Brain–Heart Interactions Reveal Consciousness in Noncommunicating Patients

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Objective: We here aimed at characterizing heart–brain interactions in patients with disorders of consciousness. We tested how this information impacts data-driven classification between unresponsive and minimally conscious patients.

Methods: A cohort of 127 patients in vegetative state/unresponsive wakefulness syndrome (VS/UWS; n = 70) and minimally conscious state (MCS; n = 57) were presented with the local–global auditory oddball paradigm, which distinguishes 2 levels of processing: short-term deviation of local auditory regularities and global long-term rule violations. In addition to previously validated markers of consciousness extracted from electroencephalograms (EEG), we computed autonomic cardiac markers, such as heart rate (HR) and HR variability (HRV), and cardiac cycle phase shifts triggered by the processing of the auditory stimuli.

Results: HR and HRV were similar in patients across groups. The cardiac cycle was not sensitive to the processing of local regularities in either the VS/UWS or MCS patients. In contrast, global regularities induced a phase shift of the cardiac cycle exclusively in the MCS group. The interval between the auditory stimulation and the following R peak was significantly shortened in MCS when the auditory rule was violated. When the information for the cardiac cycle modulations and other consciousness-related EEG markers were combined, single patient classification performance was enhanced compared to classification with solely EEG markers.

Interpretation: Our work shows a link between residual cognitive processing and the modulation of autonomic somatic markers. These results open a new window to evaluate patients with disorders of consciousness via the embodied paradigm, according to which body–brain functions contribute to a holistic approach to conscious processing.

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Patients with disorders of consciousness (DOC) are characterized by preserved wakefulness in the absence of clear evidence of awareness such that they remain unable to communicate with their surroundings.1 For example, patients in a vegetative state/unresponsive wakefulness syndrome (VS/UWS) open their eyes, but they do not show conscious responses to sensory stimulation.2 When patients exhibit signs of fluctuating yet

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reproducible remnants of nonreflex behavior, such as visual pursuit, they are considered to be in a minimally conscious state (MCS). The diagnostic assessment of patients with DOC is mainly based on the observation of motor and oculomotor behaviors at the bedside. The evaluation of nonreflex behavior, however, is not straightforward, as patients can fluctuate in terms of vigilance, and may suffer from cognitive and/or sensory impairments, from small or easily exhausted motor activity and pain, which may lead in the underestimation of the level of consciousness. Previous work employing data-driven analyses with neuroimaging and neurophysiological tools suggest relatively accurate patient diagnosis and prediction of clinical outcome.

Aside from this neurocentric approach, classic and more recent studies in interoception in healthy subjects demonstrate that brain modulation of peripheral body functions can be affected by concomitant cognitive processes. Therefore, such brain–body interaction might be relevant to evaluate consciousness states in DOC patients. Cardiac activity is a peripheral body signal that has been linked to cognitive processes. For example, “bradycardia of attention” refers to the effect of heartbeat frequency deceleration when the subject is engaged in an active cognitive task (such as target detection or auditory oddball counting). As regards patients with DOC, previous work has shown that cardiac autonomic markers, such as heart rate (HR) and heart rate variability (HRV), are markers of autonomic system malfunction (dysautonomia) after traumatic brain injury. However, the link between these autonomic markers and conscious cognitive processing in DOC patients remains unknown.

Here, we aimed at (1) characterizing the conscious state of DOC patients by means of heart–brain interactions and (2) determining whether the electrocardiogram (EKG)-extracted information may complement the single patient electroencephalographic (EEG) diagnosis of consciousness state. For the first objective, we quantified the modulation of cardiac cycle during an auditory stimulation protocol, known as the local–global paradigm, which is designed to test 2 hierarchical levels of processing of auditory regularities. For the second objective, we contrasted the performance of multivariate patient classification of the state-of-consciousness at the single patient level using solely EEG markers, and combining EEG and cardiac cycle modulation markers. We hypothesized a cardiac cycle modulation in the group of MCS patients and that this modulation will carry partially independent information about the state of consciousness, reflected in the form of an enhanced classification performance.

Subjects and Methods

Subjects and Patients

Patients admitted for consciousness evaluation at the Neurological Department of the Pitié-Salpêtrière Hospital, Paris between February 2008 and April 2015 were included. Informed consent was signed by the patients’ legal representatives. The protocol conformed to French regulations and the Declaration of Helsinki and was approved by the ethics committee CPP Ile de France 1 (Paris, France). The neurological evaluation of the patients’ disorders of consciousness was performed by trained clinicians, including the Coma Recovery Scale–Revised (CRS-R). CRS-R scoring ranges from 0 to 23 and is based on the presence or absence of response on a set of hierarchically ordered items testing auditory, visual, motor, otoromotor, communication, and arousal functions. Behavioral evaluations were performed systematically before each EEG recording.

In the present study, we aimed at characterizing the cardiac cycle in relation to the state of consciousness as a post hoc analysis. No EKG was available during the EEG evaluations. As a consequence, EKG time series were obtained using independent component analysis (ICA) on the EEG recordings for each patient. The current analysis only used the temporal location of the R wave peaks.

From the 259 patients originally assessed with EEG (130 VS/UWS, 129 MCS), 132 patients (51%; 60 VS/UWS, 72 MCS) were rejected due to the lack of a clear EEG recording or EKG reconstructed source that produced at least 40 samples for each stimulation block type. There were no differences between the included and excluded patients in terms of diagnostic state (χ²[1, n = 259] = 2.07, p = 0.15) and sex (χ²[1, n = 259] = 0.21, p = 0.64). Included patients were older than excluded patients (48 ± 18 vs 44 ± 17 years; W = 6701, p = 0.04), and more patients suffered from anoxic as compared to traumatic injuries in the included group compared to the excluded group (χ²[4, n = 259] = 12.84, p = 0.01).

A final cohort of 127 (49%) patients remained: 70 in VS/UWS (20 females, mean age = 45 ± 19 years, range = 17–80, 12 traumatic, 21 assessed in a chronic setting [ie, >2 months posts insult]), and 57 in MCS (17 females, mean age = 52 ± 16 years, range = 21–79, 13 traumatic, 17 assessed in a chronic setting). Patient groups did not differ in terms of gender (χ²[1, n = 127] = 6.2e−31, p = 1), etiologies (χ²[4, n = 127] = 9.4, p = 0.051), and chronicity (χ²[1, n = 127] = 2e−36, p = 1). MCS patients were older than VS/UWS patients (52 ± 16 vs 45 ± 19; W = 2435, p = 0.03). No patient had any history of cervical spinal cord injury or symptoms of autonomic dysfunction (eg, hemodynamic instability, abnormal HRV) at the time of EEG recording.

Auditory Stimulation and EEG

Cognitive processing was prompted by means of the EEG-based auditory local–global paradigm. The local–global paradigm is characterized by 2 embedded levels of auditory
regularities (Fig 1A). Each trial is formed by 5 consecutive sounds lasting 50 milliseconds, with a 150-millisecond gap between the sounds’ onsets and an intertrial interval ranging from 1,350 to 1,650 milliseconds. The fifth sound can be either equal to or different from the first 4; this defines whether the trial is standard or deviant at a local level. The second level of regularities is defined across trials (or at a global level); frequent trials (80%) define the regularity, and rare ones (20%) violate this regularity. Two types of stimulation blocks are played to the subjects; in the XX blocks, the frequent stimulus corresponds to 5 equal sounds (local standard and global standard [LSGS]). In contrast, the infrequent stimulus corresponds to 4 equal sounds followed by a fifth different sound (local deviant and global deviant [LDGD]). In the XY blocks, the frequent stimulus corresponds to 4 equal sounds and a fifth different sound (local deviant and global standard [LDGS]). The infrequent stimulus corresponds to 5 equal sounds (local standard and global deviant [LSGD]; see Fig 1A). The local effect is quantified by contrasting all local deviant (LD) trials (LDGS + LDGD) versus all local standard (LS) trials (LSGS + LGSD). The global effect is quantified by contrasting all global deviant (GD) trials (LSDG + LDGD) versus all global standard (GS) trials (LSGS + LDGS). Patients were stimulated vocally between each stimulation block (/C24/3.5 minutes, task instruction) and with tactile stimulations if patients appeared asleep (pressure as recommended in the “arousal facilitation protocol” in the CRS-R). A mask was applied on the eyes to normalize the eyes open/closed across patients. EEG recordings were performed using a NetAmps 300 amplifier (Electrical Geodesics, Eugene, OR) at a sampling rate of 250Hz with a 256-electrode HydroCel Geodesic Sensor Net (Electrical Geodesics) referenced to the vertex.

**EKG Extraction from EEG**

In the absence of direct recordings of cardiac activity, EKG was extracted from the EEG using ICA. The independent components (ICs) corresponding to the EKG were selected by visual inspection based on the spatial and temporal representation of the QRS complex. Raw EEG data were first filtered using an eight-order low-pass Butterworth filter at 45Hz and a fourth-order high-pass filter at 0.5Hz (Fig 2A). Second, we computed 3 different ICA decompositions: (1) FastICA\textsuperscript{23} parametrized to obtain the components that explain 99% of the variance and computed from raw filtered data, (2) INFOMAX\textsuperscript{24,25} parametrized to obtain 256 components from raw filtered data, and (3) INFOMAX in combination to artifact channels rejection. Individual channels were removed when the temporal variance was $>3$ standard deviations away from the mean of the variance of the rest of the channels. The IC with the EKG information was selected based on the time series and the weights’ topographies by visual inspection (see Fig 2B). The selected time series had to clearly contain the R peak corresponding to the QRS complex. The R peak had to be easily detected by using a simple threshold. The corresponding topography had to concentrate the mixing weights on the frontal right and posterior left electrodes. These electrodes are located in the right cheek, left maxillary junction, and underneath the left mastoid, as depicted by previous studies on cardiac electrical fields.\textsuperscript{26} We then picked the algorithm that presented the clearest decomposition, usually the one with the highest rank in
descending order of explained variance. Finally, R peak onsets were obtained automatically by the algorithm described in Elgendi. Subjects for whom the EKG component was unclear were excluded from the analysis. Exclusion criteria were set to any of the following: EKG reconstructed signal with no clear R peaks, detection failure by the automatic algorithm, or

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**FIGURE 2.**
Baseline Cardiac Activity
The overall HR was computed by averaging the differences between consecutive R peaks (RR intervals; see Fig 1B) during the whole recording. Following the method described in Deboer et al., HRV spectral variables were obtained by computing the power spectrum decomposition on the point events time series from the detected R peaks. Power spectral density was estimated in whole recording using Welch’s method with 32,768 samples (131.072 seconds) per segment and 28,672 samples (114.688 seconds) overlap using a Hanning window. HRV variables were extracted from the sum of the spectral power in 3 frequency bands: (1) very low frequency (VLF; HRV variables were obtained by computing the power spectrum decomposition on the point events time series from the detected R peaks. Power spectral density was estimated in whole recording using Welch’s method with 32,768 samples (131.072 seconds) per segment and 28,672 samples (114.688 seconds) overlap using a Hanning window. HRV variables were extracted from the sum of the spectral power in 3 frequency bands: (1) very low frequency (VLF; range = 0–0.04Hz), (2) low frequency (LF; range = 0.04–0.15Hz) and (3) high frequency (HF; range = 0.15–0.4Hz).

R Peak Locked EEG Evoked Responses
EEG recordings were filtered as previously described, segmented from −200 milliseconds to 600 milliseconds relative to the onset of the R peak and baseline corrected using the 200-millisecond long window before R peak. Bad channels and trials were rejected based on peak-to-peak amplitude exceeding 100 µV. Bad channels were interpolated. The remaining trials were averaged. We performed a group analysis and obtained the mean evoked response for each group, and contrasted the VS/UWS mean evoked activity to the MCS one. Statistics on EEG responses were done using nonparametric cluster corrected permutation test.

Markers of Cardiac Cycle Modulation Induced by the Auditory Stimulation Protocol
To evaluate potential phase shifts in the cardiac cycle associated with the processing of different types of auditory stimuli, 2 intervals temporally locked to the onset of the fifth sound were defined (see Fig 1B): (1) the PRE interval, that is, the interval between the heartbeat (defined by the location of the R peak) preceding the stimulus and the onset of the auditory stimulation; and (2) the POST interval, that is, the interval between the stimulus onset and the following heartbeat. All time intervals were then labeled according to the contained auditory stimulation following the local–global paradigm (XX block: LSGS or LDGD; XY block: LDGS or LSGD). Finally, in order to avoid using peaks without a clearly defined temporal association to a given heartbeat (and not the previous or following one), we restricted the analysis to the trials in which both the PRE and POST intervals were between 20 and 600 milliseconds. A mean of 520 ± 150 trials per subject were included, whereas 135 ± 100 trials were rejected (20 ± 13%). A repeated measures Bayesian analysis of variance (ANOVA) was computed for each interval using the ratio of rejected trials as the study variable and the trial label and clinical state as factors. All the models including the clinical state factor presented evidence for no difference (PRE BF01 ≥ 2.35, POST BF01 ≥ 2.61). When the models included the trial type factor, the test showed strong evidence for no difference (PRE BF01 ≥ 39.19, POST BF01 ≥ 45.17).

To test whether conscious processing of auditory regularities affects the ongoing cardiac activity, we analyzed the PRE and POST stimulus intervals for each group of subjects in relation to the type of trials. For the local effect, each subject mean of the PRE and POST intervals corresponding to LD trials was subtracted from the mean of the LS ones. Similarly, for the global effect, the mean of the PRE and POST intervals corresponding to GD trials was subtracted from the mean of GS ones.

Multivariate Pattern Analysis
To analyze the relevance and independence of the markers regarding the diagnosis of DOC, we used multivariate pattern analysis (MVPA) in combination with \textit{wrappers} algorithms for feature selection. This method consists on training classifiers with different sets of features and comparing the obtained performance. Based on the performance comparisons, a set of features can be defined as (1) strongly or weakly relevant, when they are partially independent and contribute to an optimal classification; or (2) irrelevant, when they do not contribute to the classification.

MVPA were done using 120 EEG-extracted markers (corresponding to quantification of power spectrum and complexity in individual EEG sensors and information sharing

FIGURE 2: Electrocardiographic (EKG) independent components (ICs) present no difference in the QRS complex between clinical groups. (A) Time series from 7 typical electroencephalographic (EEG) sensors from 1 unresponsive wakefulness syndrome (UWS) patient. (B) Corresponding time series of 7 independent component analysis (ICA) components extracted from the previous EEG recording and the respective weight topographies. The IC with cardiac information is shown in red. Dotted lines represents the automatically detected R peak. (C) Mean weight topographies for each clinical group (top). A sensor-wise Bayesian t test shows evidence for no difference in the topographies between groups (bottom). (D) Mean and standard error of the mean (SEM) for each clinical group QRS complex from the ICA-extracted EKG. A single channel cluster permutation test indicated significant differences (p = 0.017) only between 184 and 344 milliseconds after the R peak, consistent with the location of the T wave. (E) We evaluated 2 independent groups of healthy (H) controls (n = 12) and patients (n = 12) using simultaneous EEG and EKG recordings. For each subject, EKG was also extracted using the described ICA method. We then computed the differences between each R peak onset detected in the direct EKG and the corresponding R peak detected using ICA (left). Right panel shows the mean difference and 95% confidence interval for each type of trial and subject as measured in samples (1 sample = 4 milliseconds). Using Bayesian analysis of variance, we found no evidence for a difference as an effect of the trial type (BF01 > 4.27) and strong evidence for no difference for the interaction between the type of trial and the clinical state (BF01 > 15). LDGD = local deviant and global deviant; LDGS = local deviant and global standard; LSGD = local standard and global deviant; LSGS = local standard and global standard; MCS = minimally conscious state; VS = vegetative state.
between EEG sensors) as described in Sitt et al7 (see supplementary material in the same paper) and 8 EKG-extracted markers. We trained a support vector classifier (SVC) to distinguish between the VS/UWS and the MCS patients with a penalization parameter equal to 1. The SVC was repeatedly cross-validated with randomized stratified k-folding (k = 8). Previously to the training of the classifier, relevant features were automatically selected keeping the highest 20% of the ANOVA F scores. Performance of the classifier was measured using area under the curve (AUC) scores. We defined 3 sets of features: (1) EKG markers of cognitive processes, corresponding to the PRE and POST intervals for the local and global contrasts (termed EKGreg); (2) EKG markers of baseline vegetative function (termed EKGveg), corresponding to the HR and HRV at the 3 frequencies previously defined; and (3) EEG markers. We estimated the accuracy of the classification algorithm with 6 different combinations of these sets of markers: (1) EKG + EKGreg + EKGveg, (2) EEG + EKGreg, (3) EEG + EKGveg, (4) EKG markers only, (5) EEG with both EKGreg and EKGveg markers shuffled, and (6) EKGreg + EKGveg markers only. To minimize the effect of the random selection of folds, the AUC scores were averaged across 250 repetitions.

Statistical Analysis
Statistical analysis encompassed correlations using Pearson product–moment correlation coefficient (r) and Spearman rank correlation coefficient (rho) with corresponding probability values. Pearson chi-square and Wilcoxon rank sum test were used to test for independence between the diagnosis and the demographic information of the patients. Bayesian ANOVA was performed to test the differences between groups using the BayesFactor R package (JZS Bayes factor with “medium” default prior setting r = 0.5; Rouder et al.31 Morey et al.32 R Development Core Team33). Bayes factor (BF) interpretation was done according to the Raftery scale.34 Differences across stimulation blocks were tested using 2-sided paired samples signed tests. MVPA models were tested using nonparametric Kruskal–Wallis test adjusted for multiple comparisons.

All these steps were performed with custom-made software made in Python 3.4 in combination with Scikit-Learn35 and MNE-Python.36,37 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines38 were followed thoroughly.

Results

EKG Extraction Method Validation
To test the homogeneity of the EKG-related ICA decompositions between groups, we computed the mean IC weights across subjects for the selected components. A sensor-wise Bayesian t test showed evidence for no difference in the weights between the MCS and VS/UWS groups (see Fig 2C). We then averaged the cardiac cycle locked to the QRS complex at the group level and contrasted the obtained time series between clinical groups (see Fig 2D). A single channel cluster permutation test found only 1 significant difference (p = 0.017) between 184 and 344 milliseconds after the R peak, consistent with the location of the T wave. No difference was found in the QRS complex.

Finally, we aimed at ensuring that the results obtained in terms of phase shifts of the cardiac cycle induced by the processing of the auditory stimulation paradigm were not a side effect of the EKG extraction methodology. In other words, we focused on testing that EEG-ICA extraction methodology was not injecting relevant EEG-related activity into the EKG extracted signal. For this objective, we compared pure EKG to EEG-extracted EKG. We performed simultaneous EEG-EKG recordings in an independent group of 24 healthy subjects and 32 patients (14 VS/UWS, 18 MCS). We applied the same EKG extraction method previously described and obtained 12 (50%) healthy subjects and 12 (37.5%); 3 VS/UWS, 9 MCS) patients with both direct EKG and indirect EEG-extracted EKG. We contrasted the 2 corresponding EKG time series in each trial, by subtracting the timing of the R peaks in the direct EKG signal from EEG-extracted signal (see Fig 2E). A repeated measures Bayesian ANOVA was computed using the R^EKG–R^EEG time differences as the study variable, and trial types (LSGS, LDGD, LDGS, and LGSD) and clinical state as factors. All the models including the trial type as a factor presented positive evidence in favor of no difference (BF01 ≥ 4.27). Furthermore, the model that tested the interaction between clinical state and trial type presented even stronger evidence of no difference (BF01 ≥ 15). Given that the only information used in this study was the timing of the R peaks (automatically extracted and analyzed within subjects), the validation results presented here strongly suggest that no effect was induced by the adopted EKG extraction methodology.

Baseline Cardiac Activity
Overall HR was similar in patients across the 2 diagnostic groups (BF10 = 0.73; Fig 3A). When patients with overlapping behavioral CRS-R scores (CRS-R = 6 or 7; 10 MCS, 20 VS/UWS) were excluded from the analysis, there was evidence for faster heart frequencies in the VS/UWS group (BF10 = 8.80). In the VS/UWS group, a positive correlation was identified between the HR and the CRS-R scores (rho = 0.27, p = 0.02). No such correlation was found for the MCS patients. Similarly, HRV markers were comparable in both diagnostic groups (see Fig 3B–D; HF HRV, BF10 = 0.62; LF HRV, BF10 = 0.36; VLF HRV, BF10 = 0.21). In the VS/UWS group, a positive correlation was identified between the CRS-R and the HRV markers at HF (rho = 0.40, p = 0.0007) and at LF (rho = 0.27, p = 0.02). No such
correlations were identified for the MCS group at either frequency.

R Peak Locked EEG Evoked Responses
In terms of evoked responses to the cardiac activity as measured by EEG, a sharp peripheral bipolar topography was observed at the R peak for both clinical groups (Fig 4A, B). Between 0 and 250 milliseconds after the R peak, both groups presented topographies following the pattern of the cardiac field artifact (CFA). A cluster-level permutation test revealed a single significant cluster ($p < 0.034$; see Fig 4C) located between 144 and 540 milliseconds after the R peak, with 2 spatial patterns, one similar to the CFA associated with the T wave between 144 and 340 milliseconds, and the second central spatial pattern after 340ms.

Cardiac Cycle Modulation Induced by the Auditory Stimulation Protocol
There was no evidence for difference in cardiac cycle modulation between groups due to the processing of the local regularities in either the PRE (BF$_{10} = 0.19$) or the POST (BF$_{10} = 0.19$) intervals (Fig 5). Within the groups, neither the VS/UWS nor the MCS patients presented significant differences between LS and LD trials (sign test LD-LS trials: VS/UWS, $p > 0.7$; MCS,
In the case of the global effect, there was no evidence of modulation difference between groups due to the global auditory processing in the PRE interval ($BF_{10} = 0.21$). On the contrary, in the POST interval, there was strong evidence for a difference between the MCS and VS/UWS groups ($BF_{10} = 43.07$; see Fig 5).
This result is explained by a shortening of the POST intervals in the GD trials compared to the GS trials in the MCS patients (sign test GD-GS trials, \( p = 0.007 \)) and no difference between GD and GS in the VS/UWS patients (sign test GD-GS trials, \( p = 0.55 \)). The small sample of healthy controls included in this study presented a pattern of results similar to the MCS subjects (although not statistically significant). Each dot represents a patient in vegetative state/unresponsive wakefulness syndrome (VS/UWS, \( n = 70 \)) or in minimally conscious state (MCS, \( n = 57 \)), or a healthy control (Healthy, \( n = 12 \)). Boxplots with interquartile range, median (black line), and mean (dashed line) represent the distribution of data in the clinical groups. [Color figure can be viewed at www.annalsofneurology.org]

**Correlation between EEG and EKG Markers of Consciousness**

We tested the relationship between cardiac cycle modulation markers and EEG markers that previously were reported to distinguish VS/UWS and MCS patients. These markers include quantifications of the spectral distribution of the EEG (such as power in the different frequency bands), the complexity of the EEG (such as Kolmogorov complexity and spectral entropy), and the information sharing between EEG electrodes (such as weighted symbolic mutual information [wSMI]). The modulation of the POST interval due to the global effect significantly correlated with EEG Kolmogorov complexity \((r = -2.31, p = 0.02)\), permutation entropy \((r = -2.63, p = 0.01)\), spectral entropy \((r = -2.3, p = 0.02)\), wSMI \((r = -0.19, p = 0.02)\), and normalized delta power \((r = 0.2, p = 0.02)\). No correlation was found between EEG evoked responses to the global effect and the phase shifts computed in the EKG (see Table for all markers). Nevertheless, none of the computed correlations survived a false discovery rate correction from multiple comparisons.
Multivariate Patient Classification by Means of EKG and EEG Markers

To determine whether the EKG-extracted information is partially independent of the consciousness-related information extracted from the EEG, we trained classifiers to distinguish clinical groups and compared the performance of using as features EEG markers alone or combinations of EEG and EKG markers. Multivariate analysis combining EEG and EKG cog showed better performance compared to EEG and EKG veg markers and EEG markers alone (Fig 6). Combining the EKG cog and EEG markers led to an improvement of the performance (EEG + EKG\textsubscript{cog}, AUC = 76.1; EEG + EKG\textsubscript{cog} + EKG\textsubscript{veg}, AUC = 75.7). Conversely, when EKG\textsubscript{cog} markers were not included in the MVPA, the performance did not differ from EEG alone (EEG only, AUC = 73.7; EEG + EKG\textsubscript{veg}, AUC = 73.3). As a control test for the effect of the number of features, classification was also computed combining EEG and label-shuffled EKG markers; in this case, the AUC was estimated at 73.6.

Using solely cardiac markers, the classifier performed above chance, with a mean AUC of 60.1. When we compared the performance of MVPA\textsubscript{s} that included EEG features, we only found significant differences when the MVPA\textsubscript{s} also included EKG cog versus when the MVPA\textsubscript{s} did not include these cardiac features ($p < 1e^{-9}$, Kruskal–Wallis test, corrected for multiple comparisons).

The inclusion of EKG\textsubscript{veg} features did not significantly change the performance of the tested MVPA classifiers ($p > 0.1$).

Discussion

We here aimed at characterizing consciousness state in patients with DOC by means of baseline heart activity and heart–brain interactions. We tested whether cardiac-extracted information can complement single patient EEG-based classification performance. When we contrasted behaviorally nonoverlapping VS/UWS and MCS patients, we found higher HR and HRV in the VS/UWS than MCS group, in accordance with a recent study.39 This comparison included MCS patients who were at the higher end of the CRS-R scale in comparison to the VS/UWS patients, who were at the lower end of the CRS-R. When all DOC patients were included to reflect clinical reality, we did not find group differences of overall cardiac autonomic markers between the groups. This suggests a common underlying baseline cardiac function across patients. Interestingly, we found a positive correlation between CRS-R total scores and 3 autonomic markers (HR, HF HRV, and LF HRV) only in the VS/UWS patients.

| Marker        | $R$ Statistic | $p$   | $p$, FDR Corrected |
|--------------|--------------|------|-------------------|
| CNV          | 0.45         | 0.649 | 0.744             |
| K            | -2.31        | 0.022*| 0.138             |
| PE           | -2.63        | 0.009*| 0.138             |
| Alpha        | -1.30        | 0.192 | 0.412             |
| Alpha N      | -1.31        | 0.190 | 0.412             |
| Beta         | -1.91        | 0.058 | 0.251             |
| Beta N       | -1.53        | 0.128 | 0.349             |
| Delta        | 0.85         | 0.393 | 0.562             |
| Delta N      | 2.40         | 0.017*| 0.138             |
| Gamma        | -1.67        | 0.096 | 0.321             |
| Gamma N      | -1.43        | 0.154 | 0.385             |
| SE           | -2.30        | 0.023*| 0.138             |
| Theta        | -0.50        | 0.616 | 0.744             |
| Theta N      | -0.94        | 0.345 | 0.562             |
| MSF          | -1.61        | 0.108 | 0.324             |
| SEF90        | -1.87        | 0.062 | 0.251             |
| SEF95        | -1.84        | 0.067 | 0.251             |
| wSMI         | -2.34        | 0.020*| 0.138             |
| GD-GS        | 0.54         | 0.589 | 0.744             |
| LD-LS        | 0.43         | 0.662 | 0.744             |
| LSGD-LDGS    | -0.31        | 0.756 | 0.810             |
| LSGS-LDGD    | -0.99        | 0.320 | 0.562             |
| MMN          | -0.86        | 0.387 | 0.562             |
| Delta P3a    | -0.02        | 0.979 | 0.979             |
| Delta P3b    | -0.42        | 0.669 | 0.744             |
| P1           | -0.22        | 0.820 | 0.848             |
| TOPO P3a     | -0.86        | 0.387 | 0.562             |
| TOPO P3b     | -1.11        | 0.265 | 0.501             |
| DECOD global | 0.62         | 0.532 | 0.726             |
| DECOD local  | 1.11         | 0.267 | 0.501             |

For detailed information, see Sitt et al7 (including supplementary material). *Statistically significant.

CNV = Contingent Negative Variaton; DECOD = Decoding; FDR = false discovery rate; GD = global deviant; GS = global standard; K = Kolmogorov complexity; LD = local deviant; LS = local standard; MMN = Mismatch Negativity; MSF = Median Power Frequency; PE = permutation entropy; SE = spectral entropy; SEF90 = Spectral Edge 90; SEF95 = Spectral Edge 95; TOPO = Topography; wSMI = weighted symbolic mutual information.

Multivariate Patient Classification by Means of EKG and EEG Markers

To determine whether the EKG-extracted information is partially independent of the consciousness-related information extracted from the EEG, we trained classifiers to distinguish clinical groups and compared the performance of using as features EEG markers alone or combinations of EEG and EKG markers. Multivariate analysis combining EEG and EKG\textsubscript{cog} showed better performance compared to EEG and EKG\textsubscript{veg} markers and EEG markers alone (Fig 6). Combining the EKG\textsubscript{cog} and EEG markers led to an improvement of the performance (EEG + EKG\textsubscript{cog}, AUC = 76.1; EEG + EKG\textsubscript{cog} + EKG\textsubscript{veg}, AUC = 75.7). Conversely, when EKG\textsubscript{cog} markers were not included in the MVPA, the performance did not differ from EEG alone (EEG only, AUC = 73.7; EEG + EKG\textsubscript{veg}, AUC = 73.3). As a control test for the effect of the number of features, classification was also computed combining EEG and label-shuffled EKG markers; in this case, the AUC was estimated at 73.6. Using solely cardiac markers, the classifier performed above chance, with a mean AUC of 60.1. When we compared the performance of MVPA\textsubscript{s} that included EEG features, we only found significant differences when the MVPA\textsubscript{s} also included EKG\textsubscript{cog} versus when the MVPA\textsubscript{s} did not include these cardiac features ($p < 1e^{-9}$, Kruskal–Wallis test, corrected for multiple comparisons). The inclusion of EKG\textsubscript{veg} features did not significantly change the performance of the tested MVPA classifiers ($p > 0.1$).

Discussion

We here aimed at characterizing consciousness state in patients with DOC by means of baseline heart activity and heart–brain interactions. We tested whether cardiac-extracted information can complement single patient EEG-based classification performance. When we contrasted behaviorally nonoverlapping VS/UWS and MCS patients, we found higher HR and HRV in the VS/UWS than MCS group, in accordance with a recent study.39 This comparison included MCS patients who were at the higher end of the CRS-R scale in comparison to the VS/UWS patients, who were at the lower end of the CRS-R. When all DOC patients were included to reflect clinical reality, we did not find group differences of overall cardiac autonomic markers between the groups. This suggests a common underlying baseline cardiac function across patients. Interestingly, we found a positive correlation between CRS-R total scores and 3 autonomic markers (HR, HF HRV, and LF HRV) only in the VS/UWS patients.
Our results are consistent with previous findings showing a relationship between the level of consciousness and dysautonomia in DOC after traumatic brain injuries. Specifically, low CRS-R scores were related to tachycardia in patients with low scores on the Glasgow Coma Scale and to lower HRV (at both HF and LF), which was considered a symptom of a neurological disconnection syndrome. Taken together, these results suggest that the diversity of behaviors characterizing conscious states (associated with cortical processing) does not necessarily translate into strong correlations with autonomic markers, such as HR and HRV. Therefore, the observed differences in these markers in VS/UWS patients on the lower end of the CRS-R scale seems to be associated with an overall deterioration of clinical condition, rather than to cognitive processing.

Our analysis of the heart evoked potentials revealed 2 results. First, we observed a statistical difference between VS/UWS and MCS in the CFA corresponding to the T wave but no difference in association with the QRS wave. The differences observed in the T wave between VS/UWS and MCS patients, in the shape of a
dipole with a left-posterior positivity and a right-frontal negativity, are similar to the reported cardiac repolarization changes induced by mental stress and neurodegeneration or stroke. Although previous works depict a main modulation during the time window corresponding to the T wave with frontal negativities, in our study the differences between the groups of DOC patients are highlighted by the cluster statistic in the posterior positive side of the dipole. Second, we found differences between VS/UWS and MCS patients in a time window after the T wave. Crucially, this difference had a different topography to the previously described CFA. The maximal differences in the EEG were obtained in the central electrodes. Taken together, these results further suggest differences in heart–brain interaction between VS/UWS and MCS patients.

In terms of cognitive processing, we analyzed the cardiac activity while patients were evaluated with the local–global paradigm aiming to probe cognitive-related responses on cardiac markers. Such brain–heart interactions have been previously shown in protocols where, by quantifying neural events locked to heartbeats, one could predict whether a subject would report a fast flashing visual stimulus as perceived. In addition, during complex cognitive processing, such as when playing chess, the heartrate dynamics, as measured before players made a move, could predict the likelihood of them eventually committing an error. Heartbeat-evoked cortical responses were further shown to differ in auditory interoceptive learning tasks and emotional states. Taken together, these studies suggest a bidirectional interaction between brain and heart that can be modulated by cognitive processes.

In our protocol, we found that the cardiac cycle was modulated by the processing of global auditory regularities only in the MCS group. Specifically, MCS patients showed an acceleration of the heartbeat following the auditory stimulus (shortening of the POST interval), which disrupted the global regularity. No such modulation of cardiac cycle was found in the VS/UWS patients, nor was any effect found in either group for the local irregularities. No modulations of the PRE intervals were found; this suggests that the only observed modulation is a direct effect of the cognitive process of the stimulation. It is important to compare these results with previous works that analyzed the evoked responses in the EEG using the same protocol. These studies show that the violations of local regularities (in the form of a mismatch negativity response) can be detected in healthy and awake controls but also during unconscious conditions such as in subjects during sleep, coma, and VS/UWS. In contrast, disruptions of the global regularities (eliciting a P3b response) are only present in conscious and attentive subjects (although see Tzovara et al and Naccache et al for ongoing discussions). That a cardiac cycle modulation effect was present only in association with global irregularities (which requires maintaining conscious attention) and only in the MCS patients (who are generally characterized by more complex brain function compared to VS/UWS patients) suggests that the source of this effect is a brain-driven indirect modulation due to the conscious processing of information.

A recent study demonstrated a link between conscious perception and cardiac activity in normal subjects. Specifically, in a visual detection task, subjects’ heart rate decreased during a warning cue and increased immediately after reporting or not reporting the perception of the stimuli following the cue. When subjects responded correctly, following RR intervals were significantly shorter than the ones corresponding to an incorrect response. This indicates an interaction between conscious perception and the modulation of cardiac activity. Interestingly, previous studies showed that the characterization of the modulation depends on the stimulation intertrial interval. With short intervals, this cardiac slowing is reversed within the same cycle that the target is detected. In our work, we depict a shortening of the RR interval containing the stimuli, although only when the stimulus is known to produce neural modulations and only in patients with higher level of consciousness. Our attention-driven effect is consistent with these previous results and characterizes the modulation in relation to the subjects’ overall level of consciousness.

Having a proficient test at the single subject level is a clinical necessity to reduce the diagnostic uncertainty in each case. The modulation of the heart cycle within each subject was not powerful enough to have a significant effect distinguishing the clinical state of individual subjects. With the aim of improving the single case performance of diagnostic tests, and particularly in terms of EEG, we have shown that multivariate classification performance of the combination of 120 EEG markers (such as quantifications comprising connectivity analysis, information complexity, spectral analysis, and evoked related potentials) outperformed the univariate classification accuracy, when markers were considered individually. This combination of EEG markers allowed an enhanced classification of conscious state at the single patient level. Although the cardiac measures alone did not allow a single subject diagnosis, combining information from both neural and cardiac sources increased significantly the accuracy of the classification of these patients. This indicates that the information extracted from the
modulations of cardiac activity due to cognitive processing is partially independent from the neural correlates of consciousness as measured by EEG. To our knowledge, this is the first time that body-related signals have been considered as contributing factors in data-driven diagnosis in patients with DOC. We think that such an embodied approach to cognition52 paves the way for further investigations of body–brain interactions in DOC that might be informative not only for clinics but also for tracing the neural correlates of consciousness. In the future, and with the aim of improving the single case performance of this test, we will introduce novel versions of the stimulation paradigm (with stimulations contextually locked to the ongoing cardiac cycle).

In conclusion, we show a relation between autonomic nervous system function and a stimulation paradigm exposing subjects to violations of auditory regularities in MCS patients. Our results suggest that cardiac cycle modulation is relevant for the assessment of patients with DOC because it potentially carries partially independent information when taken together with neural correlates of consciousness. We think that our work opens a window to the study of DOC via the embodied paradigm, according to which body–brain functions contribute to a holistic approach to conscious processing.

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Author Contributions
F.R., M.S., D.F.S., L.N., and J.D.S. contributed to the conception and design of the study; F.R., B.R., M.V., and J.D.S. contributed to the acquisition and analysis of data; F.R., A.D., M.S., D.E., L.N., and J.D.S., contributed to the drafting of the manuscript and/or figures.

Potential Conflicts of Interest
Nothing to report.

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