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ORIGINAL ARTICLE

Abnormal Effective Connectivity of the Anterior Forebrain Regions in Disorders of Consciousness

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Abstract A number of studies have indicated that disorders of consciousness result from multifocal injuries as well as from the impaired functional and anatomical connectivity between various anterior forebrain regions. However, the specific causal mechanism linking these regions remains unclear. In this study, we used spectral dynamic causal modeling to assess how the effective connections (ECs) between various regions differ between individuals. Next, we used connectome-based predictive modeling to evaluate the performance of the ECs in predicting the clinical scores of DOC patients. We found increased ECs from the striatum to the globus pallidus as well as from the globus pallidus to the posterior cingulate cortex, and decreased ECs from the globus pallidus to the thalamus and from the medial prefrontal cortex to the striatum in DOC patients as compared to healthy controls. Prediction of the patients' outcome was effective using the negative ECs as features. In summary, the present study highlights a key role of the thalamo-basal ganglia-cortical loop in DOCs and supports the anterior forebrain mesocircuit hypothesis. Furthermore, EC could be potentially used to assess the consciousness level.

Keywords Mesocircuit · Basal ganglia · Posterior cingulate cortex · Spectral dynamic causal modeling · Connectome-based predictive modeling

Introduction

Disorders of consciousness (DOCs) have always been difficult to understand; yet studies have advanced our understanding of both responsiveness and consciousness after severe brain injuries. Recent neuroimaging studies have made much progress in this field and have consistently revealed that impaired consciousness is associated with damaged mid-line regions of the frontal and parietal cortices [1–4] as well as abnormal thalamus- and basal ganglia-modulated connectivity [5–7]. Based on the evidence that traumatic brain injuries in these subcortical and cortical regions as well as in their complex interactions may cause impaired consciousness, a mesocircuit hypothesis [8] has been proposed to predict the dynamic process that occurs in the anterior forebrain during the restoration of consciousness. According to the circuit-level mechanism, DOCs can be attributed to widespread reductions in the thalamocortical and thalamostriatal outflows followed by a failure in the firing state of the striatum. These events result in excessive inhibition of the thalamus and then the cortex [9].

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The thalamus acts as a transfer station that transmits information through its broad connectivity [10] between the cortex and subcortical nuclei (the basal ganglia, for example) [11–15]. The thalamo-frontal circuit [16–18] as well as the cortico-thalamo-basal ganglia-cortical circuit [8, 16] are crucial in the recovery from DOCs, and atrophy of the thalamus has been found to be proportional to the severity of DOCs [8]. In addition to its function in DOCs, some studies have also shown that the thalamus is important in many aspects of forebrain function through various projection patterns, such as those that control alertness, attention, and awareness, and those that regulate sleep-wake rhythms [19, 20].

In contrast, several studies in both humans and animals have indicated that the thalamo-cortical loop may have a lesser, or only an indirect, effect on limited wakefulness after brain lesions [21] and in propofol-induced unconsciousness [22–24]. According to the mesocircuit hypothesis, other subcortical structures (e.g. the striatum and globus pallidus) in the basal ganglia also contribute to consciousness through interactions with the thalamus [8], and may be controlled by the thalamus [25]. Reciprocal connections between the thalamus and basal ganglia as well as other basal ganglia connections may jointly contribute to arousal, attention [26–28] and movement [29, 30]. Another study suggested that regions in the basal ganglia can mediate consciousness, bypassing the thalamus [21].

While subcortical regions including the thalamus and basal ganglia have been shown to be important in DOCs, how they interact with cortical regions and the exact locations of these regions remain under debate. Crone *et al.* [31] have compared various existing models and inferred that recovery of consciousness is a dynamic process involving changes in the cortico-thalamo-basal ganglia-cortical connectivity, and cortical regions such as the medial prefrontal cortex (mPFC) and posterior cingulate cortex (PCC) are particularly emphasized. These two regions have also been frequently found to be important in impaired consciousness in multimodal imaging studies [32–34] and literature reviews [4, 35] on DOCs.

Apart from the specific regions, the mechanisms and systems underlying consciousness, including the subcortico-cortical and cortico-cortical connectivity, have gained increasing attention [19, 36, 37]. Structural [13] and functional [31] imaging studies have both shown that pathways connecting the thalamus, basal ganglia, and PCC jointly contribute to consciousness. Despite the revealed impairments in fiber tracts and functional connectivity in DOC patients, a mechanistic understanding of the specific effective couplings (ECs) and their directionality in DOCs remains unclear.

The present study was designed to investigate how regions of the anterior forebrain are related to DOCs. We first compared the ECs among the thalamus, striatum, globus pallidus, and two midline regions in the default mode network (DMN) between DOC patients and healthy controls using spectral dynamic causal modeling (spDCM). Then, a machine-learning method was used to inspect the indexes that performed best in predicting the Coma Recovery Scale-Revised (CRS-R) scores.

Materials and Methods

Participants

A total of 40 patients with severe brain injury (30 M/10 F, aged 20–64 years, mean ± SD, 40.9 ± 13.9) were recruited to this study. The inclusion criteria before the scanning were as follows: (1) disease course <1 year; (2) no history of psychological disorders; (3) no previous alcohol or drug abuse; and (4) either in a vegetative state/unresponsive wakefulness syndrome (VS/UWS) or a minimally conscious state (MCS). The clinical severity was evaluated based on the Coma Recovery Scale-Revised [38]. To decrease diagnostic error, each patient was evaluated by 2–3 doctors together on the day of scanning. During data processing, 8 patients who had excessive head motion (translation >3 mm in any of the planes or rotation >3° in any of the x, y, and z axes) and 7 with severe brain lesions were excluded. Thus, 19 DOC patients (8 in MCS and 11 in VS/UWS) were included for further analyses. The detailed clinical information of these patients are listed in Table 1.

We also recruited 19 gender- and age-matched healthy participants (13 M/6 F, aged 20–50 years, 35.6 ± 10.0) as controls (Table 2). None of them had a history of neurological or psychiatric illnesses or brain injury. Written informed consent was given by each healthy participant and by the legal surrogate of each patient. The protocols were approved by the Research Review Board of The General Hospital of Guangzhou Military Command of The PLA.

Data Acquisition

All MRI data were acquired on a 3T GE MRI scanner with an eight-channel phased-array head coil. Two hundred and forty resting-state functional volumes were acquired for each participant. Resing state fMRI data were obtained using a gradient-echo echo-planar imaging sequence with

Table 1 Demographic and clinical characteristics of the patients with disorders of consciousness.

Patient index	Gender	Age (years)	Months post-icuts	Etiology	Diagnosis	CRS-R scores	
						Au/V/M/O/C/Ar	Total
P01	M	36	2	HIE	VS/UWS	1/0/0/0/0/1	2
P02	M	62	1	HIE	VS/UWS	0/0/1/0/0/2	3
P03	M	48	1	HIE	VS/UWS	0/0/1/0/0/2	3
P04	M	43	1	HIE	VS/UWS	0/0/1/1/0/2	4
P05	M	64	2	TBI	VS/UWS	0/0/1/1/0/2	4
P06	M	21	1	HIE	VS/UWS	1/0/2/1/0/1	5
P07	M	39	2	TBI	VS/UWS	0/0/2/1/0/2	5
P08	M	39	1	HIE	VS/UWS	0/0/2/1/0/2	5
P09	M	32	9	HIE	VS/UWS	1/0/1/1/0/2	5
P10	M	36	9	TBI	VS/UWS	0/0/1/2/0/2	5
P11	M	51	1	TBI	VS/UWS	1/0/2/1/0/2	6
P12	M	47	3	HIE	MCS	1/3/0/1/0/2	7
P13	M	41	3	TBI	MCS	1/1/3/0/0/2	7
P14	F	46	2	TBI	MCS	1/0/3/1/0/2	7
P15	M	41	3	HIE	MCS	2/3/2/1/0/1	9
P16	F	59	1	TBI	MCS	1/0/5/1/0/2	9
P17	F	27	1	TBI	MCS	1/0/5/1/0/2	9
P18	M	30	1	TBI	MCS	1/1/3/2/0/2	9
P19	F	20	2	TBI	MCS	2/3/3/1/0/2	11

MCS, minimally conscious state; VS/UWS, vegetative state/unresponsive wakefulness syndrome; TBI, traumatic brain injury; HIE, hypoxic-ischemic encephalopathy; M, male; F, female; CRS-R, Coma Recovery Scale-Revised; Au, auditory; V, visual; M, motor; O, oromotor; C, communication; Ar, arousal.

Table 2 Demographic and clinical characteristics of the patients with disorders of consciousness (DOCs) and the healthy controls (HCs).

Characteristics	DOCs	HCs	Statistics	*P*-value
Gender (male/female)	15/4	13/6	$\chi^2 = 0.71$	0.71[a]
Age (years)	42.3 ± 13.0	35.6 ± 10.0	$t = 1.78$	0.08[b]
Months post-ictus	1.1 ± 0.3	–	–	–
Etiology (HIE/TBI)	9/10	–	–	–
Diagnosis (MCS,VS/UWS)	8,11	–	–	–
CRS-R scores	6.1 ± 2.5	–	–	–

HIE, hypoxic ischemic encephalopathy; TBI, traumatic brain injury; MCS, minimally conscious state; VS/UWS, vegetative state/unresponsive wakefulness syndrome; CRS-R, Coma Recovery Scale-Revised.

[a]χ^2-test.

[b]Two sample *t*-test.

the following parameters: repetition time (TR) = 2,000 ms, echo time (TE) = 26 ms, flip angle (FA) = 90°, field of view (FOV) = 240 × 240 mm^2, data matrix = 64 × 64, voxel size = 3.75 × 3.75 × 3.6 mm^3, 36 sequential slices, and 240 volumes obtained in ~8 min. In addition, high-resolution structural images were acquired using a T1-weighted 3D fast spoiled gradient recalled sequence (TR = 8.86 ms, TE = 3.52 ms, FA = 90°, FOV = 240 × 240 mm^2, data matrix = 256 × 256, voxel size = 0.94 × 0.94 × 1 mm^3, and 176 sagittal slices).

Data Preprocessing

Functional images were preprocessed using statistical parametric mapping (SPM12, http://www.fil.ion.ucl.ac.uk/spm) and data preprocessing and analysis for brain imaging (DPABI, http://rfmri.org/dpabi). First, we removed the initial 10 volumes of functional images and then performed slice-timing correction and motion correction. Afterwards, the high-resolution structural and functional images were both manually reoriented to the standard position parallel

to the anterior commissure-posterior commissure line. Functional images were individually co-registered with the high-resolution structural images and then spatially normalized to the group template, which was made using diffeomorphic anatomical registration through exponentiated Lie algebra [39]. Finally, the resulting data were spatially smoothed with a Gaussian kernel of 4 mm full-width at half-maximum.

Region selection

The ROIs were determined according to the Brainnetome atlas (http://atlas.brainnetome.org/bnatlas.html), which divides the whole brain into 105 cortical regions and 18 subcortical regions in each hemisphere [40]. The ROIs in our study were directly extracted and combined using these segmentations implemented in MarsBaR (http://marsbar.sourceforge.net). The posterior part of the cingulate cortex (BA 23) was extracted as the ROI for the PCC. Two parts of the basal ganglia (caudate and putamen) were combined as the striatum. Segmentation of the thalamus and mPFC (8 individual parts for the thalamus and 2 parts for mPFC) were separately combined into one ROI. The globus pallidus was also extracted directly from the atlas. Every ROI was further checked visually using the Automated Anatomical Labeling [41] and Brodmann atlases. These regions have been previously described in other consciousness-related studies using spDCM [31, 42]. Figure 1 illustrates the distributions of these ROIs.

Spectral Dynamic Causal Modeling

Using DCM12 implemented in SPM12, we performed spDCM analysis. The specific calculation steps were as follows: (1) General linear model (GLM) definition: A GLM was defined using the preprocessed images and the confounding time-series from the white matter and cerebrospinal fluid were regressed out as nuisance variables. (2) Time series extraction: The first eigenvectors for the 5 ROIs were extracted for each participant. (3) Model specification and estimation: The fully and reciprocally connected models, including the self-connections, between the 5 ROIs were specified. Thus $2^5 = 32$ free parameters were produced and then estimated for each model. (4) Bayesian *post-hoc* model selection routine: A *post-hoc* model optimization routine [43] was used to determine the model that had the best fit for each group.

Machine-Learning Approach in Brain-Behavior Prediction

To predict the clinical performance of the DOC patients from their brain connectivity, we used a recently launched machine-learning approach termed connectome-based predictive modeling [44]. The calculation included the following steps: (1) Feature selection. The correlation between the effective connectivity and the total CRS-R score for each patient was analyzed using Spearman's correlation. By taking the correlation coefficients as matrix elements, we produced a 19-by-19 matrix, in which each element ranged from -1 to 1. Only the significant coefficients ($P < 0.05$) were selected as features. Positive and negative correlations were analyzed separately. All 19 patients were divided into a training set and a testing set to perform leave-one-subject-out cross-validation. (2) Feature summarization. The significant features (coefficients) were simply summed for further computations. In the calculations, we separately analyzed the positive and negative EC values. (3) Model building and evaluation. The summary value of the features and the total CRS-R score for each participant in the training set were built into a linear model using the least squares method. The predicted CRS-R scores were generated by applying this linear model to the testing set. Finally, we evaluated the predictive performance by computing the Spearman's correlation between the predicted CRS-R scores and the real scores. In this way, we produced the correlation coefficients and their *P*-values.

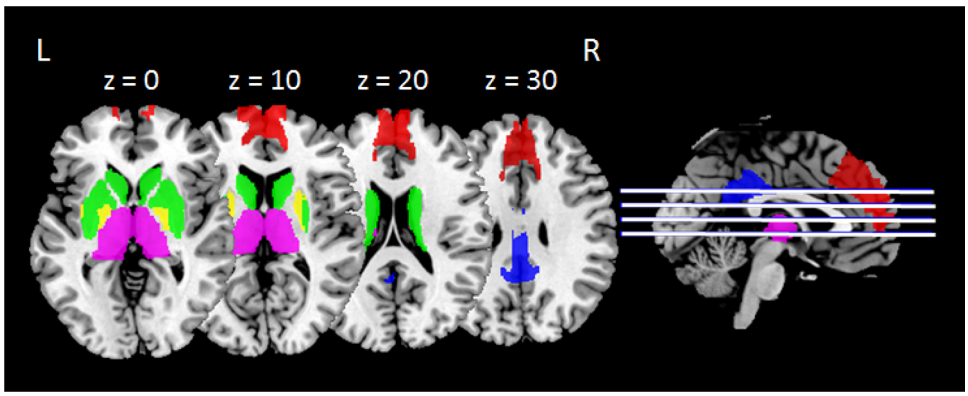

Fig. 1 Regions of interest selected in the current study.- Red, medial prefrontal cortex; blue, posterior cingulate cortex; green, striatum; yellow, globus pallidus; purple, thalamus. L, left hemisphere; R, right hemisphere.

Statistics

Comparisons of Effective Connections

Differences in EC strength between patients and controls were determined with permutation tests. The ECs were also compared among patients with different etiologies (HIE and TBI) to calculate the influence of the heterogeneity. All the parameters of intrinsic connectivity (shown as DCM.Ep.A in Matlab files) were calculated. In addition, the connection strengths for the corresponding winning models within each group were analyzed using one-sample *t*-tests. All the resulting *P*-values were corrected for a false-discovery rate (FDR < 0.05), and another significance threshold, $P < 1/$(number of comparisons) [42], was also taken into consideration when analyzing the differences between the groups. Of note, positive values of the EC indicate excitatory influence from one region to another, and negative values indicate inhibitory influence, whereas self-connections of regions are assumed to be always inhibitory [42].

Brain-Behavior Prediction

The predictive significance was assessed using permutation testing. By preserving the connectivity matrices but randomly reassigning the behavioral scores a thousand times, an empirical null distribution was generated. The resulting *P*-value was the proportion of sampled permutations that are greater than or equal to the real predictive correlation.

Results

Dynamic Causal Modeling

The Bayesian optimization procedure searched all the reduced versions of the full model for each group. The fully-connected model was the winning one for both the patients and controls and had a posterior probability of almost 1 (Fig. 2A). After the optimized model was selected, the parameter inference of connection strength within the same group was compared using one-sample *t*-tests. The connectivity strengths and directions between the 5 ROIs are shown in Fig. 2B. In the healthy controls, all the self-connections within the nodes were significant and the thalamo-globus pallidus-posterior cingulate cortex circuit was also significantly connected. In the patient group, however, almost all the reciprocal connections between regions disappeared except for a driving influence from the striatum to the globus pallidus. The self-connection within the globus pallidus also disappeared, but the self-inhibition of the other 4 regions remained. The ECs were not influenced by etiology.

Significant changes in effective connectivity strength were found in the DOC patients compared to the controls (Fig 3). Specifically: (1) The patients showed a significant increase in the influence from the striatum to the globus pallidus ($P = 0.039$) and also a significant decrease in the opposite direction ($P = 0.006$). This went along with a reduction in the influence from the globus pallidus to the thalamus ($P = 0.011$) and an increase in the opposite direction ($P = 0.0007$). (2) In the DOC patients, the PCC negatively influenced the globus pallidus ($P = 0.032$) and received a positive influence ($P = 0.0006$). There was also a significant reduction in the driving influence from the mPFC to the striatum ($P = 0.021$). The effective connectivity strengths and *P*-values for both groups are listed in Table 3.

Brain-Behavior Prediction

The valid features used in the predictive procedures are shown in Fig. 4. Given the 25 ECs in the optimized model (that is, 20 inter-regional connectivities between the 5 ROIs and 5 recurrent connectivities within the 5 ROIs) and using a threshold of $P < 0.05$, four out of 25 features (correlations) were retained. These four consisted of only one positive feature, a significantly positive correlation between the CRS-R scores and the mPFC-to-PCC EC ($r = 0.456$, $P = 0.049$), and three negative features. The latter were significantly negative correlations between the CRS-R scores and self-connections within the PCC ($r = -0.566$, $P = 0.012$), self-connections within the GP ($r = -0.462$, $P = 0.047$), and the thalamus-to-PCC EC ($r = -0.512$, $P = 0.025$). When taking the negative effective connections as features, we found a significant correlative relationship ($r = 0.55$, $P = 0.014$) between the real CRS-R scores and the predicted performance. The nonparametric permutation testing showed that the statistical significance of this performance was $P = 0.018$. No significant correlation was found when taking the positive effective connections as features (Fig. 5).

Discussion

In this work, we investigated the relationships among 5 key regions within the anterior forebrain using spDCM. We identified disrupted ECs in the thalamo-basal ganglia-posterior cingulate cortex circuit of a group of DOC patients compared to the healthy group. Using connectome-based predictive modeling, we found that the predicted CRS-R scores had a significant correlation with the real scores when negative correlation coefficients were selected

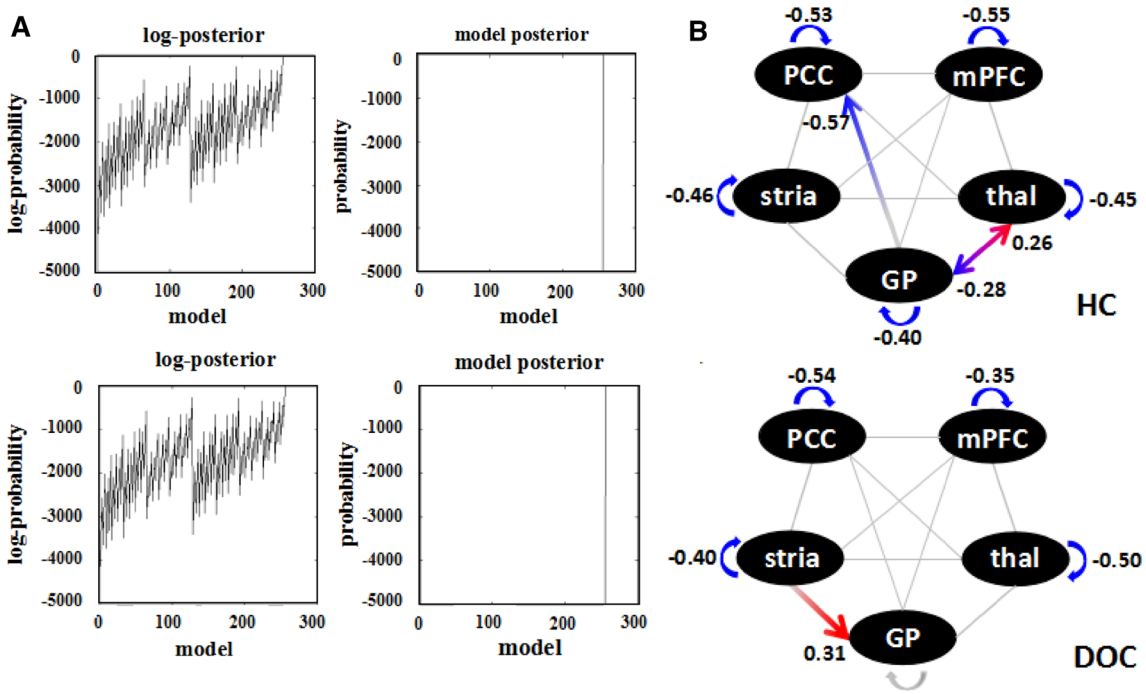

Fig. 2 Results of DCM *post-hoc* selection and one-sample *t*-tests. **A** Bayesian model optimization. The full model was optimized for both the patients and the controls with a posterior probability of almost one. **B** Results of one-sample *t*-tests. Red arrows, significant positive inputs or outputs; blue arrows, significant negative values ($P < 0.05$, FDR-corrected for multiple comparisons); and grey, insignificant values. The digits represent the mean EC within the group. mPFC, medial prefrontal cortex; PCC, posterior cingulate cortex; thal, thalamus; GP, globus pallidus; stria, striatum; HC, healthy control; DOCs, disorders of consciousness.

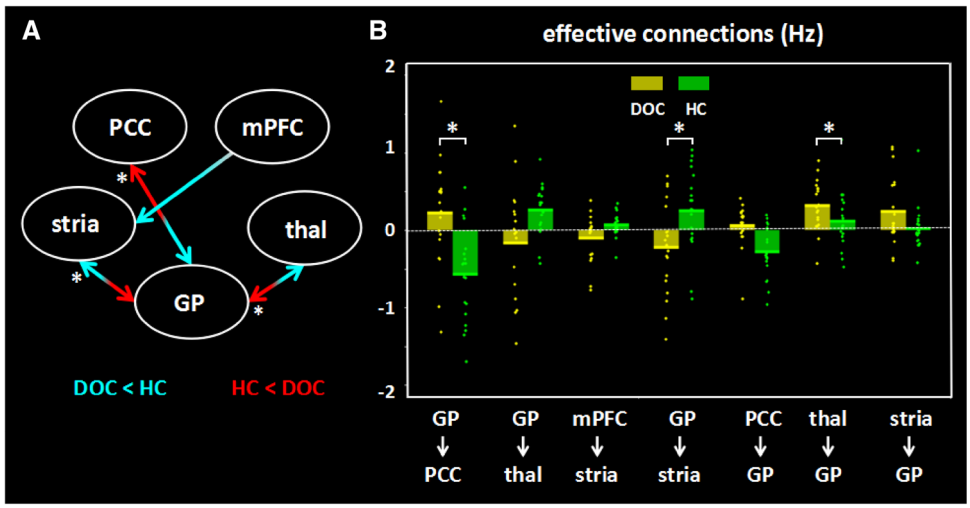

Fig. 3 Significant changes in effective connections (Hz) in the DOC patients. **A** Differences between patients and controls derived from permutation tests. Red arrows, connections with significant increases; blue arrows, connections with significant decreases ($P < 0.04$ = 1/25 uncorrected); asterisks, significant results after FDR correction ($P < 0.006$). **B** Mean effective connections of significantly changed pairs of regions. PCC, posterior cingulate cortex; mPFC, medial prefrontal cortex; thal, thalamus; GP, globus pallidus; stria, striatum.

as features. Importantly, the PCC and the globus pallidus appeared to play a key role in this brain-behavior prediction.

Changes in Effective Connections within the Mesocircuit in DOC Patients

Our results indicated that, compared to controls, patients with DOCs had disrupted connectivity between key regions within the anterior forebrain mesocircuit. In these patients, the striatum first seemed to lose its inhibition of the globus pallidus, then the globus pallidus had a decrease in self-inhibition, and finally the globus pallidus had an inhibitory influence on the thalamus (see Figs 2 and 3). Our results are in line with the mesocircuit hypothesis in that the globus pallidus was released from striatal inhibition and was overactive, resulting in excessive inhibition of the thalamus [8]. Another DCM study [31] also reported the

Table 3 Effective connectivity strengths in patients and controls

Regions of interest	Within groups				Between groups	
	DOC		HC		DOC-HC	
	Mean ± SD	*P*-value	Mean ± SD	*P*-value	Mean differences	*P*-value
From mPFC to						
mPFC	− 0.35 ± 0.38*	0.001	− 0.55 ± 0.41*	0.000	0.20	0.065
PCC	− 0.08 ± 0.54	0.555	0.09 ± 0.27	0.152	− 0.17	0.126
Thalamus	0.01 ± 0.33	0.900	0.12 ± 0.82	0.536	− 0.11	0.299
Striatum	− 0.17 ± 0.49	0.157	− 0.13 ± 0.79	0.489	0.04	0.435
Globus pallidus	− 0.09 ± 0.54	0.467	− 0.13 ± 0.67	0.399	0.04	0.424
From PCC to						
mPFC	0.24 ± 0.44	0.028	0.02 ± 0.29	0.753	0.22	0.041
PCC	− 0.54 ± 0.34*	0.000	− 0.53 ± 0.28*	0.000	− 0.01	0.450
Thalamus	− 0.06 ± 0.19	0.225	0.10 ± 0.65	0.503	− 0.16	0.165
Striatum	− 0.10 ± 0.41	0.324	− 0.21 ± 0.54	0.101	0.12	0.228
Globus pallidus	0.22 ± 0.68	0.180	− 0.57 ± 0.61*	0.001	0.79#	0.001
From thalamus to						
mPFC	− 0.03 ± 0.34	0.686	0.01 ± 0.07	0.597	− 0.04	0.324
PCC	− 0.02 ± 0.45	0.877	0.00 ± 0.11	0.909	− 0.01	0.455
Thalamus	− 0.50 ± 0.26*	0.000	− 0.45 ± 0.25*	0.000	− 0.05	0.277
Striatum	0.08 ± 0.64	0.599	− 0.03 ± 0.45	0.794	0.11	0.279
Globus pallidus	− 0.17 ± 0.70	0.317	0.26 ± 0.32	0.002	**− 0.43**	0.011
From striatum to						
mPFC	− 0.10 ± 0.30	0.164	0.06 ± 0.15	0.091	**− 0.16**	0.020
PCC	− 0.03 ± 0.28	0.612	0.01 ± 0.14	0.873	− 0.04	0.305
Thalamus	0.04 ± 0.43	0.652	− 0.13 ± 0.26	0.037	0.17	0.064
Striatum	− 0.40 ± 0.39*	0.000	− 0.46 ± 0.32*	0.000	0.06	0.304
Globus pallidus	− 0.23 ± 0.60	0.113	0.25 ± 0.54	0.054	− 0.48#	0.006
From globus pallidus to						
mPFC	0.03 ± 0.30	0.623	0.02 ± 0.18	0.691	0.02	0.418
PCC	− 0.12 ± 0.33	0.131	0.04 ± 0.17	0.310	**− 0.16**	0.032
Thalamus	0.06 ± 0.28	0.389	− 0.28 ± 0.33	0.002	0.34#	0.001
Striatum	0.31 ± 0.32*	0.001	0.11 ± 0.37	0.201	**0.20**	0.039
Globus pallidus	− 0.22 ± 0.25	0.001	− 0.40 ± 0.39*	0.000	0.18	0.042

*Significant effective connectivity at the group level ($P < 0.05$, FDR-corrected); #significant differences between groups ($P < 0.05$, FDR-corrected); significant differences ($P < 0.04$, uncorrected) between groups are shown in bold. PCC, posterior cingulate cortex; mPFC, medial prefrontal cortex

loss of striatal output to the globus pallidus in healthy individuals under propofol-induced sedation, and this influence returned after the recovery of consciousness, suggesting that the striatopallidal inhibition is modulated by the level of consciousness. We found a significantly decreased and negative influence from the globus pallidus to the thalamus in the DOC patients. Several current models suggest that striatal projection neurons modulate the inhibitory pallido-thalamic connectivity [8, 45, 46] in both direct and indirect pathways [47–49]. These existing models have been proposed to be linked to basic motor functions essential for survival [50], an aspect which might explain the impaired motor function of DOC patients.

Differences in the Connectivity between the Basal Ganglia and the Cortex between the Two Groups

In addition to the disrupted subcortico-subcortical connections (the striato-pallido-thalamic loop) in the DOC patients, two significantly changed subcortico-cortical pathways were found between the basal ganglia and cortical regions. First, we detected a significantly decreased

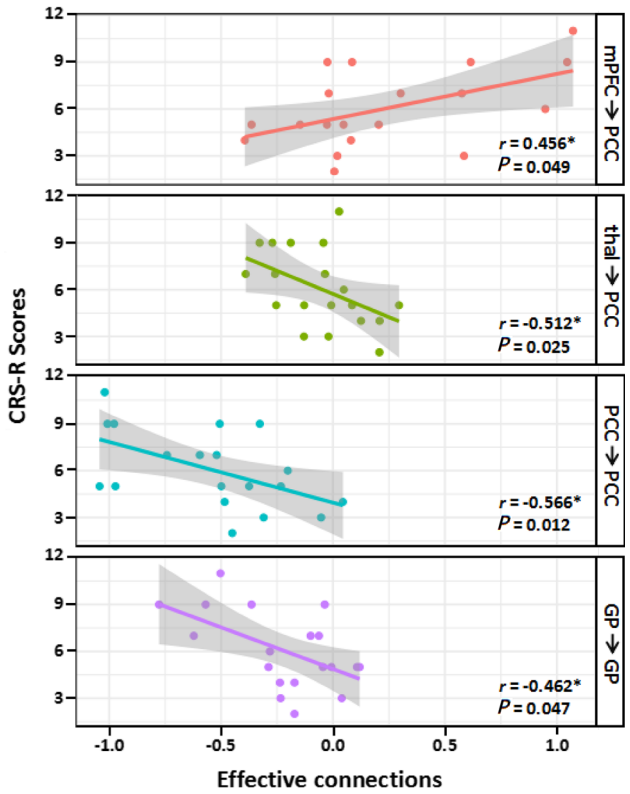

Fig. 4 Significant Spearman's correlation between the CRS-R scores and the effective connections (EC) in the DOC patients. Scatter plots showing the linear fits between ECs and CRS-R scores. Each dot represents a patient. Each colored line represents the fitted line in each correlation. The grey shaded areas show the 95% confidence interval ($P < 0.05$). mPFC, medial prefrontal cortex; PCC, posterior cingulate cortex; thal, thalamus; GP, globus pallidus.

and negative influence from the mPFC to the striatum in the DOC patients. Previous studies [42, 51–53] have suggested that, as an important part of the DMN, the mPFC is engaged in modulating consciousness levels. In addition, the striatum receives frontal influence from the mPFC through different types of connectivity, which are proposed to influence information processing [54], sensorimotor functions [55], self-related social cognition [56, 57], and decreased dopamine [58, 59]. Second, we found a significant difference between the two groups in the connection between the globus pallidus and the PCC. Our results agree with previous findings implying that pallido-cortical connectivity [31] is modulated by the level of consciousness. Significant differences in the mPFC-striatum-globus pallidus loop between groups are also in line with a previous study [5], implying that the basal ganglia might regulate consciousness and cortical activation *via* the cortico-striato-pallidal loop. Taking these studies together, we can reasonably infer that the basal ganglia-cortical loop is a prominent neural circuit in regulating consciousness. Animal models have shown that the basal ganglia are critical for motion with its direct and indirect neural circuits [29, 30]. This evidence may explain the movement disorders in DOC patients.

Impaired Thalamic and Extrathalamic Circuits in Disorders of Consciousness

In contrast to the widespread deafferentation across the thalamo-cortical system that is hypothesized in the meso-circuit theory [8], we did not find a significant difference in thalamo-cortical connectivity between the patients and the controls. A growing body of literature has suggested that the direct connectivity of the thalamo-cortical loop may be less involved in loss of consciousness [7, 31, 60, 61] and that thalamic input may be neither necessary nor sufficient to produce wakefulness [21]. Our results might support those studies indicating that thalamo-cortical deafferentation is mainly associated with higher cognitive functions and motor responsiveness [8], whereas the basal ganglia-cortical loop seems to play a more important role in regulating consciousness [5, 21, 25]. Although no significant difference was detected in thalamo-cortical connectivity, the thalamo-globus pallidus-posterior cingulate

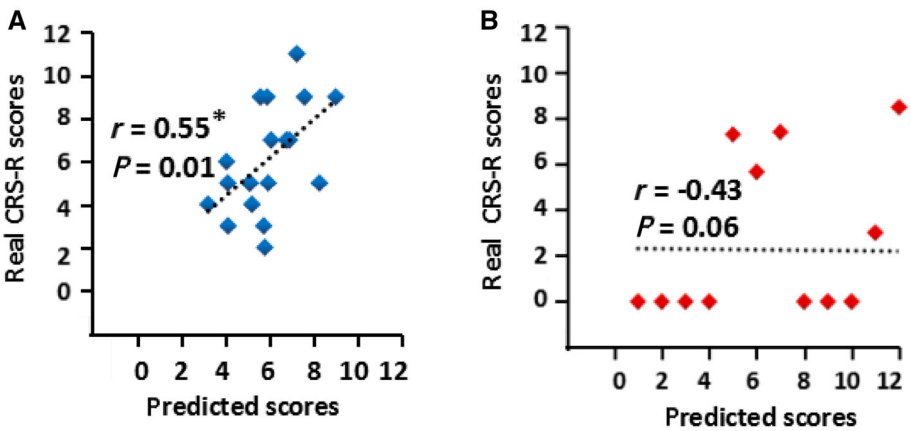

Fig. 5 Spearman's correlation between real and predicted CRS-R scores using machine learning. **A** Features selected only from negative effective connections. **B** Features selected only from positive effective connections. Each dot represents a patient (*$P < 0.05$).

cortex loop was found to differ between the two groups. Our results support the view that, at least in DOCs, different aspects of consciousness can be mediated by both thalamic and extrathalamic circuits including the basal ganglia-cortical circuit [25].

Negative Correlations Predicted CRS-R Scores

From the machine-learning analysis based on the correlative relationship between the patients' ECs and their clinical assessments, we found that negative correlations predicted the patients' CRS-R scores (Fig. 5A). Three significant features played a joint role in this prediction (Fig. 4). First, as the level of consciousness decreased, self-connection within the PCC approached zero. This suggests that self-inhibition of the PCC is related to consciousness levels. A close relationship between the activity of the PCC and the levels of DOCs has consistently been found across different kinds of imaging studies [1, 3, 62, 63]. Similarly, PCC activity is an important index for distinguishing different types of DOC, such as between MCS and VS/UWS [3]. Second, patients with higher CRS-R scores also had increased self-inhibition in the globus pallidus. This seems to support the mesocircuit hypothesis [8] by implying that dynamic changes in the globus pallidus occur during recovery from DOCs. Third, the EC from the thalamus to the PCC was negatively correlated with the CRS-R scores. Previous evidence [35, 64] has shown that functional connectivity between the thalamus and the PCC is associated with the degree of impaired consciousness. Our findings indicate that the EC might be a useful marker of a patient's state of impairment.

Although the positive feature made a non-significant prediction of the CRS-R scores, a significant correlation was detected before the prediction step. We found a positive correlation between the CRS-R scores and the EC from the mPFC to the PCC. A previous study [65] has implied that functional coupling within the midline DMN may be useful for detecting different levels of DOCs. The DMN also has particularly interesting implications for consciousness-level studies ranging from pathological alterations of consciousness [1–3, 32, 33, 63, 66] to sleep-wake cycles [60, 67, 68] and pharmacological changes (e.g., anesthesia) [69–71]. Our results support a previous study [65] that suggested that the decoupling of these two spontaneous, synchronized regions of the DMN could account for the varied states of consciousness. Notably, this positive feature (correlation) failed to build a significant model for the DOC patients in the subsequent prediction step. This supports previous studies [65, 72] showing that patients with extreme consciousness disruption can maintain a preserved DMN network. More interestingly, none of the four effective connections with a significant correlation with the CRS-R scores of the DOC patients showed any group difference from the healthy controls. Our results imply that the EC-based predictive model is able to predict patient outcome. However, the changes within the midline DMN, the self-inhibition of the globus pallidus, and the thalamo-PCC connectivity may not be a definite diagnostic biomarker for DOC patients.

Limitations

A number of limitations should be considered when interpreting our results. First, although we recruited 40 patients, we discarded more than half based on relatively strict procedures of quality control. A larger cohort is needed to validate our results, especially the cross-validation results. Second, previous studies [73–75] have shown that specific losses of neurons in the thalamus are associated with different kinds of disease (e.g. the central thalamus with DOCs [76]). In our study, to fully utilize this region, the entire thalamus was extracted; therefore, further studies are needed to correlate different parts of the thalamus with brain or behavioral functions. Third, because our signals were acquired from fMRI rather than neuron-level experiments, our interpretation of the results must be limited to the meso-system level. Particularly importantly, the positive or negative signs of the ECs should be kept in mind when explaining our findings. We may not conclude that the EC is an inhibitory or an excitatory signal in the biological sense. Last, we performed an FDR correction to correct for multiple comparisons, and we also expected <1 false-positive at P <1/25 uncorrected for the between-group differences, which accordingly increased the probability of type-I (false-positive) errors. In this way, we were able to investigate more specific influences between each pair of regions. Importantly, our main findings among the thalamus, the basal ganglia, and the PCC persisted after correction. Further studies with a finer-grained hypothesis for every brain region, unlike this one that used the fully-connected model in an exploratory manner, could reduce the number of connections in the model and the false-positive error rate.

Conclusion

In the current study, we investigated the effective connectivity between the anterior forebrain regions in DOC patients and matched controls. Our findings were primarily in line with the mesocircuit hypothesis, showing a dysfunctional thalamo-basal ganglia-cortical connection loop in the DOC patients. Self-connections within the PCC and the globus pallidus seemed to be less variable in the DOC patients and were highly effective when used to predict the

patients' clinical scores. The current findings suggest that the effective couplings among anterior forebrain regions have potential value for the clinical assessment of DOCs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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