The heartbeat-evoked potential in young and older adults during attention orienting

ABREVIATED TITLE Attention orienting and the neural responses to heartbeats

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Abstract

Cardiac deceleration occurs when individuals orient their attention in anticipation of a sensory stimulus they might have to respond to (attentive anticipation). Cardiac deceleration might be important to optimize sensory processing. However, it is unclear which mechanism connects heart rate with the neuronal processing of external stimuli.

In this study, we investigated if cardiac deceleration evoked by attentive anticipation as well as ongoing fluctuations in cardiac cycle duration were associated with changes in the heartbeat-evoked potential (HEP), a cortical response evoked by the heartbeat associated with sensory sensitivity. We studied these phenomena in young and older people [N = 33 (26 women) and 29 (23 women); mean age 23 and 61 years], by analysing electroencephalograms (EEG), electrocardiograms (ECG), and pupilograms simultaneously acquired during a cued simple reaction time and a cued go/no-go task.

The HEP was measured in young and older participants with similar amplitude and topography. In the older group, higher HEP was associated with slower average reaction time. Attention orienting, associated with cardiac deceleration, was not related with significant changes in the HEP. Nevertheless, fluctuations in cardiac cycle duration (not locked with the warning cue) affected the HEP in a task and age group dependent manner. Reaction time was, however, independent from these changes.

In conclusion, HEP amplitude was associated with average reaction time in the older group, yet we found no evidence that attention orienting affected the HEP, or that, on a trial-by-trial basis, fluctuations in cardiac cycle duration were associated with sensorimotor efficiency.

Introduction

Attentive anticipation, or the preparation to respond to a sensory signal, is a cognitive state associated with cardiac deceleration (Jennings et al., 1998; Gladwin et al., 2016; Ribeiro and Castelo-Branco, 2019a; Skora et al., 2022). Warning stimuli evoke this state of attentive anticipation, leading simultaneously to cardiac deceleration, pupil dilation, and frontal cortex activation (Jennings and van der Molen, 2005). Across participants, the amplitude of this preparatory cardiac deceleration correlates negatively with average reaction time (Jennings et al., 1998; Reyes et al., 2015; Ribeiro and Castelo-Branco, 2019a). Moreover, periods of slower heart rate are associated with higher visual sensitivity (Sandman et al., 1977). These findings suggest that the strength of the cardiac deceleration response and absolute heart rate are associated with the efficiency of sensory processing, yet the link between these two processes remains to be understood.

Cardiac activity might interfere with neural processes due to an inhibitory influence of the heartbeat on the brain that hinders sensory processing (Skora et al., 2022). The neural responses to heartbeats can be measured non-invasively in humans using scalp electroencephalography (EEG) or magnetoencephalography (MEG). These responses, locked to the R or the T peaks of the electrocardiogram (ECG), form the heartbeat-evoked potential (HEP). Shifts in attention from internal bodily sensations to external sensations are associated with a decrease in HEP amplitude suggesting that weaker HEPs are associated with attention to external stimuli and more efficient sensory processing (Mai et al., 2018; Petzschner et al., 2019). We hypothesize that the mechanism that connects heart rate with sensory processing is associated with a modulation in HEP amplitude where slower heart rates are associated with reduced HEP amplitude.

Aging affects heart rate and the preparatory cardiac deceleration. Older people have slower, less variable heart rate (Umetani et al., 1998), and weaker preparatory cardiac deceleration responses (Jennings et al., 1990; Ribeiro and Castelo-Branco, 2019a). Interestingly, HEP amplitude measured during quiet rest is more positive in older people (Kamp et al., 2021). This increase in HEP amplitude with aging might reflect a stronger effect of heartbeats on neural processing, suggesting that heartbeats might have a more deleterious effect in older people. Moreover, as heart rate fluctuations are reduced in older people, the facilitating effect of cardiac deceleration might be attenuated in this population.

In this study, we investigated if preparatory cardiac deceleration and ongoing fluctuations in cardiac cycle duration [interbeat interval (IBI)] have an impact on the HEP amplitude in a way that might elucidate the mechanism through which longer cardiac cycles are associated with more efficient sensorimotor processing. As

older people show reduced heart rate fluctuations and weaker cardiac deceleration responses, we included both young and older people in our study, hypothesizing that any impact of IBI on the HEP would be weakened in older people. To explore these questions, we re-analysed a previously described dataset containing EEG, ECG and pupillography data from young and older adults, acquired during an auditory cued simple reaction time (RT) task and an auditory cued go/no-go task (Ribeiro and Castelo-Branco, 2021). In previous analyses of this dataset, we showed that while task accuracy was equivalent in both groups of participants, older people showed increased reaction time difference across tasks (Ribeiro and Castelo-Branco, 2019b). In each trial, the warning cue evoked a cardiac deceleration response that was stronger in the young group than in the older group and stronger in the go/no-go than the simple RT task (Ribeiro and Castelo-Branco, 2019a). Across-participants correlation analysis revealed that, in the young group, stronger cardiac deceleration correlated with faster reaction time suggesting an association with sensorimotor processing. Finally, task-related pupil responses were stronger in the go/no-go than the simple RT task but were equivalent across age groups. In the current study, we investigated if the cue evoked cardiac deceleration responses and ongoing fluctuations in (IBI) affected HEP amplitude and if HEP amplitude and its hypothesized association with heart rate explain sensorimotor processing.

METHODS

Participants

Thirty-six young adults and thirty-nine older adults were included in this study. Participants' characteristics were reported elsewhere (Ribeiro & Castelo-Branco, 2019a). One young and four older participants presented a high number of ectopic beats in the ECG recordings and were excluded from the analyses. In addition, two young and six older participants presented ECG recordings with poor quality, difficult to segment. These were also excluded from analyses. We included 33 young adults (mean age ± SD = 23 ± 3 years; 26 women; 3 left-handed) and 29 older adults (mean age ± SD = 61 ± 5 years; 23 women; 1 left-handed). All participants had normal hearing and normal or corrected to normal vision; had no history of neurological, psychiatric, or vascular disease; and were not taking any psychotropic medications or beta-blockers. Five older participants were taking other types of medication to control blood pressure (angiotensin receptor blockers, angiotensin-converting

enzyme inhibitors or calcium blockers). All participants were screened for dementia and scored above the cutoff in the Montreal Cognitive Assessment – MoCA (Freitas et al., 2011), and gave written informed consent (Ethics Committee of the Faculty of Medicine of the University of Coimbra, No. CE-002-2016).

Experimental design

Participants performed two auditory tasks; a cued simple reaction time task and a cued go/no-go task presented sequentially (Fig. 1). The order of presentation was pseudorandomised and counterbalanced across participants. In the simple reaction time task, trials started with the presentation of an auditory cue (1500 Hz) followed by an auditory go stimulus (1700 Hz). In the go/no-go task, the trial started with the auditory cue (1500 Hz) followed by either the auditory go stimulus (1700 Hz, 80 trials) or an auditory no-go stimulus (1300 Hz, 20 trials) (Figure 1) (Ribeiro and Castelo-Branco, 2019a, 2019b). In each task, 17 % of the trials were cue-only trials, where only the auditory cue was pseudo-randomly presented (20 trials). The interval between the cue and the target and between the target and the onset of the next trial were drawn from a nonaging distribution, $-W*\ln(P)$, where W is the mean value of the interval distribution and P is a random number between 0 and 1 (Jennings et al., 1998). The cue-target interval was $1.5-0.25*\ln(P)$ in seconds, and the interval between the target and the next trial (cue) was $5.2-1*\ln(P)$ in seconds (median = 7.6 s, min= 6.7 s, max= 19.6 s).

Each task had 120 trials, with a short break halfway through the task. All auditory stimuli were suprathreshold and were presented for 250 ms. Participants were instructed to press a button with their right index finger as soon as the go stimulus was presented, and to withhold the response in the cue-only trials and in the go/no-go task when the no-go stimulus was presented. The tasks were presented with the Psychophysics Toolbox, version 3 (Brainard, 1997) in MATLAB (The MathWorks Company Ltd).

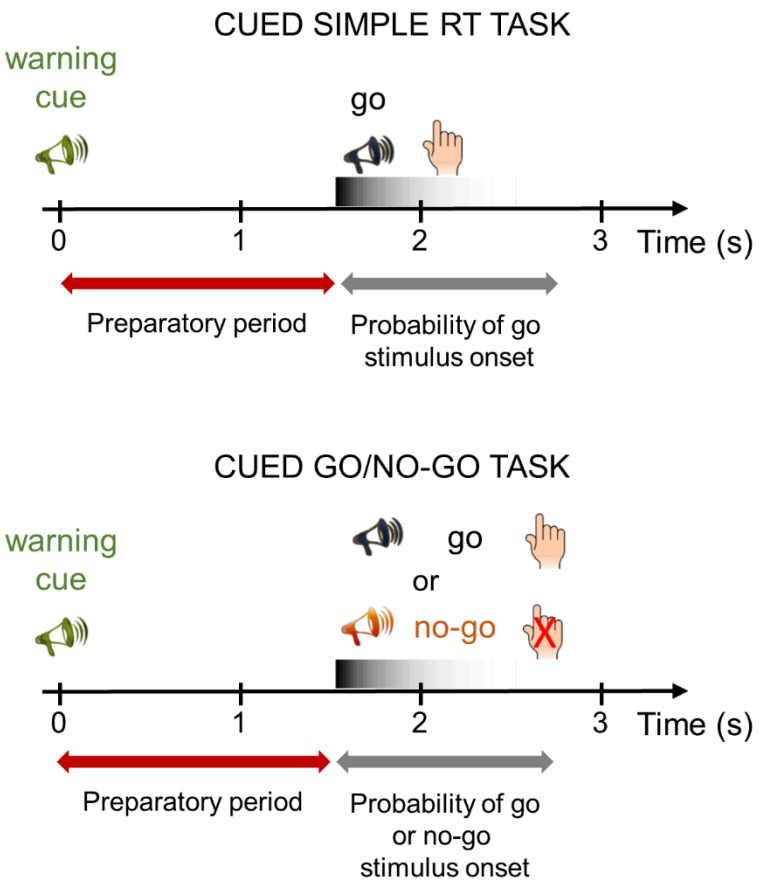

Figure 1. Behavioural task design. Participants performed two task conditions: a cued simple reaction time (RT) task and a cued go/no-go task. In the simple RT task, the warning cue presented at the beginning of the trial was followed by a go stimulus, while in the go/no-go task the warning cue was followed by a go stimulus in 67 % of the trials or a no-go stimulus in 17 % of the trials. In both task conditions, 17 % of the trials were cue-only trials, where the target was not presented after the cue. Participants were instructed to respond with their right index finger as fast as possible upon detection of the go stimulus and to withhold the response in no-go trials and cue-only trials. Image adapted from (Ribeiro and Castelo-Branco, 2022).

ECG and EEG recording and analyses

The ECG data was recorded with bipolar electrodes attached to the participants' chest (500 Hz sampling rate) and downsampled offline to 250 Hz for further processing. The R and T peaks were identified using an in-house set of ECG analysis algorithms (Henriques et al., 2015), developed by the Adaptive Computation Group of CISUC, that offers a set of functionalities for the examination and interpretation of the ECG. The analysis process comprises a set of stages, including pre-processing and segmentation, data transformation, feature extraction, modeling, and validation. Particularly for the segmentation phase, a morphological transform

approach was implemented (Sun et al., 2005), enabling the determination of the most important ECG fiducial points. These points enable the characterization of the main waves in the ECG, namely the QRS complex, the P and T waves, as well as the most relevant ECG intervals and segments.

The data was visually inspected to ensure correct peak detection and segmentation. In two participants, the in-house algorithm failed to detect the R and T peaks and instead these peaks were detected using the findpeaks.m function implemented in MATLAB.

The EEG signal was recorded using a 64-channel Neuroscan system (Compumedics EUROPE GmbH) with scalp electrodes placed according to the International 10–20 electrode system (500 Hz sampling rate). The reference was located between electrodes CPz and Cz and the ground between FPz and Fz. Eye movements and blinks were monitored using vertical and horizontal electrooculograms. Participants placed their heads in a forehead rest to allow for recording of pupillographic data (see Ribeiro and Castelo-Branco, 2019b) compromising the signal in channels FP1, FPz, and FP2, which were excluded from further analysis. Offline EEG analysis was performed with the EEGLAB (v2022.2, Delorme and Makeig, 2004) and MATLAB custom scripts. The data was digitally band-pass-filtered between 0.5-45 Hz and re-referenced to linked earlobes. Physiological artefacts such as eye blinks, saccades and cardiac activity were corrected by means of independent component analysis. Cardiac independent components which time courses correlated significantly with the ECG signal were removed. In the individual datasets where no component correlated significantly with the ECG signal, then the component that presented the highest correlation coefficient was removed.

Heartbeat-evoked potentials (HEPs)

We focused our analyses on the HEP locked with the peak from the T wave as performed in previous studies (Park et al., 2014). There were two reasons behind this choice. First, after the T wave of the ECG, during the heart relaxation period, the cardiac field artefact is minimum allowing for a better characterization of the neural response. Second, the QT interval of the ECG increases with ageing (Rabkin et al., 2016; Satpathy et al., 2017) and therefore, in the HEPs locked with the R peak, the T wave occurs at different times in the different age groups resulting in differences in the timing of the artefact associated with the T wave. Locking our analyses to the T peak allowed us to look at the HEPs of both groups independent of differences in the cardiac field artefact.

For these reasons, we focused our statistical comparisons on the time window between 51 and 399 ms after the T peak (the heart relaxation period) where the cardiac field artefact is minimal (Park and Blanke, 2019).

To compute the HEP, we segmented the data in 0.8 s segments, starting -0.2 s before the onset of the T peaks without baseline correction (number of epochs are detailed in Table 1). We chose to calculate the HEP without baseline correction because, given the cyclical nature of the HEP, any baseline period would contain activity from the previous HEP affecting its amplitude (Azzalini et al., 2019; Park and Blanke, 2019).

Table 1. Across-participants' mean ± standard deviation of number of cardiac cycles used in each of the analyses presented.

	Young group (N = 33)		Older group (N = 29)	
	Simple RT	Go/no-go	Simple RT	Go/no-go
HEP and HEP vs IBI	1104 ± 148	1093 ± 153	998 ± 128	1008 ± 117
HEP vs IBI only long IBIs	879 ± 260	841 ± 327	992 ± 119	954 ± 162
Before the cue	88 ± 13	88 ± 14	80 ± 13	81 ± 11
After the cue	88 ± 13	87 ± 13	79 ± 13	80 ± 9

To ensure that the HEP calculated represent neural activity truly locked to the participants' heartbeat, we created surrogate T peaks by adding a random number between 0 and 1 to each peak latency value (Babo-Rebelo et al., 2016).

To compare the HEP before cue onset with the HEP after cue onset, it was necessary to correct for the baseline drift associated with the preparatory potential evoked by the cue (Azzalini et al., 2019; Ribeiro and Castelo-Branco, 2019b). With this aim, we calculated the surrogate HEPs in a time window before cue onset (-1.65 s before cue onset) and a time window after cue onset (from .350 s up to 2 s after cue onset, an interval that avoided the cue and target evoked potentials). Then, we subtracted the trial-averaged surrogate HEP from the corresponding HEPs calculated in the same time window before or after cue-onset. To avoid adding noise to the HEP during the subtraction process, we first smoothed the trial averaged surrogate HEPs by filtering the data with a Gaussian window using the MATLAB function smoothdata.m with a moving window length of 150 points.

Calculation of power of EEG alpha oscillations

We calculated baseline alpha power using the FOOOF algorithm (version 1.0.0) using the MATLAB wrapper (Donoghue et al., 2020) to fit the EEG power spectral densities (PSDs) estimated via the Welch's method, as described previously (Ribeiro and Castelo-Branco, 2022). PSDs were estimated in single trials in 3.5 s long pre-cue epochs. PSDs were then averaged across trials for each electrode and fit using the FOOOF algorithm with the following settings: peak width limits = [1 8]; peak threshold = 1; aperiodic mode = 'fixed'; minimum peak height = 0.1; max number of peaks = 4; frequency range = [1 35]. Peaks with peak frequency between 7 and 14 Hz were considered within the alpha range. Average alpha power was determined as the average of the nine EEG channels with highest alpha power across both groups of participants and the two task conditions (PO3, POz, PO4, P3, P1, Pz, P2, P4, and P6).

Pupillography data acquisition and analysis

The pupil diameter of the right eye was measured by an infrared eye-tracker (iView X Hi-Speed 1,250 system from SMI) with a sampling rate of 240 Hz, as described previously (Ribeiro and Castelo-Branco, 2019b). Analysis of pupil data was performed using MATLAB custom scripts and the EEGLAB toolbox. Artefacts and blinks were corrected using the blink correction MATLAB script by Greg Siegle (stublinks.m) available in http://www.pitt.edu/~gsiegle/ (Siegle et al., 2003). Briefly, artefacts, including blinks, were identified as large changes in pupil dilation occurring too rapidly to signify actual dilation or contraction. Linear interpolations replaced artefacts throughout the data sets. Data were smoothed using a 3-point unweighted average filter applied twice. Pupil baseline values were extracted as the average pupil size in a 200 ms time window just before cue onset. Pupil epochs were visually inspected for artefacts not adequately corrected by the automatic linear interpolation procedure. Epochs with remaining artefacts were manually rejected. In addition, we discarded all error trials and correct trials that immediately followed error trials. We calculated the amplitude of the task-related pupillary responses by finding the amplitude and latency of the maximum value at the single trial level. Trial-by-trial jittering in the latency of the peaks can significantly affect amplitude measurements (Ribeiro et al., 2016). This single-trial analysis allowed for a 'jitter-free' estimation of pupil dilation amplitude thereby reflecting more faithfully the amplitude of the signal. The peak was identified automatically using MATLAB as the maximum value in cue-locked data between 0 and 6 s after cue onset. Trials with measured peak latency at the beginning or the end of the search window were excluded from analysis, as these were not

likely to be associated with a local maximum. For each participant, we then calculated the median of the amplitude measurements.

General linear modelling of EEG data

Statistical analyses were performed by analysing all time points and all EEG channels using a hierarchical linear model approach, as implemented in the LIMO EEG toolbox, an open-source MATLAB toolbox (Pernet et al., 2011). Second level analysis (group level) were run using robust trimmed means methods. Results are reported corrected for multiple testing using spatial-temporal clustering with a cluster forming threshold of p = 0.05 (Pernet et al., 2015).

To study the effect of task condition and group on the HEP amplitude, we run a first level analysis (subjects level), by setting up a general linear model with two categorical regressors corresponding to the two task conditions, and processing all subjects single trials automatically (Bellec et al., 2012). In these first level analyses, all trials for each time point and channel were modelled as the sum of a constant term and the coefficients for the two experimental conditions (simple RT and go/no-go). Parameter estimates were obtained using Ordinary Least Squares. At the second level analysis (group level), we run two analyses. First, we run a one-sample *t*-test on the trial averaged HEP amplitude to reveal where and when the HEP amplitude was significantly different from zero across all participants. Second, we run a robust repeated measures ANOVA testing the effect of task (within-subject factor) and the effect of group (between-subjects factor).

To compare the HEP amplitude before and after the cue, we modelled the data using only the second level analysis and the trial averaged HEP. This was because the correction for the baseline drift was done on the trial averaged HEP and therefore, we did not have single trial values to model at the first level. At the second level, we run a robust repeated measures ANOVA testing the effect of time (before *versus* after cue onset – within subject factor), the effect of task (simple RT *versus* go/no-go - within-subject factor) and the effect of group (between-subjects factor) on the HEP amplitude values.

To study the effect of IBI on the HEP amplitude, at the first level, a general linear model was set up with two categorical regressors corresponding to the two task conditions (isolating the effect of task) and two continuous regressors, one with the z-scored IBIs for the simple RT task and zeros in the go/no-go trials and the other with the go/no-go z-scored IBIs and zeros for the simple RT trials (Rousselet, 2011). With this design, we were able

to compare the effect of IBI on HEP amplitude across tasks. Parameter estimates were obtained using Ordinary Least Squares. At the second level (group level), we run two analyses. First, we run a one-sample *t*-test on the coefficients for the continuous IBI predictors from the first level analysis. This revealed where and when the effect of IBI on HEP amplitude was significantly different from zero for the simple RT and go/no-go task conditions. Second, we run a robust repeated measures ANOVA testing the effect of task (within-subject factor) and the effect of group (between-subjects factor) on the coefficients from the IBI predictors. Similar analyses were run testing the effect of IBI on the amplitude of the ECG measured with our bipolar derivation.

To study the association between the task difference in pupil dilation responses and the task differences on the impact of IBI on the HEP, we run, at the second level (group level), a regression analysis including as predictor the across tasks difference in pupil dilation response and as dependent variable the across task difference in regression coefficients linking IBI with HEP amplitude at each time point and each channel.

Software for statistical analyses

Repeated measures ANOVAs for heart rate, pupil data, and alpha power were run using IBM SPSS Statistics software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Other analyses were run in MATLAB (The MathWorks Company Ltd).

Results

HEP does not differ across age groups or task conditions

The HEP showed a significant positive potential in a cluster encompassing central, parietal, and occipital channels (Fig. 2). One-sample *t*-test analysis across all time points and EEG channels revealed that the HEP was significantly different from zero in one spatiotemporal cluster starting at 51 ms and ending at 399 ms after the T peak, with average amplitude of .193 ± .503 μV (mean ± across participants standard deviation; cluster *p* =.001; Fig. 2A). Scalp topographies of the HEP revealed a midcentral positive potential in both groups of participants (Fig. 2B). Repeated measures ANOVA revealed no significant group or task effect or group x task

interaction (Fig. 2C and D). Notably, randomizing the timing of the ECG events and recalculating the HEP resulted in an ERP (surrogate HEP) that was not significantly different from the baseline in any spatiotemporal cluster (Fig. 2E).

The lack of significant group differences in HEP amplitude contrasts with Kamp et al (2021) results that suggested that the HEP was stronger in older than young adults. Visual inspection of the HEP waveforms suggests that the older group presents stronger HEP around the time of the T peak, a time window where the HEP might be contaminated by the cardiac field artefact. Notably, the T peak is delayed in older people (Rabkin et al., 2016; Satpathy et al., 2017) implying differential impact of the cardiac field artefact across age groups. Visual inspection of the ECG signal locked to the R peak confirms T peak delay (Fig. 2F), while in the ECG locked to the T peak, we can observe that the T peak in older people is slightly stronger and longer lasting (Fig. 2F). Although these differences were not statistically significant in our ECG derivation, after controlling for multiple comparisons, they might explain at least in part the HEP age group differences previously observed.

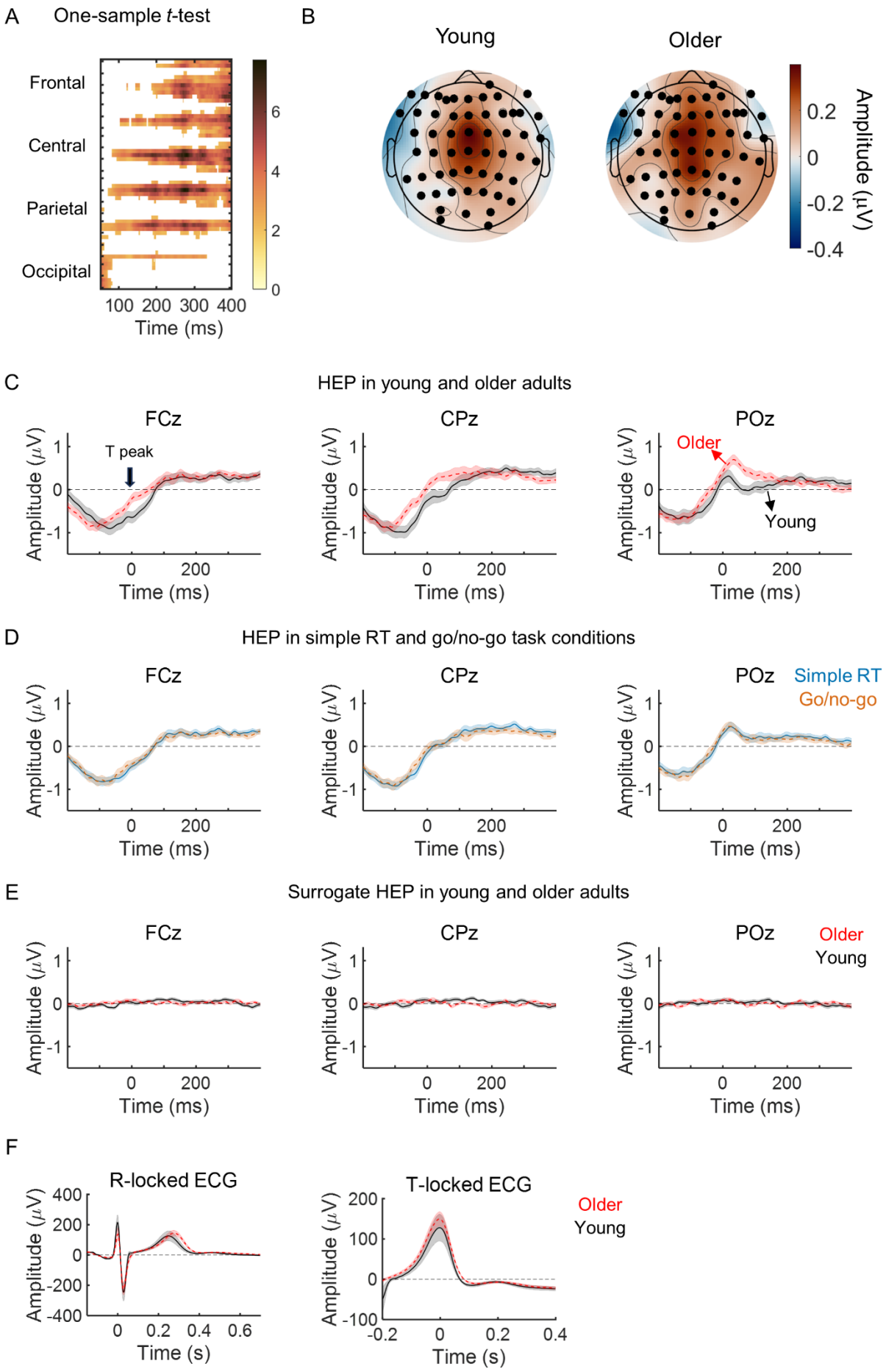
A
One-sample t-test
Frontal
Central
Parietal
Occipital
100 200 300 400
Time (ms)
6
4
2
0
B
Young
Older
0.2
0
-0.2
-0.4
Amplitude (μV)
C
HEP in young and older adults
FCz
CPz
POz
T peak
Older
Young
Amplitude (μV)
Time (ms)
D
HEP in simple RT and go/no-go task conditions
Simple RT
Go/no-go
E
Surrogate HEP in young and older adults
Older
Young
F
R-locked ECG
T-locked ECG
Time (s)

Figure 2. The HEP presented a positive potential with a midcentral topography that was not significantly different across age groups or task conditions. **(A)** One-sample *t*-test *t*-values within the spatiotemporal cluster where the amplitude of HEP was significantly different from zero, including all participants. **(B)** Scalp topographies of the grand average HEPs averaged within the time window 51-399 ms after the T peak. Black circles highlight the channels that belong to the significant spatiotemporal cluster where the HEP is significantly different from zero. **(C)** HEP in three midline channels measured in the young (black continuous line) and the older (red dashed line) groups. **(D)** HEP in three midline channels measured during the simple RT (blue continuous line) and the go/no-go (orange dashed line) task conditions, averaged across all participants. **(E)** Surrogate HEP (locked to randomized ECG events) in three midline channels for the young (black continuous line) and the older (red dashed line) groups. **(F)** ECG locked with the R peak (left) or the T peak (right) measured in the young (black continuous line) and older (red dashed line) participants. **(C-F)** The graphs plot the mean across participants ± standard error of the mean.

HEP amplitude at the end of the cardiac cycle is associated with reaction time in older people

To determine if HEP amplitude interferes with sensorimotor processing, we investigated the association between average HEP amplitude and average reaction time (averaged across task conditions) across participants using regression analyses separately for each age group. We found that reaction time was significantly associated with HEP amplitude in the older group in two spatiotemporal clusters located at the end of the cardiac cycle (cluster 1 starting at 295 ms and ending at 399 ms after the T peak, p = .001; cluster 2 starting at 351 ms and ending at 369 ms after the T peak, p = .015; Fig. 3A). In the older group, HEP amplitude was more positive in the participants responding with slower reaction times compared with the participants presenting faster reaction times (Fig. 3B). No significant clusters were observed in the young group.

HEP spatiotemporal dynamics was associated with average heart rate (Fig. 3C). Within each age group, participants with faster heart rate presented HEPs that peaked earlier and returned to baseline earlier (Fig. 3D). This effect was significant at the end of the cardiac cycle in the young group (cluster starting at 359 ms and ending at 399 ms; p = .010) where participants with slower heart rate presented HEPs with higher amplitude, and, in the older group, in two small frontal clusters (cluster 1 starts at 273 ms and ends at 287 ms, p = .046; cluster 2 starts at 219 ms ends at 235 ms, p = .030) where participants with faster heart rate showed higher HEP (Fig. 3). In contrast, the effect of heart rate on the ECG was not significant within the time window analysed, indicating that the heart rate effects on the HEP are probably independent of the cardiac field artefact. Notably, heart rate did not correlate with reaction time in either group of participants (young: r = .048, p = .791; older: r =

.128, p = .508), suggesting that across participants heart rate differences do not explain the association between HEP and reaction time observed in the older group.

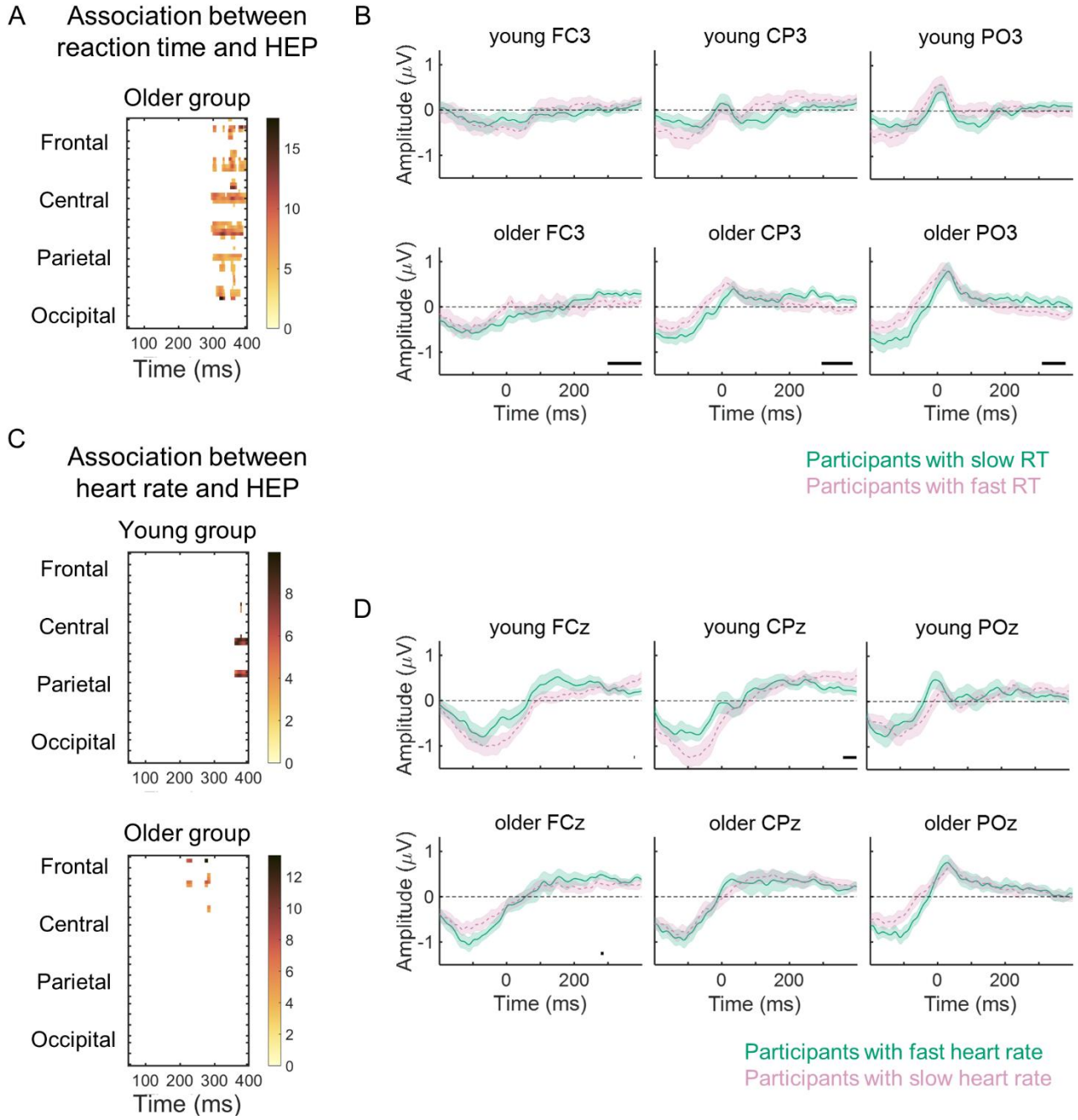

Figure 3. The HEP shows significant associations with average reaction time and average heart rate. **(A)** Regression model *F*-values within the spatiotemporal cluster where the amplitude of HEP was significantly associated with average reaction time, in the older group. **(B)** HEP in three channels measured in the young (top row) and the older (bottom row) groups. The horizontal black line shows the time windows where the HEP was significantly associated with reaction time in the older group. Green continuous line represents data from participants that responded with slow reaction times and pink dashed

line from participants that responded fast reaction times (median split within each group). **(C)** Regression model *F*-values within the spatiotemporal clusters where the amplitude of HEP was significantly associated with average heart rate, in the young group (top) and in the older group (bottom). **(D)** HEP in three midline channels measured in the young (top row) and the older (bottom row) groups. The horizontal black line shows the time windows where the HEP was significantly associated with heart rate. Green continuous line represents data from participants that present fast heart rates and the pink dashed line from participants that present slow heart rates (median split within each group). **(B** and **D)** The graphs plot the mean across participants ± standard error of the mean.

Attentive anticipation is not associated with HEP changes

Next, we investigated if attention orienting induced by the warning cue was associated with changes in the HEP in a way that might facilitate sensorimotor processing. We measured the HEP after cue onset, in a time window from .35 up to 2 s after the cue, and the HEP before the cue, in a time window with equal duration (1.65 s) placed just before cue onset (from -1.65 s up to cue onset). These time windows were chosen to minimize the event related activity time-locked to the cue and to the target. Heart rate measured in these time windows was significantly lower after the cue than before the cue [mean ± standard deviation (SD): young simple RT before cue 70.9 ± 9.7, after cue 70.2 ± 9.8; young go/no-go before cue 71.3 ± 10.6 after cue 70.6 ± 10.3; older simple RT before cue 64.5 ± 7.8, after cue 64.1 ± 7.5; older go/no-go before cue 65.5 ± 7.7, after cue 65.1 ± 7.5]. Repeated measures ANOVA of heart rate with within-subject factors time (before cue onset *versus* after cue onset) and task (simple RT *versus* go/no-go), and between-subjects factor group (young *versus* older group) showed a main effect of time [$F_{(1, 63)} = 33.2$, $p < .001$] that did not differ between tasks [task x time interaction: $F_{(1, 63)} = .369$, $p = .546$] or participant groups [group x time interaction: $F_{(1, 63)} = .963$, $p = .330$] reflecting an overall cardiac deceleration after cue presentation. In addition, there was a main effect of group reflecting the fact that heart rate was slower in the older versus the young participants [$F_{(1, 63)} = 5.41$, $p = .023$] and no overall effect of task [$F_{(1, 63)} = 3.21$, $p = .078$].

We then examined if this preparatory state induced by the warning cue led to changes in HEP amplitude. To compare HEP amplitude changes before and after the cue onset, it was necessary to correct the baseline drift observed in the EEG signal after the cue due to the slow negative preparatory potential, the contingent negative variation (CNV) (Azzalini et al., 2019; Ribeiro and Castelo-Branco, 2019b). To this aim, we first computed the HEP in the time windows of interest (Fig. 4A). It was noticeable that the HEP after the cue appeared on top of a slowly decreasing baseline. To correct for this drift, we calculated the surrogate HEP obtained before and after

the cue by randomizing the T events and therefore breaking the link between the EEG and the ECG (see Methods). Visual inspection of the surrogate HEPs calculated before and after the cue revealed that after the cue there was a slowly evolving negative potential (a baseline drift) not locked to the ECG (Fig. 4B). We used this surrogate HEP to calculate the baseline drift evoked by the cue and subtracted it from the HEP to obtain the baseline drift corrected HEP containing only the activity locked to the T peak. To avoid adding noise to the HEP, we first smoothed the surrogate HEP before subtracting it (Fig. 4C). The baseline drift corrected HEPs before and after the cue were not significantly different (Fig. 4D). Repeated measures ANOVA across all time points and EEG channels did not reveal any spatiotemporal cluster presenting a significant effect of time (before *versus* after the cue), suggesting that, at least under our task conditions, the cardiac deceleration evoked by the cue did not result in significant changes in the HEP.

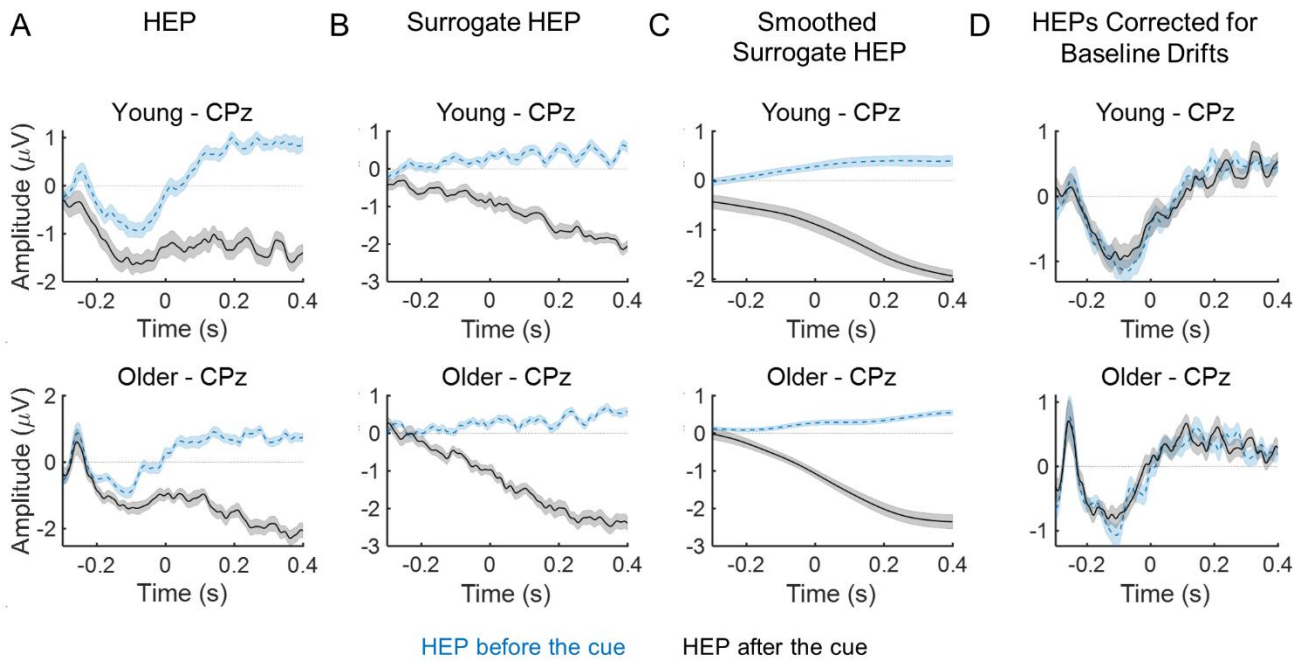

Figure 4. Heartbeat-evoked potential (HEP) measured after the cue is not significantly different from the HEP measured before the cue. **(A)** HEP before and after cue onset **(B)** Surrogate HEP before and after cue onset. **(C)** Smoothed surrogate HEP before and after cue onset. **(D)** HEP before and after cue onset corrected for baseline drift by subtracting the HEP from the smoothed surrogate HEP for each participant. **(A-D)** HEP measured at channel CPz in the young and older groups. The graphs plot the mean across within group participants ± standard error of the mean. HEP after the cue plotted in black continuous line. HEP before the cue plotted in blue dashed line.

Within-subject HEP amplitude fluctuations with IBI differ across age groups and across task conditions

Absolute IBI, as opposed to preparatory cardiac deceleration, has also been associated with the efficiency of sensorimotor processing (Sandman et al., 1977). One possibility is that ongoing fluctuations in cardiac cycle duration (IBI) might be associated with fluctuations on the neural responses to heartbeats. We investigated the effect of IBI fluctuations on the neural responses to heartbeats during the performance of the two tasks using within-subject regression analyses including IBI as a predictor and HEP as response variable. One-sample *t*-test analyses revealed that the regression coefficients were significantly different from zero in a cluster encompassing most EEG channels in a time window from 51 ms up to 175 ms after the T peak in the simple RT task condition (p = .001) and in a time window from 51 ms up to 183 ms after the T peak in the go/no-go task condition (p = .001; Fig. 5A). In both task conditions, HEP amplitude was found to be more negative in longer cardiac cycles than in shorter cycles starting before the T peak up to around 200 ms after the T peak (Fig. 5A, C and D). Interestingly, the effect of cardiac cycle duration on the HEP was also significant in a second cluster in the simple RT task from 333 up to 385 ms after the T peak that also showed negative regression coefficients (p = .028; Fig. 5A). This second cluster was not apparent in the go/no-go task condition. Repeated measures ANOVA with within-subject factor task (simple RT *versus* go/no-go task) and group as between-subject factor (young *versus* older group), performed on the regression coefficients from the IBI predictors, revealed that the coefficients were significantly more negative during the simple RT task in comparison to the go/no-go task in a cluster that starts at 335 ms and ends at 397 ms after the T peak (p = .011) (Fig. 5B). We also observed a significant group effect in a frontocentral cluster that starts at 183 ms and ends at 367 ms (p = .003), reflecting the fact that in frontal channels the regression coefficients were more negative in the young group (Fig. 5D). We found no significant group x task interaction.

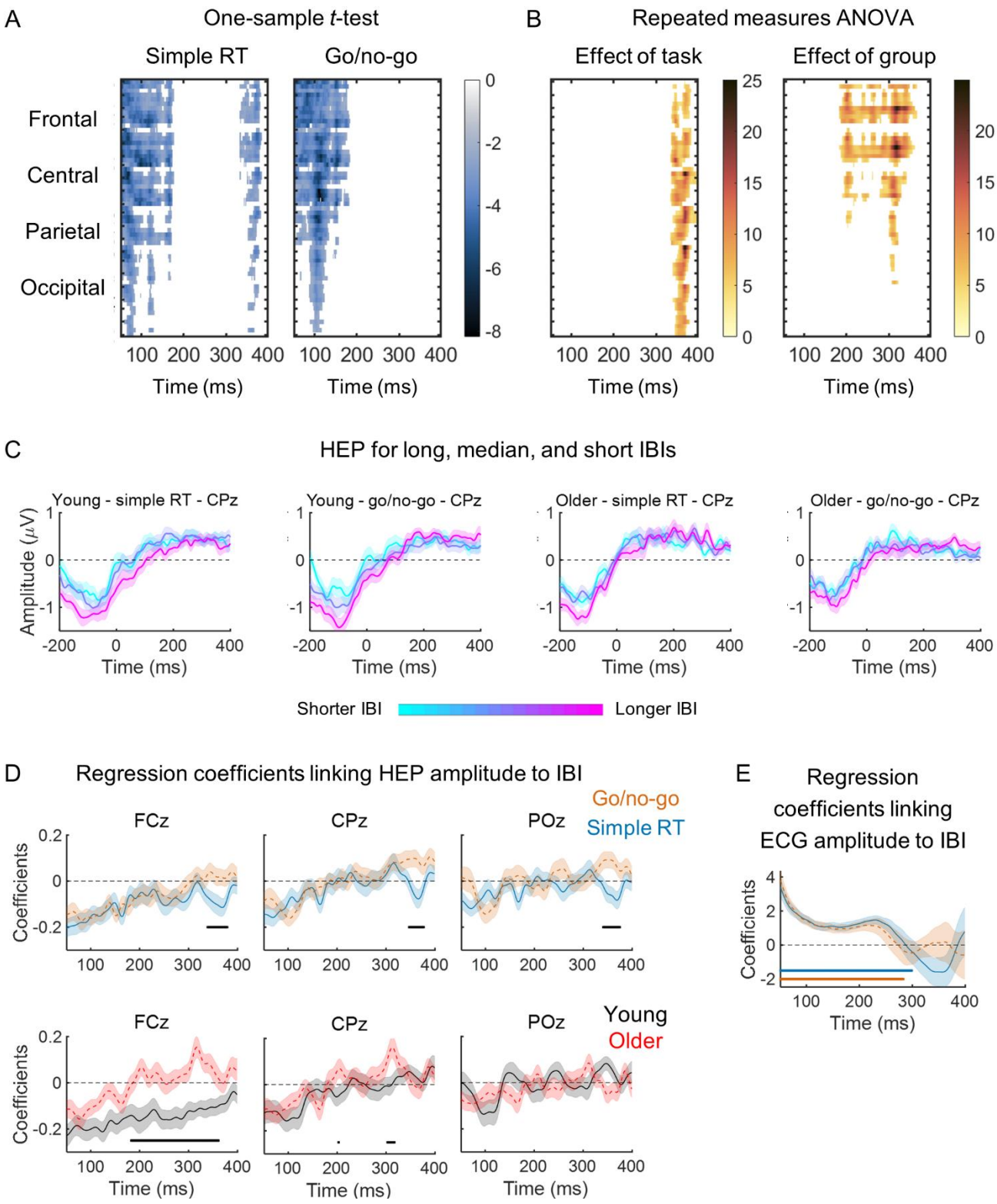

Figure 5. The amplitude of the heartbeat-evoked potential (HEP) is significantly related with cardiac cycle duration [interbeat interval (IBI)]. **(A)** One-sample *t*-test *t*-values within the spatiotemporal cluster where the amplitude of HEP presented a significant linear relation with IBI. **(B)** Repeated measures ANOVA *F*-values within the spatiotemporal cluster where the regression coefficients linking the amplitude of HEP to IBI presented a significant effect of task (left) and a significant effect of group (right). **(A)** and **(B)** EEG channels are stacked up along the y-axis. **(C)** HEP measured in channel CPz divided

within each participant in tertiles according to IBI. Longer IBIs (slower heart rate) were associated with a more negative HEP. **(D)** Top row: Time course of the regression coefficients linking HEP amplitude to IBI for the simple RT (blue continuous line) and go/no-go (orange dashed line) task conditions in the midline channels FCz, CPz and POz. The horizontal black line shows the time windows where the coefficients presented a significant effect of task condition. Bottom row: Time course of the regression coefficients linking HEP amplitude to IBI for the young (black continuous line) and older (red dashed line) groups in the midline channels FCz, CPz and POz. The horizontal black line shows the time windows where the coefficients presented a significant effect of group. **(E)** Time course of the regression coefficients linking ECG amplitude to IBI for the simple RT (blue continuous line) and go/no-go (orange dashed line) task conditions. The blue and orange horizontal lines indicate the time windows where the coefficients are significantly different from zero (significant temporal clusters from one-sample *t*-tests, $p < .001$). **(C-E)** Graphs plot the mean across all participants ± standard error of the mean.

To investigate if the observed relationship between cardiac cycle duration and HEP amplitude is a result of signal contamination by the cardiac field artefact, we mimicked the same analysis on the activity recorded on the ECG channel. We found a significant cluster where the regression coefficients were significantly different from zero starting at 51 ms and ending 299 ms after the T peak in the simple RT task and starting at 51 ms and ending 283 ms after the T peak in the go/no-go task condition ($p = .001$; Fig. 5E). Notably, we found no significant task effect, no group effect, and no group x task interaction for the coefficients obtained for the ECG data. Thus, the IBI effect at the beginning of the cardiac cycle might reflect at least in part contamination from the electrical cardiac artefact, while the differential task effect at the end of the cardiac cycle is likely to reflect changes in the neuronal responses where the HEP in response to longer IBIs is more positive in the go/no-go task than the simple RT task (Fig. 5C). Also, the observed group effect suggests that the effect of IBI on the HEP is stronger in the young group, particularly in frontal channels, and this is not reflected in differences in the ECG suggesting a neural origin.

Table 2. Heart rate parameters, pupil-linked arousal measures, and power of alpha oscillations across task conditions and group

Mean ± standard deviation

	Young		Older	
	Simple RT	Go/no-go	Simple RT	Go/no-go
Heart rate average (bpm)	71 ± 10	71 ± 11	64 ± 7.8	65 ± 7.7
Heart rate standard deviation (bpm)	4.5 ± 1.4	4.6 ± 1.6	2.6 ± .9	2.7 ± .8
Heart rate range (bpm)	28 ± 9.4	29 ± 9.4	17 ± 5.8	17 ± 4.9
Amplitude of pupil response (%)	11.2 ±3.1	13.1 ± 3.6	11.1 ± 2.9	12.3 ± 3.4
Pupil baseline (mm)	3.74 ± .61	3.75 ± .65	3.15 ± .46	3.17 ± .48
Alpha oscillations power	.95 ± .50	.94 ± .50	.61 ± .49	.63 ± .51

Repeated measures ANOVA

	Effect of task	Effect of group	Task x group interaction
Heart rate average	$F_{(1, 63)} = 1.88$; $p = .175$	**$F_{(1, 63)} = 7.45$; $p = .008$**	$F_{(1, 63)} = .860$; $p = .357$
Heart rate standard deviation	$F_{(1, 63)} = .465$; $p = .498$	**$F_{(1, 63)} = 42.9$; $p < .001$**	$F_{(1, 63)} = .162$; $p = .689$
Heart rate range	$F_{(1, 63)} = .632$; $p = .430$	**$F_{(1, 63)} = 43.9$; $p < .001$**	$F_{(1, 63)} = .373$; $p = .544$
Amplitude of pupil response	**$F_{(1, 58)} = 68.3$; $p < .001$**	$F_{(1, 58)} = .349$; $p = .557$	$F_{(1, 58)} = 3.67$; $p = .347$
Pupil baseline	$F_{(1, 58)} = .458$, $p = .501$	**$F_{(1, 58)} = 16.5$, $p < .001$**	$F_{(1, 58)} = .058$, $p = .810$
Alpha oscillations power	$F_{(1, 60)} = .047$, $p = .829$	**$F_{(1, 60)} = 6.56$, $p = .013$**	$F_{(1, 60)} = .915$, $p = .343$

It is possible that the differential effect of IBI on HEP amplitude across tasks observed at the end of the cardiac cycle, may be influenced by artefactual noise from trials with very short cardiac cycles. However, several factors make this possibility unlikely. First, we did not observe any task difference on average heart rate, heart rate standard deviation or heart rate range (Table 2). Moreover, the effect of heart rate on the ECG did not show an effect of task (Fig. 5E). Nevertheless, to discard this possibility, we run the general linear model including only cardiac cycles longer than 750 ms (Fig. 6). Under these conditions, we still observed a significant cluster in the simple RT task starting at 343 ms and ending at 381 ms after the T peak that was not observed in the go/no-go task condition, however, the effect of task was no longer significant possibly due to reduced power by the lower number of cycles included (Table 1; Fig. 6).

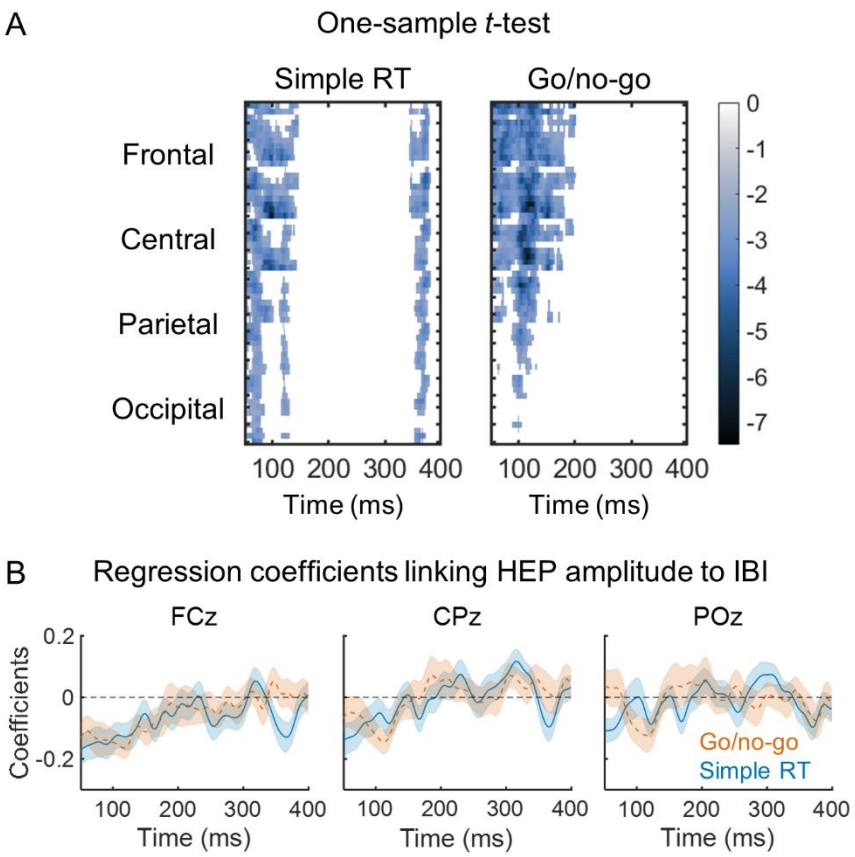

Figure 6. Association between the heartbeat-evoked potential (HEP) amplitude and the interbeat interval (IBI) after excluding cardiac cycles with short IBI (< 750 ms). **(A)** One-sample *t*-test *t*-values within the significant spatiotemporal clusters where the amplitude of the HEP presented a significant linear relation with IBI, in the simple RT and the go/no-go task conditions. EEG channels are stacked up along the y-axis. **(B)** Time course of the regression coefficients linking HEP amplitude to IBI for the simple RT (blue continuous line) and go/no-go (orange dashed line) task conditions in the midline channels FCz, CPz and POz. The graphs plot the mean across all participants ± standard error of the mean.

Differences across tasks in pupil dilation responses are related to the effect of IBI on the HEP at early stages of the cardiac cycle

Our previous analyses of the pupil-linked arousal responses in this dataset revealed that phasic pupil responses evoked by the warning cue were stronger in the go/no-go than in the simple RT task (Ribeiro and Castelo-Branco, 2019b; Table 2). Baseline pupil size (a measure of tonic arousal level) was not significantly different across tasks nor was the baseline alpha oscillations power associated with modulation of cortical excitability (Bergmann et al., 2019) (Table 2).

We investigated if the differences in the pupil-linked arousal responses across tasks were related to the differences in the effect of heart rate on the HEP. We found four significant clusters within the time window between 65 ms and 197 ms after the T peak (cluster 1 starts at 113 ms and ends at 129 ms, p = .020; cluster 2 starts at 91 ms and ends at 107 ms, p = .005; cluster 3 starts at 65 ms and ends at 89 ms, p = .001; cluster 4 starts at 175 ms and ends at 197 ms, p = .001; Fig. 7A). Participants with higher difference in the pupil responses across tasks showed more positive regression coefficients (Fig. 7B). However, as all the significant clusters were located at early stages of the cardiac cycle, this relationship does not explain the task difference observed at the end of the cycle.

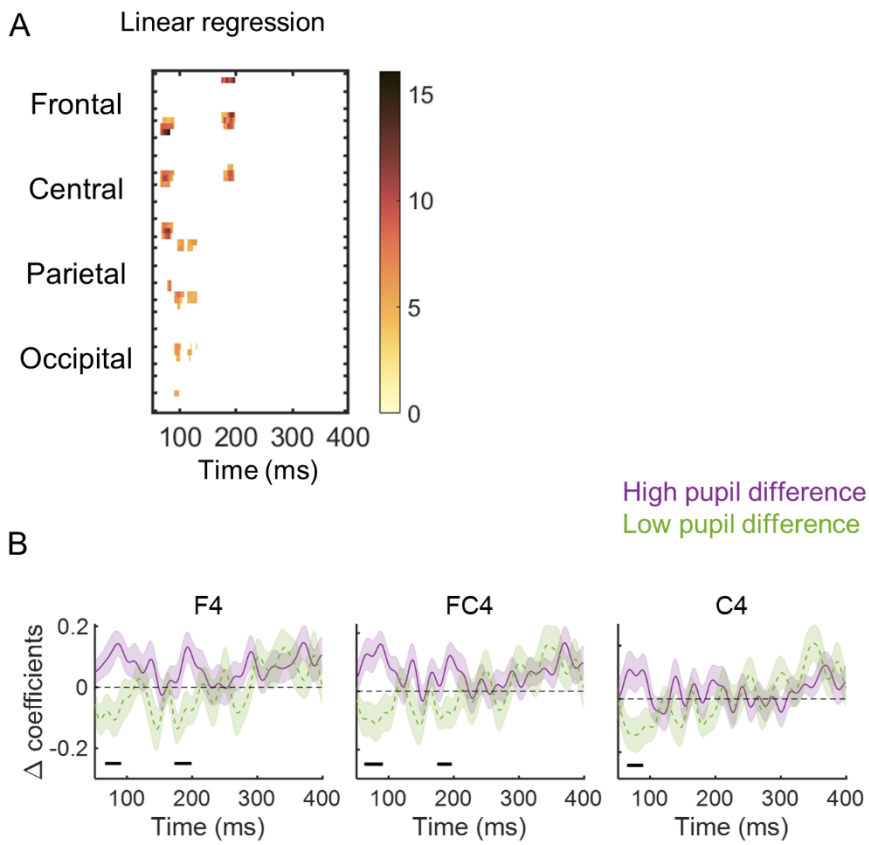

Figure 7. Across participants regression analysis revealed that differences across tasks in pupil dilation responses were related to the task differences in the effect of cardiac cycle duration (IBI) on the HEP. **(A)** Regression model *F*-values within the significant spatiotemporal clusters where the across tasks difference in the pupil response was related to the across tasks difference in the effect of IBI on the HEP. **(B)** Time course of the task difference in regression coefficients linking HEP amplitude to IBI for participants with higher across task difference in pupil dilation response (purple continuous line) and participants with lower pupil dilation difference (green dashed line). The graphs plot the mean across all participants ± standard error of the mean. The horizontal black line shows the time windows where the regression linking the across tasks difference in the pupil response to the across tasks difference in the effect of IBI on the HEP was significant.

IBI at the time of target onset does not predict reaction time

If, as we hypothesized, the HEP interferes with sensorimotor processing and, as our results suggest, HEP amplitude is affected by IBI during task performance, then IBI at the time of target onset might be associated with the efficiency of sensorimotor processing. We investigated this hypothesis using within-subject correlation analyses between IBI at the time of target onset and reaction time. At the group level, we tested the correlation coefficients against zero using one-sample *t*-tests for each task and each age group separately. The correlation coefficients were not significantly different from zero after controlling for multiple comparisons with Bonferroni correction (mean ± SD of within subject correlation coefficients: young simple RT = -0.02 ± 0.10; young go/no-go = -0.02 ± 0.17; older simple RT = 0.07 ± 0.15; older go/no-go = -0.03 ± 0.15; one-sample *t*-test: young simple RT $t_{(33)}$ = -1.30, p = .202; young go/no-go $t_{(33)}$ = -.817, p = .429; older simple RT $t_{(30)}$ = 2.41, p = .023; older go/no-go $t_{(30)}$ = -.941, p = .354).

Discussion

In this study, we investigated if in cued reaction time tasks where the warning cue induces cardiac slowing, we could also observe cue induced changes in the neural responses to heartbeats in a way that might facilitate the processing of external sensory stimuli. We studied these responses in young and older adults, as older adults present weaker cardiac deceleration responses to warning cues (Ribeiro and Castelo-Branco, 2019a). We studied the HEP locked with the T peak of the ECG, during the heart relaxation period to avoid contamination by the cardiac field artifact. We could detect the HEP in both young and older participants and found no group differences in HEP amplitude or topography. In the older group, participants with higher HEP amplitude, responded slower in the cued reaction time tasks supporting the idea that the HEP interferes with sensorimotor processing. Nevertheless, we found that the warning cues did not induce changes in the HEP during the period between the cue and the target. Exploratory analyses of the impact of fluctuations in IBI throughout the recordings (not locked to the warning cue) indicated that the HEP amplitude was affected by IBI. Interestingly, at the later stages of the cardiac cycle, the HEP was modulated by IBI in a task dependent manner. Significant group differences, particularly at frontal channels, suggest that the effect of IBI on the HEP was stronger in the young group. Nevertheless, we found no evidence of any association between reaction time and IBI at the time

of target onset in our dataset suggesting that the impact of IBI on the HEP might not significantly affect sensorimotor processing in our task conditions.

The quantification of HEP amplitude and comparison across studies is hindered by the fact that ERP amplitude is affected by the choice of reference electrode and baseline (Coll et al., 2021). Having these limitations in mind, converging evidence suggests that the HEP amplitude changes with the locus of attention, with the modulation of arousal, and with the ability to sense the heart (Coll et al., 2021). HEP amplitude is also modulated by visual stimulation (Sel et al., 2017; Gentsch et al., 2019) and performance outcome (Fouragnan et al., 2022) suggesting that different components of cognitive tasks can have an impact on the neural integration of heartbeats. Paying attention to the heart rhythm appears to increase the amplitude of the HEP particularly at the end of the cardiac cycle (Petzschner et al., 2019). This finding suggests that higher HEP amplitude is associated with processing of the heartbeats possibly in detriment of the processing of other sensory stimuli, reflecting an inhibitory influence of the heartbeat on cortical processing. Our findings further support this hypothesis. We found that, while young and older adults had comparable HEP amplitude, in older adults, participants with stronger HEP at the end of the cardiac cycle responded slower. This suggests that, particularly in older adults, HEP might interfere with sensorimotor efficiency.

Warning cues facilitate sensorimotor processing (Dietze et al., 2023) and induce cardiac deceleration (Skora et al., 2022). However, the role of the increase in IBI for sensorimotor processing is not completely understood. Here, we explored the hypothesis that HEP amplitude might be affected by ongoing fluctuations in IBI in a way that modulates the processing of external sensory stimuli. Contrary to our hypothesis, we did not find any changes in HEP during the period immediately following the warning cue. One limitation of our task design was the relatively small interval between the warning cue and the target. In fact, the peak of the cardiac deceleration occurred after target onset at a time point where we did not evaluate the HEP as it overlaps with target processing mechanisms. It is possible that given longer cue-target intervals associated with stronger cardiac deceleration responses, changes in HEP would become evident. In fact, the cardiac deceleration observed between the cue and the target was small in comparison with the ongoing fluctuations in IBI observed during the whole task recordings. As such, fluctuations in IBI throughout the task recording and not locked with the warning cue were associated with significant changes in HEP amplitude particularly at the early stages of the cardiac cycle before and immediately after the T peak. However, at these time points in the cardiac cycle, the ECG amplitude also changes with IBI, and it is possible that this effect reflects, at least in part, contamination by the cardiac field artefact that might have survived signal cleaning during preprocessing. Later in the cardiac

cycle during the quiet ECG period where we did not observe any effect of IBI on the ECG, we also observed a significant relationship between HEP amplitude and IBI but only in the cued simple RT task and not in the cued go/no-go task. The two tasks did not differ in cardiac parameters and together with the fact that the ECG does not depend on IBI at these later stages of the cardiac cycle suggest that this effect is independent of the cardiac field artefact. However, we measured the ECG in a single bipolar derivation, and it is possible that ECG signals from different derivations will show a dependence on IBI at different time intervals (Buot et al., 2021).

Although these findings suggest that the HEP might be affected by IBI in a task dependent manner, we found no evidence that IBI at the time of target onset affects stimulus processing under our task conditions. One reason for this lack of evidence might be because we used suprathreshold auditory stimuli, while previous studies that found an effect of IBI or HEP amplitude on sensory processing used stimuli at the threshold of detection and assessed detection threshold rather than reaction time (Sandman et al., 1977; Park et al., 2014; Al et al., 2020).

The observation that under different task conditions the HEP responds differently to changes in IBI, suggests that each task is associated with a different neuronal gain modulating the input-output function. Besides not differing in cardiac parameters, the two task conditions also did not differ in the amplitude of alpha oscillations (a measure of neural excitability), or baseline pupil size. The conditions did differ in the amplitude of task-related pupil responses, a measure of mental effort (van der Wel and van Steenbergen, 2018) and an indirect measure of activity in the ascending neuromodulatory pathways of the brainstem, like noradrenaline and acetylcholine, which are associated with variations in arousal and changes in neuronal gain in cortical areas (Mineault et al., 2016; Reimer et al., 2016; McCormick et al., 2020). However, the task differences in the pupil dilation responses were not related to differences in the association between HEP and IBI at the later stages of the cardiac cycle where we observed a task effect. Thus, although differences in cognitive state might affect the association between IBI and HEP further studies are needed to confirm these findings and understand their impact.

Although, fluctuations in IBI might influence the HEP as our findings suggest, the cardiac deceleration observed immediately after the warning cue was not associated with changes in HEP or with the efficiency of sensorimotor processing. Does this cardiac modulation play a role in sensory processing? In our previous study, we observed an across-participants correlation that suggested that individuals with stronger cardiac deceleration on average also responded faster (Ribeiro and Castelo-Branco, 2019a). However, the lack of trial-by-trial correlation

indicates that the brain mechanism that induces the cardiac deceleration is associated with sensorimotor efficiency but the slowdown of the heart itself might not directly affect sensorimotor processing.

At the time of the T peak, older adults presented a stronger HEP than young adults as had been observed before by Kamp et al. (2021). This difference, however, is not significant during the later stages of the cardiac cycle when the cardiac field artefact is minimal. Comparison of the ECG amplitude across age groups suggested that the HEP differences observed previously might have been confounded by differences in the electrical cardiac output and that the neural responses to the heartbeat might not change significantly with aging.

In conclusion, in older adults, the HEP is associated with sensorimotor processing, with older participants that present higher HEP amplitude responding slower in the cued reaction time tasks. This finding supports the hypothesis that the HEP interferes with external stimulus processing. However, we also found that the attentive anticipation state induced by warning cues and associated with cardiac deceleration is not associated with changes in HEP amplitude. Thus, the facilitation of sensorimotor processing by warning cues, while involving cardiac deceleration, does not involve changes in HEP. However, ongoing fluctuations in cardiac cycle duration (not locked with the warning cue) affect the HEP amplitude in a task and age group dependent manner suggesting an effect of cognitive state.

Data and code availability

The dataset used in this study is available in Open Neuro Repository under the https://doi.org/10.18112/openneuro.ds003690.v1.0.0. All analyses and figures presented in the manuscript resulted from analyses of this dataset. MATLAB code for data analyses is available on GitHub on the following link

https://github.com/mariajribeiro/2024_Ribeiro_Effect_of_heart_rate_on_heartbeat-evoked_potential.

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Conflict of interest

The authors declare no competing financial interests.

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