Analysis of Subcortical Arousal System Connectivity in Temporal Lobe Epilepsy

By

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Dedication

This thesis is dedicated to the people who have supported me throughout my life and helped me get where I am today. To my family for their love and wonderful upbringing that encouraged me to love learning. To Dario for being an incredibly supportive mentor, for taking me on as his first student, and being a great friend. To the latest addition to my family Leonardo González-Narasimhan, for being the best Christmas gift ever. Last and most importantly, to Saramati Narasimhan. Saramati, from the day I met you, you have pushed me to grow and become a better person, and I will always be thankful for that. Without your help it would have been impossible for me to get to this point, and without your unbelievable support over the last years and especially the last few weeks I could not have completed writing this dissertation. Thank you for being the best partner in lab, in life, and in love.

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CHAPTER I

I Specific Aims

Epilepsy is a debilitating neurological disorder that affects more than 50 million people in the world.[8] In temporal lobe epilepsy (TLE), a focal epilepsy syndrome, seizure onset is typically localized to an epileptogenic zone (EZ) in the mesial temporal lobe.[9] For most patients antiepileptic drugs are sufficient to control seizures, however, medically refractory seizures occur in about 30-40% of these individuals.[9,10] This leads to patients with TLE between seizures exhibiting broad neurocognitive deficits and neuropsychiatric comorbidities.[11-14] Some resultant neurocognitive deficits, including verbal or visuospatial memory decline, could be explained by abnormalities of the temporal lobe. However, patients with TLE also exhibit a wide array of neurocognitive deficits, such as attention and executive function problems, not explained by problems in the temporal lobe.[15-19] Epilepsy surgery, which primarily encompasses surgical resection or laser ablation of the EZ, results in seizure freedom in about 60-80% of patients with TLE.[20,21] Other epilepsy surgeries like neuromodulation rarely result in seizure freedom, but can still result in significant improvements in quality of life.[3,22-24] In the long term, successful surgery is often accompanied by improvements in neurocognitive domains such as executive function, processing speed, and concentration.[25-28] A critical but unanswered question is: Why does TLE, a <u>focal</u> epilepsy, cause <u>global</u> structural and functional damage, such as widespread gray matter atrophy, diffuse neocortical hypometabolism, and broad neurocognitive impairments?[29-34] No one has bridged the knowledge gap between how *ictal* (during seizures) network effects result in long-term *interictal* (between seizures) brain connectivity and neurocognitive problems, and this knowledge may be crucial to improving surgical treatment of TLE.

The work in this thesis seeks to address these questions through neuroimaging studies of brain connectivity over long-term (interictal, resting-state) in patients with TLE. Resting-state functional connectivity (RSFC) is a relatively modern analysis that estimates similarity of neurophysiological signals arising from spatially distinct brain regions.[35-37] Prior work by our group, and others, has demonstrated RSFC changes both distal to and around the EZ in focal epilepsy.[31,38-43] Building on results of previous works alongside our group's prior rodent studies of abnormal subcortical activity during focal limbic seizures,[44,45] **our central hypothesis is that recurrent seizures in**

TLE result in altered connectivity between deep arousal structures and the neocortex, leading to decreased neocortical connectivity and impaired neurocognitive function.

This novel hypothesis will be explored by examining the effects of TLE on arousal system connectivity. Patients with TLE often develop neurocognitive and psychosocial deficits interictally that are not related to EZ location,[32,46-48] but may instead be related to subcortical connectivity disturbances.[31,49] Most prior works attempted to investigate these effects primarily by study of the limbic structures (which, in TLE, often include the EZ).[40,50-54] Other studies have attempted to understand epilepsy through the study of more commonly examined cortical intrinsic connectivity networks such as the default mode network or the attention networks.[55-58] We propose that to better understand, and possibly prevent, these broad neurocognitive comorbidities, it is necessary to appreciate the clinical implications of arousal center RSFC alterations. Functional connectivity studies of TLE have largely ignored smaller subcortical structures in part due to technical difficulties.[59,60] Functional magnetic resonance imaging (fMRI) allows us to study deep brain structures better than other modalities commonly used to study epilepsy, like electroencephalography or magnetoencephalography.[31] Using fMRI, this work proposes to measure arousal center RSFC changes in patients with TLE before and after surgery, and investigate whether RSFC perturbations improve in patients with post-operative seizure improvements. We will focus on three of the most important arousal structures: the brainstem ascending reticular activating system (ARAS), the intralaminar thalamic nuclei, and the cholinergic basal forebrain nucleus basalis of Meynert (NBM).[1,61-63] Neurostimulation of these structures in rodent epilepsy models and patients with disorders of consciousness have shown some promise in ameliorating arousal deficits in these disorders.[64-67] Therefore, elucidating the role of arousal structures in TLE pathophysiology through this proposed work may provide novel targets for therapeutic intervention.

<u>Aim 1:</u> Examine brainstem ARAS RSFC in pre-operative and post-operative patients with TLE. Previous work has shown that broad functional and structural brain changes are present in patients with TLE,[29,40,68-70] however, it is unknown how connectivity of brainstem ARAS nuclei is affected by TLE. By quantifying fMRI RSFC between brainstem arousal centers and neocortex, we can compare RSFC patterns in patients with TLE before versus after epilepsy surgery, compared to healthy matched controls. We expect to observe diminished connectivity between

neocortex and ARAS structures in patients with TLE before surgery versus matched controls. Finally, we hypothesize that ARAS connectivity disturbances in patients with TLE may improve towards normal after successful epilepsy surgery.

Aim 2: Delineate thalamic arousal network RSFC in patients with TLE before and after epilepsy surgery, using a custom atlas of thalamic nuclei. While it has been shown that the anterior thalamus plays important roles in the pathophysiology of TLE,[71,72] the connectivity of thalamic arousal nuclei has not been well studied in patients with TLE. In this Aim, we examine thalamic arousal network RSFC in pre-operative and post-operative patients with TLE compared to matched controls, using a participant-specific thalamic hierarchical active shape model atlas based on the Morel atlas.[73] We anticipate that connectivity in intralaminar thalamic nuclei will be perturbed in pre-operative patients with TLE. Additionally, we expect that thalamic arousal network connectivity may recover in post-operative patients with improved seizure control after epilepsy surgery.

Aim 3: In patients with TLE, investigate RSFC of the NBM with fMRI using advanced network analysis techniques. NBM plays prominent roles in arousal, influences neurocognition, has dense anatomic projections to limbic structures and ARAS structures, and may help regulate functional networks.[74-78] In rodent TLE models, focal seizures lead to diminished activity in NBM, resulting in neocortical deactivation,[79] however the role of NBM in human TLE has not yet been explored. Given its role in both regulation of functional networks and relationship to focal seizures, we will employ network-based analyses, including community detection and network-based statistic, to examine how the NBM may be affected in patients with TLE. We anticipate that given its broad neocortical projections, association with other arousal regions, and intimate limbic connectivity the NBM may be one of the most perturbed structures in TLE.

Aim 4: Characterize RSFC between arousal structures and intrinsic connectivity networks in patients with TLE and relate to neurocognitive deficits. Others studies of patients with epilepsy have observed connectivity disturbances within intrinsic connectivity networks (e.g. default mode network),[55-57] that were associated with measures of disease severity including neurocognitive impairment.[58,80,81] However, RSFC between arousal structures and intrinsic

connectivity networks (default mode network, central executive network, and salience network) has not yet been examined in patients with TLE. Here, we use directed and non-directed network connectivity measures to elucidate how RSFC between arousal structures and intrinsic connectivity networks may be involved in TLE and its comorbidities. We anticipate that connectivity between arousal structures and intrinsic connectivity networks will be perturbed, that more abnormal RSFC will be associated with worse neurocognition, and that successful epilepsy surgery may lead to recovery of both connectivity and neurocognitive performance.

CHAPTER II

II Background and Significance

II.1 Clinical Significance

II.1.1 Disease Burden

Epilepsy is one of the most common neurological disorders,[8] and the most common form of epilepsy is temporal lobe epilepsy (TLE).[82-87] Patients can have either unilateral or a bilateral seizure onset. An epilepsy with unilateral onset involves seizures originating from an epileptogenic zone (EZ) in one brain hemisphere, while a bilateral onset has seizures originating from both hemispheres.[82] Specifically, for mesial TLE (mTLE), which is the primary disease presentation studied in this thesis, the EZ resides in mesial temporal structures including the hippocampus and/or amygdala.[8,82,83,87] As expected with seizures originating in the mesial temporal lobe, patients with mTLE often experience problems with verbal or non-verbal memory when their dominant or non-dominant mesial temporal structures, respectively, are affected.[19,88-90] Paradoxically, while TLE is classified as a focal epilepsy disorder, it leads to widespread brain problems that cannot be explained by abnormalities in the temporal lobe alone. These problems include broad neurocognitive deficits, progressive diffuse gray matter atrophy, and widespread connectivity perturbations.[17,29,31,69,89] While seizures are the most apparent symptom of TLE, these broad and insufficiently understood comorbidities significantly impact individuals with TLE.

Approximately 30-40% of these individuals who suffer from TLE specifically experience drug resistant epilepsy (DRE), which means they continue to experience seizures despite treatment with anti-epileptic medications.[11,12,20,91-93] In general, patients with poorly controlled epilepsy are at higher risk for sudden unexpected death in epilepsy (SUDEP), which ranks second only to stroke in years of life lost among all neurologic diseases.[94] Epilepsy represents as much as 0.8% of disability-adjusted life years (DALY) globally or 20.6 million DALYs per year.[84] For patients with DRE, epilepsy surgery is a highly efficacious treatment that can reduce/eliminate seizures,[95] improve quality of life, and halt some of the progressive global neural deficits seen in those with untreated epilepsy.[17,28,96] Unfortunately, between 33-60% of patients who undergo surgery, continue to experience recurrent, disabling, post-operative seizures as well as the broad neural

deficits incurred by continued seizures.[86,97] Focusing on persons with drug resistant unilateral mTLE, in this thesis we seek to address the incomplete understanding of these understudied observed global network deficits and how they respond to epilepsy surgery.

II.1.2 Clinical Presentation and Diagnostic Evaluation

Accurate diagnosis of TLE is central to formulating a successful treatment plan these steps include confirming the patient's clinical presentation as TLE, determining their susceptibility to medical treatment, and possibly a surgical workup and surgery if they have DRE.[93,98] About 9% of all individuals will have an isolated epileptic seizure at some point during their lifetime, however they are not diagnosed with epilepsy.[99,100] Epilepsy is defined as a syndrome where a patient experiences two or more unprovoked seizures, occurring more than 24 hours apart.[101,102] Seizures can present with a variety of symptoms (semiology) depending on the epilepsy syndrome and origin of the epileptic activity.

An accurate description of the seizure semiology is the first step towards diagnosing TLE.[101] For mTLE, the most common form of seizure is focal impaired consciousness seizures (FICS).[103] These typically present with behavioral arrest where patients are unresponsive and/or may be amnestic to concurrent events. Additionally, about one third of patients with mTLE have focal to bilateral tonic clonic seizures (FBTCS).[83] Common characteristics of isolated auras (focal aware consciousness seizures (FACS)) in mTLE include a rising epigastric sensation, déjà vu, auditory hallucinations, manual automatisms, and oral automatisms. In some cases these auras may precede FICS/FBTCS.[103] Seizure semiology not only assists in diagnosing TLE, but can also even help localize seizure focus in specific instances (for example hand automatisms).[103] Unfortunately, many individuals are amnestic to their seizures or these seizures may go unobserved. Therefore, while seizure semiology and patient history is central to initial diagnosis of TLE, they alone are often insufficient to definitively diagnose TLE.

The next common steps in any work-up of suspected TLE, after obtaining patient history, include scalp electroencephalography (EEG) and anatomical magnetic resonance imaging (MRI). Scalp EEG measurements of patients with TLE often reveals ictal and interictal epileptiform abnormalities that are maximum in the anterior temporal electrodes or inferior frontal electrodes.[104] Non-epileptiform abnormalities, like focal slowing, may also help localize the EZ in TLE. With

repeated and/or prolonged inpatient monitoring of EEG, the yield of observing EEG abnormalities in mesial TLE has been reported as high as 90%.[105] In addition to these focal electrophysiological abnormalities, which are attributable to the EZ, patients with focal epilepsy also exhibit more global abnormalities. One example of these global abnormalities is slowing of the posterior-dominant rhythm, which has been associated with widespread neural dysfunction.[106,107] Broad interictal electrophysiological disturbances like slowed posterior-dominant rhythm, cannot be explained solely by focal mesial temporal abnormalities.

After electrophysiological evaluation with EEG, anatomical MRI is an integral part of the diagnosis of TLE.[108] Identification of a focal lesion, that corresponds with electrophysiological findings, increases confidence in the diagnosis of TLE and portends a great probability of having a positive outcome following epilepsy surgery.[109] The most typical focal structural lesion found in mTLE is mesial temporal sclerosis which is characterized by atrophy, loss of internal structure, and increased T2 signal in the hippocampus.[108,110] Other common focal lesions in TLE include encephalocele, atrophy of the fornix, atrophy of the mamillary body, atrophy of the temporal pole, or malrotation of the hippocampus.[108] A focal radiographic lesion which corresponds to electrophysiological EZ increases probability of favorable outcome from epilepsy surgery (i.e. seizure freedom).[86] In addition to these focal structural abnormalities, individuals with TLE also exhibit non-focal, widespread, and often progressive structural perturbations, including cortical and subcortical atrophy and white matter abnormalities.[17,29,40,68,111-119] These broad anatomic disturbances outside of mesial temporal and limbic structures are present both in those who appear seizure-free through medical management and those with DRE.[69] In some individuals, this combination of patient history, electrophysiology, and anatomic neuroimaging is sufficient to begin medical treatment of TLE and may mark the conclusion of their evaluation. However, in other individuals, including those with DRE whose seizures are refractory to treatment with anti-epileptic drugs (AEDs), further workup is often pursued for reasons such as confirmation of epilepsy or to increase confidence in a surgical treatment plan.

Further non-invasive diagnostic evaluation methods include various types of neuroimaging and further clinical evaluations. One example is interictal positron emission tomography (PET) scan. For patients with mTLE, interictal PET with focal hypometabolism in the mesial temporal lobe may portend a successful surgical outcome and may even suggest good outcome without MRI

visible mesial temporal sclerosis. In addition to focal lesions attributable to the EZ, PET studies have also shown that patients with TLE also have broad extratemporal hypometabolism as well.[29] The international league against epilepsy (ILAE) has also recommended at minimum a screening for cognitive and behavioral difficulties in all children and adults with newly diagnosed epilepsy.[98,120,121] Neuropsychological evaluation can serve as a baseline against which to measure neurocognitive changes associated with the disease.[120] Neuropsychological evaluation can also help localize the EZ by observing the presence of certain deficits and help determine risk of neurocognitive performance deficits after surgery.[122-124] Beyond diagnostics, neuropsychological evaluations also indicate the pervasive network changes associated with TLE; in these patients it often reveals broad nontemporal deficits such as attention problems and executive function problems in TLE patients. [48,88,125] Other less commonly used modalities include ictal single photon emission computerized tomography (SPECT), subtraction ictal SPECT coregistered to MRI (SISCOM), MEG, and localization of eloquent cortex by functional MRI (fMRI) or intracarotid sodium amobarbital procedure (more commonly known as Wada testing).[98] These diagnostics are often highly effective at localizing the EZ in TLE and provide in depth information for surgical planning.

Finally, for individuals whose EZ cannot confidently be localized through non-invasive means, invasive, intracranial monitoring consisting of either stereo electroencephalography (SEEG) or subdural grids and strips placed based on strong *a priori* hypothesis regarding possible EZ can be used for invasive EZ localization.[126] As with non-invasive scalp EEG evaluations, after placement of intracranial EEG, the patient remains in the epilepsy monitoring unit for days of video-EEG monitoring. Once an EZ is identified based on ictal activity, surgical treatment options for the patient's specific disease presentation can be discussed. In summary, the diagnosis and localization of TLE presentations is an extensive, multidisciplinary approach that often requires multiple modalities.[98,127,128] Furthermore, based on observations made during diagnosis (broad atrophy, general neurocognitive decline, etc.), there is a plethora of evidence indicating that deficits in TLE are not focal but rather global.

II.1.3 Treatments

II.1.3.1 Non-Surgical Treatment: Anti-Epileptic Drugs

In general, the treatment of TLE aims to stop all seizures and the initial treatment of choice consists of pharmacological drug treatment, often referred to as AEDs.[129] As many as half of patients may respond to a single AED.[103] Two AEDs in combination may improve seizure control, but few individuals become completely seizure free as more medications are progressively tried.[103] In individuals who fail to achieve seizure-freedom after two medications, 23.6% of those who try a 3rd medication became seizure free, 15% accomplished seizure freedom after trying a 4th medication, 14.1% after a 5th medication, and 14% after trying a 6th medication.[130-132] While medical management can control seizures for some patients, treatment with these medications often come with a downside.[133] As dosage and number of medications increase, so do adverse effects caused by these medications. Such side effects include fatigue, worse sleep quality, dizziness, cognitive slowing, teratogenicity for fetus, potential cytotoxicity, and drug-drug interactions.[103,134,135] Unfortunately, complete seizure freedom in all individuals with epilepsy with medical treatment alone remains out of reach despite the development of multiple new AEDs.[132] Individuals with TLE, like those examined in this thesis, can experience drug resistant epilepsy. Drug resistant epilepsy is defined as individuals who continue to experience seizures despite adequate trial of at least two tolerated, well-chosen medications, at appropriate dosage.[93] Patients with drug resistant epilepsy are best treated surgically.[93,98]

II.1.3.2 Surgical Treatment: Resection and Ablation

We specifically studied individuals with TLE who had DRE. In this patient population epilepsy surgery, which includes resection,[20,86] ablation,[136,137] and neurostimulation,[138,139] targets the EZ(s) of the patient with the intention of reducing or eliminating their seizures.[96] In DRE mTLE, the gold standard for treatment is surgical resection.[140] Anterior temporal lobectomy (ATL) and selective amygdalohippocampectomy (SAH) are the most common surgical procedures for TLE. Efficacy of ATL for TLE has been shown in two randomized controlled trials as compared to AEDs alone. These randomized controlled trials showed 58-73% seizure freedom in the surgical group, but 0-8% seizure freedom in the best medical therapy group.[20,95] Additionally, long term follow-up studies show about 50-60% of patients achieve seizure remission when evaluated up to 10 years after resective surgery.[12,98,141-143] Resective procedures have predictable side effects

including memory problems, naming, and visual field cuts. These resective surgeries are safe and have a high probability of success in adequately chosen patients.

MRI guided laser interstitial thermal therapy (LITT) and stereotactic radiosurgery (SRS) are two minimally invasive destructive therapies that have been developed to expand the patient population who can be treated surgically and/or reduce side effects associated with resective surgeries. LITT surgery employs a fiberoptic laser probe inserted along the mesial temporal structures and performs an ablation with real-time MRI thermography monitoring.[144] This allows intra-operative evaluation of ablated tissue and monitoring of adjacent structures. Compared to resection, LITT appears to have a slightly lower rate of seizure freedom at 41-54% 1-year after surgery. However, some studies have preliminarily shown better neurocognitive outcomes (e.g. better naming and object recognition).[145-147] The Radiosurgery for Open Surgery for Epilepsy (ROSE) trial was completed evaluating SRS versus anterior temporal lobectomy (ATL). Seizure freedom was achieved in 52% of individuals who were treated with SRS compared to 78% of those who underwent ATL; both groups had similar memory and language outcomes.[148] Follow up studies of ROSE showed that SRS and ATL had similar financial costs. While SRS cost less initially, it had larger follow-up costs due to higher rates of adverse events.[149] The greatest downside of SRS treatment is that it takes 18 months to achieve seizure reduction.[148] Generally, these ablative surgeries for mTLE achieve seizure freedom at lower rates than resective surgery, but may provide an option for patients who are not eligible for open, resective surgery.

In summary. for patients with DRE TLE, epilepsy surgery is a highly efficacious treatment that can result in seizure freedom/reduction as well as other benefits.[17,28,96] Seizure freedom often leads to improved quality of life,[150] improved neurocognition,[25,27] and decreased mortality risk[151] after surgery. Untreated or insufficiently treated epilepsy results in progressive deficits, and early surgery for TLE results in improved seizure outcomes as well as improved comorbidities.[152] Despite this knowledge, surgery is often underutilized, and the time between onset of epilepsy to surgery is often more than 20 years.[12,127] Interestingly, focal epilepsy is associated with broad cortical atrophy, and TLE patients with successful resective epilepsy surgery had this progressive atrophy prevented.[28] While there can be no doubt about the benefits of resective surgery for TLE, there remain questions about what is driving these improvements. Are the broad benefits of epilepsy surgery accompanied by post-operative connectivity reorganization? How does post-

operative connectivity change in TLE?[153,154] Also what role does arousal structure connectivity play in this? To our knowledge, the connectivity of arousal structures has not been well evaluated in TLE. This thesis tries to address these questions by examining a possible common source for the broad pre-operative deficits of this focal disease and the post-operative improvements.[49]

II.1.3.3 Surgical Treatment: Neuromodulation

Neuromodulation techniques are promising treatments for individuals with DRE TLE who either fail resective/ablative surgery or are not good surgical candidates. Although neuromodulation rarely yields complete seizure freedom, there are still significant benefits to this therapy including frequency/intensity reduction, quality of life improvements, lower risk of SUDEP, and/or treatment of neuropsychological comorbidities. Neuromodulation involves closed-loop (responsive neurostimulation (RNS)) and open-loop (deep brain stimulation (DBS) and vagus nerve stimulation (VNS)).

RNS is the only truly closed-loop neuromodulation device, and it can be employed for multiple purposes: including for the treatment of epilepsy and/or long-term seizure data collection in patients with possible bilateral mTLE. Like resective/ablative surgeries, the closed-loop RNS requires localization of one or two EZ(s) to be targeted with the device. There was a long-term observation study of RNS in mTLE that examined 111 patients with mesial TLE treated with RNS, including 72% with bilateral onset and 28% with unilateral onset.[155] In general, this study showed that with a mean follow-up of 6 years, a 70% median decrease in seizure frequency was observed in unilateral and bilateral TLE patients. RNS can also be used to collect long term ambulatory intracranial EEG recordings for patients hypothesized to have bitemporal epilepsy. One retrospective study of 157 patients treated with bitemporal RNS eventually identified 25 patients for mesial temporal resection, and all nine patients who had unilateral mTLE were completely free of disabling seizures at their latest follow-up.[156]

On the other hand, the two open-loop neurostimulation methods do not require precise localization of EZ(s). Most VNS uses open-loop stimulation of the left vagus nerve.[157] However, some VNS use closed-loop stimulation in response to ictal tachycardia.[158] In general, long-term follow up of VNS shows 50% median seizure decrease in 50% of individuals after 2 years.[157] DBS of the anterior nucleus of the thalamus was tested in the Stimulation of the Anterior Nucleus of the

Thalamus (SANTE) trial. This randomized controlled trial examining DBS for epilepsy showed a 40% reduction in seizure frequency[159] with about 70% of individuals responding to DBS in the long term.[160]

None of these neuromodulation-based treatments have a rate of seizure freedom comparable to ablation or resection with seizure freedom rates of 8% for VNS, 16% of individuals with DBS were seizure free for at least 6 months, and 18% of individuals with RNS achieving a seizure free period of greater than 1-year.[160,161] Despite the lower rate of seizure freedom, these neuromodulation treatments exhibit other improvements. For example, RNS showed improved quality of life, reduced risk of SUDEP, and improved verbal learning, visuospatial ability and memory at follow-up.[3,24,162,163] Some of these broader effects suggest a neuromodulatory effect on widespread networks in the brain versus only focal seizure control. Despite the long study and knowledge/ treatment of TLE as a focal disease, there are many aspects of this disease which cannot be fully explained by only focal abnormalities.

II.2 Network Inhibition Hypothesis

II.2.1 Unanswered Question in TLE

In TLE, patients experience ictal (during seizures) and interictal (between seizures) effects that cannot be explained solely by diseased tissue of the EZ.[31,32] During focal seizures, TLE patients can experience impaired consciousness despite a lack of seizure propagation to widespread cortical regions.[1,45,164] Traditionally, impaired consciousness is thought to arise from lesions or abnormal activity in bilateral fronto-parietal neocortex or subcortical arousal structures.[1,13] Additionally, from historical patient H.M., we know that even bilateral removal of mesial temporal structures does not result in impaired consciousness.[165] So how do we explain ictal impaired consciousness during focal TLE seizures? Furthermore, between seizures TLE patients exhibit broad neurocognitive deficits, widespread neocortical hypometabolism, higher risk of SUDEP, and progressive diffuse gray matter atrophy, none of which can be attributed to abnormal limbic structures.[11,12,29,68,69,90,112,114] Contrary to traditional stereotyping of TLE as a focal disease, cerebral dysfunction outside of EZ implies that TLE is a disorder that widely affects brain networks,[31,166] but underlying mechanisms of global brain dysfunction are poorly understood.[49] We hypothesize that deep arousal structures, while under-studied in TLE, play a critical role in

producing diffuse network perturbations, contributing to interictal broad functional and structural disturbances in TLE.

II.2.2 Functional Connectivity Network Analyses in TLE

Recent advances in functional neuroimaging and electrophysiology techniques provide new methods to better study the broad brain network abnormalities in TLE.[37,39,41,167] Resting-state functional connectivity (RSFC) analysis, based on the presumption that regions with similarly fluctuating neuronal activity are likely functionally related, allows for the study of connectivity in TLE during the interictal period.[166-168] However, it is historically argued that TLE is a type of focal epilepsy, so why should we use network connectivity to study this disease? More specifically, is TLE a network disorder or a focal disorder? It has recently become evident that these perspectives are not mutually exclusive. Traditionally, focal epilepsy is considered a regional (focal) brain disorder, meaning seizures originate in one location and propagate. The EZ was first defined as the region where seizures originate by Talairach, Bancaud, and Jasper.[169,170] Lüders specified the EZ as an "area of cortex that is necessary and sufficient for initiating seizures and whose removal is necessary for complete abolition of seizures."[171] Since surgery on localized EZs are successful in seizure freedom for some, especially in TLE, there is some justification to consider epilepsy a focus disorder.

In TLE, seizures originate in the limbic and temporal lobe structures; therefore, most past neuroimaging research has focused on limbic and temporal structures, including amygdala and hippocampus, and their associated functions.[31,40-43,50-54] In our mTLE patient population, we also have found that the connectivity of the limbic network (bilateral anterior cingulate, amygdala, hippocampus, insula, and orbitofrontal cortex) with the rest of the brain is lower in pre-operative TLE patients as compared to controls bilaterally (**Fig. II.1**). We also examined cross brain limbic network connectivity (ipsilateral limbic network connectivity with contralateral limbic structures) and found that patients

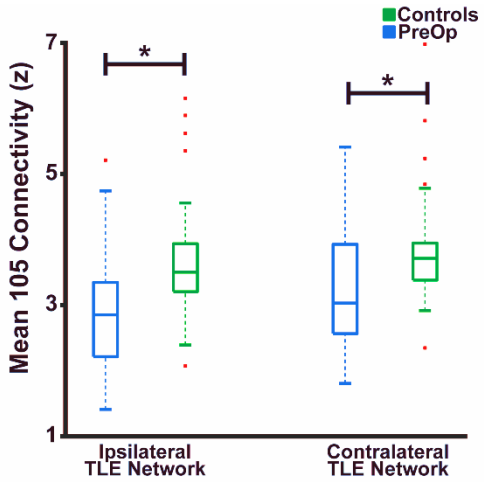

Figure II.1 Connectivity of TLE network (limbic network) with the rest of the brain is lower bilaterally in patients with TLE. *p<0.05 t-test Bonferroni-Holm corrected. (González, et al. Unpublished).

have lower connectivity than controls ($p < 0.001$, t-test, uncorrected). These functional connectivity findings reinforce the idea that these limbic structures represent focal pathological tissue in this disorder. Additionally, consistent findings of altered limbic connectivity may explain deficits in verbal memory (dominant temporal lobe) and visuospatial memory (non-dominant temporal lobe). These focal abnormalities, however, do not explain the broad neurocognitive deficits typical of TLE patients, including impairment of executive function, cognitive processing, attention, and concentration.[51-54,172-175] Therefore, this thesis takes the perspective that TLE, and focal epilepsy, is a network disorder and that network connectivity beyond limbic structures is fundamental to understanding epilepsy.[31,166,176]

II.2.3 Extended Network Inhibition Hypothesis in TLE

In this thesis, we thought it was necessary to expand the scope of study beyond the limbic network to overcome the limitations of prior studies of TLE and uncover network-based perturbations associated with these broad comorbidities in TLE. Previous studies of TLE, beyond those that study limbic regions, have shown interictal widespread decreases in RSFC of neocortical regions and intrinsic connectivity networks (e.g. default mode network, salience network, dorsal/ventral attention networks, and central executive network) in patients with TLE versus healthy. controls.[56,70,177-180] Other studies have related RSFC decreases in intrinsic connectivity networks to neurocognitive impairments in epilepsy.[53,55,56,175,181,182] A major limitation of these works is that more broad network dysfunctions that may be associated with these neocortical connectivity perturbations remain understudied. Prior research in TLE are limited, in that they have not: (i) evaluated connectivity of deep arousal structures, or how these patterns change after surgery (**Aims 1, 2, 3, 4**), (ii) examined how characteristics subcortical arousal functional connectivity networks may relate to disease characteristics (including disease severity and neurocognitive measures) (**Aims 1, 2, 3, 4**), (iii)

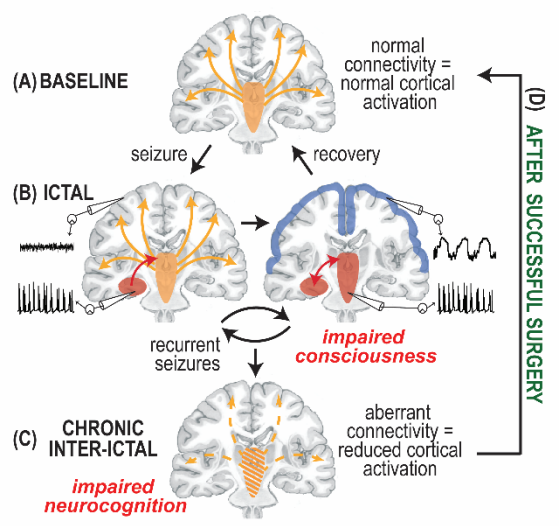

Figure II.2 Extended Network Inhibition Hypothesis.
(Englot and Blumenfeld 2009; González et al. 2020 Neurosurgery).[1,2]

applied advanced network measures to examine TLE as a network disorder (**Aims 3**), or (iv) examined how arousal system connectivity may be related to connectivity of cortical intrinsic connectivity networks (**Aim 4**).

TLE affects neocortical connectivity far beyond the EZ, but the mechanisms of distal effects remain unknown.[31] The "Network Inhibition Hypothesis," (**Fig. II.2**) first introduced by Blumenfeld, proposes that focal seizures may influence widespread neocortex by affecting deep brain structures important for cortical activation.[44-46,183,184] Subcortical arousal structures – including the brainstem ascending reticular activating system (ARAS), basal forebrain, intralaminar thalamus, and posterior hypothalamus – keep the cortex activated at wakeful baseline (**Fig. II.2A**).[5,13,61,63] TLE seizure activity typically begins in the mesial temporal lobe, but it can spread to and cause abnormal activity in subcortical arousal structures (**Fig. II.2B** left).[1] This interferes with normal function of the subcortical structures which are important for neocortical activation. The neocortex then defaults to a deactivated state resulting in a focal impaired consciousness seizure (**Fig. II.2B** right).[46,185] This network inhibition, during the ictal state, has been supported by our group's human intracranial electroencephalography (iEEG) studies,[46,183,186] and rodent electrophysiology and functional magnetic resonance imaging (fMRI) investigations.[45,46,79,164] Specifically, in a previous human intracranial EEG study by our group ictal neocortical inhibition was observed during focal impaired consciousness seizures, but not during consciousness-sparing focal seizures.[46] Additionally, in previous rodent studies by our group ictal neocortical inhibition (as measured by decreased neuronal firing rate, decreased cerebral metabolism, and decreased excitatory neurotransmission) was observed only when seizure activity spread to subcortical activating structures.[44,45,79]

As an extension of this hypothesis, regarding cerebral activity during seizures, this thesis proposes that recurrent focal impaired consciousness seizures in TLE lead to long-term interictal connectivity decreases between subcortical arousal structures and the neocortex, which may lead to long-term diminished interictal neocortical connectivity and neurocognitive problems (**Fig. II.2C**). In support of our proposed Extended Network Inhibition Hypothesis, our group has shown that neocortical connectivity reductions are quantitatively related to frequency of focal impaired consciousness seizures and epilepsy duration.[38,70] However, systematic studies of arousal center connectivity are needed to fully address this hypothesis. Additionally, we expect that successful epilepsy surgery resulting in seizure freedom may allow postoperative connectivity recovery (**Fig.**

II.2D).[2,32,48,89,150] The goals of this dissertation work are to: (i) define arousal structure-cortical RSFC disturbances in TLE with fMRI using two novel approaches to defining the regions of interest (**Aims 1, 2**), (ii) employ novel network based connectivity analyses to determine how network properties in TLE are perturbed and which structures may be most affected (**Aim 3**), and (iii) examine how connectivity between arousal structures and intrinsic connectivity networks alterations are related to neurocognitive deficits and surgical outcomes (**Aim 4**).

II.3 Subcortical Activating Systems in Epilepsy

II.3.1 Healthy and Disrupted Functions of Subcortical Activating Systems

TLE is classically defined as a "focal" epilepsy, where seizures originate from a discrete brain region, usually localized in the hippocampus and/or amygdala.[187] Accordingly, patients with TLE experience focal problems such as impaired verbal and visuospatial memory associated with mesial temporal abnormalities.[9,188,189] For this reason, most studies of TLE have primarily focused on mesial temporal structures.[14,43,51-54,56,190] Recently, TLE has been recognized as a more broad brain network disorder with pathological sequelae not explained solely by diseased tissue in the temporal lobe.[31,167] For example, in addition to focal deficits associates mesial temporal structures patients with TLE also experience neurocognitive impairments associated with frontoparietal neocortex or subcortical brain regions. These extratemporal neurocognitive deficits include impaired attention, diminished executive function, and disrupted concentration.[49,173,191] These impairments may interfere with activities of daily living, make obtaining and maintaining employment difficult, and overall impair quality of life.[192,193] In addition, patients with TLE experience consciousness impairing seizures which are not explained by seizures in mesial temporal structures, but may be explained by aberrations in subcortical arousal structures.[1,46] These consciousness impairing seizures also result in decreased quality of life.[194-196] Therefore, to improve upon other studies, which focus on mesial temporal structures, in this thesis we propose to examine the role of subcortical arousal networks in TLE to examine global deficits in TLE.

What are subcortical arousal systems? Subcortical arousal systems are defined as deep nuclei and brain networks with ascending excitatory projections needed to keep the neocortex in an awake activated state.[63,197] Some responses incurred when these systems stimulate the neocortex include rousing individuals from sleep in response to stimuli, enhancing arousal, and generating and maintaining wakefulness.[198] Subcortical arousal structures include brainstem ascending reticular

activating system (ARAS), nucleus basalis of Meynert (NBM), intralaminar thalamic nuclei, posterior hypothalamus, and pulvinar.[4,5,44,63,197-202] As described in the Network Inhibition Hypothesis (II.2.3), multiple animal studies have shown that when seizure activity propagates to these subcortical arousal structures this disrupts their normal neocortical activating functions and results in a deactivated neocortex and impaired consciousness seizure.[185,203,204] Additionally, some studies have shown that neurostimulation of these structures may be a treatment option for cognitive dysfunction and disorders of consciousness in traumatic brain injury.[64,205,206] In this section we will discuss the healthy functions of subcortical arousal networks examined in this thesis and evidence of their disturbed activity in epilepsy.

II.3.2 Brainstem Ascending Reticular Activating System

The brainstem ascending reticular activating system (ARAS) is responsible for arousal of the neocortex, a crucial requirement for human consciousness.[5] The role of the brainstem as the fundamental seat of arousal was first proposed by Moruzzi and Magoun.[207] In these classic feline model experiments neurostimulation of a region in the upper brainstem termed the reticular formation (specifically cuneiform/subcuneiform nucleus and pontis oralis) induced bihemispheric activation of the neocortex on electroencephalography (EEG).[207,208] In addition to the reticular formation (named for the netlike appearance of its neurons) modern definitions of ARAS structures include structures such as raphe subnuclei, locus coeruleus, ventral tegmental area, pedunculopontine nucleus, laterodorsal tegmental nucleus, and parabrachial complex.[5,197,198] While all brainstem ARAS structures are involved in arousal they also have other functions. For example, some studies of pedunculopontine nucleus (PPN) emphasize its importance in the initiation of movement,[209] while other studies have suggested it plays a role in sensory feedback to the cerebral cortex.[206,210] Additionally, it also participates in initiation and maintenance of rapid eye movement (REM) sleep.[211] Another ARAS structure, the dopaminergic ventral tegmental area has ascending projections that are important for sustained attention and reward circuits.[198] Brainstem ARAS structures are important subcortical activating structures which issue broad excitatory ascending projections to the neocortex and abnormalities in these structures caused by epilepsy play a key role in broad brain network effects in patients with TLE.

As historically important structures, the role of ARAS structures in normal arousal physiology has been thoroughly studied; however, their role in epilepsy requires more attention. Early classical

studies by Moruzzi and Magoun, Jasper and Droogleever-Fortuyn, and Penfield suggested that brainstem structures markedly alter cortical synchronicity and therefore may be involved in the generation of seizures.[207,212,213] In studies of patients with infantile spasms, a type of generalized epilepsy, PET scans demonstrated brainstem activation and brainstem atrophy.[214,215] Involvement of ARAS structures in generalized epilepsy seem to provide evidence that arousal structures may be central to generalized seizures. Interestingly, there is also evidence of the involvement of brainstem ARAS structures in focal epilepsy syndromes including TLE. For example, one group found that loss of volume in brainstem autonomic nuclei and disruption of normal brainstem connectivity in TLE may be related to sudden unexpected death in epilepsy (SUDEP).[113,216] Others have shown that stimulation of the amygdala, a common epileptogenic zone in mTLE, was associated with apnea in patients with epilepsy.[217] There findings and others suggest that stimulation or seizure activity in this region may spread to subcortical brainstem respiratory centers engendering ictal/post-ictal apnea.[1,217] These respiratory centers include ARAS structures such as ventral lateral medulla, nucleus of the solitary tract, periaqueductal gray, and parabrachial complex. Additionally, SPECT studies of temporal lobe seizures have shown bilateral increased perfusion of the brainstem and thalamus during seizures and suggest ictal impaired consciousness is correlated with increased perfusion of these structures.[183,218-220] These exemplar studies of the role of the brainstem ARAS in epilepsy suggest brainstem arousal regions merit further examination to better understand the broad pathological deficits in TLE.

II.3.3 Intralaminar Thalamus

The thalamus is a crucial hub for cortical synchrony and information exchange and it is the main source of input to the neocortex and receives many reciprocal connections from the cortex to the thalamus (thalamus comes from the Greek word for chamber).[204] The thalamus is commonly thought of as a relay station, for example every sensory system (except for olfaction) includes a thalamic nucleus that receives these signals and relays them to the cortex.[13] Reciprocal thalamocortical connections are of particular interest to this work as they are central to thalamus function in modulating brain rhythms, including sleep wake transitions.[198,221] One such well known brain rhythm is the posterior dominant α-rhythm (8-13Hz) in EEG.[222] The α-rhythm was first discovered by Hans Berger and is considered a hallmark of the EEG of healthy resting-state eyes-closed adults. [222-226] Brain rhythms such as these may be influenced by thalamic arousal networks

including anatomic connections between intralaminar thalamic nuclei and other arousal structures.[199] Broadly, the whole thalamus orchestrates interactions between brain regions and has been shown to be involved in some aspects of epilepsy.

Prior studies have indicated that thalamic networks are involved in generalized and focal epilepsies. For example, research in generalized epilepsy has suggested that thalamocortical networks are central to absence seizures.[227] Specifically, these studies have shown that the thalamus is vital in the 3-Hz spike-and-wave seizures pathognomonic of this disease.[204] Additionally, abnormalities of brain rhythms in epilepsy may be associated with thalamic deficits. For example, EEG and fMRI studies of resting-state healthy adults have shown negative correlations between the thalamus and occipital cortex during the resting-state α-rhythm.[224,228,229] We also know, that epilepsy patients may exhibit slowing of the α-rhythm,[106,107] and studies have suggested that dysfunction of the α-rhythm is associated with various neuropsychiatric and neurological disease states, including epilepsy.[230,231] Ictal involvement of thalamus in TLE is supported by both neuroimaging studies and intracranial EEG which show interictal thalamic atrophy in mTLE and elevated ictal activity in the thalamus respectively.[232-235] In this work we are most interested in thalamic arousal structures and their role in TLE.

Intralaminar thalamic nuclei project broadly to the cortex and are known to be central for maintenance of arousal and various neurocognitive functions.[61,221,236] These thalamic nuclei receive particularly dense ascending input from brainstem ARAS nuclei.[198] For example, the central lateral (CL) thalamic nucleus is the largest of the intralaminar thalamic nuclei and plays an important role in awareness and visuospatial attention.[199,237] In rodent epilepsy models, aberrant activity in CL is associated with behavioral arrest and abnormal neocortical activity during limbic seizures.[65,238] Case studies have shown that deep brain stimulation of CL in minimally conscious traumatic brain injury patients may result in increased arousal.[64] Also, in a rodent model of focal limbic seizures, others have shown that simultaneous stimulation of CL and pontine nucleus oralis during focal seizures prevented deleterious neocortical and behavioral effects seen without stimulation.[65] These studies suggest that intralaminar thalamic arousal structures may play a role in both the detrimental effects of epilepsy and potential novel therapeutic approaches, however, long-term effects of seizures on thalamic arousal networks and their potential clinical implications remain poorly understood in TLE.

II.3.4 Cholinergic Basal Forebrain – Nucleus Basalis of Meynert

Cholinergic cell groups in the basal forebrain include areas that are important for arousal. Mesulam originally delineated the cholinergic regions in the basal forebrain into 4 groups Ch1-4, where the Ch nomenclature designated cholinergic neurons (defined as containing choline acetyltransferase (CHAT)) neurons.[74,75,239] These include medial septum (Ch1), diagonal band of Broca (Ch2, Ch3) and Nucleus Basalis of Meynert (NBM, Ch4). In this thesis we focused on the NBM which is known to provide the major source of cholinergic innervation to the entire cortex.[74,75] Historically, NBM was synonymous with the substantia innominata ("Ungenante Mark-Substanze" as noted by Reil in 1809[240]). Studies in humans and animal models have shown that NBM plays a central role in arousal and sleep-wake cycles. For example, sea mammals are known to employ unihemisphereic sleep, which is characterized by slow waves in only one hemisphere and these are modulated by cholinergic basal forebrain nuclei.[72] During unihemispheric sleep, sea mammals show differences in cholinergic neurotransmitter levels between hemispheres, specifically the "sleeping" side has more synchronous brain activity and shows low acetylcholine levels and "awake" side has more asynchronous brain activity with high acetylcholine levels.[239,241] Additionally, other studies have shown that NBM influences various neurocognitive functions including attention, learning, memory, reward, and plasticity.[75-78,242,243] Studies of non-human primates have found that pharmacological inhibition of NBM resulted marked changes to cortical resting-state fMRI.[78] This finding suggests that NBM may be a particularly interesting arousal structure to examine in this work. Through its broad cholinergic projections NBM plays an influential role in arousal and neurocognition.

These significant neuromodulatory abilities of the NBM can also be inferred from its anatomic connectivity. Interestingly, Mesulam has previously proposed that based on its anatomical connections, NBM is closely related to both limbic system and brainstem ARAS.[75,244] As part of the limbic system, NBM receives its primary cortical input from limbic and paralimbic cortices.[75,244,245] Cholinergic projections to the hippocampus appear to modulate cellular memory functions like synaptic plasticity, long term potentiation, short term depression, and long term depotentiation.[239] In rodent models NBM has been shown to receive projections from brainstem ARAS regions such as ventral tegmental area, brainstem raphe nuclei, and locus coeruleus.[75] Additionally, NBM also has a similar morphology to brainstem reticular nuclei, leading some to consider the NBM a telencephalic extension of the brainstem ARAS.[75,246] TLE is a disease which

originates in limbic structures and in this thesis we propose that it also causes interictal abnormalities of arousal structures, therefore, NBM, which sits at the intersection of these two systems may play a crucial role in this disease.

The NBM has been shown to be involved in neurological disorders including epilepsy. NBM most notoriously is affected in the pathophysiology of Alzheimer disease where both the hippocampus and NBM are thought to be part of a network central to the cognitive dysfunction in Alzheimer.[247] Neuroimaging studies have shown that NBM volume can differentiate patients with Alzheimer from those with mild cognitive impairment, suggesting that structural and functional abnormalities of NBM may be related to neurocognitive deficits in a spectrum of disorders.[248] In rodent models of TLE, focal seizure activity leads to diminished neuronal activity in NBM cholinergic neurons, resulting in neocortical deactivation,[79] but preventing seizure spread from hippocampus to basal forebrain using white matter lesions can mitigate these effects.[45] However, targeting this inhibition is complicated by recent rodent models showing that inhibition of NBM during focal consciousness impairing limbic seizures may occur either through the lateral septum or the thalamic parataenial nucleus.[249] Increased elucidation of connectivity of NBM may illuminate novel therapeutic targets and deeper understanding of the role of the NBM in broad network deficits of TLE.

II.4 Clinical Impact

In this thesis, we employed an innovative approach to study network effects of TLE beyond the scope of previous studies. Historically, studies of TLE have relied upon the traditional lesion-based theory of clinical neurology, which has biased research towards investigations of the temporal lobe. However, lesion-based studies are limited as recently many neurological diseases have been determined to truly be network based.[166] The need to study the effects of TLE beyond the temporal lobe is further demonstrated by the increasingly recognized pernicious effects of focal seizures beyond the temporal lobe.[31,167] The key innovations of this thesis include:

1. Novel evaluation of subcortical arousal structure connectivity after epilepsy surgery in temporal lobe epilepsy patients.
2. Use of novel techniques to study functional connectivity of arousal structures in TLE.

3. Understanding neurocognitive deficits in TLE by searching beyond the temporal lobe.

This proposed work will test the Extended Network Inhibition Hypothesis (**Fig. II.2**) through analyses of arousal system connectivity in patients with TLE. By employing hypothesis driven methods to calculate RSFC of arousal networks, this proposal will elucidate relationships between connectivity patterns, disease severity, and neurocognitive measures in TLE.

Possible clinical impacts of this work include: (i) identification of new neuromodulation targets to counteract damaging network effects of focal seizures and address comorbidities of the disease, (ii) understanding arousal network dysfunction that has been proposed to contribute to sudden unexpected death in epilepsy (SUDEP),[184,250] and (iii) understanding the importance of early surgery,[11,21,86] to prevent progressive network damage from repeat seizures.

CHAPTER III

III Evaluating Brainstem ARAS Connectivity After Epilepsy Surgery

III.1 Summary and Contributions

This chapter details how structural and functional connectivity of individual brainstem ascending reticular activating system (ARAS) structures are affected in temporal lobe epilepsy (TLE) patients before and after epilepsy surgery in order to understand how these connectivity perturbations are quantitatively related to disease-related factors. In this work, functional and structural connectivity of brainstem ARAS structures were calculated for all participants. Participants included TLE patients pre- and post-operatively and matched healthy controls. Connectivity was calculated with resting-state functional magnetic resonance imaging (fMRI) and diffusion weighted imaging (DWI). For TLE patients, we also collected measures of disease severity and neurocognitive performance. We hypothesized that greater disease burden was related to more abnormal connectivity; we tested this by relating disease measures and surgical outcome to connectivity.

The results of this work provided strong evidence that patients who achieved long-term post-operative seizure freedom demonstrated increases in functional connectivity between ARAS structures and fronto-parietal-insular neocortex compared to their pre-operative baseline. The same was not true of patients with continued post-operative seizures, nor were there any changes in their structural connectivity post-operatively. Furthermore, our results showed that patients with more severe disease (greater pre-operative frequency of consciousness impairing seizures and worse neurocognition) had greater recovery of functional connectivity with post-operative seizure freedom.

This study highlights two novel concepts in the investigation of TLE: (i) the importance of examining the role of arousal structures to understand global effects of this focal disease, and (ii) arousal structure connectivity after surgery. Brainstem ARAS structures are some of the earliest structures noted to influence broad brain functions by modulating arousal and consciousness.[61,63,207] Blumenfeld first showed the important role of subcortical arousal structures in ictal neocortical deactivation and focal impaired consciousness seizures.[1,45,204] Despite this

knowledge, few other groups have studied subcortical arousal structures in TLE.[251,252] Therefore, study of arousal structures in patients with focal epilepsy is a relatively novel concept. Furthermore, few have performed studies measuring functional connectivity before and after epilepsy surgery. The existing studies were limited to limbic structures or structures in the default mode network.[153,154,253] Until our study, subcortical arousal structures after epilepsy surgery had yet to be interrogated.[31,251] This novel work combined these ideas and for the first time, to our knowledge, to investigate the effects of epilepsy surgery on arousal structure functional connectivity in TLE. To address the challenge of studying small subcortical structures, which poses a significant challenge in fMRI, we employed a brainstem ARAS structure atlas (derived from post-mortem 7T MRI and histology) in order to interrogate our innovative hypothesis about the roles of subcortical structures in TLE. To the best of our knowledge, this work was one of the first to utilize an atlas of brainstem ARAS structures to study functional connectivity in TLE. Additionally, the results of this aim were the first to show diminished ARAS connectivity in TLE and improved ARAS connectivity after successful epilepsy surgery.

III.2 Brainstem Functional Connectivity Disturbances in Epilepsy May Recover After Successful Surgery

The work in this section appears in:

González, H.F.J., Goodale, S.E., Jacobs, M.L., Haas, K.F., Landman, B. A., Morgan, V.L., Englot, D.J. (2020). "Brainstem functional connectivity disturbances in epilepsy may recover after successful surgery," Neurosurgery, 86(3), 417-428.

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Abstract

Background: Focal seizures in temporal lobe epilepsy (TLE) are associated with widespread brain network perturbations and neurocognitive problems.

Objective: As recent work has demonstrated decreased brainstem connectivity in TLE that is related to disease severity and neurocognitive profile, we asked whether these connectivity disturbances improve with successful epilepsy surgery.

Methods: We evaluated 15 adult TLE patients before and after (> 1-year; mean, 3.4 years) surgery, and 15 matched control subjects, using magnetic resonance imaging to measure functional and structural connectivity of ascending reticular activating system (ARAS) structures, including cuneiform/subcuneiform nuclei (CSC), pedunculopontine nucleus (PPN), and ventral tegmental area (VTA).

Results: TLE patients who achieved long-term post-operative seizure freedom (10 of 15) demonstrated increases in functional connectivity between ARAS structures and fronto-parietal-insular neocortex compared to pre-operative baseline ($p = 0.01$, Kruskal-Wallis), with post-operative connectivity patterns resembling controls' connectivity. No functional connectivity changes were detected in five patients with persistent seizures after surgery ($p = 0.9$, Kruskal-Wallis). Among seizure free post-operative patients, larger increases in CSC, PPN, and VTA functional connectivity were observed in individuals with more frequent seizures before surgery ($p < 0.05$ for each, Spearman's Rho). Larger post-operative increases in PPN functional connectivity were seen in patients with lower baseline verbal IQ ($p = 0.03$, Spearman's Rho) or verbal memory ($p = 0.04$, Mann-Whitney U). No changes in ARAS structural connectivity were detected after successful surgery.

Conclusions: ARAS functional connectivity disturbances are present in TLE but may recover after successful epilepsy surgery. Larger increases in post-operative connectivity may be seen in individuals with more severe disease at baseline.

III.2.1 Introduction

Temporal lobe epilepsy (TLE) is a debilitating disorder, and seizures are often medication-resistant.[11,20,91,92] While seizures typically begin in the hippocampus, TLE leads to widespread brain problems that cannot alone be explained by abnormalities in this focal region, including broad neurocognitive deficits, gray matter atrophy, and connectivity pertubations.[29,31,69,89] We hypothesize that recurrent seizures may cause abnormalities in deep brain regions important for arousal, leading to reduced connectivity between these structures and neocortex, which may contribute to neuropsychological problems. Recent work has reported reductions in connectivity between brainstem ascending reticular activating system (ARAS) nuclei and neocortex in TLE that were related to disease severity and certain neurocognitive deficits.[38,254] It remains unknown how ARAS connectivity might be influenced by epilepsy treatment.

In TLE, surgery leads to seizure freedom in approximately two-thirds of patients.[20,255] Seizure freedom often leads to improved quality of life,[150] improved neurocognition,[25,27] and decreased mortality risk[151] after surgery. Are these benefits accompanied by connectivity reorganization? Few groups have evaluated post-operative connectivity in TLE,[153,154] and to our knowledge, connectivity of arousal structures has not been evaluated. Here we examine brainstem connectivity in 15 TLE patients before and after surgery, alongside 15 controls. We focus on networks previously found to be most perturbed prior to surgery,[38,254] including connections between ARAS structures to fronto-parietal-insular neocortex. In post-operative patients, we analyze brainstem ARAS connectivity changes before and after surgery to determine the effects of epilepsy surgery on connectivity in TLE.

III.2.2 Methods

III.2.2.1 Participants

We evaluated 15 adult TLE patients who presented for epilepsy surgery evaluation from 2012 to 2016 and received surgery and had >1-year post-operative follow-up. In these 15 patients, the diagnosis of TLE was established according to standard clinical care at our institution by a multidisciplinary process including neurologists, neurosurgeons, and neuropsychologists. This process included a detailed patient history, analyzing the semiology of seizures, anatomical magnetic resonance imaging (MRI), inpatient video electroencephalography (EEG) monitoring, positron emission tomography (PET) scan, localization of language and memory by functional MRI (fMRI) or Wada testing, and testing by a licensed neuropsychologist. Based on all these tests, the multidisciplinary epilepsy committee felt confident in proceeding to surgery with a diagnosis of TLE without intracranial EEG monitoring for all 15 patients.[98] Overall, post-operative patients were re-imaged 33.6 ± 11.6 (mean ± SD) months after surgery. 15 healthy control participants were individually matched to patients by age, sex and handedness, except for one control who was not handedness-matched (**Table III.1**). Informed consent for this study was obtained, and all procedures were approved by the Institutional Review Board.

	Patients	Controls	P value
Age, years	39.4 ± 14.2	40.2 ± 13.6	0.85
Gender, female	8 (53.3)	8 (53.3)	0.99
Handedness, right	13 (86.6)	14 (93.3)	0.99
Epilepsy duration, years	22.6 ± 16.5		
Seizure frequency, monthly			
FACS	0.2 ± 0.6		
FICS	3.0 ± 2.5		
FBTC	0.3 ± 1.1		
History of FBTC, yes	7 (46.6)		
Epileptogenic side, right	10 (66.6)		
MTS on MRI, yes	12 (80.0)		
Non-lesional on MRI, yes	3 (20.0)		
Time between pre-operative MRI & surgery, months	1.87 ± 3.5		
Range time between pre-operative MRI & surgery, months (min, max)	(0,14)		
Time between surgery & post-operative MRI, months	33.6 ± 11.6		
Range time between surgery & post-operative scan, months (min, max)	(14,52)		
Surgery type			
SAH	8 (53.3)		
ATL	5 (33.3)		
Laser	2 (13.3)		
Operative Specimen pathology			
MTS	11 (73.3)		
Gliosis	2 (13.3)		
No specimen	2 (13.3)		
Seizure-free after surgery	10 (66.6)		

Table III.1 Patient and control participant demographics.
For continuous variables, data are mean ± standard deviation and the statistical test is Mann-Whitney U. For categorical variables, data are N (%) and the statistical test is Chi-square. N = 15 patients and 15 controls. ATL: anterior temporal lobectomy; FACS: focal aware conscious seizures; FBTC: focal to bilateral tonic clonic (secondarily-generalized) seizures; FICS: focal impaired consciousness seizures; Laser: laser ablation of amygdala and hippocampus; MRI: magnetic resonance imaging; MTS: mesial temporal sclerosis; SAH: selective amygdalohippocampectomy.

III.2.2.2 Imaging

MRI was performed using a Philips Achieva 3T scanner (Philips Healthcare, Best, Netherlands) and 32-channel head coil. Images acquired included (1) three-dimensional T1-weighted whole-brain images for inter-participant normalization and tissue segmentation (gradient echo, TR = 9.1 ms, TE = 4.6 ms, 192 shots, flip angle=8 degrees, matrix = 256x256, 1x1x1 mm^3), (2) two-dimensional, T1-weighted axial images for functional images to structural images coregistration (1x1x4 mm^3), (3) two 10-minute $T2^*$-weighted blood oxygenation level dependent (BOLD) fMRI images at rest with eyes closed (FOV = 240 mm, TE = 35 ms, TR = 2 s, 34 axial slices, slice thickness = 3.5 mm/0.5 mm gap, matrix = 80x80, 3x3x4 mm^3), with 300 volumes acquired during each 10-minute acquisition, (4) diffusion weighted imaging (b = 1600 s/mm^2, 92 directions, 2.5x2.5x2.5 mm^3). Physiological respiratory and cardiac rates were recorded at 500 Hz.

III.2.2.3 Connectivity Regions

Regions for connectivity analyses included three ARAS structures (cuneiform/subcuneiform nuclei: CSC, pedunculopontine nucleus: PPN, and ventral tegmental area: VTA) from the Harvard Ascending Arousal Network Atlas (https://www.martinos.org/resources/aan-atlas)[5] and 105 cortical/subcortical regions from the Harvard-Oxford atlas (http://www.fmrib.ox.ac.uk/fsl/). Atlas coregistration details have been previously reported.[254]

III.2.2.4 Functional Connectivity Analysis

SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and MATLAB 2016a (The MathWorks, Natick, Massachusetts, USA) were used to preprocess fMRI. FMRI preprocessing included slice-timing correction, segmentation into white matter, gray matter, and cerebrospinal fluid, and spatial normalization to the Montreal Neurological Institute template. Additionally, signal fluctuation resulting from movement and physiological noise were minimized using standard protocols across all subjects. Motion correction was accomplished by framewise displacement correction, and physiological noise correction was accomplished using a RETROspective Image CORrection (RETROICOR)[256] algorithm. We used SPM to normalize and coregister fMRI through T1 images to the cortical/subcortical atlas. Finally, fMRI images were band-pass filtered between 0.0067 and 0.1 Hz. For each of the two fMRI sessions in each participant, functional connectivity was computed between each ARAS region (CSC, PPN, VTA) and each of 105 cortical/subcortical areas by partial Pearson correlation between each region's

time series, with six motion time series and mean white matter BOLD signal serving as confounds. Fisher Z scores for each participant were averaged across both fMRI sessions. We evaluated functional connectivity differences in patients before and after surgery between ARAS and frontal, parietal, and insular neocortical regions which showed large decreases in TLE patients in prior work.[38,254] These "frontoparietal" cortical regions – a term used henceforth – included bilateral inferior frontal gyrus pars opercularis and pars triangularis, precentral gyrus, postcentral gyrus, superior parietal lobule, and insula. For visualization of ARAS functional connectivity differences between participant groups we employed CONN toolbox 17 (https://www.nitrc.org/projects/conn/).[257] Patients' functional connectivity image laterality was oriented according to epileptogenic side, and images of matched controls were flipped accordingly.

III.2.2.4 ALFF Measurements

Functional connectivity measurements between two regions do not allow insight into which of the two regions (if any) is "driving" connectivity differences. To further understand patterns of ARAS and frontoparietal fMRI signal fluctuations, we measured amplitude of low frequency fluctuations (ALFF) from fMRI in ARAS structures and frontoparietal regions in patients who achieved post-operative seizure freedom, alongside matched controls. Preprocessing of fMRI data proceeded as above (low-pass filter, 0.1 Hz). ALFF was calculated by transforming the time-series BOLD signal to frequency domain using the MATLAB fast Fourier transform function. Then we measured the averaged square root of the absolute value of the transformed signal in 0.01-0.08 Hz frequency band,[258] and divided by mean ALFF of the brain (**equation III.1**).

$$ALFF = \frac{mean(sqrt(fft(0.01Hz-0.08Hz)))}{mean\ ALFF\ of\ brain} \qquad \textbf{(eq. III.1)}$$

III.2.2.5 Structural Connectivity Analysis

DTI was processed with FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) and estimates of voxel-wise diffusion measurements were conducted using the BEDPOSTX algorithm's Bayesian approach.[259] PROBTRACKX, a probabilistic fiber-tracking algorithm with crossing fibers, was used to examine tracts seeded from each of three ARAS structures - CSC, PPN, and VTA - to 105 cortical/subcortical target regions. PROBTRACKX used 5,000 trials from all voxels in each seed region and tracked a streamline until exceeding limits set for: number of steps per sample = 2,000

steps; step-length = 0.5 mm; or curvature = 0.2 or ± 80°. Structural connectivity tractography was corrected for distance from ARAS seed and calculated as the sum of all tracts from all voxels in the seed region that went through each of the target regions. In patients who achieved post-operative seizure freedom, structural connectivity was compared to pre-operative baseline between ARAS structures and 1) frontoparietal neocortical regions, defined above, and 2) the 10 areas of greatest structural connectivity decreases in patients vs. controls, which included thalamus, caudate, putamen, pallidum, posterior cingulate, precuneus, middle frontal gyrus, frontal pole, supplementary motor area, and precentral gyrus. One patient was excluded from structural connectivity analysis given absent post-operative DTI, and the matched control was also excluded, both the patient and control were still included in all other analyses. In example participants, we employed the BrainSuite Diffusion Pipeline (http://brainsuite.org)[260] to visualize deterministic diffusion tractography seeded from the three ARAS regions.

III.2.2.6 Disease Measures and Neurocognitive Testing

Participant demographics and patient disease measures including seizure frequency, epilepsy duration, history of focal to bilateral tonic-clonic (secondarily-generalized) seizures, epileptogenic side, and MRI results were collected from treating epileptologists' clinical assessments (**Table III.1**). Seizure outcomes were defined at the time of post-operative MRI using Engel classification, with Engel 1 indicating freedom from disabling seizures, and Engel 2-4 indicating persistent seizures.[82]

A licensed neuropsychologist administered a standardized battery of neurocognitive examinations to pre-operative patients. Given that previous work suggested relationships between ARAS connectivity disturbances and verbal ability in TLE,[38,254] we related increases in post-operative connectivity in seizure free patients to pre-operative verbal IQ and verbal memory. Verbal IQ was established using the Verbal Comprehensions Index, Wechsler Adult Intelligence Scale, Fourth Edition and verbal memory was established using the California Verbal Learning Test, part II, and Wechsler Memory Scale. Verbal memory performance was categorized as average/above average (40-100th percentile) or below average (0-40th percentile) as compared to a standard normative population.

III.2.2.7 Statistical Analyses

Non-parametric tests were employed for non-normally distributed data determined using the Anderson-Darling test.[261] Participant demographics were compared with Mann-Whitney U-test for continuous variables and chi-square for categorical variables. Kruskal-Wallis, with post hoc Dunn where appropriate, was used to compare functional connectivity, ALFF, and structural connectivity between groups: pre-operative patients, post-operative patients, and controls. For all groups compared with Kruskal-Wallis, Levene's test was used to ensure homogeneity of variances between groups prior to statistical comparison.[262] Spearman's rho was used to compare continuous disease measures and verbal IQ to functional connectivity differences between post-operative and pre-operative values. Performances on verbal memory tests and categorical disease parameters were dichotomized and compared with Mann-Whitney U-test. Statistical analyses were performed with MATLAB 2016a and SPSS23 (Armonk, New York, USA). Significance was prospectively defined as $p < 0.05$ for all tests, and the Bonferroni-Holm correction method was used for multiple comparisons where indicated.

III.2.3 Results

III.2.3.1 ARAS Functional Connectivity in TLE Patients Increases After Successful Surgery

At the time of post-operative scan, at least one year after surgery, 10 patients had achieved seizure freedom and five patients continued to experience seizures. For the 10 seizure free patients, seven patients were Engel 1a, and one patient each had an Engel 1b, 1c, and 1d outcome, respectively. As previous work demonstrated functional connectivity decreases between ARAS structures and fronto-parietal-insular neocortex in TLE,[38,254] we first examined whether this connectivity changes after successful epilepsy surgery. Compared to pre-operative baseline, patients who achieved post-operative (> 1-year) seizure freedom (10 of 15) demonstrated increases in connectivity between ARAS structures and bilateral fronto-parietal-insular cortical regions on voxel-wise whole-brain connectivity maps (**Fig. III.1**). Connectivity increases seeded from PPN appeared most prominent (**Fig. III.1B**), followed by CSC (**Fig. III.1A**), and VTA (**Fig. III.1C**). Next, we specifically analyzed ARAS functional connectivity to frontoparietal cortex.

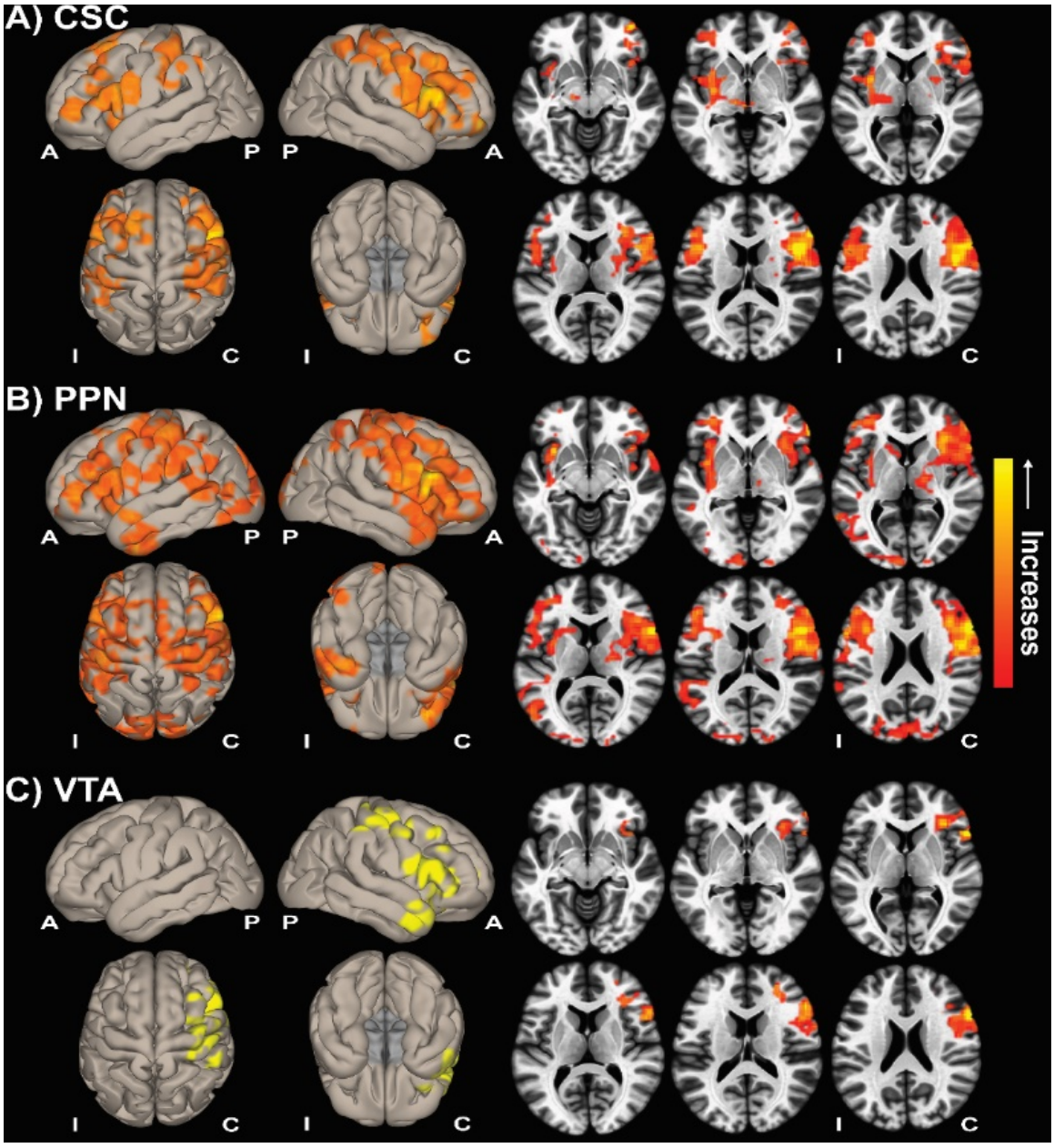

Figure III.1 Functional connectivity increases in seizure-free TLE patients after surgery.
Cortical surface (left) and axial slice (right) views are shown, demonstrating functional connectivity increases in patients with TLE who achieved seizure freedom after surgery, seeded from CSC (**A**), PPN (**B**), and VTA (**C**). Data represent seed-to-voxel functional connectivity (bivariate correlation) maps comparing post-operative vs. pre-operative fMRI (paired t-test, cluster threshold level $p < 0.05$, FDR correction) generated using the CONN toolbox (https://www.nitrc.org/projects/conn/). Positive contrasts are shown, and no connectivity decreases were observed on negative contrasts. Images are oriented across all patients with respect to the epileptogenic side. N = 10 patients before surgery and > 1-year after surgery. A: anterior; ARAS: ascending reticular activating system; C: contralateral; CSC: cuneiform/subcuneiform nuclei; FDR: false discovery rate; I: ipsilateral; P: posterior; PPN: pedunculopontine nucleus; VTA: ventral tegmental area.

Mean connectivity between ARAS structures and frontoparietal cortex was higher in post-operative patients who achieved seizure freedom, with post-operative connectivity more closely resembling controls (**Fig. III.2A**). Specifically, increases in post-operative connectivity were observed for PPN and CSC, while no increase was observed for VTA (**Fig. III.2B**). Notably, the increase in post-operative ARAS-frontoparietal connectivity was comparable in patients who had stopped or reduced pre-operative epilepsy medications (N = 6, 1.38 ± 1.78, mean ± SD) vs. those continuing similar medication regimens (N = 4, 2.44 ± 1.29; p = 0.35, Mann-Whitney U-test) at the time of second scan. Additionally, given that motion during fMRI has been shown to introduce artifact in results, we conducted an analysis of maximum translation and rotation. In seizure-free group and controls (N = 10) no difference in maximum translation was detected among pre-operative patients (0.77 ± 0.36 mm, mean ± SD), post-operative patients (0.65 ± 0.22 mm), or controls (0.53 ± 0.23 mm, p = 0.32, Kruskal-Wallis). Nor, in this group, was there any difference detected in maximum rotation for pre-operative patients (0.74 ± 0.32 degrees), post-operative patients (0.62 ± 0.21 degrees), or controls (0.44 ± 0.23 degrees, p = 0.06, Kruskal-Wallis). These analyses suggest that post-operative connectivity increases detected were not driven primarily by medication changes or motion artifacts

Examination of patients with continued post-operative seizures was limited by sample size (5 of 15), and Engel outcomes for these patients were Engel 2c in two patients and Engel 3a in three patients at the time of post-operative scan. In these individuals there were no differences detected in ARAS-frontoparietal post-operative connectivity (2.82 ± 2.24, mean ± SD) compared to pre-operative baseline (2.71 ± 3.14; p = 0.59, Kruskal-Wallis). With analysis of voxel-wise connectivity maps seeded from CSC, PPN, or VTA we did not detect any altered connectivity post-operatively compared to before surgery (not shown). Motion analysis was also performed for the non-seizure free group and we were unable to detect any difference in motion between groups. Specifically, for the non-seizure free group (N = 5), maximum translation did not differ between pre-operative patients (0.89 ± 0.47 mm), post-operative patients (0.61 ± 0.25 mm), or controls (0.43 ± 0.11 mm, p = 0.28, Kruskal-Wallis). There was no detectable difference in maximum rotation among pre-operative patients (0.65 ± 0.36 degrees), post-operative patients (0.57 ± 0.23 degrees), or controls (0.35 ± 0.15 degrees, p = 0.26, Kruskal-Wallis) for patients with continued post-operative seizures. Overall, these results suggest that ARAS-frontoparietal connectivity may increase in patients who achieve post-operative seizure freedom.

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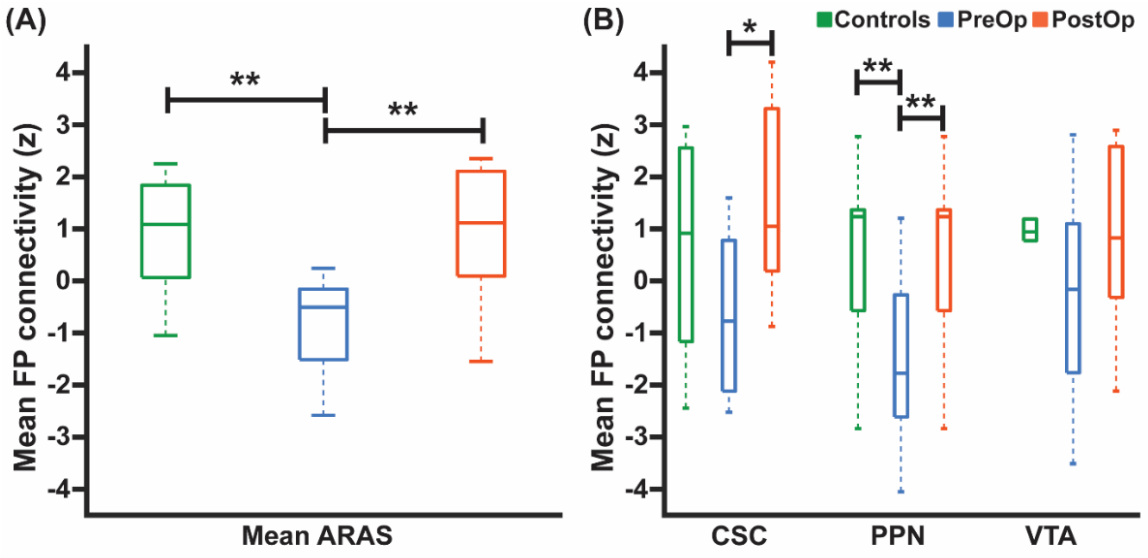

Figure III.2 ARAS-frontoparietal functional connectivity in TLE patients before and after surgery and controls.
(**A**) Mean functional connectivity between ARAS and frontoparietal and insular neocortex is reduced in pre-operative patients with TLE compared to controls. However, connectivity in the same TLE patients is increased > 1-year after surgery, resembling connectivity in controls. (**B**) Examining ARAS regions individually, increases in frontoparietal connectivity are seen after surgery in CSC and PPN, but not VTA. N = 10 patients before surgery and > 1-year after surgery who ultimately achieved seizure freedom vs. 10 matched controls. *p = 0.05, Kruskal-Wallis, with post hoc Dunn, **p-value range = 0.01 - 0.04, Kruskal-Wallis, with post hoc Dunn. Central bar shows median, bottom and top edges of box indicate 25th and 75th percentiles, and whiskers indicate data extremes. ARAS: ascending reticular activating system; CSC: cuneiform/subcuneiform nuclei; FP: frontoparietal; PostOp: post-operative patients; PPN: pedunculopontine nucleus; PreOp: pre-operative patients; VTA: ventral tegmental area.

III.2.3.2 ARAS ALFF is Altered in Patients but Does Not Change After Surgery

We also examined ALFF in ARAS structures and frontoparietal cortex in patients who achieved seizure freedom, to better understand ARAS-frontoparietal connectivity. Mean ARAS ALFF was higher in TLE patients – both pre- and post-operative – compared to controls (**Fig. III.3A**). However, no differences in frontoparietal ALFF were noted between controls, pre-operative patients, and post-operative patients (**Fig. III.3B**). No differences in ARAS or frontoparietal ALFF were observed in post-operative patients compared to pre-operative baseline (**Fig. III.3A, B**). These results suggest that ARAS-frontoparietal connectivity disturbances in epilepsy may be driven more by ARAS alterations than by frontoparietal changes.[263] However, unlike ARAS functional connectivity, ARAS ALFF does not appear to change after successful surgery.

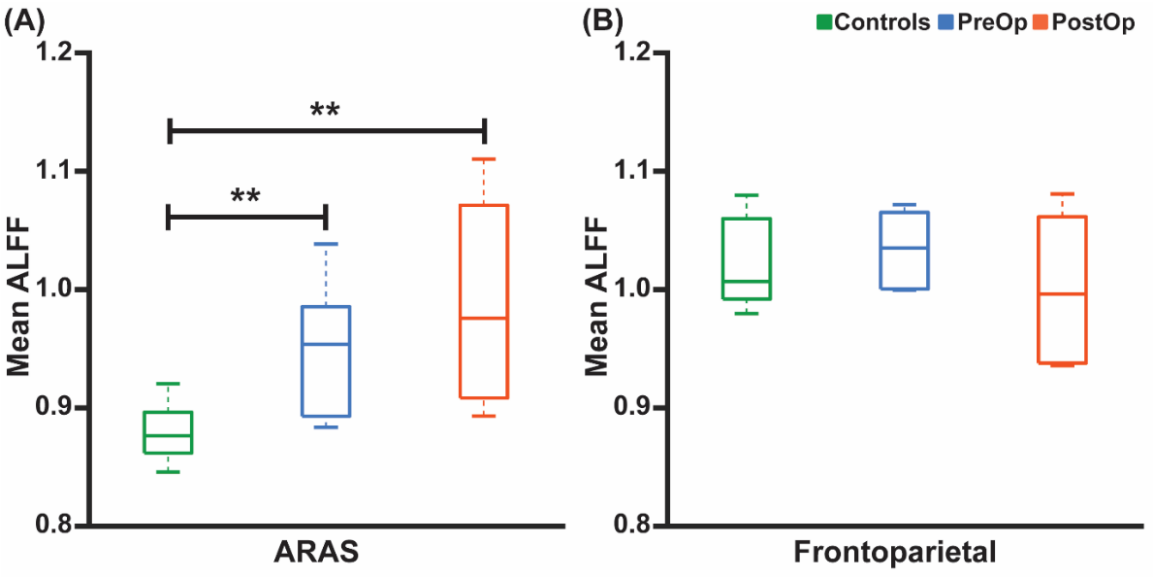

Figure III.3 ALFF in ARAS, but not frontoparietal neocortex, differs between patients and controls.
(**A**) Differences in mean ALFF values in ARAS between controls and both pre-operative and post-operative patients. No ARAS ALFF differences are noted between pre-operative and post-operative patients. (**B**) No differences in mean ALFF values in bilateral frontoparietal/insular neocortical regions are observed between controls, pre-operative patients, or post-operative patients. N = 10 patients before surgery and > 1-year after surgery who ultimately achieved seizure freedom vs. 10 controls. **p < 0.05, Kruskal-Wallis, with post hoc Dunn. Central bar shows median, bottom and top edges of box indicate 25th and 75th percentiles, and whiskers indicate data extremes. ALFF: Amplitude Low Frequency Fluctuations; ARAS: Ascending Reticular Activating System; CSC: cuneiform/subcuneiform nuclei; PostOp: post-operative patients; PPN: pedunculopontine nucleus; PreOp: pre-operative patients; VTA: ventral tegmental area.

III.2.3.3 Relating ARAS Functional Connectivity Changes to Disease and Neurocognitive Variables

Prior work demonstrated larger ARAS functional connectivity reductions in patients with more frequent focal impaired consciousness seizures (FICS).[38] We asked whether pre-operative FICS frequency was related to post-operative connectivity change in patients who achieved seizure freedom. Larger post-operative connectivity increases between CSC, PPN, and VTA and frontoparietal cortex were observed in patients with more frequent pre-operative FICS (**Fig. III.4A**). This suggests that individuals with greater disease burden have larger connectivity increases after successful surgery. Examining whether these connectivity changes are related to time elapsed since surgery prior to the second MRI scan, we observed a marginal relationship between CSC connectivity and time, and no relationship for PPN or VTA (**Fig. III.4B**). No relationship was observed between ARAS-frontoparietal connectivity change after surgery and

duration of epilepsy in these individuals (rho = 0.17 – 0.56, p = 0.63 - 0.09 for CSC, PPN, and VTA).

It has been previously reported that ARAS connectivity reductions are associated with impaired performance on verbal testing in pre-operative TLE patients.[38,254] Therefore, we next evaluated potential relationships between pre-operative verbal IQ and memory and post-operative change in ARAS-frontoparietal connectivity in seizure-free patients. A marginal relationship was observed between lower pre-operative verbal IQ and increase in post-operative PPN functional connectivity, while no relationships were seen for CSC or VTA (**Fig. III.4C**). Furthermore, compared to individuals with average or above verbal memory, patients with pre-operative below average verbal memory experienced larger increases in post-operative PPN connectivity (**Fig. III.4D**), but not CSC (**Fig. III.4E**) or VTA (**Fig. III.4F**) functional connectivity. This suggests that individuals with worse verbal IQ and memory may experience greater increases in post-operative PPN functional connectivity.

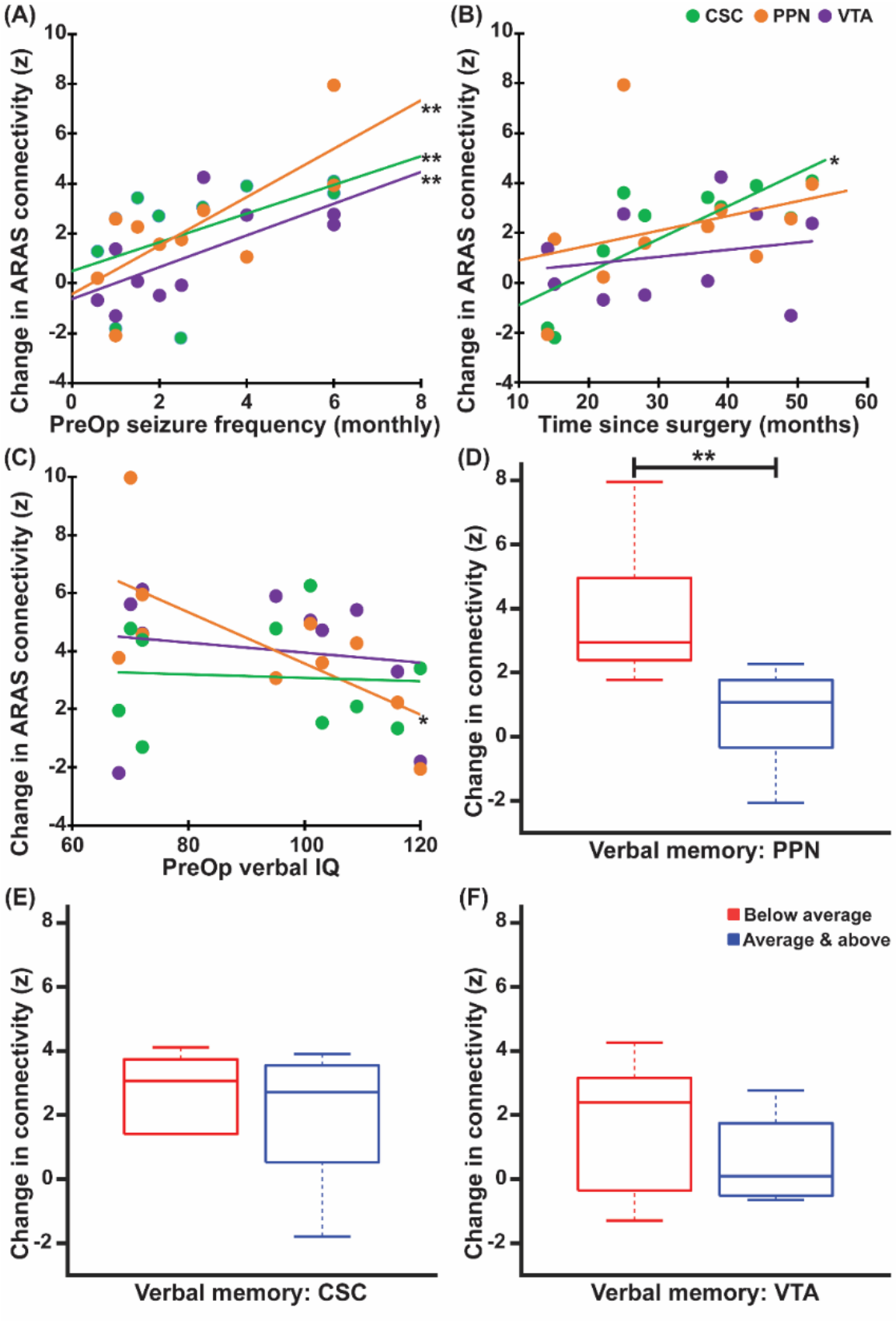
(A)
Change in ARAS connectivity (z)
PreOp seizure frequency (monthly)
**
**
**
(B)
CSC
PPN
VTA
Change in ARAS connectivity (z)
Time since surgery (months)
*
(C)
Change in ARAS connectivity (z)
PreOp verbal IQ
*
(D)
**
Change in connectivity (z)
Verbal memory: PPN
(E)
Change in connectivity (z)
Verbal memory: CSC
(F)
Below average
Average & above
Change in connectivity (z)
Verbal memory: VTA

Figure III.4 Relationships between ARAS post-operative functional connectivity changes and disease measures in seizure-free patients.
(**A**) Larger increases in functional connectivity between each ARAS structure (CSC, PPN, VTA) and frontoparietal neocortex are associated with higher pre-operative focal impaired consciousness seizure frequency. (**B**) Larger increases in functional connectivity between CSC and frontoparietal neocortex is associated with longer time between surgery and the post-operative scan, while no similar relationship is observed with changes in PPN or VTA connectivity. (**C**) Patients with lower pre-operative verbal IQ before surgery demonstrate a larger post-operative increase in functional connectivity between PPN and frontoparietal neocortex, although no similar relationship is noted for CSC or VTA. (**D, E, F**) Patients with worse pre-operative verbal memory performance show a larger increase in PPN post-operatively (**E**), but no such relationship is noted for CSC (**D**) or VTA (**F**). N = 10 patients who ultimately achieved seizure freedom after surgery. *p-value range = 0.02-0.03, uncorrected, **p-value range = 0.03-0.04 after Bonferroni-Holm correction for Spearman's Rho (**A-C**) or Mann-Whitney U-Test (**D-F**). Central bar shows median, bottom and top edges of box indicate 25th and 75th percentiles, and whiskers indicate data extremes. ARAS: Ascending Reticular Activating System; CSC: cuneiform/subcuneiform nuclei; PPN: pedunculopontine nucleus; PreOp: pre-operative patients; VTA: ventral tegmental area.

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III.2.3.4 ARAS Structural Connectivity Does Not Change After Epilepsy Surgery

We next asked whether ARAS structural connectivity changes after surgery, as prior work demonstrated both ARAS functional and structural connectivity decreases in TLE.[254] Diffusion tractography in example participants (**Fig. III.5**) reveal fewer tracts reaching targets seeded from CSC, PPN, and VTA in patients compared to controls (**Fig. III.5A**). Additionally, no obvious differences between patient structural connectivity patterns before surgery (**Fig. III.5B**) vs. after surgery (**Fig. III.5C**) were observed. Overall, when evaluating the 10 regions of greatest ARAS structural connectivity decreases in TLE patients ($3.3x10^5 \pm 1.0x10^5$ tracts; mean ± SD) compared to controls ($4.5x10^5 \pm 1.1x10^5$ tracts), no changes in structural connectivity were observed after surgery in patients who achieved seizure freedom ($3.3x10^5 \pm 7.8x10^4$ tracts; $p > 0.99$ Kruskal-Wallis, with post hoc Dunn). Furthermore, no differences in structural connectivity from ARAS to frontoparietal neocortex were observed between pre-operative patients ($2.1\ x10^5 \pm 1.0x10^5$ tracts), post-operative patients ($2.4x10^5 \pm 8.3x10^4$ tracts), or controls ($2.3x10^5 \pm 7.9x10^4$ tracts; $p = 0.63$, Kruskal-Wallis). Thus, unlike functional connectivity, structural connectivity alterations in TLE may not change after successful surgery.

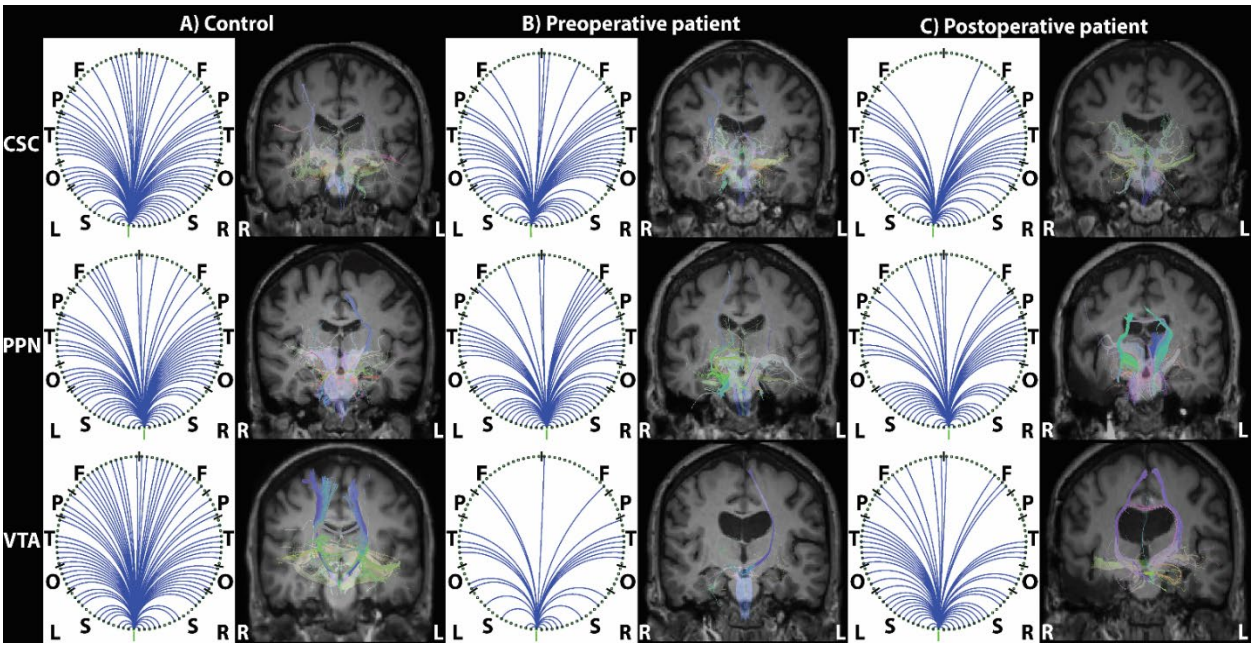

Figure III.5 Example ARAS structural connectivity in a patient before and after surgery, and a matched control.

Example ARAS diffusion tractography seeded from CSC (top row), PPN (middle row), and VTA (bottom row) in an example matched control. (**A**), pre-operative patient (**B**), and the same patient post-operative (**C**) for each region. Figures are generated using the BrainSuite Diffusion Pipeline (BDP; http://brainsuite.org). On the left in each column (**A-C**), are circle graphs that summarize projections seeded from ARAS regions to cortical and subcortical regions in BrainSuite's SVReg atlas. On the right in each column, (**A-C**), are estimated diffusion tensors overlaid onto T1-weighted coronal anatomical images using a rigid mutual information-based registration. Overall, for the three ARAS seed regions, the most tracts are seen in the controls (**A**) compared to patients (**B, C**). Additionally, visually, there are no differences in estimated tracts between pre-operative patients (**B**) and post-operative patients (**C**). BrainSuite settings: 1 seed-per-voxel, step-size = 0.25 mm, max. steps = 500, angle-threshold = 10 degrees, fractional anisotropy threshold = 0.05, orientation distribution function sampling = 20, generalized fraction anisotropy/lambda 2 threshold = 0.01. CSC: cuneiform/subcuneiform nuclei; F: frontal; L: left; O: occipital; P: parietal; PPN: pedunculopontine nucleus; R: right; S: subcortical; T: temporal; VTA: ventral tegmental area.

III.2.4 Discussion

Previous investigations have suggested that recurrent seizures may lead to decreased ARAS connectivity, which may contribute to broad neurocognitive problems in TLE.[38,254] Might these connectivity perturbations "improve" in patients who achieve seizure freedom after epilepsy surgery? Our present findings suggest that post-operative functional connectivity between ARAS and frontoparietal cortex may increase after successful surgery (10 of 15 patients), more closely resembling connectivity patterns in controls. While we did not see functional connectivity increases in patients with continued post-operative seizures, only five individuals were included in this analysis. Why does diminished ARAS functional connectivity in TLE matter? It is possible that ARAS connectivity reductions contribute to neurocognitive deficits or may be related to

sudden unexplained death in epilepsy (SUDEP). SUDEP has been proposed to involve dysfunction of ARAS networks,[250] and risk of SUDEP decreases after epilepsy surgery.[264]

Why might ARAS-frontoparietal functional connectivity be disturbed in TLE, and why might it recover after successful surgery? In our working model (**Fig. III.6**) built upon prior work by Blumenfeld,[1,204] normal cortical activation is maintained during interictal baseline through normal connectivity from subcortical activating structures (**Fig. III.6A**). During the ictal period, seizure activity begins in the hippocampus (**Fig. III.6B**), and may spread to subcortical activating structures (**Fig. III.6C**), resulting in focal seizures with impaired consciousness (FICS) and neocortical depression given absent subcortical excitation (**Fig. III.6C**). This transient network inhibition is associated with sleep-like neocortical rhythms and diminished cortical blood flow in TLE patients,[46,183,186] and is supported by rodent studies showing reduced neocortical activity and behavioral arrest that only occurs if limbic seizure activity propagates to subcortical activating structures.[44,45,79] While neocortical activation transiently recovers after the post-ictal period (**Fig. III.6C→6A**), it is possible that over time, recurrent FICS lead to decreased connectivity between subcortical activating structures and neocortex that persists during the interictal, resting-state (**Fig. III.6D**). This may reflect an evolutionarily advantageous phenomenon to prevent secondary generalization of seizure activity, or may simply result from cumulative damage to neural networks from seizures. After successful epilepsy surgery, cessation of recurrent FICS may allow ARAS-frontoparietal functional connectivity to normalize (**Fig. III.6E**). Supporting this hypothesis, prior work has observed larger functional connectivity decreases in TLE patients with more frequent FICS, and we now observe that these individuals may also have larger functional connectivity increases after successful surgery.

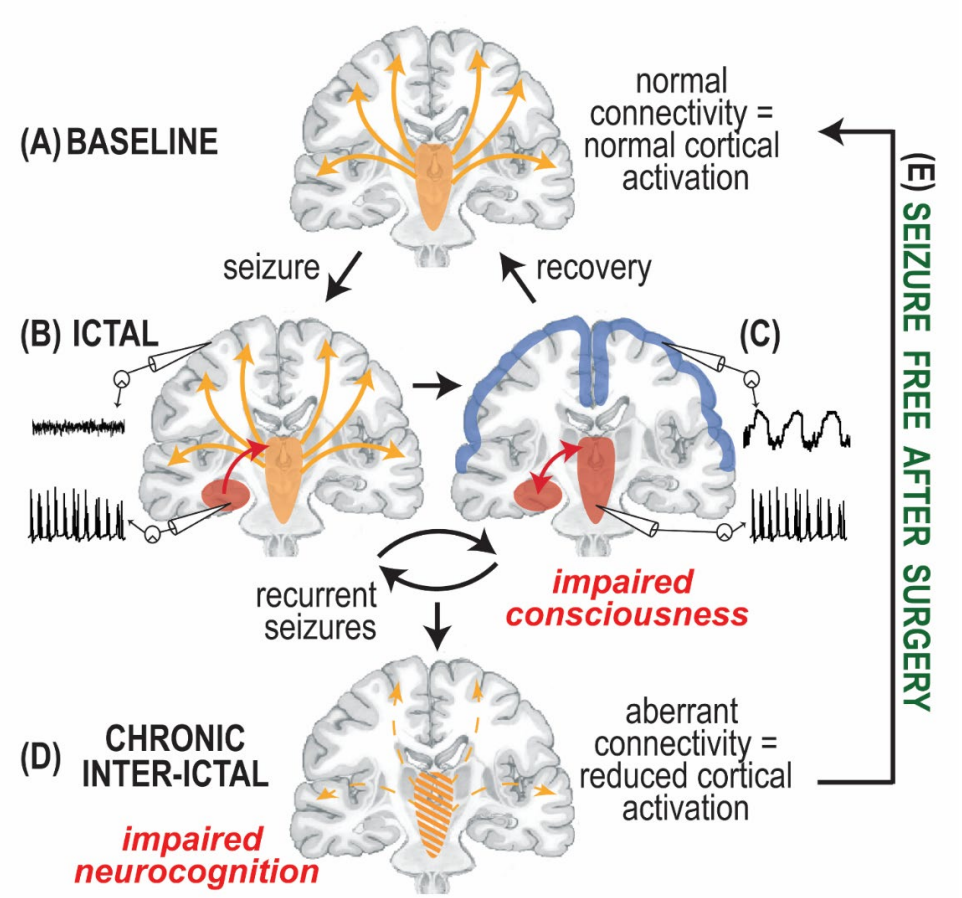

Figure III.6 Model for subcortical-cortical connectivity disturbances and recovery in TLE.

(**A**) At wakeful baseline, neocortical activation is maintained via direct and indirect excitatory projections from subcortical activating structures, including ARAS, intralaminar thalamus, and basal forebrain. (**B**) During the transition to the ictal period, seizure activity begins in the mesial temporal lobe, and may remain confined there without disturbing cortical activity, generating a small consciousness-sparing focal seizure, or aura. (**C**) When seizure activity spreads to involve subcortical activating structures, the normal excitatory input from the subcortical regions to the neocortex is perturbed, and the neocortex defaults to a sleep-like inhibited state, resulting in a consciousness-impairing focal seizure. (**D**) Over time, recurrent consciousness-impairing focal seizures may lead to progressive dysfunction of subcortical activating structures and aberrant connectivity between these regions and the neocortex, leading to a chronic state of reduced neocortical activation and impaired neurocognition. (**E**) Seizure freedom after successful epilepsy surgery may allow recovery of certain subcortical-cortical functional connectivity perturbations. Modified with permission from Englot and Blumenfeld, 2009[1], and courtesy of Hal Blumenfeld.

Next, it has been reported that relationships exist between ARAS connectivity decreases and diminished verbal abilities, and here we observe larger PPN functional connectivity increases in patients with worse pre-operative verbal IQ and memory. This may suggest greater potential for improvement in those with more significant neurocognitive deficits at baseline. Interestingly, PPN deep brain stimulation in Parkinson's disease has been associated with improvements in neurocognition,[265,266] and PPN stimulation in rats can help prevent deleterious neocortical and behavioral effects of limbic seizures.[65]

Prior work noted diminished ARAS structural connectivity in TLE patients, albeit to different structures than functional connectivity changes.[254] It is also known that functional connections can exist absent direct axonal connections, presumably because functional connectivity may reflect indirect/polysynaptic pathways.[267,268] Perhaps it is therefore not surprising that we did not observe ARAS structural connectivity increases after surgery in this study, as new axonal growth is not likely the source of functional connectivity improvements. The lack of post-operative structural change in ARAS may be further supported by our observation that while fMRI ALFF in ARAS was altered in TLE patients compared to controls, ARAS ALFF did not change after successful surgery. Further comparison of functional connectivity, structural connectivity, and ALFF in TLE may lead to a better understanding of disease-related and treatment-related network alterations.

This study has other limitations worth discussing. First, this work must be considered a preliminary analysis of ARAS connectivity changes after surgery, due to the small sample size and the heterogeneity of the patient population. The study is therefore not sufficiently powered to evaluate potential confounders, such as pathology results and surgery type, using multivariate analysis. Therefore, our results must be validated in a larger patient cohort with appropriate subgroup analyses in future studies. Nevertheless, this is the first study to evaluate brainstem arousal connectivity changes with epilepsy surgery, and includes patients with long-term (mean 33 months) post-operative imaging. Additionally, of the two ARAS regions in which we observed significant increases in post-operative functional connectivity, increased connectivity was observed in 9 of 10 patients for PPN and 8 of 10 patients for CSC. It is also important to note the limitations of statistical tests used in this study, and that a lack of significance differences between participant groups does not imply that the groups are equal. We utilized non-parametric statistical tests given that the Anderson-Darling test suggested that our data were not normally distributed, and results may differ using various statistical tests. However, repeating our analyses using

parametric tests (e.g., ANOVA with post-hoc Fisher's least significant difference procedures; not shown), our findings remained consistent.

Another limitation of this study is that long-term post-operative neuropsychological assessments were not available, as our center previously performed post-surgical cognitive assessments immediately after surgery. Prior work has demonstrated that patients who achieve long-term seizure freedom after epilepsy surgery often also have improvements in various neurocognitive domains.[25,26,269] Although we hypothesize that connectivity improvements may be accompanied by improvements in certain neurocognitive domains in seizure free patients, this particular hypothesis could not be tested in this study. While the goal of this preliminary study was to first determine whether ARAS connectivity may improve towards control values after surgery, and relate connectivity changes to various pre-operative clinical variables, future studies should include long-term post-operative neuropsychological assessments to relate connectivity to cognitive changes after surgery. Finally, another important future direction will be to perform serial measurements of connectivity at various time points after surgery, to determine the potential effects of evolving seizure outcome and time since last seizure(s) on connectivity patterns.

III.2.5 Conclusion

In summary, connectivity perturbations of certain subcortical arousal networks are present in TLE and may be related to disease severity and neurocognitive function. After successful epilepsy surgery, some brainstem functional connectivity patterns may recover and more closely resemble connectivity in healthy control. These findings may have important implications for treatment selection and timing, and for future investigations into neuromodulation targets, neuropsychological outcomes, and the risk of SUDEP in TLE.

Funding and Acknowledgements

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Brain Research (CONN toolbox), and the Leahy/Shattuck neuroimaging collaboration (BrainSuite).

CHAPTER IV

IV Employing Novel Participant-Specific Thalamic Atlas to Evaluate Thalamic Arousal Networks

IV.1 Summary and Contributions

This chapter details our application of participant-specific thalamic atlases to investigate how resting-state functional connectivity of the thalamus, particularly the intralaminar thalamus, is affected in patients with TLE and how this functional connectivity is impacted by epilepsy surgery. Prior literature has established that the thalamus, particularly the intralaminar thalamic nuclei, plays an important role in arousal and awareness.[13,61,199] Based on this, we hypothesized that thalamic arousal nuclei were affected by recurrent seizures and that interictal abnormalities in thalamic connectivity may play an important role in the pathophysiology of TLE. We expected that pre-operative thalamic functional connectivity would be perturbed, and that after successful surgery, patients would regain healthy functional connectivity (closer to controls). We used resting-state fMRI and a custom participant-specific thalamic atlas to calculate functional connectivity networks and test our hypothesis.

From the results of this study, we observed that intralaminar thalamic nuclei have altered connectivity with occipital regions in patients with TLE and that after successful epilepsy surgery, this connectivity recovered. We also noted that patients had abnormal brainstem-intralaminar thalamic connectivity, which was more abnormal in patients with impaired visuospatial attention. Both thalamo-occipital and brainstem-thalamic connectivity recovered after epilepsy surgery in patients who had positive surgical outcomes (reduced seizure burden).

This work highlights the importance of using novel methods to interrogate regions previously not thought to be involved in TLE. Broadly, the thalamus is an important structure that operates as a waystation for connections in the brain and synchronizes/orchestrates brain rhythms. In particular, intrathalamic nuclei are central to consciousness. Specifically, the central lateral thalamic nucleus is notable as the largest of these nuclei. Despite their importance, intralaminar thalamic nuclei remain understudied. This is largely because they are impossible to visualize at 3T without specialized scans. Additionally, there exist few segmentations of thalamic nuclei for

functional connectivity calculation and until our investigation, none had been used to study intralaminar thalamic nuclei in TLE. To address this challenge our study employed an atlas of small subcortical structures to improve deep brain stimulation (DBS) planning projected into patient-specific MRI volumes (developed by Dr. Dawant).[270-272] Few groups have examined functional connectivity of intrathalamic structures in TLE patients[231,273] and to our knowledge this is the first work to examine have examined functional connectivity of thalamic nuclei after epilepsy surgery.

IV.2: Thalamic Arousal Network Disturbances in Temporal Lobe Epilepsy and Improvement after Surgery

The work in this section appears in:
González, H.F.J., Chakravorti, S., Goodale, S.E., Gupta, K., Claassen, D.O., Dawant, B.M., Morgan, V.L., Englot, D.J. (2019). "Thalamic Arousal Network Disturbances in Temporal Lobe Epilepsy and Improvement after Surgery," Journal of Neurology, Neurosurgery, & Psychiatry, (90)10, 1109-1116.
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Abstract

Objective: The effects of temporal lobe epilepsy (TLE) on subcortical arousal structures remain incompletely understood. Here we evaluate thalamic arousal network functional connectivity in TLE and examine changes after epilepsy surgery.

Methods: We examined 26 adult TLE patients and 26 matched control participants and used resting-state functional magnetic resonance imaging (fMRI) to measure functional connectivity between the thalamus (entire thalamus and 19 bilateral thalamic nuclei) and both neocortex and brainstem ascending reticular activating system (ARAS) nuclei. Post-operative imaging was completed for 19 patients >1-year after surgery and compared to pre-operative baseline.

Results: Before surgery, TLE patients demonstrated abnormal thalamo-occipital functional connectivity, losing the normal negative fMRI correlation between the intralaminar central lateral (CL) nucleus and medial occipital lobe seen in controls ($p < 0.001$, paired t-test). Patients also had abnormal connectivity between ARAS and CL, lower ipsilateral intrathalamic connectivity, and smaller ipsilateral thalamic volume compared to controls ($p < 0.05$ for each, paired t-tests).

Abnormal brainstem-thalamic connectivity was associated with impaired visuospatial attention (ρ = -0.50, $p = 0.02$, Spearman's rho), while lower intrathalamic connectivity and volume were related to higher frequency of consciousness-sparing seizures ($p < 0.02$, Spearman's rho). After epilepsy surgery, patients with improved seizures showed partial recovery of thalamo-occipital and brainstem-thalamic connectivity, with values more closely resembling controls ($p < 0.01$ for each, ANOVA).

Conclusions: Overall, TLE patients demonstrate impaired connectivity in thalamic arousal networks that may be involved in visuospatial attention, but these disturbances may partially recover after successful epilepsy surgery. Thalamic arousal network dysfunction may contribute to morbidity in TLE.

IV.2.1 Introduction

Temporal lobe epilepsy (TLE) is the most common form of epilepsy, and 40% of patients have debilitating medication-resistant seizures originating in mesial temporal limbic structures.[11] In drug resistant TLE, surgery results in seizure-freedom in 60-70% of patients.[11,86] While TLE is a focal epilepsy, it engenders widespread deleterious functional and structural brain changes that cannot be explained by abnormalities in temporal or limbic structures.[29,31,38,89] We hypothesize that recurrent focal seizures in TLE may incite aberrant changes in subcortical arousal networks.[1] This may lead to perturbed connectivity between arousal structures, such as the thalamus, and neocortex, which may contribute to neuropsychological deficits common in TLE.[15,38,89] Recently, we have shown using magnetic resonance imaging (MRI) that TLE is associated with connectivity perturbations between brainstem ascending reticular activating system (ARAS) structures and neocortex, which in turn may be associated with neurocognitive problems.[38,254] We have also shown that TLE patients who achieve seizure-freedom after epilepsy surgery may demonstrate recovery of connectivity between ARAS and neocortex.[2] However, other subcortical arousal centers with ascending excitatory projections, such as the thalamic arousal network, have not yet been well studied in pre- or post-operative TLE patients.

Intralaminar thalamic nuclei project broadly to the cortex and are known to be central for maintenance of arousal and neurocognitive functions.[221,236] Of particular interest, is the central lateral (CL) thalamic nucleus which is the largest of the intralaminar thalamic nuclei and plays an important role in awareness and visuospatial attention.[199,237] The CL receives dense anatomic input

from two brainstem ARAS structures, median raphe (MR) and parabrachial complex (PBC),[5,61] and the CL projects heavily to medial occipital regions, including visual cortex.[199,237] Interestingly, thalamo-occipital connectivity is associated with posterior dominant rhythm which is a hallmark of healthy resting-state adult electroencephalography,[222-225] but has been shown to be slowed in epilepsy patients.[106,107] Furthermore, in rodent epilepsy models, aberrant activity in CL is associated with behavioral arrest and abnormal neocortical activity during limbic seizures.[65,238] While there are some indications of abnormalities in thalamic arousal system function in epilepsy, long-term effects of seizures on thalamic arousal networks and their potential clinical implications remain poorly understood in TLE. In this study, we will examine thalamic arousal network functional connectivity in TLE patients using MRI, relate network parameters to important clinical variables such as visuospatial attention, and then evaluate how thalamic arousal network connectivity may change after epilepsy surgery.

IV.2.2 Materials and Methods

IV.2.2.1 Participants

Participants included 26 adult TLE patients who received evaluation for epilepsy surgery at Vanderbilt University Medical Center from 2012 to 2016. Details of these patients have been described previously.[254] In these patients, diagnosis of mesial TLE was established according to standard clinical care at our institution by a multidisciplinary process including neurologists, neurosurgeons, neuropsychologists, and other practitioners. This process included a detailed patient history, analysis of seizure semiology, anatomical MRI, inpatient video electroencephalography (EEG), positron emission tomography (PET), eloquent function localization by functional MRI (fMRI) or Wada testing, and neuropsychological testing by a licensed neuropsychologist. Based on these tests, the multidisciplinary epilepsy committee felt confident in recommending proceeding to surgery with a diagnosis of TLE without intracranial EEG monitoring. Then 25 patients elected to undergo epilepsy surgery, and 19 of these patients underwent a second research MRI 35.7 ± 13.8 (mean ± SD) months after surgery. Additionally, 26 healthy controls were recruited and individually matched to patients by age (± 3 years), sex, and handedness (Table 1). One to one matching is performed at a date prior to any analyses, and therefore investigators are blinded to results of any analyses while matching controls. All

participants gave written informed consent to participate in this study and all procedures were approved by the Vanderbilt University Institutional Review Board.

	Patients (mean ± SD)	Controls (mean ± SD)	P value
Age, years	37.3 ± 12.5	38.4 ± 12.6	0.75
Gender, female	13 (50)	13 (50)	0.99
Handedness, right	23 (88.4)	23 (88.4)	0.99
Epilepsy duration, years	20.1 ± 14.5		
Seizure frequency, monthly			
FACS	2.8 ± 11.4		
FICS	6.7 ± 7.8		
FBTC	0.4 ± 1.0		
History of FBTC, yes	14 (53.8)		
Epileptogenic side, right	18 (69.2)		
MTS on MRI, yes	19 (73.0)		
Post-operative patients: Time between surgery & post-operative MRI, months	35.7 ± 13.8		
Surgery type			
SAH	12		
ATL	7		

Table IV.1 Participant demographics and disease factors.
For continuous variables, data shown are: mean ± standard deviation and the statistical test is the paired t-test. For categorical variables, data are N (%) and the statistical test is Chi-square. N = 26 pre-operative TLE patients and N = 26 controls, and N = 19 post-operative patients with repeat imaging. ATL: anterior temporal lobectomy; FACS: focal aware conscious seizures; FBTC: focal to bilateral tonic-clonic (secondarily-generalized) seizures; FICS: focal impaired consciousness seizures; MRI: magnetic resonance imaging; MTS: mesial temporal sclerosis; SAH: selective amygdalohippocampectomy.

IV.2.2.2 Imaging

MRI was acquired using Philips Achieva 3T MRI scanner (Philips Healthcare, Best, Netherlands) with 32-channel head coil. As in previous studies,[254] imaging performed consisted of (i) three-dimensional T1-weighted whole-brain images for inter-participant normalization and tissue segmentation (gradient echo, TR = 9.10 ms, TE = 4.60ms, 192 shots, flip-angle = 8.0°, matrix = 256x256, 1.0x1.0x1.0 mm^3), (ii) two-dimensional, T1-weighted axial images for functional to structural image coregistration (1.0x1.0x4.0 mm^3), (iii) two resting-state eyes closed 10-minute T2*-weighted blood oxygenation level dependent (BOLD) fMRI (FOV = 240.0 mm,

TE = 35.0 ms, TR = 2.0 s, 34 axial slices, slice thickness = 3.50 mm/0.50 mm gap, matrix = 80x80, 3.0x3.0x4.0 mm^3), with 300 volumes acquired during both 10-minute acquisitions.[38] Identical resting-state conditions for all participants included instructions prior to fMRI acquisition to lay at rest with eyes closed for the entire scan. Physiological signals, respiratory and cardiac rates, were acquired at 500 Hz.

IV.2.2.3 Regions of Interest

Regions of interest (ROIs) for connectivity measurements included 105 cortical and subcortical regions from Harvard-Oxford atlas (http://www.fmrib.ox.ac.uk/fsl), which includes a mask of the entire thalamus. This Harvard-Oxford atlas whole thalamus mask was used when calculating connectivity of the entire thalamus. For occipital regions, the medial occipital lobe was defined to include bilateral cuneal cortex, intracalcarine cortex, lingual gyrus, occipital fusiform gyrus, occipital pole, and supracalcarine cortex, while the lateral occipital lobe included bilateral inferior lateral occipital cortex and superior lateral occipital cortex. In addition, a hierarchical active shape model[274] guided by the Morel stereotactic atlas[73] was used to segment 23 bilateral (46 total) intrathalamic nuclei on T1 MRI and create a custom thalamic atlas for each participant.[275] Masks for four of these intrathalamic nuclei were smaller than two fMRI voxels (mean across participants), and were therefore excluded, leaving 19 bilateral (38 total) intrathalamic nuclei. Finally, we also examined an ROI incorporating the median raphe (MR) and parabrachial complex (PBC) ARAS nuclei from the Harvard Ascending Arousal Network Atlas (https://www.martinos.org/resources/aan-atlas),[5] given known projections between MR/PBC and intralaminar thalamus. Coregistration processes for these regions have been published.[254]

IV.2.2.4 Functional Connectivity Analysis

SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and MATLAB 2017a (The MathWorks, Natick, MA, USA) were used to preprocess fMRI data. Preprocessing steps included slice-timing correction, motion correction, correction for respiratory and cardiac noise using RETROspective Image CORrection,[256] segmentation into white and grey matters and cerebrospinal fluid, and spatial normalization to the Montreal Neurological Institute template. SPM was used to normalize and coregister fMRI through T1 MRI to the cortical/subcortical atlas.

FMRI images were band-pass filtered between 0.0067 and 0.1 Hz. For each of the two fMRI sessions in each participant, functional connectivity was computed between ROIs outlined above by partial Pearson correlation between each region's time series, with six motion time series (three measures of translation: x, y, and z and three measures of rotation: roll, pitch, and yaw) and mean white matter BOLD signal serving as confounds. Pearson correlations were transformed using Fisher z-transformation for each participant and were averaged across both fMRI sessions. For visualization of functional connectivity differences between participant groups we employed CONN toolbox 17 (https://www.nitrc.org/projects/conn/).[257] Patients' functional images were oriented according to epileptogenic side, and images of matched controls were reoriented accordingly.

IV.2.2.5 Volume Calculations

The T1-weighted images were parcellated into regions using a Multi-Atlas approach, for which technical details have previously been described.[276] All resulting segmentations were visually inspected for accuracy and no conspicuous flaws were found. From this analysis, volume of the thalamus was calculated.

IV.2.2.6 Neuropsychological Performance and Epilepsy Measures

Participant demographics and patient epilepsy measures - including seizure type and frequency, duration of epilepsy, history of focal to bilateral tonic-clonic (secondarily-generalized) seizures, epileptogenic side, antiepileptic medication doses, and MRI evidence of mesial temporal sclerosis - were determined using treating epileptologist's clinical assessments (**Table IV.1**). For patients with post-operative imaging, seizure outcomes were designated at time of post-operative MRI by epileptologist's clinical assessment using Engel classification.[9] Additionally, a licensed neuropsychologist administered a pre-operative standardized set of neuropsychological testing to patients. While there was some variability in tests administered to patients (see our previous full description[38]), the trail-making test part A was administered to all patients, and was of specific interest given the potential role of the thalamic arousal network in visuospatial attention. The neuropsychological report for one patient was not available, therefore this patient was excluded from neurocognitive analyses.

IV.2.2.7 Statistical Analyses

Parametric tests were utilized for normally distributed data, as defined using Anderson-Darling test. Demographics in pre-operative patients vs. controls were compared with paired t-tests for continuous variables and chi-square for categorical variables. Paired t-tests with post hoc Bonferroni-Holm correction for multiple comparisons were used to compare functional connectivity in pre-operative patients vs. controls, to compare connectivity between each patient and their individually matched control. ANOVA, with post hoc Fisher's least significant difference procedure (LSD), was used to compare functional connectivity between pre-operative patients, post-operative patients, and control participants. Spearman's rho was used to compare functional connectivity, neuropsychological testing, disease measures, and thalamic volume. Statistical analyses were performed with MATLAB 2017a and SPSS 23 (Armonk, NY, USA). Statistical significance was prospectively defined as $p < 0.05$ for all tests.

IV.2.3 Results

IV.2.3.1 Normal Thalamo-Occipital Functional Correlations Are Lost in TLE Patients

Prior to evaluating thalamic arousal networks, we first examined whether overall functional connectivity between the entire thalamus and frontal, occipital, parietal, or temporal lobes differed between pre-operative TLE patients and healthy control participants. While a negative correlation between thalamic and occipital fMRI signals is typically expected during the resting-state,[223,225,228,229] and was observed in controls, this negative connectivity was not present in patients either ipsilateral or contralateral to the epileptogenic side (**Fig. IV.1A**; $p < 0.01$ for each side, paired t-tests with Bonferroni-Holm correction). In a voxel-wise comparison of thalamic functional connectivity between patients and controls, it was noted that altered thalamic connectivity in patients was primarily observed in medial occipital lobe bilaterally, while no connectivity differences were seen in lateral occipital lobe or other lobes (**Fig. IV.1B**). These findings suggest overall aberrant connectivity between the thalamus and medial occipital cortex in TLE.

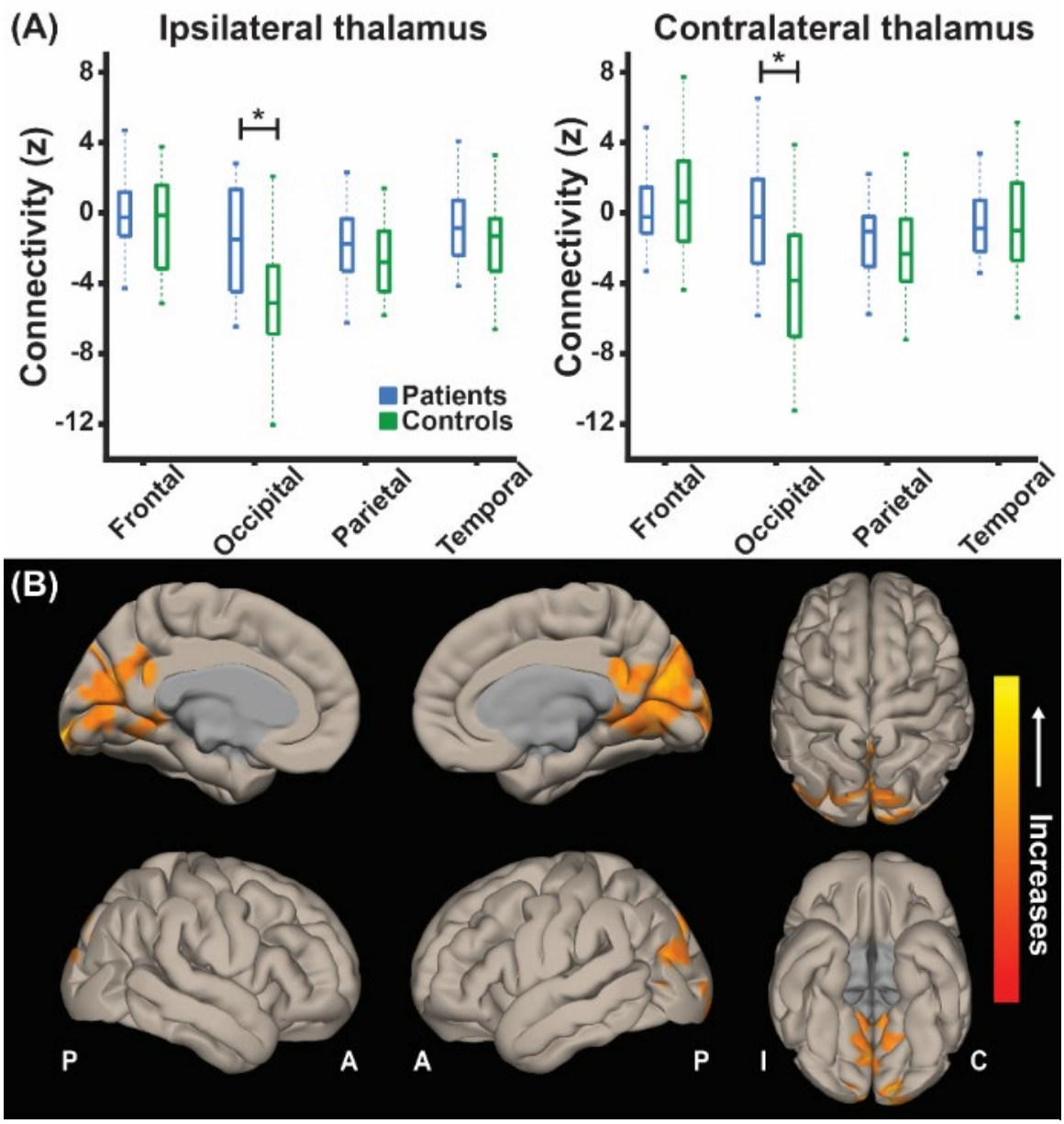

Figure IV.1 Patients lose negative thalamo-occipital functional connectivity seen in controls.
(**A**) Mean thalamo-occipital functional connectivity is more positive in TLE patients compared to control subjects, while no difference in thalamo-cortical connectivity is detected for frontal, parietal, or temporal lobes. (**B**) Cortical surface views are shown, demonstrating functional connectivity increases in the medial occipital lobe in patients with TLE seeded from bilateral thalami. Data represent seed-to-voxel functional connectivity maps (bivariate correlation) comparing pre-operative patients vs. matched control subjects fMRI (paired t-test, cluster threshold level $p < 0.05$, FDR correction). Positive contrasts are shown, no connectivity decreases were observed in grey matter on negative contrasts. FMR images are oriented with respect to epileptogenic side for TLE patients and matched controls were flipped accordingly. N = 26 TLE patients before surgery and 26 matched control subjects. *$p < 0.01$ paired t-test with Bonferroni-Holm correction. Center bar shows median value, bottom and top of box designate 25th and 75th percentiles respectively, and whiskers indicate data extremes. A: anterior; C: contralateral; I: ipsilateral; P: posterior; TLE: temporal lobe epilepsy.

IV.2.3.2 TLE Patients Exhibit Perturbed Connectivity in the Thalamic Arousal Network

We next investigated connectivity between individual thalamic nuclei and the medial occipital lobe, with a particular focus on the intralaminar thalamic nucleus CL (highlighted in **Fig. IV.2**), given its potential role in visuospatial attention[237] and possible involvement in limbic epilepsy.[238] For these analyses, we utilized a custom atlas of 19 bilateral (38 total) thalamic nuclei (**Fig. IV.2**). We found that while controls demonstrated a normal negative functional correlation between CL and medial occipital lobe, this was lost in TLE patients (**Fig. IV.3A**; $p < 0.001$, paired t-test, uncorrected). Overall, of the bilateral thalamic nuclei, CL demonstrated the largest difference in connectivity with medial occipital lobe between patients and controls. Given that the CL is an intralaminar nucleus[199] important for maintaining cortical activation and arousal,[5] with major afferent projections originating in the MR and PBC brainstem ARAS nuclei,[62] we next measured functional connectivity between MR/PBC and CL in all participants. Functional connectivity between MR/PBC and CL was found to be altered in TLE patients compared to controls, with more positive connectivity observed in patients (**Fig. IV.3B**; $p = 0.003$, paired t-test, uncorrected). Together, these findings suggest abnormal connectivity in thalamic arousal networks in TLE.

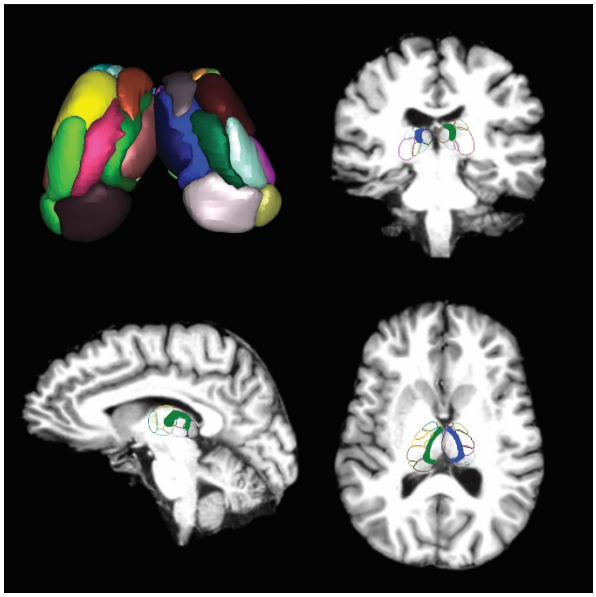

Figure IV.2 Thalamic atlas.
Shown here is the active shape model thalamic atlas with all 23 bilateral intrathalamic nuclei (46 total nuclei) volumetrically rendered in the top left. Four nuclei with masks smaller than 2 voxels (mean across participants) were excluded, and 19 bilateral (38 total) nuclei were used for analyses. MRI coronal, sagittal, and axial slices show the same atlas overlaid on a standard Montreal Neurological Institute (MNI) space brain with each nucleus outlined in color. In the coronal, sagittal, and axial slices the central lateral intralaminar thalamic nuclei are highlighted in solid blue and green. For analyses involving the whole thalamus the Harvard-Oxford atlas entire thalamus was used, whereas for connectivity analyses using CL the highlighted nuclei were used. CL: central lateral.

Furthermore, to ensure robustness of our findings we examined correlations of functional connectivity values calculated for the first and second resting-state epochs in pre-operative patients

and matched controls. We found that all thalamic connectivity measures (whole thalamus and CL) from each resting-state were correlated ($\rho = 0.54\text{-}0.78$, $p < 0.01$ for all, Spearman's rho). We also repeated all comparisons of pre-operative patients vs. matched controls connectivity measures independently for each resting-state epoch and found the same relationships as the averaged comparisons for thalamic (whole thalamus and CL) connectivity ($p < 0.01$ for all, paired t-tests).

IV.2.3.3 Ipsilateral Intrathalamic Connectivity and Thalamic Volume Are Reduced in TLE Patients

We next evaluated intrathalamic connectivity, which we defined as mean functional connectivity between all 19 individual thalamic nuclei in each side of the thalamus (**Fig. IV.2**). Intrathalamic connectivity was reduced in TLE patients compared to controls on the side ipsilateral to the epileptogenic zone ($p = 0.04$), but there was no discernible difference in intrathalamic connectivity on the contralateral side (**Fig. IV.3C**; $p = 0.10$, paired t-tests with Bonferroni-Holm correction). We also examined volume of the thalamus. We found that thalamic volume on the ipsilateral side was smaller in patients than in controls ($p = 0.02$), but there was no difference detected in contralateral thalamic volume (**Fig. IV.3D**; $p = 0.26$, paired t-tests with Bonferroni-Holm correction). As prior studies have described differences in thalamic atrophy in TLE patients with mesial temporal sclerosis (MTS) vs. those without MTS we compared thalamic volume between these groups.[234] We found no differences between ipsilateral thalamic volume in patients with MTS ($N = 19$, 6986 ± 838.7 mm^3, mean ± SD) vs. those without MTS ($N = 7$, 6922.9 ± 596.1 mm^3, $p = 0.85$, unpaired t-test). Also, we did not find differences in contralateral thalamic volume between patients with MTS (7310.2 ± 888.0 mm^3) vs. those without MTS (7098.7 ± 691.7 mm^3, $p = 0.57$, unpaired t-test). Interestingly, in both the ipsilateral (**Fig. IV.3E**) and contralateral (**Fig. IV.3F**) thalamus, higher intrathalamic connectivity was correlated with greater thalamic volume in patients ($\rho = 0.48$, $p = 0.02$ ipsilateral; $\rho = 0.46$, $p = 0.03$ contralateral; Spearman's rho with Bonferroni-Holm correction) but not in controls ($\rho = 0.13$, $p = 0.52$ ipsilateral; $\rho = 0.04$, $p = 0.81$ contralateral; Spearman's rho with Bonferroni-Holm correction). Neither ipsilateral nor contralateral thalamic volume were correlated with occipital lobe connectivity ($\rho = 0.13\text{-}0.17$, $p = 0.39\text{-}0.51$, Spearman's rho). In addition, to ensure intrathalamic connectivity is not simply a reflection of thalamic volume we examined correlation of intrathalamic connectivity with thalamic volume in an expanded group of 40 controls. In this larger group of healthy participants left thalamic volume was not correlated with left intrathalamic connectivity ($\rho = 0.20$, $p = 0.19$,

Spearman's rho) nor was right thalamic volume correlated with right intrathalamic connectivity ($\rho = 0.27$, $p = 0.09$, Spearman's rho)." These observations further suggest functional and structural thalamic abnormalities in TLE, particularly ipsilateral to the epileptogenic zone.

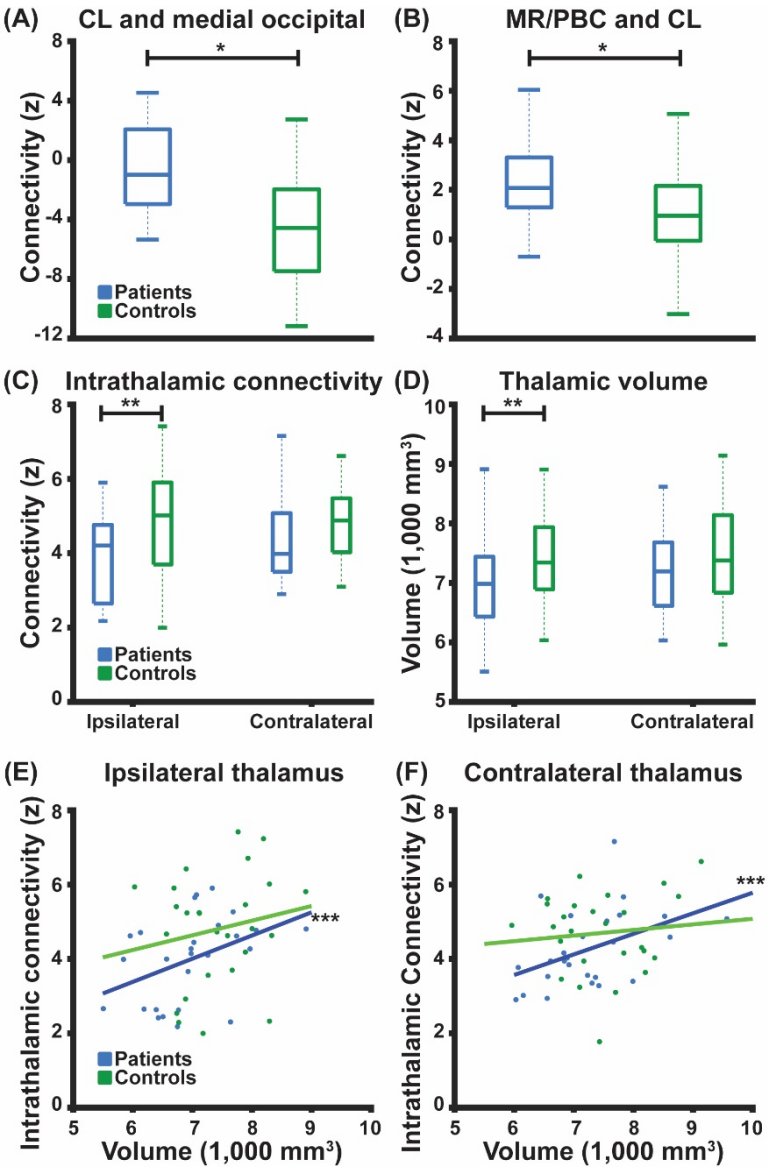

Figure IV.3 TLE patients exhibit perturbed thalamic connectivity and decreased ipsilateral thalamic volume.
(**A**) Patients exhibit loss of negative connectivity between CL intralaminar thalamic nucleus and medial occipital lobe when compared to control subjects. (**B**) Patients exhibit abnormally increased functional connectivity between CL and MR/PBC as compared to control subjects. (**C**) Compared to control subjects, patients exhibit decreased intrathalamic connectivity and (**D**) decreased thalamic volume, on the side ipsilateral to the epileptogenic temporal lobe but not the contralateral side. (**E, F**) For patients, but not control subjects, higher thalamic volume is correlated with higher intrathalamic connectivity. N = 26 TLE patients before surgery and 26 matched control subjects. *p < 0.01 paired t-test, **p < 0.05 paired t-test with Bonferroni-Holm correction, and ***p < 0.05 Spearman's Rho with Bonferroni-Holm correction. Center bar shows median, bottom and top of box designate 25th and 75th percentiles respectively, and whiskers indicate data extremes. CL: central lateral thalamic nucleus; MR: median raphe; PBC: parabrachial complex; TLE: temporal lobe epilepsy.

IV.2.3.4 Clinical Correlates of Network Alterations

Next, we examined potential associations between thalamic network disturbances in pre-operative TLE patients and clinical variables related to visuospatial attention and epilepsy severity.

Greater positive connectivity between MR/PBC and CL (i.e., more abnormal connectivity further from healthy control values) was associated with lower percentile score on trail-making part A test of visuospatial attention (ρ = -0.50, p = 0.02), although connectivity between CL and medial occipital lobe was not associated with trail-making score (ρ = -0.09, p = 0.67, Spearman's rho, Bonferroni-Holm correction). In examining disease severity, frequency of neither focal aware conscious seizures (FACS) nor focal impaired consciousness seizures (FICS) was related to connectivity between MR/PBC and CL nor between CL and medial occipital lobe (ρ = -0.29-0.08, p = 0.28-0.78, Spearman's rho, Bonferroni-Holm correction). However, a higher frequency of FACS was associated with lower intrathalamic connectivity in the ipsilateral (ρ = -0.55, $p < 0.01$) and contralateral thalamus (ρ = -0.58, $p < 0.01$, Spearman's rho, Bonferroni-Holm correction), and smaller volume of the ipsilateral (ρ = -0.50, p = 0.02) and contralateral thalamus (ρ = -0.50, p = 0.01, Spearman's rho, Bonferroni-Holm correction). No relationships were observed between frequency of FICS or duration of epilepsy and the aforementioned thalamic parameters ($p > 0.60$ for each, Spearman's rho). Additionally, there were no differences detected in any of the parameters in patients with vs. without a history of secondarily-generalized (focal to bilateral tonic-clonic) seizures ($p > 0.05$, unpaired t-tests). Overall, these findings demonstrate some negative associations between perturbed thalamic arousal network properties and disease severity.

IV.2.3.5 Thalamic Arousal Network Connectivity Perturbations May Improve After Epilepsy Surgery

Post-operative fMRI data were available in 19 patients 35.7 ± 13.8 (mean ± SD) months after epilepsy surgery. At time of the post-operative scan, seizure outcome was Engel I-A in seven patients, Engel I-B in two patients, Engel I-D in two patients, Engel II-C in two patients, Engel III-A in four patients, Engel III-B in one patient, and Engel IV-C in one patient. As we are most interested in the potential relationship between seizure improvement and connectivity changes after surgery, we excluded the one patient who did not improve after surgery (Engel IV) and included the remaining 18 patients for post-operative connectivity analyses. We first examined voxel-wise maps of functional connectivity changes, seeded from the entire bilateral thalami, in post-operative patients compared to their own pre-operative values. We noted connectivity decreases between the thalamus and medial occipital cortex after surgery (**Fig. IV.4A**), in areas showing connectivity increases in pre-operative patients compared to controls (**Fig. IV.1B**).

Connectivity increases between the thalamus and fronto-parietal association cortex were also observed (**Fig. IV.4A**). Examining functional connectivity between CL and medial occipital cortex, which was altered in pre-operative patients (**Fig. IV.3A**), we saw a decrease in post-operative connectivity towards normal connectivity values in controls (**Fig. IV.4B** left; $p < 0.001$, ANOVA with Fisher's LSD). Similarly, connectivity between MR/PBC and CL in post-operative patients more closely resembled connectivity in controls than the patients' pre-operative baseline (**Fig. IV.4B** right; $p < 0.01$, ANOVA with Fisher's LSD). While we saw overall differences between pre-operative and post-operative thalamic connectivity in all patients with seizure improvement (Engel I-III), no preoperative connectivity differences or post-operative connectivity changes were noted between patients with Engel I vs. Engel II-III seizure outcome ($p = 0.2$-0.9, unpaired t-test). Of note, changes in connectivity after surgery between CL and the medial occipital lobe, and between MR/PBC and CL, were similar in patients who were still on the same or similar anti-epileptic medication regimens ($N = 9$) compared to those who were off of medications or were on reduced medications ($N = 9$, $p = 0.46$-0.47, unpaired t-tests, uncorrected). Overall, these findings suggest partial "recovery" of thalamic arousal network connectivity after epilepsy surgery in patients with seizures that are eliminated or reduced.

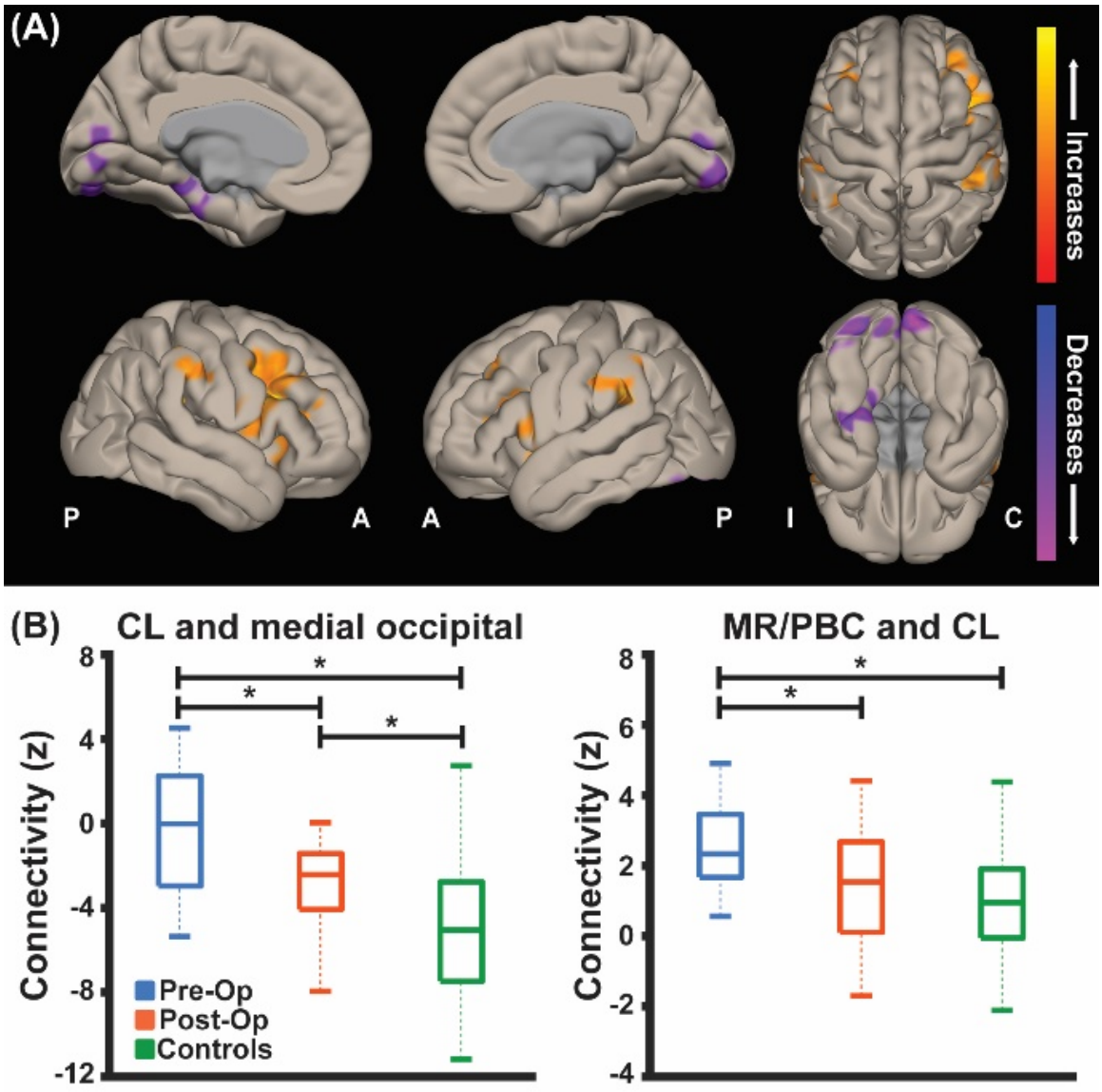

Figure IV.4 Post-operative TLE patients with improved seizures exhibit partial recovery of thalamic arousal network connectivity.

(**A**) Cortical surface views are shown, demonstrating functional connectivity decreases in the medial occipital lobe in post-operative TLE patients seeded from bilateral thalami. Data represent seed-to-voxel functional connectivity maps (bivariate correlation) comparing post-operative vs. pre-operative TLE patient's fMRI (paired t-test, cluster threshold level $p < 0.05$, FDR correction). Two sided contrasts are shown. FMR images are oriented with respect to epileptogenic side for TLE patients and matched controls were flipped accordingly. (**B**) Post-operative patients were found to have some recovery of functional connectivity between CL and medial occipital lobe but connectivity values did not quite reach control subject values (left). However, post-operative patients' functional connectivity between MR/PBC and CL are decreased compared to their pre-operative connectivity with no difference found between post-operative and control subject connectivities (right). N = 18 post-operative TLE patients with improved seizures after surgery, the same 18 patients before surgery, and 18 matched control subjects. *$p < 0.01$ ANOVA with post-hoc Fisher's least significant difference procedure. Center bar shows median, bottom and top of box designate 25th and 75th percentiles respectively, and whiskers indicate data extremes. A: anterior; C: contralateral; CL: central lateral thalamic nucleus; I: ipsilateral; MR: median raphe; P: posterior; PBC: parabrachial complex; Post-Op: post-operative patients; Pre-Op: pre-operative patients.

IV.2.4 Discussion

In this study, we observed disturbances in the thalamic arousal network in TLE patients that may be associated with disease severity, and which may partially improve after seizures are reduced or eliminated with epilepsy surgery. For instance, the intralaminar thalamus is known to have dense projections to occipital lobe, and we observed a loss of normal connectivity between CL and medial occipital neocortex in TLE. We also found that connectivity between MR/PBC and CL was abnormal in TLE patients compared to controls. CL is the largest rostral intralaminar thalamic nucleus,[199] and it receives the greatest input from MR and PBC in the brainstem ARAS.[5] While we previously demonstrated abnormal connectivity between ARAS nuclei and neocortex in TLE,[38,254] brainstem-thalamic connectivity has not previously been examined in this disorder, to the best of our knowledge. Together, these results suggest abnormal thalamic arousal network connectivity in TLE.

The thalamus is a crucial hub for cortical synchrony and modulation of brain rhythms,[221] such as the posterior dominant α-rhythm (8-13 Hz) in EEG.[222] The α-rhythm was first discovered by Hans Berger and is considered a hallmark of the EEG of healthy resting-state eyes-closed adults.[226] EEG and fMRI studies of resting-state healthy adults have shown negative correlations between the thalamus and occipital cortex during the resting-state α-rhythm,[224,228,229] congruent with what we found in our healthy control participants. Epilepsy patients may exhibit slowing of the α-rhythm,[106,107] and studies have suggested that dysfunction of the α-rhythm is associated with various neuropsychiatric and neurological disease states.[230] Furthermore, previous fMRI studies have demonstrated abnormal connectivity between the thalamus and the posterior neocortical quadrant in TLE patients.[231] EEG recordings were not performed in the present study, but correlating EEG patterns including the posterior dominant rhythm with thalamo-occipital connectivity may be interesting in future studies of TLE. Thalamic arousal networks connectivity changes in TLE may be related to the widespread functional changes typically seen in TLE patients.

What are potential clinical implications of abnormal thalamic arousal network connectivity in TLE? We have previously hypothesized that aberrant connectivity of arousal networks may be associated with the broad extratemporal deleterious effects seen in TLE.[31] For instance, TLE

patients demonstrate deficits that are not explained solely by temporal lobe and limbic network problems, such as impaired attention.[173] Previous studies have suggested that projections of the intralaminar thalamic nuclei may play a role in attention and visual awareness.[221,237] In this investigation, we show that connectivity between brainstem ARAS (MR/PBC) and thalamus (CL) is associated with worse performance on the trail-making test part A, which may reflect impaired visuospatial attention. In addition, the thalamus is also known to play a role in seizure propagation in TLE and prior studies have shown thalamic atrophy in TLE.[71,234,238,277,278] In this study, we observed greater reductions in both bilateral thalamic volume and intrathalamic connectivity were related to a higher frequency of focal aware conscious seizures (FACS), but not focal impaired consciousness seizures (FICS). This differs from our previous study where we found that aberrant connectivity between ARAS and frontoparietal cortex was associated with increased frequency of FICS but not FACS.[254] In general, this may suggest that these two seizure types have distinct influences on different arousal centers.

How does perturbed thalamic arousal connectivity respond to successful epilepsy surgery? Our present results suggest that post-operative thalamic network functional connectivity may partially recover after epilepsy surgery. We found that patients who experienced reduction or cessation of seizures demonstrated improvements in both thalamo-occipital connectivity and brainstem-thalamic connectivity, with connectivity values more closely resembling those in controls. These results are consistent with our recent work, in which we showed recovery of abnormal connectivity between ARAS and fronto-parieto-insular neocortex after successful epilepsy surgery.[2] Next, how might studies of subcortical connectivity in TLE lead to novel treatment options? Identification of subcortical networks involved in TLE pathophysiology may allow the identification of novel targets for neuromodulation treatments, such as deep brain stimulation. Some work has already been done to study the effects of stimulating the CL across disorders of consciousness. Case studies have shown that deep brain stimulation of CL in minimally conscious traumatic brain injury patients may result in increased arousal.[64] Also, in a rodent model of focal limbic seizures, others have shown that simultaneous stimulation of CL and pontine nucleus oralis during focal seizures prevented deleterious neocortical and behavioral effects seen without stimulation.[65] Ultimately, neurostimulation of subcortical arousal networks may play a role in reducing morbidity in epilepsy when seizure-freedom cannot be achieved.

This study has limitations worth discussion. While pre-operative connectivity patterns were associated with visuospatial attention problems, potential associations between post-operative connectivity improvement and changes in attention scores could not be evaluated, as our patients did not undergo long-term post-operative neuropsychological testing. As is typical in TLE patients, our cohort is heterogeneous in various ways including: (i) patients underwent two different surgeries: selective amygdalohippocampectomy and standard anterior temporal lobectomy, (ii) not all of our patients displayed findings of mesial temporal sclerosis on either MRI or operative specimen pathology, and (iii) not all of our patients had a history of focal to bilateral tonic-clonic seizures. Unfortunately, our present patient cohort is too small to perform subgroup analyses testing for each of these variables. Future work should include a larger number of patients allowing multivariate subgroup analyses to be performed.

Next, in this work we obtain one post-operative scan (ranging 14 to 60 months after surgery) showing improved thalamic arousal network connectivity in TLE patients with improved seizures. In future work it would interesting to perform serial post-operative fMRI and observe any possible evolution of connectivity with time after surgery. Further, we did not collect information regarding length of time since most recent seizure prior to fMRI, and in future studies the possible relationships between timing of the last seizure and connectivity should be investigated. Additionally, although all participants' resting-state fMRI are acquired under identical conditions, in which they are told to remain awake, we do not directly measure level of arousal during the scans. This limitation could be addressed in future studies by incorporating quantitative measures of arousal such as simultaneous EEG-fMRI or eyes open resting-state with tracking of eye movements. Finally, thalamic nuclei are impossible to visually identify on 3T anatomical imaging, which may affect the accuracy of our analyses. Nevertheless, we were able to obtain patient-specific maps of thalamic nuclei by using an active shape model that fit to each patient. Furthermore, in order to mitigate the susceptibility of small structures to motion and noise, our functional connectivity analyses were corrected for movement and physiological measures as a part of our preprocessing.

IV.2.5 Conclusion

In conclusion, while others have studied the role of the thalamus in TLE, this work employs an innovative approach to this topic that merits highlighting, including i) novel examination of the thalamic arousal network in epilepsy, ii) investigation of connectivity changes after epilepsy surgery, and iii) utilization of a custom subject-specific thalamic atlas. We observed significant perturbations of brainstem-thalamic and thalamo-occipital connectivity in TLE patients, and certain connectivity patterns may be related to seizure frequency and visuospatial attention problems. Thalamic arousal network connectivity disturbances partially recovered in patients who achieved seizure-freedom or reduced seizure frequency with epilepsy surgery. Further study of thalamic arousal network disturbances in TLE may improve our understanding of broad neural and cognitive effects of chronic seizures, and aid in the identification of neuromodulation targets to treat this devastating disorder.

Funding and Acknowledgements

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CHAPTER V

V Investigating the Role of the Nucleus Basalis of Meynert Using Advanced Network Analyses

V.1 Summary and Contributions

This chapter details how we employed network analyses of nucleus basalis of Meynert (NBM) functional connectivity networks in temporal lobe epilepsy (TLE) patients to determine the role of the NBM with respect to broad TLE network pathophysiology. In order to understand extra-temporal deficits in found in TLE, we believe that we must use a network-based approach rather than treating TLE as a focal disorder. In this work we acquired resting-state functional magnetic resonance imaging (fMRI) in TLE patients and healthy matched controls. In this investigation, we calculated functional connectivity networks and employed a comprehensive network analysis of NBM connectivity in resting-state fMRI for both the TLE patients and their matched healthy controls. We also related certain connectivity measures to disease-related variables to determine if the NBM may be an underappreciated, yet key network node of altered functional connectivity in TLE.

The results of this study indicated that the NBM may be one of the most altered network nodes in patients with TLE. We found large decreases in functional connectivity between the NBM and the rest of the brain in TLE patients; some of these connectivity values were more perturbed in individuals with higher frequency of consciousness impairing seizures. Interestingly, we found strong evidence that the NBM ipsilateral to the epileptogenic zone (seizure generating brain region) is part of a central network of nodes with altered connectivity, and the NBM also has altered community structure relative to healthy controls. Perhaps most significantly, our findings suggested that the NBM may have more abnormal connectivity network properties than any other brain structures in TLE, including limbic regions, specifically mesial temporal regions, traditionally central to the pathophysiology of TLE.

This work highlights the importance of applying rigorous network analyses to TLE, which enables the investigation of this seemingly focal disorder as a network-based disease. Network analyses, specifically, in epilepsy, may be used to elucidate seizure origination and propagation

pathways,[279,280] as well as probing disease effects on distal brain networks not involved in seizure generation. In this paper we explicitly considered several hypotheses that could have driven the observed complementary and correlated changes in our analyses. We accomplished this by implementing a novel studying leveraging null models generated from the patient and control data. Utilizing such an integrative network modeling framework allowed us to select the least complicated plausible model that explained the observed effects in a principled way. Our network modeling approach to investigating the role of the NBM in TLE prevented overly comprehensive analyses, prevalent in basic and translational neuroscience,[281] preventing the danger of circular analyses in this work. Overall, this study demonstrated the key role of NBM in TLE network pathophysiology.

V.2: Role of the Nucleus Basalis as a Key Network Node in Temporal Lobe Epilepsy

The work in this section appears in:
González, H.F.J., Narasimhan, S., Johnson, G.W., Wills, K.E., Haas, K.F., Konrad, P.E., Chang, C.**,** Morgan, V.L., Rubinov, M**.,** Englot, D.J. (2021) "Role of the nucleus basalis as a key network node in temporal lobe epilepsy," Neurology, (96)9, 31334-e1346.
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Abstract

Objective: To determine if nucleus basalis of Meynert (NBM) may be a key network structure of altered functional connectivity in temporal lobe epilepsy (TLE), we examined fMRI with network based analyses.

Methods: We acquired resting-state fMRI in 40 adults with TLE and 40 matched healthy control participants. We calculated functional connectivity of NBM and used multiple complementary network based analyses to explore the importance of NBM in TLE networks without biasing our results by our approach. We compared patients to controls and examined associations of network properties with disease metrics and neurocognitive testing.

Results: We observed marked decreases in connectivity between NBM and the rest of the brain in patients with TLE (0.91 ± 0.88, mean ± SD) versus controls (1.96 ± 1.13, $p < 0.001$ t-test). Larger decreases in connectivity between NBM and fronto-parietal-insular regions were associated with higher frequency of consciousness-impairing seizures ($r = -0.41$, $p = 0.008$, Pearson). A core network of altered nodes in TLE included NBM ipsilateral to the epileptogenic side and bilateral limbic structures. Further, normal community affiliation of ipsilateral NBM was lost in patients, and this structure displayed the most altered clustering coefficient of any node examined (3.46 ± 1.17 controls versus 2.23 ± 0.93 patients). Abnormal connectivity between NBM and subcortical arousal community was associated with modest neurocognitive deficits. Finally, a logistic regression model incorporating connectivity properties of ipsilateral NBM successfully distinguished patients versus control datasets with moderately high accuracy (78%).

Conclusions: These results suggest that while NBM is rarely studied in epilepsy, it may be one of the most perturbed network nodes in TLE, contributing to widespread neural effects in this disabling disorder.

V.2.1 Introduction

Temporal lobe epilepsy (TLE) is the most common epilepsy syndrome.[11] While TLE is a focal epilepsy, where seizures originate in hippocampus or amygdala of the limbic system, it leads to widespread neural effects. Patients experience ictal loss of consciousness, even when seizures do not propagate beyond these limbic structures.[282] Between seizures, patients demonstrate neurocognitive deficits in domains not typically related to mesial temporal function.[172,173] These observations may suggest a common subcortical source of global network dysfunction in TLE.[49]

It has been demonstrated that disruption of subcortical arousal circuits may contribute to ictal loss of consciousness in TLE.[1] We have hypothesized recurrent seizures might incur long-term subcortical arousal network disruptions.[2,49] In patients with TLE, we uncovered abnormal interictal fMRI connectivity of brainstem ascending reticular activating system (ARAS) and intralaminar thalamus.[254,283] Currently, most TLE studies focus on limbic network and while few studies examine arousal networks in TLE, they may be central in its interictal pathophysiological sequelae.[49] Other important subcortical arousal networks have not yet been explored in human TLE.

Nucleus basalis of Meynert (NBM) plays prominent roles in arousal, influences neurocognition, has dense anatomic projections to limbic structures, and may help regulate functional networks.[74-78] In rodent TLE models, focal seizures lead to diminished activity in NBM, resulting in neocortical deactivation.[79] Might recurrent seizures in TLE produce long-term NBM connectivity disruptions, leading to broader network dysfunction? Here we employ complementary network analyses to determine if NBM may be an underappreciated, yet key network node of altered connectivity in TLE.

V.2.2 Methods

V.2.2.1 Participants

Participants included 40 consecutive adult patients with unilateral mesial TLE who underwent epilepsy surgery evaluation and agreed to participate in our study and undergo an additional research MRI at Vanderbilt University Medical Center. A multidisciplinary team established diagnosis of mesial TLE by assessing patient history, structural MRI, seizure semiology, ictal/interictal video-EEG, neuropsychological examination, and mesial temporal hypometabolism on PET. We also included 40 healthy control participants from a larger population of previously

recruited controls. Prior to analyses, we individually matched controls to patients by age (typically ± 3 years, maximum ± 5 years) and sex (**Table V.1**).

	Patients	Controls	P value
Age, Years	38.5 [18, 68]	38.5 [18, 69]	0.99
Gender, female	21.0 (52.5%)	21.0 (52.5%)	0.99
Epilepsy duration, years	21.1 [2, 50]		
Seizure frequency, monthly			
FACS	9.1 [0, 195]		
FICS	6.4 [0, 48]		
FBTC	0.6 [0, 8]		
History of FBTC, yes	22.0 (55.0%)		
Epileptogenic side, right	30.0 (75.0%)		
MTS on MRI, yes	27.0 (67.5%)		
Video-EEG results			
Well localized (ictal), yes	35 (87.5%)		
Lateralized (interictal), yes	26 (65.0%)		
Invasive recording (SEEG)	6 (15.0%)		
Mesial temporal hypometabolism on PET	31 (77.5%)		

Table V.1 Participant demographics.
For continuous variables, data shown are mean [minimum, maximum] and statistical testing is performed using paired t-test. For categorical variables, data shown are N (%) and statistical testing is performed using McNemar's test. Seizure type and frequency were determined from clinical visit most proximal to fMRI and documented in the final epilepsy conference note. N = 40 patients with TLE and N = 40 controls. FACS: focal aware conscious seizures; FBTC: focal to bilateral tonic-clonic seizures; FICS: focal impaired-consciousness seizures; MRI: magnetic resonance imaging; MTS: mesial temporal sclerosis; SD: standard deviation; SEEG: stereotactic-electroencephalography.

V.2.2.2 Standard Protocol Approvals, Registrations, and Patient Consents

All procedures were approved by Vanderbilt University Institutional Review Board. All participants gave written informed consent for study.

V.2.2.3 Imaging

As in prior studies,[254] we performed imaging with Philips Achieva 3T MRI (Philips Healthcare, Best, Netherlands) and 32-channel head coil. MRI sequences included: (1) 3D T1-weighted whole brain images for tissue segmentation and inter-participant normalization (1.0x1.0x1.0 mm^3), (2) 2D T1-weighted axial images for functional to structural image coregistration (1.0x1.0x4.0 mm^3), (3) two resting-state eyes-closed 10-minute T2*-weighted blood oxygenation level dependent (BOLD) fMRI (TE = 35.0 ms, TR = 2.0 s, 34 axial slices, slice thickness = 3.5 mm with 0.5 mm gap, 3.0x3.0x4.0 mm^3). All resting-state fMRI sessions included instructions to lay at rest with eyes-closed for the scan. We performed physiological monitoring of respiratory and cardiac rates at 500 Hz.

V.2.2.4 Regions of Interest

We defined 133 regions of interest (ROIs) for functional connectivity analyses. These included 108 ROIs from Harvard-Oxford atlas,[284] which we call "whole brain." From a subset of Harvard-Oxford regions, we defined a collection of frontal, parietal, and insular regions ("frontoparietal") which previously showed decreased connectivity with arousal structures in TLE.[38,254] These regions included bilateral inferior frontal gyrus pars opercularis and pars triangularis, precentral gyrus, postcentral gyrus, superior parietal lobule, and insula. We included 8 participant-specific intralaminar thalamic nuclei whose implementation we previously described.[275,283,285] We also incorporated 13 brainstem ARAS nuclei from Harvard Ascending Arousal Network Atlas.[5] Finally, we analyzed 2 bilateral ROIs consisting of cholinergic basal forebrain nuclei obtained by previous groups from postmortem stereotaxic probabilistic mapping of magnocellular cells.[74,242] The first of these ROIs included diagonal band of Broca and septal nuclei. The second included NBM. We acquired Montreal Neurological Institute (MNI) space masks for these ROIs from SPM Anatomy toolbox with a probabilistic threshold > 50%.[286] Lastly, all non-midline ROIs were designated as either ipsilateral or contralateral to epileptogenic side of the brain per patient, and ROIs of matched controls were defined accordingly.

V.2.2.5 Functional Connectivity Analysis

We employed CONN toolbox 17 to visualize seed-to-voxel NBM bivariate correlation functional connectivity differences between patients with TLE and controls.[257] We aligned patients' imaging laterality based on epileptogenic side, and realigned images of matched controls accordingly.

For detailed analysis of connectivity differences, we calculated functional connectivity matrices for each participant. We preprocessed fMRI with MATLAB 2017a (MathWorks, Natick, MA, USA) and SPM12.[287] Preprocessing included slice-timing correction, motion correction, retrospective correction of physiological motion effects (RETROICOR),[256] tissue segmentation (white matter, grey matter, and CSF), and spatial normalization to MNI template. We temporally band-pass filtered fMRI between 0.0067 Hz and 0.1 Hz. Each ROI's average time-series was calculated with participant-specific CSF and white matter segmentations to exclude these signals. For each individual fMRI acquisition per participant, we calculated functional connectivity between ROIs by partial Pearson correlation between each region's mean BOLD time-series, with six motion time-series (three degrees of translation: x, y, and z dimensions, and three degrees of rotation: roll, pitch, and yaw) and mean white matter BOLD serving as confounds. We transformed these correlations using Fisher z-transformation per participant. We averaged connectivity across both fMRI resting-state sessions. This yielded a symmetric 133x133 functional connectivity matrix with self-connections along diagonal set to zero. We calculated clustering coefficient of all ROIs with Brain Connectivity Toolbox.[288] Clustering coefficient assesses connectivity of a node with its neighbors; high clustering coefficient indicating a node's neighbors are more connected with each other. This connectivity matrix was also used for comparison of seed-based NBM connectivity, community detection, and network-based statistic described below.

V.2.2.6 Community Analyses and Network-Based Statistic

To complement seed-based NBM connectivity comparisons, we analyzed how community structure of NBM may change in TLE with community detection. Community detection algorithms identify groups of brain nodes that are most strongly functionally connected to each other. First, we calculated community structure in healthy controls by optimization of modularity.[289] To detect stable community modules, we performed community detection with a parameter sweep of

resolution parameter γ_M=1.30, 1.31, 1.32, […], 2.50 using Louvain algorithm with iterative adjustments.[290-292] We selected community partitions in controls at γ_M exhibiting maximum normalized mutual information across partition pairs.

To compare community structure between patients and controls, we used control community structure to calculate a community-based property in all participants: node strength to module. Node strength to module is defined for each node as the sum of connectivity to all nodes within a module divided by size of that module. For a particular node, node strength to module should be maximum to its home community as defined from healthy control community structure. Maximal node strength to module outside of control home community represents abnormal community structure. We focused our analyses on node strength to module of NBM with its home community.

We also employed the network-based statistic to identify core networks of connected nodes with connectivity decreases in patients compared to controls.[293] The network-based statistic is a non-parametric method that controls family-wise error rate when performing mass univariate testing on network connections. We used a range of primary *t*-test thresholds (t-statistic > 4.2, 4.4, 4.6) for each link to define a set of suprathreshold component networks and their size (number of links). Next, we calculated a family-wise error correction for the component network by randomly permuting patient and control labels 10,000 times and repeating calculations per permutation. This generated a null distribution of component sizes and allowed identification of thresholded network components showing significant connectivity decreases in patients versus controls with family-wise error corrected significance $p < 0.01$. We employed BrainNet viewer for decreased connectivity network visualization.[294]

V.2.2.7 Integrative Explanatory and Predictive Network Modeling

We next sought to integrate our findings by postulating several biologically motivated hypotheses for what may be driving our observed effects, operationalizing these hypotheses in four explanatory network models, and comparing these models. We wanted to determine if NBM properties were driving observed differences, or if these differences resulted from other network properties. Each of the four network models isolated individual network differences between patient and control community structures. We used unbiased network-sampling methods[295] to compare models to find the simplest model that explains central connectivity network difference between patients and controls.

The four network models were:

Model 1 - Posits that main network differences observed can be explained by non-specific, global changes in connectivity between control and patient populations. We operationalized this model by only constraining overall connectivity density of all nodes.

Model 2 - Posits a primary role for non-specific disruptions within limbic system only. We operationalized this model by constraining average connectivity between nodes in limbic system module only.

Model 3 - Assumes an important role for connectivity of NBM ipsilateral to epileptogenic side of the brain, as well as the entire limbic system. We operationalized this model by constraining connectivity density within limbic system module and constraining ipsilateral NBM strength.

Model 4 - Full model that preserves the average connectivity of all individual nodes, and all individual modules. We considered this our full explanatory model of observed effects.

We compared individual models by generating 1,000 random networks for each individual participant that preserved specific constraints of each model but were otherwise maximally random.[295,296] These networks were generated using simulated annealing, a popular optimization algorithm that near-uniformly samples networks with specified constraints.[295]

Explanatory power of each model was assessed by considering extent to which the models explained all observed connectivity changes. Thus, for all networks generated in the four models, we calculated the main network metrics analyzed: NBM connectivity with the whole brain, NBM clustering coefficient, and node strength to module of ipsilateral NBM to limbic community. Either non-parametric Wilcoxon rank-sum test (for non-normally distributed data) or *t*-test (for normally distributed data) was used to evaluate differences between original participant data and data calculated from models. First, rank-sum statistic or t-statistic was calculated, comparing original patient and original control metric. The test statistic was also calculated for the same metric per network model, comparing randomly perturbed patient networks (4,000 total networks) to the randomly perturbed control networks (4,000 total networks). A network model was considered to capture observed differences in patients with TLE if the network model differences were not distinguishable from differences between patients and controls seen in the empirical data.

V.2.2.8 Neurocognitive Testing and Epilepsy Measures

We also asked how NBM network properties may be related to clinical measures of disease severity and neurocognition. We determined patient demographics and epilepsy measures using treating epileptologists' assessments (**Table V.1**). Disease measures included: MRI evidence of mesial temporal sclerosis, epilepsy duration, seizure type and frequency, and history of focal to bilateral tonic-clonic (secondarily-generalized) seizures. A licensed neuropsychologist administered comprehensive neuropsychological testing to patients which we summarized into six neurocognitive categories as in prior work.[38] We evaluated attention and concentration using Trail Making Test Part A, Digit Span Forward, Digit Span Backward, and WAIS IV Digit Span Sequencing. Visual memory testing included Brief Visuospatial Memory Test-Revised, Continuous Visual Memory Test, and Rey-Osterrieth Complex Figure Test. We tested cognitive processing with Working Memory Index. We tested language using Boston Naming Test, Neuropsychological Assessment Battery Naming Test, or Animal Naming. We evaluated verbal memory using California Verbal Learning Test, part II, and Wechsler Memory Scale (third and fourth edition). We evaluated executive function with Wisconsin Card Sorting Test, F-A-S Words, and Trail Making Test Part B. Overall performance per patient per neurocognitive domain was obtained by converting score on each test to a z-score and then averaging performance on all tests in each domain.

V.2.2.9 Logistic Regression

To further elucidate reliability of NBM connectivity alterations in TLE, we asked whether connectivity properties of ipsilateral NBM alone could accurately identify whether each participant's dataset belonged to a patient or control. For comparison, we evaluated connectivity of ipsilateral hippocampus in identifying patients versus controls, as it is known that connectivity of this limbic structure is markedly altered in TLE.[43,51,53] Three key network properties were selected to generate two combinatory, binary logistic regression models. These measures, calculated for NBM and hippocampus, included (i) functional connectivity to the whole brain, (ii) clustering coefficient, and (iii) node strength to module. We trained logistic regression models on all patient and control data. To ascertain model performance variability, we used bootstrapping with five-fold cross validation to subsample total participant population. We also calculated receiver operating characteristic (ROC) curves and their associated measurements.

V.2.2.10 General Statistical Approaches

We utilized parametric tests for normally distributed data, as defined using Anderson-Darling test,[261] or non-parametric tests otherwise. We compared demographics in patients with TLE versus controls with paired *t*-tests for continuous variables and McNemar's test for categorical variables. Paired t-tests were utilized to compare functional connectivity between patients and controls. We used Mann-Whitney U-test to compare network properties (clustering coefficient and node strength to module) between participant groups. We employed Pearson correlation to relate functional connectivity to continuous variable disease measures. We used Spearman's rho to relate network properties to neuropsychological testing, as these properties were not normally distributed. Statistical analyses were performed with MATLAB 2017a and SPSS 23 (Armonk, NY, USA). We prospectively defined statistical significance at $p < 0.05$ for all tests and used post hoc Bonferroni-Holm to correct for multiple comparisons where indicated.

V.2.2.11 Data Availability

Due to restrictions from participant informed consent, data will not be made freely available in a public repository. Anonymized data can be made available upon request if approved by the Vanderbilt University Institutional Review Board.

V.2.3 Results

V.2.3.1 NBM Functional Connectivity is Decreased in Patients with TLE

We first asked whether overall functional connectivity of NBM differs between 40 patients with unilateral mesial TLE versus 40 healthy matched controls (demographics in **Table V.1**). In a voxel-wise comparison of bilateral NBM connectivity, we observed that patients displayed connectivity decreases between NBM and broad neocortical regions versus controls (**Fig. V.1A**).

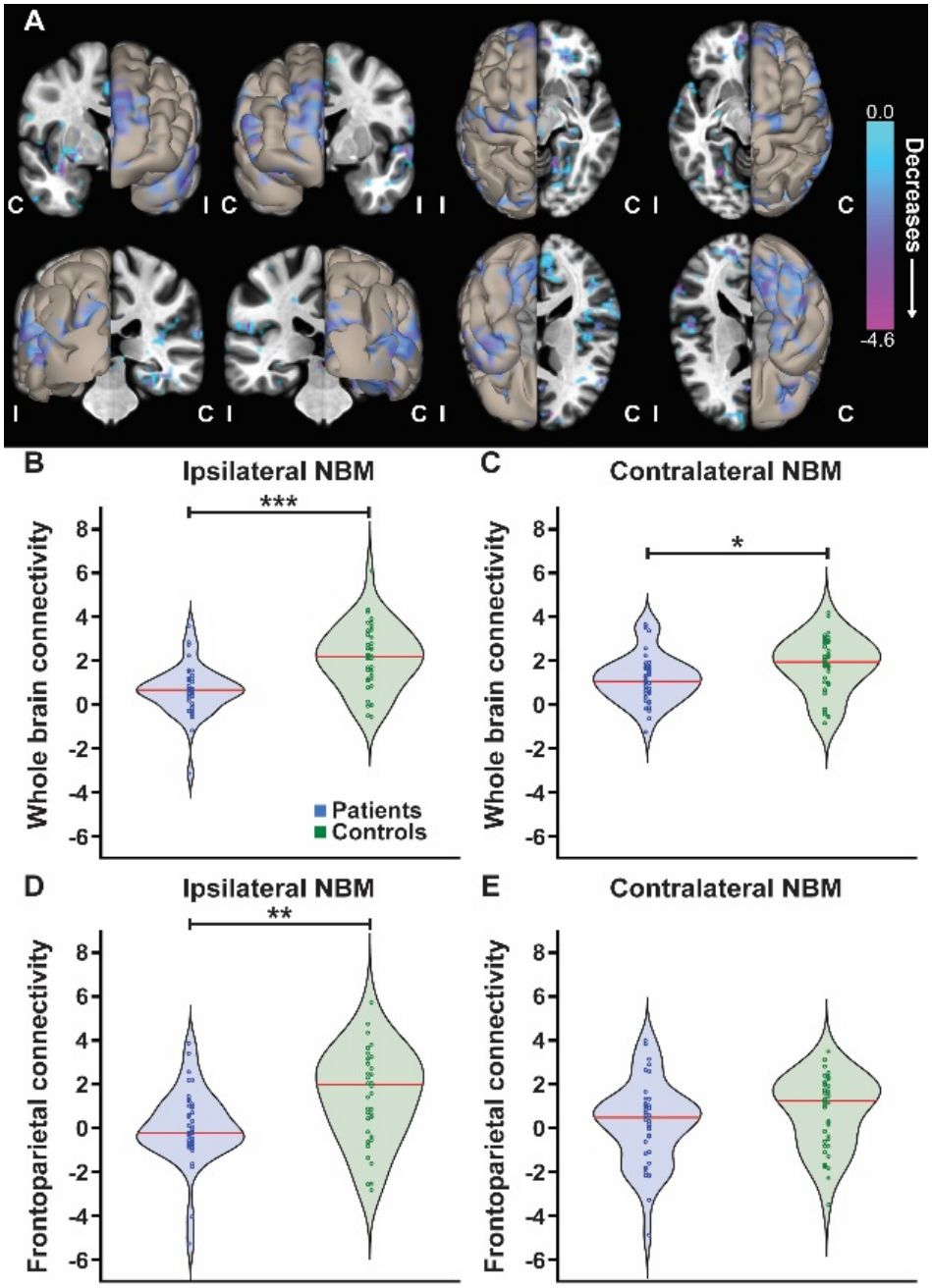

Figure V.1 Functional connectivity of the NBM is decreased in patients with TLE vs. controls.
(**A**) Data represent seed-to-voxel fMRI functional connectivity maps (bivariate correlation) seeded from bilateral NBM, comparing patients with TLE versus control participants (paired t-test, cluster threshold level $p < 0.05$, FDR correction). These seed-to-voxel group level comparisons are projected onto an average brain template. Negative contrasts are shown, and no connectivity increases were seen in patients. FMRIs are oriented with respect to the side of seizure onset for patients with TLE, and matched controls images are flipped accordingly. In evaluating functional connections between NBM and all other regions in the brain (excluding cerebellum), NBM connectivity reductions in patients with TLE versus controls are observed both ipsilateral (**B**) and contralateral (**C**) to the side of seizure onset. Restricting the analysis to selected frontoparietal regions, large reductions in NBM connectivity are noted in patients versus controls on the ipsilateral (**D**) but not contralateral (**E**) side. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, paired t-tests with Bonferroni-Holm correction. Red line shows median. N = 40 patients with TLE and 40 matched healthy control participants. C: contralateral; FDR: false discovery rate; I: ipsilateral; NBM: nucleus basalis of Meynert.

Overall, connectivity between NBM and the whole brain was reduced in patients on both ipsilateral (0.65 ± 1.11 patients, 2.13 ± 1.43 controls; mean ± SD) and contralateral side (1.18 ± 1.10 patients, 1.79 ± 1.25 controls), with respect to side of seizure onset (**Fig. V.1B, C**; $p = 9x10^{-6}$ and p = 0.03 respectively, paired t-tests, Bonferroni-Holm correction). We noted large decreases in connectivity between ipsilateral NBM and frontoparietal cortex in patients (0.04 ± 1.66 patients, 1.47 ± 2.05 controls; **Fig. V.1D**, p = 0.004), but observed no differences in contralateral NBM (0.20 ± 1.88 patients, 0.73 ± 1.63 controls; **Fig. V.1E**, p = 0.18, paired t-tests, Bonferroni-Holm correction). We observed greater connectivity decreases between NBM and frontoparietal cortex in patients with higher frequency of consciousness-impairing focal seizures (r = -0.41, p = 0.008), but saw no relationship between NBM-frontoparietal connectivity and frequency of consciousness-sparing focal seizures (r = -0.13, p = 0.418, Pearson correlation, uncorrected). Overall, these results demonstrate impaired NBM connectivity in TLE that are larger on the epileptogenic side of the brain, and which may be related to consciousness-impairing seizure frequency.

V.2.3.2 Patients with TLE Exhibit Altered NBM and Limbic Network Community Structure

We also interrogated network connectivity by comparing community structure in controls with community structure in patients. First, in healthy controls we identified nine anatomically symmetric communities (**Fig. V.2A**, regions per community in **Table V.2**). In controls, all brainstem ARAS and intralaminar thalamic nuclei clustered into one community we termed "subcortical arousal," while ipsilateral and contralateral NBM clustered into a community with limbic structures, including hippocampus and amygdala (**Fig. V.2A**). This may suggest closer functional connections between NBM and limbic structures than with other subcortical arousal nuclei in controls.

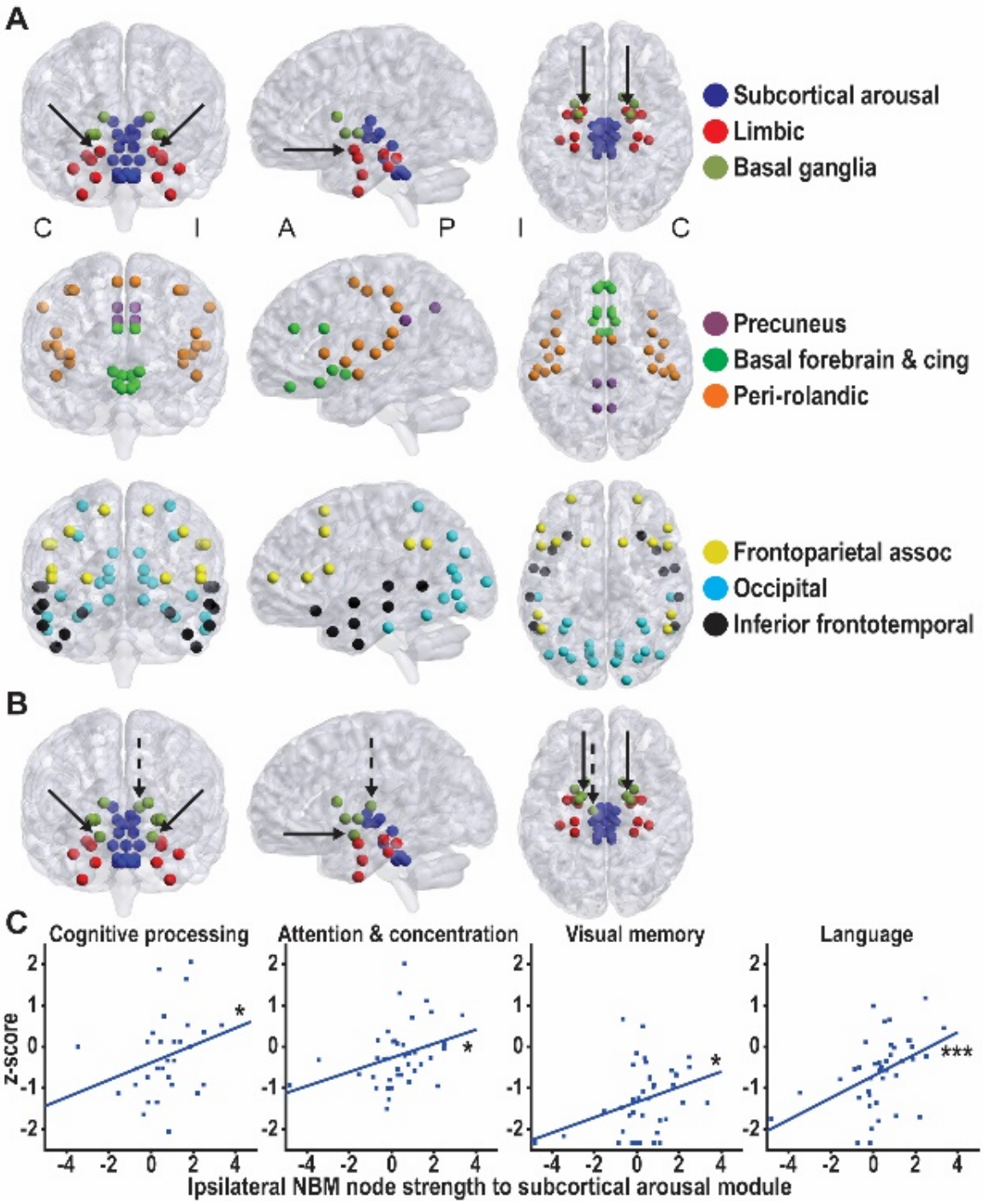
A
C
I
A
P
I
C
Subcortical arousal
Limbic
Basal ganglia
Precuneus
Basal forebrain & cing
Peri-rolandic
Frontoparietal assoc
Occipital
Inferior frontotemporal
B
C
Cognitive processing
Attention & concentration
Visual memory
Language
z-score
Ipsilateral NBM node strength to subcortical arousal module

Figure V.2 Community structure of NBM is altered in TLE.
(**A**) Communities were first defined in healthy control participants, and nine communities were detected. We named these communities based on anatomic and functional similarities between regions. Both ipsilateral and contralateral NBM (solid arrows) clustered together with limbic structures (red) and did not cluster within a community including other subcortical arousal nuclei, including ARAS and intralaminar thalamic nuclei (blue). A list of all communities and nodes is provided in **Table V.2**. N = 40 healthy control participants. (**B**) When this community structure was applied to patients with TLE, and node strength to module was calculated, ipsilateral and contralateral NBM (solid arrows) in patients did not cluster (i.e., did not demonstrate maximum node strength to module) with the limbic community as in controls, but instead these nodes clustered with the basal ganglia community. Other nodes that clustered with different communities in patients compared to controls included ipsilateral central lateral thalamic nucleus (dashed arrow), as well as ipsilateral frontal operculum cortex, ipsilateral orbitofrontal cortex, and contralateral orbitofrontal cortex (not shown). N = 40 patients with TLE. Assoc: association cortex; Cing: cingulate; NBM: nucleus basalis of Meynert. (**C**) We compared performance on neurocognitive testing in patients with ipsilateral NBM node strength to subcortical arousal module to determine if there were any associations. On y-axis for each plot is z-score performance in each domain in the title and on x-axis in each plot is ipsilateral NBM node strength to subcortical module. We observed trends towards increasing node strength to module between ipsilateral NBM and subcortical arousal module was associated with improved performance on tests measuring cognitive processing (N = 31 patients), attention and concentration (N = 40 patients), visual memory (N = 40 patients), and language abilities (N = 40 patients). ***$p < 0.001$, *$p < 0.05$, Spearman's rho, uncorrected.

To determine if communities differ in patients, we then calculated node strength to module, which should be maximal to a node's home community module. While in controls, both ipsilateral and contralateral NBM had maximal node strength to module with limbic community, in patients both NBM demonstrated abnormal maximal node strength to module with basal ganglia community (**Fig. V.2B, solid arrows**). This suggests that NBM no longer clusters with its home community in TLE. Other structures that did not cluster with their home community in patients included ipsilateral central lateral thalamic nucleus (**Fig. V.2B, dashed arrow**), ipsilateral frontal operculum cortex, and ipsilateral and contralateral orbitofrontal cortex. All other 127 (95.5%) regions clustered with their home communities in TLE. Notably, all limbic regions except for NBM clustered in their home limbic community, suggesting NBM may be among the most affected limbic structures.

Next, we asked whether functional connectivity disturbances of subcortical arousal communities are related to neurocognitive dysfunction in TLE. We measured node strength to module of ipsilateral NBM with subcortical arousal community and detected a clinically interesting, but nonsignificant trend towards lower strength in this connection in patients (0.35 ± 1.45, mean ± SD) versus controls (0.91 ± 1.37; $p = 0.07$, Mann-Whitney U-test). In patients, we noted modest trends towards improved performance on multiple neurocognitive measures with increasing (i.e., closer to controls) node strength to module between ipsilateral NBM and

subcortical arousal community (**Fig. V.2C**). These included cognitive processing (r = 0.37, p = 0.035), attention and concentration (r = 0.39, p = 0.011), visual memory (r = 0.32, p = 0.039), and language abilities (r = 0.53, p < 0.001, Spearman's rho, uncorrected). There was no relationship between this node strength to module and either executive function or verbal memory (r = 0.22-0.29, p = 0.067-0.163; Spearman's rho, uncorrected). These findings suggest aberrant connectivity between ipsilateral NBM and subcortical arousal network may be moderately related to worse neurocognitive performance in TLE.

Subcortical Arousal	**Basal Forebrain & Cingulate**	**Occipital**	**Inferior Frontotemporal**
Cuneiform/Subcuneiform	Diagonal Band of Broca	Cuneal Cortex	Frontal Orbital Cortex
Dorsal Raphe	Anterior Cingulate Gyrus	Intracalcarine Cortex	Temporal Pole
Locus Coeruleus	Accumbens	Lingual Gyrus	Inferior Temporal Gyrus, Anterior Division
Median Raphe	Frontal Medial Cortex	Occipital Fusiform Gyrus	Middle Temporal Gyrus, Anterior Division
Parabrachial Complex	Paracingulate Gyrus	Occipital Pole	Superior Temporal Gyrus, Anterior Division
Pontine Nucleus Oralis	Subcallosal Cortex	Supracalcarine Cortex	Middle Temporal Gyrus, Posterior Division
Pedunculopontine Nucleus	**Peri-Rolandic**	Superior Parietal Lobule	Superior Temporal Gyrus, Posterior Division
Ventral Tegmental Area	Central Opercular Cortex	Temporal Occipital Fusiform Cortex	Middle Temporal Gyrus, temporoOccipital part
Centre Median Intralaminar Thalamic Nucleus	Frontal Operculum Cortex	Lateral Occipital Cortex, Inferior Division	**Frontoparietal Association**
Parafascicular Intralaminar Thalamic Nucleus	Heschl's Gyrus	Inferior Temporal Gyrus, Posterior Division	Angular Gyrus
Central Lateral Intralaminar Thalamic Nucleus	Insular Cortex	Lateral Occipital Cortex, Superior Division	Frontal Pole
Central Medial Intralaminar Thalamic Nucleus	Parietal Operculum Cortex	Inferior Temporal Gyrus, temporoOccipital, part	Inferior Frontal Gyrus, pars opercularis
Limbic	Planum Polare	**Basal Ganglia**	Inferior Frontal Gyrus, pars triangularis
Nucleus Basalis of Meynert	Planum Temporale	Caudate	Middle Frontal Gyrus

Amygdala	PostCentral Gyrus	Pallidum	Superior Frontal Gyrus
Hippocampus	PreCentral Gyrus	Putamen	Supramarginal Gyrus, Posterior Division
Parahippocampal Gyrus, Anterior Division	Supplementary Motor Cortex	**Precuneus**	
Temporal Fusiform Cortex, Anterior Division	Supramarginal Gyrus, Anterior Division	Posterior Cingulate Gyrus	
Parahippocampal Gyrus, Posterior Division		Precuneus	
Temporal Fusiform Cortex, Posterior Division			

Table V.2 Healthy community structure.
Members of each of the nine communities shown in **Fig. V.2A**, for healthy control participants. Read each column top to bottom. Cells with thick borders and bold text indicate the start of a new community list (9 total), and the non-bold text are the regions contained within each community.

V.2.3.3 Ipsilateral NBM is a Key Node in a Central Network of Altered Connectivity in TLE

We next employed the network-based statistic to expand upon seed-based and community-based connectivity analyses, and to define a network of functional connections that are reduced in patients with TLE compared to controls (**Fig. V.3**).

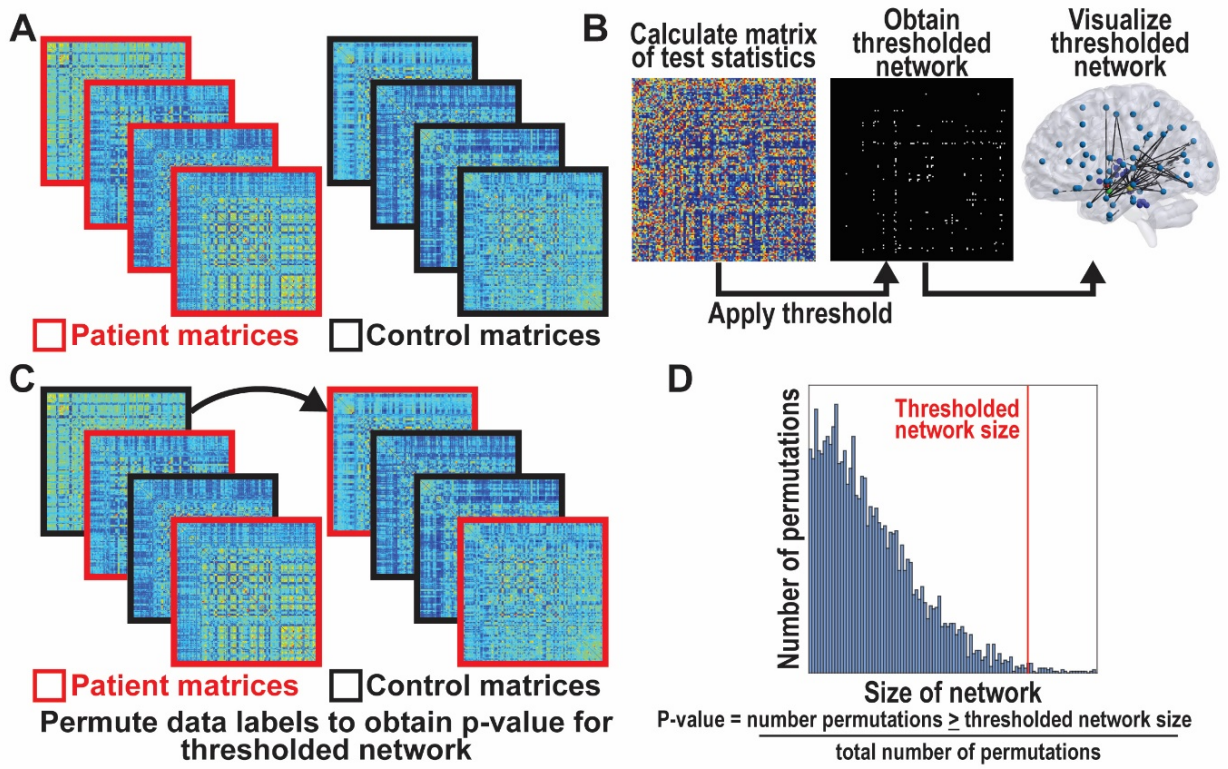

Figure V.3 Calculation of the network-based statistic in patients with TLE and controls.
(**A**) Functional connectivity matrices for each participant are calculated. (**B**, left) A test statistic (t-test) is calculated at each cell of the connectivity network. Then network components of interest are identified using a primary threshold of t-statistic > 4.2, 4.4, 4.6. This thresholded network can be visualized in a matrix (**B**, middle) where white cells represent suprathreshold links or in a schematic diagram (**B**, right) where connected nodes represent the suprathreshold network components. (**C**) Random permutation of data labels (10,000 permutations) across participants is then applied to calculate family-wise error. (**D**) After repeating the calculations on every permutation, a null distribution is generated, and family-wise error rate at desired level of $p < 0.01$ is controlled in the final result.

At multiple thresholds, we identified a network of decreased connections involving ipsilateral but not contralateral NBM, bilateral hippocampi and amygdalae, and other structures (**Fig. V.4**). At primary threshold of *t*-statistic > 4.6, 27 edges remained suprathreshold in the network out of 8,778 possible altered connections. These included 2 of 27 edges involving ipsilateral NBM, and 13 of 27 edges involving ipsilateral hippocampus. Interestingly, though we have previously shown that other subcortical arousal structures have altered connectivity in TLE, ipsilateral NBM was the only subcortical arousal structure in this decreased connectivity network.

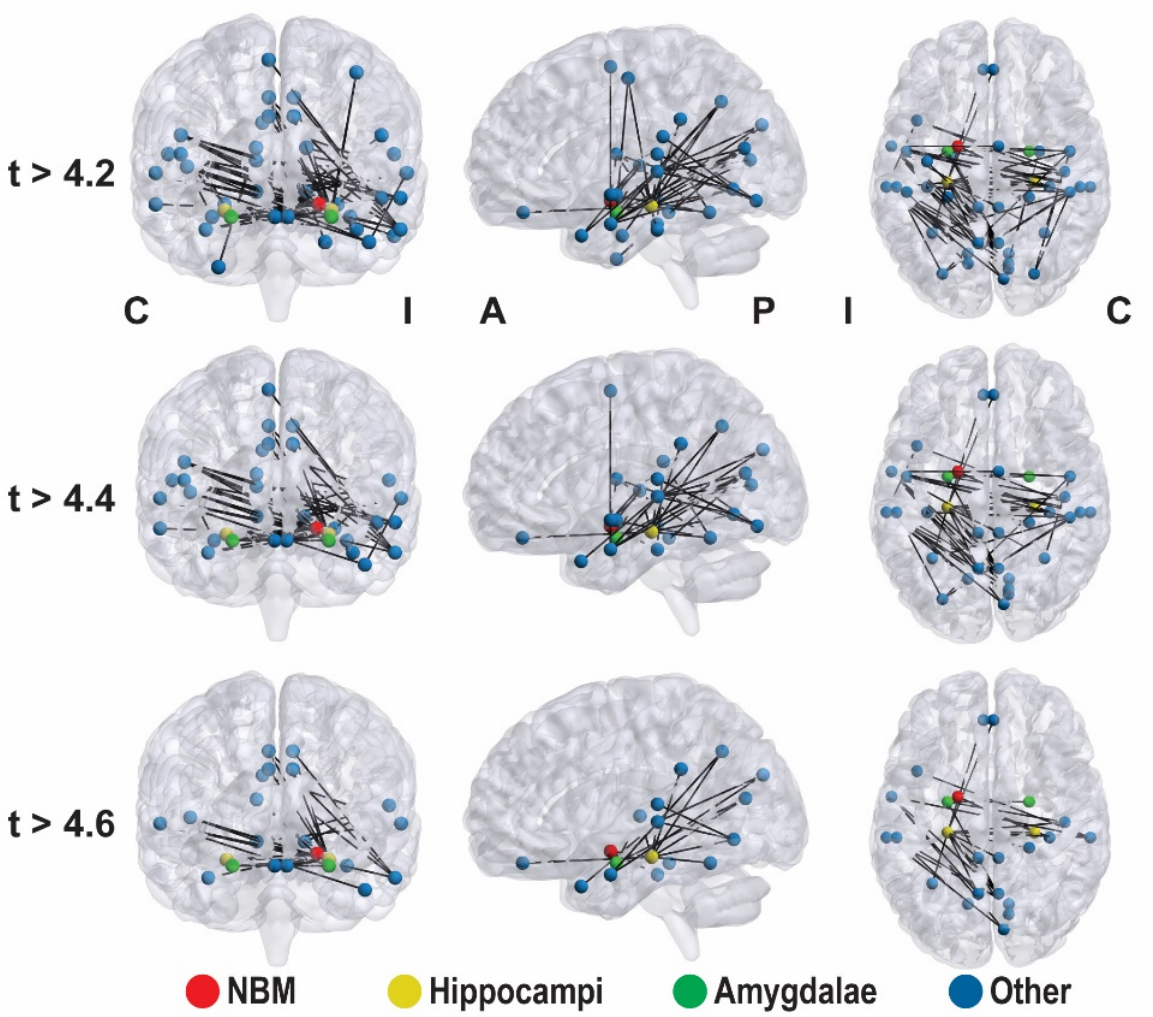

Figure V.4 A core network of altered connectivity in TLE includes bilateral mesial temporal structures and ipsilateral NBM.
The network-based statistic reveals a central network of nodes and edges that are altered in patients versus controls. This component network was tested at multiple t-statistic thresholds and the network contained 43 nodes and 66 edges at $t > 4.2$, 35 nodes and 49 edges at $t > 4.4$, and 24 nodes and 27 edges at $t > 4.6$. At multiple t-statistic thresholds, this network included ipsilateral but not contralateral NBM, and included bilateral hippocampi and amygdalae, but did not include other subcortical arousal nuclei. Network-based statistic is performed at $p < 0.01$ with family-wise error correction. N = 40 patients with TLE and 40 matched healthy control participants. A: anterior; C: contralateral; I: ipsilateral; NBM: nucleus basalis of Meynert; P: posterior.

To further probe altered networks in TLE, we calculated clustering coefficient of all nodes across all participants. We found that NBM exhibited lower clustering coefficients in patients than in controls on both ipsilateral (2.23 ± 0.93 patients, 3.46 ± 1.17 controls; $p = 3.38 \times 10^{-6}$) and contralateral sides (2.63 ± 0.85 patients, 3.32 ± 1.12 controls; $p = 0.005$, Mann-Whitney U-test, Bonferroni-Holm correction). Notably, ipsilateral NBM exhibited the single greatest decrease in

clustering coefficient in patients out of all 133 regions examined. By comparison, ipsilateral hippocampus and ipsilateral amygdala demonstrated third and twelfth largest decreases in clustering coefficient, respectively, in patients compared to controls. Overall, these results suggest that NBM ipsilateral to epileptogenic zone represents a key structure in the altered TLE network.

V.2.3.4 A Cohesive Network Model Explains Key Role of NBM and Limbic System in TLE Networks

We next used an integrative modeling framework to identify the simplest model to cohesively account for key network connectivity abnormalities in patients (**Fig. V.5**). Model 1 (controlling connectivity density) assumed that network differences can be explained by non-specific, global network changes present in TLE. Model 2 (controlling limbic only) posited that network disruptions in limbic system alone would account for connectivity differences observed. Model 3 (controlling limbic and NBM) assumed that disruptions in both limbic system and ipsilateral NBM play important roles in explaining observed findings. Model 4 (controlling nodes and modules) was a full explanatory model preserving average connectivity of all individual nodes and modules.

When considering reduced connectivity between ipsilateral NBM and the whole brain, models 1 and 2 were rejected and thus did not explain the finding, whereas models 3 and 4 were not rejected and could explain observed differences (**Fig. V.5A**). This suggests that widespread alterations of ipsilateral NBM functional connectivity are most simply explained by the combination of network changes in both limbic community and ipsilateral NBM, as model 3 is less constrained than model 4. In contrast, when considering reduced connectivity between contralateral NBM and the whole brain, none of the four models were rejected and thus all could explain the result (**Fig. V.5B**). Thus, reduced connectivity of contralateral NBM might simply be reflective of non-specific, global network changes in TLE. In evaluating two other major findings in our study, diminished clustering coefficient of ipsilateral NBM (**Fig. V.5C**) and altered strength between ipsilateral NBM and limbic module (**Fig. V.5D**), models 1 and 2 were rejected, while models 3 and 4 were not. Thus, model 3 was the simplest model to account for both these findings. Together, these results suggest that abnormalities in both ipsilateral NBM and limbic community may be central to core network perturbations we observed in TLE, and that these findings are not simply related to non-specific, global network changes.

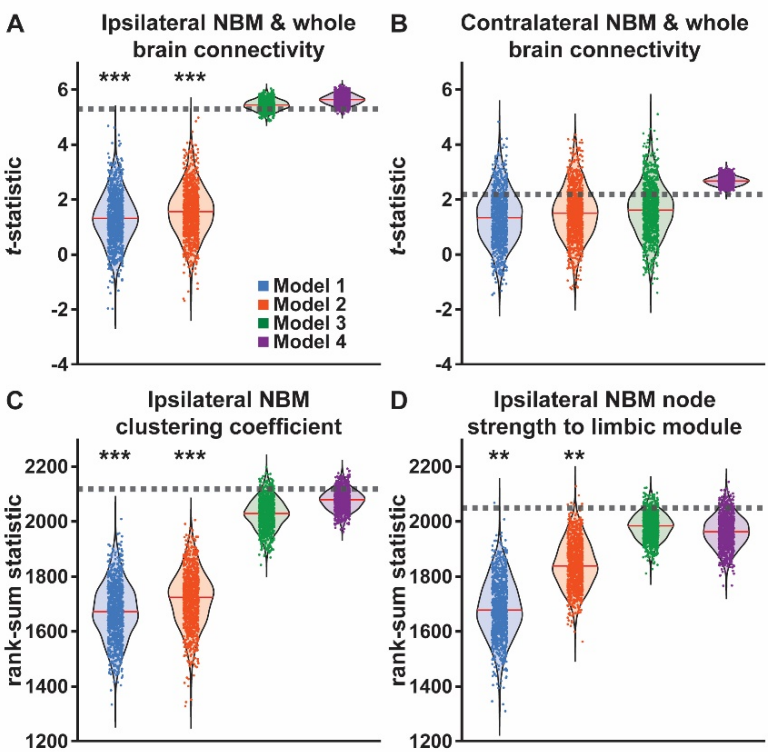

Figure V.5 Network models explain connectivity differences between patients and controls. Data points represent test statistics (t-statistic for normally distributed data or rank-sum statistic for non-normally distributed data) quantifying differences between patient and control null models (1,000 data points per null model). Dashed line represents the original data test statistic. Models with sufficient data points above the line represent network models that capture the difference between patients and controls observed in empirical data. Reduced connectivity of ipsilateral NBM with the whole brain (**A**) is best explained by Models 3 and 4, while reductions in contralateral NBM connectivity with whole brain (**B**) are explained equally well by all models. Decreases in ipsilateral NBM clustering coefficient (**C**) and ipsilateral NBM node strength to limbic module (**D**) are best explained by Models 3 and 4. The four models are defined in the Methods. ***p < 0.001, **p < 0.01, t-statistic or rank-sum statistic. Red line shows median. N = 1,000 comparisons per model. NBM: nucleus basalis of Meynert.

V.2.3.5 Ipsilateral NBM Network Properties Alone May Distinguish Patients with TLE From Controls

Finally, we investigated whether ipsilateral NBM network measures alone are sufficient to predict whether each participant's dataset belonged to a patient or control. For comparison, we evaluated whether ipsilateral hippocampus network measures could identify patient versus control datasets. For each structure, a logistic regression model was generated incorporating three key connectivity measures. From bootstrapping analysis, we quantified variability in area under the curve (AUC) and accuracy of both models based on subsampling total participant data. Using all participant data, ipsilateral NBM model demonstrated an AUC of 0.83 and an overall accuracy of

78% in accurately identifying patients versus controls (**Fig. V.6A**). The sensitivity and specificity of this model (at maximum sensitivity plus specificity) were 75% and 83%, respectively. The bootstrapped analysis revealed an accuracy of 76% ± 11% (mean ± SD) and AUC of 0.84 ± 0.05. Conversely, with all participant data, ipsilateral hippocampus model demonstrated an AUC of 0.85 and overall accuracy 76% (**Fig. V.6B**), with a sensitivity and specificity of 78% and 83%, respectively (at maximum sensitivity plus specificity). The bootstrapping analysis showed an accuracy of 77% ± 11% and AUC of 0.86 ± 0.05. These results suggest that ipsilateral NBM connectivity patterns may identify patients with moderately high accuracy, and that NBM connectivity perturbations are specific to patients. Furthermore, performance of ipsilateral NBM model was similar to that of ipsilateral hippocampus.

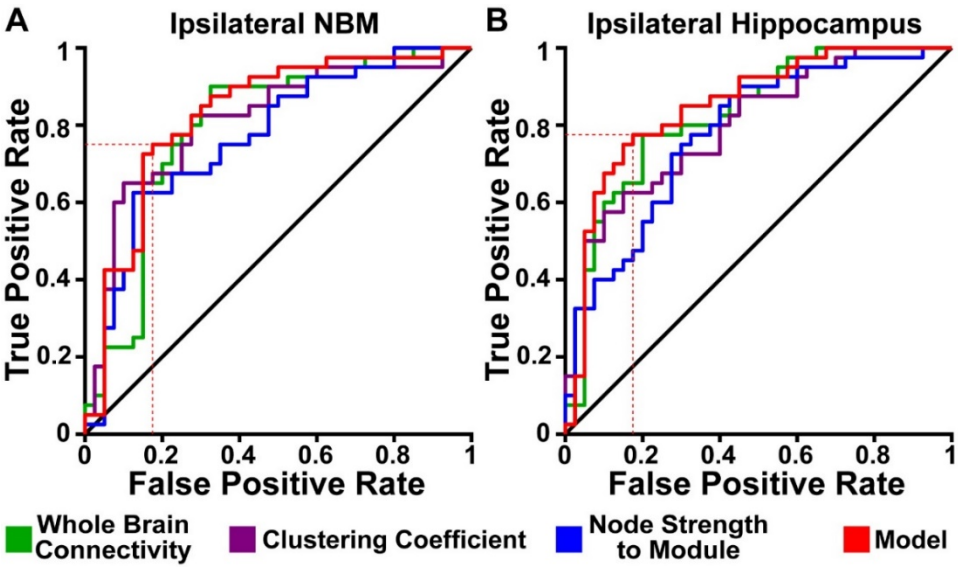

Figure V.6 Network properties of ipsilateral NBM, similar to ipsilateral hippocampus, may distinguish patients with TLE from healthy controls.
(**A**) The receiver operator characteristic (ROC) curves demonstrate true positive rate and false positive rate of three individual connectivity measures of ipsilateral NBM in correctly distinguishing patients from controls. In addition, a model resulting from logistic regression analysis incorporating the three network properties is shown. The area under the curve (AUC) of the ipsilateral NBM model is 0.83 with an overall accuracy of 78%, and at the maximum sensitivity plus specificity (dashed red line), the model exhibits sensitivity of 75% and specificity of 83%. (**B**) ROC curves of ipsilateral hippocampus connectivity properties are shown for comparison. The AUC of the ipsilateral hippocampus model is 0.85 with an overall accuracy of 76%, and at the maximum sensitivity plus specificity (dashed red line), the model exhibits sensitivity of 78% and specificity of 83%. NBM: nucleus basalis of Meynert.

V.2.4 Discussion

In this work, we found that NBM ipsilateral to the side of seizure onset may be one of the most disturbed brain network nodes in TLE. With the network-based statistic, we identified a central network of altered connectivity in patients including ipsilateral NBM and limbic structures known to be affected by TLE. Notably, despite having previously shown other arousal structures are perturbed in TLE,[2,254,283] in this analysis ipsilateral NBM was the sole subcortical arousal structure included. Ipsilateral NBM also exhibited largest decrease in clustering coefficient of all regions examined. Together these findings suggest that NBM may be more strongly affected than other arousal structures in TLE.

It has been proposed that based on its anatomical connections, NBM is closely related to both limbic system and brainstem ARAS.[75,244] Supporting this, NBM clustered with limbic community in healthy participants. In patients, NBM was the only limbic structure to no longer group with its home community. Our network model comparisons indicated that connectivity changes of limbic system together with ipsilateral NBM are central to explaining broad network alterations observed in TLE and that NBM connectivity alterations are not simply reflective of global network changes in this disorder. Finally, we found that network measurements in ipsilateral NBM alone were sufficient to differentiate between patients and controls with comparable accuracy to ipsilateral hippocampus connectivity. Overall, these observations evidence that NBM may be among the most profoundly altered network structures in TLE, with connectivity perturbations comparable to limbic regions.

Loss of integration of NBM from limbic community may be an adaptive response to prevent seizure propagation beyond limbic structures. This builds upon the "network inhibition hypothesis,"[1] which postulates that TLE seizures may cause dysfunction of subcortical arousal structures.[75,297] Previous studies have suggested that brainstem atrophy and disrupted brainstem arousal connectivity in focal epilepsy may be related to consciousness-impairing seizure frequency, worse verbal neurocognition, and risk of sudden unexpected death in epilepsy (SUDEP).[38,113,216] Likewise, we observed larger decreases of NBM-frontoparietal connectivity were associated with more frequent consciousness-impairing seizures. While studies in rodent models of TLE have examined ictal NBM activity,[79] to the best of our knowledge, interictal network properties of NBM in human TLE have not previously been examined. The relationships between these observed network changes and effects of drugs including antiepileptic (increase

seizure threshold) and anticholinergic medications (decrease seizure threshold) have not been investigated. Future studies in animal models evaluating cholinergic neurotransmission in these networks would be central to parsing out these relationships. Additionally, we found some neurocognitive measures were moderately associated with strength of connectivity between NBM and other subcortical arousal structures. Further study of NBM and downstream networks may help elucidate certain broad yet unexplained deficits in TLE.

For epilepsy patients who continue to experience frequent disabling seizures despite maximal medical therapy, neuromodulation may incur quality of life improvements, including amelioration of ictal impaired-consciousness, treatment of neuropsychological comorbidities, or reduced SUDEP risk.[3,22,24,298] NBM has been proposed as a viable neurostimulation target to prevent consciousness-impairment during seizures.[64,298] Supporting this hypothesis, studies in anesthetized rats demonstrated that stimulating ARAS structures may increase functional connectivity between NBM and paralimbic structures, potentially restoring normal functional relationship of that community.[299] While neurostimulation of NBM has not been explored in human epilepsy, small trials of NBM neurostimulation for Parkinson's disease dementia[300] and Alzheimer's disease[301] indicated safety and tolerability. NBM may ultimately warrant investigation as an innovative neuromodulation target to treat deleterious brain network effects of TLE.

This study has limitations that should be discussed. Our patient cohort is not uniform: not all individuals exhibited hippocampal sclerosis on MRI, duration of epilepsy varied, not all patients had a history of tonic-clonic seizures, and medication dosage could not be controlled. This variability might influence applicability of our results to other patient populations. Additionally, during resting-state fMRI acquisition, participants are reminded to stay awake with eyes-closed, but we cannot positively ascertain whether participants become drowsy during scans, which may influence arousal network functional connectivity. In future studies, it may be interesting to employ quantitative arousal measures, like simultaneous EEG, to account for this variable. Similarly, we cannot account for possible influences of interictal discharges on BOLD signal. Simultaneous EEG-fMRI would also allow us to examine how interictal electrophysiology may affect these subcortical arousal structures. Additionally, while examining all possible relationships between subcortical arousal structures and neurocognition was beyond the scope of the present study, in subsets of this cohort we have previously examined relationships between brainstem ARAS and broad areas of neurocognitive testing,[38] and between intralaminar thalamus and visual

attention.[283] Future studies powered for examining relationships between connectivity of arousal structures and neurocognition would help discriminate which arousal networks are most associated with specific domains. Furthermore, NBM is a small structure in which fMRI signal may be susceptible to motion and other noise, although our connectivity analyses did incorporate correction for physiological artifact and movement.

NBM connectivity is markedly perturbed in patients with TLE, and it may be a key network node involved in the broad pathophysiology of this disorder. Through network based analyses, we found profound disturbances of NBM connectivity patterns in patients with TLE and observed subcortical arousal community properties which may be related to neurocognition and disease severity. This work may have important implications to help understand, treat, and prevent widespread deleterious effects of TLE on cognition, alertness, and cortical function.

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CHAPTER VI

VI Examining how Functional Connectivity Between Arousal Structures and Intrinsic Connectivity Networks May Impact Neurocognition

VI.1 Summary and Contributions

This chapter details our findings regarding functional connectivity between subcortical arousal structures and intrinsic connectivity networks (ICNs) in patients with temporal lobe epilepsy (TLE). It has been previously shown by other groups that ICNs including default mode network (DMN), salience network (SN), and central executive network (CEN) have abnormal connectivity in patients with epilepsy. These three particular ICNs form the "triple network model" and have been shown to be abnormal in various other neurological diseases and psychiatric disorders. We calculated directed and non-directed functional connectivity between subcortical arousal structures and ICNs in patients with TLE. We related these connectivity measures to neurocognitive performance to determine if connectivity between ICNs and subcortical arousal structures were associated with neurocognitive deficits in TLE. Additionally, we employed an fMRI only measure of vigilance to determine if vigilance changed over the period during which we acquired our fMRI and if this affected connectivity of arousal structures in TLE.

The results of this study indicated that there are directed and non-directed functional connectivity abnormalities between subcortical arousal structures and ICNs in patients with TLE. We also found abnormal connectivity withing both the DMN and SN. These abnormal connectivity values were associated with neurocognitive deficits before epilepsy surgery. We also noted some recovery of connectivity after epilepsy surgery. Last, we preliminarily noted that although vigilance decreased in patients during fMRI acquisition, these decreases were not associated with changes in resting-state functional connectivity of arousal structures.

VI.2 Abnormal FMRI Connectivity Between Arousal Structures and Intrinsic Connectivity Networks in Patients with Temporal Lobe Epilepsy

González, H.F.J., Narasimhan, S., Goodale, S.E., Johnson, G.W., Wills, K.E., Haas, K.F., Konrad, P.E., Morgan, V.L., Chang, C., Englot, D.J. "Abnormal fMRI connectivity between arousal structures and intrinsic connectivity networks in patients with temporal lobe epilepsy."

Abstract

Objective: To determine if patients with temporal lobe epilepsy (TLE) have perturbations in functional connectivity (FC) between arousal structures and intrinsic connectivity networks (ICNs).

Methods: We acquired resting-state fMRI in 50 adults with TLE and 50 controls. We calculated non-directed FC (correlation) and directed FC (Granger causality laterality index: GC_{LI}) within ICNs (default mode network: DMN, salience network: SN, and central executive network: CEN) and between arousal structures and ICNs. We compared FC in patients vs. controls, and associated FC with disease metrics and neurocognitive testing. Finally, we used an fMRI-based vigilance measurement to preliminarily relate vigilance changes to resting-state FC of arousal structures.

Results: We noted decreased non-directed FC within DMN in patients (5.73 ± 1.44) vs. controls (6.75 ± 1.38, p = 0.0008) and within SN in patients (9.27 ± 2.19) vs. controls (10.40 ± 2.33, p = 0.0008). We found decreased FC between arousal network and SN in patients (1.12 ± 1.03) vs. controls (2.04 ± 1.27, p = 0.0001). Larger decreases in non-directed FC between nucleus basalis of Meynert (NBM) and SN were associated with worse processing speed index (r = 0.251, p = 0.033). Lower non-directed FC between pedunculopontine nucleus (PPN) and SN associated with worse verbal comprehension index (r = 0.350, p = 0.015) and full-scale intelligence quotient (FSIQ) (r = 0.296, p = 0.043). We noted abnormal GC_{LI} between arousal network and SN in patients (-0.095 ± 0.21) vs. controls (-0.26 ± 0.24, p = 0.0012), meaning SN exerts influence on arousal structures in controls, but not patients. After surgery, we noted some recovery of non-directed FC between NBM and SN. Last, in a preliminary analysis, we found that patients, but not controls, may exhibit decreased awareness during fMRI, but that FC did not change with vigilance.

Conclusions: These results suggest that abnormal FC between subcortical arousal structures and ICNs may partially underlie neurocognitive deficits typically seen in patients with TLE, and that these networks may represent novel neuromodulation targets to treat neurocognitive comorbidities in TLE.

VI.2.1 Introduction

Temporal lobe epilepsy (TLE) is the most common form of epilepsy and has broad and devastating neuronal network disturbances.[11] Although TLE is a focal epilepsy, which originates in the temporal lobe, patients with TLE experience functional and structural abnormalities that cannot be explained solely by focal deficits in the temporal lobe.[31] For example, patients with TLE display diffuse neocortical hypometabolism, broad connectivity perturbations, and widespread gray matter atrophy.[29,31,69,113,302] They also experience neurocognitive deficits that are unrelated to temporal lobe functions; these include problems with executive function, social cognition, and concentration.[15,173] In addition to these global structural abnormalities, pervasive functional perturbations, and widespread neurocognitive deficits, patients with TLE exhibit problems with alertness, attention, and experience increased somnolence.[15,303,304] These broad deficits suggest an extratemporal common source for these widespread network disturbances in TLE.[49]

Recent work by our group supports the idea that interictal abnormalities in subcortical arousal structures underlie these pervasive neural disturbances in TLE.[49] We have previously shown that focal limbic seizures with impaired consciousness are associated with ictal neocortical deactivation, which can be explained by the network inhibition hypothesis.[46] Blumenfeld originally proposed the network inhibition hypothesis, which posits that ictal suppressed cortical activity is resultant from seizure spread to subcortical regions important for neocortical activation such as brainstem ascending reticular activating system (ARAS), basal forebrain cholinergic nuclei, and intralaminar thalamic nuclei.[46,204] We have proposed, in the extended network inhibition hypothesis, that ictal inhibition of activity leads to long-term interictal abnormal connectivity between subcortical arousal structures and neocortical networks.[2] We found that patients with TLE exhibit altered functional magnetic resonance imaging (fMRI) functional connectivity of subcortical arousal structures with neocortical regions, and that these connectivity abnormalities relate to neurocognitive deficits and seizure frequency.[2,254,283,305] Overall, we suggest that abnormal resting-state connectivity of subcortical arousal structures, central to vigilance, engender broad network deficits in TLE leading to impaired neurocognition and other global deficits in this disease.[49] While studying connectivity of arousal structures in TLE is a relatively new concept, prior research by others has shown that TLE alters connectivity in other important brain networks.[56,58,306]

Previous studies approached understanding these broad network deficits by examining connectivity within and between intrinsic connectivity networks (ICNs).[58] ICNs, also called resting-state networks, are large-scale networks of interdependent and functionally distinct brain regions that are repeatedly and reliably observed in subjects at rest (e.g. default mode network).[307] Here we examine connectivity among three ICNs that compose the "triple network model." The triple network model has been proposed to underly cognitive dysfunction in psychiatric and neurological disorders.[307,308] The three ICNs that form this triumvirate include: the default mode network (DMN), the central executive network (CEN), and the salience network (SN) (**Fig. VI.1**):

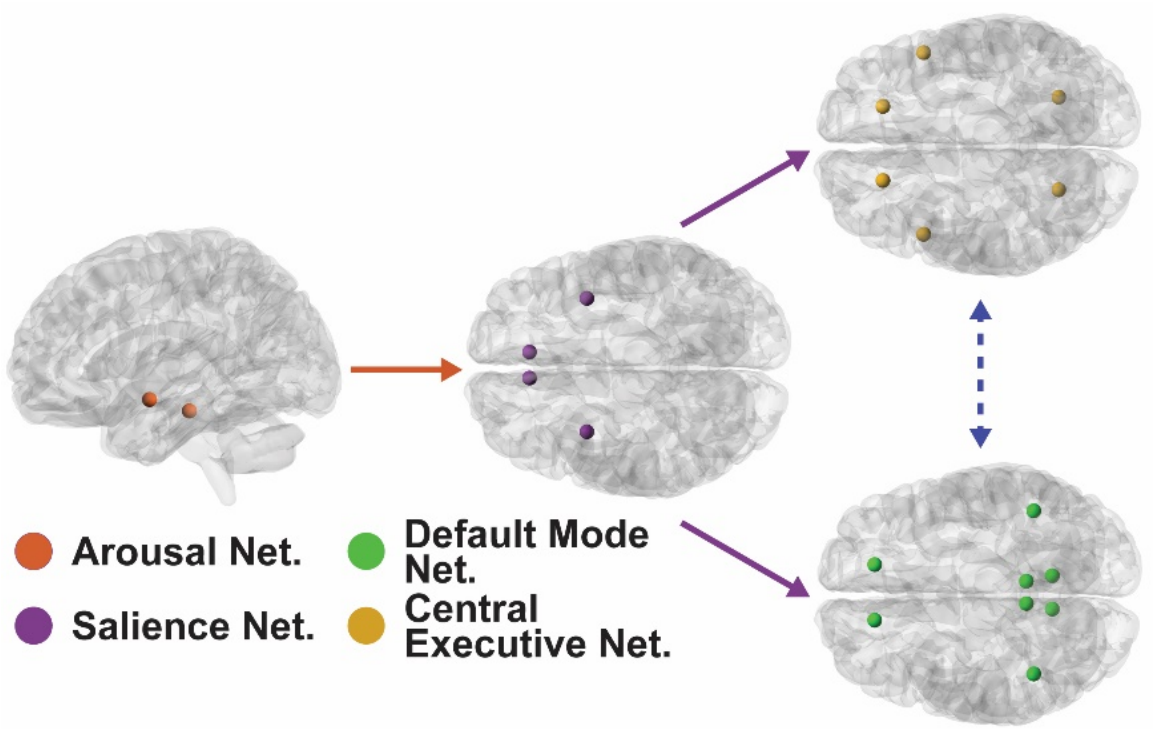

Figure VI.1 Proposed relationship between subcortical arousal networks and three ICNs.
The arousal network (burnt orange; includes brainstem ARAS nuclei, intralaminar thalamic nuclei, and NBM) anatomically projects to cortical networks and their core function is modulation of brain wide arousal levels.[3-5] The salience network (royal purple) is composed of the insula and anterior cingulate cortex, and controls switching between the default mode network (lime green) and central executive network (mustard yellow).[6] The default mode network is centered around the posterior cingulate cortex, medial prefrontal cortex, precuneus, and angular gyrus/lateral parietal cortices.[7] The central executive network is a frontoparietal intrinsic connectivity network centered around dorsolateral prefrontal cortex and lateral posterior parietal cortex. Activity in the default mode network and the central executive network are known to be anticorrelated, indicated by the blue dashed arrow. ARAS: ascending reticular activating system; ICN: intrinsic connectivity network; NBM: nucleus basalis of Meynert; Net: network.

DMN is a network primarily active when a person is in quiet repose, during self-referential tasks, and/or with introspective activity.[7] The DMN consistently is deactivated during stimulus-driven, attention-demanding activities.[307] CEN is a brain network whose functions include higher level neurocognition, decision making, and goal-directed activities.[307] Prior work has shown that DMN and CEN are anticorrelated, which is consistent with their antithetical functions.[6] The main role of the SN is to detects and orient attention to salient stimuli[307] and subsequently engage frontoparietal systems, such as the CEN, for task positive neurocognitive functions.[309] Multiple connectivity studies have consistently shown that SN coordinates brain network switching between DMN and CEN by causally influencing these two anticorrelated networks.[6,310] These three networks form a core set of cortical networks that are important for understanding healthy and disordered cognitive function. Interestingly, these cortical networks are modulated by broadly projecting subcortical arousal networks.[307]

Some TLE studies have shown altered connectivity within ICNs[55-58,180] and our prior work has shown connectivity abnormalities in arousal structures;[2,38,49,254,283,305,311] however, none have examined functional connectivity between subcortical arousal structures and ICNs. Subcortical arousal structures (**Fig. VI.1**) are known to project to and modulate key structures within these ICNs.[4,5,78,312] Here we employ non-directed and directed functional connectivity network analyses to examine connectivity differences between arousal structures and ICNs. We also relate connectivity to measures of disease severity and neurocognition, and examine how epilepsy surgery affects connectivity between arousal structures and ICNs. Additionally, the structures under investigation are known to be fundamental to vigilance.[31,49] Therefore, we also asked how short-term changes in vigilance might affect these long-term resting-state functional connectivity measures. In a preliminary investigation of this question, we employed a novel fMRI-based measure of alertness to examine changes in vigilance and their relationship to connectivity of arousal structures.[313] We hypothesize that there will be abnormal connectivity between arousal structures and ICNs which may relate to measures of disease severity, neurocognition, and abnormal connectivity within ICNs.

VI.2.2 Methods

VI.2.2.1 Study Participants

Participants consisted of 50 adults with unilateral mesial TLE who underwent evaluation for epilepsy surgery at Vanderbilt University Medical Center from 2012 to 2020. Our clinical group diagnosed mesial TLE via a presurgical epilepsy assessment performed by a multidisciplinary healthcare team whose members included epileptologists, functional neurosurgeons, clinical neuropsychologists, and other providers. Prior to presentation for discussion, all patients had the following evaluations: acquisition of detailed patient history (including seizure semiology, history of treatment, initial presentation), anatomical magnetic resonance imaging (MRI), inpatient video electroencephalography (EEG), complete neuropsychological testing, positron emission tomography (PET), and localization of eloquent cortex function by fMRI and/or intracarotid sodium amobarbital procedure (Wada test). In 29 patients, we acquired a repeat fMRI scan at least 1-year after surgery (31.5 ± 15.9 months after surgery, mean ± standard deviation, max = 60 months, min = 12 months). In all patients who elected to undergo epilepsy surgery, we obtained an Engel outcome from their epileptologist's clinical evaluations at their latest follow-up. Other participants included 50 healthy adult controls. Before any investigators conducted any analyses, we individually matched these controls to patients by age (typically ± 3 years, maximum ± 5 years) and sex (**Table VI.1**). All participants gave written informed consent to participate in this study. All study procedures were in accordance with the Declaration of Helsinki and authorized by Vanderbilt University Institutional Review Board.

VI.2.2.2 Imaging

We performed all imaging sessions with a Philips Achieva 3T MRI scanner (Philips Healthcare, Best, Netherlands) and a 32-channel head coil as in previous studies.[254] Neuroimaging MRI data acquired included: (i) a three-dimensional (3D) T1-weighted whole brain image for tissue segmentation and inter-participant normalization (gradient echo, repetition time (TR) = 9.10 ms, echo time (TE) = 4.60 ms, 192 shots, flip-angle = 8.0°, matrix = 256 x 256, 1.0 mm^3), (ii) two-dimensional (2D) T1-weighted axial images acquired in the same slice orientation as the functional images for functional to structural image coregistration (1.0 mm x 1.0 mm x 3.5 mm, with 0.5 mm gap), (iii) two resting-state eyes-closed 10-minute T2*-weighted blood oxygenation level dependent (BOLD) fMRI (field of view (FOV) = 240.0 mm, TE = 35.0 ms, TR = 2.0 s, 34 axial

slices, slice thickness = 3.50 mm with a 0.50 mm gap, matrix = 80 x 80, 3.0 x 3.0 x 4.0 mm^3), with 300 volumes acquired during each resting-state acquisition. For the remainder of this paper, we will refer to the two pre-operative fMRI acquisitions as 'fMRI 1' and 'fMRI 2.' Resting-state fMRI sessions in all participants included instructions to lay at rest with their eyes-closed for the entire scan. We conducted respiratory and cardiac physiological at 500 Hz sampling rate with the MRI scanner's respiratory belt and pulse oximeter.

VI.2.2.3 Regions of Interest

We defined regions of interest (ROIs) for this study as follows. First, we defined ROIs for the cortical ICNs: salience network, central executive network, and default mode network.[58] Using FreeSurfer (https://surfer.nmr.mgh.harvard.edu/fswiki/CorticalParcellation), we performed automatic cortical surface parcellation in a participant-specific manner. These cortical parcellations were based on the Desikan-Killiany atlas.[314,315] The ROIs we included in these three ICNs are as follows. The salience network included the insula, rostral anterior cingulate, and caudal anterior cingulate. The default mode network included the lateral orbitofrontal cortex, medial orbitofrontal cortex, inferior parietal cortex, precuneus, and posterior cingulate. The central executive network included superior frontal cortex, pars opercularis, and superior parietal cortex. Next, we included 2 bilateral participant-specific intralaminar thalamic nuclei ROIs, the centre median (CM) and central lateral (CL), whose implementation we have previously described.[275,283,285] We incorporated 1 brainstem ARAS nuclei from the Harvard Ascending Arousal Network Atlas (https://www.martinos.org/resources/aan-atlas)[5] described in prior research. The brainstem nuclei we included was the pedunculopontine nucleus (PPN) as this ARAS nucleus showed profound changes in our prior investigations.[2,254] The last ROI we included was the nucleus basalis of Meynert (NBM), a bilateral cholinergic basal forebrain nucleus obtained from stereotaxic probabilistic mapping of Mesulam's designations,[74,242,316] whose use we have previously published.[305] Lastly, all non-midline ROIs were designated as either ipsilateral or contralateral to the epileptogenic side of the brain for each patient, and ROIs of the matched controls were defined accordingly.

VI.2.2.4 Non-Directed Functional Connectivity Analysis

We first wanted to examine if there were differences between patients and controls in non-directed connectivity of arousal structures and ICNs. We measured connectivity within ICNs by calculating average connectivity between members of each ICN. We also measured connectivity between arousal structures and ICNs. For analysis of non-directed connectivity differences, we calculated Pearson partial correlation functional connectivity matrices for each participant. We preprocessed fMRI data using MATLAB 2019a (MathWorks, Natick, Massachusetts, USA) and SPM12.[317] Preprocessing included slice-timing correction, motion correction, retrospective correction of physiological motion effects (RETROICOR),[256] tissue segmentation (white matter, grey matter, and cerebrospinal fluid (CSF)), and spatial normalization to Montreal Neurological Institute (MNI) template. We temporally band-pass filtered fMRI between 0.0067 Hz and 0.1 Hz. We calculated an average time series for each ROI describe above. Additionally, we calculated participant-specific CSF and white matter segmentations to exclude these signals. For each individual fMRI acquisition per participant, we calculated functional connectivity between ROIs by partial Pearson correlation between each region's mean BOLD time-series, with six motion time-series (three degrees of translation: x, y, and z dimensions, and three degrees of rotation: roll, pitch, and yaw) and mean white matter BOLD serving as confounds. For each participant, we then transformed the correlations with Fisher z-transformation and averaged connectivity of both fMRI resting-state sessions, fMRI 1 and fMRI 2.

VI.2.2.5 Network-Based Statistic

We also employed the network-based statistic to identify a core network of connected nodes from the arousal structures and ICNs with connectivity decreases in patients compared to controls.[293] The network-based statistic is a non-parametric method that controls family-wise error rate when performing mass univariate testing on network connections. We used a primary *t*-test threshold t-statistic > 2.7 obtained heuristically, for each link to define a set of suprathreshold component networks and their extent (number of links). Next, we calculated a family-wise error correction for the component network by randomly permuting patient and control labels 10,000 times and repeating calculations for every permutation. This resulted in a null distribution of component extents and allowed discrimination of thresholded network components that exhibited connectivity decreases in patients as compared to controls with family-wise error corrected

significance $p < 0.001$. We employed BrainNet viewer to visualize the decreased connectivity network and components obtained from network-based statistic.[294]

VI.2.2.6 Directed Functional Connectivity Analysis

After examining non-directed functional connectivity, we wanted to know if there were directed connectivity disturbances in patients with TLE. For directed functional connectivity analysis, we calculated Granger causality and Granger causality laterality index. Granger causality states that a time series x Granger-causes time series y if knowing the past value of time series x helps predict future values of y better than using past values of y alone.[318] As with non-directed connectivity, preprocessing included slice-timing correction, motion correction, RETROICOR,[256] tissue segmentation, and spatial normalization to MNI template. Then fMRI was high-pass filtered above 0.0067 Hz.[319] Then we calculated participant-specific white matter timeseries from this new high-pass filtered fMRI data to be used as a regressor when obtaining the average time series per ROI. For all ROIs described above, we removed 7 nuisance regressors (mean white matter BOLD timeseries and 6 degrees of motion) from the fMRI by subtracting residuals from a linear fit by sum square error. Then average time series was obtained per ROI, this was then z-scored (by subtracting the mean and dividing by standard deviation) to improve stationarity,[319] yielding an average z-scored timeseries per ROI for each fMRI acquisition.[320] After this preprocessing, we performed Granger causality analysis using the multivariate Granger causality (MVGC) toolbox. Specifically, we calculated pair-wise Granger causality for each 10 minute resting-state acquisition (fMRI 1 and fMRI 2), between all ROIs outlined above.[321] This yielded an asymmetric square matrix of time-domain Granger causality, per participant, per resting-state fMRI acquisition (fMRI 1 and fMRI 2). Then we integrated spectral causalities to obtain an average Granger causality value within the 0.0067-0.1 Hz frequency band (same as that used for non-directed connectivity). We then averaged Granger causality values per participant over both fMRI 1 and fMRI 2.

In order to determine which structure influenced which more strongly, we calculated Granger Causality laterality index (GC_{LI}) (**equation VI.1**) from Granger Causality (GC) as Morgan et al did in prior work.[54]

$$GC_{LI} = \frac{GC(X \rightarrow Y) - GC(Y \rightarrow X)}{GC(X \rightarrow Y) + GC(Y \rightarrow X)} \qquad \textbf{(eq. VI.1)}$$

GC_{LI} is positive when arousal structures are more strongly influencing cortical ICNs. When GC_{LI} is negative cortical ICNs are influencing subcortical arousal structures, and when GC_{LI} is zero the direction of influence is equal between ICNs and arousal structures.

VI.2.2.7 Neurocognitive Testing and Epilepsy Measures

We also asked how connectivity of arousal structures and ICNs may be related to clinical measures of disease severity and neurocognition. We obtained patient demographics and TLE measures from each patient's epileptologists (**Table VI.1**). Measures of epilepsy severity which we recorded included: MRI evidence of mesial temporal sclerosis, duration of TLE, seizure type, seizure frequency, and history of focal to bilateral tonic-clonic (secondarily-generalized) seizures. A licensed neuropsychologist administered neuropsychological testing to patients as in prior work.[38] We evaluated how connectivities may relate to these measures of neurocognition: full scale intelligence quotient (IQ), verbal comprehension index (VCI), processing speed index (PSI), and working memory index (WMI).

VI.2.2.8 FMRI-Based Alertness Index

We employed an fMRI-based template estimate of alertness as calculated in Goodale et al. and followed the approach in previous work by this group.[313,322,323] Briefly, this template of alertness was constructed from a set of resting-state fMRI scans that were voxel-wise temporally correlated with each subjects' EEG alertness index. The average 'vigilance template' over all subjects was obtained and yielded a 3D template. This template could then be spatially correlated with each TR and yield an fMRI alertness index. We calculated this alertness index over all 600 TR's obtained over the 20 minutes of resting-state fMRI data (fMRI 1 and fMRI 2). We then average the alertness index for each subject within fMRI 1 and fMRI 2 to obtain an average alertness index in the first half of the scan (fMRI 1) and the second half of the scan (fMRI 2). We then compared these values between groups (patients vs. controls) and within groups (patients fMRI 1 vs. fMRI 2 and controls fMRI 1 vs. fMRI 2). To determine if there were differences in alertness between patients and controls and to determine if either group became less alert over the course of the fMRI acquisition.

VI.2.2.9 General Statistical Approaches

We utilized parametric tests for normally distributed data or non-parametric tests otherwise. We tested normal distribution with the Anderson-Darling test. We compared demographics in TLE patients versus controls with paired t-tests for continuous variables and chi-square for categorical variables. Paired t-tests were utilized to compare directed and non-directed functional connectivity and alertness index between participant groups. We used an ANOVA to compare pre-operative versus post-operative versus control functional connectivity with Tukey's honest significant difference criterion for multiple comparison corrections as needed. We employed Pearson correlation to relate functional connectivity to continuous variable disease measures and neuropsychological testing performance. Statistical analyses were performed with MATLAB 2019a and SPSS 27 (Armonk, NY, USA). We prospectively defined statistical significance at $p < 0.05$ for all tests and post hoc Bonferroni-Holm was used to correct for multiple comparisons where necessary.

VI.2.3 Results

VI.2.3.1 Patient Characteristics

All patients but one elected to have epilepsy surgery (N = 49): 36 had a selective amygdalohippocampectomy (SAH), 11 had an anterior temporal lobectomy (ATL), and 2 underwent responsive neurostimulation (RNS) implantation. Engel outcomes were assigned for all patients who had at least 1-year follow-up (N = 46). Although Engel outcome is not traditionally used for neuromodulation, the one RNS patient who had > 1-year follow up had no improvement in seizures after RNS implant and therefore was included in the Engel IV category. The other RNS patient was less than 1-year out from implantation, so no Engel outcome was assigned. In those individuals that we obtained a post-operative scan ≥ 1-year after surgery (N = 29), the outcomes were: 17 Engel I, 5 Engel II, 6 Engel III, and 1 Engel IV.

	Patients	Controls	P value
Age, years	39.9 [18, 68]	39.8 [18, 69]	0.99
Gender, female	28.0 (56.0%)	28.0 (56.0%)	0.99
Epilepsy duration, years	19.7 [2, 50]		
Seizure frequency, monthly			
FACS	6.8 [0, 195]		
FICS	7.7 [0, 63]		
FBTC	0.6 [0, 8]		
History of FBTC, yes	25.0 (50.0%)		
Epileptogenic side, right	36.0 (72.0%)		
MTS on MRI, yes	29.0 (58.0%)		
Video-EEG results			
Well localized (ictal), yes	44.0 (88.0%)		
Lateralized (interictal), yes	33.0 (66.0%)		
Invasive recording (SEEG)	13.0 (26.0%)		
Mesial temporal hypometabolism on PET	37.0 (74.0%)		
History of status epilepticus	4.0 (8.0%)		
Surgery			
ATL	11		
SAH	36		
RNS	2		
Months between surgery & post-op fMRI	31.5 [12, 60]		
Engel outcome latest ≥ 1-year post-op			
Engel I	29		
Engel II	7		
Engel III	6		
Engel IV	4		

Table VI.1 Demographics for patients before and after epilepsy surgery, and matched healthy controls.
For continuous variables, data shown are mean [minimum, maximum] and statistical testing is performed using paired t-test. For categorical variables, data shown are N (%) and statistical testing is performed using McNemar test. Seizure type and frequency were determined from clinical visit most proximal to fMRI and documented in the final epilepsy conference note. N = 50 patients with TLE before surgery, N = 46 patients with outcomes after surgery, N = 29 patients with fMRI after surgery, and N = 50 healthy matched controls. ATL: anterior temporal lobectomy; FACS: focal aware conscious seizures; FBTC: focal to bilateral tonic-clonic seizures; FICS: focal impaired-consciousness seizures; MRI: magnetic resonance imaging; MTS: mesial temporal sclerosis; RNS: responsive neurostimulation; SAH: selective amygdalohippocampectomy; SEEG: stereotactic-electroencephalography.

VI.2.3.2 Patients with TLE Exhibit Abnormal Pre-Operative Non-Directed Functional Connectivity in Intrinsic Connectivity Networks and Arousal Structures

We first wanted to know if there was abnormal non-directed functional connectivity within any of the ICNs. We found that as compared to controls patients had lower average within non-directed functional connectivity in SN (**Fig. VI.2A;** patients 9.27 ± 2.19; controls 10.40 ± 2.33; mean ± standard deviation (STD); $p = 0.042$; paired t-test Bonferroni-Holm correction) and in DMN (**Fig. VI.2B;** patients 5.73 ± 1.44; controls 6.75 ± 1.38; mean ± STD; $p = 0.00081$; paired t-test Bonferroni-Holm correction). There were also many differences in functional connectivity among the individual members of SN (**Fig. VI.2C**) and DMN (**Fig. VI.2D**) when comparing patients and controls. Overall, these results indicated decreased connectivity within two of the three ICNs we examined.

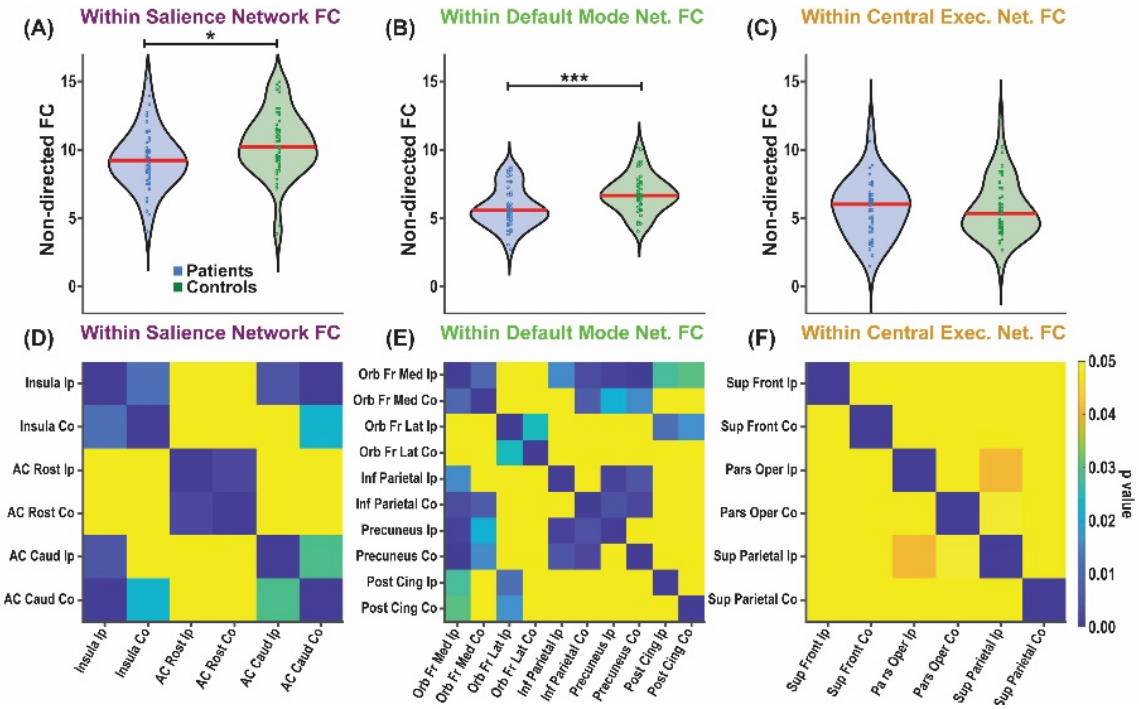

Figure VI.2 Patients with TLE exhibit decreased non-directed functional connectivity within ICNs as compared to controls.

On the y-axis (**A-C**) is average non-directed functional connectivity (Fisher z transformed Pearson correlation) between all components of each ICN. In patients with TLE average connectivity within SN (**A**) and within DMN (**B**) were decreased as compared to controls. We detected no differences within CEN connectivity in patients as compared to controls (**C**). Paired t-test, Bonferroni-Holm correction. *p < 0.05, **p < 0.01, ***p < 0.001. Red line shows the median. (**D-F**) Each location value in the figure shows the p-value (t-test, uncorrected) comparing patients and controls for every connection between all members of each network. The diagonals (**D-F**) represent connectivity within an individual brain region and were set to zero. N = 50 patients with TLE and 50 healthy controls. AC: anterior cingulate; Caud: caudal; Cing: cingulate; Co: contralateral; Exec: executive; FC: functional connectivity; Fr: frontal; ICN: intrinsic connectivity network. Inf: inferior; Ip: ipsilateral; Lat: lateral; Med: medial; Net: network; Oper: opercularis; Orb: orbital; Post: posterior; Rost: rostral; SN: salience network; Sup: superior; and TLE: temporal lobe epilepsy.

Next, we examined connectivity between arousal structures and each of the ICNs. We found that average connectivity between all arousal structures and entire salience network was decreased in patients (1.12 ± 1.03) versus controls (2.04 ± 1.27; p = 0.00014, paired t-test; Bonferroni-Holm correction). To further dissect this abnormal relationship, we inspected connectivity between individual arousal structures and the whole salience network. We found that non-directed functional connectivity between PPN and SN (**Fig. VI.3A**) was lower in patients with TLE (-0.01 ± 1.48) compared to controls (1.09 ± 1.70, p = 0.0036, paired t-test, Bonferroni-Holm correction). We also observed that connectivity between CM ipsilateral to the epileptogenic side of the brain

and SN (**Fig. VI.3B**) was lower in patients (1.55 ± 1.79) compared to controls (2.82 ± 2.00, p = 0.0028, paired t-test, Bonferroni-Holm correction). Although in prior studies we noted that CL exhibited altered non-directed functional connectivity with posterior quadrant regions in TLE,[283,311] here we detected no differences in connectivity between either CL and the SN (not shown; p > 0.05, paired t-test, Bonferroni-Holm correction). Last, we examined NBM connectivity with SN. We were particularly interested in these relationships because we had previously observed NBM may be one of the most perturbed network nodes in patients with TLE.[305] Here, we found that ipsilateral NBM (**Fig. VI.3C**), had lower non-directed functional connectivity with SN in patients (1.24 ± 1.82) compared to controls (2.51 ± 2.16, p = 0.015, paired t-test, Bonferroni-Holm corrected). Notably, only CM and NBM ipsilateral to the epileptogenic temporal lobe displayed significantly lower connectivity with SN, suggesting greater pathological impact on arousal structures ipsilateral to seizure onset zone.

Additionally, previous work has indicated that abnormal connectivity among ICNs may be related to neurocognitive problems,[307] so we asked if abnormal connectivity between arousal structures and ICNs may impact neurocognition. We found that more abnormal non-directed connectivity between ipsilateral NBM and SN was related to worse performance on processing speed index (r = 0.351, p = 0.033, Pearson correlation, uncorrected, N = 37). We also found that lower connectivity (further from healthy controls) between PPN and SN was associated both lower full-scale IQ (r = 0.296, p = 0.043, N = 47, Pearson correlation, uncorrected) and associated with lower verbal comprehension index (r = 0.350, p = 0.015, N = 48, Pearson correlation uncorrected). This suggests that neurocognitive deficits in TLE may be related to dysfunctional relationships between arousal structures and ICNs.

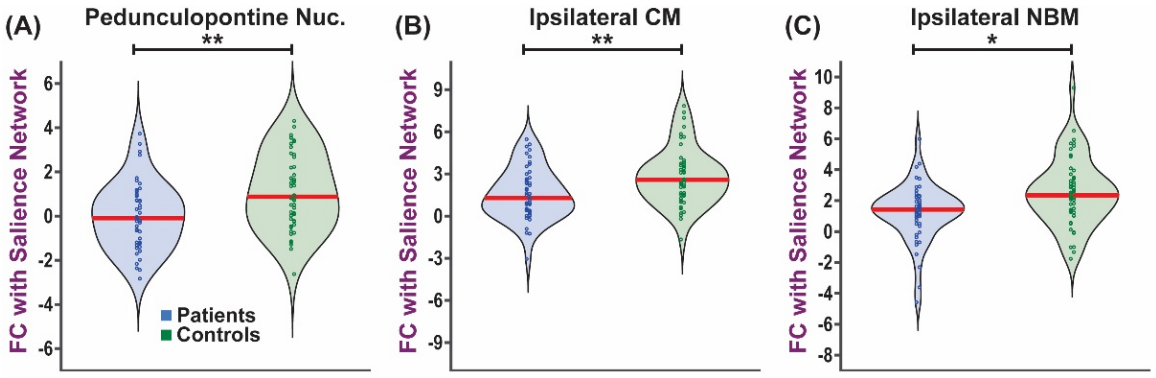

Figure VI.3 In patients with TLE salience network exhibits decreased non-directed connectivity decreases with subcortical arousal structures.
On the y-axis in each panel (**A-C**) is non-directed functional connectivity (Fisher z transformed Pearson correlation) between the SN and three arousal structures. In patients with TLE PPN (**A**), CM ipsilateral to seizure onset zone (**B**), and NBM ipsilateral to seizure onset zone (**C**) all have decreased connectivity with the SN. Paired t-test, Bonferroni-Holm correction. *p < 0.05, **p < 0.01, ***p < 0.001. Red line shows the median. N = 50 patients with TLE and 50 healthy controls. Exec.: executive; FC: functional connectivity; ICN: intrinsic connectivity network; Net: network; Nuc.: nucleus; PPN: pedunculopontine nucleus; SN: salience network; TLE: temporal lobe epilepsy.

VI.2.3.3 Core Network of Nodes with Decreased Connectivity in TLE is Composed Primarily of Nodes from Arousal Structures, Salience Network, and Default Mode Network

Next, we used network-based statistic to determine which nodes among the arousal structures and ICNs exhibited key decreases in TLE. At $t > 2.7$, the network-based statistic identified one network of nodes with decreased connectivity that included of 21 nodes and 32 edges. We found that 18 of the 21 nodes in this key network of decreased connectivity were members from either arousal structures, SN, or DMN, and all but one member of the SN were in included. More specifically, 7/32 (21.8%) edges in this network involved differences in connectivity between arousal structures and SN structures, and 6 of those edges involved ipsilateral NBM, PPN, and ipsilateral CM which individually exhibited decreased connectivity with average SN. Additionally, 3/32 (9.3%) edges in this network were within SN connections, and above we found decreased within connectivity in SN in patients with TLE. Likewise, 8/32 (25%) edges were connections within the DMN which also displayed decreased average connectivity in patients as shown above. Overall, these findings with the network-based statistic provide additional evidence that functional connectivity between arousal structures and ICNs is decreased, and when examining connectivity between and within ICNs, SN and DMN show marked disturbances in TLE.

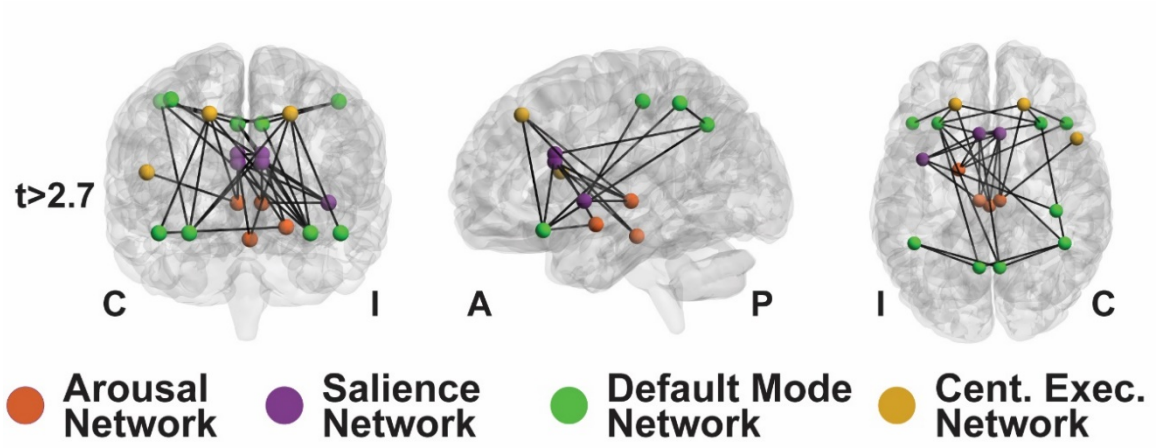

Figure VI.4 In patients with TLE, a key network of decreased connectivity includes nodes from the arousal network, SN, and DMN.
The network-based statistic reveals a network of nodes and edges that are decreased in patients with TLE as compared to controls. This component network was tested at t-statistic $t > 2.7$ and the network contained 21 nodes and 32 edges. Network-based statistic was performed at $p < 0.001$ with family-wise error correction. $N = 50$ patients with TLE and 50 healthy controls. A: anterior; C: contralateral to epileptogenic temporal lobe; Cent: central; Exec: executive; I: ipsilateral to epileptogenic temporal lobe; P: posterior.

VI.2.3.4 Patients with TLE May Have Lost Directed Connectivity Influence from ICNs to Subcortical Arousal Structures

We also wanted to determine if patients with TLE demonstrated altered directed connectivity influence between subcortical arousal structures and ICNs. To measure this, we calculated Granger causality laterality index (GC_{LI}). In this context, a positive GC_{LI} indicates greater influence from arousal structures to ICNs, and a negative GC_{LI} demonstrates greater influence from ICNs to arousal structures. We first examined if average GC_{LI} between arousal network, SN, DMN, and CEN was abnormal. As we found above with non-directed connectivity, only GC_{LI} between arousal structures and SN (**Fig. VI.5A**) was more positive in patients (-0.095 ± 0.21) versus controls (-0.26 ± 0.24, $p = 0.0012$, paired t-test, Bonferroni-Holm corrected). We then examined GC_{LI} between individual arousal structures and salience network. We observed that GC_{LI} between PPN and SN (**Fig. VI.5B**) was less negative in patients (-0.05 ± 0.37) versus controls (-0.33 ± 0.37, $p = 0.0028$, paired t-test, Bonferroni-Holm corrected). These findings suggest that while healthy controls have mostly negative GC_{LI} between PPN and SN, indicating predominantly top-down influence to the PPN, patients have approximately equal influence between these structures, with GC_{LI} values approaching zero.

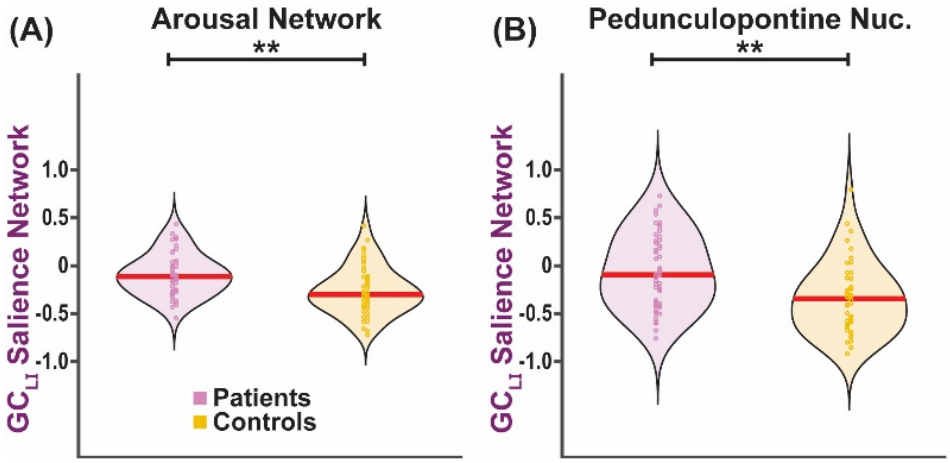

Figure VI.5 Direction of connectivity influence between arousal structures and SN is lost in patients with TLE.
On the y-axis in each panel (**A-B**) is directed functional connectivity as measured by Granger causality laterality index (GC_{LI}) between (**A**) average arousal structures and SN or between (**B**) PPN and SN. Positive values of GC_{LI} indicate greater directed connectivity influence from arousal structures to ICNs. Negative values of GC_{LI} indicate greater influence from ICNs to arousal structures. We found that in healthy controls SN exhibits influence on average arousal structures in general (**A**) and PPN specifically (**B**). However, in patients this directionality of influence is lost with GC_{LI} values approaching zero. Paired t-test, Bonferroni-Holm correction. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Red line shows median. N = 50 patients with TLE and 50 healthy controls. GC_{LI}: Granger causality laterality index; ICN: intrinsic connectivity network; Nuc.: nucleus; PPN: pedunculopontine nucleus; SN: salience network; TLE: temporal lobe epilepsy.

VI.2.3.5 Some Non-Directed Functional Connectivity Between Arousal Structures and ICNs Recovers After Epilepsy Surgery

We were interested in seeing if pre-operative abnormal connectivities between arousal structures and ICNs recovered (moved towards healthy control values) after surgery. We examined the post-operative directed and non-directed connectivity in 29 patients with post-operative fMRI ≥ 1-year after surgery. Of the abnormal connectivities we found in pre-operative patients, we found two that recovered in post-operative patients with TLE. First, we found that non-directed connectivity between arousal structures and SN (**Fig. VI.6A**) in post-operative patients (1.54 ± 1.08) was not significantly different from that of controls (2.08 ± 1.30, $p = 0.21$ ANOVA, post-hoc correction Tukey's Honest Significant Difference Criteria (HSD)) after surgery, suggesting a trend towards recovery. When we examined post-operative connectivity between individual

arousal structures and SN, we found that non-directed connectivity of the NBM ipsilateral to seizure onset zone with the SN (**Fig. VI.6B**) in post-operative patients (2.03 ± 2.20) was not distinguishable from connectivity of healthy controls (2.95 ± 2.20, p = 0.23 ANOVA, post-hoc HSD).

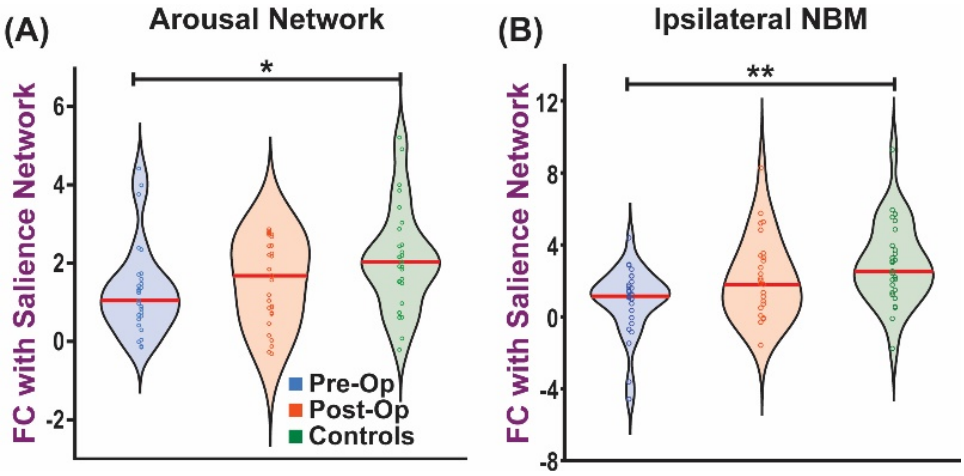

Figure VI.6 After surgery non-directed connectivities between arousal structures and SN recovers in patients with TLE.
On the y-axis in panels (**A**) and (**B**) is non-directed functional connectivity (Fisher z transformed Pearson correlation) between the arousal structures (**A**) or the NBM ipsilateral to the epileptogenic side of the brain (**B**) and SN. We found that non-directed connectivity of both average arousal structures and ipsilateral NBM with SN after surgery was not different from healthy controls. ANOVA, multiple correction with HSD. *p < 0.05, **p < 0.01, ***p < 0.001. N=29 patients with TLE with ≥ 1-year post-operative and 29 healthy controls. FC: functional connectivity; NBM: nucleus basalis of Meynert; Pre-Op: pre-operative patients with TLE; Post-Op: post-operative patients with TLE; TLE: temporal lobe epilepsy.

VI.2.3.6 Although Vigilance Decreases in Patients With TLE, FMRI Connectivity Disturbances of Arousal Structures Are Stable

Last we wanted to determine if problems with vigilance known to plague patients with TLE may affect resting-state functional connectivity of subcortical arousal structures. To obtain preliminary insight into this question we calculated "alertness index," an fMRI only measure to monitor vigilance developed by our collaborators.[313] We found no differences in alertness index between patients and controls in either fMRI 1 or fMRI 2 (p > 0.05 for both, paired t-test, uncorrected). This suggested that overall, there were no differences in vigilance between patients and controls during the duration of our fMRI acquisition. We then wanted to determine if either participant group (patient or controls) had decreased vigilance in their second fMRI acquisition,

fMRI 2, as compared to their own first fMRI acquisition, fMRI 1. We found that patients (**Fig. VI.7A**) had decreased alertness index in fMRI 2 as compared to the fMRI 1 ($p = 0.024$, paired t-test, Bonferroni-Holm corrected). Controls (**Fig. VI.7B**) did not have decreased alertness index in the fMRI 2 versus fMRI 1 ($p = 0.43$, paired t-test, Bonferroni-Holm corrected).

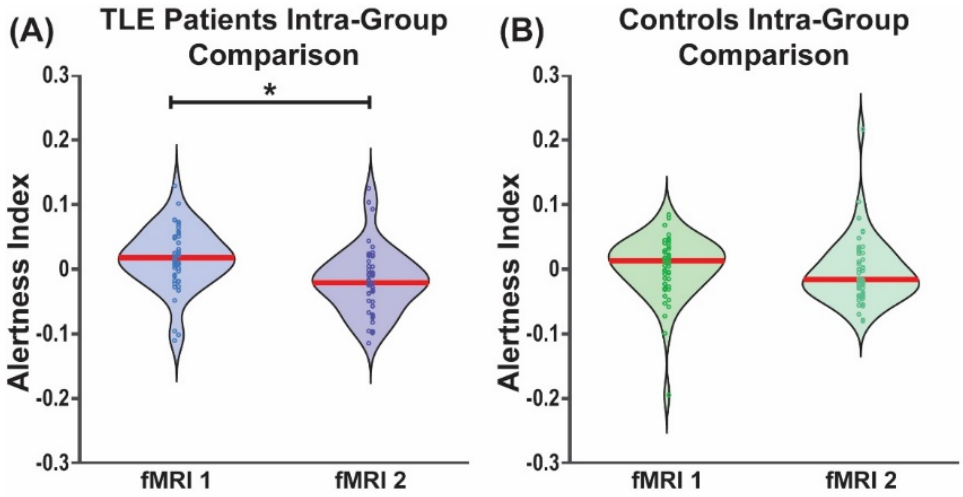

Figure VI.7 Patients, but not controls, have decreased alertness in the second fMRI acquisition (fMRI 2) as compared to the first acquisition (fMRI 1).
On the y-axis is the alertness index which ranges from -1 to 1. On the x-axis, fMRI 1 indicates the first 10-minute resting-state acquisition, and fMRI 2 indicates the second 10-minute resting-state acquisition. (**A**) Patients with TLE display decreased alertness in fMRI 2 as compared to fMRI 1. (**B**) Controls exhibit no difference in alertness index over the course of the experiment. *$p < 0.05$, paired t-test, Bonferroni-Holm corrected. N = 50 patients with TLE and N = 50 healthy controls.

Last, we then asked whether this vigilance decrease in patients with TLE may impact the resting-state directed and non-directed functional connectivity differences discussed above. To answer this question, we conducted intra-group comparisons of functional connectivity in fMRI 1 as compared to fMRI 2. We were able to detect no differences in either patients or controls when comparing fMRI 1 and 2 for non-directed or directed functional connectivity values of interest ($p > 0.05$ for all, paired t-tests, uncorrected). To summarize, these findings suggest that patients with TLE, but not controls show decreased vigilance over a relatively short 20-minute time interval. Conversely, connectivity abnormalities of subcortical arousal structures remained stable, despite changes in vigilance in patients with TLE.

VI.2.4 Discussion

In this study, we found that in patients with TLE there exist functional connectivity disturbances in subcortical arousal structures and ICNs. First, we found that both DMN and SN exhibited decreased within network non-directed connectivity in patients as compared to controls. This is significant as these structures represent two-thirds of the triple-network of ICNs, whose abnormalities have repeatedly been shown to be associated with psychiatric and neurological disease.[307] We also observed that in patients, as compared to healthy controls, PPN, CM, and NBM all exhibited perturbed non-directed functional connectivity with SN that was worse on the side of seizure onset. Worse connectivity with DMN on the side ipsilateral to the EZ was also found in a prior study of unilateral mTLE that observed decreased connectivity between mesial temporal structures (hippocampus and amygdala) and DMN that were worse on the side ipsilateral to the EZ.[56] However, our study appears to be the first to show not only abnormal connectivity between arousal structures and ICNs in TLE, but also suggests that worse connectivity ipsilateral to EZ extends to these arousal structures. Additionally, abnormal connectivity between arousal structures and ICNs was also moderately associated with worse neurocognitive performance. The association between perturbed arousal connectivity and impaired neurocognition is supported by our prior work,[2,254] and other studies have shown association between abnormalities in ICNs and neurocognitive deficits in TLE and other diseases.[180]

We also found that arousal structures exhibited abnormal directed connectivity (Granger causality laterality index) with the SN in patients with TLE. Specifically, in patients, both PPN and NBM connectivity showed a loss of influence from SN to arousal structures. Traditionally, we think of arousal structures projecting to ICNs, so why did we find "healthy" directed connectivity is greater from ICNs to arousal structures? Prior studies of cholinergic modulation (NBM and PPN both contain broadly projecting cholinergic neurons[5,74,324]) found that the direction of cholinergic modulation of sensory cortex, frontoparietal regions, and task positive networks is actually dependent on top-down influences (e.g. from ICNs to arousal structures).[325] These studies also found associations between poor memory encoding and abnormal cholinergic stimulation of neocortex and hippocampal regions.[325] This reinforces the association between impaired neurocognitive performance and non-directed connectivity abnormalities of these cholinergic regions.

Another question addressed in this work was how these connectivity relationships are affected by epilepsy surgery. We had previously found that connectivity between subcortical arousal structures and certain neocortical regions recovered, moved towards healthy control values, after successful epilepsy surgery.[2,283,311] In this study we examined connectivity in all patients after surgery, regardless of surgical outcome, and found that key non-directed functional connectivity abnormalities recovered after surgery. However, future work should include analyses that account for surgical outcome. In preliminary studies, not shown here, within this patient cohort, we limited analyses to 22 patients with Engel 1 or Engel 2 outcomes. In these patients with well controlled seizures after surgery we found both directed and non-directed connectivity between ipsilateral NBM and SN shows more marked recovery with strong differentiation from pre-operative values. The connection between surgical outcome and arousal connectivity is not fully understood. For example, a study by Maccotta et al. that examined effects of epilepsy surgery on ICNs in patients with TLE, observed no recovery of connectivity.[154] Another study, showed that in patients with mTLE after epilepsy surgery, DMN connectivity increases to the remaining hippocampus and that this correlated with postsurgical performance on a memory task.[180] No other studies, to our knowledge, have examined the interactions of arousal structures with ICNs, making our observations unique in the field. Our early findings, that there exists some recovery of connectivity with ICNs after surgery, posits the question how these connectivity improvements may relate to symptomatic improvement including neurocognitive performance. Clinical studies have shown that patients who experience seizure-freedom after surgery reap neurocognitive benefits as well.[326] Therefore, future studies, to determine whether postoperative neurocognitive improvement correlates with recovery of arousal-ICN connectivity are needed.

Although other studies have examined connectivity within ICNs in patients with TLE,[55,57,58,180,306] there is incomplete knowledge about how these networks may be modulated by subcortical arousal structures in TLE. Blumenfeld et al, showed that the DMN has decreased activity as measured by fMRI and electrophysiological data during FICS, FBTCS, absence seizures, and generally seizures with loss of consciousness.[327] This ictal decreased activity within ICNs can be explained by the network inhibition hypothesis, originally proposed by Blumenfeld.[46,204] We have recently expanded upon the network inhibition hypothesis in prior work that showed interictal abnormal connections of subcortical arousal structures in patients with TLE.[2,254,283,305,311] Some of these structures that we have previously examined such as the

pedunculopontine nucleus (PPN), nucleus basalis of Meynert (NBM), and intralaminar thalamic nuclei are known to anatomically project to members of these ICNs.[328] Subcortical arousal structures are known to modulate alertness, and abnormal alertness in patients with TLE may be associated with some neurocognitive deficits. Although it is known that patients with TLE have problems with arousal and alertness, there have been no studies to date that have examined how changes in alertness may affect connectivity of subcortical arousal structures. Here, in a preliminary assessment of this question, we found that connectivity between subcortical arousal structures and ICNs did not change with changes in alertness index. These initial findings, suggest that abnormal connectivity of arousal structures are long-term resting-state abnormalities that are not modulated by short-term changes in vigilance.

Finally, how might knowledge of abnormal connectivity between arousal structures and ICNs be used clinically? Not all patients with focal epilepsy are candidates for resective surgery. In these individuals, treatment with neuromodulation can yield significant improvements in quality of life, decreased risk of sudden unexpected death in epilepsy (SUDEP), and improved neurocognition;[3,22,24] despite the low probability of complete seizure freedom. Interestingly, a case study showed that vagus nerve stimulation balanced perturbed relationships between DMN and SN in a patient who failed resective epilepsy surgery.[329] Some studies have shown that the modulation of activity in the insular cortex, a SN structure, is possible both via vagus nerve stimulation and stimulation of the PPN.[206] Given the association we and others have found between neurocognitive impairment in TLE and abnormal connectivity involving ICNs, neuromodulation treatments targeting these networks via arousal structures may be particularly beneficial in treating neurocognitive comorbidities of TLE.[330]

This study has some limitations to discuss. First, we do not know whether patients were having interictal spikes or inter-epileptic discharges during their fMRI acquisition, nor do we know how this might have affected our results. Prior simultaneous EEG-fMRI studies in patients with TLE have shown that there may be alterations in connectivity within members of the DMN, SN, and dorsal attentional network immediately prior to spikes.[306] Furthermore, the alertness index we employed was developed in a group of healthy controls with simultaneous EEG-fMRI.[313] This is the first study to examine how this awareness index generalizes to TLE patients; unfortunately, we did not have an independent measure of arousal available. Future studies with concurrent

measurement of EEG and fMRI could address both effects of interictal spikes and accuracy of vigilance template simultaneously. Another limitation, is that our patients are on varying amounts of antiepileptic medications, and it is incompletely understood how these drugs may affect functional connectivity and awareness. It is well established that antiepileptic drugs have neurocognitive and arousal side effects.[331,332] However, prior research found that neither psychomotor slowing nor attentional deficits in TLE can be fully accounted for by medication side effects alone. Additionally, these symptoms are found in patients not on antiepileptic medications.[326,332,333] This suggests that the changes in alertness index and functional connectivity we observed in patients cannot be solely explained by medication effects. We believe these limitations represent interesting areas for future research.

VI.2.5 Conclusions

Patients with TLE exhibit altered directed and non-directed functional connectivity between subcortical arousal structures and ICNs. In particular, abnormal connectivity between arousal structures and SN was associated with worse neurocognition, however, this may represent an opportunity for treatment of neurocognitive comorbidities of TLE with neuromodulation. Additionally, we found preliminary evidence that perturbed connectivity of arousal structures are long-term pathological consequences of TLE, not affected by short-term changes in vigilance. Connectivity between subcortical arousal structures and ICNs should be further studied to gain further insight into broad comorbidities of TLE including problems with arousal and neurocognition and potentially develop novel therapeutic targets for TLE.

Chapter VII

VII Future Directions and Conclusions

VII.1 Summary

This thesis expands upon prior work, that explained global ictal deficits of this focal epilepsy, by examining how abnormal connectivity of subcortical arousal structures may relate to interictal effects of TLE and how these networks respond to epilepsy surgery. In this work, the central hypothesis was that reduced neocortical connectivity in TLE is caused by recurrent consciousness-impairing seizures that over time engender perturbed connectivity between subcortical activating structures and neocortical regions. This may result in decreased neocortical activity contributing to impaired neurocognitive abilities in TLE. First, we examined brainstem ARAS RSFC in pre-operative and post-operative patients with TLE (Chapter III). Next, we used a novel participant-specific atlas of thalamic nuclei to delineate thalamic arousal network RSFC in TLE before and after epilepsy surgery (Chapter IV). Then we used advanced network analyses to examine RSFC of the NBM with fMRI in patients with TLE (Chapter V). Last, we examined how RSFC abnormalities of subcortical arousal structures may influence RSFC of intrinsic connectivity networks in patients with TLE (Chapter VI). This work showed for the first time in human TLE abnormal connectivity of subcortical arousal networks, recovery of abnormal arousal network connectivity after epilepsy surgery, and associations between abnormal arousal connectivity, disease severity, and neurocognitive impairments.

VII.2 Limitations and Future Directions

These studies increased understanding of perturbations of subcortical arousal structure functional connectivity in TLE and how they relate to global interictal deficits of this focal disorder. However, there are certain limitations in these studies which may serve as guidance for future investigations.

VII.2.1 How Does Post-Operative Functional Connectivity Relate to Neurocognitive Improvement?

In this thesis, we found that after patients with TLE underwent epilepsy surgery, their functional connectivity of brainstem ARAS, thalamic nuclei, and NBM often moves towards values resembling healthy controls. This was most often true in patients who achieved improved seizure control with surgery.[2,283,311] The intent in performing these experiments was to determine how pre-operative connectivity perturbations would be affected by epilepsy surgery. In other words, would epilepsy surgery cause arousal connectivity networks to move further from healthy control values or recover by moving towards healthy control values? Interestingly, we found that greater post-operative connectivity recovery was often associated with worse pre-operative neurocognitive deficits, higher pre-operative seizure frequency, and better post-operative seizure control.[2,283,311] Our findings from these experiments reinforced our hypothesis that repeated seizures engender pre-operative connectivity differences of subcortical arousal structures. However, these studies did not elucidate in detail how connectivity after epilepsy surgery might relate to non-seizure outcomes after surgery.

Future studies could expand upon this dissertation by performing a more in-depth examination of the relationship between post-operative arousal structure connectivity and post-operative neurocognitive performance. Based on the results of this dissertation, we hypothesize that neurocognitive outcomes after epilepsy surgery will be accompanied by recovery of functional connectivity which can be quantified with changes in fMRI. For example prior studies in patients with mTLE after epilepsy surgery have shown that DMN connectivity increases to the remaining hippocampus and that this correlated with postsurgical performance on a memory task.[180] We expect that functional connectivity of arousal networks will recover after surgery and that this may be related to improvements in neurocognitive domains not related to mesial temporal lobe such as attention and executive function. Future longitudinal studies comparing changes in post-operative neurocognitive performance with post-operative connectivity in patients who do and do not achieve seizure-freedom could test this hypothesis. The execution of this experiment requires repeat post-operative resting-state fMRI scans with paired repeat neurocognitive testing. Repeat longitudinal testing is vital to achieve an accurate assessment of post-operative neurocognitions as some neurocognitive measures in those who achieve seizure-freedom worsen temporarily after surgery, but then may improve as compared to pre-operative performance.[334] Practical

considerations in these experiments would include the length of these additional evaluations and the length of time after epilepsy surgery necessary to capture these long term effects. Despite the difficulty in gathering this patient data, these studies would allow us to determine how changes in post-operative subcortical arousal structure connectivity relates to longitudinal changes in neurocognitive outcomes.

VII.2.2 How Does Neuromodulation Surgery for Epilepsy Impact Functional Connectivity of Arousal Structures?

Another hypothesis that was tested in this work was that seizure-freedom after epilepsy surgery results in recovery of arousal structure functional connectivity resembling that of healthy controls. The post-operative studies in this thesis, however, were limited to individuals who underwent resective epilepsy surgery. This means that patients who received neuromodulation-based treatments were not included in our post-operative investigations. Generally, these include patients that despite careful presurgical evaluation, still fail to achieve seizure freedom or significant seizure reduction post resection.[97] In these individuals and those who did not qualify for resection, neuromodulation though rarely resulting in seizure freedom, remains an excellent treatment option that can yield substantial benefits.[139,335] Despite the low probability of seizure freedom, individuals treated with neuromodulation have shown decreased seizure frequency, lower risk of SUDEP, possible improvements in quality of life (QOL), and improved neuropsychological performance.[3,22,24,155,160,162,336] Options for neuromodulation in epilepsy surgery include: vagus nerve stimulation (VNS), responsive neurostimulation (RNS), and deep brain stimulation (DBS). Neuromodulatory effects regarding both seizure control and treatment of comorbidities of these treatments are incompletely understood and may involve modulation of subcortical arousal system functional connectivity, and therefore merit further investigation. A compelling further study, building upon the existing work presented in this thesis, is expanding the connectivity studies to patients who receive neuromodulation epilepsy surgery rather than resection.

A simplified interpretation of the effects of neuromodulation can be split into two temporal scales: 1) acute response to stimulation (for example termination of detected seizures in RNS) and 2) chronic modulation of the network (> 1-year post implantation). Unlike in neuromodulation of movement disorder where the most obvious effects fall into that first category, most neuromodulatory treatments of epilepsy display their benefits over a long period of time, falling

into the second chronic category. Both DBS and RNS have improved efficacy over time.[23,160] A recent study of RNS suggested this longitudinal improvement of efficacy is tied to two effects from stimulation: direct and indirect. The direct effects included ictal inhibition, but only the indirect effects, those occurring distant from stimulation, were associated with clinical benefit.[23] Therefore, an interesting experiment would be to perform a longitudinal study periodically evaluating these indirect effects of neuromodulation on RSFC of subcortical arousal structures and compare these arousal network connectivity changes to improvements (or lack thereof) in seizure frequency and comorbidities. A possible timeline for scans in this experiment would be (1) 1st scan concurrent with initial testing of stimulation, (2) 6-months post implantation, (3) 1-year post implantation, and (4) 2-years post implantation.

This study would be most interesting on intracranial neuromodulation, either RNS or DBS, as there may be the least understood about their complete mechanism of action in treatment of epilepsy. However, currently RNS was only recently approved for 1.5T MRI conditional labeling.[337] Therefore, DBS for epilepsy would be the most feasible initial neuromodulation treatment to evaluate in an initial investigation. Speaking towards the feasibility of such a study, similar investigations examining the effects of DBS on networks in movement disorders using fMRI have already been conducted.[338-340] Additionally, it has been shown in a phantom and *in vivo* study that DBS both on and off can be studied with 3T MRI for a broad range of scan types including T1-MPRAGE, T2*EPI, DTI, SWI, ASL and T2 TSE.[341] The phantom study showed that temperature increases were safe (< 1° Celsius), the DBS exhibited minor deviations in voltage (< 3 volts), and had safe specific absorption rates (SAR). FMRI study of DBS for epilepsy is safe and would allow us to examine indirect modulation of subcortical arousal structures.

The timing of the proposed scans would allow us to address certain questions. The object of the first fMRI, at time point 1, would be to evaluate how functional connectivity of arousal structures is or is not immediately impacted by acute neuromodulation. This would allow comparison to long-term cumulative effects of neuromodulation on the brain networks. To avoid interfering with initial clinical programming of the device, this first scan could be performed intra-operatively. Many institutions perform placement of their DBS in anesthetized patients under intraoperative MRI which creates an opportunity to obtain an fMRI evaluating the acute effects of neurostimulation on the arousal network during a type of impaired consciousness. Multiple studies

have shown the feasibility of intraoperative resting-state fMRI.[342-344] An immediate post-operative awake fMRI would establish a baseline awake resting-state against which to compare future awake resting-state scans. All subsequent fMRI scans would enable the quantification of chronic neuromodulation effects on the brain networks, specifically those that involve the arousal structures studied in the body of this work. Not only would such a study address the limited patient population (those who underwent resection), but also would possibly begin to clarify the different mechanisms of action that occur between resective and neuromodulatory epilepsy surgery in a broader sense.

VII.2.3 How Do Subcortical Arousal Network Abnormalities in Temporal Lobe Epilepsy Compare to Arousal Network Abnormalities in Other Epilepsies?

This dissertation examined functional connectivity of arousal structures in patients with unilateral drug resistant mesial TLE undergoing evaluation for epilepsy surgery. They represented an ideal first population for in which to perform the first ever set of analyses in people with epilepsy on connectivity of subcortical arousal structures before and after surgery for multiple reasons. First, these individuals have a single, unilateral, nearly uniform seizure onset zone in mesial temporal limbic structures (amygdala/hippocampus). Second, TLE is the most common form of epilepsy, meaning that we had a large population to draw from. Furthermore, the surgical treatment of these individuals is successful in about two-thirds of patients, and the majority of patients with unilateral TLE who receive surgery at our institution undergo either temporal lobectomy or selective-amygdalohippocampectomy.[95] This enables our post-operative studies to be performed on a relatively homogenous population. However, it should be noted that even in this cohort with relatively comparable individuals there exists heterogeneity that was not fully studied in this thesis. Examples of this heterogeneity include presence of mesial temporal sclerosis on MRI, history of status epilepticus, and various surgical pathology findings. For example, in our population about 75% of individuals who underwent resective surgery had pathology findings consistent with hippocampal sclerosis. Other studies have shown that 54.4% of specimens from temporal lobe epilepsy surgery exhibited hippocampal sclerosis.[345] While we selected this patient population for our investigations, only studying this subset of epilepsy patients is a notable limitation of this work. There are other forms of epilepsy that we did not examine, including other focal epilepsies such as focal neocortical epilepsy and generalized epilepsies like juvenile myoclonic epilepsy.[346]

Studying these other epilepsy syndromes could provide broader insight into arousal network abnormalities in epilepsy in general.

By expanding the patient population to other forms of epilepsy that are treatable by surgery, two potential simultaneous future studies become apparent. First, similarities and differences between arousal connectivity in patients with TLE versus connectivity differences in other forms of epilepsy can be quantified. Second, using these comparisons, there is the potential for using resting-state fMRI to delineate between different epilepsy types, which can aid in surgical planning.

Beginning with the first study, other groups have performed some studies providing initial evidence of arousal structures abnormalities in other types of epilepsy. One study of patients with juvenile myoclonic epilepsy (JME) found that ARAS functional connectivity is increased in JME.[347] As opposed to our findings of decreased connectivity, this group found that posterior thalamus, locus coeruleus, and mesencephalic reticular formation exhibited hyperconnectivity in JME patients. Another group examining new-onset JME in a pediatric population for dissociation between subcortical regions (including the brainstem) and cortical regions.[348] In order to execute such a study, resting-state fMRI scans of patients with other epilepsy presentations would need to be collected pre- and post-operatively. One potential difficulty in studying these groups, however, is their lack of uniformity which makes it difficult to perform group-wise analysis. This would most likely require a larger patient population to adequately compare types of epilepsy and would most likely necessitate cooperation between multiple institutions to gather sufficient data. Based on our results in patients with TLE, we anticipate that there would be functional abnormalities in arousal structures in patients with other epilepsy types relative to controls, but these abnormal connectivities would exhibit different network properties relative to TLE.

Using the same data set, we could also address the second question of whether these arousal connectivity differences can be used to differentiate between different types of epilepsy. During current clinical practice for surgical evaluation patients undergo a battery of noninvasive and possibly invasive testing for epileptogenic zone localization. While some of this localization is specified (i.e. which one brain region is causing seizures), there are also larger questions being asked. These include if the seizures are bilateral versus unilateral and if they are mesial or neocortical. The potential to unveil network differences related to arousal structures between these

different classifications could provide improvement in quality of care for patients with epilepsy who are undergoing surgical evaluation.

VII.2.4 How are Neurocognitive Deficits and Abnormal Subcortical Arousal System Connectivity Related to Impaired Vigilance in TLE?

Throughout this work, we examined structures which may underlie global neurocognitive deficits in TLE that cannot be explained solely by abnormalities in the temporal lobe; these deficits include impairment of executive function, cognitive processing, attention, and concentration.[172-174] In this dissertation and in previous work, we have shown that there exist subcortical activating network connectivity abnormalities including the brainstem ascending reticular activating system (ARAS), basal forebrain, and intralaminar thalamus.[2,157,254,283,305] Additionally, we have found relationships between these arousal network changes and neuropsychological deficits.[2,38,44,45,254,283,305,311] We also know, from prior studies, that patients with TLE exhibit psychomotor slowing, excessive sleepiness, and difficulty with sustained attention.[304,349-351] The subcortical activating structures we have examined here are also involved in vigilance regulation.[49] Both vigilance and arousal refer to aspects of neocortical activation. Here, arousal most refers to the physiological sleep-wake cycle (or in terms used in this thesis: consciousness versus impaired-consciousness), and vigilance is exemplified by sustained attention.[352,353] Recurrent consciousness-impairing seizures in TLE may lead to the abnormal connectivity between subcortical activating structures and neocortex, which may engender vigilance deficits leading to impaired neurocognitive abilities in TLE. Below we will outline possible investigations which examine associations between vigilance, functional connectivity, and neurocognition in TLE may improve our understanding of global network deficits in TLE.

We can begin to assess these topics by employing objective measures of vigilance and arousal simultaneous to fMRI resting-state baseline and outside of the fMRI. In chapter VI, we showed a preliminary study using a measure of alertness that is based solely on fMRI activity.[313] However, the results in this chapter are limited, as the alertness index we employed was based off of simultaneous EEG-fMRI and was derived from brain activity of healthy individuals. Therefore, it is difficult to know how well this alertness template might generalize to patients with TLE without an experiment employing similar objective measures of arousal in the TLE population for confirmation. Additionally, problems with vigilance and excessive sleepiness can be assessed

outside of the scanner, to allow assessment of chronic problems in these domains for patients with TLE. By assessing functional neuroimaging, neurocognitive assessments, and measures of vigilance we may shed light on the role of subcortical activating networks in vigilance problems in TLE.

Specifically, first, we can measure extent of vigilance and arousal problems experienced by patients with TLE with neuropsychological evaluations. It is well established that patients with TLE experience progressive neurocognitive decline;[17,125,354] however, it is incompletely understood how these declines may interact with problems of vigilance and connectivity.[49] Patients with TLE exhibit increased daytime sleepiness and sleep-wake problems that suggest problems with subcortical activating structures.[355-357] We can evaluate daytime sleepiness by administering the Epworth sleepiness scale.[304,355-359] The Epworth sleepiness scale (ESS) is a questionnaire which measures daytime sleepiness and has been broadly validated.[360-362] Next, vigilance can be measured by the psychomotor vigilance task (PVT).[363] The most commonly used PVT is a ten-minute test that measures reaction time to random interval stimuli.[363] Last, we could evaluate neurocognitive domains and relate performance in these domains to vigilance. Particular neurocognitive deficits we would focus on would include deficits seen in TLE not explained by temporal lobe problems, such as attention, cognitive processing, and executive function.[88,123] Problems with these neurocognitive domains suggest impairment of frontoparietal regions or subcortical structures.[49] Evaluation with PVT, ESS, and neuropsychological testing would enable objective measurement of the severity of vigilance deficits in patients with TLE. These measures could then be related to functional connectivity.

Next, simultaneous measurement of fMRI with measures of vigilance would allow discrimination of arousal connectivity networks that are modulated by changes in vigilance. There are multiple ways to dynamically evaluate arousal while acquiring fMRI. These include eye tracking, galvanic skin response, heart rate, respiratory rate, and EEG.[323,364-366] EEG is the gold-standard for non-invasive measurement of vigilance, and therefore would be the first-choice for this experiment.[367] Clinically EEG has long been used to establish wakefulness through NREM and REM sleep stages.[368,369] Broad categories of arousal that are well established by EEG changes include low amplitude desynchronized EEG, such as beta rhythm, often seen during active cognitive engagement. Awake relaxed with eyes-closed leads to appearance of a posterior-

dominant alpha rhythm on EEG.[222,226,370-372] As patients become drowsier delta and theta power become more prominent.[104] Specifically, we would want to evaluate functional connectivity of subcortical activating structures, like those we studied in this thesis, simultaneous to these independent objective vigilance measures. We would expect that connectivity of some arousal networks will fluctuate with changes in vigilance, while other connectivity networks will not. For example, from our results in Chapter VI, we might expect that connections between arousal regions and the salience network will move away from healthy controls during decreased vigilance but move towards healthy control values with increased vigilance. Acquiring resting-state fMRI along with simultaneous measures of vigilance state could allow determination of which fMRI arousal network connectivity is most associated with changes in vigilance and which remain unaffected by fluctuations in vigilance levels.

In prior work, it has been shown that studies combining electrophysiology, behavioral measures, and neuroimaging can evaluate changes in vigilance while tracking cortical regions and/or subcortical activating structures.[323] In non-human primates, it has been shown that pharmacological inactivation of nucleus basalis of Meynert, an important vigilance structure, engendered remarkable changes to cortical resting state fMRI.[78] To summarize, the experiments proposed here would integrate electrophysiology, neurocognitive evaluation, neuropsychological measures, and fMRI to improve our understanding of vigilance problems in TLE and their relation to abnormalities of subcortical arousal structures and neurocognition. These proposed future studies will allow us to better understand which arousal connectivity networks are modulated by vigilance changes. The results should elucidate relationships between specific neurocognitive function and vigilance measures in TLE. Finally, they will allow us to relate connectivity abnormalities to neurocognitive deficits in TLE.

VII.3 Conclusions

In this work we showed that there exist interictal abnormalities in functional connectivity networks of subcortical arousal systems in patients with TLE. While there were some limitations in our experiments, the limitations highlighted here lay the foundation for multiple intriguing future investigations. Longitudinal connectivity studies with reference to neurocognitive changes,

not only can further the understanding of long-term connectivity changes in response to epilepsy, but also can also elucidate if arousal connectivity could be a biomarker for patient response to epilepsy surgery. Resection is only a portion of the ever-expanding set of surgical options for patients with epilepsy and studying those who receive neuromodulation surgery could help better understand the mechanisms by which neuromodulation results in improved seizure control with longer duration of therapy. While patients with TLE represent the most common epilepsy syndrome, expanding the work in this dissertation to include other epilepsy syndromes can help answer numerous mechanistic and clinical questions regarding this disease. These future directions build upon the work in this thesis.

The central findings of this dissertation were that patients with TLE exhibit altered connectivity between deep arousal structures and the neocortex, which may be related to disease severity, seizure frequency, and neurocognitive impairments. We found that connectivity of some arousal structures, such as the nucleus basalis of Meynert, may be as abnormal as connectivity of mesial temporal epileptogenic structures. This suggests that disrupted arousal networks may be as central to the pathophysiology of TLE as seizure onset brain regions. We also found that some functional connectivity abnormalities in arousal structures move towards healthy control connectivity after successful epilepsy surgery. Post-surgical recovery of arousal structures may indicate that these regions are important nodes to target to treat global network deficits of TLE. Further study of subcortical arousal networks may help elucidate pathophysiological changes in epilepsy that affect broad neural networks and ultimately result in novel interventions to treat this disease.

Appendix

A.1 Examining Causal Functional Connectivity Between Subcortical Arousal Structures and Basal Ganglia

A.1.1 Introduction

In this work we examined functional connectivity between intralaminar thalamic nuclei and the basal ganglia, using a novel method of measuring "causal functional connectivity" of resting-state functional magnetic resonance imaging (fMRI). A recent study by He et al.[280] suggested that abnormal connectivity between basal ganglia and thalamus may disturb the inhibitory function of the basal ganglia which could then lead to focal to bilateral tonic clonic seizures in patients with temporal lobe epilepsy (TLE). In this study they particularly noted that ipsilateral medial dorsal thalamus had more abnormal connectivity in patients with a history of generalized seizures. However, intralaminar thalamic nuclei also project to the basal ganglia and we wondered whether three may be abnormal connectivity between these structures as well.

In this thesis we employed a few different techniques to study subcortical arousal structures in TLE by using resting-state fMRI to either examine the signal in an individual brain region or examine relationships of signals between regions of interest (ROIs). In the main experiments for this thesis, we performed both these types of analysis. For example, we examined amplitude of low frequency fluctuations (ALFF) in chapter III, which allowed us to examine activity in brainstem arousal regions and frontoparietal regions. ALFF measures fluctuations in BOLD fMRI signal for a region without relating it to other regions.[258] We also examined relationships between ROIS by calculating resting-state functional connectivity in chapters III-VI. Functional connectivity is defined as a statistical relationship between two neural signals and can broadly be divided into either directed or non-directed connectivity[168] In this work we primarily focused on non-directed connectivity by calculating bivariate functional connectivity. In Chapter VI we also calculated a measure of directed connectivity called Granger Causality.[321] In addition to the connectivity measures explored in the main body of this dissertation in this work we calculated the combinedFC value developed by Romero and Cole.[373] The combinedFC measures proposes to simultaneous calculate bivariate correlation and partial correlation in order to determine causal

connectivity between neural signals of interest. Specifically, combinedFC was developed to avoid three types of interpretation errors that hide the true causal mechanism in commonly used connectivity measures: confounders, chains, and colliders. In this work we applied combinedFC to examine the causal connectivity between intralaminar thalamic nuclei and basal ganglia in patients with TLE.

A.1.2 Examination of Subcortical FMRI Causal Connectivity Patterns in Temporal Lobe Epilepsy

This work was submitted to AES and accepted as poster.

González HFJ, Narasimhan S, Treuting RL, Johnson GW, Wills KE, Chang C, Morgan VL, Englot DJ. Examination of subcortical fMRI causal connectivity patterns in temporal lobe epilepsy. American Epilepsy Society; 2020 December; Seattle, WA, USA. (Virtual conference).

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A.1.2.1 Rationale

Temporal lobe epilepsy (TLE) is a focal epilepsy, yet it engenders broad connectivity abnormalities in arousal networks. We previously found that central lateral (CL) thalamic nucleus exhibits abnormal fMRI connectivity with occipital lobe in TLE. Intralaminar thalamic arousal nuclei like centre médian (CM) and CL project to the cortex and basal ganglia, particularly dorsal striatum (caudate and putamen).[13,199] While others have shown disrupted basal ganglia-thalamic connectivity in TLE,[280] to our knowledge none have examined connectivity between intralaminar nuclei and basal ganglia. Most resting-state functional magnetic resonance imaging (rsfMRI) studies utilize connectivity measures that cannot detect directed connections or casual interactions. Here, we applied a novel method of measuring causal connectivity[373] of thalamic nuclei (CM and CL) and basal ganglia, and anticipated that causal connectivity of these structures would be decreased in TLE.

A.1.2.2 Methods

We acquired rsfMRI in 53 patients (29 female, 39 ± 12 years) with unilateral TLE and 53 controls (29 female, 39 ± 12 years). FMRI was corrected with RETROICOR and standard preprocessing as in previous studies.[254] We obtained participant-specific brain segmentations based on the Desikan-Killiany atlas for basal ganglia regions and a participant-specific active-shape model for thalamic nuclei.[285,314] We used these segmentations to generate average time series for the regions of interest. We calculated causal connectivity of intralaminar nuclei and striatum with the combinedFC metric developed by Sanchez-Romero and Cole.[373] CombinedFC between two regions first calculates bivariate correlation between two regions and then partial correlation between these regions using all other regions as confounds. By combining results of bivariate and partial correlation, strength of causal interactions between brain regions is determined.

A.1.2.3 Results

Patients with TLE exhibit overall decreased causal connectivity between thalamic nuclei (CM and CL) and dorsal striatum (**Fig.A.1.1**). In individual nuclei, we observed in patients left CL displayed decreased causal connectivity with right striatum vs. controls ($p = 0.02$, t-test). We also found in patients right CM and CL had decreased connectivity with left striatum vs. controls ($p = 0.04$ and $p=0.01$ respectively, t-test). Interestingly, greater causal connectivity between right CM and left striatum (i.e. closer to control values) associated with later age of TLE onset ($r = 0.3$, $p = 0.029$, Pearson correlation). We measured striatal-cortical causal connectivity and found that left striatum in patients had lower causal connectivity with both left parietal and right frontal lobe ($p = 0.01$ and $p = 0.02$ respectively, t-test).

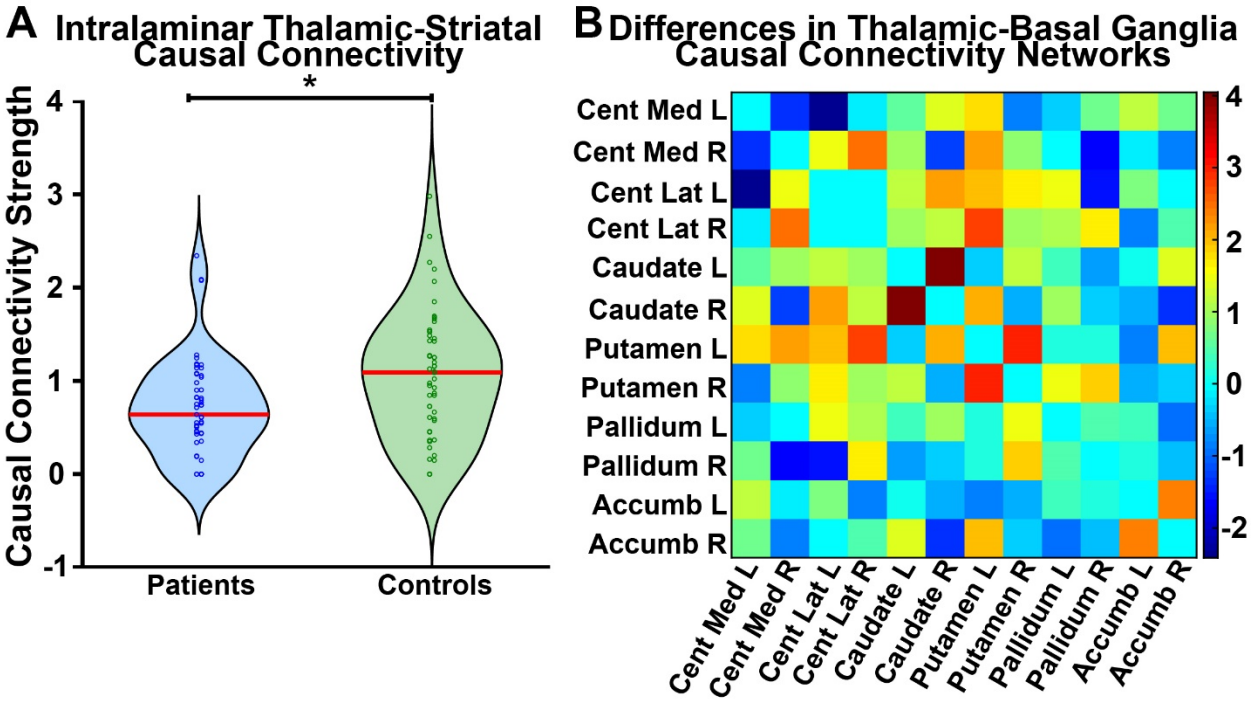

Figure A.1.1. Patients with TLE exhibit decreased causal connectivity strength in intralaminar thalamic nuclei and basal ganglia networks.
(**A**) In patients with TLE, causal connectivity strength between intralaminar thalamic nuclei (CM and CL) and the dorsal striatum was decreased as compared to healthy controls. *p=0.006 paired t-test. The red line indicates the median of each group, and each circle indicates the data of one participant. N=53 patients with TLE and N=53 healthy matched controls. (**B**) Overall, healthy controls exhibit increased causal connectivity strength across key regions of the thalamic-basal ganglia network. This figure shows the group difference between control minus patient causal connectivity strengths summed over each group. Cent Med=centre médian; Cent Lat=central lateral; Accumb=accumbens area; L=left; R=right.

A.1.2.4 Conclusions

Patients with TLE exhibit decreased causal interactions among subcortical regions, including between intralaminar thalamic nuclei and dorsal striatum. Disrupted causal interactions among subcortical arousal nuclei may contribute to broad brain network deficits typically seen in patients with TLE. Further work studying causal connectivity in brain networks may improve our understanding of this devastating disorder.

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