Appendix e-3. Supplement to the Discussion

Specificity of functional activity changes identified in patients with DOC

A fundamental aspiration is to discover specific neural mechanisms associated with DOC. We acknowledge that the differences in functional activity identified in this meta-analysis may not be specific to patients with DOC. The identified studies did not make direct comparison between patients with DOC and patients with other neurological disorders. Research is needed to determine which configurations of node/edge/network impairment are more specifically linked to DOC (and its different subtypes), differentiating them from changes observed in other neurological phenotypes.

Analysis according to DOC phenotype and etiology

The term DOC encompasses a heterogeneous group of patients with coma, VS or MCS and with diverse etiologies. In the systematic review, we reported the types of DOC assessed in each study. Wherever possible, differences between functional neuroimaging results in MCS and in VS patients are extensively discussed in the systematic review section of the Results. In many studies, however, this approach was not possible because authors combined MCS and VS patients for analysis. In the coordinate-based meta-analysis, we used several approaches to help distinguish resting functional activity in MCS and VS. First, we analyzed all groups of DOC together. Then, we analyzed data in MCS and VS groups separately. Finally, we ran a conjunction analysis which did not yield any significant between-group differences, likely because of the small number of studies (usually this requires more than 15 studies on each arm).
Regarding the two most frequent etiologies of DOC (anoxic brain injury and TBI), the majority of the studies enrolled mixed samples of patients with anoxia, TBI, as well as patients with less common causes; these studies did not group patients by etiology for analytical purposes. A small number of studies enrolled and/or ran analyses of patients grouped by etiology. Results of these studies are summarized in the systematic review. Coordinate based meta-analysis according to DOC etiology (TBI or anoxia) was possible with anoxia (6 studies met inclusion criteria) but not with TBI (only one study available).

**Analysis according to functional neuroimaging approach**

In the systematic review we report the results of studies which used PET or SPECT and those using fMRI separately. However, in the meta-analysis, we chose to pool data from PET and fMRI studies. Multiple studies have demonstrated that estimates of spatial uncertainty are comparable in PET and fMRI \(^{e1-e4}\). Furthermore, extensive evidence supports pooling of data from studies using either technique is appropriate for meta-analytical purposes \(^{e5-e9}\). ALE-based meta-analysis models coordinates derived from both methodologies similarly and looks for consistency. This approach allows larger sample size and higher power. Segregating fMRI and PET studies and performing two meta-analyses (as opposed to pooling data) may or may not generate different results; however, the trade-off in sample size and power would increase the likelihood of errors of inference. We therefore preferred work with a larger sample especially since the scientific and methodological rationale for pooling modalities has been demonstrated elsewhere.
Variability across studies

Our main objective in performing this systematic review and meta-analysis was to be inclusive of all relevant studies and simultaneously to limit heterogeneity as much as possible. We reported on all studies that met our inclusion criteria for the systematic review (result section, table e3). We did not exclude any study or limit our reporting to results that were convergent. The brain regions which the paper focuses on were identified through coordinate-based meta-analysis, which was done in attempt to limit variability. To limit variability, we used rigorous inclusion criteria for the systematic review. To be included in the study, the report should have at least 5 subjects with a control arm and to further include the report in the meta-analysis, the analysis should have been performed using data driven model (ICA, ALFF, GLM) at the brain level thus limiting bias from pre-defining regions of interest. ALE coordinate-based meta-analysis aims to find consistencies among studies. The regions which the meta-analysis identifies were found to be consistently impaired in DOC subjects compared to controls. To identify these regions, we used all the reported coordinates from all studies that reported difference between DOC and control. The results of the meta-analysis were significantly consistent, suggesting that these areas are almost always involved in DOC patients.

Test-retest reliability in functional connectivity measurements

Resting-state neuroimaging studies conducted in healthy controls suggest that significant changes occur in resting functional connectivity over the course of a single recording e10-e12. However, the degree to which such intra-subject changes might confound interpretation of
resting state activity in patients with neurological disorders has not been addressed in published
studies e13. We speculate that this type of confounding will be limited by the inclusion of
multiple datasets. The relatively consistent findings we report support this prediction, however
this question needs to be explored in future studies.

Inferences from the BOLD signal

The BOLD signal is a hemodynamic surrogate whose accuracy and reliability in mapping neural
activity may be confounded by multiple variables including the balance of regional excitatory
and inhibitory activity within the brain, physiological changes, pharmacological changes, and
neurological disease e14. Overall, the degree to which the BOLD signal reflects differences across
brain regions is a fundamental concern which requires further study. However, fMRI remains
arguably the best available tool to map human brain function in vivo e15. Measurements obtained
from the available studies assessing DOC are probably our best representation of brain function
in this condition. The methodology we used evaluated consistency across studies regarding
regional BOLD signal impairment when contrasting healthy controls and DOC; we did not
analyze the degree of functional impairment (this point is brought out in the Discussion section).
In summary, the results of this coordinate-based meta-analysis show significant consistencies
across studies which were conducted using the best available tools for mapping the human brain
function in vivo.
References


e15. Logothetis NK. What we can do and what we cannot do with fMRI. Nature 2008;453:869-878.