The cortical heartbeat-evoked potential is modulated by heart rate in a task and mental effort dependent manner

ABREVIATED TITLE Heart rate and the neural responses to heartbeats

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Conflict of interest

The authors declare no competing financial interests.

Abstract

Periods of slow heart rate are associated with increased sensory sensitivity. Accordingly, 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 heart rate were associated with changes in the heartbeat-evoked potential (HEP), a cortical response evoked by the heartbeat which amplitude modulations are 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], using a previously described dataset including electroencephalograms (EEG), electrocardiograms (ECG), and pupilograms which were acquired during an auditory cued simple reaction time task, an auditory cued go/no-go task, and a passive task condition.

While the period of attentive anticipation between the cue and the target characterized by cardiac deceleration was not related with significant changes in the HEP, ongoing heart rate fluctuations affected HEP amplitude. Interestingly, the effect of heart rate on the HEP increased with task difficulty and was associated with the amplitude of task-related pupil responses suggesting a link with mental effort.

Thus, the impact of ongoing heart rate fluctuations on the HEP depends on cognitive state and this effect might link heart rate with the neural processing of external stimuli.

SIGNIFICANCE STATEMENT

These findings suggest that arousal level and mental effort modulate the input-output function that links heart rate to the strength of the cortical responses to the heartbeat. As such, the modulation of the heartbeat-evoked potential by heart rate emerges as a marker of cortical gain that is relatively easy to measure non-invasively in humans. Further studies should explore if this effect of mental effort extends to other sensory modalities, for example, the visual cortex neuronal gain. Moreover, this study suggests that the effect of heart rate on the neuronal processing of external stimuli is stronger during periods of high mental effort, raising a new hypothesis which exploration will further elucidate the connection between the heart and cognitive processing.

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 heart rate fluctuations have an impact on the HEP amplitude in a way that might elucidate the mechanism through which slower heart rates 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 changes in heart rate 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, an auditory cued go/no-go task, and a passive task condition (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 heart rate affected HEP amplitude and if HEP amplitude and its hypothesized association with heart rate explain sensorimotor processing.

Materials and 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

The experiment started with a passive task where the participants were fixating on a white cross in the centre of the computer screen and listened to pure tones (250 ms at 1500 Hz, 30 repetitions) presented at random intervals (median of 7.6 s) during a 4-minute period. The frequency of presentation of the pure tones in the passive task was identical to the frequency of presentation of the cue stimuli in the following tasks. Thereafter, 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).

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. First, the data was digitally band-pass-filtered between 0.1-45 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 were identified and removed as the components which time courses correlated significantly with the ECG signal or, alternatively, the component that presented the highest correlation coefficient.

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 electrical cardiac 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 wave allowed us to look at the heartbeat evoked responses of both groups independent of differences in the electrical cardiac artefact. We focused our statistical comparisons on the time window between 51 and 400 ms after the T wave (the heart relaxation period).

To compute the HEP, we used a baseline placed before the R peak and epoched the data locked to the T peak, as follows. First, we segmented the EEG signal into 1.65 s epochs, starting 0.15 s before the R peak, and baseline corrected by subtracting from each epoch the average amplitude computed between -0.15 and -0.5 s. We then re-segmented the data in 0.8 s segments, starting -0.2 s before the onset of the T peaks (number of epochs are detailed in Table 1).

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 three tasks	293 ± 42	293 ± 42	257 ± 42	257 ± 42
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. We first shifted the onset of the original R peaks creating a surrogate R peak by adding a random number between 0 and 1 to each peak latency value (Babo-Rebelo et al., 2016). Surrogate T peaks were then created by adding to the surrogate R peaks the average RT interval computed for each participant.

We subsequently applied the same criteria for calculating the HEP time-locked to the T peak as previously described.

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 smoothed the trial-averaged surrogate HEP and subtracted it from the corresponding HEPs calculated in the same time windows before or after cue-onset. To avoid adding noise to the HEP during the subtraction process, we first smoothed the trial averaged random HEPs by filtering the data with a Gaussian window using the MATLAB function smoothdata.m with a moving window length of 150 points.

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).

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 using robust trimmed means methods. 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 including the passive task condition including three categorical and three continuous predictors at the first level, and testing the effect of IBI on the amplitude of the ECG measured with our bipolar derivation.

Results are reported corrected for multiple testing using spatial-temporal clustering with a cluster forming threshold of $p = 0.05$ (Pernet et al., 2015).

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.

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 version R2021a (The MathWorks Company Ltd).

Results

HEP is stronger in older people

To describe the HEP evoked in our dataset and its dependence on age group and task demands, we calculated the T-locked HEP averaged across all the cardiac cycles in the recordings for each of the two active task conditions: simple RT and go/no-go (Fig. 1). We included in our HEP analyses all time points and EEG channels and corrected for multiple testing using spatial-temporal clustering.

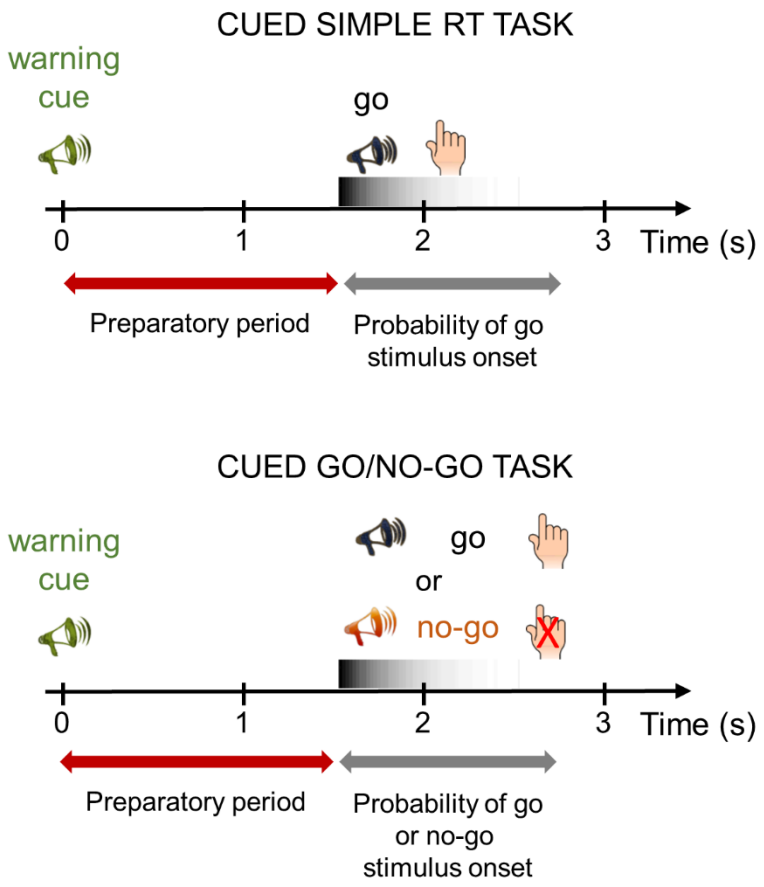

Figure 1. Behavioural task design. Participants performed two task conditions: a cued simple reaction time (RT) task and a cued go/no-go task. In the simple RT task, the warning cue presented at the beginning of the trial was followed by a go stimulus, while in the go/no-go task the warning cue was followed 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).

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 the baseline in one spatiotemporal cluster starting at 51 ms and ending at 329 ms after the T peak, with average amplitude of .312 μV (p = .024; Fig. 2A). In older adults, the HEP was significantly more positive than in young adults. Repeated measures ANOVA revealed a spatiotemporal cluster where the effect of group was significant, encompassing central, parietal, and occipital channels and spanning the time window from 51 up to 175 ms after the T peak with an average amplitude difference across groups of .531 μV (p = .048; Fig. 2B-D). Scalp topographies of the HEP revealed a midcentral positive potential with more positive amplitude in older people (Fig. 2C). There was no significant task effect (Fig. 2E). 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. 2F).

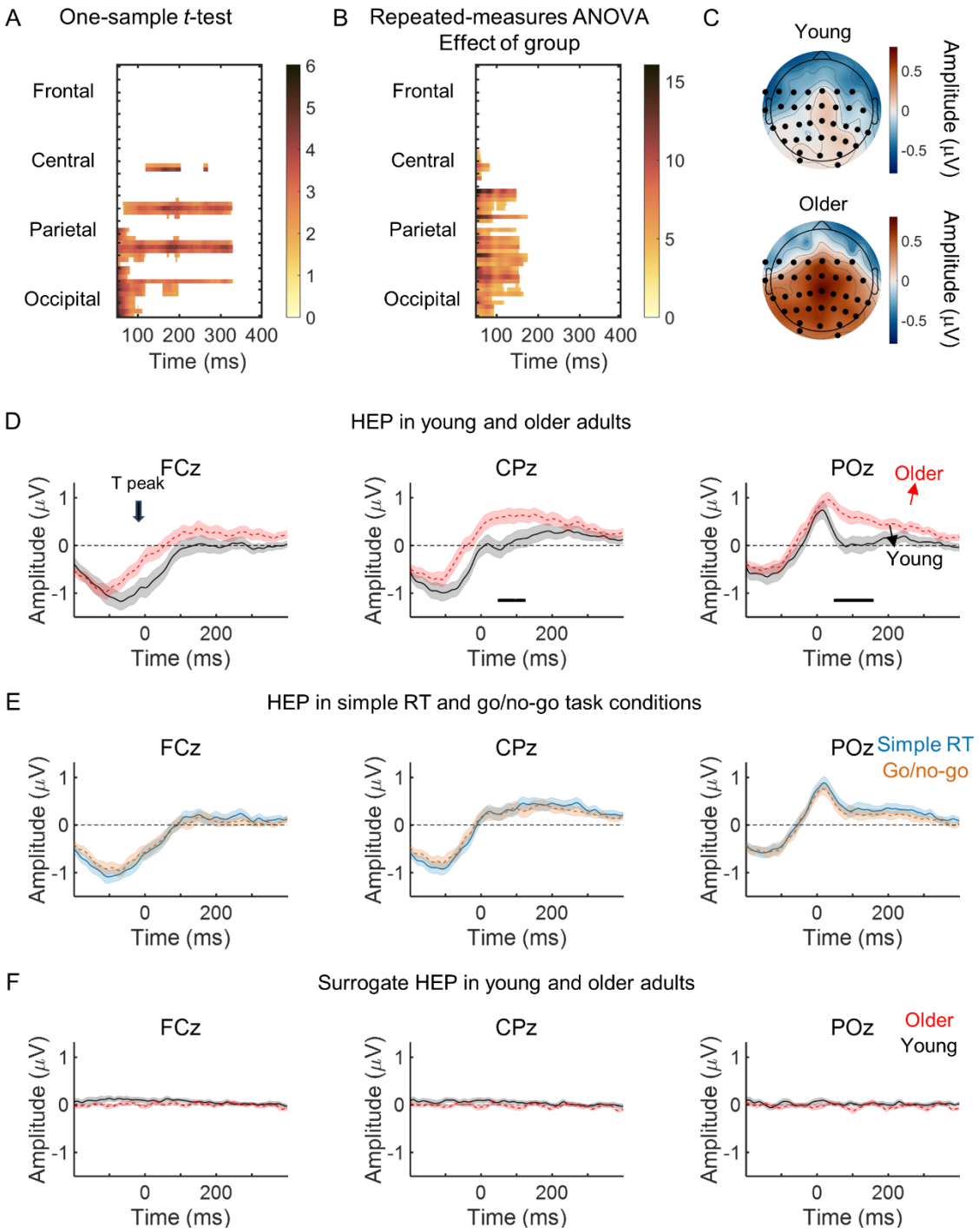

Figure 2. The HEP presented a positive potential with a midcentral topography that was significantly more positive in older adults than in young adults. **(A)** One-sample *t*-test *t*-values within the spatiotemporal cluster where the amplitude of HEP was significantly different from zero, including all participants. **(B)** Repeated measures ANOVA *F*-values within a spatiotemporal cluster where the amplitude of HEP showed a significant effect of group. **(A-B)** EEG channels are stacked up along the y-axis. **(C)** Scalp topographies of the grand average HEPs averaged within the time window 50-150 ms after the T peak. Black circles highlight the channels that belong to the significant spatiotemporal cluster presenting a group effect. **(D)** HEP in three midline channels measured in the young (black continuous line) and the older (red dashed line) groups.

The horizontal black line shows the time windows where the HEP was significantly different across groups. **(E)** 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. **(F)** Surrogate HEP (locked to randomized ECG events) in three midline channels for the young (black continuous line) and the older (red dashed line) groups. **(D-F)** The graphs plot the mean across participants ± standard error of the mean.

Attentive anticipation is not associated with HEP changes

To determine if the cardiac deceleration evoked by the warning cue was associated with changes in the HEP, 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 and after cue onset) and task (simple RT, go/no-go), and between-subjects factor group (young and 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 anticipatory cardiac deceleration 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. 3A). 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 Materials and Methods). When we compared the surrogate HEPs calculated before and after the cue, it was clear that after

the cue there was a slowly evolving negative potential (a baseline drift) not locked to the ECG (Fig. 3B). 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. 3C). The baseline drift corrected HEP before and after the cue were not significantly different as determined by repeated measures ANOVA across all time points and EEG channels (Fig. 3D). Repeated measures ANOVA 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.

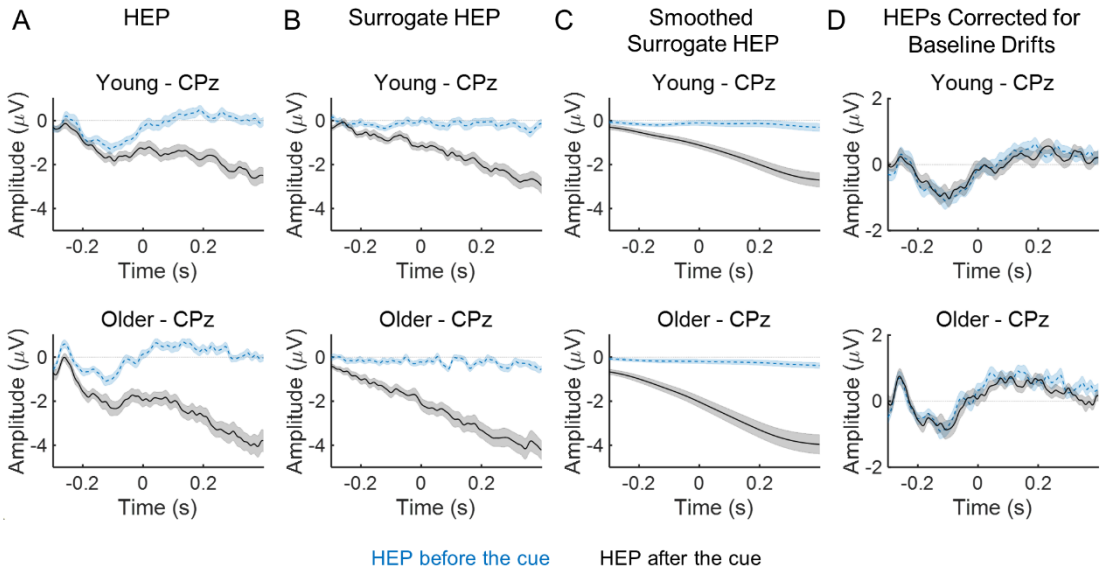

Figure 3. Heartbeat-evoked potential (HEP) measured after the cue is not significantly different from the HEP measured before the cue. **(A)** HEP before and after cue onset **(B)** Surrogate HEP before and after cue onset. **(C)** Smoothed surrogate HEP before and after cue onset. **(D)** HEP before 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.

HEP amplitude changes with heart rate and this effect is stronger in the go/no-go than in the simple RT task

As absolute heart rate has also been associated with the efficiency of sensorimotor processing (Sandman et al., 1977), we investigated the effect of ongoing heart rate fluctuations on the neural responses to heartbeats during the performance of the two tasks using within-subject regression analyses including the length of the cardiac cycle [interbeat interval (IBI)] as a predictor. One-sample *t*-test analyses revealed that the regression coefficient was significantly different from zero over occipital and parietal channels in a time window from 51 ms up to 400 ms after the T peak ($p < 0.001$) (Fig. 4A). In both task conditions, HEP amplitude was found to be more positive in longer cardiac cycles (slower heart rates) than in shorter cycles (faster heart rates). This relationship was stronger at the end of the cardiac cycle (Fig. 4A, C and D). Interestingly, the effect of heart rate on the HEP was stronger in the go/no-go task than in the simple RT task. A 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 heart rate predictors, revealed that the coefficients were significantly stronger during the go/no-go task in comparison to the simple RT task between 319 ms and 399 ms after the T peak ($p = .014$) (Fig. 4B). We found no significant group effect or group x task interaction, suggesting a similar effect of heart rate on HEP amplitude and morphology across both age groups.

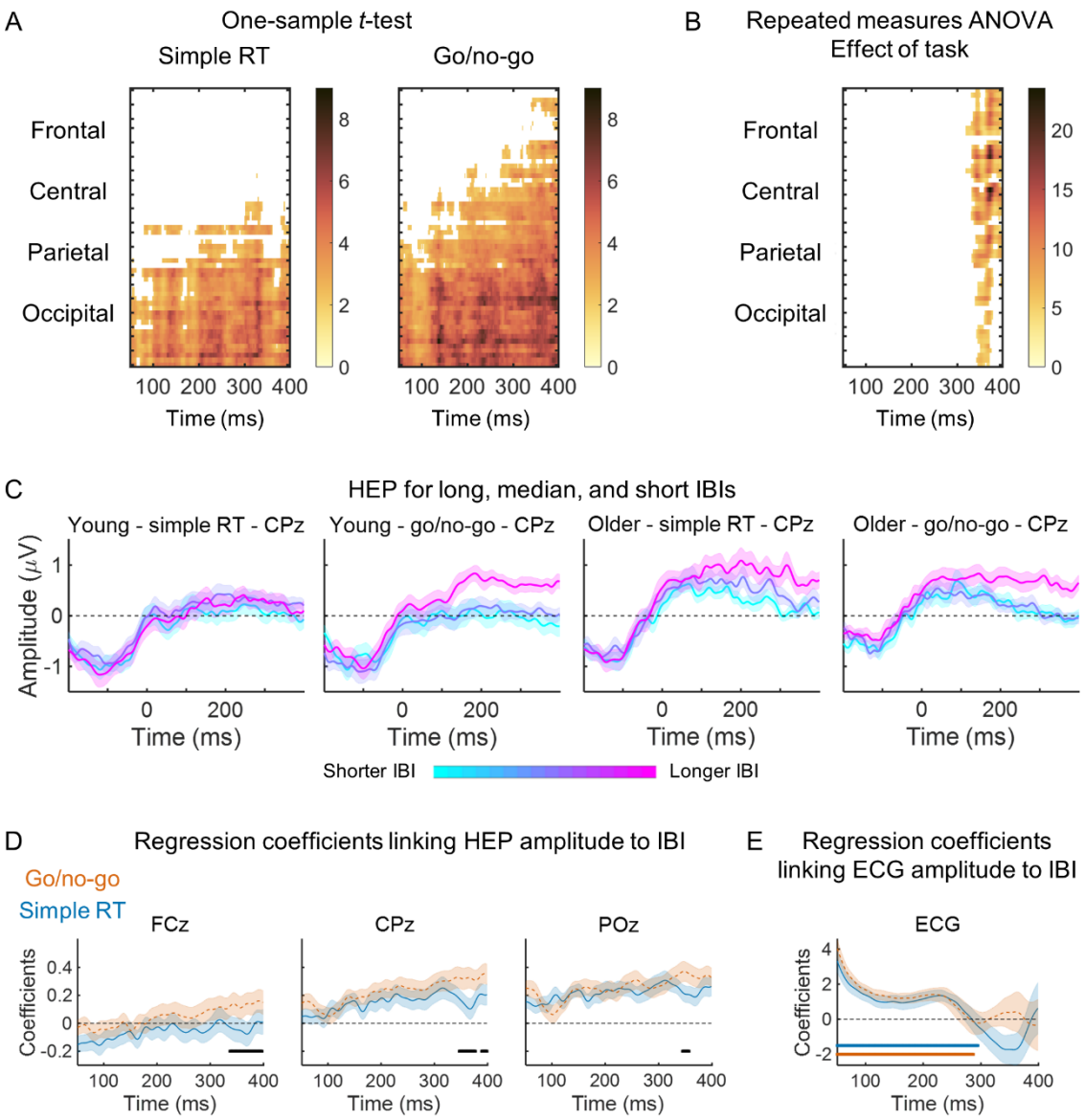

Figure 4. The amplitude of the heartbeat-evoked potential (HEP) is significantly related with heart rate [interbeat interval (IBI)]. **(A)** One-sample *t*-test *t*-values within the spatiotemporal cluster where the amplitude of HEP presented a significant linear relation with IBI. **(B)** Repeated measures ANOVA *F*-values within the spatiotemporal cluster where the regression coefficients linking the amplitude of HEP to IBI presented a significant effect of task. **(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 positive HEP. **(D)** 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. **(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)** The graphs plot the mean across all participants ± standard error of the mean.

To rule out that the observed relationship between heart rate and HEP amplitude is not a result of signal contamination by the electrical cardiac artefact, we mimicked the same analysis on the activity recorded on the ECG channel. We found that the regression coefficients were significantly different from zero in a time window from 51 ms to 300 ms after the T peak ($p < .001$; Fig. 4E). It is important to note that the coefficients emerging from the analysis performed in the ECG channel were stronger at the beginning of the cardiac cycle and weaker at the end of the cardiac cycle, while for the HEP amplitude the opposite pattern emerged, i.e. the coefficients increased towards the end of the cardiac cycle. This pattern of results suggests that the enhanced HEP amplitude found in trials with slower heart rate cannot be explained by peripheral physiological changes associated with changes in heart rate. Notably, we found no significant task effect, no group effect, and no group x task interaction for the coefficients obtained for the ECG data.

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 stronger effect of heart rate on HEP amplitude between 319 ms and 399 ms after the T peak amplitude in the go/no-go task in comparison to the simple RT task, 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. 4E). Nevertheless, to discard this possibility, we run the general linear model including only cardiac cycles longer than 750 ms (Fig. 5) and found that the effect of task was still present at the end of the cardiac cycle (time window between 335 ms and 400 ms after the T peak in a single spatiotemporal cluster with a p = .083 corrected for multiple comparisons using a threshold of p = .1; Fig. 5B).

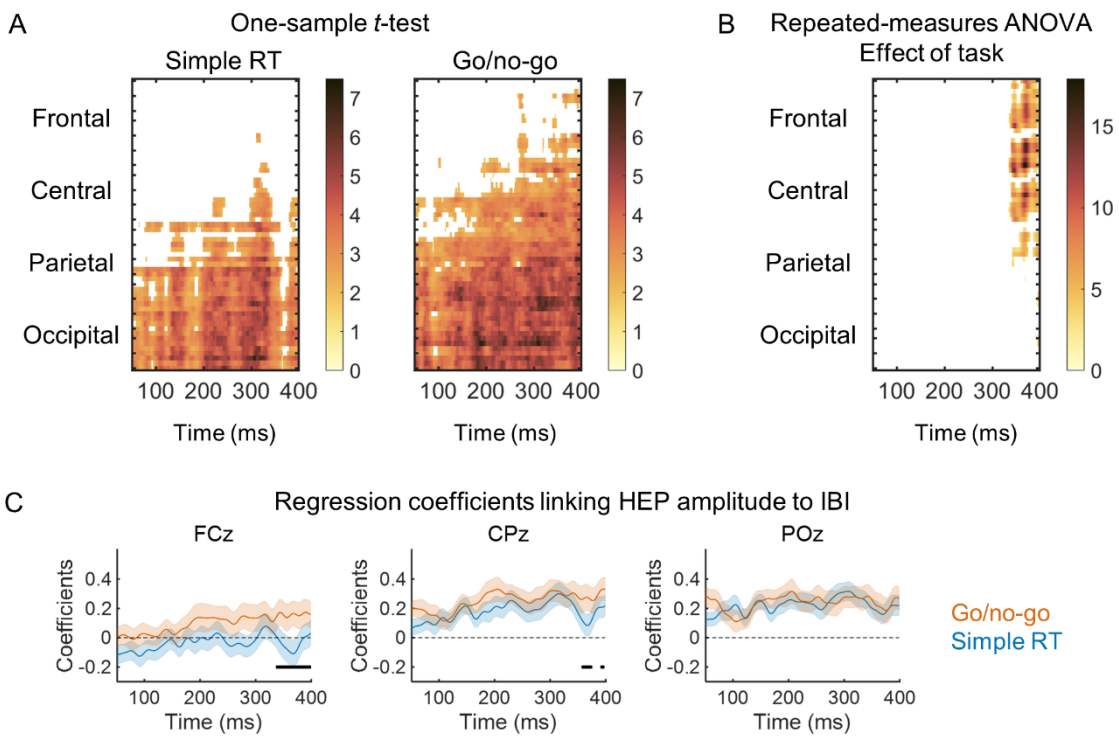

Figure 5. The amplitude of the heartbeat-evoked potential (HEP) is still significantly related with the interbeat interval (IBI) after excluding cardiac cycles with short IBI (< 750 ms). **(A)** One-sample *t*-test *t*-values within the significant spatiotemporal clusters where the amplitude of the HEP presented a significant linear relation with IBI, in the simple RT and the go/no-go task conditions. **(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. **(A)** and **(B)** Correction for multiple comparisons was obtained by spatiotemporal clustering with a cluster forming threshold of p = .05 **(A)** or p = .1 **(B)**. EEG channels are stacked up along the y-axis. **(C)** Time course of the regression coefficients linking HEP amplitude to IBI for the simple RT and go/no-go task conditions in the midline channels FCz, CPz and POz. The graphs plot the mean across all participants ± standard error of the mean. The horizontal black line shows the time windows where the coefficients presented a significant effect of task with a cluster forming threshold of p = .1.

Differences across tasks in pupil dilation responses are related to the task differences in the effect of heart rate on the HEP

The observation that under different task conditions the HEP responds differently to changes in heart rate, suggests that each task is associated with a different neuronal gain modulating the input-output function. Neuromodulators, like noradrenaline and acetylcholine, 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). With this in mind, we investigated if task differences in the engagement of the pupil-linked arousal systems might explain the task differences in the response of the HEP to heart rate fluctuations.

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).

Next, 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 that participants with higher difference in the pupil responses across tasks showed higher difference in the effect of heart rate on the HEP across tasks (Fig. 6).

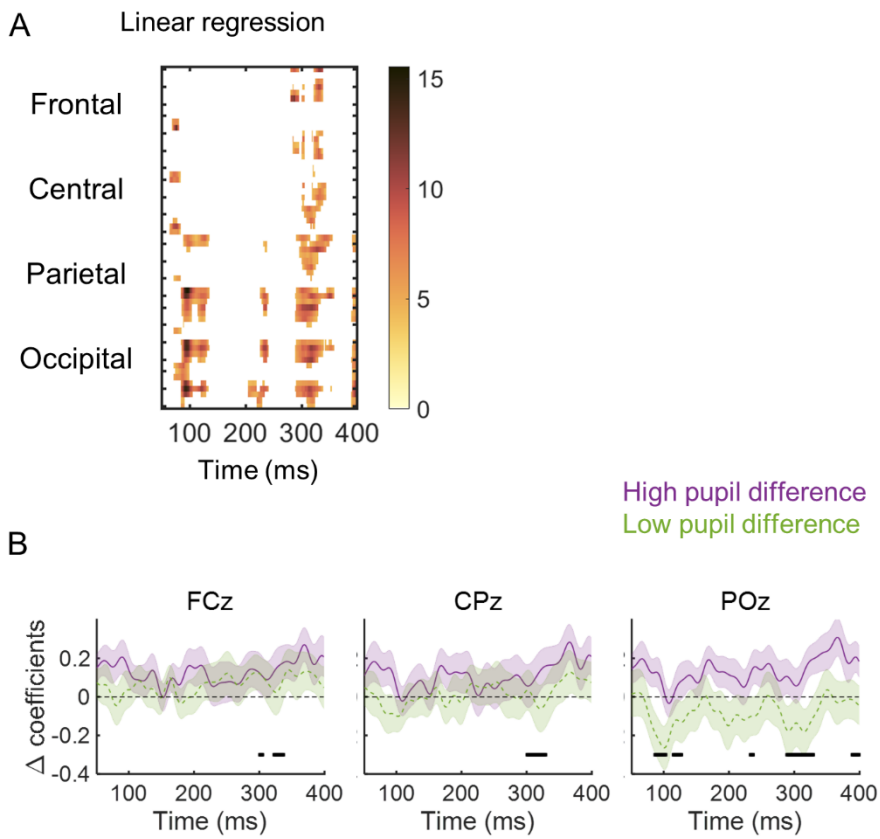

Figure 6. Across participants regression analysis revealed that differences across tasks in pupil dilation responses were related to the task differences in the effect of heart rate on the HEP. **(A)** Regression model *F*-values within the significant spatiotemporal clusters where the across tasks difference 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.

HEP amplitude does not change with heart rate in a passive task condition

The finding that the amplitude of the pupil response is related to the effect of heart rate on the HEP suggests that engaging in a task that requires a higher level of cognitive effort (the go/no-go task) makes the HEP more sensitive to heart rate. To explore this possibility, we took advantage of the fact that, at the beginning of the EEG session, we recorded four minutes of data while the participants were passively listening to the auditory stimulus used as cue in the other tasks (passive task condition). In this passive condition, the pupil response evoked by

the cue was markedly reduced (see Fig. 5A in Ribeiro and Castelo-Branco, 2019b). Interestingly, in the passive condition, we did not find a significant effect of heart rate on HEP amplitude (no significant cluster was observed). Importantly, if we include only the first trials from the simple RT and go/no-go tasks so that these had the same number of trials as the passive task for each participant, the regression coefficients between the HEP amplitude and heart rate were still significantly different from zero (Fig. 7A; both simple RT and go/no-go task conditions presented one significant cluster starting at 51 ms and ending at 399 ms after the T peak, $p < .001$). Moreover, repeated measures ANOVA including the three tasks as within-subject factors presented a significant effect of task at the end of the cardiac cycle (one significant cluster starting at 233 ms and ending at 399 ms, $p = .005$; Fig. 7B). The regression coefficients were more positive at the end of the cardiac cycle in central, parietal, and occipital channels in the active tasks than in the passive task (Fig. 7C).

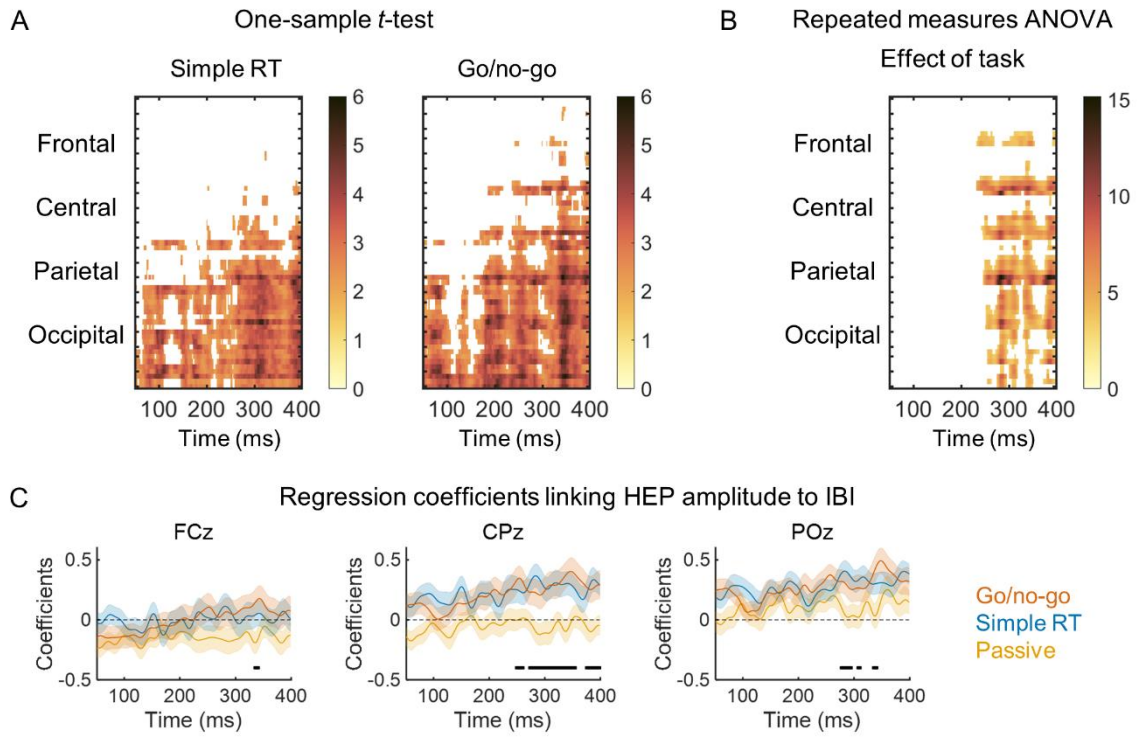

Figure 7. The amplitude of the heartbeat-evoked potential (HEP) is not significantly related with the interbeat interval (IBI) in a passive task condition. **(A)** One-sample *t*-tests *t*-values within the significant spatiotemporal clusters found in the simple RT and go/no-go task conditions using the same number of trials as in the passive condition. **(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. **(C)** Time course of the regression coefficients linking HEP amplitude to IBI for the passive, simple RT, and go/no-go task conditions in the midline channels FCz, CPz and POz. The graphs plot the mean

across all participants ± standard error of the mean. The horizontal black line shows the time windows where the coefficients presented a significant effect of task.

No evidence of any association between reaction time, HEP, heart rate or cardiac phase at the time of target onset

If, as we hypothesized, the HEP interferes with sensorimotor processing and, as our results suggest, HEP amplitude is affected by heart rate during task performance, then heart rate at the time of target onset might be associated with the efficiency of sensorimotor processing. Moreover, as the effect of heart rate on the HEP depends on the phase of the cardiac cycle, this effect might be modulated by the cardiac phase. We investigated this hypothesis using within-subject multiple regression analyses where we included as response variable reaction time and as predictors heart rate at the time of target onset, the sine and cosine of cardiac phase at the time of target onset [capturing the effect of cardiac phase as a circular variable (Nurhab et al., 2017)], and interaction terms between heart rate and sine and cosine of cardiac phase. Given that the effect of heart rate on the HEP did not differ across age groups, we analysed both groups together. We found that, at the group level, the regression coefficients were not significantly different from zero for any of the predictors suggesting that reaction time was not affected by heart rate on a trial-by-trial basis.

To determine if HEP amplitude interferes with sensorimotor processing, we investigated the correlation between average HEP amplitude and average reaction time across participants separately for each age group and task condition. We found no spatiotemporal cluster where this correlation was significant. Note that the HEP amplitude at the time of target onset is challenging to estimate, particularly at the single-trial level, given the overlap with preparatory and target evoked potentials and we did not attempt this.

Finally, as HEP varies with heart rate it is possible that participants with slower heart rate present a HEP with more positive amplitude values. In fact, as older people have slower heart rates (Table 2) this might explain why older people present larger HEP amplitude. However, we found that across participants, average heart rate was not significantly related to average HEP amplitude for any of the age groups or any of the task conditions.

Discussion

In this study, we investigating the impact of heart rate on the neural responses to the heartbeat (the HEP). Slow heart rates were associated with stronger HEPs. Interestingly, heart rate only affected the HEP during active tasks that engaged pupil-linked arousal phasic responses, a measure of mental effort (van der Wel and van Steenbergen, 2018), and this effect was related to the task difficulty and the amplitude of the task-related pupil responses. Thus, the modulation of the HEP by heart rate emerged as a marker of neuronal gain modulated by arousal and associated with mental effort. These findings suggest that the impact of heart rate fluctuations on the brain depends on cognitive state and that fluctuations in the amplitude of the HEP might link heart rate with the neural processing of external stimuli particularly during periods of high mental effort.

The HEP is evoked by the activity in the arterial baroreceptors that in turn is sensitive to stroke volume and heart rate (Garfinkel and Critchley, 2016; Buot et al., 2021). Buot et al (2021) studied the effect of stroke volume (the volume of blood ejected from the left ventricle at each cardiac cycle) on the HEP. Their study found that under resting conditions, stroke volume did not affect HEP amplitude. Thus, indirectly, the study of Buot et al (2021) suggests that the HEP is not affected by heart rate. This is what we found on the analysis of the passive task condition. In contrast, the HEP was affected by heart rate in the recordings obtained while the participants were engaged in sensorimotor tasks. As the passive task condition was recorded at the beginning of the session, the difference between this task condition and the active conditions is confounded with the effect of time on task. However, the order of the two active tasks was counterbalanced across participants and these showed significant differences in the strength of the effect of heart rate on the HEP. The more challenging task, the cued go/no-go task, that required greater motor control, stimulus discrimination, choice between two types of responses, and was associated with larger task-related pupil dilation was associated with a stronger effect of heart rate on the HEP in comparison with the cued reaction time task. This difference was not related to differences in average heart rate, heart rate variability or power of alpha oscillations as these did not vary across tasks. However, it was related to differences in the engagement of the arousal system during task performance as indexed by the amplitude of the task-related pupil dilation responses suggesting that higher mental effort is associated with a brain state where neural responses to heartbeats are more sensitive to fluctuations in the strength of the input. In other words, task engagement modulates the neuronal gain that maps input to output in the brain. In fact, higher pupil-linked arousal has been shown to result in increased neuronal gain (Mineault et al., 2016; McCormick et al., 2020). Thus, engagement of the neuromodulatory arousal systems of the

brainstem reflected in pupil responses (Murphy et al., 2014; Joshi et al., 2016; Reimer et al., 2016; Gee et al., 2017; Lloyd et al., 2023) might cause the HEP to be more sensitive to changes in input in effortful task conditions associated with stronger pupil dilation responses (van der Wel and van Steenbergen, 2018). In alternative, given the lack of causality assessment, it is possible that other aspects of brain function change with mental effort that both explain the increase in pupil dilation responses and the increase in the effect of heart rate on the HEP.

Enhanced sensory sensitivity is associated with periods of slower heart rates (Sandman et al., 1977). We found that slower heart rates were associated with more positive HEP amplitudes, particularly at the end of the cardiac cycle. It is still unclear if the HEP itself affects sensory processing. Previous studies suggest that reduced HEP amplitude is associated with more sensitive sensory detection indicating that the HEP interferes negatively with neural processing of external stimuli and might reflect the inhibitory influence of the heartbeat on cortical processing (Petzschner et al., 2019). Our findings showing that slower heart rates are associated with stronger HEP go against the idea that slower heart rates reduce the impact of the heartbeat on the cortex. Notably, we found no evidence that heart rate at the time of target onset affected sensorimotor efficiency as assessed by reaction time. One reason for this lack of evidence might be because we used suprathreshold auditory stimuli, while previous studies that found an effect of heart rate on sensory processing used stimuli at the threshold of detection and assessed detection threshold rather than reaction time (Sandman et al., 1977), but it appears to indicate that under our task conditions heart rate does not affect sensory processing.

Despite the fact that the warning cue induced a reduction in heart rate, we did not observe changes in the HEP during the period between the cue and the target. However, it is important to note that the cardiac deceleration observed between the cue and the target was small in comparison with the ongoing fluctuations in heart rate observed during the whole task recording. 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.

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). 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. 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. In our study, we found no evidence of differences in HEP amplitude across task conditions. We found instead that mental effort increased the effect of heart rate on the HEP. This could suggest that heart rate will have a stronger effect on task performance during periods of higher mental effort, like tasks where participants are required to detect threshold stimuli for example (Sandman et al., 1977).

Older adults presented a stronger HEP than young adults as had been observed before by Kamp et al. (2021). The cause behind this increase in HEP amplitude with aging is unclear. In our within-subject analyses, periods of slow heart rates were associated with larger HEPs; thus, larger HEPs in older people could be associated with the fact that older people had slower heart rates on average. In fact, Kamp et al. (2021) found a negative correlation between heart rate and HEP amplitude. However, in our dataset, this correlation was not significant and further studies are needed to clarify the impact of average heart rate on HEP amplitude.

The impact of higher HEP amplitude in older people is also unclear. Stronger HEP amplitude could suggest better cardiac interoception; however, older people appear to have reduced ability to sense their own heart (Murphy et al., 2018). Moreover, in older people, metacognitive judgments of everyday cognitive functioning are negatively correlated with HEP amplitude (Kamp et al., 2021) suggesting that higher HEP amplitude might be associated with poorer cognition.

In conclusion, ongoing heart rate fluctuations affect the HEP amplitude in a mental effort dependent manner associated with the engagement of the pupil-linked arousal system. These findings suggest that heart rate affects neural processing particularly during challenging tasks.

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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