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Does stroke volume influence heartbeat evoked responses?

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Abstract

We know surprisingly little on how heartbeat-evoked responses (HERs) vary with cardiac parameters. Here, we measured both stroke volume, or volume of blood ejected at each heartbeat, with impedance cardiography, and HER amplitude with magneto-encephalography, in 21 male and female participants at rest with eyes open. We observed that HER co-fluctuates with stroke volume on a beat-to-beat basis, but only when no correction for cardiac artifact was performed. This highlights the importance of an ICA correction tailored to the cardiac artifact. We also observed that easy-to-measure cardiac parameters (interbeat intervals, ECG amplitude) are sensitive to stroke volume fluctuations and can be used as proxies when stroke volume measurements are not available. Finally, interindividual differences in stroke volume were reflected in MEG data, but whether this effect is locked to heartbeats is unclear. Altogether, our results question assumptions on the link between stroke volume and HERs.

Highlights (3-5 bullet points, 85char max)

- Beat-to-beat fluctuations in stroke volume mostly affect the cardiac/pulse artifact
- A specific ICA correction strongly attenuates the influence of stroke volume on HER
- Interbeat intervals and ECG amplitude are sensitive to stroke volume fluctuations
- Inter-individual differences in stroke volume affect MEG data

Keywords:

Interoception; Heartbeat-evoked response; stroke volume; baroreceptors;

Introduction

Interoception refers to how the brain perceives and represents visceral signals – whether consciously or unconsciously (Azzalini et al., 2019). In the field of cardiac interoception, the heartbeat-evoked response (HER) is used to quantify how the brain transiently responds to an incoming interoceptive signal, the heartbeat. HERs correspond to electrophysiological (EEG or MEG) data averaged locked to one peak of the electrocardiogram, for instance the R peak that corresponds to ventricular contraction. Simultaneously revealed in 1986 by the group of Schandry (1986) and Jones (1986), HERs have attracted a lot of interest in the recent years as they offer a window on heart-to-brain communication and its potential role in perception, emotion, cognition and consciousness (for reviews: Azzalini et al., 2019; Coll et al., 2021; Park and Blanke, 2019; Park and Tallon-Baudry, 2014). However, despite this growing interest, little is known about the origin of HERs and their dependence on cardiac parameters.

HERs are responses to an internal, cyclical neuro-muscular event resulting in blood ejection. The volume of blood ejected from the left ventricle at each cardiac cycle is called the stroke volume. Blood ejection transiently stretches the walls of the aorta and carotid arteries, inducing neural activity in baroreceptors at each cardiac cycle (Charkoudian et al., 2005; Vaschillo et al., 2012). It is often assumed that HERs are caused by the transient aortic and carotid baroreceptor responses to blood ejection, with some delays due to transduction and neural conduction times (Garfinkel and Critchley, 2016; Gray et al., 2007). Under this hypothesis, HER amplitude should co-vary with stroke volume on a beat-to-beat basis. However, some cardiac mechanoreceptors discharge at each heartbeat but appear insensitive to stroke volume (Bishop et al., 1983). It thus seems equally plausible that HERs are independent from stroke volume. The primary aim of this paper is to test how beat-to-beat fluctuations in stroke volume affect HERs. To that aim, we measured beat-to-beat fluctuations in stroke volume using impedance cardiography (Figure 1) while also recording the electrocardiogram (ECG) and magnetoencephalographic (MEG) data in 21 young healthy participants at rest with eyes open.

A specificity of neural responses to heartbeats measured non-invasively is that they are contaminated by the cardiac artifact, because MEG and EEG sensors detect the electrical activity of the heart itself (Jousmäki and Hari, 1996; Dirlich et al., 1997). We thus have to consider a third hypothesis: HER amplitude measured non-invasively is related to stroke

volume, but this effect is mediated by a modulation of cardiac electrical activity detected by scalp sensors, rather than by mechanoreceptor discharges in the heart or blood vessels that are relayed, with some delay, to the brain. In practice, Independent component analysis (ICA, Jung et al., 2001; Makeig et al., 1996) is efficient to attenuate, but probably not to remove completely, the cardiac artifact in MEG or EEG data. We systematically analysed the contribution of beat-to-beat fluctuations in stroke volume to HERs in ICA-uncorrected as well as ICA-corrected MEG data.

Additionally, two previous EEG studies suggested that interindividual differences in stroke volume (Schandry and Montoya, 1996) or a closely related measure (Gray et al., 2007) were reflected in interindividual differences in HERs. We thus also analysed whether interindividual differences in stroke volume contribute to interindividual differences in HERs measured with MEG.

Finally, most recent studies relating HERs with perceptual, emotional or cognitive variables compared HERs between two experimental conditions, observed HERs differences and verified that neither the electrocardiogram (ECG) nor parameters derived from the ECG such as inter-beat interval (IBI) and inter-beat interval variability differed between conditions. The difference in HERs along perceptual, emotional or cognitive variables was thus attributed to a difference in the neural processing of heartbeat related to the task at hand, rather than to a difference in cardiac input (e.g., Babo-Rebelo et al., 2019, 2016; Canales-Johnson et al., 2015; Müller et al., 2015; Park and Tallon-Baudry, 2014; Pollatos and Schandry, 2004; Schulz et al., 2015; Sel et al., 2017; Petzschner et al., 2019). However, stroke volume was not measured in those experiments - and is unlikely to be routinely measured, because it requires dedicated equipment and the injection of a high frequency current. We thus investigated the link between stroke volume and cardiac parameters commonly acquired, such as ECG amplitude and interbeat intervals, to determine whether they are sensitive to fluctuations in stroke volume and can be used as proxies to test whether stroke volume varies between experimental conditions.

Materials and Methods

Participants

24 right-handed volunteer were included in the study after giving written informed consent and were paid for their participation. The study was approved by the ethics committee CPP Ile de France III. Inclusion criteria were: age between 18 and 30 years old, right-handedness, no psychiatric or neurological disorder, no cardiac disorder, no ongoing medical treatment, normal or corrected vision, as well as absence of claustrophobia or the absence of metallic objects in the body to allow MEG recordings. Three participants were excluded from the study for extra systole (n=1), noisy impedance cardiography data (n=1) and outlying stroke volume values (n=1). The 21 remaining participants (10 males; age: mean±std = 23±2.14 years, range [19 28]) were included in the analysis.

Procedure

Five minutes recordings were acquired during resting-state with eyes open. Participants were seated in front of a grey screen with a black fixation point. They were asked to remain silent, to relax, to avoid large eye or body movement, as well as repetitive physical or mental activity such as finger taping or counting.

Recordings

Continuous MEG data were acquired using a whole-head MEG system with 102 magnetometers and 204 planar gradiometers (Elekta Neuromag TRIUX, Elekta AB, Stockholm, Sweden; sampling rate of 1000 Hz, online low-pass filtered at 330 Hz). The ground electrode was located on the left costal margin.

ECG data were acquired on a separate amplifier (BIOPAC Systems, Inc., Goleta, United States; sampling rate of 1000 Hz, online band-pass filters 0.05 to 35 Hz) using 4 electrodes placed around the base of the neck (2 electrodes over the left and right clavicles, 2 electrodes above the left and right supraspinatus muscle) and referenced to a left abdominal location. Three different leads (I, II and III) were computed offline (Figure 1A).

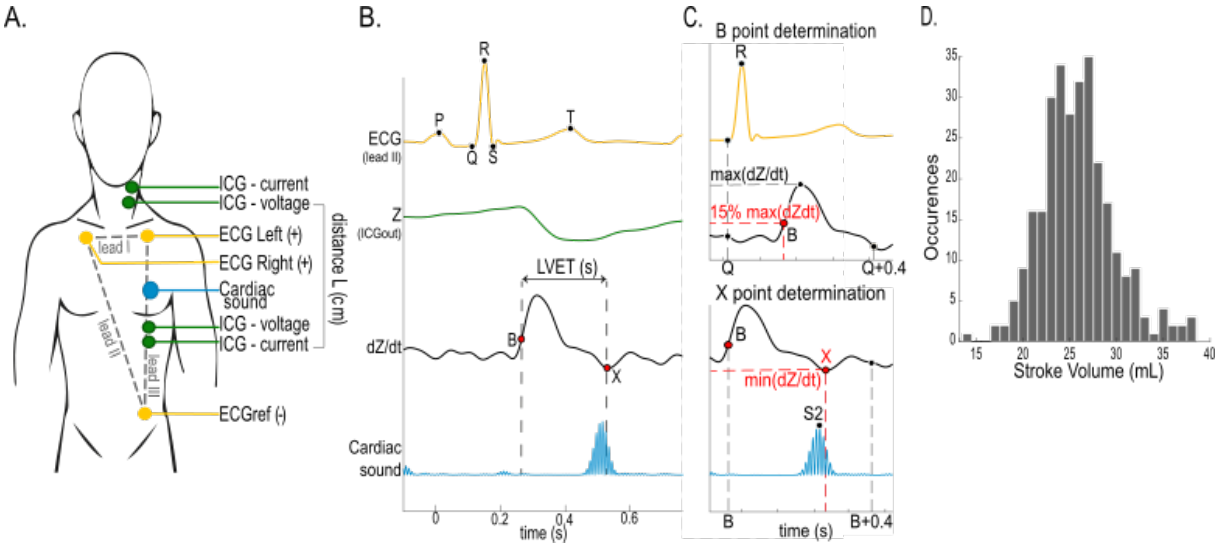
Impedance cardiography data were acquired using the Biopac EBI100C module using four spot electrodes (Figure 1A; Sherwood(Chair) et al., 1990). Two sources electrodes placed on the left side of the abdomen and neck, separated by a distance L (range [27 34cm]), were used to inject a low intensity (400 µA rms) high frequency (12.5 kHz) current.

Two monitoring electrodes placed 4 cm above and below the sources electrodes were used to measure the voltage across the tissue. The distance L, which is important when computing SV based on impedance cardiography data, was precisely measured in each subject. For the validity of SV values obtained using impedance cardiography, see Raaijmakers et al., 1999.

Cardiac sounds were recorded by placing an a-magnetic homemade microphone (online band-pass filter 0.05-300 Hz) on the chest of the subject.

Gaze location was monitored using an eye-tracker (EyeLink 1000, SR Research Ltd., Mississauga, Canada).

Figure 1. Experimental set up and stroke volume computation



A. *Experimental set up for recording ECG (yellow), ICG (impedance cardiography, green), and cardiac sound (blue). Briefly, impedance cardiography consists in applying a constant current at the low level of the abdomen (through the ICG-current electrodes) and in recording the voltage below and above the heart (through the ICG-voltage electrodes). Because the current is held constant and the body tissues with the least resistivity are the blood and plasma, the voltage drop occurring during left ventricular ejection time (LVET) is proportional to the amount of blood being ejected from the left ventricle after each heartbeat.* ***B.*** *The different signals used in impedance cardiography, with the ECG, are time-varying impedance Z, its first derivative dZ/dt, and cardiac sound. The LVET is indicated by the arrow.* ***C.*** *Methodology to compute LVET using ECG, ICG and cardiac sound.* ***Upper panel****: B point (LVET onset) determination. The B point marks ejection onset and is defined as the time point when the 1st derivative of the impedance reaches 15% of its maximum in the time interval between Q and Q+400ms.* ***Lower panel****: X point (LVET offset) determination. The X point marks the end of the ejection and is defined as the time point when the 1st derivative of the impedance reaches a minimum in the time interval [B to B+400ms].* ***D.*** *Stroke volume distribution in one subject.*

R peak detection

A two-step procedure was used to label the R peaks of the ECG: We first created a subject-specific template QRS complex, and then used this template to detect all R peaks. To create the template, a band-pass filtered (1-100 Hz) version of the ECG was submitted to threshold detection, such that enough (>20) R peaks would be detected. The average QRS complex was computed as the average ECG signal locked to these peak detections. The full list of R-peaks was then detected by correlating the ECG with the template QRS complex and identifying the local maximum within the episodes of correlation >0.6. R-peak detection was visually verified in all participants. Q-peak was then identified as the minimum within 50ms before the R-peak, S-peak as the minimum within 100ms post R-peak, and T-wave as the maximum in the interval [S S+0.4s].

IBI consisted of the average time distance between two R-peaks and the heart rate variability corresponded to the standard deviation of the IBIs.

SV computation

For each participant, we calculated the beat-to-beat SV from the impedance cardiography data using the following Kubicek's formula (Kubicek et al., 1970):

$$SV = rho \times \frac{L^2}{Zo^2} \times LVET \times \left(\frac{dZ}{dt}\right) max$$

- Rho is blood resistivity, considered here to be 135 ohms.cm (Berntson et al., 2007). Note that blood resistivity is taken here to be a constant, although it might vary from one participant to the other, and depend on current injection frequency (Berntson et al., 2007).
- L is the distance in cm between the two recordings electrodes
- Z_0 is the average impedance, in ohm, during the left ventricular ejection
- LVET is the left ventricular ejection time in second.
- (dZ/dt)max is the maximum value of the first derivative of the impedance measure in ohm/sec.

To estimate LVET (duration of the left ventricular ejection) at each beat, we identified for each heartbeat, the beginning (B-point) and end (X-point) of the ejection. The procedure followed to determine these key points is illustrated in figure 1B and C.

B point identification: Impedance cardiography data were low pass filtered at 15 Hz and the first derivative dZ/dt was computed. dZ/dt was baseline corrected by removing the average signal in the [Q-point Q-point+0.05sec] interval (Sherwood et al., 1990), to limit respiratory influences on impedance measurements (Cybulski, 1988). The B point was then identified as the time point at which dZ/dt reaches 15% of its maximum in the [Q-point Q-point+0.4sec] window (Figure 1C upper panel).

X point identification: The end of the ejection was defined as the time point at which dZ/dt reaches a minimum in the [B-point B-point+0.4sec] time window (Figure 1C lower panel). If multiple local minima were observed in the time window of interest, we chose the one closest in time to the second heart sound that mainly reflects the closing of the aortic valve (Sherwood et al., 1990).

The identification of the B and X-point were performed using a homemade Matlab script (source code available online, Fieldtrip toolbox required, Oostenveld et al., 2011), which included a visual verification step and detected outlying values for the ejection time, the delay between the R peak and the B point, and the delay between S2 (second heart sound) and the X point. Across the 21 participants, the mean±std LVET was 0.264±0.034 sec (range [0.185 0.311sec]). An illustration of LVET determination is represented in Fig 1B.

MEG/ECG data preprocessing

Continuous MEG data were denoised using temporal signal space separation (as implemented in MaxFilter) and band pass filtered between 0.5 and 40 Hz using fourth-order Butterworth filter. MEG data contaminated by artifacts were detected through visual inspection and excluded from further analysis. MEG data contaminated by blink or saccade with amplitude >3degrees as indicated by the EyeLink were also excluded (https://www.sr-research.com).

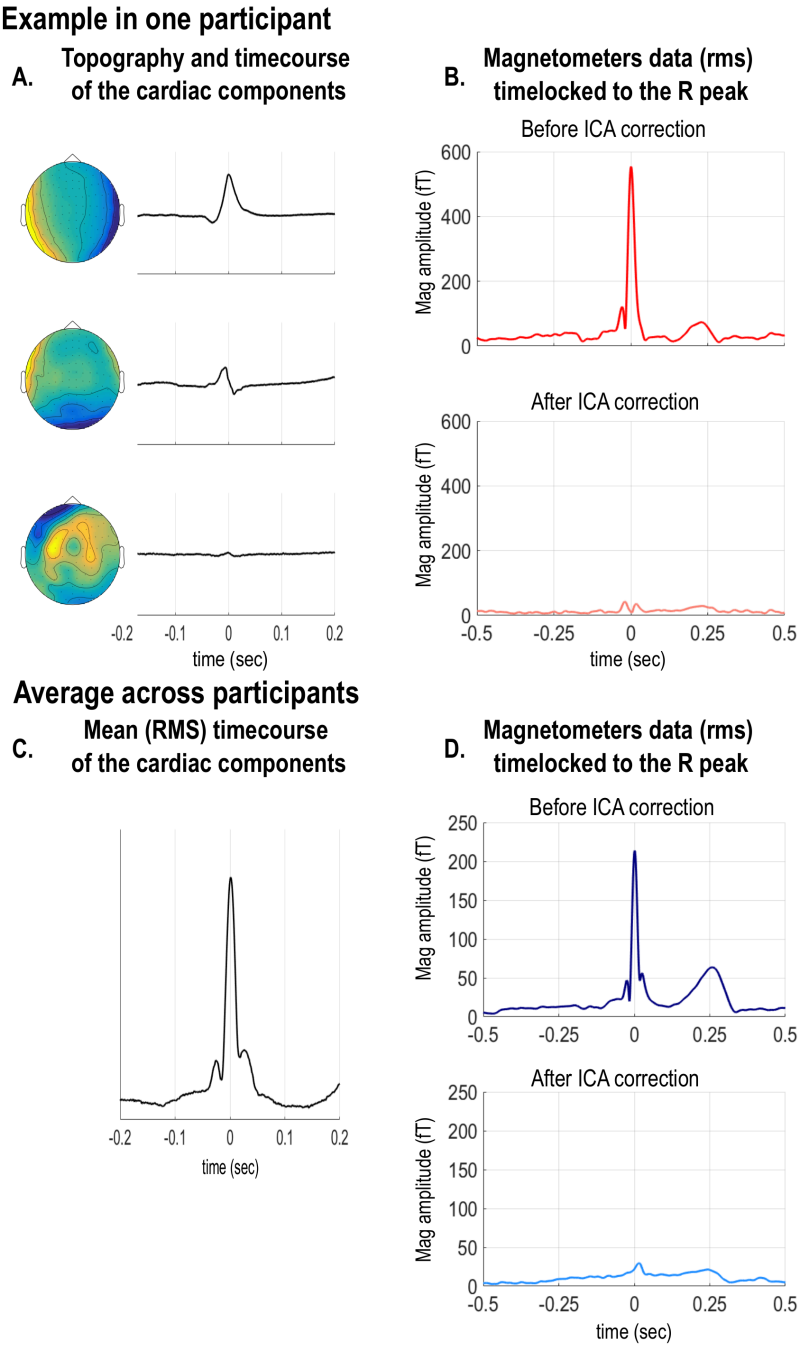
ICA procedure to correct for the cardiac artifact

Independent component analysis (ICA), as implemented in the Fieldtrip toolbox (Oostenveld et al., 2011), was used to attenuate the cardiac artifact, for both magnetometer and gradiometer signals. MEG and ECG data were first high-pass filtered at 0.5 Hz using fourth-order Butterworth filter and then epoched in [-200 to +200 ms] segments centered on the R-peaks. MEG data segments free from artifact, blink and large saccade were then

decomposed in independent component using the ft_componentanalysis fieldtrip function (which is using the runica function from EEGlab, (Delorme and Makeig, 2004)). Because temporal signal space separation induces rank deficiency, we defined the maximum number of ICA components as the rank of the dataset. To detect the components corresponding to cardiac activity, we computed the mean pairwise phase consistency (ppc; Vinck et al., 2010) between each independent component and the lead II ECG signal, in the 0–25 Hz range. Components with a ppc superior to the mean+3std across ppcs were selected as cardiac components. The component selection process was repeated iteratively until no ppc was exceeding the mean+3std threshold, or until the maximum number of 3 components was reached. The cardiac components (between 1 and 3 per subject) were then removed from MEG data using the ft_rejectcomponent fieldtrip function, resulting in ICA-corrected MEG data. Before removing the cardiac components, their topographies and time-courses were visually checked. Across our participants, the rank of the data (i.e maximum number of components allowed when decomposing the data) was mean±std = 71±10 (range [67 116]). The number of cardiac components removed from the data was mean+std = 2.9±0.3 (range [2 3]).

Cardiac components topographies and time-courses, as well as magnetometers data before and after ICA correction, for an individual participant and at the group level, are shown on Figure 2.

Figure 2: **ICA correction for the cardiac artifact**



A. Example of cardiac components in one participant. **Left panel**: Topographies of the components. **Right panel**: Time-courses of the components time-locked to the R peaks (t=0 sec) and averaged across heart cycles. **B.** Magnetometer data time-locked to the R peak and averaged across heart cycles, before and after ICA correction, in the same participant (root mean square, RMS, across sensors). **C.** Time-courses of the RMS cardiac components averaged across heart cycles, components and participants. **D** represents the same analysis as in B but averaged across participants.

Cardiac component extraction

To isolate the time-course of the cardiac component, we used the same ICA decomposition as described above, selected all components that were not correlated to cardiac activity and removed them from the ICA-uncorrected data using the ft_rejectcomponent fieldtrip function. This resulted in one continuous signal at each sensor corresponding to the cardiac components identified based on their similarity to the ECG and extracted from initial data.

HERs

To perform the analysis on HERs, MEG data were epoched in segments of [-0.1 to 0.7sec] around the R peak. Epochs containing an artifact, a blink or a large saccade, epochs containing a cardiac cycle shorter than 0.7 s, or with corresponding SV values exceeding a [mean+/-3std] threshold, were discarded from further analysis. On average, 37% of HER was discarded (min: 5%, max: 74%), resulting in a mean number of valid HER per subject of 205 (min: 108; max: 314). On average, 3% (min:0% max: 19%) of the trials were discarded due to artifact contamination, 26% (min: 1% max: 59%) due to blink, 2% (min: 0% max: 11%) due to saccade, 5% (min: 0% max: 47%) due to cardiac cycle duration and 1% (min: 0% max: 2%) due to outlying SV values. Gradiometers data were then recombined using the fieldtrip function ft_combineplanar. The exact same selection procedure was applied to ECG data.

Correlation analysis

For each subject, each channel and each time point, the Pearson correlation coefficient between HER and SV values was calculated and Fisher-transformed. To estimate chance level correlation in each subject, channel and time point, we created shuffled data where we permuted the association between SV and HER (e.g., SV in trial i re-allocated to HER in trial j) and recalculated the Fisher-transformed correlation coefficient. This operation was performed 100 of times and the median value of the random correlation coefficient distribution was extracted. This procedure resulted in one empirical correlation and one chance level correlation, at each channel and time point in each participant. Empirical and chance level correlation could then be statistically compared at the group level with a cluster-based permutation procedure (Maris and Oostenveld, 2007) detailed below.

Median-split analysis

For each subject, the median SV value was extracted. For each channel, high (above median) and low-SV (below median) HERs were then computed by averaging MEG data across segments according to their categories. Here again, the significance of the difference was assessed at the group level using a cluster-based permutation t test.

Group-level statistics: Cluster-based permutation procedure

Group level statistics relied on a cluster-based permutation procedure (Maris and Oostenveld, 2007). This method does not require the definition of any a priori spatial or temporal regions and intrinsically corrects for multiple comparisons in time and space. Briefly, for each test (correlation/median-split), a statistical value t that quantifies the effect is computed between the two distributions being compared (empirical versus random r for the correlation, high versus low-SV HERs for the median-split). Individual samples with a statistical value corresponding to a P value below a selected threshold ($P<0.05$, two tailed) are clustered together based on temporal and spatial adjacency. The cluster is characterized by the sum of the t values of the individual samples. To establish the likelihood that a cluster was obtained by chance, we shuffled the labels (empirical versus random or high versus low-SV) 10,000 times and repeated the clustering procedure selecting the maximum positive and the minimum negative cluster-level statistic across the tests. The Monte Carlo P value corresponds to the proportion of elements in the distribution of maximal (or minimal) cluster-level statistics that exceeds (or is inferior to) the originally observed cluster-level test statistics.

Effect size

Effect size was estimated using Cohen's d definition for paired samples $d=t/\sqrt{N}$, where N is the number of participants and t the paired t-test statistics obtained when comparing high versus low-SV IBIs, ECG and HERs.

Interindividual analysis

To compute the interindividual correlation, we averaged MEG signal in the 450 to 550ms window post R-peak using heart cycles free from artifact, blink or saccade above 3 degrees. MEG signal and SV were then averaged across cycles for each subject, and mean

HERs were correlated to mean SVs using Pearson correlation. To look at the evolution of the correlation coefficient and its topography across time, the same procedure was performed using MEG signal at each time-point of the cardiac cycle.

Surrogate analysis

We first shuffled the IBI series for each subject. We then created surrogate R-peaks timing using the shuffled IBI series, with the first surrogate R-peak being placed 350ms before the first real R-peak. The SV associated to each surrogate cycle was the one corresponding in time to the surrogate R-peak. As for the initial analysis, we removed cardiac cycle contaminated by artifacts, blinks or saccades above 3 degrees. The procedure was repeated 150 times. To check whether our procedure succeeded in unlocking data from the real R-peak, we plotted for each subject the averaged ECG locked to the surrogate R-peaks. Each plot was visually inspected.

Results

Validating the stroke volume measure in the MEG at 12.5 kHz

Impedance cardiography requires the injection of a small high-frequency current to quantify impedance changes due to blood ejection at each cardiac cycle. We found that the most commonly employed current frequency (100 kHz) is not compatible with good quality MEG recordings, because current injection at 100 kHz generated large spectral peaks at subharmonics of stimulation frequency. We thus estimated beat-to-beat stroke volume with a 12.5 kHz current, where spectral perturbations were not visible to the naked eye. Using the Kubiceck formula (see Materials and Methods), we obtained a mean SV of 29.98 ± 1.74mL (SE) and a mean SV-variability of 3.25 ± 0.24mL. Mean and standard deviation of SV values for each participant is reported in table 1. An example distribution of the stroke volume in one subject (S12) is also represented on figure 1D.

Table 1. Mean and standard deviation of SV values for each participant

Mean SV (mL)	Std SV (mL)
20.15	1.98
29.67	4.24
20.78	3.39
34.51	3.15
24.61	2.24
35.33	3.33
48.93	5.03
27.83	3.04
23.43	2.53
25.82	3.92
39.98	5.28
26.88	4.70
21.10	1.76
25.02	3.28
28.24	2.61
44.51	4.66

23.16	1.19
39.82	3.19
27.03	3.60
32.90	1.93
29.95	3.23

In our sample, SV magnitudes were lower compared to those reported in textbooks (e.g., 60-100 mL in Turner, 2000). We suspected an effect of the stimulation frequency, notably because impedance depends on injected current frequency. We thus recorded, in five participants, five minutes at rest with 100 kHz stimulation and with 12.5 kHz stimulation, and compared the values of stroke volume thus obtained. In all five participants, the measured stroke volume was smaller at 12.5 kHz (30.6±2.8 ml) than 100 kHz (48.2 ± 4.0 ml). We then verified the incidence of stimulation frequency on each parameter used to compute the stroke volume. Left ventricular ejection time was similar at 12.5 and 100 kHz (mean LVET ± SE at 12.5 kHz = 0.288±0.007 s; at 100 kHz = 0.283±0.008 s), but the mean body impedance (mean Z_0±SE at 12.5 kHz = 59.45±4.05 Ohms; at 100 kHz = 39.74±2.35 Ohms) and the peak value of the first derivative of the impedance (max(dZ/dt) ± SE at 12.5 = 3.40±0.34 $Ohm.s^{-1}$; at 100 kHz = 2.47±0.26 $Ohm.s^{-1}$) were systematically larger at 12.5 than 100 kHz. The distance between the electrodes remaining constant in each subject across stimulation frequencies, this confirmed that the stimulation frequency influenced the basal level of the impedance, leading to smaller stroke volume magnitude without affecting ejection time. Since all further analysis are based on beat-to-beat fluctuations in stroke volume, or comparison between participants with the same acquisition parameters, rather than on the absolute value of stroke volume, this systematic underestimation of stroke volume has little incidence.

Influence of beat-to-beat fluctuations of stroke volume on HERs depends on ICA correction

We then evaluated whether variations of stroke volume were associated with modulations of HER amplitude computed on ICA-uncorrected magnetometers data, on the cardiac component isolated with ICA, which can be considered as reflecting the cardiac

artifact affecting MEG sensors, and on ICA-corrected data. We performed two types of analysis in each participant: a correlation between single-beat HER at each timepoint and SV (one value per cardiac cycle), and a median-split analysis in which we directly compared HERs computed in high- versus low-SV cardiac cycles. In both analyses, we identified clusters of channels and timepoints on which the correlation or the difference was significant at the group level using cluster-based permutation tests (Maris and Oostenveld, 2007) .

ICA-uncorrected magnetometer data revealed a significant negative correlation between HER and SV at late latencies, from 534 to 617ms after the R-peak in a large cluster of 41 bilateral occipital and occipito-temporal channels (figure 3A; sum(t)=-2886, Monte Carlo p=0.037). The median-split analysis showed very similar results, with a significant difference between high- and low-SV HERs ranging from 569 to 694ms post R-peak in a cluster of 39 channels, with a similar topography (figure 3B; sum(t)=-4113, Monte Carlo p=0.002). A second negative subthreshold cluster (not shown) was observed at earlier latencies (472-536 ms post R peak) at posterior locations, but the difference between the high- and low-SV HERs did not reach significance (sum(t)=-1991, Monte Carlo p=0.07).

The cardiac component identified with ICA and locked to heartbeats showed a pattern of correlation with SV overlapping with the one observed in ICA-uncorrected magnetometer data. The cardiac component identified with ICA negatively correlated with SV from 489 to 700ms post R-peak in a cluster of 38 left-lateralized channels (figure 3C; sum(t)=-9889, Monte Carlo p=0.002). A significant difference between the high- and low HERs was also observed from 595 to 700ms post R-peak, in a cluster including 39 channels (figure 3D; sum(t)=-4419, Monte Carlo p=0.030).

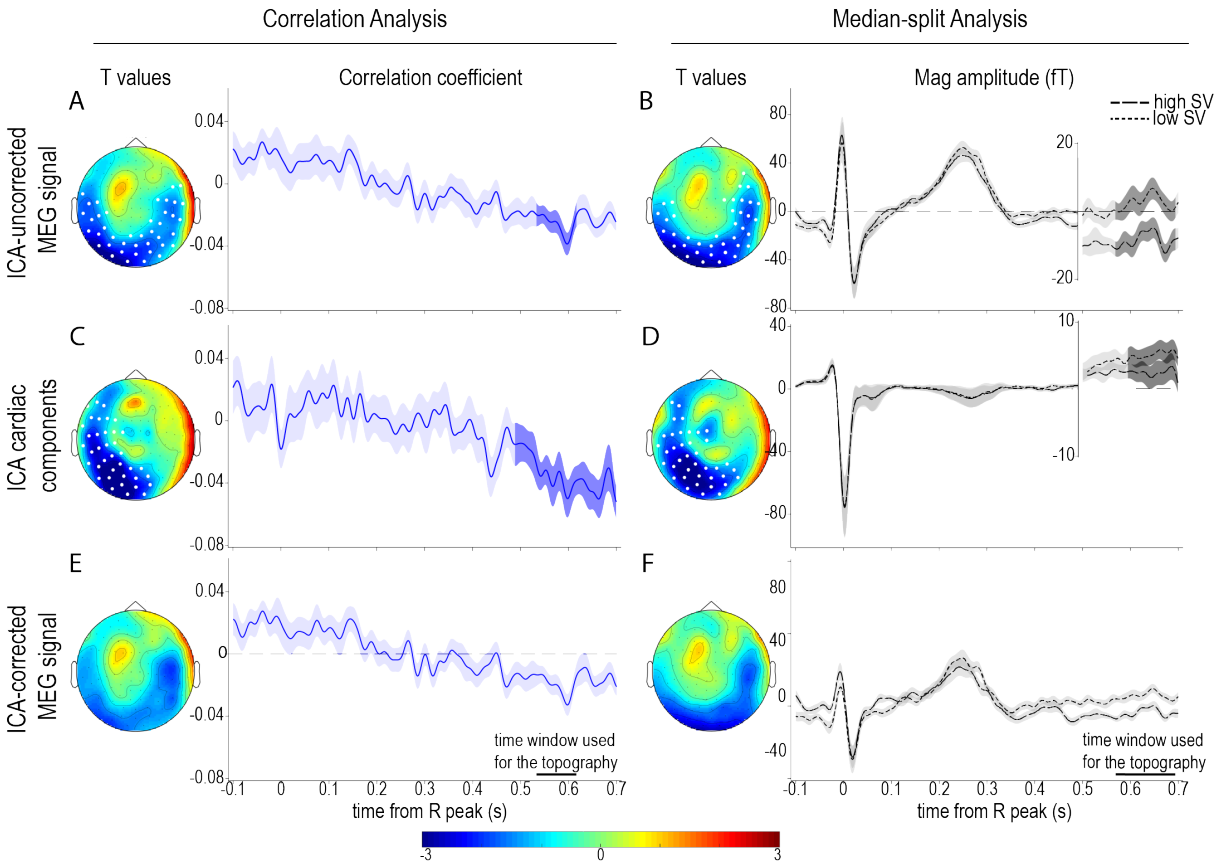
Finally, HERs computed on ICA-corrected magnetometer data did not significantly correlate with stroke volume (figure 3E), and the difference between high- and low-SV HERs was no longer significant (figure 3F). This suggests that ICA successfully removed the cardiac artifact related to stroke volume. Still, this conclusion has to be tampered down by the existence of a subthreshold cluster (not shown) on the difference between high- and low-SV HERs found in ICA-corrected data (464-532ms post R-peak, sum(t)=-1820, Monte Carlo p=0.069).

The exact same analysis was performed on HERs computed from the combined gradiometers. As opposed to magnetometers, we observed no significant correlation with SV

or significant difference between the low- and high-SV HERs on any type of data (ICA-uncorrected data, cardiac component, ICA-corrected data).

In summary, HERs computed on combined gradiometers are not sensitive to stroke volume, but HERs computed on magnetometer data are sensitive to fluctuations in stroke volume. An ICA correction tailored to target the cardiac artifact strongly attenuates the contribution of stroke volume to HERs computed on magnetometers but might not entirely remove it.

Figure 3. Correlation and median-split analysis of the MEG data



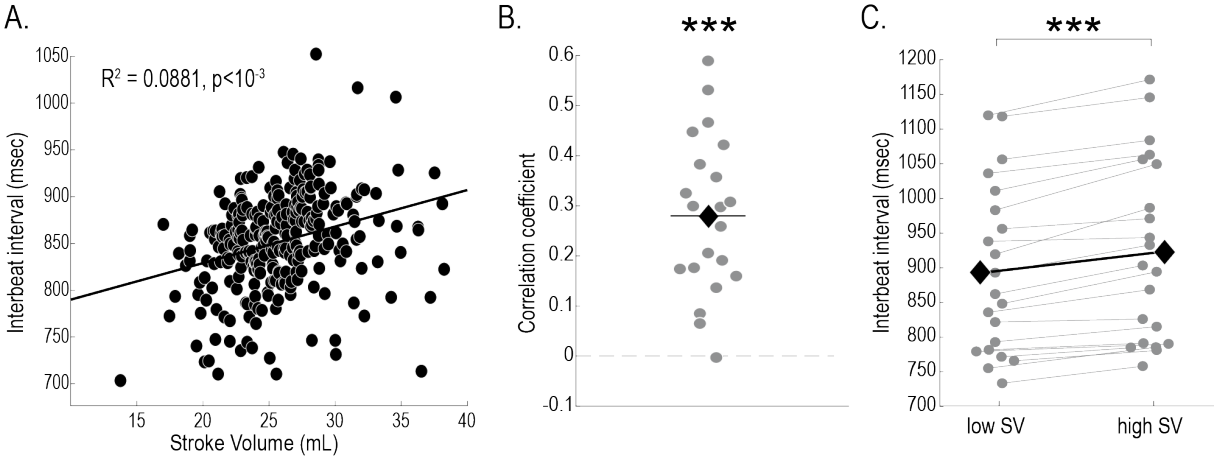
A. *Beat-to-beat correlation analysis between HERs computed from ICA-uncorrected MEG signal and SV, grand average across participants. Left: Topography of the cluster with a significant correlation, with significant channels highlighted in white. Right: Time course of the correlation averaged across significant channels, with significant time-points in darker shading.* ***B.*** *Comparison between low- and high-SV HERs computed from ICA-uncorrected MEG signal, grand average between participants. Left: Topography of the cluster with a significant difference, with significant channels highlighted in white. Right: Time courses of the HERs averaged across significant channels, with significant points in darker shading.* ***C and D*** *represent the same analysis performed on the cardiac ICA components.* ***E and F*** *represents the same analysis performed on the ICA-corrected MEG signal. Since no significant effect was observed, the time window and the channels used for the figure are the same than the one used for ICA uncorrected MEG signal.*

Electrocardiogram and heart rate co-vary with stroke volume

Recording stroke volume requires a specific equipment and current injection might create artifacts on MEG/EEG data. We thus tested whether stroke volume fluctuations are reflected in fluctuations of classical and more readily accessible measures of cardiac activity, namely ECG itself and interbeat intervals (IBIs).

At the group level, we observed a significant positive correlation between IBI and stroke volume (figure 4B, mean correlation coefficient ± sd = 0.28±0.16, one-sample t test comparing the Fischer-transformed correlation coefficients against 0, two-sided $t(20)=7.61$, $p<10^{-6}$). The correlation was confirmed at the individual level with a significant positive correlation observed in 86% (18 out of 21) of the participants. One example of co-variation between SV and IBI in one subject (S12) is shown on figure 4A. The comparison between high and low-SV trials analysis revealed a similar pattern with significantly longer IBIs in cardiac cycles with a larger stroke volume (figure 4C, mean difference between the high- and low-SV cardiac cycles ±sd = 29.70±18.45ms; one-sample t test, $t(20)=7.37$, $p<10^{-6}$). Individual data revealed a significant difference of IBI between high- and low-SV cycles in 81% (17 out of 21) of the participants.

Figure 4. Cardiac parameters: Stroke volume and IBI

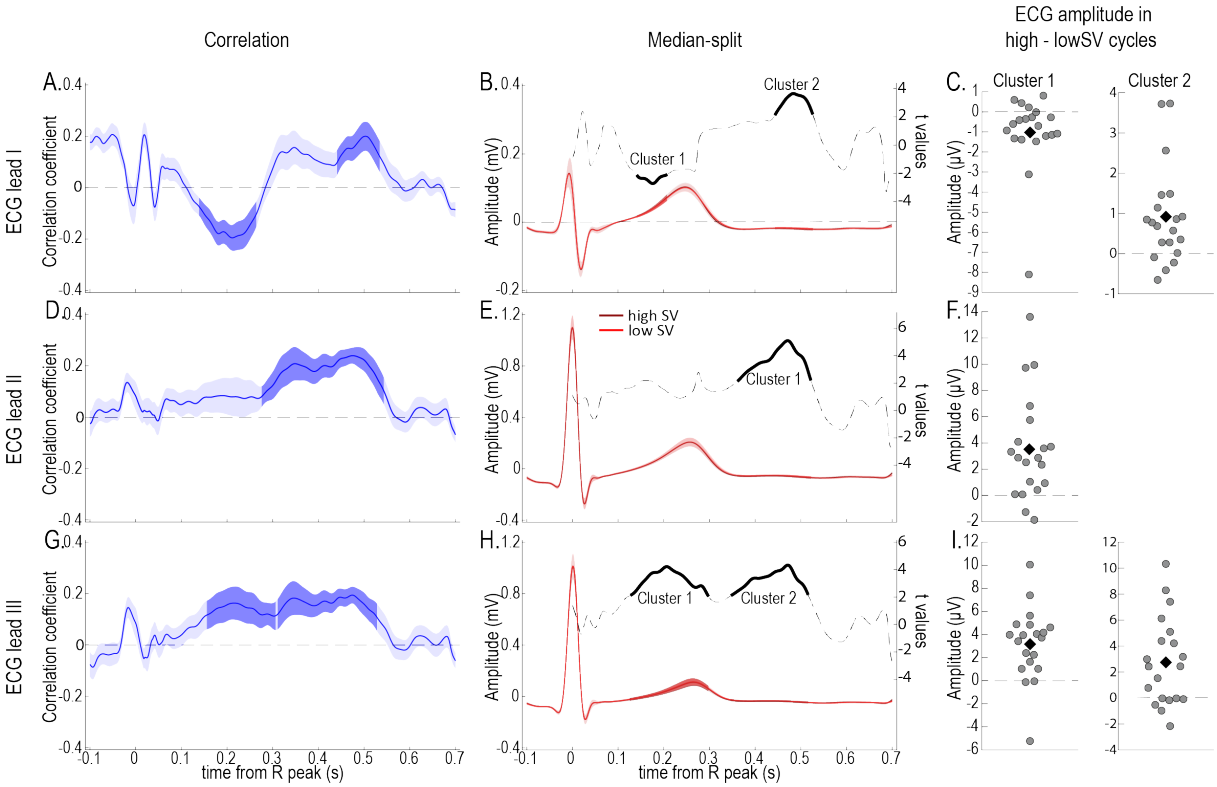


A. *Correlation between IBI and stroke volume in one subject with a correlation representative of the group mean (R^2=0.0881, $p<10^{-3}$).* ***B****. Correlation coefficients between IBI and stroke volume in each subject (grey dots) and mean across participants (black diamond) (mean±sd = 0.28±0.16, One sample t test on Fischer-transformed correlation coefficients against 0: t(20)=7.61, $p<10^{-3}$).* ***D.*** *Mean IBI in low and high-SV cardiac cycles in each subject (grey dots and thin lines) and average across participants (black diamonds and thick line) (median-split based on stroke volume; mean IBI in low-SV cardiac cycles ±se = 894±27ms, mean IBI in high-SV cardiac cycles ±se = 923±29ms, Paired t test: t(20)=7.37, $p<10^{-3}$). *** indicates $p<10^{-3}$.*

We then assessed the relationship between stroke volume and ECG, using the same clustering procedure previously used for MEG data, on three different ECG derivations, named lead I, lead II and lead III in reference to the Einthoven triangle (Figure 1A). We report results for three different ECG leads because each of them captures different features of cardiac activity and given the difference in ECG waveforms according to the leads, stroke volume effect might vary depending on the lead being studied.

As shown in detail in table 2 and illustrated on figure 5, all ECG leads significantly correlated with stroke volume at the group level, at different latencies within 125-540ms post R peak. In other words, spontaneous fluctuations in stroke volume are associated with modulations of ECG amplitude, during the ejection of the blood from the left ventricle, but also later in the cardiac cycle during diastole.

Figure 5. Correlation and median-split analysis of ECGs



A, D, C. *Time-course of the correlation coefficient between the stroke volume and ECG data (A, ECG lead I; D, ECG lead II; G, ECG lead III), grand average across participants. Significant clusters are indicated in darker shading.* ***B, E, H.*** *ECG data locked on the R peak, grand-averaged across participants, in low (light red) and high (dark red) SV trials, and t-values (black) of the comparison between the two. Significant clusters are indicated in bold. The difference between high and low SV is small compared to ECG amplitude, but consistent between participants and thus gives rise to a significant statistical effect.* ***C, F, I.*** *Difference in ECG amplitude in high – low-SV trials for each subject (grey dots), in each cluster, and grand average across participants (black diamond).*

Table 2. Summary of the correlation (top rows) and median-split (bottom rows) analysis on the three ECG leads

	Lead I			Lead II			Lead III		
	Latency (ms)	Sign of the correlation/ difference	Cluster statistics (sum(t), MonteCarlo p)	Latency (ms)	Sign of the correlation/ difference	Cluster statistics (sum(t), MonteCarlo p)	Latency (ms)	Sign of the correlation/ difference	Cluster statistics (sum(t), MonteCarlo p)
Correlation with SV	139-264	-	sum(t)=-399 p=0.01	276-543	+	sum(t)=-1126 $p<10^{-3}$	156-306	+	sum(t)=367 p=0.036
	441-534	+	sum(t)=294 p=0.026				316-527	+	sum(t)=821 p=0.02
SV High vs. Low	141-207	-	sum(t)=-160 p=0.044	364-522	+	sum(t)=599 p=0.001	126-297	+	sum(t)=563 p=0.005
	444-527	+	sum(t)=285 p=0.007				347-516	+	sum(t)=561 p=0.005

In conclusion, larger stroke volumes are significantly associated with longer cardiac cycles, and stroke volumes fluctuations are reflected in the ECG itself, during both systole and diastole.

Interbeat intervals and ECG are more sensitive to stroke volume than MEG data

We have shown that HERs computed from magnetometers, as well as IBIs and ECG are sensitive to stroke volume. Because measuring stroke volume by impedance cardiography requires a specific set-up and induces additional noise on electrophysiological data, it would be interesting to be able to use ECG or IBIs as proxies to detect stroke volume differences between experimental conditions. To explore this possibility, we computed the effect size (Cohen's d, see formula in the methods) for the difference between high- and low-SV variables (Table 3).

The largest Cohen's d was observed for IBI with an effect size of 1.61. The second largest Cohen's D was observed for ECG-lead II with an effect size twice smaller (0.83). Effect sizes on HERs computed from magnetometers were systematically smaller, reaching at most 0.64 when selecting the channel showing the largest effect. In conclusion, IBIs are twice more sensitive to stroke volume fluctuations than MEG or ECG data.

Table 3. Influence of stroke volume on the different variables tested: effect size (Cohen's d) of the difference between high and low SV trials.

MEG data show very similar effect sizes in ICA-uncorrected data and cardiac component identified with ICA, while no significant effect persists in ICA-corrected data. The effect observed on ICA-uncorrected data is thus fully captured by the cardiac components identified with ICA, and disappears from ICA-corrected data. For ECG, effect sizes pertain to the different clusters of significant difference between high and low SV trials reported in Table 2. ECG lead II is the most sensitive ECG lead to detect fluctuations in SV, and IBI is by far the most sensitive indirect measure of stroke volume.

	MEG (high vs low SV)		ECG (high vs low SV)			IBI (high vs. low SV)
	ICA-uncorrected	Cardiac components	Lead I	Lead II	Lead III	

	signal	identified with ICA				
Mean across all channels	0.61	0.62	0.54 (cluster 1) 0.70 (cluster 2)	0.83 (cluster 1)	0.74 (cluster 1) 0.73 (cluster 2)	1.61
Value on max channel	0.64	0.64				

Interindividual correlation between SV and HER amplitude

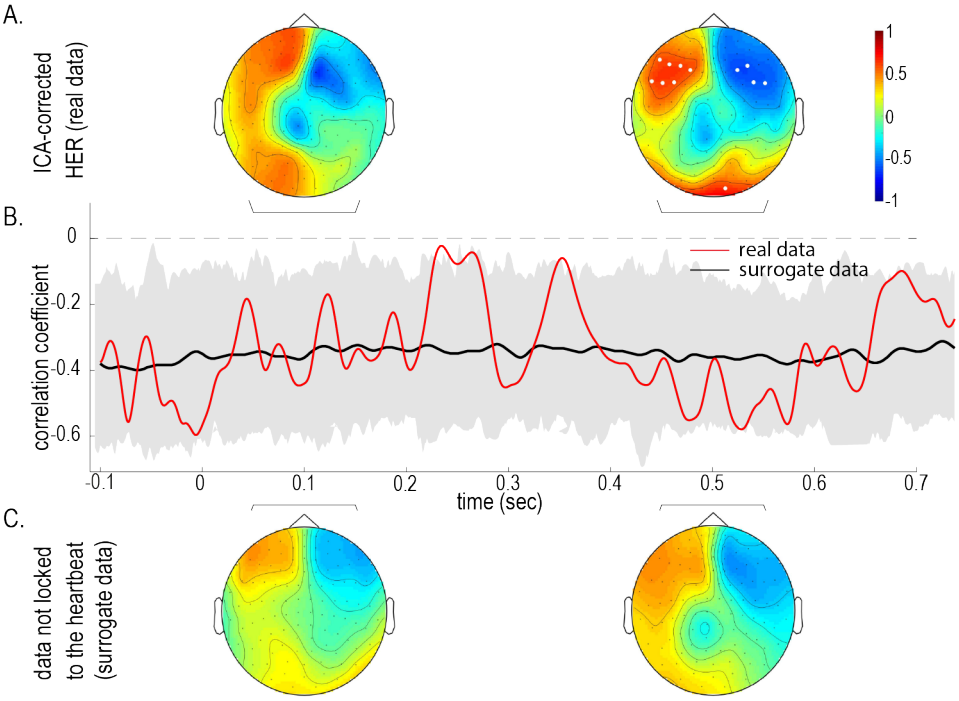
Given the previous report of an interindividual correlation between mean SV and mean HER's amplitude as measured with EEG in the 450 to 550ms window post Rpeak (Schandry and Montoya, 1996), we tested for an interindividual correlation between mean SV and HER's amplitude averaged in the same time window in MEG. Note that this analysis consists in correlating, across participants, the HER averaged across all heartbeats with the SV averaged across all heartbeats, i.e., very different from the beat-to-beat fluctuations reported so far in this article.

The HER amplitude averaged between 450 and 550ms in each participant on ICA-corrected magnetometers data significantly correlated with each participant's mean SV (Fig 6A right panel) on 12 channels (FDR-corrected $p<0.05$). Four of the 12 channels located over the right fronto-temporal regions showed a negative correlation (mean R=-0.61, all FDR corrected $p<0.05$), and 7 of the 12 channels, located over left fronto-temporal regions, showed a positive correlation (mean R=0.60, all FDR corrected $p<0.05$). An isolated channel over median occipital regions also showed a negative correlation (R=0.64, FDR-corrected $p=0.04$). Our results thus reveal a significant interindividual correlation between SV and HER amplitude in ICA-corrected magnetometer data, in the time-window in which Schandry and Montoya reported a correlation with HEP amplitude measured on EEG data.

However, and to our surprise, we observed that neither the topography (Fig 6A) nor the time-course of the correlation between SV and HER varied much over time (Fig 6B). This pattern of results does not seem compatible with the hypothesis of a transient HER driven

by aortic baroreceptors activity indexed by SV. We thus performed an additional analysis to probe whether at least part of the interindividual correlation between SV and HER was specifically locked to the heartbeat. To this end we performed the same analysis on surrogate datasets created by randomizing Rpeaks latency. We repeated this operation 150 times, and found that both the magnitude and the topography of the correlation between SV and surrogate HER data were similar to that of the correlation between SV and HER locked to the real heartbeats (Fig 6C). The results thus reveal the existence of a sustained, ongoing component in the correlation between MEG data and SV, which is not locked to heartbeats. The results do not exclude the possibility of an additional transient interindividual correlation between HER and SV locked to heartbeats since the empirical correlation coefficient in the 450-550ms time window reported by Schandry and Montoya (1996) tends to be larger than surrogate correlation coefficients.

Figure 6. Interindividual correlation between meanHERs and meanSV



A. *Topography of the interindividual correlation between mean SV and mean HER computed from ICA-corrected data in the 50 to 150ms time-window (left), and 450-550ms window post Rpeak (right). Channels with a significant correlation in the 450 to 550ms time-window are highlighted in white.* ***B.*** *Time-evolving interindividual correlation between mean SV and mean HER in real and surrogate data (data not locked to the heartbeat). The correlation coefficient computed from real data, averaged across the 4 channels showing a significant negative correlation in the 450 to 550ms time-window is shown in red. The correlation coefficient computed from surrogate data, averaged across the 150 simulations and the 4 channels with a significant correlation in the 450 to 550ms time-window is shown in black, with the 95% confidence interval shown in light grey. C. Topography of the interindividual correlation between mean SV and mean HER computed from surrogate data in the 50 to 150ms (left), and 450-550ms time-window post Rpeak (right).*

Discussion

HERs are often held to be related to the phasic arterial baroreceptor response to blood flow increase at each heartbeat, and should hence covary with stroke volume. However, because some mechanoreceptors in the heart are insensitive to stroke volume, HERs could also be independent from stroke volume magnitude – those two hypotheses not being exclusive. The question of the link between HERs and stroke volume is further complicated by the presence of the cardiac artifact, attenuated by ICA correction. Here, we evaluated the link between beat-to-beat changes in stroke volume and HERs. We found that in healthy participants at rest, stroke volume contributed to HER amplitude in ICA-uncorrected MEG data, over occipital and bilateral occipito-temporal areas with a late latency (500-700 ms post R-peak), as well as to the electrocardiogram in a wide, but earlier, latency range (150-550ms post R-peak). Using an ICA procedure designed to remove signal components covarying with the ECG, we found that SV influence disappeared from ICA-corrected MEG data. As further discussed below, this indicates that at least part of the HER (the one that remains after ICA correction) is unrelated to stroke volume and thus from transient arterial baroreceptor activity. In addition, we show that IBIs have a tight link with stroke volume, and are actually twice more sensitive to stroke volume fluctuations than ICA-uncorrected MEG data, which might be useful since stroke volume requires a specific equipment to be recorded, and can induce noise in electromagnetic recordings. Finally, we further explored, in ICA-corrected MEG data, the between-participant correlation between HERs and SV as originally reported in EEG (Schandry and Montoya, 1996). We replicated the correlation between mean HER's amplitude in the 450 to 550ms time-window post R-peak and mean SV across participants, but found that this correlation is actually sustained, not transient, with a stable topography over the whole cardiac cycle.

What is the origin of beat-to-beat fluctuations in HER related to SV?

Before ICA correction, HERs did co-vary with stroke volume. This effect could reflect transient responses to blood ejection in arterial baroreceptors transmitted with rather long delays to the brain. In this scenario, the ICA would overcorrect HERs by removing genuine neuronal activity.

But the ECG, which is directly captured in MEG data and contributes to the cardiac artifact, also varies with SV. It follows that the influence of SV on ICA-uncorrected MEG data

might come from the cardiac artifact. This is coherent with the observation that the ICA procedure is effective at removing the SV effect from MEG data, since the cardiac ICA components in MEG data are identified on the basis of their correlation with the ECG. Another argument in favor of this interpretation is that SV did not affect gradiometers data, which are less sensitive to distant sources than magnetometers data. However, if SV affected MEG data through the cardiac artifact, we would expect SV to co-vary with MEG at the same latencies than with ECG, which was not the case in our study. It is thus difficult to determine whether the influence of SV on ICA-uncorrected MEG data is due to a genuine neural response dependent on SV, or to the cardiac artifact. Our results also show that ICA removes from the data both the cardiac artifact and the SV influence (whether corresponding to neural activity or mediated by the cardiac artifact).

Whichever interpretation is correct, our results indicate that the source of SV influence on ICA-uncorrected data is statistically dependent on ECG signal. The ICA correction, which was computed on small epochs of data ranging from -200 to +200ms around the R peak, affected the SV effect occurring late in the cardiac cycle, starting 500ms after the R-peak. In other words, removing MEG components identified early in the cardiac cycle also modified MEG signal late in the cardiac cycle, suggesting some statistical dependencies between MEG signals at different phases of the cardiac cycle.

Another candidate to account for the SV effect on ICA-uncorrected MEG data is the pulse artifact. This artifact is related to the cardiac cycle but its exact origin remains unclear. It has been related to different factors such as small movement of the head triggered by blood vessels pulsation, as well as the movement of blood particles at the time of blood ejection (Debener et al., 2010), which have different magnetic properties depending on their oxygenation level (Tank et al., 1992). From what has been described in EEG studies, the pulse artifact seems to share similar characteristics with the SV effect since it varies within participants across cardiac cycles, and also with the duration of the cardiac cycle (Debener et al., 2010). Whether MEG data are affected by the pulse artifact is not clear. Whereas one study suggested that MEG data were not contaminated by blood flow (Jousmäki and Hari, 1996), another study suggested that a late component might be related to the flow of blood hitting the aortic arch (Rodin et al., 2005). However, in both MEG and EEG data, the pulse artifact seems to occur around 200ms after the R-peak (Allen et al., 1998; Rodin et al., 2005),

at the time of the ejection of blood from the left ventricle, which does not correspond to the latencies of the SV effect.

Arterial baroreceptors cannot be the only source of HER fluctuations

Whatever the origin of the SV influence on HERs in ICA-uncorrected data, our results show that after ICA correction, HERs do not depend on stroke volume. It follows that at least part of the beat-to-beat fluctuation in HER is independent from beat-to-beat fluctuations in the volume of blood ejected at each heartbeat. Hence, arterial baroreceptors should not be considered as the sole sources of HERs.

The attribution of HERs to arterial baroreceptor transient activity dates back to the correlation initially described by Schandry et al in EEG (1996), where participants with a small mean SV had smaller HERs in the 450 to 550ms post R-peak time-window. While we replicate this interindivual correlation in this time window, we show that the correlation between SV and HER and its topography are relatively constant during the entire cardiac cycle, suggesting the existence of a basal correlation between MEG data and SV not related to the heartbeat. Since the basal level of correlation was constant across time, it cannot be triggered by a discrete cardiac event such as the ejection of blood from the left ventricle. Still this observation has to be considered with caution for multiple reasons. First, we did not carefully test the correlation at each time-point with a proper statistical analysis. In fact interindividual analyses of stroke volume are not recommended when using impedance cardiography (Sherwood et al., 1990). Indeed, between participants variations in thoracic density, erythrocyte orientation and electrode placement may influence stroke volume in an unpredictable manner (Miller and Horvath, 1978). Second, we cannot rule out the existence of an additional transient response to the heartbeat since the correlation in the 450 to 550ms was slightly higher in magnitude than during the rest of the cycle. However, the topography of the correlation that is not uniform across channels suggests that it does not come from differences in measurements between participants such as different level of noise affecting both impedance cardiography and MEG data.

Altogether, the fact that within-subject correlation with SV disappeared after ICA correction, as well as the fact that the inter-subject correlation with SV was constant across the entire cardiac cycle is not in favor of the hypothesis that HER amplitude only depends on

the transient activity of arterial baroreceptors, itself shaped by the amount of blood ejected from the left ventricle.

Since our results question the hypothesis that HERs are driven by transient responses in arterial baroreceptors after blood ejection, other pathways might be considered. First, a great variety of baroreceptors exists, with both myelinated and non-myelinated pathways (Shepherd, 1985) and various number of synapses (Malliani et al., 1986). Additionally, baroreceptors are not only present in the arteries but also in the heart atria and ventricles (Armour, 1973). Activation of these baroreceptors could occur not only at the end of the systole when the blood is ejected from the left ventricle but at any time during the heart cycle, while the heart is filling. Finally, some mechanoreceptor activity is independent from blood pressure (Bishop et al., 1983). Beyond cardiac and arterial mechanoreceptors, additional pathways could also be recruited such as the skin somatosensory pathway (Khalsa et al., 2009) or more hypothetically the recently suggested vasculo-neuronal coupling (Kim et al., 2016). Finally, HERs during resting-state might reflect more cognitive parameters resulting from cortico-cortical interactions, such as content of spontaneous thoughts (Babo-Rebelo et al., 2016a, 2016b), than a direct ascending influence of cardiac parameters.

Implication and recommendation for future HER studies

The modulation of HERs amplitude according to stroke volume also means that a difference in HERs amplitude between two conditions, even if it occurs late in the cardiac cycle when the heart is electrically relatively silent, can still be related to a difference in cardiac activity and not solely to a difference in the way cardiac information are integrated at the level of the central nervous system. To eliminate or at least lower the influence of cardiac activity on HER amplitude, correction methods such as ICA are often used. Here, we showed that the stroke volume effect was present on the ICA cardiac components removed from the data and not anymore on the ICA corrected data. This highlights the importance of applying a correction procedure on brain data when studying HERs and shows the efficiency of the ICA in removing stroke volume effects. However it must be kept in mind that the ICA is a statistical procedure, with results that might slightly vary from one computation to the other. Additionally, the ICA procedure used in our study is not the standard textbook procedure since we segmented the data in 400ms time-window centered on the R-peak. Applying the same filters on ECG and MEG data might also be important to select the cardiac

components, even more in case of noisy datasets. Thus we cannot rule out that in some instances, depending on ICA parameters and efficiency, an effect of stroke volume might remain in ICA-corrected data. Finally, other artifact correction methods have been used to remove the cardiac field artifact from EEG or MEG data, such as template matching algorithms, spectral analyses or a combination of multiple procedures. It is thus important to remind the reader that the results presented here hold only for the specific ICA procedure that we used in this study and that stroke volume effects on HER might persist when using other correction methods.

Fortunately, stroke volume-related modulations were also observed on ECG and IBI, with significant correlations as well as differences in high- versus low-SV trials. The highest effect size was observed for the correlation between SV and IBI, followed by the correlation between SV and ECG lead II. This means that in the absence of stroke volume monitoring, a statistical comparison between ECG waveforms (with a preference for the lead II of the Einthoven triangle) and IBI durations between two experimental conditions can be used as a proxy to evaluate stroke volume differences between the two conditions. In the absence of differences in IBI or in ECG, it seems very unlikely that differences in brain response would be linked to a difference in cardiac activity and especially in stroke volume. It must be noted that the stroke volume effect on the ECG is observed both early and late in the cardiac cycle, and that SV-related difference in ECG amplitude is fairly small and undetectable by eye. Thus, to rule out an effect of cardiac parameters between two conditions using the ECG, we recommend performing statistical test on the entire ECG time-series to rule out a differential effect of experimental conditions on heart activity. Finally, the existence of a between-participant correlation between HER and SV, even after cardiac artifact attenuation with ICA, indicates that any comparison of HERs between groups of participants should carefully control for cardiac parameters.

As many HER studies are using EEG and not MEG to monitor brain activity, it would be important to also assess the stroke volume effect on HER computed from EEG data. It is difficult to speculate on this since the precise origin of the stroke volume effect on MEG data remains unknown. However, since the estimation of cardiac parameters such as IBI duration and ECG waveform do not vary between EEG and MEG recordings, our recommendations on carefully controlling for differences in cardiac parameters between conditions still hold in the case of EEG studies.

Conclusion

Whereas numerous recent studies point at a role of cognition in the modulation of HERs (Azzalini et al., 2019; Park and Blanke, 2019), the link between cardiac parameters and HERs has remained understudied. Our results call for a reconsideration of the hypothesis that HERs are driven by arterial baroreceptors activity and stroke volume fluctuations, at least when HERs are measured with MEG, since after the specific ICA correction applied here, HERs no longer co-vary with stroke volume. Still, the small within-subject correlation being observed between HER's amplitude and SV in ICA-uncorrected data highlights the need for a careful artifact rejection procedure, as well as rigorous assessment of cardiac parameters when comparing HERs between conditions.

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Data and code accessibility

Data and code available at:
https://osf.io/xr6zk/?view_only=002003ae99b947b4933322e2b80c6126

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