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Visual fixation in disorders of consciousness: Development of predictive models to support differential diagnosis

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ABSTRACT

The visual fixation represents a doubtful behavioral sign to discriminate Vegetative from Minimally Conscious State (MCS). To disentangle its meaning, we fitted univariate and multivariable logistic regression models matching different neurophysiological and neuroimaging data of 54 patients with Disorders of Consciousness to select the best model predicting which visual performance (visual blink or pursuit) was shown by patients and the best predictors set. The best models found highlighted the importance of the structural MRI and the visual evoked potentials data in predicting visual pursuit. Then, a qualitative pilot test was made on four patients showing visual fixation revealing that the obtained models correctly predict whether the patients' visual performance could support/correlate to a cognitively mediated behavior. The present pilot models could help clinicians to evaluate if the visual fixation response can support the MCS diagnosis.

List of abbreviations (in alphabetical order)

AICc	Akaike information criterion for small sample sizes
aOR	Adjusted odd ratio
AUC	Area under the curve
CRS-r	Coma recovery scale-revised
DOC	Disorders of consciousness
FDG-PET	Positron emission tomography with 2-deoxy-2-[*fluorine*-18] fluoro-D-glucose
fVEPs	Flash visual evoked potentials
gVIF	Generalized Variance Inflation Factor
IQR	Interquartile range
LR +/-	Positive and negative likelihood ratio
MCS	Minimally conscious state

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MRI Structural magnetic resonance imaging data
MRI_optrad_right Structural mri score for the right optic radiation
MRI_V1_right Structural mri score for the right primary visual cortex v1
N2/P2 area The area expressed in mv*ms under n2/p2 component up to the return to the isoelectric line after p2 component (from fveps)
NPV Negative predictive values
OR Odds ratio
PPV Positive predictive values
ROC Receiver operating characteristic
SUV Standardized uptake value
SUVr SUVratio of the cluster resulted statistically significant from between-groups analysis
VS/UWS Vegetative State/Unresponsive Wakefulness Syndrome

1. Introduction

Disorders of Consciousness (DoCs) after acquired brain injuries are classically split into two clinical categories, namely the Vegetative State, also known as Unresponsive Wakefulness Syndrome (VS/UWS), and Minimally Conscious State (MCS). The differentiation between these clinical conditions is of uttermost importance for both rehabilitative and caregiving reasons. Specifically, when a patient is diagnosed with DoCs it is challenging to know if the environmental stimuli elaboration takes place; in this scenario, finding some signs able to shed light on the real abilities of the patients represents a fundamental step to plan tailored rehabilitative interventions on the one hand, and to have a prognostic indicator that greatly affects the caregivers' reactions on the other hand [1,2]. This clinical differentiation grounds on the behavioral responses to tailored stimuli administered through the Coma Recovery Scale-revised (CRS-r) which represents the recommended tool to diagnose DoCs [3]. However, the clinical diagnosis still represents a challenge for clinicians studying DoCs; indeed, the misdiagnosis rate is around 40% when comparing medical consensus to standardized behavioral scales such as the CRS-r[4,5]. For this reason, neuroimaging and neurophysiological tools could play a pivotal role in supporting the clinical diagnosis besides the behavioral scales.

One of the first behavioral signs of the transition from VS/UWS to MCS is represented by the visual response to the items included in the visual subscale of the CRS-r[6,7]. Specifically, visual pursuit, i.e., tracking a moving target, and visual fixation, i.e., a movement of the eyes from an initial fixation point with a re-fixation on the new target location for more than 2 s, have been considered as signs of consciousness emergence[3]. Conversely, VS/UWS patients manifest only non-cognitively mediated behaviors, such as visual blink occurring after a visual threat[8]. Nevertheless, the evidence attesting a close link between visual fixation and cognitively mediated processing is still controversial[8–10]. For instance, the UK guidelines reported that visual fixation is "*an isolated fragment of behavior*" that "*appears to reflect the survival of 'islands' of cortex which are no longer part of the coherent thalamo-cortical system required to generate awareness*"[11]. In contrast, the Aspen Neurobehavioral Conference considered sustained fixation as an indicator of MCS diagnosis[12]. Furthermore, the American Academy of Neurology declared that clinicians should be extremely cautious in making a diagnosis of VS/UWS if there is any degree of consistent and reproducible visual fixation[13]. Hence, when and in which way visual fixation should be considered as an evidence of consciousness associated with the diagnosis of MCS is still debated[14,15].

One possible way to shed light on the association between visual fixation and an aware cognitively mediated processing would be to identify a clinical benchmark for the aware visual behavior which could be tested on the visual fixation. This will also help to explore if visual fixation could have a prognostic value in determining the outcome of DoCs. In a previous study, we identify some markers associated with consciousness by comparing neurophysiological and neuroimaging measures related to the visual system of DoC patients manifesting visual blink and visual pursuit[16]. Specifically, patients with visual pursuit showed better preservation of the visual system functioning as attested by a higher area under the N2 and P2 peaks, i.e., one of the most consistent and robust components of flash Visual Evoked Potentials (fVEPs)[17], as well as a greater metabolic and structural integrity of the primary visual areas in the right hemisphere[16].

To build on our previous work, in the present study, we explored whether and how much the neurophysiological and neuroimaging measures previously identified[16] could be predictive of the visual behavior of DoC patients developing a series of predictive models. Furthermore, we examined if the obtained best models could suggest whether to consider the visual fixation more probably related to visual pursuit, hence a cognitively mediated behavior, or to visual blink, and so a reflexive visual behavior. To this aim, we tested the best models on 4 patients not previously considered but who showed the same behavioral features of the sample of patients of our previous study[16] except for the visual fixation as the best performance in the visual subscale of the CRS-r.

With the present study, we intend to help clinicians in deciding if the visual fixation response should be considered behavioral evidence of the MCS diagnosis when it is the only indicator during the clinical evaluation with CRS-r. Importantly, our aim was not to explore the real nature of the visual fixation per se, but instead to give useful hints to better define the clinical significance of the visual fixation when the diagnosis of MCS grounds on this single behavioral sign.

2. Materials and methods

2.1. Patients and setting

Data is derived from a study conducted between 2011 and 2013[16]. As reported in this previous study, we screened 153 adults with a diagnosis of DoC following the standard diagnostic criteria[3], enrolled for a 1-week hospitalization program in a single-center, by cross-sectional study design.

We enrolled patients with age $\geq$18 and with a CRS-r subscales scores corresponding to a VS/UWS diagnosis except for the visual function subscale. Indeed, by considering the visual subscale, both patients obtaining a score of 1, i.e., visual blink, indicative of VS/UWS diagnosis and patients obtaining scores of 2, i.e., visual fixation, and 3, i.e., visual pursuit, associated with MCS diagnosis have been enrolled. Patients were excluded if they had a premorbid history of psychiatric, neurodegenerative diseases, severe visual deficits, and ocular trauma affecting ocular movements.

58 patients fulfilled the above-mentioned criteria; the sample differed from that considered in the previous study by the same authors [16] as we here additionally included 4 patients obtaining a score of 2 in the visual subscale of the CRS-r. All the included patients had the same behavioral profile consistent with a diagnosis of VS/UWS following the CRS-r criteria[3], except for the visual behaviors. The diagram showing the participants selection for each step of the selection process is reported in the supplemental materials.

2.2. Standard protocol approvals, registrations, and patient consents

The present study was conducted after approval of the local Ethics Committee and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from the legal representatives of all of the patients.

2.3. Procedure

Patients were hospitalized for a week's service with diagnostic purposes. During hospitalization, all patients were evaluated by 1 neurologist, 2 neuropsychologists, and 1 neuro-ophthalmologist. Furthermore, due to a lack of economic resources useful to re-hospitalize all the 58

patients, only those showing visual fixation were followed up 12 months after the first evaluation using CRS-r.

For the aim of the present study, we considered all the variables already described in Sattin et al.[16].. Specifically, they included socio-demographic and clinical data (age, sex, etiology and time from the acute event); neuro-ophthalmology variables (pupillary light reflex, presence of strabismus, presence of nystagmus, presence of eyes deviation, and pupillary diameters); CRS-r total and sub-scores; flash Visual Evoked Potentials (fVEPs) data; structural Magnetic Resonance Imaging (MRI) data of the visual system; Positron Emission Tomography with 2-deoxy-2-[*fluorine*-18]fluoro-D-glucose (FDG-PET) variables including Standardized Uptake Value (SUV) maps of striate and extra-striate visual cortices and SUVmean plus SUVratio (SUVr) of the cluster emerged as statistically significant in the General Linear Model between-groups analysis[16] as reported in the statistical analyses section.

2.4. Behavioral assessment

Each patient was independently assessed with the Italian version of the CRS-r[3] 4 times in a week by 2 experienced neuropsychologists. The behavioral assessment was conducted while patients were in sitting position, awake, with open eyes, at least 2 h from post-prandial time, and without environmental interference or factors affecting and modulating brain state or patient's activation, such as the presence of noisy sound and presence of more than two persons in the room.

The total score of the CRS-r was determined considering the best performance scores obtained in each subscale according to standard guidelines[3]. Both visual blink and visual pursuit were assessed following the standard procedures, as already described in Sattin et al. [16]..

Visual fixation was evaluated by presenting a mirror (rectangular, 15 × 21 cm) in front of the patient's face (15–20 cm) and then rapidly moved above and below the horizontal and vertical midlines, so that the stimulus moved once in each direction (4 trials). The mirror was used as it is the most appropriate object for testing the visual items of the CRS-r [18]. The order of presentation was randomized. We scored the visual fixation item whenever the patient showed at least 2 episodes of visual fixation during a single assessment, according to the standard guidelines [3]. All the patients showing visual fixation during the evaluation were re-tested in a follow-up assessment after 12 months to evaluate their clinical/behavioral status by the same neuropsychologists who performed the first evaluation.

2.5. fVEPs data

The fVEPs recording procedure was the same described in Sattin et al.[16].. Specifically, fVEPs were recorded using the Galileo Mizar System (EBNeuro, Florence, Italy) during multiple trials for each test[16, 19]. According to the EEG 10–20 system, Ag/AgCl electrodes were placed at Oz, O1 and O2, with the reference electrode on Fz and keeping the impedance below 5 kΩ.

Stimuli consisted of flashes of light at frequency of 0.9 Hz delivered in a dichoptic way for each eye separately. 100 responses without artifacts for each eye were filtered (bandpass 1–100 Hz) and averaged. We collected the initial negative peak (N1), first positive peak (P1), second negative peak (N2), and the second positive peak (P2) as indicators of peak amplitude and latency of waveform following a stimulus. Given the N2 and P2 peaks are most consistent and robust components of fVEPs in typical adults[17], and to minimize the effect of both the dispersion and the desynchronization over the value of N2/P2 amplitude, we calculated the area under N2/P2 component (expressed in μV*msec) up to the return to the isoelectric line after P2 component, recorded by Oz-Fz in the dichoptic stimulation condition (frequency of stimulation 0,9 Hz; intensity of light 1 Joule; bandpass filters 1–100 Hz[20]) as the best neurophysiological indicator of the cortical response to visual stimuli in DOC patients. Mean normative reference value was 723 mV*ms + 112 mV*ms, lower limit 498 mV*ms.

The patients were not sedated during any of the neurophysiological evaluations, and all of the tests were carried out at the patients' bedsides. All of the measures and scores were independently determined by an expert neurophysiologist.

2.6. MRI data

The MRI acquisition was performed through a 3T scanner with a 32-channel head coil (Achieva TX; Philips Healthcare, Best, the Netherlands). A volumetric 3D Turbo Field Echo (TFE) T1-weighted (voxel size = 1 mm3)), sagittal T1-weighted turbo spin echo (TSE) inversion recovery (IR), axial T2-weighted TSE, and coronal fluid attenuated inversion recovery (FLAIR) were collected; for 2D sequences in-plane resolution was 0.9 mm with 4 mm slice thickness.

For the aim of the present study, we considered the visual rating score indicating the severity of gross anatomical and signal abnormality in the following structures: Optic nerves, chiasma, optic tracts, lateral geniculate bodies, optic radiations, visual primary cortex V1, and cerebral areas from V2 to V8 both for right and left hemispheres. The visual rating scores ranged from 0 (severely damaged) to 4 (normal appearing; see[21] for details on this procedure).

2.7. FDG-PET data

Image acquisition was performed with a Biograph Truepoint 64 PET/CT scanner (Siemens, Erlangen, Germany). Patients rested in a quiet, dimly lit room during FDG uptake (140 6 30MBq). During this period, all patients had their eyes open as indirectly reported by the operators. No sedation drugs were used before or during the acquisitions. PET imaging was obtained for 10 min at least 40 min after FDG administration (mean 146MBq). Each acquisition included a transmission scan followed by a 3-dimensional static emission for 15 min. PET sections were reconstructed using iterative ordered-subset expectation maximization (6 iterations, 8 subsets), corrected for scattering and attenuation, then reconstructed to in-plane voxel size = 1.3 mm, thickness = 3.0 mm. Standardized uptake value (SUV) maps were derived as SUV = AC/(FDGdose/BW), where AC represents activity concentration in kilobecquerels per milliliter in a given voxel, FDG dose is the injected radiotracer dose in megabecquerels corrected for residual activity in the syringe, and BW is the body weight in kilograms (reference values in Britz-Cunningham & Gerbaud[22]). SUV maps were thereafter coregistered using SPM12 to individual volumetric T1 series, which were segmented to generate the normalization deformation field to be applied to the coregistered FDG-PET scan.

2.8. Statistical analysis

Descriptive statistics are expressed as mean ± standard deviation or median and interquartile range (IQR) for continuous variables, and frequencies for categorical variable.

2.8.1. Preliminary clinical and instrumental markers selection

Firstly, we considered the results that emerged from groups comparison (visual blink vs visual pursuit) in the previous work[16] to select those factors with possible predictive value for visual behaviors in DoC patients. Specifically, we considered the N2/P2 area as we previously found a significant difference in this measure between patients manifesting visual blink and patients manifesting visual pursuit[16]. Following the same reasoning, we also considered the MRI scores for the right V1 area and optic radiation as patients manifesting visual pursuit obtained greater rating scores in these areas than patients manifesting visual blink[16] (see supplemental materials for a further analysis concerning the lesions' extent). As for FDG-PET data, we computed the ratio value (SUVr) of the significant clusters that emerged in the previous work, i.e., clusters localized in the right calcarine cortex and the

right lingual gyrus[16]. Specifically, for each patient, the mean of SUV in the significant clusters was extracted and it was normalized for the SUV mean obtained from a reference region comprising all the gray matter areas of the Automated Anatomical Labeling (AAL) using the Wake Forest University (WFU) PickAtlas 3.0.5 software[23].

2.8.2. Statistical analysis

The sample size kept by the statistical analysis was 52 because of missingness on structural MRI data; accounting for this, 2 patients were excluded by listwise deletion.

In the beginning, to evaluate the crude effects of each predictor (previously identified) on the dichotomous outcome (blink/visual pursuit groups, ref. "blink"), detached ordinary logistic regression models were fitted (see *preliminary univariate analysis* in the supplemental materials).

We performed multiple logistic regression models with Firth's correction[24] to evaluate the conditional effects (in adjusted OR terms) of the prognostic factors on the outcome. Firth's correction was considered to provide a bias-reduction for the small sample size. At this stage, the effects of the predictors on the outcome variable are conditional, that is, we obtain the expected outcome variation per unit increase of predictor, keeping fixed the others in the built-in model.

Hence, we carried out a multi-model inference procedure to select the best predictors' set for the outcome, among the significant predictors achieved by ordinary models. We used an information-theoretical approach[25,26]: All the possible models were run and ranked based on their AICc, and their normalized Akaike weights (Aw), whose formulas are reported in[25]. The full model included all the significant variables achieved by ordinary models. Accounting for this, we used a (conditional) model averaging method as reported in *supplemental materials* (model averaging details).

Notably, the models ran by the model averaging do not need multiple testing corrections because information theoretic approaches provide an attractive alternative to the traditional presentation of T-tests, ANOVA (analysis of variance), and multiple comparisons (based on separation statistical tests) as specified by Burnham et al.[27]..

Multicollinearity and potential confounding were checked using Spearman's correlation coefficients (r) and the generalized Variance Inflation Factor (gVIF[28]): predictors with gVIF >2.5 were discarded from the analysis. Finally, the ROC curve and relative indexes were also computed to assess the goodness of fit and predictive ability for each model.

Finally, it is worth to point out that this study may be included in a small sample size framework because it applies an approach used in machine learning (i.e., features selection) where the statistical units (n) are poor in relation to the variable (p), often presented as $p > n$ problem [29,30]. Indeed, the classical logistic regression model is often plagued with degeneracies when $p>n$, and exhibits wild behavior even when n is close to p.

2.8.3. Validation analysis

In a distinct phase, we have also applied a deviance leave-one-out cross-validation, by a LASSO penalized logistic regression model[31], to internally validate the selected models and compare the coefficients. It is worth noting that cross-validation is a powerful empirical approach to minimize the risk of double-dipping (see Ball et al.[32].).

Next, to provide a useful suggestion about the development of a diagnostic/prognostic algorithm (including clinical and instrumental parameters), we have probed the best predictive model on 4 validation cases by comparing the follow-up empirical outcome, i.e., visual pursuit or fixation. This could be of help in clinical practice to establish which relevance has to be given to visual fixation and visual pursuit in disentangling VS/UWS from MCS. In the final part of the paper, we showed results of the predictive analysis on 4 patients out of 58 who showed visual fixation as the best performance in the CRS-r visual subscale, and who were followed-up, by generating a nested prospective case series sub-group. These results have been described only to explain the potential usefulness of this work for both clinicians and readers, thus it should be considered a description of single cases only, without any causal determination.

The statistical analysis was performed on R 3.3.2 (R Core Team 2013) using R/epitools (Tomas and Aragon Developer 2017), R/pROC [33], R/ROCR[34], R/OptimalCutpoints[35], R/brglm[36], R/MuMIn [37] R/fmsb[38] and R/glmnet[39] packages. No part of the study procedures or analyses was pre-registered prior to the research being undertaken.

3. Results

42 (72%) patients out of 58 showed only visual blink reflex in the CRS-r visual function subscale, 12 (21%) manifested visual pursuit, and 4 (7%) showed visual fixation. Among non-traumatic etiologies, 18 (31%) patients had hemorrhagic etiology, 2 (3.4%) ischemic, and 21 (36.2%) showed post-anoxic damage. Details of sociodemographic and clinical features are reported in Table 1. No statistical differences were found between groups when clinical and socio-demographic variables are considered, except for the CRS-r score.

3.1. Preliminary univariate analysis

Table 2 shows the effects of the prognostic factors (selected from the significant results after the comparison between patients showing visual blink and patients showing visual pursuit described in[16] on outcome in terms of crude ORs (with p-values and 95%CIs). We found that for a unit increase of SUVratio in the Significant Cluster, the expected outcome odds were 33.187 times bigger, while when the N2/P2area increases to 100 units, the expected outcome odds was 1.349 times bigger ($=1.003^{100}$) and both statistically significant. Remarkably, table 2 also shows ROC indexes with AUC values >0.77 and standard cut-offs for each significant predictor including Likert scales. [*see online supplemental materials for descriptive statistics and associations between predictors; Tables A.1, A.2 respectively*].

3.2. Multivariate analysis

To improve the analysis, we fitted Firth's multiple logistic regression models by also including the 2 significant continuous predictors (by

Table 1
Demographic and neuro-ophthalmology features of each group of patients (fixation, blink, and pursuit) at study entry expressed either as mean and SD (Age and Months from acute events), number and percentage (Sex and Aetiology), or median and interquartile range (CRS-R score). The summary of univariate statistical analysis comparing groups of patients showing blink and visual pursuit is reported in the last column of the table.

	Fixation	Blink	Visual pursuit	*P-value*
Age – mean (SD)	66.72 (25.07)	49.36 (18.18)	54.24 (26.28)	*0.486* [a]
Sex – n (%)				
M	1 (25.0)	27 (64.3)	5 (41.7)	*0.194* [b]
F	3 (75.0)	15 (35.7)	7 (58.3)	
etiology – n (%)				
TBI	0	13 (31.0)	4 (33.3)	*1.0* [b]
nTBI	4 (100)	29 (69.0)	8 (66.7)	
CRS-R score – median (IQ)	9 (1)	7 (1)	9.5 (1)	*<0.001* [a]*
Months from acute event – mean (SD)	71.85 (90.25)	29.33 (28.71)	29.06 (51.58)	*0.868*[a]

Abbreviations: TBI: traumatic brain injury; nTBI: anoxic, ischemic and hemorrhage etiology; CRS-R: Coma Recovery Scale–Revised. [a]Mann Whitney U test [b]Fisher's exact test (two-sides); *$p<0.001$(p values referred to differences between Blink and visual pursuit groups only). The table has been modified from Sattin et al. (2020) previous work.

Table 2
Preliminary results from crude statistics.

Potential prognostic factors (predictors)						
N2/P2 area (mV*ms)	**SUVr Significant Cluster**	**SUV Significant Cluster**	**MRI_v1_right (5-points Likert scale)**	**Dichotomous MRI_v1_right (high:≥2;low: <2) [Ref: low]**	**MRI_optrad_right (5-points Likert scale)**	**Dichotomous MRI_optrad_right (high:≥3;low:<3) [Ref: low]**
OR= 1.003	OR= 33.187	OR= 1.299		OR= 11.428		OR= 6.999
P = 0.046*	*P* = 0.005*	*P* = 0.121	*P*>0.05	^*P* = 0.002*	*P*>0.05	*P* = 0.009*
95%CI=[1.000; 1.006]	95%CI=[2.936; 375.0]	95%CI=[0.932; 1.811]	°no trend is significant	95%CI=[1.386; 450.838]	° no trend is significant	95%CI=[1.608; 30.479]
AICc = 54.33	AICc=49.16	AICc=57.74	AICc= 53.31		AICc=57.03	
ROC indexes						
AUC= 0.832 (95%CI= 0.701; 0.963)	AUC=0.816 (95%CI= 0.695; 0.936)	AUC=0.715 (95%CI= 0.560; 0.869)	AUC= 0.771 (95%CI=0.656; 0.885)	AUC= *N*/A (95%CI= *N*/A)	AUC=0.776 (95%CI=0.642; 0.910)	AUC= *N*/A (95%CI= *N*/A)
Accuracy = 0.731	Accuracy = 0.712	Accuracy = 0.615	Accuracy = 0.615	Accuracy = 0.615	Accuracy = 0.712	Accuracy = 0.712
Sensitivity = 0.917	Sensitivity = 0.917	Sensitivity = 0.917	Sensitivity = 1	Sensitivity = 1	Sensitivity = 0.75	Sensitivity = 0.75
Specificity = 0.675	Specificity = 0.650	Specificity = 0.525	Specificity = 0.5	Specificity = 0.5	Specificity = 0.70	Specificity = 0.70
PPV = 0.458	PPV = 0.44	PPV = 0.366	PPV = 0.375	PPV = 0.375	PPV =0.428	PPV = 0.428
NPV= 0.964	NPV= 0.963	NPV= 0.954	NPV= 1.00	NPV= 1	NPV=0.903	NPV= 0.903
LR+ = 2.820	LR+ = 2.619	LR+ = 1.929	LR+ = 2.000	LR+ = 2	LR+ = 2.5	LR+ = 2.5
LR- = 0.123	LR- = 0.128	LR- = 0.159	LR- = 0.000	LR- = 0	LR- = 0.357	LR- = 0.357
Cutoff =56.5	*Cutoff =1.18*	*Cutoff =2.947*	*Cutoff = 1.5*	*Cutoff = 2*	*Cutoff = 2.5*	*Cutoff = 3*

Note: Outcome: visual pursuit vs blink (ref: blink), MRI_V1_right=score on the right primary visual cortex area from structural MRI; MRI_optrad_right= score on the right optic radiations tract from structural MRI; SUVr Sign Cluster= Standardized uptake value ratio of the significant cluster in visual area; SUV Sign Cluster= Standardized uptake value of the significant cluster in visual area; N2/P2area=the area expressed in mV*ms under N2/P2 component up to the return to the isoelectric line after P2 component from Visual evoked potentials; OR=crude odds ratio. *P* = *P*-value. 95%CI= 95% Confidence Interval. AICc=Akaike Information Criterion for small samples. *$P<0.05$. °Testing for odds trends across 5-Likert points by orthogonal polynomial contrasts (i.e., linear, quadratic, cubic and quartic trends). ^Fisher's exact test. ROC= Receiver Operating Characteristic; AUC= Area under the curve; PPV= Positive predictive value; NPV= Negative predictive value; LR+= positive likelihood ratio; LR-= negative likelihood ratio; cutoff= optimal Youden Index cut-off; N/A: not achieved.

ordinary models), namely SUVr and N2/P2 area, in dichotomized form by their cutoffs. Accounting for this, we run 2 model averaging procedures by considering different full models: One including all the 4 dichotomized predictors, the other with 2 continuous and 2 dichotomized predictors. In this step, the models run by model averaging procedures were compared by AICc.

The final results of the model averaging (table 3) show that the best model has an AICc equal to 42.05 (Aw=0.219). The best model encompasses the dichotomized N2/P2 area (β* = 2.163, 95%CI= 0.196; 4.129, RI=0.91) and the dichotomized MRI_V1_right (β* = 2.209, 95% CI=−1.148; 5.566, RI=0.55) that provided the highest standardized effect sizes and/or RI weights. Specifically, the dichotomized N2/P2 area had the highest RI value (Fig. 1). In table 3 are also reported the adjusted standard error (ASE) and the 95%CI because it was incorporated in the model selection uncertainty. Concerning the adjusted effects of the selected model, the dichotomized N2/P2 area had a significant effect on outcome (OR=9.132; *P* = 0.020). In other words, adjusting for the dichotomized MRI_V1_right, the odds of visual pursuit were 9.132 times higher among subjects with N2/P2 area bigger (or equal) than 56.5 (cut-off value). Similarly, adjusting for the dichotomized N2/P2 area, the odds of visual pursuit was 13.36 (*P* = 0.092) times higher among subjects with MRI_V1_right area bigger (or equal) than 2 (cut-off value).

Furthermore, table A.3 (see *online supplemental materials*) shows the 35 Firth logistic regression models fitted and considered by two AICc model averaging procedures, with their predictive capacity indexes. In particular, the best pilot predictive model was the model n°34 including all the 4 dichotomized predictors. It provided an AUC equal to 0.902, an accuracy of 0.808, a sensitivity of 0.916, and a specificity of 0.775 [*see online supplemental materials, Figure A.1*], although the conditional effects (adjusted-ORs) were not significant. Regarding the multicollinearity, all the predictors returned a gVIF less than 2.5, thereby confirming the adequacy of the model regarding its parameter estimates.

Table 3
Results of the model averaging on Firth's logistic regression models.

Selected multivariable model by AICc model averaging on all four predictors dichotomized (by cut-offs)		
	Dichotomized MRI_V1_right (high:≥2; low :<2) [Ref: low]	**Dichotomized N2/P2 area (high:≥56.5;low:<56.5) [ref: low]**
Selected model results	OR= 13.36 (*P* = 0.092) 95%CI= 0.655; 272.652	**OR= 9.132 (*P* = 0.020) 95%CI= 1.396; 59.711**
Model Averaging Results AICc= 42.05 Aw=0.219	β*= 2.209 ASE= 1.712 95%CI= −1.148; 5.566 RI= 0.55	**β*= 2.163 ASE= 1.003 95%CI= 0.196; 4.129 RI= 0.91**
Selected multivariable model by AICc model averaging with two continuous and two dichotomized predictors		
	Dichotomized MRI_V1_right (high:≥2; low :<2) [Ref: low]	**SUVr Sign Cluster**
Selected model results	OR = 11.671 (*P* = 0.118) 95%CI= 0.534; 254.694	OR = 6.509 (*P* = 0.120) 95%CI= 0.613; 69.042
Model Averaging Results AICc= 46.54 Aw=0.240	*β*= 2.678 ASE= 1.645 95%CI= −0.546; 5.903 RI= 0.81	*β*= 2.035 ASE= 1.362 95%CI= −0.634; 4.706 RI= 0.6

Note: OR= adjusted odds ratio; 95%CI= 95% Confidence Interval. *P* = *P*-value; AICc= Akaike Information Criterion for small samples; Aw=Akaike weight; β*=standardized effect size; ASE= adjusting standard error; RI= relative importance weights. In **bold** are shown the significant results ($P<0.05$). In Italic are shown the suspect results ($0.05<P<0.10$).

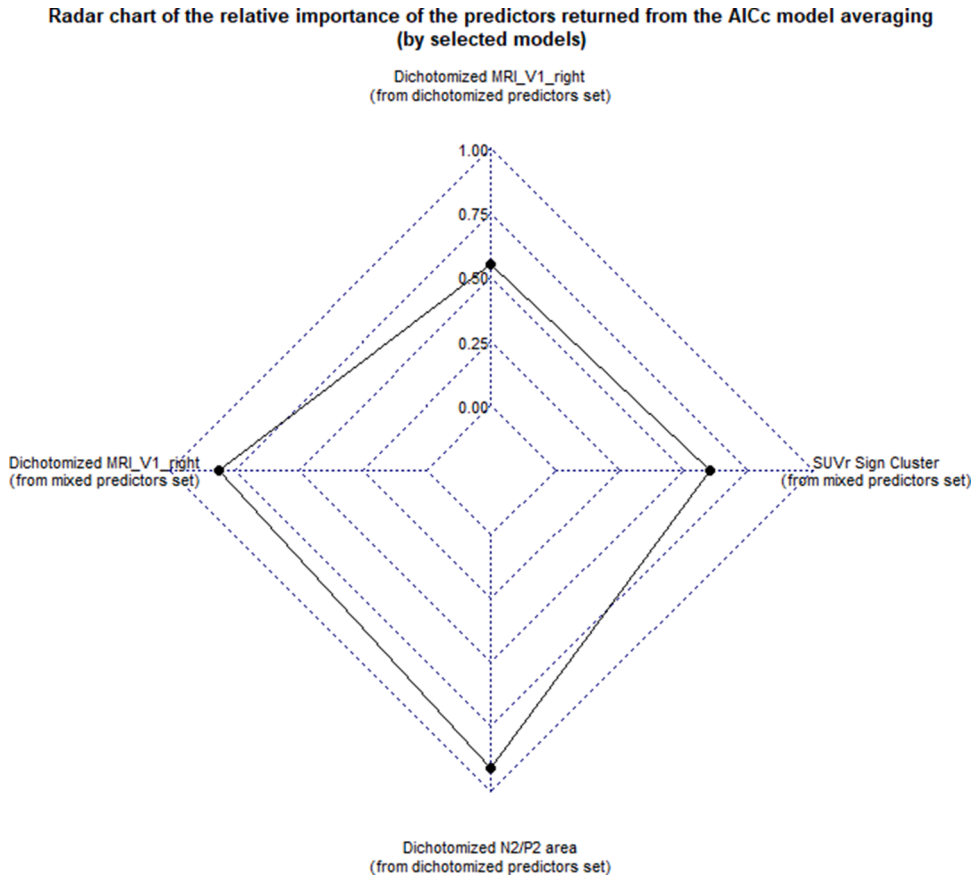

Fig. 1. Radar chart of the relative importance of the predictors returned from the AICc model averaging. The relative importance weights are the weights of evidence for each predictor: they vary from 0: useless to 1: very important.

Table 4
Application of the 'best' predictive model (model 34) on the four patients with follow up.

	Patient			
	# 1	# 2	# 3	# 4
Data at first assessment				
Age (years)	82	51	70	63
Sex	F	M	F	F
Time from acute event (days)	107	133	36	37
Aethiology	Ischemic and Hemorrhagic Strokes	Post anoxic	Hemorrhagic Strokes	Hemorrhagic Strokes
CRS-r total score	9	8	9	9
Diagnosis	MCS	MCS	MCS	MCS
Dichotomized N2/P2 area	*Not available*			
(high :≥56.5; low :<56.5)		Low (0)	High (232)	High (171)
[Ref: low]				
Dichotomized MRI_V1_right				
(high:≥2; low :<2)	High (3)	Low (1)	High (4)	Low (1)
[Ref: low]				
Dichotomized				
MRI_optrad_right (high :≥3; low :<)	Low (2)	Low (1)	High (3)	Low (0)
[Ref: low]				
SUVr at first evaluation	1.57	0.74	1.58	0.74
Predicted probability of visual pursuit	0.132*	0.512*	0.010	0.636	0.064
[95% PI]	[0.007; 0.764]*	[0.073; 0.933]*	[0.000; 0.289]	[0.352; 0.849]	[0.002; 0.724]
Predicted outcome	*Fixation**				
(optimal probability cut-off°=0.376)		***Visual pursuit****	***Fixation***	***Visual pursuit***	*Fixation*
Empirical follow up outcome				
(CRS-r visual item score)	**Visual pursuit****		**Fixation**	**Visual pursuit**	Visual pursuit

MRI V1/optrad=structural magnetic resonance score data for V1 area and optic radiations; N2/P2= area under N2/P2 component recorded during VEPs by Oz-Fz in the dichoptic stimulation; SUVr cluster= relative ratio of Standardized Uptake Value between the selected cluster in V1 area and the global cortex; CRS-*r*=coma recovery scale revised;*The N2/P2 area on the patient #1 has not been estimated so we hypothesized the two scenarios by imputing both "Low" and "High". **Imputing dichotomized N2/P2 area as "High". 95%PI = 95% Prediction Interval. °Cut-off= optimal Youden Index cut-off: if the predicted probability of visual pursuit is bigger than 0.376 then predicted outcome is "Visual pursuit". The outcomes predicted correctly are in bold.

3.3. Validation analysis

The internal validation performed by a binomial deviance leave-one-out cross-validation returned the results shown in the table A.4 in the supplemental materials (models 36–39). In detail, four cross-validations were carried out to account for the different sets of the predictors, concerning the two dichotomizations applied to the markers SUVr significant cluster and N2/P2 area. Notably, the best model, in terms of predictive abilities, was the number 38 that kept all the 4 predictors. In particular, the predictors of model #38 were the same as model #34 (see the table A.3 on the model averaging in the supplementary), and the ORs were similar. Concerning the predictive abilities of the models #34 (AUC=0.902, probability Youden cut-off=0.376) and #38 (AUC=0.906, probability Youden cut-off=0.347), the indexes were equal and both confusion matrices built by crossing the actual and predicted outcomes, returned the following values: 11 true positives (visual pursuit predicted as visual pursuit), 31 true negatives (blink predicted as blink), 1 false negative (visual pursuit predicted as blink) and 9 false positives (blink predicted as visual pursuit).

Finally, concerning the validation cases, we reported in Table 4 both i) the clinical and fVEPs, MRI and FDG-PET data of the 4 patients with visual fixation response only at the first evaluation, as well as ii) the results derived from the predictive model compared to clinical evidence found during follow-up evaluation. Follow-up time was greater than 12 months for 2 patients due to infections that limited behavioral evaluation. In Fig. 2 the structural imaging of the neural visual pathways of the 4 patients was also shown.

The current diagnostic criteria classified all 4 patients as MCS (visual fixation for the CRS-r is associated to an MCS diagnosis using standard guidelines but in one of them the visual fixation was not supported by neural structure or activity in our analysis; specifically, all instrumental data of patient #2 were under the marker cut-offs values).

Comparing the results from our predictive model (n°34) with empirical clinical outcomes at follow-up, we found that for patients #2 and #3 there was matching. Moreover, regarding the detached markers, the agreement between the number of instrumental data below and above cut-off points and the score of 2 and 3 at the CRS-r visual subscale at follow-up was excellent. [*see clinical description of patients (Sub-group) in supplemental materials*].

4. Discussion

Differentiating between VS/UWS and MCS is of uttermost importance for both managing and prognostic reasons. Evidence attested that visual behaviors could be particularly informative of a changing from VS/UWS to MCS as they represent one of the first clinical signs of consciousness emergence[40,41]. However, among the visual behaviors usually assessed in DoC patients, visual fixation deserves attention given its doubtful meaning in determining the presence of an aware behavior. Indeed, some evidence considered visual fixation more similar to reflexive behavior like the visual blink[11,13], whilst others considered visual fixation as indicative of MCS, in the same way as visual pursuit [42]. Given the power the visual behaviors have in inferring the awareness level of DoC patients, it appears useful to disentangle the meaning of visual fixation especially when it is the sole clinical indicator of the state of the patient.

For these reasons, we investigated whether previously identified features of the visual system of patients with DoCs can predict their visual behaviors developing a series of predictive models. Furthermore, we showed results of our predictive analysis on a pilot small sample of patients who showed visual fixation as the best performance in the CRS-r visual subscale, and who were followed up, by generating a nested prospective case series sub-group.

The results from logistic regression analysis, in terms of information processing (by AICc) and predictive performance (by AUC), revealed that the model encompassing MRI and fVEPs data in a dichotomous form, and the model including the same predictors along with the FDG-PET data of the cluster centered in V1 and the MRI data concerning the right optic radiation were more suitable in detecting the visual pursuit than the other models encompassing different sets of predictors [*see online supplemental materials for all the models, Table A.3*]. Our results suggest that the role of the primary visual cortex in differentiating between reflexive (visual blink) and aware (visual pursuit) visual behaviors could be significant. Indeed, fVEPs, structural MRI data on V1, and FDG-PET data on the primary visual areas (in this order) have a good relevance in predicting visual pursuit. Furthermore, the same

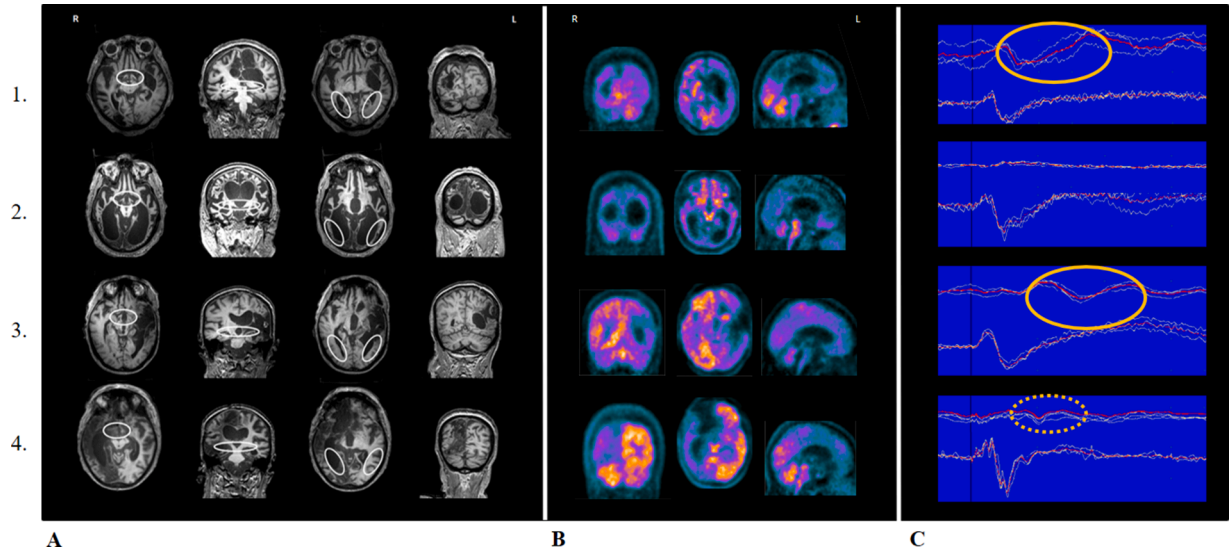

Fig. 2. Patients with visual fixation: MRI, FDG-PET, and fVEPs data. Each row corresponds to a single patient showing visual fixation (from 1 to 4 on y-axis). Both MRI and FDG-PET images have been selected from those most representative. (A) The panel shows the MRI data of the retrochiasmatic structures; each circle highlights the optic tracts (1st column), lateral geniculate body (2nd column), optic radiation/primary visual cortex (3rd column), and the visual cortex (V1-V2/V8; 4th column) in the four patients showing visual fixation. (B) The panel shows the metabolic activations derived from FDG-PET data in the four patients showing visual fixation. (C) The panel shows the visual evoked potentials (VEPs) at Oz-Fz (upper traces) and electroretinograms of the four patients showing visual fixation. Red lines represent grand averages of three different averages (yellow lines). Orange circles highlight the VEPs obtained in patients 1, 3, and (albeit largely abnormal) 4. Patient 2 showed no cortical response to flash.

multimodal instrumental data turn out to be reliable in discriminating between visual pursuit and visual fixation showing predictive power when the empirical outcome of the patients is considered.

To explain the present results, it must be considered that we already acknowledged the difference between patients showing visual pursuit and patients showing visual blink in both fVEPs, MRI, and FDG-PET data [16]. With the present study, we proved how the above-mentioned data have a predictive value on the visual behaviors of DoC patients. Among all, the primary visual cortex (V1) seems to play a pivotal role in determining an aware visual behavior. The result is in line with what we have stated in our previous study showing greater integrity of V1 for patients manifesting visual pursuit than patients manifesting visual blink, a data further supported by the FDG-PET data[16]. However, literature about the role of V1 in visual awareness is still controversial. Some studies highlighted the crucial role of the extrastriate areas in supporting visual pursuit and conscious processing of stimuli, converging their results in important theories on visual consciousness which did not directly link visual awareness to V1 activity[43,44]. On the other hand, it has been suggested that visual functions related to the perceptual organization are pivotal for the transition from unconscious to conscious[45]. In this challenging scenario, we here support the notion that V1 area structural and metabolic integrity seems to be a valid marker supporting the visual pursuit (inferred as a cognitively mediated behavior) more than V2-V8 areas, even considering recent results on healthy subjects[46,47] and patients with blindsight[48,49] that reached our same conclusions. However, different perspectives and considerations about the neural areas candidates with a crucial role for the conscious elaboration of a stimulus are proposed in the last years[50, 51] and our results could be analyzed in the future also using some of these proposals. For example, some theories defined the role of a brain "space" useful for the conscious elaboration of stimuli defining the features needed to allow this process involving different areas. Considering our study, it is difficult to explain our results using this perspective mainly because we limited our analysis to the structural components of the visual system, and, as reported in the supplementary materials, to a preliminary check on the correlation of the metabolic activity of the calcarine area with the prefrontal ones, highlighting a substantial association only in the visual blink group. Another interesting recent theory, called temporo-spatial theory of consciousness (TTC)[52], has focused its attention on the temporal and spatial dimensions of the brain activity, postulating four neural mechanisms accounting for the different levels of consciousness (e.g. temporo-spatial (t-s) nestedness, t-s alignment, t-s expansion, and globalization) from the predisposition of consciousness to the late activity linked to a cognitive elaboration. We analyze all the structures of the visual systems and in this sense our results could be analyzed in the future using this perspective, trying to explore short-term and long-term spatial alignment as well as the suppression of spontaneous activity fluctuations associated with the spatial elaboration of the visual stimulus in a long-time exposition. In parallel, other theories such as the predictive coding approaches (PCT)[53,54] emphasized the sharing of information among different neurons/regions as a hierarchical activity mutually interdependent and fundamental for the predictions of more cognitive aspects of consciousness. Contrary to the TTC, the PCT focused on the role of feedback-feedforward processes (and the relative "error" during the "prediction" of behavior) as constituting upon which sensory inputs ride for consciousness appearance[55]. However, our work did not analyze the prediction value in this sense, but the predictive role that some neural areas/activities can have in defining visual behaviors within a diagnostic perspective. As it is perceivable, the literature is controversial on this topic, and there are no solid data to determine if there are minimal features of a stimulus able to elicit a visual pursuit behavior to our knowledge. In other words, pragmatically, our real problem is that we do not know if a subject with a diagnosis of DoC is able to perform a visual pursuit due to the recognition of his/her own mirrored image (a behavior that implies a high elaboration of the presented stimulus) moved in the proximal space, or simply due to the perception of the stimulus (that could be undefined) that cover a great part of the subject' visual field. Nevertheless, this problem is quite different from those related to the prediction value derived from studies based on the feedback-feedforward processes.

Therefore, we preferred to limit our conclusions supporting the idea that future studies are needed to analyze in deeper all the controversial issues on this topic. Moreover, in combining our results with studies that attributed the pivotal role of extrastriate areas to conscious visual processing, an analysis of the outcome measure must be considered. Indeed, previous works relied on the verbal reportability and differentiation between similar responses[44] instead of the observation of the visual response as it happens in the clinical setting. These measures are clearly different; indeed, a person could have a peripheral problem that limited visual pursuit, but this problem could not influence his consciousness of something as well as his ability to verbally report it. In this sense, we tried to offer a model to predict the visual responses of patients after collecting a series of data on their visual system rather than inferring a particular conscious ability after the behavioral observation only.

Considering the challenge to classify visual fixation in patients with DoCs and its relationship to neural activity, few studies are available in the literature and not all of them reported data that were comparable to our results. One study from Bruno et al.[56]. reported that "*no difference in metabolism in visual areas and no difference in cortico-cortical connectivity between patients without and with visual fixation were found*"; none of the patients in that study showed a change in the visual outcome after one year. This result seems in line with our qualitative analysis on the small group of patients manifesting visual fixation, supporting the idea that low metabolism in V1 (and V2 in the study by Bruno et al.[56].) could be a negative marker for the improvement in visual functions. Our analysis wants to help clinicians in disentangling VS/UWS from MCS diagnosis that is one of the hardest challenges for professionals when it relies on visual fixation. Our pilot model (that must be validated and tested in the future) will allow clinicians to insert their instrumental data, and then predict if the visual fixation they observed is more probably associated with visual pursuit rather than visual blink responses. We analyzed and explained the visual item variability by statistical modeling, focusing on the dichotomy between visual blink and pursuit responses, and by going beyond the differences between DoC patients with and without fixation. This is a crucial point because our results are based on the visual pursuit response as a possible benchmark for the interpretation of the observed visual fixation.

The statistical models have been developed by taking into account data derived from a relatively homogeneous sample; indeed, all the CRS-r sub-items scores were in line with VS/UWS diagnosis, except for the visual item's subscore that confirmed the VS/UWS diagnosis when it was equal to 1, i.e., visual blink (42 patients), or changed the diagnosis in MCS when it was equal to 3, i.e., visual pursuit (12 patients). Importantly, we did not include patients with scores equal to 4 (corresponding to Object Localization: Reaching) and 5 (corresponding to Object Recognition) in the visual subscale to guarantee the homogeneity of the sample. In other words, if we had included patients obtaining a visual score of 4 or 5 it would have meant to include patients who probably show higher profiles also in the other subscales of the CRS-r[57], and this would have implied a real difficulty in finding patients with comparable profiles corresponding to VS/UWS diagnosis.

In the last part of the present study, we probed the predicting value of our pilot model using the data of 4 patients showing visual fixation only during the first assessment with the CRS-r. This subsample was taken as a case series with a follow-up in which the visual responses were re-tested after 1 year. We found a perfect matching between the outcomes of our pilot predictive model and the empirically observed behavioral performance in 3 out of 4 patients with visual fixation during the first assessment. Specifically, the analysis of the 4 cases showed that in 2 patients (#2 and #3) there were almost complete associations between fVEPs, MRI, and FDG-PET data and visual outcomes after 1 year (during the follow-up, a patient showed visual fixation and the other one

improve to visual pursuit). In one patient (#1), instead, fVEPs were not included in the analysis due to the very high latency of the cortical response. Hence, we hypothesized 2 possible scenarios by imputing both below and above N2/P2 area cut-off values in our pilot model. Imputing the N2/P2 "High" value, the predictive outcome of the model was in line with the observed behavior at the follow-up. Conversely, the profile of another patient (#4) showed controversial results. The fVEPs data was above cut-off value but not the FDG-PET and MRI data; furthermore, the outcome of our model was different from the real behavior of the patient during the follow-up. We hypothesized that our pilot model could lose sensitivity if there is an asymmetrical lesion of the visual cortex, as attested by this case, also considering that, albeit altered, fVEPs could be bilaterally evoked even in case that only one of the visual pathways has been spared. Interestingly, the literature points out that an early component of visual response, Visual Awareness Negativity, is linked to the development of visual consciousness[58]. However, we are not sure if the visual response to fVEPs could be a marker of consciousness as itself. In this work, we want to use fVEPs along with other techniques, to disentangle VS/UWS from MCS in patients who differ only in one item on the CRS-r as reported in the analysis on the 4 patients with visual fixation.

A lateral consideration for this last part of our study should be made. In our work, only 4 patients showed visual fixation as the best performance in the CRS-r visual subscale, and previous articles debated on what is the best stimulus to elicit visual fixation (e.g., a mirror rather than an illuminated object[18]) by adopting different settings. Our aim did not concern the best stimulus to obtain visual fixation and we adopted the standard CRS-r criteria making our results not comparable with the previous ones. On this point, it could be useful to test several stimuli for each subscale to validate a subset of "standard" objects for the CRS-r assessment in the next future. Finally, it is worth to point out that these results have not the presumption to provide a validated diagnostic/prognostic model or clinical guidelines, given the very small sample size (and power); they shed just a light on the importance of specific clinical and instrumental parameters that could be useful to develop a stable algorithm (tool) that can be of help for the clinical practice.

4.1. Limitations and conclusions

The present study has some limitations. First, due to the low number of followed-up patients with visual fixation, we could not validate the statistical models. However, despite the small sample size, 3/4 of them improved their visual functioning after 1 year, a fortuitous coincidence that allowed to study the possible role of the primary visual cortex as a marker to sustain cognitively mediated and conscious visual behaviors. Some studies with similar aims analyzed other neural systems, such as the fronto-parietal network[59–61], but further studies are needed to link all these results. Another issue concerns the lesions occurring in V1-V2 areas. The so-called Riddoch phenomenon in blindsight (a patient reports that he does not see, but rather has a 'feeling' that something had moved within his blind field, indicating some level of awareness[62,63]), as well as oculomotor impairments and other visual deficits (difficult to test in unable to communicate patients) or performance fluctuation[64] are all factors that could imply an underestimation in the number of patients who preserved conscious processes. In this sense, the impossibility to test/verify the presence of these problems in patients with DoCs could determine an error during the clinical evaluation of patients, for example classifying their visual performances as reflexive behavior due to the effect of the above-reported confounding variables. Another limitation of our study could be due to the fact that patients with DoCs usually have diffuse brain impairments and one could argue that the same analysis of the relationships between structures and functional properties on somatosensory area, for instance, could also show a positive correlation with visual behavior without any direct physiological relation. To overpass this limit, future studies are planned in which the model we proposed will be checked to verify validity, specificity, and sensibility.

Moreover, our models were developed using MRI, PET, and VEPs data but collecting and analyzing these data required tools and techniques that are not present in all rehabilitation centres. Consequently, as reported above, future studies are needed to highlight the power of our models with respect to other results obtained using a single technique.

Finally, we described only the outcomes of 4 patients with visual fixation after 12 months and we did not report outcomes of the overall sample. Unfortunately, the economic resources for the project were limited and we were able to re-hospitalize only a small part of the sample. This is the main reason why we retested only patients who showed visual fixation.

As a final consideration, we think that clinical assessment performed with ad-hoc assessment tools remains the gold standard method to evaluate patients with DoC and their visual performances, but the instrumental parameters can help professionals during this clinical decision-making process. However, the number of data derived from instrumental tools can be cautiously used and our preference was directed toward the most simple and replicable information that can be translated into clinical practice. This is the principle that guided us in the development of our study and that differentiates it from other research with different aims, like to develop the best tools to overpass the human decision-making process. In this sense, in a previous study, we proposed that information from 3 techniques (fVEPs, MRI, and PET) could be useful for clinicians when they are uncertain about the interpretation of visual fixation response[16]. This is particularly important when the visual fixation is the best performance in the visual subscale of the CRS-r, as it is fundamental in differentiating between VS/UWS and MCS when patients do not show other cognitively mediated behaviors in other CRS-r subscales. However, as we only considered the variables for which there was a significant difference between patients manifesting visual blink and patients manifesting visual pursuit in our previous study [14], future research could better explore the role of other structures such as the LGN and extrastriate areas in a larger sample of patients. Moreover, the subcortical structures including the LGN could be taken into account for the future validation of the model given its role as a subcortical relay station for the visual system[65,66].

Our study suggests clinicians to use our pilot model to test the predictive value of their data. Of course, the small sample size and the consequent use of the leave-one-out cross-validation might make vary widely the model accuracy estimates; the small sample size of this study did not allow us to validate our model, and so, for instance, the cut-off values reported for the data derived from each technique analysis should be used cautiously, although the strict inclusion criteria allowed us to homogenize our sample and to develop our pilot model using a rigorous and replicable methodological approach. Anyway, the study might be underpowered and there might be any lack of generalizability (concerning this, a post-hoc power analysis is reported in the supplementary materials). Finally, we are aware that the results of the present study are not conclusive but a subsequential study has been planned to perform an external validation on different and more data, to check the performance and robustness of the selected model. However, by stating the explorative nature of the study, we consider valuable to share this preliminary evidence. Notwithstanding such limitations, the present study demonstrated that our approach, combining fVEP, MRI, and FDG-PET data can provide useful and easily available information to weight the probability to consider visual fixation as a marker for MCS, and so limiting doubts in clinical interpretation. The role of the primary visual cortex for the differentiation between visual blink and visual pursuit seems to be important, but future research is needed on this topic. AUC results of fVEP and FDG-PET were high (>0.80), as well as the sensitivity, therefore, the probability to detect information if visual performance is supported by selected cortical activity appeared good.

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Declaration of Competing Interest

All authors claim that there are no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2021.113310.

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