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Ascending Arousal Network Connectivity in Disorders of Consciousness: A Diffusion MRI Study

Darwin Li[1]

[1]Upper Canada College, Toronto, Ontario, Canada

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ABSTRACT: The human brain gives rise to a great variety of conscious experiences. Patients with disorders of consciousness, a state characterized by the dissociation of awareness and wakefulness, are particularly noteworthy. This study attempts to find key biomarkers of the disorder of consciousness state and discover key regions of the brain that govern consciousness. The focus is on the ascending arousal network—a network of nodes and edges representing connections from the brainstem to subcortical (thalamus, hypothalamus, basal forebrain) nuclei and reaching the cerebral cortex. Previous studies using animal models have demonstrated a high prognostic value of the ascending arousal network in relation to consciousness. This study conducts a diffusion tensor imaging analysis and generates a tract count plot to illustrate differences in connectivity between (N=6) healthy controls and (N=6) patients with chronic disorders of consciousness. Each region of interest was isolated to investigate its specific role and impact on consciousness. A principal component analysis was performed to assess the separability of the two cohorts. The results found each of the regions of interest to be significantly ($p<0.05$) disrupted in patients with disorders of consciousness. They contributed equally to the linear separability of the two cohorts. This is consistent with previous research and hints at the importance of the ascending arousal network in governing consciousness. These changes are likely associated with the many pathological deteriorations associated with an impaired cognitive state, such as neuronal loss, gliosis, and the degeneration of white matter tracts that connect critical areas of the brain involved in consciousness.

INTRODUCTION

The mechanisms of consciousness are characterized by the dynamically changing interplay of neural activities underpinning two factors: human awareness and wakefulness.[1] A myriad of previous studies have attempted to isolate the precise neurobiology and function of the human brain that governs consciousness. Yet, given the brain is an immensely complex system, this realm of neuroscience remains poorly understood.

An increasingly promising biomarker of the conscious state is the structural connectivity within the ascending arousal network (AAN).

Knowledge of the AAN used to be largely based on past studies using animals with experimentally induced lesions.[2,3,4] Recent research reveals similarities between the human AAN and that of animals. A mapped neuroanatomic connectivity of the human AAN revealed definite nuclei connections that mediate wakefulness. The AAN is now believed to consist of neuronal projections from the brainstem that extend to subcortical (thalamus, hypothalamus, basal forebrain) nuclei before reaching the cerebral cortex. Thalamic pathways are a key relaying center through which brainstem neurons pass to the cerebral cortex, and the thalamus helps to integrate and modulate these nuclei interactions.[5]

The hypothalamus, along with the basal forebrain further mediates wakefulness by regulating various autonomic functions and circadian sleep-wake cycles. In the end, the AAN is a network of nodes representing gray matter nuclei, beginning with arousal (wakefulness) pathways in the brainstem and connecting to awareness networks in the cerebral cortex.[5,6,7]

The human brain gives rise to a great diversity of conscious experiences based on the varying extents of awareness and wakefulness. In this spectrum, patients in pathological or pharmacological coma experience the least conscious state. They are characterized by a loss of both awareness and wakefulness to the surrounding environment, usually as a result of severe brain injuries.[1] Among those who survive, 44-45% remain in a disorder of consciousness (DoC), such as a vegetative state (VS) or minimally conscious state (MCS).[8] These are unique instances where awareness and wakefulness are disassociated. Patients with DoC have their eyes wide open and are awake but are not aware of and cannot voluntarily interact with their surrounding environment.[1] This is supported by several functional MRI-based approaches, which found preserved cognitive processing and willful modulation of brain activity in DoC patients, indicating that behavioral unresponsiveness might not always equate to a complete absence of cognitive function.[9,10] This unique dissociation of awareness and wakefulness allows studies to isolate awareness from wakefulness and identify specific correlates of each. For instance, there has been a postulation that the default mode network is compromised in DoC patients due to a loss of awareness while structures integral to wakefulness appear to be maintained.[11]

The prognostic importance of the AAN has become particularly evident in comatose patients. Acute damage to the brainstem is a hallmark of these patients, likely a consequence of a lesion centered in the upper pons or in the midbrain which disrupts AAN pathways.[12] Consistent with this, traumatic coma has met the criteria for a disconnection syndrome with a complete disconnection of brainstem arousal nuclei from previously mentioned subcortical nuclei.[7,8] In addition, connectivity between the temporal lobe and the medial thalamus and rostral brainstem seems to be linked to the loss of consciousness associated with temporal lobe epilepsies.[13] A disassociation of awareness and wakefulness is detected during seizures, although this is a much more transient example.[1]

Here, we further test the AAN as a regulator of consciousness using a cohort (n=6) of patients suffering from chronic DoC following traumatic brain injury (TBI) and comparing them with a cohort of (n=12) healthy controls. We use previously published high-angular resolution diffusion imaging (HARDI) data to evaluate AAN connectivity in each cohort.[8] HARDI captures the architecture of white matter fibers in the brain and offers a detailed representation of neural pathways including key intersecting and diverging regions. With the HARDI data, we conducted diffusion tensor imaging (DTI) and tractography analysis to determine differences in AAN connectivity between patients with chronic post-traumatic DoC and healthy controls. Finally, we performed a principal component analysis (PCA) to highlight the axes accounting for the most variance between the cohorts.

METHODS

Patients

Previously published HARDI data of six patients with chronic DoC was obtained from OpenNeuro dataset 003367.[14] Among them, three were diagnosed with VS and three with MCS on the Coma Recovery Scale-Revised (CRS-R, total = 6.5) following TBI. Similarly, HARDI data of 12 healthy controls with no recorded psychiatric or neurological disorders were attained. Details of the HARDI sequence can be found in previous research.[14] Note specifically the 2 mm isotropic resolution, 60 diffusion-encoding directions, and contrast

of b = 2,000 sec/mm2.

Processing

All preprocessing was done using FSL and MRtrix3. Brain extraction (0.2 thresholds), eddy current, and bulkhead motion correction were performed. Linear registration was used to transform each patient's diffusion space to Montreal Neurological Institute (MNI) 152 T1 1 mm space. To guide the tractography, fiber response functions were estimated using the Tournier algorithm, and fiber orientation distributions (FODs) were computed. A white matter mask was also generated to confine the whole-brain tractography. Finally, diffusion tensor imaging (DTI) parameters were calculated specific to the entire brain and AAN regions of interest (ROIs). Metrics such as Fractional Anisotropy (FA), Mean Diffusivity, Axial Diffusivity, and Radial Diffusivity were derived.

Regions of interest

A combination of previously published ROIs, the Harvard Ascending Arousal Network Atlas, and the Harvard-Oxford cortical and subcortical structural atlases included within FSL were used.[14] The thalamus, hypothalamus, and basal forebrain were taken from publicly available ROIs. The cortical lobes were generated from the Harvard-Oxford cortical and subcortical structural atlases, and brainstem ROIs from the Harvard Ascending Arousal Network Atlas. All ROIs matched the MNI 152 T1 1 mm space.

Tractography

Whole brain tractography was first generated, guided by a white matter mask. Three million streamlines were launched and dynamic seeding based on the FODs was used. Then, a second tractography was created only including tracts connecting AAN brainstem ROIs to the thalamus, hypothalamus, and basal forebrain. Here, a threshold of 100 mm was set as the maximum length of streamlines. Otherwise, default parameters were used. Finally, a voxel-wise map representing the number of streamlines passing through each voxel in the AAN tractography was generated, normalized by the total number of streamlines launched. The exact path of the streamlines within each voxel was accounted for and the results were set to unsigned 32-bit integers. Finally, tract-specific analyses of DTI parameters were performed, and the Benjamini-Hochberg method was used to control false discovery rates.

Principal component analysis

To assess the separability of chronic DoC patients from healthy controls, a PCA was conducted in R Studio with connectivity in each AAN ROI as the dimensions. Principal components 1 (PC1) and 2 (PC2) were projected on the same axes along with the eigenvectors for each ROI to explain the major patterns in the data while preserving the maximum variance. The associations of each ROI with PC1 and PC2 were determined by computing their respective loadings using the formula $L = v \cdot \sqrt{\lambda}$, where L is the loading, v is the component of the eigenvector of the ROI on the axis of the PC, and λ the eigenvalue of the PC.

RESULTS

To visualize AAN connectivity between controls and chronic DoC patients, we generated normalized tract plots of streamlines passing between all subcortical AAN structures. The axial and sagittal views are shown (Figure 1).

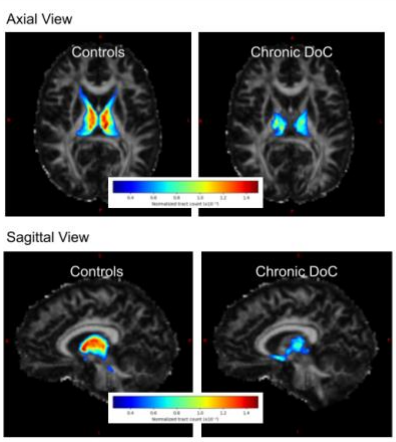

Figure 1. Differences in AAN connectivity between healthy controls and patients with chronic disorders of consciousness.

Connectivity was measured by the group-sum value of the number of streamlines between all subcortical AAN structures (brainstem to thalamus, hypothalamus, and basal forebrain), normalized by the total number of streamlines launched (3M).

With tracts from the brainstem extending fully into the thalamus and hypothalamus regions, controls exhibit much more robust and widespread connectivity than chronic DoC patients. The latter's lack of tracts indicates greatly reduced AAN connectivity, with narrower areas of activation, fewer pathways, and reduced communication between all AAN ROIs.

To determine connectivity in each AAN ROI, a boxplot was created with the tract count of each control and DoC subject (Figure 2). In all of the hypothalamus, basal forebrain, thalamus, and brainstem regions, we observed that the normalized tract counts appear to be significantly ($p<0.05$) lower in the chronic DoC cohort compared to the controls.

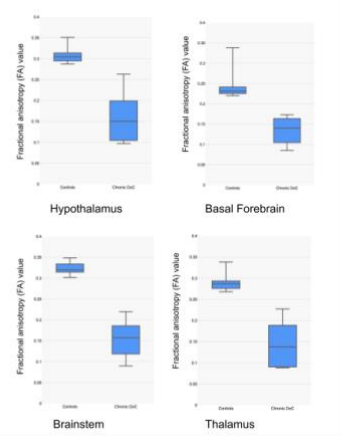

Figure 2. FA values in AAN ROIs of healthy controls and patients with a chronic DoC, $p<0.05$. This whiskers-only box plot shows connectivity, measured by the fractional anisotropy values obtained from diffusion tensor imaging analysis. The differences in all four box plots are significant for the threshold $p<0.05$.

In all four box plots and for both cohorts, the extended whiskers indicate a wide distribution of data. For healthy controls, the variability could reflect natural differences in brain architecture and connectivity that occur within a normal population. On the other hand, the variability in the chronic DoC group likely represents the diverse impacts of various pathologies associated with disorders of neural connectivity.

The box plots for the hypothalamus and thalamus show a notably lower median tract count in chronic DoC patients compared to controls, with the median line of the DoC group visibly closer to the first quartile than the third. This shift in the median indicates that the majority of chronic DoC patients have a lower connectivity profile compared to the median of the control group. Furthermore, the presence of a wider range in the chronic DoC group's data suggests that some patients retain a degree of connectivity closer to healthy norms, while others are significantly more affected.

Finally, we performed PCA with the AAN ROI tract count values to assess the separability of chronic DoC patients from healthy controls (Figure 3).

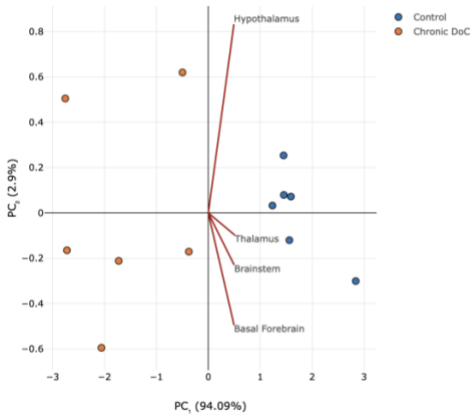

Figure 3. Principal component analysis of structural connectivity among AAN ROIs in healthy controls and patients with disorders of consciousness. The vectors represent the loadings of each AAN ROI onto the principal components. The first principal component explains 94.09% of the variance in the data while the second explains 2.89%. The third and fourth principal components account for 2.43% and 0.58%, respectively. The orange dots represent chronic DoC patients while blue are the controls. PC1 = Principal Component 1, PC2 = Principal Component 2.

With the controls clumped on the right y-axis and DoC patients spread out on the left, the results demonstrate clear linear separability between these two cohorts. The diffuse nature of the DoC patient data, in contrast to

the controls, mirrors the wide range of the box plots. The dominance of PC1, explaining 94.09% of the total variance, suggests structural connectivity differences within the AAN heavily contribute to the total variance in the data. Furthermore, we calculated the absolute loadings of each AAN ROI to determine each ROI's influence in separating chronic DoC patients from healthy controls (Table 1).

ROI	PC_1 Loading	PC_2 Loading
Hypothalamus	0.9580	0.2833
Thalamus	0.9906	-0.0333
Basal Forebrain	0.9634	-0.1686
Brainstem	0.9684	-0.0782

Table 1. Contributions of each AAN ROI to PC1 and PC2. Loadings of PC1 and PC2 for AAN ROIs were extracted from the PCA results and computed using the formula $L = v$, where L is the loading, v is the component of the eigenvector of the ROI in the axis of the PC, and the eigenvalue of the PC. Higher absolute loadings indicate a stronger contribution of the ROI to the respective PC.

The similar loadings on PC1 demonstrate approximately equal contributions from each ROI. It follows that connectivity within the AAN in DoC patients is very distinct from healthy controls, with the former exhibiting significantly fewer tracts and experiencing crucial disruptions in all AAN ROIs. On PC2, the hypothalamus is notably higher than all other ROIs. This holds little weight given PC2 accounts only for 2.89% of the total variance. The importance of PCA is simply its demonstration that there is clear separability between the two cohorts based on AAN connectivity, with each AAN ROI contributing relatively equally to this variance.

DISCUSSION

This study demonstrated that connectivity within the AAN is significantly disrupted in the chronic DoC cohort and that this separates these patients from the healthy controls. Great disruption in all subcortical structures (hypothalamus, basal forebrain, thalamus) and the brainstem was found. These results are consistent with previous research analyzing connectivity differences between these groups, as well as studies aiming to find neural correlates of consciousness.

The disruptions observed in the ROIs within the AAN in patients with DoC in this study can be attributed to the specific pathophysiological changes associated with chronic DoC states. In chronic DoC, the brain often undergoes significant structural changes that can lead to impaired connectivity. These changes may include neuronal loss, gliosis, and the degeneration of white matter tracts that connect critical areas of the brain involved in consciousness.[15]

This disruption is determined by the decrease in the number of intact white matter tracts, which could be attributed to neural damage or degeneration caused by the underlying pathology of chronic DoC. Tract counts refer to the number of neural connections, or white matter tracts, that are identified between the different regions of the brain. These tracts are made up of axons, long, thread-like parts of a nerve cell where impulses are conducted from the cell body to other cells.[16] It follows that tracts are pathways for neural signals to travel and communicate between. Higher tract counts have been found to be correlated with better cognitive and functional outcomes. Furthermore, tract counts are a common biomarker to assess the integrity of the brain's white matter.

The wide distribution of tract counts within both groups, but especially among chronic DoC patients, raises important questions about the heterogeneity of the disorder. In DoC patients, this variability may be due to differences in the etiology of the condition, the extent of brain damage, the duration of the disorder, or individual variations in the capacity for neural plasticity and recovery. Often, a result of cognitive impairments such as DoCs are axonal injuries, which are characterized by widespread damage to the white matter tracts of the brain.[17] Understanding the sources of this

variability is critical for developing personalized approaches to treatment and care for DoC patients.

Moreover, the results suggest that some chronic DoC patients retain a level of connectivity that approaches that of healthy controls. This finding opens several lines of inquiry: it may indicate the potential for recovery in some individuals, or it could reflect the presence of alternate neural pathways that compensate for lost connections. Further research is needed to explore these possibilities and to determine the clinical significance of these retained connections. Potentially, tract counts can be considered biomarkers for the recovery of DoC patients.

The investigation of the AAN has great value given the significant differences in connectivity in each AAN ROI. The hypothalamus is particularly susceptible to disruption in DoC patients due to its central role in maintaining the body's internal balance and arousal states. The hypothalamus regulates autonomic and endocrine functions, including the sleep-wake cycle.[5] Lower tract counts in the hypothalamus may be indicative of damage or dysfunction in this region, which could be a result of traumatic brain injury, or hypoxic events, in addition to a DoC. Such disruptions can directly impair the hypothalamus's ability to contribute to arousal mechanisms, thereby affecting the patient's ability to maintain consciousness. The specific vulnerability of the hypothalamus to disruption in DoC patients could also be attributed to its extensive network of connections with other brain regions involved in consciousness, including the thalamus, basal forebrain, cerebral cortex, and brainstem.

In the thalamus, lower tract counts observed in DoC patients may be related to its role as the primary relay station for sensory information to the cortex. The thalamus's involvement in consciousness extends to its participation in thalamocortical loops, which are essential for cognitive functions, including awareness.[5] Previous research has identified the thalamus as a critical neural correlate of dexmedetomidine-induced unconsciousness, with decreased rates of glucose and cerebral blood flow.[18] In addition, similar studies investigating mild cognitive impairments show a disrupted thalamus white matter anatomy.[19] Damage to the thalamus is quite commonly seen in neuropsychiatric disorders and could stem from a variety of causes, including a direct injury, degeneration of connecting white matter tracts, or secondary effects of cortical damage.[20,21,22] The vulnerability of the thalamus in DoC patients highlights the importance of intact sensory processing pathways for the maintenance of conscious awareness.

The brainstem is critical for its involvement in the reticular activating system, which regulates arousal and wakefulness.[5] Lower tract counts in DoC patients here may be because of its susceptibility to direct injury or as a consequence of secondary brain injury mechanisms such as edema or increased intracranial pressure. The brainstem's role in basic life-sustaining functions also makes its disruption common in diffuse brain injuries.[12]

The first limitation of this study is rooted in the static nature of the box plots and tractography, providing a visualization of connectivity but not capturing dynamic changes over time. Longitudinal studies would be beneficial to understand how connectivity patterns in DoC patients evolve and whether they correlate with changes in clinical status. Secondly, the normalization of tract counts allows for comparison between groups but may mask absolute differences in connectivity that could be clinically relevant. Approaches that involve additional analyses complementing the normalization are recommended as future studies.

The implications of these findings extend beyond the scientific understanding of chronic DoC. These results can inform clinical practices. From this study, the AAN is a potential biomarker for the diagnosis and prognosis of DoC. Given this information and the role each AAN ROI

plays in governing consciousness, therapeutic interventions aimed at specifically enhancing connectivity in the regions of the AAN may arise. Furthermore, there may also be a need for individualized treatment plans, hinted by the great variability in connectivity observed in the DoC cohort. A personalized medicine approach to treat DoC patients targeting specific AAN connections or neural pathways may have strong potential benefits in the path to recovery.

CONCLUSION

The results presented in this study contribute to a growing body of evidence that suggests structural connectivity is fundamentally altered in chronic DoC. These alterations have significant implications for the clinical management of the disorder and for our theoretical understanding of consciousness. Further research should first apply more rigorous statistical techniques and machine learning on a larger dataset to validate the findings of the current study and analyze the connectivity of AAN ROIs in junctions rather than treating them as independent entities.

AUTHOR INFORMATION

Corresponding Author

Darwin Li - darwin.li@ucc.on.ca

Author Contributions

Darwin Li conceptualized the study, collected and analyzed data, and wrote the manuscript.

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