

Limitations of the study

Some limitations of this study deserve consideration. Firstly, HCT performance may have been affected by confounding factors, such as HR estimation and time estimation ability.[48–50] However, it is crucial to note that our primary goal was to elicit cardiac interoceptive attention rather than assess cardiac interoceptive accuracy. Secondly, potential variations in task difficulty across tasks could have impacted HEP amplitude when comparing interoceptive and exteroceptive tasks.[16] Thirdly, the inherent ease of BCT, driven by the salience of respiratory sensations, poses a challenge when comparing it with HCT, characterized by greater ambiguity in cardiac sensations, raising questions about whether BCT qualifies as a true interoceptive task. Nonetheless, the use of BCT was necessary to ensure comparability with HCT, and previous research has suggested that breath counting can indeed elicit interoceptive attention.[34,97,98] Future studies exploring this topic could consider employing alternative tasks, such as the filter detection task,[99] respiratory resistance sensitivity task,[100] respiratory tracking task,[101] or interoceptive/exteroceptive attention task.[102]

STAR★METHODS

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AUTHOR CONTRIBUTIONS

Andrea Zaccaro: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, visualization, writing-original draft, and writing-review and editing. **Francesca della Penna**: investigation, formal analysis, data curation, and writing-review and editing. **Elena Mussini**: software, data curation, and writing-review and editing. **Eleonora Parrotta**: software, data curation, and writing-review and editing. **Mauro Gianni Perrucci**: methodology, software, data curation, resources, and writing-review and editing. **Marcello Costantini**: visualization, supervision, funding acquisition, and writing-review and editing. **Francesca Ferri**: conceptualization, methodology, resources, supervision, project administration, funding acquisition, visualization, and writing-review and editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Zenodo: Analysed ERP data	This paper	https://doi.org/10.5281/zenodo.10829954
Software and algorithms		
MATLAB (R2021a)	MathWorks Inc, Natick, MA, USA	https://it.mathworks.com/products/matlab.html
EEGLAB (v2022.1)	Delorme & Makeig, 2004[103]	https://sccn.ucsd.edu/eeglab/index.php
ERPLAB (v9.00)	Lopez-Calderon & Luck, 2014[104]	https://erpinfo.org/erplab
Kubios HRV Standard (v3.5)	Tarvainen et al., 2014[105]	https://www.kubios.com/
HEPLAB	Perakakis, 2019[106]	https://pandelisperakakis.info/heplab/
Mass Univariate ERP Toolbox	Groppe et al., 2011a, b[107,108]	https://github.com/dmgroppe/Mass_Univariate_ERP_Toolbox
Factorial Mass Univariate ERP Toolbox	Fields & Kuperberg, 2020[109]	https://github.com/ericcfields/FMUT
Reduction of Electroencephalographic Artifacts toolbox (v1.1.5)	Bailey et al., 2022; Bailey et al., 2022[110,111]	https://github.com/NeilwBailey/RELAX/releases
Original Code	This paper	https://doi.org/10.5072/zenodo.37557 Full Changelog: https://github.com/azaccaro90/HEP_attention_respiration_HCT/commits/v1.0

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Andrea Zaccaro (andrea.zaccaro@unich.it; a.zaccaro90@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Raw data reported in this paper will be shared by the lead contact upon reasonable request if data privacy can be guaranteed according to the rules of the European General Data Protection Regulation (EU GDPR 2016).
- Analysed ERP data have been deposited at Zenodo and are publicly available. https://doi.org/10.5281/zenodo.10829954.
- Original code used to produce results and statistics has been deposited at GitHub and is publicly available. https://doi.org/10.5072/zenodo.37557. Full Changelog: https://github.com/azaccaro90/HEP_attention_respiration_HCT/commits/v1.0.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Ethics statement

The Institutional Review Board of Psychology of the Department of Psychological, Health and Territorial Sciences, "G. d'Annunzio" University of Chieti-Pescara, Italy, approved the study (Protocol Number 44_26_07_2021_21016). Participants were blind to the experimental aims. The study was conducted in conformity with the Italian Association of Psychology and the Declaration of Helsinki and its later amendments. All participants read and signed an informed consent.

Human participants

Thirty-four (19 females; 1 left-handed; age: 27.48 $\pm$ 4.28 years [mean $\pm$ SD]) white healthy volunteers were recruited. The eligibility of each volunteer was confirmed based on self-reported criteria: 1) absence of personal and/or family history of psychiatric, neurological, and somatic disorders; 2) no chronic and/or acute conditions involving the respiratory tracts; 3) no use of substances interacting with the central nervous

system in the previous week; 4) no experience in mindfulness-based meditation and/or breath-control practices. All participants were of normal weight and had normal or corrected-to-normal vision. Sample size was estimated using G*Power 3.1 (v3.1.9.7).[112] Considering a 2x2 factorial design, we estimated a medium effect size of partial eta squared (ηp2) of .06, based on a recent meta-analysis on attentional effects on HEP,[16] which corresponds to a Cohen's f of .25. Significance level was set to alpha = .05, and the power (1 - beta) at .85. The total estimated sample size was 26. We excluded 2 participants from the testing of HEPs, regardless of the respiratory phase, due to noisy EEG (N = 32, 17 females; 1 left-handed; age: 27.57 $\pm$ 4.33 years [mean $\pm$ SD]). Additionally, we excluded 4 participants from the testing of HEP changes among respiratory phases due to noisy respiratory data (N = 28, 13 females; 1 left-handed; age: 27.88 $\pm$ 4.46 years [mean $\pm$ SD]).

METHOD DETAILS

Experimental procedure

After a resting-state (baseline), participants performed two interoceptive tasks and two exteroceptive tasks, requiring them to focus their attention inside or outside the body, respectively (Attention Focus factor). During the interoceptive and exteroceptive tasks, participants had to process either cardiac or respiratory signals (System factor). Participants sat comfortably in front of a computer at an optimal distance (around 60 cm) for keyboard use and screen visibility. Room lighting and noise were minimized, and the temperature and humidity were maintained at a comfortable level. Participants received written instructions (see below) presented through E-Prime 3.0 software (Psychology Software Tools, Pittsburgh, PA, USA). Subsequently, the experimenter ensured participants understood the instructions, allowing them to ask questions if any doubts arose. Baseline was always performed first. Participants were seated for 10 minutes in a resting-state condition with eyes open, watching a fixation cross at the centre of a monitor, and letting their mind wander without focusing on anything in particular.[113] Participants were also asked to breathe spontaneously.

The cardiac interoceptive task was HCT.[10,13] Participants were given the following instructions in line with Desmedt et al.[49]: "*In this task, direct your attention to your heart and the associated physical sensations. You are required to sustain your focus on your heart for various durations and count the number of heartbeats you perceive. Begin silently counting when the heart symbol appears on the screen. When the heart symbol disappears, report the number of heartbeats you are sure you felt. Only report the number of heartbeats you are sure about, without trying to estimate your heart rate. During each trial, keep your eyes open, gaze at the screen and avoid moving. Refrain from guiding your responses by checking your pulse in your wrists or neck. Breathe spontaneously and avoid changing your breathing frequency or holding your breath*". HCT consisted of three blocks, each one comprising four randomized trials of the following durations: 25, 35, 45, and 100 seconds,[114–116] for a total of 12 trials. After each trial, participants reported the number of perceived heartbeats and rated their performance (HCT confidence) on a 10-points Likert scale from 0 (very bad) to 9 (excellent). No feedback was provided about their performance. The respiratory interoceptive task was BCT.[34] Participants were given the following instructions: "*In this task, direct your attention to your breath and the associated physical sensations. You are required to sustain your focus on your breath for various durations and count the number of breaths you take. Begin silently counting your breaths when the lungs symbol appears on the screen. When the lungs symbol disappears, report the number of breaths you took. Refrain from changing the rhythm or depth of your breathing, simply stay aware of your breath without making any change*". BCT consisted of five trials of 120 seconds each. After each trial, participants reported the number of performed respiratory cycles, and rated their performance (BCT confidence). They were asked to rate the ease in performing the task without modifying the respiratory rate on a Likert scale from 0 (very difficult) to 9 (very easy). No feedback was provided about their performance. The order of tasks and blocks/trials within each task was randomized.

The exteroceptive tasks mirrored the interoceptive tasks. In C-TCT, participants were presented with digitally constructed heartbeat sounds and instructed to mentally count and report the number of heard heartbeats.[35] C-TCT consisted of three blocks of four randomized trials of different durations (25, 35, 45, and 100 seconds). Heartbeats were presented at an irregular frequency at an average frequency of 60 bpm. After each trial, participants reported the number of heard heartbeats and rated their performance (C-TCT confidence). In B-TCT, participants were presented with audio samples of respiratory sounds (adapted from[34]) comprising inhalation and exhalation sounds at irregular frequencies (mean: 18 breaths per minute). B-TCT consisted of five trials of 120 seconds each. After each trial, participants reported the number of heard respiratory cycles and rated their performance (B-TCT confidence), and the perceived ease in performing the task without modifying the respiratory rate. The order of tasks and blocks/trials within each task was randomized.

The interoceptive and exteroceptive tasks were presented in a counterbalanced order between participants. Before starting the experiment, participants had to perform a brief training session and verbally confirm that they could not feel their heartbeat through the respiratory belt, in case the belt was mounted too tight.[20] To ensure a similar number of epochs, baseline and tasks lasted 10 minutes each. EEG, ECG, and respiratory signals were recorded throughout the entire experiment. The experimental design is depicted in Figure S11. All tasks were administered using the E-Prime 3.0 software (Psychology Software Tools, Pittsburgh, PA, USA).

Electrophysiological recording

EEG signals were acquired using a 64-channel BrainAmp EEG system (BrainCap MR, BrainVision LLC, Garner, NC, USA). Electrodes were positioned on the scalp following the International 10-20 system. Electrode impedance was kept below 10 kΩ for all channels. ECG was recorded from three electrodes: two placed over the left costal margin and the right clavicle, and the ground located on the right costal margin (MP160, BIOPAC Systems Inc, Goleta, CA, USA). Three additional electrodes, serving as the backup, were integrated in the EEG recording system via an external Electrode Input Box (BrainProducts GmbH, BrainVision LLC, Gilching, Germany), and were placed over the left and right clavicles, with the ground located on the left costal margin. Breathing activity was recorded via a respiratory belt placed around the chest (respiratory

transducer TSD201, BIOPAC Systems Inc, Goleta, CA, USA). The systems running the BrainAmp EEG, MP160, and TSD201 were connected with the system running the E-Prime 3.0 software and were synchronized with a TriggerStation™ (BRAINTRENDS LTD 2010, Italy). The sampling rate was 2.5 kHz for all signals. Band-pass filtering was applied from .016 to 250 Hz, along with 50-Hz notch filtering. Subsequently, all signals were down-sampled to 512 Hz.

ECG data analysis

ECG data were processed using custom MATLAB code (R2021a, MathWorks Inc, Natick, MA, USA). ECG signal was high-pass filtered at .1 Hz to remove baseline fluctuations.[117] R-peaks were detected with the Pan-Tompkins algorithm.[118] To measure Heart Rate Variability (HRV), the time series of the distance between consecutive R-peaks (tachogram) was computed and ectopic beats were corrected using a point process model.[119] This procedure corrected very few heartbeats (baseline: range 0-4 heartbeats; HCT: 0-16; BCT: 0-9; C-TCT: 0-11; B-TCT: 0-9). Kubios software (Kubios HRV Standard, v3.5)[105] was used to pre-process the tachogram and extract average HR and frequency-domain HRV features of interest. ECG pre-processing was carried out using Kubios routines. Detrending involved removing the best straight-line fit linear trend from the ECG data. The ECG tachogram was interpolated at 4 Hz. Frequency-domain analysis utilized the Fast Fourier Transform (FFT)-based Welch's periodogram applied to the detrended RR series. In the Welch's periodogram method, the window width was set to 300 seconds, with a default overlap of 50%.[105] We calculated HR (bpm, the average number of heartbeats in one minute), High Frequency (HF: .15-.4 Hz) log power (log ms2/Hz, the log-transformed power of HF band),[120,121] HRV total power (ms2/Hz, the sum of power values in HF, Low Frequency-LF, and Very Low Frequency bands), and Low Frequency/High Frequency ratio (LF/HF).

Respiratory data analysis

Respiratory data were processed using custom MATLAB scripts (R2021a, MathWorks Inc, Natick, MA, USA). The respiratory signal was first high-pass filtered at .1 Hz to remove baseline wander. Respiratory phases were detected following a validated procedure.[61,122] Outliers (i.e., values exceeding three scaled median absolute deviations from the local median within a moving 1-second window) were linearly interpolated with their neighbouring values. The data were then smoothed with a 1-second-window filter[123] and z-scored. Inhalation and exhalation onsets were identified as the local minima (trough) and the local maxima (peaks). Peaks and troughs were required to be at least 2 seconds apart and the minimum prominence of the interquartile range of the z-scored data was multiplied by a value ranging from .3 to .8, depending on the participant's respiratory trace.[61] A respiratory cycle was defined as the interval from one inhalation onset (referred to as "trough") to the subsequent trough. Respiratory cycles with outlier durations (i.e., different from two times the median) were excluded. Rejected respiratory cycles were 6 ± 5 [mean ± SD] for the baseline, 9 ± 6 for HCT, 4 ± 3 for BCT, 12 ± 6 for C-TCT, and 8 ± 7 for B-TCT. The number of retained artifact-free respiratory cycles in the entire sample was 3997 for the baseline (143 ± 37 [mean ± SD]), 3969 for HCT (142 ± 31), 4053 for BCT (145 ± 34), 4590 for C-TCT (164 ± 28), and 4440 for B-TCT (159 ± 27). Respiratory features of interest were the breathing rate (breaths/min), average inhalation duration (sec), average exhalation duration (sec), and the Inhalation/Exhalation ratio.

EEG data analysis

EEG data were pre-processed using the EEGLAB toolbox (v2022.1).[103] EEG data were filtered using a Hamming windowed FIR filter from .5 to 45 Hz.[124,125] EEG segments with poor signal quality were manually rejected. Independent Component Analysis (ICA) was applied to remove heartbeat, ocular, and muscle artefacts (FastICA).[126] ECG, respiratory channel, and excessively noisy channels were excluded from ICA decomposition. There were a few noisy channels (less than 5), always peripheral, known not to contribute to HEP generation.[16] Independent Components (ICs) were rejected based on IC time series, scalp map, and power spectrum.[103] Particular attention was given to the Cardiac Field Artifact (CFA), as it highly impacts HEP activity.[16] ICs contaminated by CFA were identified by plotting R-peaks over IC time series. ICs with activations time-locked to ECG R-peak were removed (Figure S12).[20,124,125] To facilitate the visual inspection, the cross-correlation between each IC time course and ECG channel was calculated. Removed ICs ranged from 1 to 6 (3 ± 1 [mean ± SD]), while CFA-related ICs ranged from 0 to 3 (1 ± 1 [mean ± SD]). After ICA, previously identified noisy channels were interpolated using their neighbours.[127] Pruned EEG signals were then re-referenced to the average ref.[20] as recommended.[128] A depiction of a 10-second comparison of EEG before and after ICA in the same participant is presented in Figure S13.

HEPs were computed using ERPLAB toolbox (v9.00).[104] R-peaks were identified with HEPLAB toolbox.[106] Manual correction of mis-detected peaks was performed[20,125] and EEG was epoched between -100 and 600 msec around each R-peak.[16] To account for potential voltage drifts, a regression baseline correction was employed using the Reduction of Electroencephalographic Artifacts toolbox (RELAX v1.1.5).[110,111] The regression baseline correction method calculates the trial-based average amplitude within the baseline period and regresses out the influence of the baseline period from each time point in the HEP time window. This approach avoids introducing differences from the baseline into the HEP period of interest, contrasting with the subtraction baseline correction method.[129] Epochs followed by an R-peak by less than 600 msec were excluded to avoid overlap between HEP epoch and the following R-peak.[20,130] The number of epochs excluded was generally low (20 ± 60 [mean ± SD]). The high SD is attributed to two participants with elevated HR, resulting in a higher number of rejected epochs compared to the average. Epochs were excluded if they exceeded a peak-to-peak threshold of 100 μV[20,42,131] within a moving window (size: 200 ms; overlap: 50%).[104] The number of rejected epochs exceeding this threshold was very low (4 ± 10 [mean ± SD] epochs for the baseline, 4 ± 11 for HCT, 2 ± 6 for BCT, 3 ± 9 for C-TCT, and 3 ± 10 for B-TCT). Epochs were averaged within participants during each task, resulting in four HEP bins named as follows: HCT, C-TCT, BCT, and B-TCT. HEP epochs were then assigned to inhalation and exhalation based on the

respiratory phase in which the respective R-peak occurred. This resulted in ten HEP bins named as follows: baseline inhalation and exhalation; HCT inhalation and exhalation; C-TCT inhalation and exhalation; BCT inhalation and exhalation; B-TCT inhalation and exhalation.

Task accuracy assessment

HCT accuracy was calculated following validated procedures[44,132–134] based on the number of counted heartbeats in relation to the heartbeats recorded with ECG. The formula was:

$$\text{HCT accuracy} = 1 - \frac{|(\text{Recorded heartbeats} - \text{Counted heartbeats})|}{\text{Recorded heartbeats}}$$

In the absence of excessive over-reporting, scores range from 0 to 1, where higher scores indicate better accuracy. The accuracy of each trial was then averaged over the 12 HCT trials.[44,133,134] Similarly, C-TCT accuracy was calculated as follows:

$$\text{C} - \text{TCT accuracy} = 1 - \frac{|(\text{Presented heartbeats} - \text{Counted heartbeats})|}{\text{Presented heartbeats}}$$

Accuracy for BCT and B-TCT were calculated based on the number of counted respiratory cycles in relation to performed respiratory cycles and presented respiratory sounds, respectively. Within each task, self-reported performance (confidence) was averaged among the trials.

Psychometric questionnaires

FFMQ[135] (Italian version by[136]) and MAIA[137] (Italian version by[138]) were administered via the Qualtrics online platform (Qualtrics, Provo, UT, USA). In FFMQ, participants were asked to answer to 39 statements on a 5-point Likert scale from 1 (never) to 5 (always). FFMQ measures trait mindfulness, i.e., the tendency to stay aware of perceptions, thoughts, emotions, and actions without distraction, non-judgment, and immediate reaction. We focused on FFMQ total score to correlate HEP amplitude with trait mindfulness.[32] In MAIA, participants were asked to answer to 37 statements on a 6-point Likert scale from 0 (never) to 5 (always). It consists of eight scales assessing facets of interoceptive sensibility. Given previous association with HEP activity,[30,31] we focused on the Trusting and Not-Worrying scales of MAIA. The Trusting scale assesses the experience of the body as safe and trustworthy and the Not-Worrying scale assesses emotional distress for bodily sensations of pain and/or discomfort.

QUANTIFICATION AND STATISTICAL ANALYSIS

Heartbeat evoked potential statistical analyses

HEP changes were assessed with the Mass Univariate ERP Toolbox (MUT).[107,108] To test for interaction effects in factorial designs, we followed the procedure included in the Factorial Mass Univariate ERP Toolbox (FMUT).[109] Cluster-based permutation tests were performed with HEP amplitude as dependent variable and System (cardiac vs. respiratory) and Attention Focus (interoceptive vs. exteroceptive) as within-participant factors. The cluster mass tested clusters instead of individual electrodes or time points. Firstly, spatially (neighbouring channels) and temporally (contiguous time points) adjacent points with a p-value below .05 were included into a cluster. A cluster statistic was calculated by summing t-values within each cluster. The null distribution was estimated through Monte Carlo permutations, in which condition labels were randomly shuffled 10000 times and the largest cluster-level statistic for each randomization was entered into the null distribution. Statistical significance was determined by comparing the experimentally observed cluster-level statistics with the randomly-generated null distribution. Clusters exceeding the 1 - alpha percentile of the distribution (two-tails) were significant.[109] The maximum distance for two electrodes to be included into a cluster was 5.44 cm (*chan_hood* = .61). To test for interaction effects, we used the permutation of residuals method.[139–141] For instance, to test the 2x2 interaction (System by Attention Focus) we calculated the difference between the two levels of Attention Focus for each level of System and conducted paired cluster-based permutation t-tests comparing difference values (HCT minus C-TCT vs. BCT minus B-TCT).[108] Planned t-tests were conducted using paired cluster-based permutation t-tests implemented in MUT.[107] Specifically, we planned to compare HCT vs. C-TCT, HCT vs. BCT, BCT vs. B-TCT, and C-TCT vs. B-TCT (number of planned t-tests: 4). Based on previous research,[4,20,30,36–42] we tested HEP amplitude at each electrode and time point separately in an early (100-350 msec) and a late time window (350-600 msec) after the R-peak. We excluded external electrodes (F7, F8, FT7, FT8, T7, T8, TP7, TP8, P7, P8, PO7, PO8, O1, O2, Oz, TP9, and TP10) that are susceptible to noise contamination and contribute less to HEP modulations, in line with a recent meta-analysis.[16] We then investigated HEP differences between inhalation and exhalation across tasks. We conducted a cluster-based permutation tests with HEP as dependent variable, and System (cardiac vs. respiratory), Attention Focus (interoceptive vs. exteroceptive), and Phase (inhalation vs. exhalation) as within-participant factors. The 2x2x2 interaction (System by Attention Focus by Phase) was calculated as with the formula: [(HCT Exhalation – HCT Inhalation) – (C-TCT Exhalation – C-TCT Inhalation)] vs. [(BCT Exhalation – BCT Inhalation) – (B-TCT Exhalation – B-TCT Inhalation)]. The test was performed over the significant time window and electrodes resulting from the previous analysis. Planned t-tests were HCT inhalation vs. HCT exhalation; BCT inhalation vs. BCT exhalation; C-TCT inhalation vs. C-TCT exhalation; B-TCT inhalation vs. B-TCT exhalation; HCT exhalation vs. C-TCT exhalation; HCT exhalation vs. BCT exhalation; HCT inhalation vs. C-TCT inhalation; and HCT inhalation vs. BCT inhalation (number of planned t-tests: 8).

Trial-level cardio-respiratory control analyses

We controlled for instantaneous HR influences on HEPs between HCT and C-TCT by assessing trial-level data through linear mixed-effects models on 46903 trials clustered around 32 participants.[20,142,143] We also controlled for cardiac influences on HEPs among respiratory phases at the single-trial level during HCT with a linear mixed-effects model on 17406 trials, clustered around 28 participants. Instantaneous HR for each heartbeat was based on the trial-level inter-beat interval. Trial-based ECG amplitude was calculated by averaging ECG voltage in the significant HEP time window. Single-trial HEP activity was calculated by averaging the voltage of the significant channels and time-window of observed HEP effects. Values lying outside three standard deviations from the mean were excluded. Models were implemented with the General Analyses for the Linear Model (GAMLj) module in jamovi (v2.3.21; The jamovi project, 2022), using the Residual Maximum Likelihood (REML) method. The Akaike Information Criterion (AIC) was used to determine the best fitting model. For each parameter of the best fitting model, we reported the estimated coefficient (b), standard error (SE), t-statistic, and p-value. Degrees of freedom were estimated with the Satterthwaite's method.

Respiratory cycle analyses

Circular statistics were performed to assess HEP activity over the entire respiratory cycle, spanning from one inhalation to the subsequent one. We calculated the angular value of each R-peak using the formula:[124]

$$\text{Angular value} = \left[360 * \frac{(\text{R onset} - \text{previous inhalation onset})}{(\text{subsequent inhalation onset} - \text{previous inhalation onset})} \right]$$

R-peaks angular values ranged from 0° to 360°, with 0° indicating the onset of inhalation. Single-trial angular values were converted to radians (range 0 - 2π) and the corresponding HEP values were derived from electrodes and time window showing significant changes across the respiratory cycle in the binary analysis. Outliers exceeding three standard deviations from the mean were removed. Using the interquartile range method, HEPs were categorized into low (first quartile) and high-amplitude (fourth quartile) HEPs for each participant.[124,125] For each participant, the mean angle direction was calculated for the low and high-amplitude HEPs. At the group level, we tested whether the distribution of low and high-amplitude HEPs deviated from uniform distribution using the Rayleigh test for uniformity[144] as implemented in the Circular Statistics Toolbox.[145] To assess whether the strength of the respiratory phase-locking differed between low and high-amplitude HEPs, we applied the Watson-Williams test.[146,147]

We computed HEPs amplitude based on six equally sized respiration phase bins spanning the 0°-360° interval. A one-way repeated measures ANOVA was conducted to assess changes in HEP amplitude across bins. Planned t-tests were performed between inhalation phase bins (0°-60°, 60°-120°, 120°-180°) and exhalation phase bins (180°-240°, 240°-300°, 300°-360°) (number of planned t-tests: 9).

When appropriate, we used the Benjamini and Yekutieli procedure (FDR),[148] to correct for multiple testing with an FDR threshold of p = .05. Effect sizes were estimated with Cohen's d and partial eta squared (ηp2) indices. Statistical analyses of behavioural and physiological data were conducted using repeated measures ANOVAs. Correlations between behavioural and psychometric data with HEP values were performed using Pearson's correlation. Statistical analyses were conducted in jamovi (v2.3.21; The jamovi project, 2022).