Methodological Considerations on EEG Electrical Reference: A Functional Brain-Heart Interplay Study

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***Abstract*— The growing interest in the study of functional brain-heart interplay (BHI) has motivated the development of novel methodological frameworks for its quantification. While a combination of electroencephalography (EEG) and heartbeat-derived series has been widely used, the role of EEG preprocessing on a BHI quantification is yet unknown. To this extent, here we investigate on four different EEG electrical referencing techniques associated with BHI quantifications over 4-minute resting-state in 15 healthy subjects. BHI methods include the synthetic data generation model, heartbeat-evoked potentials, heartbeat-evoked oscillations, and maximal information coefficient (MIC). EEG signals were offline referenced under the Cz channel, common average, mastoids average, and Laplacian method, and statistical comparisons were performed to assess similarities between references and between BHI techniques. Results show a topographical agreement between BHI estimation methods depending on the specific EEG reference. Major differences between BHI methods occur with the Laplacian reference, while major differences between EEG references are with the MIC analysis. We conclude that the choice of EEG electrical reference may significantly affect a functional BHI quantification.**

I. INTRODUCTION

The central nervous system continuously receives afferences from peripheral organs and systems through anatomical, functional, and biochemical pathways to maintain the homeostasis of bodily processes [1]. Particularly, brain structures including the somatosensory cortex, insula, anterior cingulate cortex, ventromedial prefrontal cortex, and amygdala may affect the heart electrical activity [1] and, likewise, autonomic and heartbeat dynamics may influence brain dynamics [2], [3]. Thereby, these interactions are commonly referred to functional brain-heart interplay (BHI), whose quantitative assessment may provide dynamic biomarkers involved in psychological and cognitive processes and associated pathological conditions [3]–[5].

From a methodological viewpoint, previous studies quantified functional BHI through model-free and model-based approaches, including heartbeat-evoked potentials (HEP) [6], heartbeat-evoked oscillations (HEO) [7], maximal information coefficient (MIC) [8], and synthetic data generation (SDG) models [9]. HEP corresponds to the neural responses evoked by each heartbeat and has been extensively investigated with electroencephalography (EEG) [6][10] with exemplary applications in cognition [3]. Additionally, HEP-related estimates have been proposed using EEG oscillations in specific frequency bands with respect to a pre-heartbeat baseline, namely heartbeat-evoked oscillations (HEO) [7]. Furthermore, a time-varying functional linear and nonlinear coupling between EEG and heart rate variability (HRV) series may be assessed through advanced correlation measurements such as the maximal information coefficient (MIC) [8][11]. Recently, a functional BHI estimation method based on synthetic data generation (SDG) models was proposed to quantify the BHI strength and directionality, i.e. from-heart-to-brain and from-brain-to-heart [9]. Note that an SDG-based estimation may be performed between EEG- and HRV-derived power spectrum series integrated within different frequency bands [9]. To this end, while HRV-derived power series within the low frequency band (0.04-0.15 Hz) are employed as a marker of sympathovagal activity, HRV power series integrated within the high frequency band (0.15-0.40 Hz) refer to the vagal activity [12].

Despite the aforementioned evidence, the role of EEG preprocessing on a functional BHI quantification has not been investigated yet. To overcome this limitation, here we investigate functional BHI in healthy subjects during resting state sessions to quantify differences between four commonly used EEG electrical references, including the Cz electrode (or vertex reference), common average, mastoids average, and Laplacian method, as well as between SDG, HEP, HEO, and MIC methods.

Notably, previous studies demonstrated that EEG electrical reference may significantly affect EEG-derived features, such as analyses on alpha oscillations and event-related potentials [13]–[15].

II. MATERIALS AND METHODS

A. *Data acquisition*

A group of 15 young healthy adults (mean age: 26 years, 7 females) were recruited for the recording of 128-channel high-density EEG (Electrical Geodesics, Inc) and one-lead ECG during 4-minute resting state. Data were sampled at 500 Hz. All subjects signed an informed consent, and the experimental procedure was approved by the local ethical committee.

B. *EEG pre-processing*

Data were processed and analyzed using MATLAB R2017a and Fieldtrip Toolbox [16]. EEG series were bandpass filtered within the 0.5-45 Hz band using a Butterworth filter. A wavelet-enhanced independent component analysis was applied to remove large movement artefacts [17], as well as the cardiac field artefact [10]. According to the 10-10 system, a subset of 64 channels were selected for further analysis to exclude sources located over the face and neck [18]. EEG channels were marked as corrupted if their area under the curve exceeded 3 standard deviations of all channels mean, or

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if the weighted-by-distance correlation with their neighbors was below $R_2 = 0.6$. Corrupted channels were replaced using a weighted-by-distance interpolation of neighbors.

EEG data were then re-referenced to the Cz electrode (CZ), common average (CA), mastoids average (MA), and Laplacian method (LM).

EEG power spectral density (PSD) series integrated within the α-band (8-12 Hz) were computed through short time Fourier transform with 2s time windows and 1s overlap.

B. ECG pre-processing

ECG series were bandpass filtered within 0.5-45 Hz using a Butterworth approximation. An automatic R-peak detection algorithm based on template correlation was applied [19], and detections were visually inspected for further analyses.

Series of high-frequency PSD (HRV-HF within 0.15-0.4 Hz) were computed from HRV series using an adapted Wigner-Ville distribution [20].

C. Brain-Heart Interplay Assessment

A functional BHI assessment was performed through the following methods:

1) *Synthetic Data Generation* (SDG) model, which assesses the bidirectional functional coupling between EEG oscillations and HRV-derived series [9]. Here the interplay is computed from a heart-to-brain direction, considering power series in the HRV-HF and EEG-α bands.

2) *Heartbeat-evoked potential* (HEP) refers to the neural response triggered by each heartbeat [6]. For each subject, HEP is computed by averaging EEG epochs within the 200-400 ms interval following each R-peak, without a baseline correction. HEP absolute values were analyzed in order to allow comparisons with the other BHI estimation methods.

3) *Heartbeat-evoked oscillations* (HEO) refers to the neural response triggered by each heartbeat within a specific EEG band [7]. Similar to HEP, HEO is computed by averaging EEG epochs within the 200-400 ms interval following each R-peak. However, HEO accounts for a relative change with respect to a baseline value calculated in the -300 to -200 ms interval. Here, HEOs were investigated within the α-band.

4) *Maximal information coefficient* (MIC) quantifies linear and nonlinear functional coupling between EEG- and HRV-derived series [8][11]. In this study, the MIC was computed between PSD series derived from the EEG-α and HRV-HF bands.

For each method and for each EEG reference, time-varying BHI dynamics was averaged over 4-minutes for further statistical comparisons.

D. Statistical analysis

Functional BHI estimates for all reference methods were z-scored within the 64-channels spatial maps for each subject.

Statistical analysis included group-wise topographical Spearman correlation coefficient (R) on concatenated samples from all subjects and all EEG channels. The derivation of the coefficients' p-values was performed through a t-Student distribution approximation. P-values significance level was corrected in accordance with the Bonferroni rule. Correlation analysis includes a total of 24 pairwise comparisons between EEG references and between BHI estimates, hence the corrected significance threshold was set to $\alpha=0.05/24=0.0021$, with an uncorrected statistical significance set to 0.05.

III. RESULTS

For a qualitative visual evaluation, Figure 1 shows group-wise median values of z-scored functional BHI estimates from SDG, HEP, HEO, and MIC estimation methods and CZ, CA, MA, and LM references for EEG data. Spatial distribution of functional BHI estimates over different cortical regions varies between methods. SDG and HEP show a positive gradient from central to frontal scalp regions, whereas HEO and MIC seem to have an opposite behavior. Furthermore, while SDG and MIC major changes are over the central and temporal areas, HEP and HEO show changes between references over the parietal and occipital areas. HEP and HEO present similar topographies between references, whereas SDG and HEP expose similarities for CA and MA. On the other hand, while LM's major differences seem to occur between methods, MIC seems to be associated with major differences between references. SDG shares similarities with MIC, particularly for the CA reference.

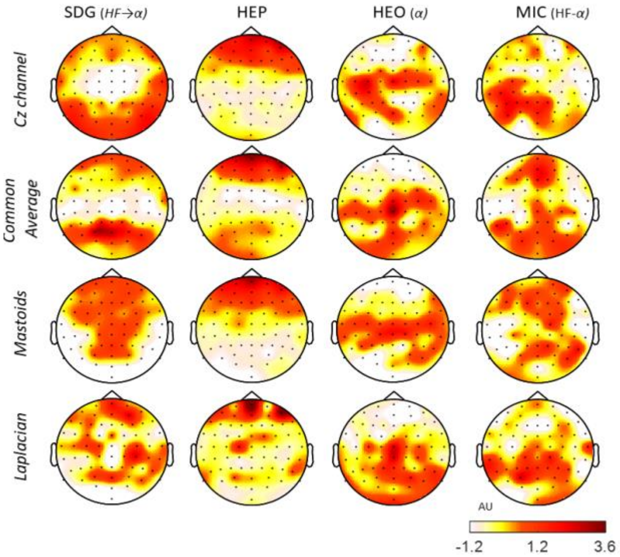

Fig 1. Group-wise median values of z-scored functional BHI estimates from SDG, HEP, HEO, and MIC methods, and EEG references including Cz channel, Common Average, Mastoids electrodes, and Laplacian method. Data refers to 4-minute grand average from N = 15 subjects. AU: Arbitrary Units.

Results from a quantitative non-parametric correlation analysis are reported in Tables I and II. Particularly, Table I shows correlation results between BHI methods for each EEG reference and confirm overall higher significant correlations between SDG and HEP for CZ and MA references, as well as between SDG and MIC for CZ, CA, MA and LM references. Method-wise, the LM reference shows lower correlation values and lowest number of significant correlations.

TABLE I. SPEARMAN CORRELATION STATISTICS BETWEEN BHI METHODS FOR EACH EEG REFERENCE METHOD.

Spearman Correlation Analysis	*CZ*	*CA*	*MA*	*LM*
SDG-HEP	**R = 0.1050** **p = 0.0011**	R = -0.0349 p = 0.2805	**R = -0.2436** **p < 0.0001**	R = -0.0205 p = 0.5256
SDG-HEO	R = 0.0268 p = 0.4062	R = -0.0252 p = 0.4359	R = 0.0187 p = 0.5618	R = 0.0153 p = 0.6358
SDG-MIC	**R = 0.2154** **p < 0.0001**	**R = 0.1215** **p = 0.0001**	**R = 0.2223** **p < 0.0001**	**R = 0.2034** **p < 0.0001**
HEP-HEO	R = 0.0242 p = 0.4542	**R = 0.1020** **p = 0.0016**	**R = 0.1004** **p = 0.0019**	R = 0.0503 p = 0.1196
HEP-MIC	**R = 0.1397** **p < 0.0001**	R = 0.0211 p = 0.5145	R = -0.0076 p = 0.8142	R = 0.0214 p = 0.5079
HEO-MIC	R = 0.0637 p = 0.0483	R = 0.0148 p = 0.6469	R = 0.0508 p = 0.1157	R = 0.0685 p = 0.0338

Bold indicates statistically significant correlation (corrected $p < 0.0021$)

Table II reports on the correlation analysis between EEG references for each BHI estimation method. A higher agreement between references is with SDG, HEP, and HEO, and higher correlation values are with HEP for comparisons not involving the LM reference. The MIC method is the most affected by the choice of EEG reference, showing the lowest number of significant correlations. Comparisons involving BHI estimates with the LM references are generally lower in magnitude, while the comparisons involving estimates from CA are higher in magnitude.

TABLE II. SPEARMAN CORRELATION STATISTICS BETWEEN EEG REFERENCE METHOD FOR EACH BHI METHOD.

Spearman Correlation Analysis	*SDG*	*HEP*	*HEO*	*MIC*
CZ-CA	**R = 0.639** **p < 0.0001**	**R = 0.999** **p < 0.0001**	**R = 0.695** **p < 0.0001**	**R = 0.165** **p < 0.0001**
CZ-MA	**R = -0.185** **p < 0.0001**	**R = 0.999** **p < 0.0001**	**R = 0.589** **p < 0.0001**	R = -0.015 p = 0.6517
CZ-LM	**R = 0.264** **p < 0.0001**	**R = 0.456** **p < 0.0001**	**R = 0.401** **p < 0.0001**	**R = 0.118** **p = 0.0002**
CA- MA	**R = 0.455** **p < 0.0001**	**R = 0.999** **p < 0.0001**	**R = 0.719** **p < 0.0001**	**R = 0.278** **p < 0.0001**
CA- LM	**R = 0.429** **p < 0.0001**	**R = 0.459** **p < 0.0001**	**R = 0.437** **p < 0.0001**	R = 0.088 p = 0.0066
MA -LM	**R = 0.341** **p < 0.0001**	**R = 0.459** **p < 0.0001**	**R = 0.428** **p < 0.0001**	R = 0.099 p = 0.0021

Bold indicates statistically significant correlation (corrected $p < 0.0021$)

IV. DISCUSSION

We reported on the role of EEG electrical reference in the assessment of functional BHI in resting state conditions. To this end, we processed EEG and ECG data gathered from 15 healthy participants to investigate similarities and differences between EEG references including CZ, CA, MA, and LM, and between BHI estimation methods including SDG, HEP, HEO, and MIC.

Overall, our findings show that a topographical agreement between BHI estimation methods may depend on the specific EEG reference. While major differences between BHI methods arises with the LM reference, major differences between EEG references may be associated with a MIC analysis.

More in detail, the significant non-parametric correlation values between SDG and MIC (see Table I) are in line with the occurrence of a linear and nonlinear functional interplay induced to the brain from heartbeat in the α-band (8-12 Hz), which is dominant in the resting state [21]. Nevertheless, while SDG shows significant correlations between EEG references, MIC seems to be mostly affected by the reference choice (see Table II). Note that in this preliminary study SDG was implemented to assess the directional interplay from the heart to the brain through specific power series in the HRV-HF and EEG-α bands. We speculate that the parametric and physiologically plausible structure of the SDG model [9] mitigates possible differences between EEG references, while a MIC analysis directly operates on EEG-derived measurements [11]. Note also that the MIC is a non-directional estimation method [8], therefore a further SDG analysis on the from-brain-to-heart direction is likely to provide additional information on the functional BHI at rest. Interestingly, HEP and HEO methods show significant correlations especially when using CA and MA references, suggesting that CZ and LM references may affect EEG activity in the -300 to -200 ms interval preceding the occurrence of a heartbeat.

We remark that different cortical regions may be differently affected by the specific EEG reference option. Indeed, previous studies reported that the specific choice of EEG reference mostly affects EEG features gathered from frontal electrodes [13]. In our study, qualitative group-wise topographical changes between EEG reference methods seem to be major with MIC and minor with HEP and HEO, whereas SDG and HEP show quite consistent activation maps (see Figure 1).

Previous studies challenged the use of a CZ reference (vertex reference) because it refers to a active cortical area [22][23]. On the other hand, the use of CA reference has been suggested because of the intrinsic independence with respect to specific scalp regions [24] and possible robustness with respect to changes in experimental conditions [25]. Nevertheless, the number of EEG electrodes used for the CA calculation might bias its estimation [25]. The MA reference may be associated with a reduced neural activity area with respect to other cephalic sites [22]. However, previous studies showed inconsistent performances for MA when compared to a CA reference [26]. Differently from other references, the LM accounts for changes in the current density across the scalp given the curvature of the brain electrical field [27], and thus the number of EEG electrodes may also affect its estimation [28]. In this study, the LM reference showed a significant influence on functional BHI estimation.

As a final note, we remark that different BHI estimation methods may refer and quantify different physiological processes underlying concurrent cortical and heartbeat potentials, which therefore may not always depend on the choice of EEG reference. A thorough comparison between BHI estimation methods and between EEG references should also be performed in experimental conditions other than resting state, including e.g. emotional or cognitive stress.

V. CONCLUSIONS

We conclude that EEG preprocessing with respect to the electrical reference may significantly affect functional BHI quantification depending on the estimation method. Our findings confirm the crucial role of EEG preprocessing procedure, which was already highlighted in case of EEG features defined in the α-band and event-related potentials. Future research directions will be directed to a larger data sample, also gathered in experimental conditions other than the resting state and EEG and HRV oscillations in different frequency bands.

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