Sedation of Patients With Disorders of Consciousness During Neuroimaging: Effects on Resting State Functional Brain Connectivity

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BACKGROUND: To reduce head movement during resting state functional magnetic resonance imaging, post-coma patients with disorders of consciousness (DOC) are frequently sedated with propofol. However, little is known about the effects of this sedation on the brain connectivity patterns in the damaged brain essential for differential diagnosis. In this study, we aimed to assess these effects.

METHODS: Using resting state functional magnetic resonance imaging 3T data obtained over several years of scanning patients for diagnostic and research purposes, we employed a seed-based approach to examine-order resting state connectivity in higher-order (default mode, bilateral external control, and salience) and lower-order (auditory, sensorimotor, and visual) resting state networks and connectivity with the thalamus, in 20 healthy unsedated controls, 8 unsedated patients with DOC, and 8 patients with DOC sedated with propofol. The DOC groups were matched for age at onset, etiology, time spent in DOC, diagnosis, standardized behavioral assessment scores, movement intensities, and pattern of structural brain injury (as assessed with T1-based voxel-based morphometry).

RESULTS: DOC were associated with severely impaired resting state network connectivity in all but the visual network. Thalamic connectivity to higher-order network regions was also reduced. Propofol administration to patients was associated with minor further decreases in thalamic and insular connectivity.

CONCLUSIONS: Our findings indicate that connectivity decreases associated with propofol sedation, involving the thalamus and insula, are relatively small compared with those already caused by DOC-associated structural brain injury. Nonetheless, given the known importance of the thalamus in brain arousal, its disruption could well reflect the diminished movement obtained in these patients. However, more research is needed on this topic to fully address the research question. (Anesth Analg 2016;00:00–00)

Survivors of severe brain injury may pass into a coma: a state of absent brain arousal (level of alertness) and awareness (content of awareness).¹ Coma usually lasts no longer than 3 weeks, after which a patient may recover some brain arousal.¹² Because still no awareness can be detected, the name unresponsive wakefulness syndrome (previously known as vegetative state; VS/UWS) is used.³ Brain awareness may return partially (minimally conscious state; MCS) or completely.⁴ Coma, VS/UWS, and MCS are collectively known as disorders of consciousness (DOC).

Neuroimaging in DOC is applied to improve accuracy of the differential diagnosis by helping to detect signs of awareness in patients with limited or absent body control. With active task paradigms, some patients, whose awareness is spared to some extent, can modulate brain activity in a way that is similar to that observed in healthy control
subjects performing the tasks. This brain activity can be picked up with functional magnetic resonance imaging (fMRI) or electroencephalography and can even be used to establish a form of limited communication.5,4 However, such active brain modulation relies on high degrees of cognitive control, which is rare in patients in MCS. Passive neuroimaging paradigms, for instance, the application of a salient sound such as the subject’s own name, test the brain’s response to the stimulus and do not need great patient cooperation. Unfortunately, common sensory problems like aphasia could render these paradigms uninformative. Task-free neuroimaging of the brain during the resting state (a nonsleep, mind-wandering state3) does not have these shortcomings. It is used to compare brain metabolism (with positron emission tomography) or functional brain connectivity (with resting state fMRI) in patients with healthy controls, inferring consciousness when high similarities are found.9

Resting state fMRI looks at spontaneous, oscillatory neuronal activity at the low-frequency range (<0.1 Hz). Brain regions that show a synchronized activity oscillation are thought to be functionally connected in a resting state network (RSN). A set of robustly detected RSNs includes the default mode network (DMN, consisting of the posterior cingulate cortex/precuneus, inferior parietal lobules, medial prefrontal cortex, medial temporal lobes, dorso-lateral frontal cortex, pontine tegmental area, and thalamus), external control networks (ECNs, consisting of the inferior parietal, dorsolateral and medial prefrontal cortices, and thalamus), and the salience network (consisting of the anterior cingulate cortex, bilateral anterior insulae, and thalamus).10–14 These higher-order RSNs are associated with internal awareness, external awareness, and saliency detection, respectively.11 Lower-order RSNs include the auditory RSN (consisting of the bilateral insulae/superior temporal cortices and thalamus), sensorimotor RSN (consisting of bilateral sensorimotor cortices and thalamus), and visual RSN (consisting of bilateral visual cortices and thalamus).10,11 The integrity of higher-order RSNs, and especially the DMN, has been shown to be indicative of the level of consciousness during sleep15 and anesthesia14,15,16 and in DOC.12,13,15 Current diagnostic examinations of DOC also include the other RSNs to gain a more complete picture of disturbed brain function.12,20 Additionally, thalamocortical connectivity changes have also been shown to play a role in DOC.19,21,25

Acquisition of resting state fMRI in patients with DOC is a challenging operation. One of the main problems is restraining patient head motion, because (resting state) fMRI is exceptionally sensitive to movement. This could induce false-positive “activations” when motion artifacts are correlated with neuronal activation. False-negative “activations” can result from reduced detection sensitivity due to motion-induced noise.26 Furthermore, head displacement leads to an altered head position in the scanner, changing slice orientation. Post-scan preprocessing steps cannot fully repair such severely damaged data, although this has become an active field of research.27–30 The problem of movement is especially present in patients with DOC.31,32 Traditional physical head restraint techniques include the placement of foam cushions around the head. Unfortunately, this precaution is often unable to cope with strong motion impulses in patients with DOC. Therefore, an often applied method for head motion reduction is sedation.33 However, little is known about the possible effects of sedation on resting state connectivity, which is important for differential diagnosis.

In the present study, we examined the effect of sedation on long-range resting state connectivity in patients with DOC, using resting state fMRI. We chose propofol, because this is one of the most well-studied, most applied, and safest anesthetic agents available.34 It is the drug of choice for immobilization purposes in MRI research, because it has short induction and recovery times, and does not usually require additional sedatives.35 We compared RSN integrity in 8 unsedated patients with DOC that had limited movement in the scanner with 8 patients with DOC who had been sedated to reduce their high-intensity movement. These groups were matched for age at onset, etiology, time spent in DOC, diagnosis, and the Coma Recovery Scale Revised (CRS-R) total score.36 Equality of structural brain injury was also assessed (using voxel-based morphometry (VBM8 [http://dbm.neuro.uni-jena.de/vbm/] for SPM8 [www.fil.ion.ucl.ac.uk/spm]), as was movement intensity.25 RSN integrity was furthermore compared with 20 age-matched healthy controls. Although a comparison of brain connectivity in unsedated patients with brain connectivity in the same patients during sedation would have allowed for a more powerful analysis, it would have relied on sedating patients who would not need the sedation. As the patients themselves were not in a condition to make an informed decision, in contrast to previous propofol studies with healthy subjects,11,14,17,18 we deemed such an approach unethical.

We expected patients with DOC to have severely disrupted higher-order RSNs.12,20 Given previous examinations of the effect of mild propofol sedation on RSN connectivity in healthy controls,13,14,17 and the fact that patients with DOC might already have severe disruption of RSNs,7,19–21 we predicted that propofol sedation would not greatly affect RSN integrity in patients with DOC.

METHODS

Subjects

Of an initial resting state fMRI database of 180 patients with DOC (coma, VS/UWS, and MCS) and patients with the locked-in syndrome and those that had recently recovered from the MCS (exit MCS), constructed over several years of scanning patients for diagnostic and research purposes, 126 patients were diagnosed as being in the VS/UWS or MCS. Of these, 36 patients had been scanned while being sedated solely with propofol, and 49 patients had been scanned without any sedative. Eventually, resting state fMRI data from only 8 unsedated patients with DOC (mean age = 44 ± 16 years; time in DOC = 695 ± 1169 days, median = 91 days, interquartile range = 23–693 days; 2 traumatic MCS and 6 nontraumatic VS/UWS; mean CRS-R total score = 7 ± 3), and 8 patients with DOC sedated with propofol (mean age = 43 ± 21 years; time in DOC = 867 ± 1426 days, median = 50 days, interquartile range = 25–1086 days; 2 traumatic MCS and 6 nontraumatic VS/UWS; mean CRS-R total score = 7 ± 4) were selected (Table 1). This was a direct result of our
Sedation Protocol

The decision to sedate the patient or not was taken by an MRI scanning expert and was based on the severity of patient movement when placed in the MRI scanner. The propofol concentration was kept to a minimum (Table 1). Foam cushion head restraints were placed. Before fMRI data acquisition, all subjects fasted for at least 6 hours for solids, and 2 hours for liquids. During scanning, they wore headphones and earplugs. Propofol sedation was administered through intravenous infusion, using a target-controlled infusion system (Diprifusor, pharmacokinetic model of Marsh et al9, AlarisTM, Alaris Medical Belgium B.V., Strombeek-Bever, Belgium), to obtain constant plasma concentrations. To ensure adequate ventilation, some patients received assisted mechanical ventilation through a tracheostomy or through an endotracheal tube when already in place. Additional oxygen was delivered, either through a facemask or through the airway instrumentation device. Parameters of all patients were closely and continuously monitored during the procedure, including arterial blood pressure, electrocardiogram, breathing frequency, and pulse oximetry (SpO2). All parameters remained stable during data acquisition. When administered, sedation was titrated to achieve immobility in the scanner. Once obtained, the necessary plasma concentration of propofol was kept constant throughout the procedure. Scanning started 5 minutes after having reached the desired clinical state (immobility), hence allowing time for the equilibration of propofol concentrations between pharmacokinetic compartments. For post-hoc confirmation of propofol plasma concentrations, a blood sample was drawn in some patients during the steady-state period of sedation (before and after the sequence). Sedation characteristics are summarized in Table 1. Throughout the procedure, a certified

strict patient inclusion criteria for this study. These were: diagnosed as being in the VS/UWS or MCS, as assessed with the CRS-R; scanning occurred more than 2 weeks after initial brain injury in stabilized patients; propofol was used as the sole sedative agent (for the sedated group); an absence of large hemorrhage effects, movement artifacts, foreign body artifacts, midline shifts, acquisition artifacts, low gray–white matter contrast, or exceptionally severe structural brain injury, as assessed by careful visual inspection of the T1 images by an expert. Both DOC groups were matched for age at onset, etiology, time spent in DOC, diagnosis, CRS-R total score, and movement intensities, as assessed both parametrically and nonparametrically. The diagnosis of MCS or VS/UWS was based on behavioral analysis with the CRS-R, which was repeated several times during a week and performed by trained professionals. The CRS-R is a standardized scale that is currently considered to be the most trustworthy behavioral diagnosis tool for patients with DOC available. The control group consisted of 20 healthy, unsedated control subjects (mean age = 46 ± 18 years). The study was approved by the Ethics Committee of the Medical School of the University of Liège and the IRB. Written informed consent to participate in the study was obtained from the subjects themselves in the case of healthy controls, and from the legal surrogates of the patients.

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Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>CRS-R</th>
<th>CR-SR Total Score</th>
<th>Time in DOC (Days)</th>
<th>Target Propofol Concentration (ug/mL)</th>
<th>Heart Rate, Median (Range)</th>
<th>Target Propofol Rate (mL/min)</th>
<th>Airway Device</th>
<th>Ventilation</th>
<th>Breathing</th>
<th>Age at Onset (Years)</th>
<th>Etiology</th>
<th>CRS-R Total Score</th>
<th>CR-SR Total Score</th>
<th>Movement Intensities</th>
</tr>
</thead>
</table>
anesthesiologist and complete resuscitation equipment were present.

**Data Acquisition**

Structural MRI T1 data (T1-weighted 3-dimensional gradient echo images using 120 slices, repetition time = 2300 milliseconds, echo time = 2.47 milliseconds, voxel size = 1.0 × 1.0 × 1.2 mm³, flip angle = 9°, field of view = 256 × 256 mm²) and resting state fMRI data (Echo planar imaging sequence using 32 slices, repetition time = 2000 milliseconds, echo time = 30 milliseconds, voxel size = 3.0 × 3.0 × 3.0 mm³, flip angle = 78°, field of view = 192 × 192 mm², 300 volumes) were acquired on a 3T scanner (Siemens, Munich, Germany).

**Assessment of Equality of Movement Intensity and Brain Morphology**

Movement severity was assessed by calculating interscan movement and total head displacement using 6 rigid-body movement parameters, and a t test was applied to examine potential differences in movement severity between patient groups. Similarly, a T1-based voxel-based morphometry analysis of brain structure (VBM8 [http://dbm.neuro.uni-jena.de/vbm/] for SPM8 [www.fil.ion.ucl.ac.uk/spm]) was applied to search for potential morphological differences between both patient groups. For this analysis, we used DARTEL-based spatial normalization to allow for high-dimensional spatial normalization to increase the chance of correct normalization of the severely injured DOC brain. A DOC template made from T1 images from an independent population of 61 patients with DOC was used to further aid the normalization procedure.

**RSN Analysis**

We used SPM8 software (statistical parametric mapping, Wellcome Trust Centre for Neuroimaging [www.fil.ion.ucl.ac.uk]) to realign, normalize, smooth (8 mm), and analyze the data. Movement artifact reduction software was applied (ArtRepair, software written by Paul Mazaika, Center for Interdisciplinary Brain Science Research at Stanford University, http://cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html), and data were temporally bandpass filtered (0.007–0.1 Hz). We performed a seed-based analysis to investigate correlations between low-frequency blood oxygenation level–dependent signals in selected seed regions and the rest of the brain to examine RSN connectivity. The regions selected for the analysis were based on RSNs described in literature. These were (using MNI coordinates): the posterior cingulate cortex/precuneus (DMN; 6, −42, 32), left middle frontal gyrus (left ECN; −44, 36, 20), right middle frontal gyrus (right ECN; 44, 36, 20), right anterior insula (salience RSN; 38, 26, −10), left posterior insula (auditory RSN; −40, −22, 8), supplementary motor area (sensorimotor RSN; −2, −12, 44), and primary visual cortex (visual RSN; −4, −84, 8). Connectivity with the thalamus was also examined (−7, −16, 6 and 7, −16, 6, combined). Spheres with a diameter of 8 mm around these coordinates were used as seed regions. Movement parameters, as well as white matter and cerebrospinal fluid parameters, were used for removal of sources of noise in the resting state fMRI data. For analysis, patients and controls were organized into 3 groups: controls, unsedated patients, and sedated patients. Given the relatively modest patient sample sizes available, we had limited power to detect clinically important differences. Thus, the main aim of this study was to make a first step in understanding the effect of propofol sedation of patients with DOC. Future studies should provide further insights. Whole-brain false discovery rate (FDR)-corrected (thresholded at P = .05) spatial maps were obtained per RSN per group. Potential DOC-associated and propofol-induced brain connectivity decreases were explored using the contrasts (controls > unsedated patients; controls > sedated patients; and unsedated patients > sedated patients) for each of the 8 networks. The results of these contrasts were thresholded at an uncorrected P value of .01. To minimize the chance of false-positive results occurring at this liberal threshold, regions not belonging to the specific RSN examined in each contrast (as found at FDR-corrected P = .05 for the control group) were masked out, and results had to comply with a cluster extent threshold of 30 voxels.

**Table 2. Movement Parameters**

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<tr>
<td>Controls versus patients combined</td>
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<tr>
<td>Unsedated versus sedated patients</td>
<td>P = .72</td>
<td>P = .28</td>
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RESULTS
Assessment of Equality of Movement Intensity and Brain Morphology
A t test assessed potential differences in movement severity between the groups of unsedated and sedated patients, as well as between controls and the combined group of patients, using speed and displacement parameters. 23 No significant difference in movement severity between the patient groups was found, although a potential difference was found between controls and the combined group of patients (displacement: P < .02, speed: P = .02; Table 2).

Differences in gray matter volume (using a threshold of FDR-corrected P < .05) between healthy controls and the combined group of patients with DOC (contrast: controls > patients combined), and between unsedated and sedated patients with DOC (contrasts: unsedated DOC > sedated DOC; sedated DOC > unsedated DOC) were examined. Differences between controls and patients were found to be widespread (P < .001; Table 3). 24 No structural differences could be observed between unsedated and sedated patients with DOC.

Resting State Networks
We separately examined connectivity of each of the 8 RSNs in controls and unsedated and sedated patients. All RSNs were reliably detected in our healthy control subjects (Figure 1). However, the integrity of RSNs obtained in the patient groups appeared to be greatly diminished.

<table>
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<th>Area</th>
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<th>Z</th>
<th>P</th>
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</tr>
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<td>Putamen</td>
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<td>Caudate</td>
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<tr>
<td>Declive</td>
<td>17,−69,−9</td>
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<td>Middle temporal gyrus</td>
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<td>Precentral gyrus</td>
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<td>4.15</td>
<td>&lt;.001</td>
</tr>
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</table>

Default Mode Network
Using a threshold of FDR-corrected P < .05, the DMN consisted of the posterior cingulate cortex/precuneus, inferior parietal lobules, medial prefrontal cortex, medial temporal lobes, dorso-lateral frontal cortex, pontine tegmental area, and thalamus. In unsedated and sedated patients with DOC, connectivity was found in the posterior cingulate cortex/precuneus and inferior parietal lobe.

Salience RSN
The salience RSN consisted of the anterior cingulate cortex, bilateral anterior insulae, and thalamus in controls. In sedated and unsedated DOC, no thalamus and typical salience-network-associated anterior cingulate cortex components were observed. Instead, a more frontal anterior cingulate region was connected.

Bilateral ECNs
In controls, the bilateral ECNs mainly consisted of the inferior parietal, dorsolateral, and medial prefrontal cortices. In both sedated and unsedated DOC, connectivity of the left ECN was mostly restricted to the left dorsolateral prefrontal and inferior parietal cortices. For the right ECN, connectivity in both groups was found in the right dorsolateral prefrontal cortex.

Auditory RSN
In controls, the auditory RSN consisted of the bilateral insulae/superior temporal cortices, several regions of the sensorimotor cortex, and thalamus. In sedated and unsedated DOC, it consisted of left insula/superior temporal lobe and sparse thalamic regions.

Sensorimotor RSN
The sensorimotor RSN consisted of sensorimotor areas and thalamus in both controls and patients with DOC, although, in controls, it also included regions overlapping with the auditory RSN.

Visual RSN
The visual RSN consisted of the visual cortex in all groups, although connectivity with the thalamus was only visible in controls.

Thalamus
The bilateral medial thalamus connected to regions overlapping with those of the DMN, ECNs, and salience network in controls, which could not be observed in the DOC groups.

RSN Contrasts
For each of the 8 RSNs, we used the following 3 contrasts to examine connectivity differences between subject groups: (controls > unsedated patients), (controls > sedated patients), and (unsedated patients > sedated patients). Numerous differences in RSN connectivity were found between controls and unsedated patients (in all but the visual RSN), and between controls and sedated patients (in all but the visual RSN). Relatively limited
further connectivity decreases, concerning the thalamus and salience RSN, were found in sedated as compared with unsedated patients (Figure 2).

**Contrasts “Controls > Unsedated Patients” and “Controls > Sedated Patients”**

Unsedated patients with DOC, as compared with controls, had less connectivity in the DMN (posterior cingulate cortex/precuneus, medial prefrontal cortex, bilateral medial temporal lobes, left inferior parietal lobe, and dorsolateral prefrontal cortex), salience RSN (left insula and anterior cingulate cortex), left ECN (bilateral dorsolateral prefrontal cortices and medial frontal cortex), right ECN (left inferior parietal lobe and left dorsolateral prefrontal cortex), auditory RSN (right insula/superior temporal lobe and sensorimotor cortex), and sensorimotor RSN (right sensorimotor cortex and left medial temporal lobe). Less connectivity with the thalamus was also found (regions overlapping with the DMN, ECNs, and salience RSN). No differences were found for the visual RSN. Highly comparable results were found for the contrast (controls > sedated patients).

**Contrast “Unsedated Patients > Sedated Patients”**

Sedated patients with DOC, as compared with unsedated patients with DOC, had less long-range connectivity (connectivity with regions distant from the seed region) in the salience network (left anterior insula: x = −24, y = 23, z = −5), and with the thalamus (posterior cingulate cortex: x = −4,
y = –31, z = 34; medial prefrontal cortex: x = –8, y = 38, z = 36; left caudate: x = –12, y = 14, z = 13; right caudate: x = 15, y = 11, z = 16; medial prefrontal cortex: x = –8, y = 41, z = 32; and left dorsolateral prefrontal cortex: x = –54, y = 20, z = 13).

No differences were found in the other 6 RSNs.

DISCUSSION

In this study, we aimed to examine the effect of propofol on brain RSNs in the damaged brains of patients with DOC. Because these RSNs are used to examine the level of remaining consciousness, any disruption induced by propofol could interfere with this assessment. As expected, we observed decreases in RSN connectivity in patients compared with controls. Importantly, minor further thalamic and insular disconnections were found to be associated with propofol sedation.

RSNs in Controls and Unsedated Patients With DOC

In our healthy controls, connectivity in the 7 RSNs we examined was comparable to that found in literature.10,11 We also added a seed analysis using the thalamus as seed region, because previous studies on the effect of propofol in healthy subjects have shown thalamic involvement.13,14 The thalamus was found to connect most to regions of the higher-order RSNs. This thalamocortical connectivity was lost in DOC. We, furthermore, found strong DOC-associated connectivity decreases in all 4 higher-order networks (DMN, bilateral ECNs, salience RSN). Similar disruptions have been reported in previous resting state fMRI studies and link the functioning of cortical higher-order RSNs and their connectivity with the thalamus with the generation of consciousness.19–24 Structural brain injury in patients with
DOC was found to be widespread, which is in line with findings from previous post-mortemstudies and MRI studies. Higher-order RSNs depend heavily on long-range connectivity, and these connections might thus be especially vulnerable to structural brain injury. Similarly, long-range connectivity decreases were observed in auditory and sensorimotor RSNs and appear to further reflect the association between reduced brain integration of information and loss of consciousness.

**Possible Effects of Propofol in DOC**

Although propofol-induced loss of consciousness in healthy controls has been found to lead to decreases in connectivity in higher-order RSNs, mild propofol sedation is associated with relatively minor connectivity decreases. In healthy subjects, the quantity of propofol administered to the patients with DOC in this study would have induced a state of mild sedation. However, because little is known about the effect of propofol on RSN connectivity in the damaged brain, a direct comparison between the mildly sedated state in healthy controls and the state resulting from propofol administration to patients with DOC cannot be readily made.

Comparing sedated with unsedated patients in DOC, we found reduced long-range connectivity in the salience RSN and with the thalamus to be associated with propofol administration. As such, induction of these disconnections appears to be sufficient to reduce movement in excessively moving patients with DOC. One of the observed thalamic connectivity decreases involved the striatum, a finding previously reported for healthy subjects sedated with propofol. This underlines the close association between the thalamus and these nuclei and could partially explain differences in thalamocortical connectivity. Striatal modulation of thalamocortical connectivity has been shown to be strongly implicated in the regulation of motor control, depending on the pivotal role of the thalamus in brain arousal. Although it is unclear which are the exact causes of the uncontrolled high-intensity movement observed in a large portion of patients with DOC, some parallels might be drawn between DOC and movement disorders, such as Parkinson's disease, where known disruption of communication between thalamus, striatum, and cortex is also likely to underlie movement abnormalities.

Thalamostriatal and thalamocortical connectivity decreases thus appear to at least partly underlie propofol-induced reduction of patient movement. However, our other findings also hint at a possibly broader affection of brain function and awareness. The thalamus and striatum have been shown to be implicated in the regulation of alertness and switching behaviors. We also found propofol-induced decreased connectivity between the thalamus and (dorsal) posterior cingulate cortex, similar to findings from previous propofol studies with healthy controls. This dorsal part of the posterior cingulate cortex, a key hub in the DMN, has been suggested to play a role in orchestrating the switch between internal and external awareness. In this context, the reduced connectivity of the thalamus with regions of the salience RSN and ECNs, again found in propofol-induced loss of consciousness in healthy subjects, is also interesting. All these connectivity changes might represent minor alterations in the functioning of higher-order RSNs. Although the disruption of these higher-order networks, and their connectivity with the thalamus, could play a role in loss of patient movement control, it has also been associated with disrupted awareness in general. Therefore, propofol application might affect remaining awareness in patients with DOC.

Although the detected propofol-induced reductions include connectivity in the salience network and with the thalamus, which might be used for neuroimaging-based diagnosis, the decreases were relatively minor, especially compared with those decreases caused by DOC-associated structural brain injury. Furthermore, considering the fact that we here present group-level results, intersubject variability might very well overshadow the connectivity reductions we here associated with propofol administration. It is, however, interesting to find that long-range thalamocortical connectivity might still be affected by propofol in patients with DOC, in which only low-level or absence of consciousness is assumed. Theoretically, such a response to propofol might in the future be used as a biomarker in itself, although there are several ethical problems with this idea. Most importantly, patients will only be sedated when absolutely necessary, mostly when the patients move too much to produce analyzable data, given the fact that no unnecessary potential health risks should be taken. Therefore, no analyzable data set will be available for the unsedated state to compare with. In addition, the found reductions in connectivity appear to be too small to produce a reliable biomarker at the single-subject level. However, the great reduction of connectivity found between thalamus and regions of higher-order RSNs observed with our contrast between healthy controls and unsedated patients with DOC, as previously found between thalamus and DMN, as well as during propofol-induced anesthesia, warrants a further examination of this connectivity pattern as a biomarker of consciousness.

**Methodological Considerations**

A clear limitation in our study is the fact that brain injury differs from one patient to the other, and consequently, to some extent, so will the effect of propofol on brain resting state connectivity. This limitation was a direct consequence of our ethics-based policy of sedating patients only when absolutely necessary. However, great care was taken to try to match groups of sedated and unsedated patients for age, time spent in DOC, etiology, diagnosis, CRS-R total score, head movement severity, and structural brain injury. As such, even with our relatively modest patient group sizes, resulting from our great care taken in matching groups, we feel confident that our analysis gives insight into the effects of propofol on RSN connectivity in patients with DOC. It should be noted that, before sedation, there was a difference in the severity of movement between the patient groups. Sedation of the intensively moving group removed this movement difference. However, the initial movement difference, with its underlying mechanisms, might be a potential factor influencing our results for which we could not adjust. For ethical reasons, no PCO2 values were obtained.
during scanning. However, it has been shown that PCO₂ levels do not appear to change the BOLD response to neuronal activity.\textsuperscript{1,7} Furthermore, for our analysis, we are interested in correlations rather than specific regional effects and are therefore confident that PCO₂ levels do not significantly influence our results.\textsuperscript{5,7} Our choice of a seed-voxel approach instead of an independent component analysis was based on its proven robustness, as reflected in our finding of RSNs greatly resembling those mentioned in literature.\textsuperscript{10,11} Although independent component analysis holds promise in the field of resting state fMRI, issues involving the component creation and selection process need to be addressed.\textsuperscript{20,76,77} Finally, the use of tracheostomy or an endotracheal tube in a number of our patients may possibly have had a limited effect on brain state, because these airway devices could induce a certain level of discomfort. To our knowledge, this effect has not been studied in detail, and our modest sample sizes do not allow for an examination. However, although it could potentially increase brain arousal, we expect any influence on our results to be minor, especially knowing that these airway devices were already in place well before our neuroimaging procedure, and knowing that the distribution of the use of these airway devices was relatively even among our sedated and unsedated patient groups.

CONCLUSIONS

In this study, we examined how propofol sedation might affect RSN connectivity in patients with DOC. We found minor propofol-associated decreases in connectivity, involving thalamostraital, thalamocortical, and salience network connectivity. This indicates that these patients still have a form of brain connectivity that can be modified by propofol, and gives insight into the brain mechanisms underlying uncontrolled patient movement. However, the major differences were found between controls and (un)sedated patients with DOC, which is related to the great extent of structural brain injury in DOC. Given the known negative effects of high-intensity movement during resting state fMRI, which decreases detectability of RSNs, propofol sedation might presently be considered to be a good method to ensure analyzable data in patients with DOC with strong, uncontrolled head and body movement. Future studies should further examine safety aspects associated with this procedure.

DISCLOSURES

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