2010

AN FMRI STUDY OF DEFAULT MODE NETWORK CONNECTIVITY IN COMATOSE PATIENTS

Loretta Norton

AN FMRI STUDY OF DEFAULT MODE NETWORK CONNECTIVITY IN COMATOSE PATIENTS

(Spine Title: Default Mode Network in Comatose Patients)

(Thesis format: Monograph)

By

Loretta Norton

Graduate Program in Neuroscience

A thesis submitted in partial fulfillment
Of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

THE UNIVERSITY OF WESTERN ONTARIO
The School of Graduate and Postdoctoral Studies

CERTIFICATE OF EXAMINATION

Co-Supervisors

Dr. Melvyn Goodale

Dr. Seyed Mirsattari

Advisory Committee Members

Dr. Bruce Morton

Dr. G. Bryan Young

Dr. David Sherry

Examiners

Dr. Jody Culham

Dr. Peter Williamson

Dr. Robert Hegele

The thesis by

Loretta Norton

Entitled:

An fMRI Study of Default Mode Network Connectivity in Comatose Patients

is accepted in partial fulfillment of the
requirements for the degree of
Master of Science

Date _____

Chair of the Thesis Examination Board

Abstract

Functional connectivity within a resting state network of the brain, termed the default mode network (DMN), has been suggested to represent the neural correlate of the stream of consciousness. Altered states of consciousness where awareness is thought to be absent could provide insight into the function of the DMN. Here I examined the functional connectivity in the DMN in both reversible and irreversible coma using fMRI. Twelve healthy control subjects and thirteen comatose patients following cardiac arrest were included in the study. DMN connectivity was observed in healthy controls and two patients who regained consciousness. DMN connectivity was absent in the eleven patients who failed to regain consciousness. Functional connectivity in the DMN is preserved in the comatose patients who regained consciousness but absent in those who did not recover consciousness indicating that potentially the DMN is necessary but not sufficient to support consciousness.

Keywords: default mode network, coma, consciousness, fMRI

Acknowledgements

There are a number of people without whom this thesis might not have been written, and to whom I am greatly indebted. Firstly, I offer my gratitude and appreciation to my supervisors, Drs. Seyed Mirsattari and Dr. G. Bryan Young for their guidance and wisdom. Dr. Mirsattari developed the protocol design and guided me through data analysis techniques and Dr. Young recruited and enrolled patients into the research study. Thanks to Matt Hutchison, a graduate student of Dr. Mirsattari, for his assistance with ICA. Thanks to Robin Bluhm for her helpful insights into default mode analysis and providing a default mode template for comparison. I would also like to recognize the hard work of both ICU and MRI staff for the transport of patients and acquisition of images. Acknowledgment to Frank Bihari and Tenielle Gofton for the use of some previous data acquired from a previous coma study. I have been privileged to see the research through from beginning to end including, scanning a number of participants, preprocessing fMRI images, analyzing functional data, performing group statistics, and writing the manuscript and thesis.

Table of Contents

Table of Figures

1. Introduction

The Neurophysiology of Consciousness

Coma is characterized by the absence of consciousness (Posner et al., 2007). Clinically, consciousness is assessed by observing a patient's alertness and responsiveness or their ability to perceive, interact and communicate with their environment and others (Zeman, 2001). Arousal and awareness are the two physiological mechanisms that regulate conscious behaviour. Arousal, also called alertness, corresponds to the level of wakefulness; awareness refers to both the conscious perception of the environment (termed external awareness), and internal awareness of such things as inner speech, mental imagery, and thoughts (Boveroux et al, 2008).

Arousal to wakefulness is required for awareness to occur. Arousal is a function of the ascending reticular activating system (ARAS) which is a series of neural circuits connecting the brainstem to the cortex (Figure 1). The ARAS is stimulated by spinal and cranial nerves that carry proprioceptive, visual, and auditory information (Wijdicks, 2008). The ARAS ascends from the brainstem tegmentum rostrally from the midpons to the midline and intralaminar nuclei of the thalamus and on to the cerebral cortex (Young et al., 1998). Neural structures in the ARAS include: the cholinergic nuclei in the upper brainstem and basal forebrain, cholinergic nuclei in the locus coeruleus, histaminergic projections from the posterior hypothalamus, as well as the potential role of dopaminergic and serotongeric pathways from the brainstem (Zeman, 2001). The most notable of these neural structures are acetylcholine-producing neurons in the peribrachial

nuclei (composed of the pedunculopontine tegmental and lateral dorsal tegmental nuclei) that project rostrally in both dorsal and ventral pathways (Young, 2009b). The dorsal pathway synapses with the midline and nonspecific thalamic nuclei which send glutaminergic projections to large areas of the cerebral cortex (Young, 2009b). The ventral pathway projects to the posterior hypothalamus, basal forebrain, and then to the cortex, bypassing the thalamus (Young, 2009b) (Figure 1).

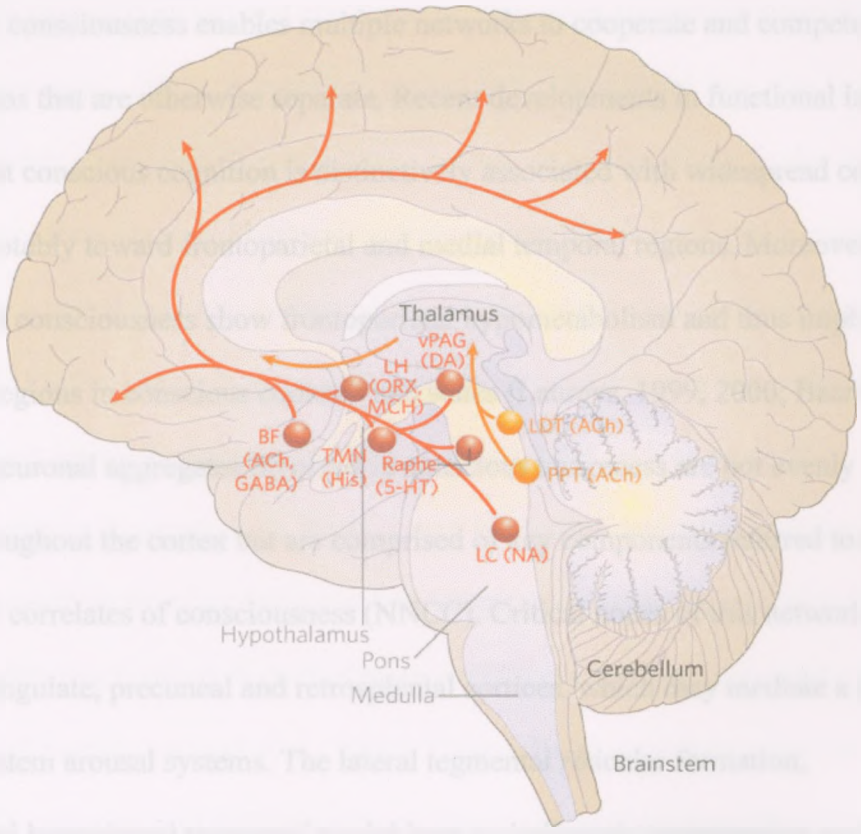

Figure 1.The nuclei and pathways of the ascending reticular activating system (ARAS). The ventral pathway, shown with red lines, involves the hypothalamus and basal forebrain in cortical activation. Yellow lines illustrate the dorsal pathway which originates from cholinergic (ACh) cell groups in the upper pons, the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT). These inputs facilitate thalamocortical transmission. Projections from the antero-ventral thalamic nucleus are thought to have important projections to the posterior cingulate cortex, potentially a critical node where arousal and awareness converge. (Adapted from Posner et al., 2007.)

The role of the cerebral cortex in governing conscious behaviour is still not largely understood. A global workspace (GW) theory of consciousness has been put forward suggesting that the primary functional role of consciousness is to allow a "blackboard" architecture to operate in the brain whereby the functioning of very large numbers of specialized networks that usually operate autonomously work together in order to integrate, provide access, and coordinate for consciousness to occur (Baars, 2005). Simply, consciousness enables multiple networks to cooperate and compete at solving problems that are otherwise separate. Recent developments in functional imaging have shown that conscious cognition is distinctively associated with widespread cortical activity most notably toward frontoparietal and medial temporal regions. Moreover, altered states of consciousness show frontoparietal hypometabolism and thus implicating frontoparietal regions in conscious contents and states (Laureys, 1999, 2000; Baars et al., 2003). Thus, neuronal aggregates involved in conscious awareness are not evenly distributed throughout the cortex but are comprised of key components referred to as the neural network correlates of consciousness (NNCC). Critical nodes of this network are the posterior cingulate, precuneal and retrosplenial cortices, which may mediate a pivotal link with brainstem arousal systems. The lateral tegmental reticular formation, parabrachial and laterodorsal tegmental nuclei have a cholinergic mesopontine projection to the anteroventral thalamic nucleus, which has a prominent projection to the retrosplenial cortex region which in turn is heavily and reciprocally connected to the posterior cingulate (Vogt, 2005). Thus, although there are two aspects to consciousness (arousal and awareness), they may have a critical junction at the posterior cingulate cortex and retrosplenial cortex (Vogt, 2005). With the knowledge of the neural

mechanisms that maintain arousal and awareness we can examine the pathologies that follow structural brain damage which cause altered states of consciousness such as coma.

Coma and Altered States of Consciousness

Coma is a state of unarousable psychological unresponsiveness with a total absence of awareness of both self and environment even when the patient is externally stimulated (Posner et al., 2007). A comatose patient lacks arousal and thus also lacks awareness. Through neurological examination, a comatose patient might display a motor response no better than simple withdrawal-type movement, a failure of eye opening to stimulation, and a verbal response no better than simple vocalization of non-word sounds (Young, 2009b). Further, coma is a persistent state of unconsciousness that endures for more than an hour which discriminates it from transient states such as syncope, concussion, or seizure (Laureys et al., 2004). Coma is a transitional state whereby a patient may succumb to an injury known as brain death (requires the loss of all brainstem reflexes and continuing apnoea) or gradual recovery of consciousness may occur, usually within a 2-4 week period (Laureys et al., 2004). Those who recover from coma can progress through a spectrum of different clinical stages of consciousness (Figure 2).

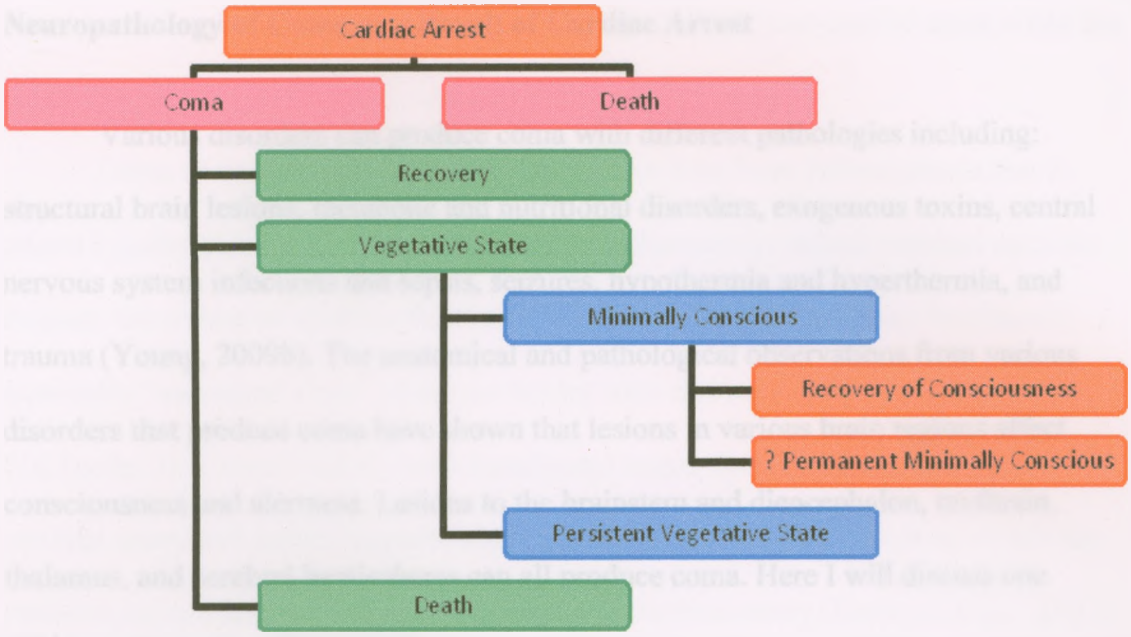

Figure 2. A flow chart of the different clinical stages that follow post-cardiac arrest coma from the recovery of consciousness. Vegetative state follows a coma state where a patient regains arousal but still lacks awareness, followed by either persistent vegetative state (a continued vegetative state after 3 months from a non-traumatic insult) or a minimally conscious state.

Patients in coma may transition to a vegetative state where they display wakefulness but maintain a lack of awareness of themselves and their environment (Posner et al., 2007). They may further transition to a minimally conscious state where they demonstrate awareness of themselves or the environment, but this awareness is limited and they are unable to communicate consistently (Laureys et al., 2004). Patients may bypass these states to full consciousness, remain in these altered states of consciousness, or progress through these states to recover after a brief or prolonged period of time.

Neuropathology of Coma as a Result of Cardiac Arrest

Various disorders can produce coma with different pathologies including: structural brain lesions, metabolic and nutritional disorders, exogenous toxins, central nervous system infections and sepsis, seizures, hypothermia and hyperthermia, and trauma (Young, 2009b). The anatomical and pathological observations from various disorders that produce coma have shown that lesions in various brain regions affect consciousness and alertness. Lesions to the brainstem and diencephalon, midbrain, thalamus, and cerebral hemispheres can all produce coma. Here I will discuss one etiology of coma, cardiac arrest.

Cardiac arrest (CA) is the cessation of cardiac mechanical activity that prevents adequate blood flow and oxygen supply to body tissues resulting in the inability to detect vital signs such as responsiveness, pulse, or respiration (Vaillancourt & Stiell, 2004). Approximately 40,000 Canadians experience cardiac arrest every year (Gardiner et al, 1999). Heart disease is the primary cause of mortality in Canada and cardiac arrest is responsible for more than half of cardiovascular related deaths (Callans, 2004). Most survivors of cardiac arrest are comatose after resuscitation following the reduced blood flow and oxygen supply to the brain and cardiac arrest has become the third leading cause of coma (Bassetti et al, 1996). Post-cardiac arrest coma is associated with the highest mortality rate among the different causes of coma, with only around 30% of patients ever regaining awareness (Lee et al., 2010). In 2010, with advancements in public education, medicine, and technology (e.g. clinical practice of hypothermia and more public access to defibrillators), more patients are surviving the initial cardiac arrest and entering ICUs in a

comatose state with unknown neurological outcome. Thus, post-cardiac arrest coma has become an important clinical syndrome.

Coma as a result of cardiac arrest is distinct from focal pathologies in that it causes a global ischemic insult to the brain and subsequently diffuse cerebral necrosis. Because the reduction in blood flow is global, all parts of the brain may be affected, especially "watershed areas" which are border zone regions to major cerebral arteries. The border zone areas include medial prefrontal cortex found between the anterior cerebral artery and middle cerebral artery and the posterior cingulate cortex which lies between the middle cerebral artery and posterior cerebral artery (Derdeyn et al., 2001). Blood flow to these areas does not necessarily stop, but may be relatively reduced to levels disposed to brain damage. Vulnerable areas of the brain are those of which that have high metabolic demand and an abundance of excitatory neurotransmitter receptors including, the large cell layers (3, 5, and 6) of the cerebral cortex especially frontoparietal cortex, hippocampus, Purkinje cells of the cerebellum, putamen, caudate, and thalamus (Young et al., 1998; Wijdicks, 2008). The brainstem is usually relatively spared in most patients with an anoxic-ischemic injury who are not brain dead after resuscitation.

At the neuronal level, ischemic injury causes oxygen stores to be depleted after 20 seconds. As a result, consciousness is lost and ATP stores become depleted within 5 minutes, creating a cascade of effects (Wijdicks, 2008). It is hypothesized that excitatory amino acids activate NMDA receptors and open voltage-gated ion channels causing an influx of sodium and calcium into the cell resulting in osmotic swelling which could be lethal (Young et al., 1998; Wijdicks, 2008). In addition, delayed neuronal death may occur with the increase of calcium which damages mitochondria and generates reactive

oxygen species, triggering apoptotic cascades in the cerebral cortex (Wijdicks, 2008) (Figure 3).

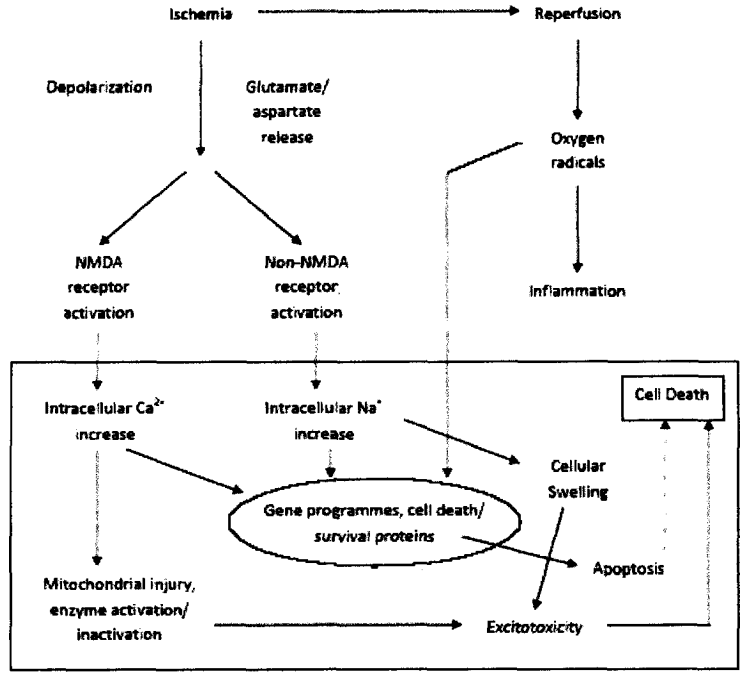

Figure 3. The sequence of events in acute brain ischemia leading to cell death. (From Young et al., 1998)

Diagnosis of Consciousness and Measures of Outcome

The level of consciousness (LOC) of a patient is determined through clinical examination using standardized scoring systems. The Glasgow Coma Scale (GCS), first published by Teasdale and Jennett in 1974, is the most commonly used "gold standard" to classify coma (Table 1). The scale, originally designed for traumatic brain injury, is calculated from the sum of three components: verbal response, motor response, and eye opening. The best response for each component is tabulated and can range from a score of 3 ("deep unconsciousness") to 15 ("fully alert and oriented"). Severe brain injury and

subsequently, the classification of coma, is determined if a patient has a GCS of eight or less (Teasdale & Jennett, 1974).

Table 1. Glasgow Coma Scale (Teasdale & Jennett, 1974)

ACTIVITY	SCORE
Eye Opening	
None	1 = Even to supra-orbital pressure
To pain	2 = Pain from sternum/limb/supra-orbital pressure
To speech	3 = Non-specific response, not necessarily to command
Spontaneous	4 = Eyes open, not necessarily aware
Motor Response	
None	1 = To any pain; limbs remain flaccid
Extension	2 = Shoulder adducted and shoulder and forearm internally rotated
Flexor response	3 = Withdrawal response or assumption of hemiplegic posture
Withdrawal	4 = Arm withdraws to pain, shoulder abducts
Localizes pain	5 = Arm attempts to remove supra-orbital/chest pressure
Obeys commands	6 = Follows simple commands
Verbal Response	
None	1 = No verbalization of any type
Incomprehensible	2 = Moans/groans, no speech
Inappropriate	3 = Intelligible, no sustained sentences
Confused	4 = Converses but confused, disoriented
Oriented	5 = Converses and oriented

While the GCS is the most common scale to grade the level of consciousness of brain injured patients, some limitations exist. Intubation and the influence of sedating and paralyzing medications make assigning an accurate GCS score more difficult and inter-rater reliability in scoring the GCS correctly has been questioned (Buechler et al., 1998, Crossman et al., 1998). Other more comprehensive scales have been developed, such as the Full Outline of UnResponsiveness (FOUR) score which has been suggested to be

superior as it tests brainstem reflexes and breathing patterns (Wijdicks et al., 2005). However, this scale and others like it are not widely used in the routine on clinical practice, as the GCS has become entrenched in clinical practice.

The Glasgow Outcome Scale (GOS) is used to predict outcome after head injury and also has applications in assessing the outcome of patients who have sustained various types of brain damage (Jennett and Bond, 1975). The GOS is a five-point scale which assesses the outcome of a patient following coma. The five categories of the original scale are: death, persistent vegetative state, severe disability, moderate disability, and good recovery (Table 2).

Table 2. Glasgow Outcome Scale (Jennett and Bond, 1975)

SCORE	DESCRIPTION
1	**Death**
2	**Persistent vegetative state** Patient exhibits no obvious cortical function.
3	**Severe Disability** (Conscious but disabled). Patient depends upon others for daily support due to mental or physical disability or both
4	**Moderate Disability** (Disabled but independent). Patient is independent as far as daily life is concerned. The disabilities found include varying degrees of dysphasia, hemiparesis, or ataxia, as well as intellectual and memory deficits and personality changes.
5	**Good Recovery** Resumption of normal activities even though there may be minor neurological or psychological deficits.

Prediction of Outcome in Comatose Survivors after Cardiac Arrest

There is a spectrum of outcomes for a comatose patient following cardiac arrest ranging from brain death to complete recovery. However, clinically predicting outcomes for comatose patients is limited and focuses only on prediction of poor outcomes, those

where a patient will do no better than persistent vegetative state (PVS). A report from the American Academy of Neurology (AAN) established practice parameter guidelines for the prediction of poor outcome in comatose survivors after cardiopulmonary resuscitation in 2006 (Figure 4). In an exhaustive review of over 391 studies, the AAN concluded that measures for predicting poor outcome rely on examination of pupillary light response, corneal reflexes, motor responses to pain, diagnosis of myoclonus status epilepticus, serum neuron-specific enolase, and somatosensory evoked potentials (Wijdicks et al., 2006).

In their meta-analysis, the AAN noted that upon neurological examination of the comatose patient, a GCS motor score of less than two where a patient exhibits extensor or absent motor responses is indicative of a poor outcome with no false predictions after 72 hours following CPR. In addition, no false predictions of poor outcomes were found for absent papillary light reflexes 24 to 72 hours after CPR or absent corneal reflexes after three days.

Electroencephalography (EEG) may have some prognostic value when certain malignant patterns are recorded, including suppression, burst suppression, alpha and theta pattern coma, and generalized periodic complexes combined (Young, 2000). These patterns are strongly but not invariably associated with poor outcome. EEG recording are vulnerable to drugs, sepsis, and metabolic conditions, all which can be reversible factors. Apart from complete EEG suppression after 24 hours from cardiac arrest no other single EEG pattern has a 100% association with an outcome no better than permanent vegetative state (Young, 2000; Wijdicks et al., 2006). In addition, continuous EEG

recording are recommended in the ICU as single recordings are limited to various probabilities but not to certainty of poor outcome (Young, 2000).

Studies have also investigated biochemical markers that may accurately predict outcome. Serum neuron-specific enolase (NSE), a gamma isomer of enolase that is located in the neurons and neuroectodermal cells, can reliably predict a poor outcome when a patient has a NSE > 33 μg/L at days 1 to 3. In a large study, it was noted that of 231 patients 60% had NSE > 33μg/L and all these patients had a poor outcome (false positive rate of 0, 95% CI) (Zandbergen, 2006). However, serum NSE testing may not be sufficiently standardized (AAN, 2006).

The AAN also recommends the use of somatosensory evoked potentials (SSEPs) to assess poor outcome. The bilateral absence of the cortical N20 component in the primary somatosensory cortex following median nerve stimulation accurately predicts a poor outcome. Recently, Young (2009a) conducted a large systematic review of predictive outcome studies and concluded that SSEPs are the most reliable indicator of poor prognosis when there is bilateral absence of the N20 response at day 3. However, unilateral or bilateral presence of SSEPs does not predict the course of coma. In fact, many patients who fail to recover from coma still have preserved N20 responses with a sensitivity of only 46% (Wijdicks et al., 2006). In addition, Young (2009a) notes that many patients who do not have any of the aforementioned unfavorable clinical features still have poor outcomes.

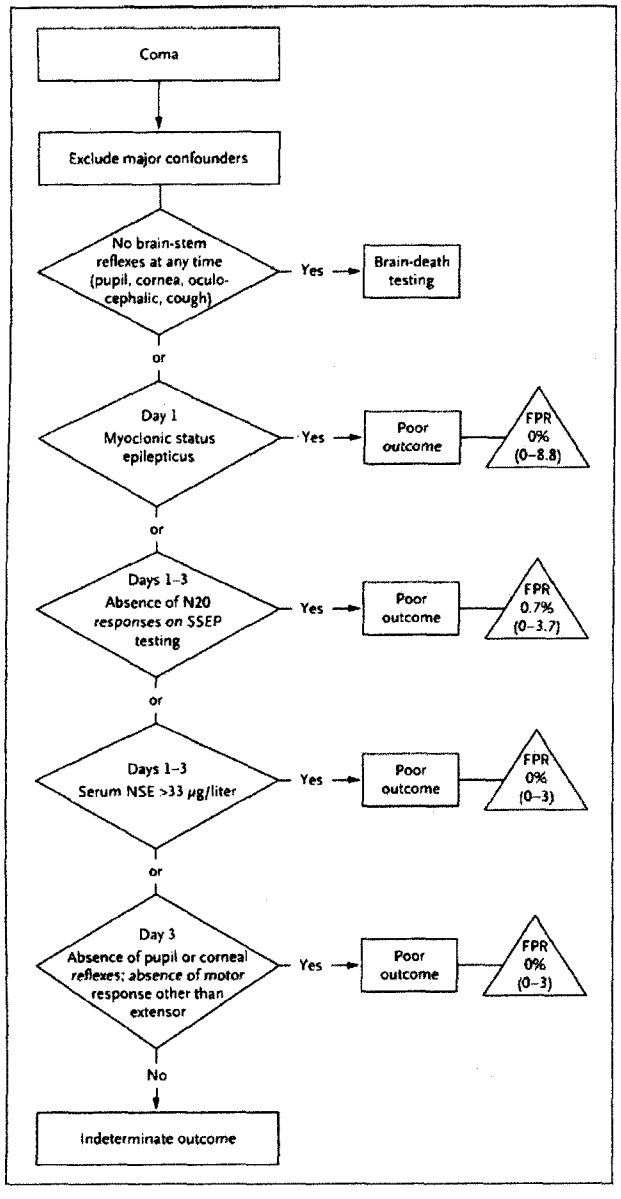

Figure 4. Decision algorithm established by the AAN for the use in prognostication of comatose survivors after cardiac arrest. The numbers in the triangles are the false positive rate (FPR) in percentages; the numbers in parentheses are exact 95% confidence intervals (CIs). From American Academy of Neurology Quality Standards Subcommittee (2006).

The studies used to determine the clinical tools for predictors of poor neurologic outcome after cardiac arrest were validated before the advent of therapeutic induced mild hypothermia (The Hypothermia After Cardiac Arrest Study Group, 2002). The

hypothermic protocol, a current standard of care used in the ICU, improves neurological outcome by reducing the cerebral metabolic rate, decreasing neurotoxicity and inflammatory responses, and preventing apoptosis of cells (Hypothermia After Cardiac Arrest Study Group, 2002; Geocadin, 2010). This method of cooling the body to 32-34°C improves the tolerance for ischemia but may alter the validity of current tools used for prognostication. Our group found that, in particular, the loss of motor responses better than extension on day 3 was not a reliable prognostic tool after the use of hypothermia (Al Thenayan et al., 2008). It may be necessary to wait until day 6 or beyond (Al Thenayan et al., 2008). In addition, neuronal markers as used thus far, including NSE may also be unreliable following hypothermia (Rossetti et al., 2010).

In addition, current clinical tools used for prognosticating outcome do not examine prediction of a positive outcome. Advances in functional MRI may hold promise in predicting favorable outcomes but preliminary studies have small sample size that limit the ability to give a precise prediction to an individual patient. Thus, with current clinical tools available it is difficult to reliably predict the chances of functional recovery.

Advances in Neuroimaging

Technological advances in functional neuroimaging may offer new ways to improve the diagnostic, prognostic, and therapeutic management of patients who suffer from disorders of consciousness (Di et al., 2008). While clinical-behavioural assessment of the unresponsive patient is impossible, functional neuroimaging allows for the examination of the integrity of cognitive functional networks even in the absence of motor output (Owen, 2007). Functional neuroimaging may possibly allow clinicians to

assess the cognitive functions in altered states of consciousness without the need for any overt response on the part of the patient (Owen, 2007).

Positron emission tomography (PET) was the first such neuroimaging technique to use visual and noxious somatosensory stimuli to show preserved higher cortical processing in patients in PVS (Menon, 1998; Laureys, 2002). Owen et al. (2002) reported two cases of patients in PVS that had regional cerebral blood flow responses during face recognition and speech perception similar to controls. These patients made a significant recovery and the authors suggested that neuroimaging could be used as a tool to detect residual cognitive function in PVS.

Case studies using functional magnetic resonance imaging (fMRI) have also demonstrated preserved cognitive function and awareness in patients with disorders of consciousness. Moritz et al.(2001) demonstrated an intact task-correlated sensory and cognitive blood oxygen level dependent (BOLD) hemodynamic response to visual, somatosensory, and auditory stimulation in one comatose patient. This patient subsequently regained consciousness and recovered many cognitive and sensorimotor functions. Furthermore, to address the question of conscious awareness in the vegetative state, Owen et al.(2006) conducted an fMRI study whereby a PVS patient was asked to perform mental imagery tasks, namely imagining playing a game of tennis or imaging visiting all the rooms of her house. The patient had cerebral responses indistinguishable from healthy control subjects confirming that the patient retained the ability to understand spoken words and, while unable to respond through speech or movement, could respond through brain activity. Bekinschtein et al.(2004) reported the response of a minimally conscious state (MCS) patient to a familiar voice, in which the activation of the amygdala

(a limbic structure involved in emotion) may indicate emotional processing in the minimally conscious state. The cerebral responses to a patient's own name spoken by a familiar voice have been further investigated by Di et al. (2007) who found that two patients diagnosed as PVS who showed the most widespread activations subsequently showed clinical improvement while another two patients with PVS failed to show any significant cerebral activation and remained in a vegetative state.

Despite initial functional neuroimaging research conducted in patients with impaired consciousness, little is known about cerebral function in patients in the acute stage of coma. Additionally, many previous studies only examine cognitive function in a small population of patients and do not compare responses to those patients who did not recover awareness. Our group recently completed an investigation comparing fMRI responses to SSEP findings (Gofton et al., 2009). It was shown that during tactile stimulation to the palm of the hand, greater BOLD signal intensity was seen in the contralateral primary somatosensory cortex (SI) of patients who retained their SSEP N_{20} waveform. In addition, there were positive correlations between BOLD signal intensity in SI with level of consciousness using the GCS as well as the patients' functional recovery and outcome following coma at three months using the GOS.

Resting-State Networks

Most neuroimaging studies investigate brain function from recordings of changes in neural activity as a result of a stimulus. This is the traditional approach used in fMRI experiments whereby the BOLD signal correlates to a stimulation timecourse. By alternating between a stimulus condition and a 'rest' condition, researchers are able to

examine the magnitude of the BOLD signal differences between the conditions to elucidate the brain regions that correspond to the stimulus or task the subject is performing. However, there are also spontaneous BOLD signal fluctuations that cannot be attributed to an experimental paradigm (Fox & Raichle, 2007). Traditionally, these spontaneous fluctuations were regarded as 'noise' and were minimized by averaging. However, the spontaneous fluctuations of the BOLD signal (specifically slow fluctuations less than 0.1 Hz) have gained increasing interest as they have been shown to reflect intrinsic brain activity (Raichle & Snyder, 2007).

The high degree of functional organization and the large amount of energy consumption of the slow spontaneous fluctuations have been two main factors in substantiating that these are indeed related to intrinsic brain activity.

The brain represents only 2% of total body mass but consumes 20% of the total body oxygen consumption and 25% of the total glucose utilization at rest (Raichle & Mintun, 2006). Approximately 60-80% of the total energy used by the brain is for neuronal communication (Fox & Raichle, 2007). The large energy consumption during rest is in marked contrast to the additional energy demands associated with task-related neural metabolism which is relatively small (0.5 to 1.0%) (Raichle & Mintun, 2006; Raichle & Snyder, 2007).

Another motivation for studying the low frequency fluctuations of the BOLD signal is related to the pattern of coherence within known brain systems. Inter-regional correlations of spontaneous signal fluctuations are believed to demonstrate 'functional connectivity' between the brain regions reflecting synchronous neural activity (Raichle & Snyder, 2007). Cordes et al. (2001) found that only frequencies below 0.1Hz contribute

to this intrinsic organization while higher frequencies relate to cardiac or respiratory factors and thus investigators studying resting-state networks usually eliminate this source of noise with low-pass frequency filters. The report of spontaneous, spatially coherent activity in the fMRI BOLD signal was conducted by Biswal and colleagues (1995) who found that spontaneous BOLD fluctuations in the left sensorimotor cortex were correlated with spontaneous BOLD fluctuations in the right sensorimotor cortex at rest. They concluded that correlations of low frequency fluctuations, which may arise from fluctuations in blood oxygenation or flow, are a manifestation of functional connectivity of the brain. Resting-state networks (RSN) have been shown to be consistent between and within subjects and groups according to functional relevance with independent spatio-temporal synchronizations (Damoiseaux et al., 2006; De Luca et al., 2006). These RSNs include regions implicated in motor function, visual processing, auditory processing, language, memory, dorsal and ventral attention, and the default mode network (Auer, 2008; Damoiseaux et al., 2006) (Figure 5).

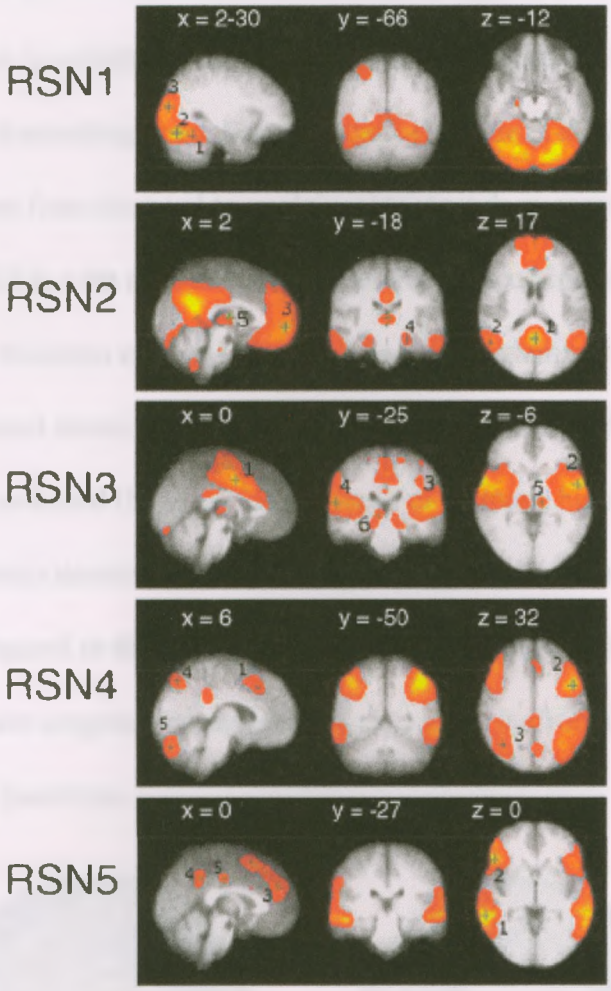

Figure 5. Group resting state networks found in awake healthy control participants. RSN 1 is thought to reflect the visual resting state network and predominantly involves the occipital cortex. RSN 2 is the default mode network including precuneus and anterior pole of the prefrontal lobe and bilaterally temporo-parietal junctions. RSN 3 includes the sensory and auditory systems with the pre and post central gyri, thalamus, and hippocampus. RSN 4 is a dorsal network that includes dorsal parietal and lateral prefrontal cortex. RSN 5 is a ventral network includes the inferior occipital parietal, temporal, and inferior prefrontal cortices. Adapted from De Luca et al., 2005.

Default-Mode Network

The default-mode network (DMN) includes cortical regions that characterize the baseline-state of the brain, in which an individual is awake and alert, but not actively involved in an attention demanding or goal-directed task (Raichle et al., 2001). The concept of the DMN arose from the need to explain consistent decreases in brain-activity measured by BOLD-fMRI in a set of areas during cognitive processing as compared to a passive resting baseline. Shulman et al. (1997) were the first to examine brain regions active during passive mental states. They compared various goal-directed task conditions with a passive task that presented the same visual words or pictures, but no directed task goals. They found consistent deactivation across a specific set of brain areas regardless of task condition when compared to the passive control condition (Figure 6). These brain areas included the posterior cingulate cortex/precuneus, medial prefrontal cortex, and bilateral temporoparietal junctions.

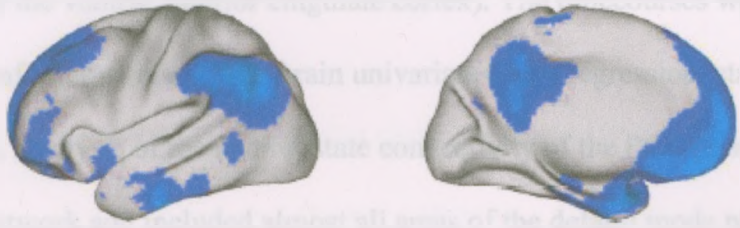

Figure 6. The default mode network originally identified by Shulman et al. (1997). Adapted from Buckner et al., 2008.

Raichle et al. (2001) explored whether or not decreases in the DMN regions during a task were activations present in the resting condition. Using PET, they identified the existence of a baseline resting-state in the adult human brain by measuring its oxygen extraction fraction (OEF). The OEF is the proportion of available oxygen that is removed from the blood. Decreases from the baseline OEF indicate increased neuronal activity

(Huettel et al., 2009). Raichle and colleagues (2001) found no decrease in the OEF within the resting brain and that the average OEF in the default mode network did not differ significantly from other areas of the brain. Thus decreases seen during the task condition are not reflective of activations present in the resting-state but are deactivations – decreases in BOLD activation during task compared to rest. These areas of the DMN which exhibit decreases from baseline suggest that there exists an organized baseline state of brain function that is suspended during externally directed behaviour which Raichle et al.(2001) coined the 'default mode of brain function.'

Greicius and colleagues (2003) demonstrated resting-state functional connectivity between brain regions implicated in the default mode network. They performed functional connectivity analysis by extracting the time series of specific regions of interest (ROI) known to be critical nodes in the default mode (specifically the posterior cingulate cortex and the ventral anterior cingulate cortex). The timecourses were then used as a covariate of interest in a whole-brain univariate linear regression, statistical parametric analysis. Analysis of the resting-state connectivity of the PCC generated a map of the larger network and included almost all areas of the default mode network originally described by Shulman et al.(1997) (Figure 7, Table 3).

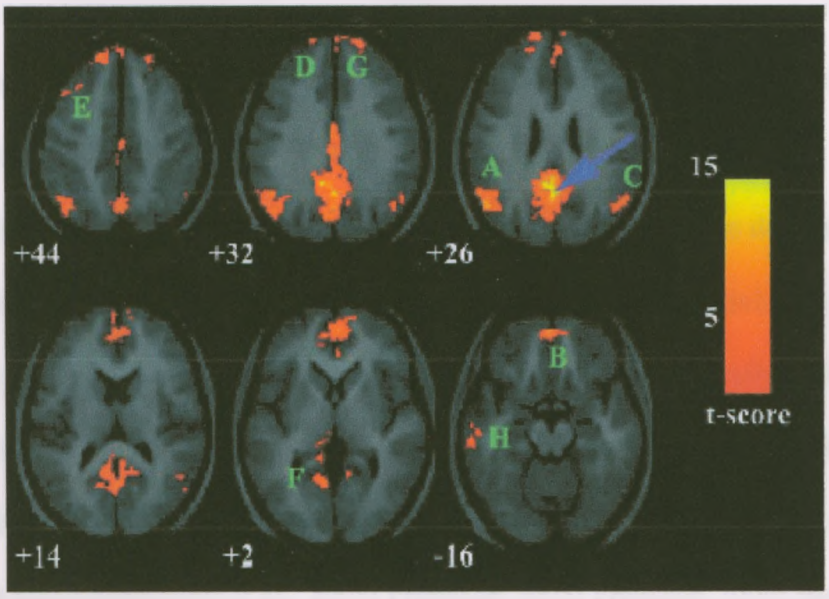

Figure 7. Map of the resting-state neural connectivity for the PCC. The blue arrow indicates the approximate location of the PCC peak [2, 51, 27]. The approximate locations of the eight significant clusters are labelled A–H in descending order of the cluster's *t* score (A corresponds to the cluster with the highest *t* score). A = left IPC. B = in the OFC, but extends superiorly into the MPFC and the vACC. C = right IPC. D = MPFC just left of midline. E = left DLPFC. F = posterior left PHG. G =, MPFC just right of midline. H = left ITC. (From Grecius et al., 2003).

Table 3. Functional connectivity of the posterior cingulate cortex (PCC) during the resting state. (From Grecius et al., 2003).

Connected regions	BA	Cluster size, voxels	Maximal z-score primary peak	Primary peak location
PCC/precuneus	23/31/7	2937	6.39	−2, −51, 27
Left IPC	39/40	742	5.55	−51, −65, 27
OFC/MPFC/vACC	11/10/32	1376	5.38	−2, 55, −18
Right IPC	39/40	229	4.79	53, −61, 27
MPFC	8	365	4.71	−16, 49, 38
Left DLPFC	8/9	106	4.59	−44, 20, 41
Left PHG	30	92	4.44	−12, −35, 0
MPFC	9	141	4.32	18, 54, 32
Left ITC	20/21	126	4.05	−18, −14

Brain regions that showed significant connectivity to the PCC ROI centered at [2, −51, 27]. The height and extent thresholds were set at $P < 0.001$. BA, Brodmann's area.

The study by Greicius and colleagues (2003) also revealed that network activity is suspended during cognitively demanding working memory tasks; resting-state activity being inversely correlated with activity in brain regions that show task-related activations. Greicius et al. concluded that the set of brain regions that have decreases in activity in active versus passive states have significant functional connectivity during the rest providing compelling evidence for the existence of a consistent, tonically active, default mode network. A wide range of fMRI studies have since shown remarkable consistency of the spatial and temporal characteristics of the DMN in the healthy, awake, and resting adult individuals (Fox et al., 2005; Fransson 2005; Fransson 2006; Bluhm et al., 2008).

Function of the Default Mode Network

DMN activity is unique to the resting-state of the brain and is suspended during goal-directed behaviours which reveal little about the functional significance of the network. While standard fMRI approaches prove difficult to investigate functional correlates of this network because DMN activity is attenuated with a defined task, there are some notable exceptions. By using a relatively challenging task in the control condition (e.g. counting syllables), the DMN can be deactivated to allow for larger relative increases when compared to the experimental conditions (Buckner et al., 2008). The few tasks that have been shown to elicit increased activity within the network as well as the anatomy involved within the system constrains its functional possibilities. Two functions of the DMN have been suggested. One postulates that the DMN is responsible for regulating internal self-directed thought and spontaneous cognition (Mason & Norton,

2007). The other suggests that the DMN monitors the external environment when focused attention is not required (Buckner et al., 2008). Regardless of the fact that one or both hypotheses might be correct, both of them relate to awareness (either of self or environment), and thus also of consciousness.

During passive resting conditions where the DMN is active, a broad low-level monitoring of the external world may possibly exist. Simply, while active tasks require acute attentional focus on the external environment, passive conditions where no directed attention to a stimulus occurs may broadly monitor the environment for unexpected events. The critical node of the network, the posterior cingulate cortex (PCC), may have a role in monitoring the external environment (Shulman et al., 1997; Gusnard & Raichle 2001, Buckner et al., 2008). Damage to the parietal cortex (including the precuneus and the posterior cingulate) causes Balint's syndrome, which includes simultagnosia or the inability to synthesize the components of an image as a whole. This syndrome suggests the posterior parietal area within the network may be responsible for information gathering and interpretation of the external environment (Raichle et al., 2001). The posterior lateral cortices implicated in the DMN (areas including the bilateral temporoparietal junctions) are also responsible for orienting attention to salient novel or familiar stimuli particularly when it contains socially relevant information (Gusnard & Raichle, 2001). It is suggested that the DMN, as a whole, may be responsible for continuously providing resources for broad and spontaneous information gathering (Raichle & Snyder, 2007). A study by Hahn et al. (2007) supports this theory in demonstrating that the network is active during a target-detection task only when the target location was unpredictable, but not when the target location was predicted by a

central cue. Thus, it is suggested that the DMN, while active in the absence of top-down-focused attention, is responsible for continuously providing resources for broad and spontaneous information gathering (Hahn et al., 2007). In addition, the DMN may not only interpret this information but may also respond to and even predict environmental demands (Raichle & Snyder, 2007).

Another hypothesized function of the DMN relates to self-reflective thought. The intrinsic fluctuations within the network are thought to relate to unconstrained, spontaneous cognition, or stimulus-independent thoughts (i.e. daydreaming) (Raichle & Snyder, 2007; Mason & Norton, 2007). Increases in activity in the dorsal medial prefrontal cortex (an area within in DMN) has been demonstrated in fMRI experiments when subjects are instructed to monitor and report one's own mental state, such as self-generated thought, intended speech and emotions, and evaluating mental states of others (Casteilli et al., 2000). Collectively, the entire network has been implicated in autobiographical remembering, envisioning the future, theory of mind, and moral decision making (Figure 8). In a large meta-analysis of PET and fMRI studies, Svoboda and colleagues (2006) found a set of regions that embody the DMN, whose activation occurs during autobiographical memory that refers to the recall of personal past experiences. Theory of mind, which refers to thinking about the beliefs and intentions of other people, is also thought to activate areas within the DMN (Buckner et al., 2008; Saxe & Kanwisher 2003). DMN structures are also preferentially activated when people are resolving personal moral dilemmas or envisioning the future (Greene et al., 2001; Schacter et al.2007).

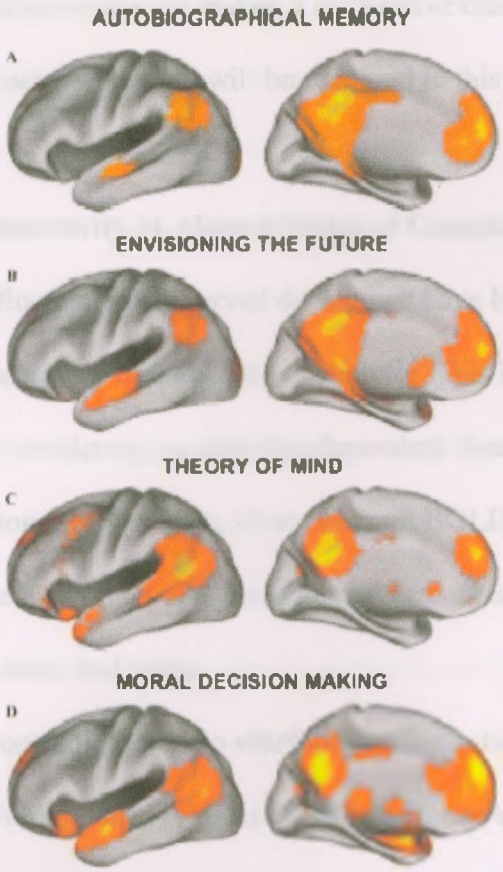

Figure 8. Default mode network connectivity as observed in tasks that require self-reflective thought or judgment which may indicate functional role in internal awareness. (Adapted from Buckner et al., 2008)

Recently, Spreng and Grady (2010) examined multiple internal cognitive processes through neuroimaging experiments and found that adults who are engaged in autobiographical remembering, prospection, and theory-of-mind reasoning showed activation in the same set of brain regions across all three conditions. These brain regions include all areas of the DMN.

The unique properties of the DMN including its functional connectivity across large associative cortical areas, activation at baseline and suspension during goal-mediated tasks, its role in internal mental processes and involvement in gathering

information about the external environment makes it a attractive candidate as the neural correlate of the stream of consciousness that will be explored in this thesis.

Default Mode Network Connectivity in Altered States of Consciousness

Spontaneous BOLD fluctuations observed during rest have been suggested to reflect unconstrained but consciously directed mental activity. This is thought to include spontaneous cognition, mind-wandering, or stimulus-dependent thoughts (Raichle & Snyder, 2007; Mason & Norton, 2007). Thus, these coherent BOLD fluctuations should be absent in conditions where there is no conscious mental activity such as in deep anesthesia, sleep, vegetative state, and coma.

Current literature is contradictory as to whether low-frequency spontaneous BOLD activity can be preserved in various states of unconsciousness due to anesthesia. Peltier et al. (2005) found that sevoflurane inhalational anesthesia reduced the number of significant voxels in functional connectivity maps of motor cortices in the human brain by 78 percent under light anesthesia and by 98 percent under deep anesthesia compared to the awake state. This work is contrasted to the findings of Vincent et al. (2007), who examined the intrinsic functional architecture in the deeply anaesthetized monkey brain. They found that resting-state networks remain highly correlated at anaesthetic levels known to produce a profound loss of consciousness. Four resting-state networks were found in the anaesthetized monkey: oculomotor, somatomotor, visual, and a homologue to the DMN seen in humans. Regions that significantly correlated with the posterior cingulate cortex in the anaesthetized monkeys included the dorsal medial prefrontal cortex, lateral temporoparietal cortex (including area 7a and superior temporal gyrus) and

the posterior parahippocampal cortex. Vincent and colleagues (2007) concluded that spontaneous BOLD fluctuations seen in the DMN cannot exclusively be a reflection of conscious mental activity, but instead reflect a more fundamental or intrinsic property of functional brain organization that is evolutionarily conserved across primate species. Our group has also shown preserved functional connectivity across networks in the anaesthetized rat (Hutchison et al., 2010). Greicius et al.(2008) has examined the effect of light sedation on the DMN in healthy adults. Functional connectivity of the DMN was compared in the same group of subjects during rest and conscious sedation with midazolam. The sedation produced anterograde amnesia and reduced levels of consciousness in which subjects were able to breathe on their own and respond to voice or gentle tactile prompts. The DMN connectivity persisted during light sedation, albeit with significantly reduced PCC connectivity. In sedated monkeys, rats, and humans spontaneous low-frequency oscillations have been shown to persist. This suggests that the DMN and/or resting state networks are somehow preserved in all forms of reversible coma. The DMN may not be exclusively the neural correlate of consciousness but focal reductions in connectivity in the specific nodes of the network may represent a stable correlate of reduced consciousness as sedation in humans shows some focal reductions of the PCC within the network (Greicius et al, 2008).

Researchers have also examined DMN during sleep, a more natural occurrence of decreased level of consciousness than anaesthesia or coma, to discern the potential role of DMN in consciousness. Correlations among brain regions involved in the DMN were shown to persist during light non-REM sleep (Horovitz et al., 2007). In a follow-up study, Horovitz et al. (2009) found a decoupling of the medial prefrontal cortex to the rest

of the cortical regions in DMN during deep sleep. The gradual decrease of conscious thought as a person falls asleep and eventually becomes absent during the deepest stage of sleep corresponds to the integrity of the DMN. Thus, the strength of the DMN correlations may be modulated by the level of consciousness, particularly by the disconnection between anterior and posterior cortical areas (Horovitz et al, 2009). It is worth noting that sleep represents a physiological process while coma is a pathological process. Therefore, absence of DMN in irreversible coma would not be an unusual prediction.

In 2009, Cauda et al. published the first case series describing the DMN in PVS patients. They described a disruption of functional connectivity of the DMN in three patients. In each case, patients had a partial impairment in the DMN compared to healthy controls. They found that the degree of functional connectivity within the network correlated with the severity of the neurological impairment. In another patient who lacked both awareness and arousal only a small portion of ventral medial prefrontal cortex and precuneus were shown to be functionally connected to the stronger oscillations of the temporal cortical areas. The patients showed reduced connectivity in right-sided areas and greater connectivity of left-sided areas in comparison to healthy controls. Cauda et al. suggest that consciousness may require a fully intact and integrated DMN including both anterior and posterior cortical areas. In addition, lateralization of the DMN may also play a role in conscious processing with right sided regions processing aspects of self-awareness (Keenen et al., 2001; Serafetinides, 1995). In another case report of a vegetative state patient who was scanned 21 years post anoxic injury an absence of the DMN was found (Tshibanda et al., 2010). In yet another case report, a vegetative state

patient, 2.5 years post cardio-respiratory arrest, had preserved DMN connectivity whereas a brain dead patient had none (Boly et al., 2009). These results suggest that the DMN cannot be responsible for spontaneous cognition because it is present in the unaware patient. The DMN may have some other intrinsic role in functional brain organization. The presence of DMN in one VS patient (Boly et al., 2009) but absence in another case of VS (Tshibanda et al., 2010) is perplexing. While the reason for the discrepancy is unknown, it may reflect the severity of the neurological impairment, the level of consciousness, or the ability to regain awareness.

Many vegetative state patients are incorrectly deemed to be vegetative when they are in fact minimally conscious as they have some level of awareness. Thus, the presence of DMN connectivity in some patients with VS may indicate that these patients may have spontaneous cognition and are in fact minimally conscious. The acute stage of coma, in which a patient lacks arousal and thus also awareness, could provide more insight into the function of DMN. Boly and colleagues (2008) reported intact DMN connectivity in one patient in coma due to a non-traumatic origin in a review paper with unpublished results. There is no mention if the connectivity within these regions were significantly reduced or found to be the same as controls. The same group in 2010 examined DMN connectivity in range of altered states of consciousness including locked-in syndrome, minimally conscious, vegetative states, and comatose patients (Vanhaudenhuyse et al., 2010). They found that in fourteen brain-damaged patients with varying levels of consciousness the DMN connectivity decreased in proportion to the degree of consciousness impairment. There were a significant exponential correlation between DMN connectivity strength and the level of consciousness in all of the brain injured patients. The PCC/precuneus was

found to be the peak area of significance of the correlation between connectivity and consciousness. Vanhaudenhuyse et al. (2010) suggest that connectivity strength within the DMN could be an indicator of a patient's level of consciousness and holds the predictive ability to differentiate between unconscious patients (as in coma and VS) versus minimally conscious or locked-in syndrome patients. While promising, the study has a few caveats. While they observed a correlation between connectivity and the Coma Recovery Scale-Revised (CRS-R) (a standardized scale for the level of consciousness) scores in the DMN, the medial prefrontal gyrus was the only area that showed a significant relationship to the scale after correction for multiple comparisons. In addition, while the outcome of these patients was documented, the authors did not examine the correlation of outcome to the DMN connectivity. In addition, SSEPs were also not performed on these patients to examine structural connectivity pathways in the cortex. The authors also only examined four comatose patients within the study, all of whom had different causes of coma including structural abnormalities with only one survivor. The authors failed to discuss why an earlier study by their own group found intact DMN connectivity in one comatose patient.

Table 4 outlines the various studies presented that examine the DMN connectivity in altered states of consciousness.

Table 4. DMN connectivity in altered states of consciousness.

Altered State of Consciousness	*Method*	*Number of Subjects*	*Findings*	*Reference*
Deep anaesthesia (in monkey)	ROI-based analysis	11	Intact DMN connectivity	Vincent et al. (2007)
Light anaesthesia	ICA	12	Focal reduction in posterior cingulate cortex	Greicius et al. (2008)
Light sleep	ROI-based analysis	14	Intact DMN connectivity	Horovitz et al. (2007)
Deep sleep	ROI-based analysis	12	Focal reduction in frontal cortex (MPFC/ACC)	Horovitz et al. (2009)
Vegetative State	?	1	Absence of entire DMN	Tshibanda et al. (2010)
Vegetative state	ICA	3	Reduced connectivity in frontal cortex; reduced connectivity on right but greater on left; the more severe the clinical condition, the more impaired the DMN.	Cauda et al. (2009)
Vegetative state	ROI-based analysis	1	Entire DMN has reduced connectivity; but DMN is intact	Boly et al. (2009)
Coma	?	1	Intact DMN connectivity	Boly et al. (2008)
Brain injured patients (coma, vegetative state, minimally conscious state)	ICA	14	DMN connectivity decreased in proportion to degree of consciousness impairment (precuneus connectivity reduced in unconscious patients)	Vanhaudenhuyse et al. (2010)

Legend: ROI – region of interest, ICA – independent component analysis, DMN - default mode network

Current Study

The objective of the current study was to use fMRI to examine the presence and integrity of the default mode network in comatose patients following cardiac arrest. Here I examined DMN connectivity in a large cohort of patients in the acute stage of coma to investigate the role of DMN in mediating the recovery of consciousness. The results of

the fMRI findings were correlated with SSEPs, which is currently used routinely to assess prognosis and to help in the decision for the level of care for comatose patients. Findings of the fMRI default mode network connectivity were also correlated with outcome using the GOS at 3 months. The correlation of the default mode network to a continuous EEG was also investigated. We hypothesized that comatose patients with bilaterally absent SSEPs and irreversible coma would have absent default mode network connectivity, while comatose patients with bilaterally present SSEPs and reversible coma would have an intact default mode network.

Methods and Materials

Participants

Twelve awake healthy control subjects with normal levels of consciousness (age: 28.4 ± 4.2 years) and thirteen comatose patients with cardiac arrest (age: 66.3 ± 11.4 years, 2 patients with reversible coma) from the University Hospital at London Health Sciences Centre in London, Ontario, Canada were included in this study. The study was approved by the Health Science Research Ethics Board at The University of Western Ontario. Written informed consent was given by all control participants and substitute decision makers of the patients. Comatose patients were enrolled in the study if they were older than 16 years of age, had no prior history of neurological impairment, and had a level of consciousness of less than eight on the Glasgow Coma Scale (GCS) and deemed to be comatose. Immediately following cardiac arrest, patients underwent standard routine hypothermic therapy for 24 hours (Hypothermia After Cardiac Arrest Study Group, 2002) and fMRI scanning, SSEP and EEG testing were performed once normothermia was re-established and within one week of arrest. The functional outcome of patients was assessed using the Glasgow Outcome Scale at hospital discharge and again at 3 months post cardiac arrest. All patients were intubated at the time of scanning. Sedation was titrated to maintain a calm and cooperative patient with minimal coughing or difficulty ventilating as per routine standard of care delivered by a treating physician not involved in the study (using propofol, fentanyl, and midazolam in preferential order). Table 5 lists the clinical profile of the patients including information of demography, presence or

absence of SSEPs, EEG findings, structural MRI results, GCS and GOS, and timing of fMRI from arrest date.

Table 5. Clinical Profile of Comatose Patients.

Patient	Sex	Age	Etiology	Day of fMRI	GCS at Scan	GOS at D/C	GOS at 3/12	SSEP result	EEG findings	Structural MRI findings
1	F	57	PEA arrest	4	8	1	1	NT	alpha coma	mild increase in signal in thalami and hippocampi
2	M	82	PEA arrest	3	4	1	1	NT	suppression	no abnormality
3	M	50	PEA arrest	2	4	1	1	NT	suppression	no abnormality
4	M	75	VSA	2	3	1	1	-	suppression evolving to alpha-theta coma	diffuse ischemic changes (thalami, occipital lobes, posterior temporal lobes, frontal and parietal lobes)
5	F	56	Asystole	3	3	1	1	-	suppression	old medial left occipital infarct
6	F	60	Asystole	1	4	1	1	-	suppression generalized sharp waves	no abnormality
7	M	86	PEA arrest	6	8	1	1	NT	burst suppression	
8	F	52	PEA arrest	4	3	1	1	NT	burst suppression	diffuse hypoxic-ischemic injury, focal acute infarction of the right insular cortex
9	M	63	PEA arrest	4	7	1	1	+	burst suppression	no abnormality
10	F	73	VF arrest	4	6	1	1	NT	epileptic burst suppression	high signal of the thalami bilaterally
11	M	74	Asystole	3	5	1	1	+	mild encephalopathy	no abnormality
12	M	64	VF arrest	3	8	3	5	+	NT	no abnormality
13	M	70	VF arrest	2	6	4	5	+	theta pattern coma	no abnormality
Mean		66.3		3.2	5.3	1.4	1.6			
SD		11.4		1.3	2	1	1.5			

Information on demography, presence (+) or absence (-) of somatosensory-evoked potentials (SSEPs), Glasgow Coma Scale (GCS), Glasgow Outcome Scale (GOS), timing of fMRI from date of cardiac arrest, electroencephalography (EEG), and structural MRI findings. Abbreviations: M = male, F = female, PEA = pulseless electrical activity, VF = ventricular fibrillations, VSA = vital signs absent, NT = not tested.

Electroencephalography (EEG) Procedures

All of the comatose patients enrolled in this study had continuous EEG monitoring (cEEG) for a duration not less than 24 hours within 7 days of the onset of coma. The cEEG was performed according to standard procedures used clinically in the intensive care unit at our hospital (Mirsattari et al., 2004; Mirsattari et al., 2009). Briefly, 23 MRI-compatible disc electrodes were placed on the scalp according to the International 10-20 system of Electrode Placement (Jasper, 1958). Electrodes were attached to the scalp using collodion adhesive (ether þ pyroxylin þ ethanol) and a conductive electrolyte gel (Redux Crème®, Parker Laboratories, Inc.) was applied underneath the electrode discs. The EEGs were recorded by a MOBEE-32 portable EEG machine (XLTEK, Oakville, ON). The EEGs were read by the consulting electroencephalographer, who was unaware of the study results, and an EEG classification system specific to the ICU was used to score the severity of the coma (Table 6) (Young et al., 1997).

Table 6. EEG classification system for coma developed by Young et al., 1997.

Category	**Subcategory**
I Delta/Theta > 50% of record (not theta coma)	A. Reactivity B. No Reactivity
II Triphasic Waves	
III Burst-Suppression	A. With Epileptiform Activity B. Without Epileptiform Activity
IV Alpha/Theta/Spindle Coma (no reactivity)	
V Epileptiform Activity (not in burst-suppression pattern)	A. Generalized B. Focal or Multifocal
VI Suppression (no reactivity)	A. <20μV, but >10μV B. ≤10μV

The EEG classification system rates the severity of coma by the electrographic recording from the cortex with: suppression as the most serious category followed by burst-suppression which is more important than the category of triphasic waves which is more significant than dysrhythmia or delta pattern (as long as they are not induced by medications deliberately administered by the intensivists). After cardiac arrest certain EEG patterns are strongly associated with poor neurological outcome including: generalized suppression, generalized burst–suppression, generalized periodic patterns (especially with epileptiform activity), and alpha or theta pattern coma (Young, 2000). Subsequently, EEG patterns were grouped into two categories, those indicative of poor neurological outcome (Category II, comprising EEG classifications III-VI) and patterns not linked to poor outcome (Category I, EEG classifications I and II). The two categories of EEG patterns that are associated with outcome were compared to the DMN of comatose patients.

Somatosensory Evoked Potential (SSEP) Procedures

Bilateral SSEPs were performed according to standard procedures used clinically in the intensive care unit at our hospital (Figure 9) (Houlden et al., 1990). SSEPs were elicited by stimulating the median nerve at the wrist. Stimulation was performed by placing one electrode 2cm proximal to the wrist crease between the tendons of the palmaris longus and the flexor carpi radialis muscles and positioning the other electrode 2cm away on the dorsal surface of the wrist. The intensity of the stimulation was set slightly above the twitch threshold for a visible thumb twitch caused by the median nerve stimulation. The stimulation rate was 4Hz with a duration of 0.2ms. Impulses ascending

along the stimulated somatosensory pathway were then recorded with electrodes placed at different levels along the pathway. A recording electrode was placed at the left and right Erb's point, located 2-3cm above the clavicle at the posterior border of the clavicular head of the sternocleidomastoid muscle, which records clavicular potential with a negative peak latency of 9msec (N_9) from stimulation of the median nerve at the wrist. The cervical potential was recorded from the neck at the level of the C2 vertebra which records a peak latency of 13msec (N_{13}). Scalp recording electrodes were placed contralateral to the stimulated arm over the parietal region, 2cm behind the C_3 and C_4 electrode position of the International 10-20 System of EEG electrode placement (termed $C_3'$and C_4'). The cortical electrodes recorded the clinically most important peak that has a latency of 20msec (N_{20}) from the stimulation of the median nerve at the contralateral wrist. Provided that peripheral responses were obtained the cortical N_{20} waveform was assessed as either present or absent. The presence or absence as well as the amplitude of the N_{20} waveform were compared to the DMN of comatose patients.

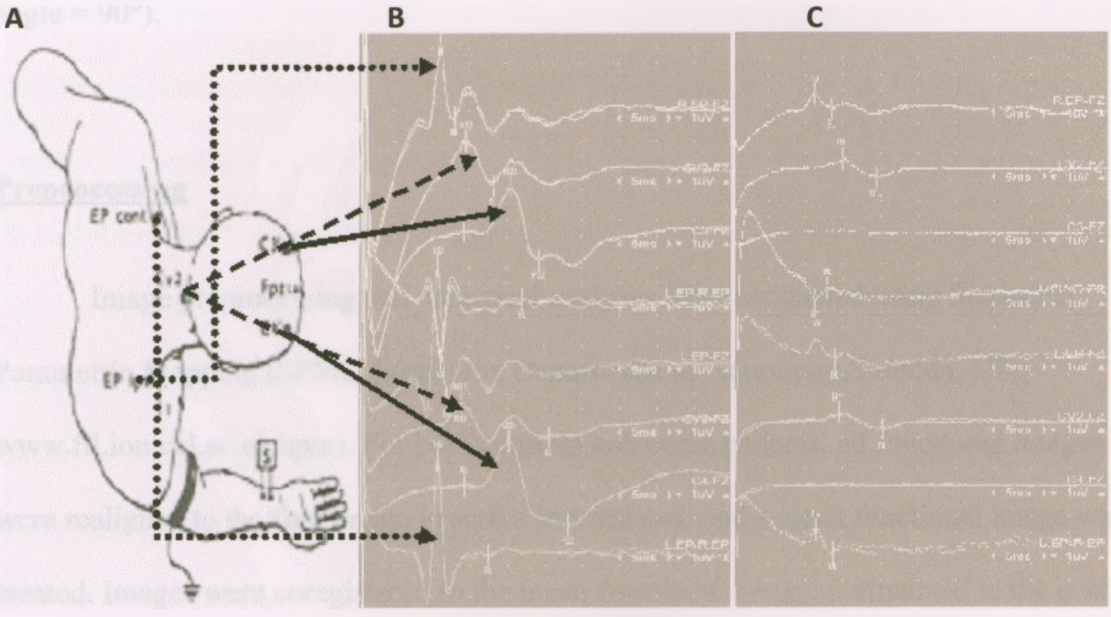

Figure 9. Somatosensory Evoked Response with Median-Nerve Stimulation. (A) Electrode placement on the body including stimulation of the median nerve at the wrist (S), Erb's point over the brachial plexus (EP), the high cervical spinal cord (Cv2), and the contralateral primary somatosensory cortex for the palm of the hand ($C_{3/4}$). (B) A normal SSEP recording displaying the N_9 response from Erb's point in channels 1,4,5,8, the N_{13} response in channels 2 and 6, \the N_{20}/P_{22} cortical potential from the contralateral C_3/C_4. (C) An abnormal SSEP recording displaying responses from the brachial plexus (N_9) and the cervical spinal cord (N_{13}) are recorded but the N_{20} response is absent bilaterally, indicative of a poor outcome following cardiac arrest.

Imaging Procedures

Anatomical and functional MRIs were acquired on a 1.5T General Electric Signa Excite MRI system (Fairfield, CT, USA) with a full-volume head-coil. A whole brain T1-weighted anatomical MRI (3d-SPGR pulse sequence, 120 slices, 1.5mm slice thickness, 0 gap, FOV 24cm x 24 cm, 256 x 256 matrix, 0.75 x 0.75 mm in-plane resolution, TR 6000 ms, TE 1500 ms, flip angles = 10°) was obtained over 12 min. The functional scans used a T2*-weighted one-shot spiral-in sequence to measure BOLD over time and acquired 118 volumes consisting of 30 slices (5 mm slice thickness, 0 gap, FOV 24cm x 24cm, 64

x 64 matrix, 3.75mm x 3.75mm in-plane resolution using TR 2500ms, TE 40ms, flip angle = 90°).

Preprocessing

Image preprocessing and statistical analyses were conducted using Statistical Parametric Mapping (SPM2, Wellcome Department of Neurology, London, UK; www.fil.ion.ucl.ac.uk/spm). For both controls and coma patients, all functional images were realigned to the first image in series and resliced, and a mean functional image was created. Images were coregistered to the mean functional image, normalized to the echo planar imaging template provided in SPM2 and smoothed using a 7.0-mm full-width half-maximum isotropic Gaussian filter. A bandpass frequency filter was applied to preserve only low-frequency oscillations between 0.01-0.08Hz thus removing lower frequency components such as scanner drift and higher frequency physiological noise such as cardiac or respiratory oscillations.

Functional Connectivity Analysis

Resting-state functional connectivity within the DMN has traditionally been examined using a region-of-interest (ROI) based analysis technique (Fox et al., 2005; De Luca et al., 2006; Greicius et al., 2003). A ROI within the network is chosen and the time course of the BOLD signal within the ROI is extracted and a correlation coefficient is calculated for all the other voxels in the brain, creating a correlation map. This method, while simple to perform, relies heavily on the selection of the region. A new, more

mathematically sophisticated approach termed independent component analysis (ICA) has become a popular method for analyzing resting-state functional connectivity. ICA is a statistical technique that separates different sources of signals that are linearly mixed together without any a priori information. This data-driven blind source separation is a powerful tool for the extraction of functional connectivity patterns of synchronized neural activity from fMRI time-series as it can decompose the entire data set into statistically independent spatial and temporal components (Calhoun et al., 2003). ICA has been shown to effectively separate spatially distributed brain networks, including the DMN, from other types of signal and noise (Bluhm et al.2008; Espositio et al., 2006; Grecius & Menon, 2004). ICA was used to identify the DMN in comatose patients instead of ROI-based connectivity analysis. Previous studies examining DMN in altered states of consciousness have identified decreased connectivity in discrete regions of the DMN based on the etiology of the impairment. It is unknown if in coma one region within the DMN would display a decrease in connectivity in comparison to the rest of the network. Thus, ROI-based connectivity analysis could provide an inaccurate representation of the entire network connectivity if the selection of a particular node, to base subsequent correlations of the connectivity on, was impaired. ICA is thought to mediate this problem. However, given the novelty of the technique, ICA was compared to the ROI-based functional connectivity analysis in the healthy controls as proof of concept that both techniques are able to readily identify the network in the healthy conscious brain. Moreover, our group has applied this technique to other conditions with reliable success (Hutchison et al., 2010; Patel et al., 2009; Mirsattari et al., 2006).

Region of Interest (ROI) Based Functional Connectivity Analysis

In healthy controls the posterior cingulate (PCC)/precuneus was used as ROI for functional connectivity analysis as it has been previously shown to be the largest cluster of voxels that remains with a threshold of the individual F-contrast images of $F>2.64(P<0.5x10^{-3}$, corrected) in a population of healthy subjects (Fransson, 2006). The PCC is the standard ROI used in functionally connectivity analysis (Bluhm et al., 2008, Fox et al., 2005, Greicius et al, 2003). For each participant the BOLD signal timecourse was extracted from a 10-mm sphere in diameter in the PCC/precuneus (centered at Montreal Neurological Institute coordinates 0, -56, 20). The extracted timecourse was then used as a covariate of interest in a whole-brain, linear regression, statistical parametric analysis. Contrast images corresponding to areas that correlate in activity with the ROI were determined in each healthy control subject. Images were then entered into a one sample t-test, (thresholded at $P< 0.001$, corrected for false discovery rate, with an extent threshold of 50 voxels) to determine brain areas that had significant functional connectivity to the PCC across subjects.

Independent Component Analysis

After preprocessing data, ICA was employed to examine the DMN using the GIFT software package (http://icatb.sourceforge.net). Components were calculated using the INFOMAX algorithm on a subject-by-subject basis. Infomax was chosen over other ICA algorithms as it is able to maximize the information transfer from the input to the output of a network using a non-linear function which is useful in fMRI data because the

sources of interest are super Gaussian in nature and the algorithm favors separation of super-Gaussian sources (Rachakonda et al., 2009; Patel et al., 2009). In addition, *Z*-scores for Infomax are higher than the other algorithms for a task-related source indicating that it has a greater contrast to noise ratio (Rachakonda et al., 2009). Minimum description length (MDL) was used to establish the number of components necessary to be generated (Calhoun et al., 2001). The ideal number of components ranged from 9 to 20 across healthy control subjects. Therefore, 20 components were generated for all subjects in order to maintain consistency (Franco et al., 2009). The component results were scaled to *Z*-scores so the component *Z*-scores reflect the degree to which the voxel's timecourse correlates with the timecourse corresponding to the specific ICA component. A standard template of the DMN was used to identify the DMN component for each subject by spatially correlating all components to the template and choosing the component with the greatest correlation. The template, previously generated by Garrity and colleagues (2007), was developed with the WFU Pickatlas from the Wake Forest Pharmaceuticals University (http://www.fmri.wfubmc.edu/). The template contained previously reported regions involved in the DMN network: the posterior parietal cortex (Brodmann's area 7), the frontal pole (Brodmann's area 10), and the lateral parietal cortices (Brodmann's area 39), and the posterior cingulate and precuneus. The component with the greatest spatial correlation to the template was chosen as the default mode network (Figure 10).

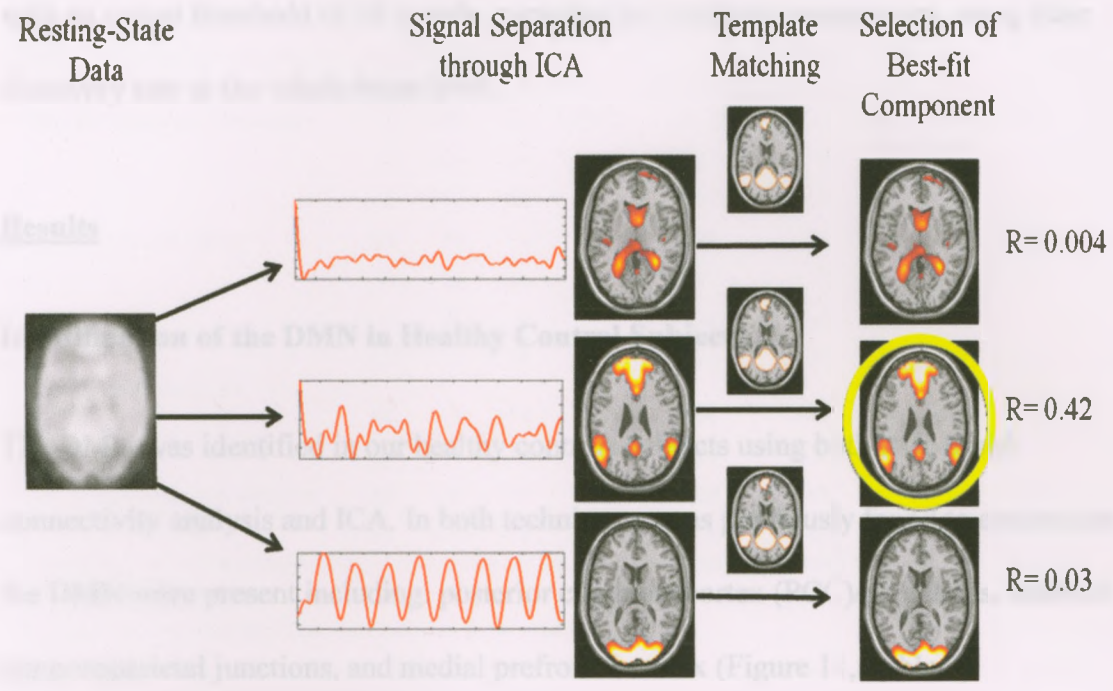

Figure 10. ICA used as an automated extraction method for functional connectivity patterns of the default mode network. Resting-state data is automatically separated into statistically independent signals using ICA and a template matching procedure is employed to select the best-fit component that has the highest correlation to a spatial template of the DMN.

In healthy control subjects, each participant's DMN component was entered into a one-sample t-test in SPM. The resulting SPM[T] map depicting the DMN component across healthy participants was thresholded at $P<0.05$, with an extent threshold of 50 voxels, corrected for multiple comparisons, using false discovery rate at the whole brain level. A one-sample t-test was also used to generate a common map of the DMN across the irreversible comatose patient group. Individual DMN maps for the two comatose patients who recovered consciousness were also identified using ICA. To compare the DMN between controls and those patients with irreversible coma, individual DMN components of both groups were entered into a two-sample t-test thresholded at $P<0.05$,

with an extent threshold of 50 voxels, corrected for multiple comparisons, using false discovery rate at the whole brain level.

Results

Identification of the DMN in Healthy Control Subjects

The DMN was identified in our healthy controls subjects using both ROI-based connectivity analysis and ICA. In both techniques areas previously found to encompass the DMN were present including: posterior cingulate cortex (PCC)/precuneus, bilateral temporoparietal junctions, and medial prefrontal cortex (Figure 11, Table 7).

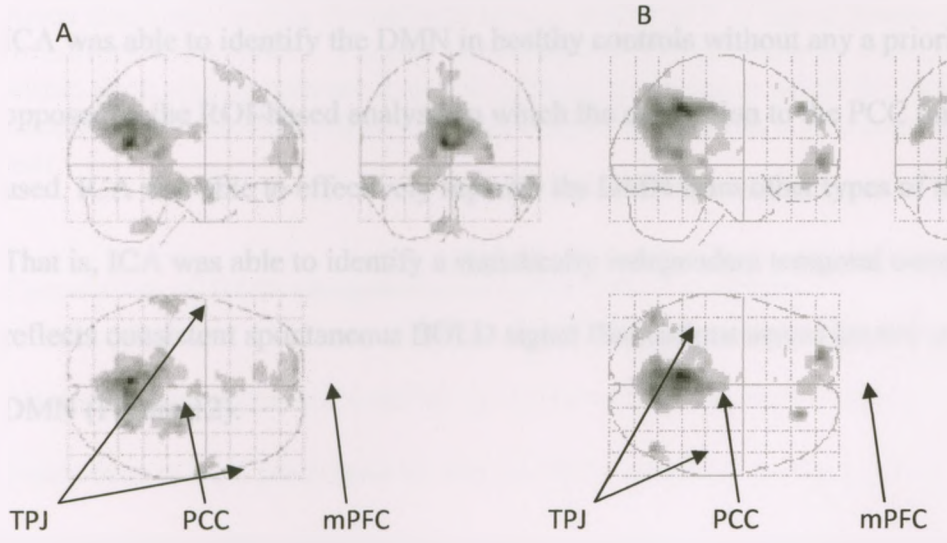

Figure 11. Coronal, sagittal, and axial glassbrain images of healthy controls depicting areas of the default mode network using both (A) ROI-based analysis from the posterior cingulate/precuneus correlations and (B) independent component analysis (ICA). Abbreviations: TPJ – temporoparietal junction, mPFC – medial prefrontal cortex, PCC – posterior cingulate/precuneus. Crosshatch placed at MNI coordinates (0,0,0).

Table 7. Areas in the default mode network of grouped healthy control participants using both ROI-based and ICA analysis

	PCC/precuneus connectivity					Independent Component Analysis				
	MNI coordinates (x,y,z)			k	z score	MNI coordinates (x,y,z)			k	z score
Posterior Cingulate/Precuneus	**-4**	**-56**	**14**	**6578**	**6.62**	**-6**	**-48**	**42**	**7533**	**5.49**
	4	-56	30		6.12	-8	-52	38		4.82
	-4	-62	28		5.97	2	-55	28		4.72
Superior Frontal Gyrus	**-14**	**66**	**10**	**681**	**4.95**	**-22**	**58**	**2**	**724**	**4.48**
	-10	66	20		4.57	4	48	-12		3.35
	-6	52	6		4.41	-5	52	8		3.15
	-10	40	50		4.54	22	44	42	85	4.33
Right Temporoparietal Junction	**62**	**-4**	**-14**	**132**	**4.84**	**48**	**-66**	**26**	**169**	**4.14**
	60	4	-24		4.78	55	-58	20		3.03
Left Temporoparietal Junction	**-62**	**-28**	**-18**	**261**	**5.03**	**-48**	**-68**	**24**	**297**	**4.2**
	-64	-20	-14		4.6	-40	-72	40		3.23

Coordinates in bold type represent global maxima for the cluster reported; coordinates below bold type are local maxima within the cluster

P-values are corrected for false discovery rate at the whole brain level

Abbreviations: MNI - Montreal Neurological Institute; PCC - posterior cingulate cortex; k - cluster size in voxels

ICA was able to identify the DMN in healthy controls without any a priori information as opposed to the ROI-based analysis in which the correlation to the PCC timecourse was used. ICA was able to effectively separate the DMN from other types of signal and noise. That is, ICA was able to identify a statistically independent temporal component which reflects consistent spontaneous BOLD signal fluctuations across known areas of the DMN (Figure 12).

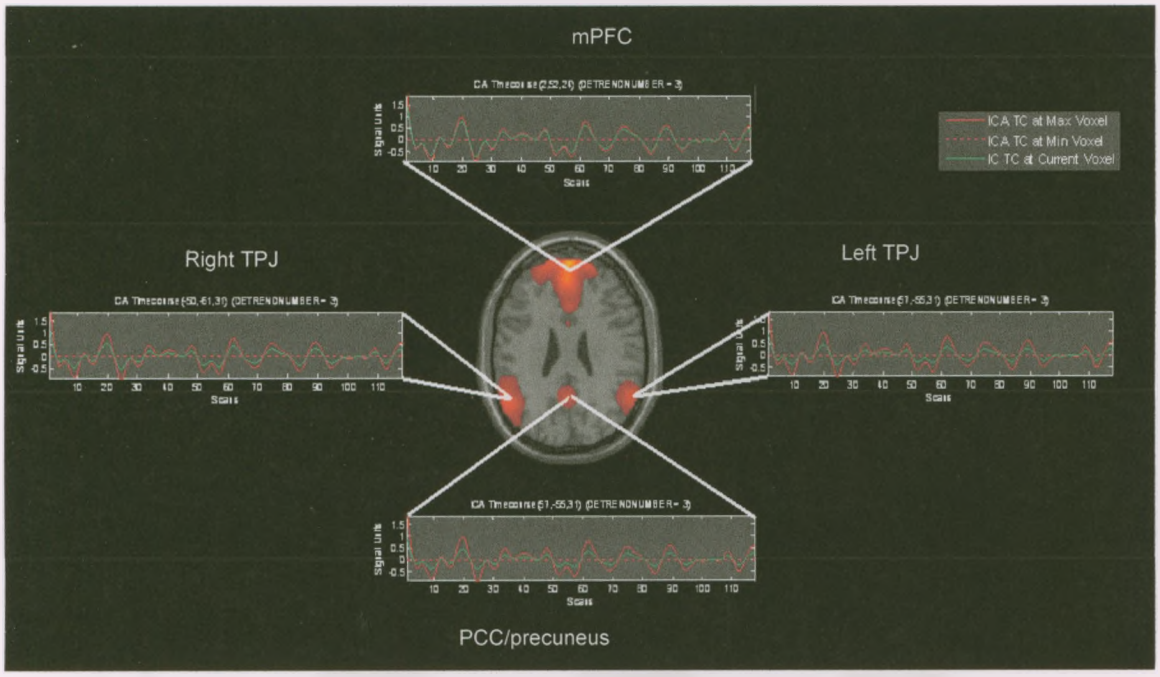

Figure 12. Default mode network as identified by independent component analysis (ICA). Areas implicated within the network have a similar BOLD signal timecourse indicating 'functional connectivity'. During the resting state the spontaneous BOLD timecourse within these regions (shown in green) reflects the degree to which the voxel's timecourse correlates with the timecourse corresponding to the specific ICA component (shown in red).

Correlation of DMN components to spatial DMN template

After ICA was performed a spatial sorting technique was used to identify the DMN component in each participant by using a DMN template. Average spatial correlation to the template for the healthy controls was 0.42 (SD=0.1), patients with reversible coma was 0.36 (SD=0.02), and patients with irreversible coma was 0.12 (SD=0.06) (Figure 13). Healthy controls subjects' DMN component had a significantly greater correlation to the spatial template than the patients who failed to regain consciousness ($t(20)=7.537$, $p<0.001$). Comatose patients who had reversible coma also had a significantly greater correlation to the spatial template than the patients who had irreversible coma

(t(11)=5.163, p<0.001). However, healthy controls subjects' DMN component did not have a significantly greater correlation to the spatial template than the patients who had reversible coma (t(11)=0.751, p=0.469).

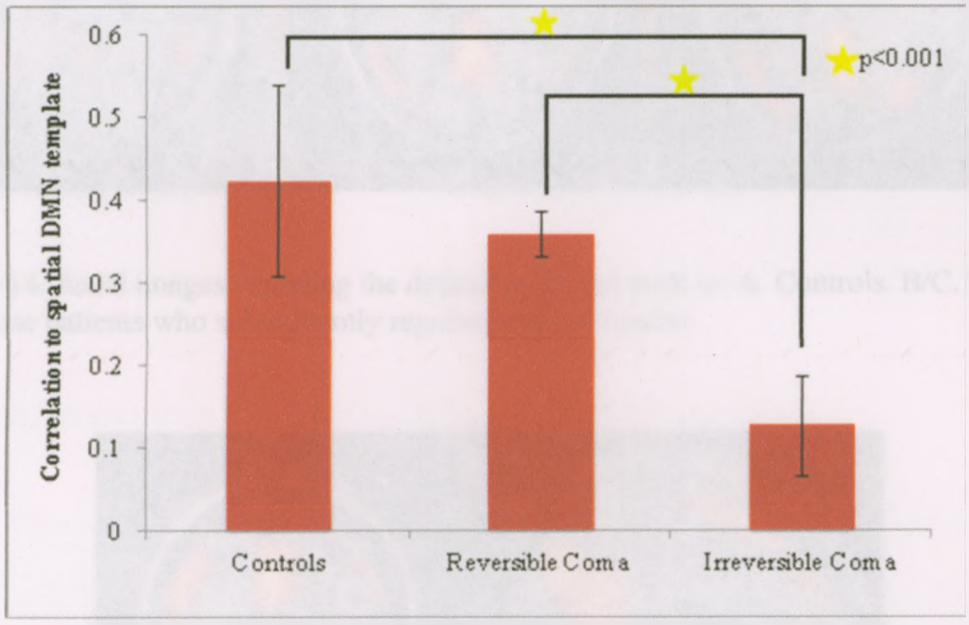

Figure 13. Correlation of the DMN best-fit component in subjects to a previously established DMN spatial template. Healthy control subjects and comatose patients with reversible coma had a significantly greater correlation to the spatial template than comatose patients with irreversible coma (p<0.001).

DMN of Patients with Reversible Coma

The DMN was readily identified in two patients who subsequently recovered from coma. ICA identified the DMN component which included medial prefrontal cortex, bilateral temporoparietal junctions, and the posterior cingulate/precuneus in both patients (Fig 14, Tables 8 & 9).

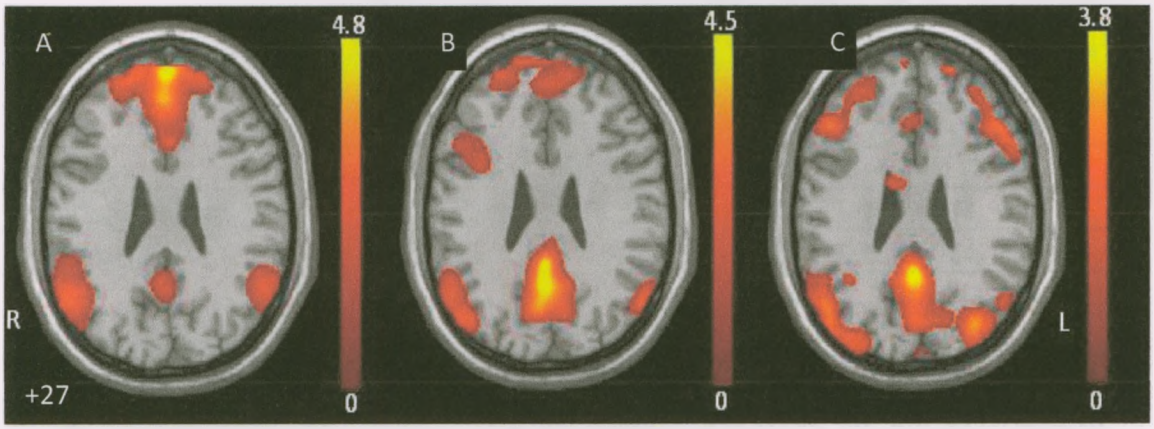

Figure 14. Axial images depicting the default mode network in: A. Controls. B/C. Two comatose patients who subsequently regained consciousness.

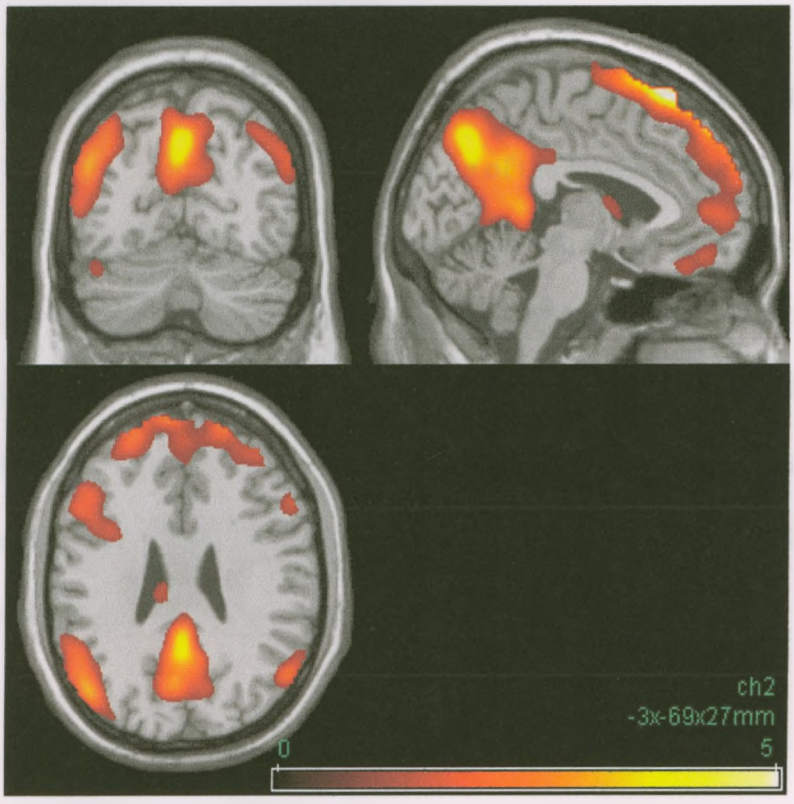

Figure 15. Group ICA depicting DMN connectivity in comatose patients who regained consciousness.

Table 8. DMN areas in two comatose patients who regained consciousness

Area	Broadman Area	R/L volume (cc)	R/L random effects: Max Value (x, y, z)
*	*	0.0/0.1	-999.0 (0, 0, 0)/5.7 (2, 26, 56)
Superior Frontal Gyrus	6, 8	1.3/0.7	6.3 (-2, 30, 57)/5.8 (6, 23, 62)
Precuneus	7, 31	2.0/0.8	4.6 (-2, -68, 40)/4.1 (2, -68, 42)
Posterior Cingulate	23, 29, 30, 31	0.4/0.6	4.1 (0, -47, 23)/3.4 (4, -48, 10)
Cingulate Gyrus	31	0.6/0.1	4.0 (0, -49, 26)/3.0 (4, -47, 26)
Cuneus	7	0.3/0.1	3.9 (-4, -68, 33)/3.0 (2, -64, 33)
Medial Frontal Gyrus	8	0.1/0.0	3.7 (-4, 49, 40)/-999.0 (0, 0, 0)
Angular Gyrus	39	0.4/0.0	3.5 (-51, -57, 34)/-999.0 (0, 0, 0)
Inferior Parietal Lobule	40	0.1/0.0	3.3 (-50, -56, 38)/-999.0 (0, 0, 0)
Supramarginal Gyrus	*	0.2/0.0	3.2 (-51, -53, 34)/-999.0 (0, 0, 0)
Middle Temporal Gyrus	*	0.2/0.0	3.2 (-46, -70, 29)/-999.0 (0, 0, 0)

DMN of Patients with Irreversible Coma

Comatose patients who did not recover awareness had a best-fit component with a significantly lower correlation to a spatial template of the DMN. A one-sample t-test of the individual best-fit component across this comatose patient group revealed no significant clusters of activation. A two-sample t-test comparing the DMN in the healthy control subjects versus the comatose patients with irreversible coma found that all areas of the network persisted when subtracting the comatose best-fit components from healthy controls components suggesting a complete absence of the default mode network in irreversible coma (Figure 15, Table 10).

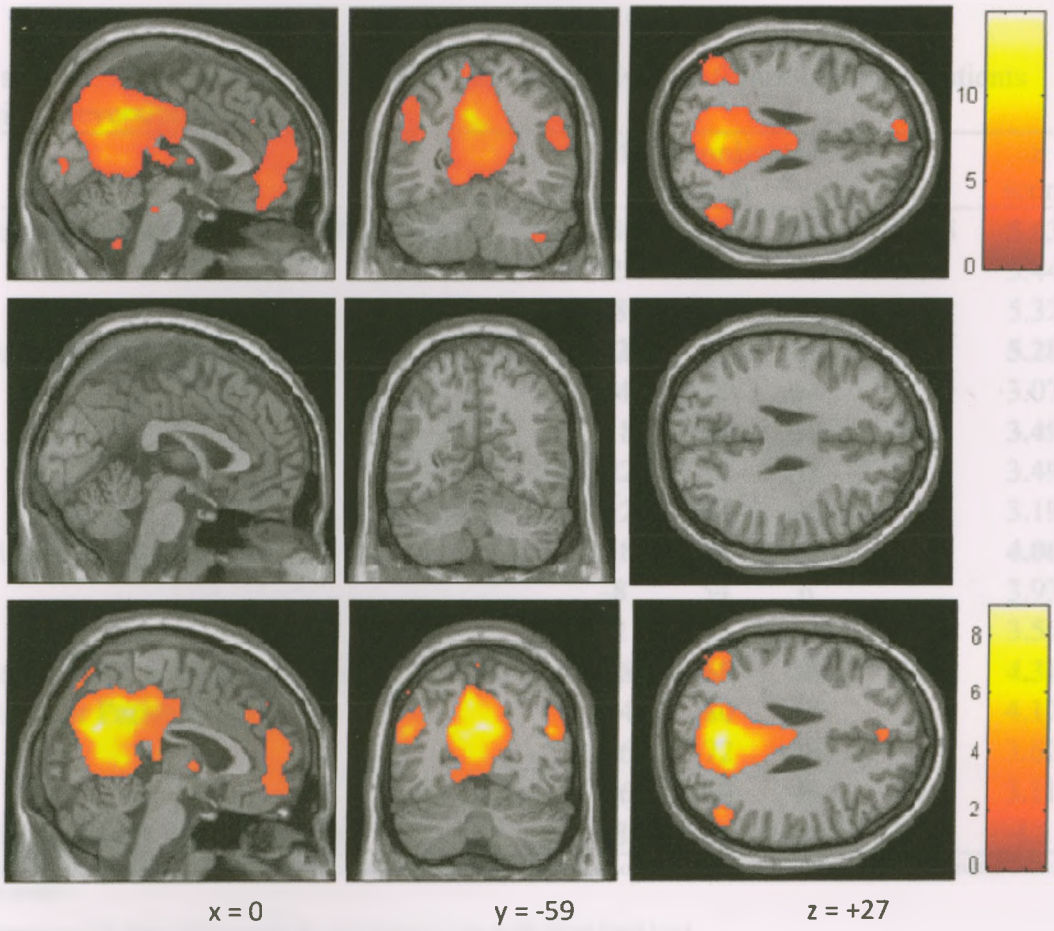

Figure 16. Disruption in DMN functional connectivity in irreversible coma. A. One sample t-test of healthy awake controls best-fit component to the DMN spatial template. B. One sample t-test of the best-fit DMN component of comatose patients who had irreversible coma. C. A two-sample t-test depicts significantly greater functional connectivity in the healthy control group versus irreversible comatose group. Thresholded at $P<0.05$, with an extent threshold of 50 voxels, corrected for multiple comparisons, using false discovery rate at the whole brain level.

Table 10. Areas with Significantly More Connectivity in Controls Versus Patients with Irreversible Coma.

	MNI coordinates (x,y,z)			k	z score
Posterior Cingulate Cortex/Precuneus	**4**	**-66**	**28**	**10075**	**5.63**
	2	-46	38		5.44
	-8	-66	22		5.32
Superior Frontal Gyrus	**22**	**44**	**42**	**512**	**5.28**
	24	28	54		3.07
	-18	**50**	**38**	**950**	**3.49**
	-22	32	48		3.49
	-12	48	46		3.19
Medial Frontal Gyrus	**-18**	**56**	**2**	**1873**	**4.06**
	-8	54	6		3.93
	-2	50	-2		3.55
Left Middle Temporal Gyrus/	**-48**	**-68**	**26**	**664**	**4.31**
Superior Parietal Lobule	-34	-80	48		4.17
	-46	-72	46		3.62
	-46	**-60**	**46**	**88**	**3.11**
Right Middle Temporal Gyrus	**48**	**-66**	**24**	**315**	**4.1**

Coordinates in bold type represent global maxima for the cluster reported; coordinates below bold type are local maxima within the cluster

P-values are less than 0.05 corrected for false discovery rate at the whole brain level

Abbreviations: MNI - Montreal Neurological Institute; k - cluster size in voxels

DMN Correlations to SSEPs and EEG

Ten patients who had a Category II EEG pattern, thought to reflect a poor neurological outcome, had a DMN component with a mean correlation of 0.16 (SD=0.79) to the DMN spatial template. The two patients who had a Category I EEG pattern, which are not associated with poor outcome, had a DMN component with a lower correlation to the spatial template of 0.05 (SD=0.01) (Figure 17).

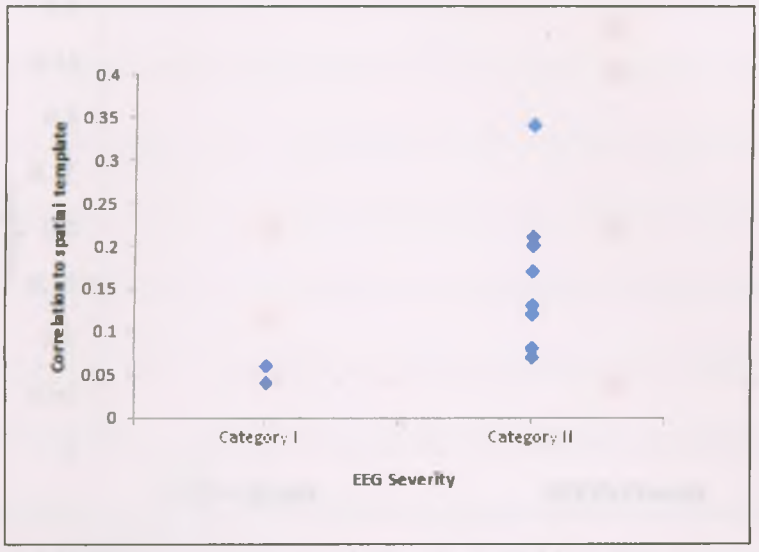

Figure 17. Correlation of the DMN best-fit component in comatose patients to the severity of EEG patterns of patients.

SSEPs were reliably performed on only seven comatose patients. Three patients were found to have bilaterally absent SSEPs and four patients had bilaterally present SSEPs. Patients with absent SSEPs, an indicator of poor outcome, had a best-fit component that had a lower correlation to the spatial DMN template then those patients who had bilaterally present SSEPs (Figure 18). The three patients with absent SSEPs who subsequently died had a DMN component with a correlation of 0.13 (SD= 0.06) to the DMN spatial template. In the four patients with present SSEPs, the DMN component had a correlation of 0.24 (SD=0.14) to the template. However, two of the four patients with present SSEPs who did not recover consciousness had a lower correlation to the template (similar to patients with absent SSEPs) then the two patients who eventually regained consciousness.

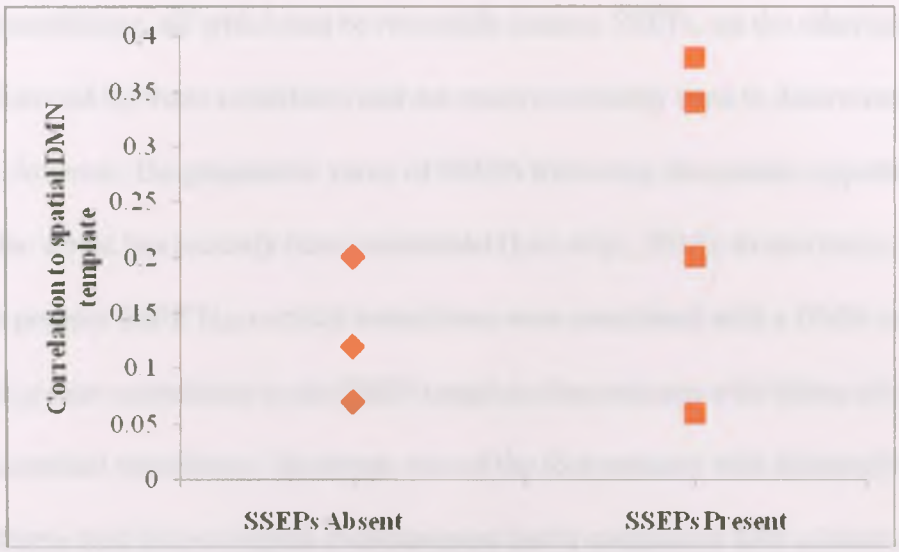

Figure 18. Patients' DMN component correlation to spatial template versus the presence or absence of SSEPs.

Discussion

In this study, I found that default mode network connectivity was intact in two cases of coma where the patients recovered consciousness but absent in comatose patients who failed to recover consciousness. These findings suggest that the presence of DMN connectivity may be an indicator of reversible coma. I demonstrated that the default mode network better correlated with the level of outcome of the patients as assessed by Glasgow Outcome Scale than the current clinically used tools including anatomical MRI findings, EEG and SSEPs. The electrophysiological measures (i.e. EEG and SSEPs) have some notable limitations which account for the inability to predict favourable outcome. Apart from complete suppression of EEG activities 24 hours after the onset of cardiac arrest, no other single EEG pattern has a 100% association with an outcome no better than permanent vegetative state (Young, 2000; Wijdicks et al., 2006). In addition, EEG activities can be influenced by reversible factors recording such as drugs, sepsis, and

metabolic conditions, all which can be reversible factors. SSEPs, on the other hand, are not as influenced by these conditions and are more commonly used to determine poor outcome. However, the prognostic value of SSEPs following therapeutic hypothermia after cardiac arrest has recently been questioned (Lee et al., 2010). In this study, bilaterally present SSEP N_{20} cortical waveforms were associated with a DMN component which had greater correlation to the DMN template than patients with bilaterally absent SSEP N_{20} cortical waveforms. However, two of the four patients with bilaterally present N_{20} waveforms that did not regain consciousness had a component with a lower correlation to the template (similar to those patients with absent N_{20} waveforms) in comparison to those patients who regained consciousness. This is in line with low sensitivity of the SSEP testing. While bilateral absence of SSEP N_{20} waveforms reliably predicts poor outcome, unilateral or bilateral presence of SSEPs does not predict the course of coma. Many patients who fail to recover from coma still have preserved N_{20} responses with a sensitivity of only 46% (Wijdicks et al., 2006). Resting state DMN connectivity was more sensitive than SSEPs in identifying a favorable outcome prognosis on an individual basis. In addition, the current resting state study examining DMN connectivity was superior to our group's fMRI paradigm of somatosensory stimulation (Gofton et al., 2009). While patients who survived cardiac arrest showed greater BOLD in S1 to somatosensory stimulation compared to patients who did not (as well as greater BOLD in S1 of patients with present SSEP N_{20} waveforms to those with absent waveforms), three out of five survivors had BOLD signals comparable to non-survivors (Gofton et al., 2009).

In this study, two patients with bilaterally present SSEP N_{20} waveforms and irreversible coma had an absence of DMN connectivity which may be a result of remaining islands of cerebral function which have been shown to exist in severely brain damaged patients (Laureys et al., 2000; Schiff et al., 2002). In patients who still have present SSEP N_{20} waveforms but who do not recover consciousness there may be a corticocortical disconnection from primary somatosensory areas to secondary sensory areas not measured by SSEPs. This is supported by Laureys et al.(2002) who found in PVS patients noxious somatosensory stimulations still activated midbrain, contralateral thalamus, and primary somatosensory cortex but had no activation of secondary somatosensory, bilateral insular, posterior parietal and anterior cingulate cortex. The activated primary somatosensory cortex was functionally disconnected from secondary somatosensory, bilateral posterior parietal, premotor, polysensory superior temporal and prefrontal cortices. This suggests that the isolation and dissociation of the primary somatosensory cortex from higher-order associative areas in PVS is indicative of a lack of conscious processing and awareness of the somatosensory stimuli (Laureys et al. 2002). DMN connectivity analysis used in the study overcame the limitations of fMRI SSEP activation outlined by Laureys et al. (2002) because it assesses corticocortical connections across multiple sites in both cerebral hemispheres.

New Insights into the Function of Default Mode Network

The DMN activity is unique to the resting-state of the brain and is suspended during goal-directed behaviours (Shulman et al., 1997; Raichle et al., 2001, Grecius et al., 2003). It has been suggested to represent the intrinsic environment including self-directed thought and spontaneous cognition which are anti-correlated to the extrinsic system of the

brain that engages sensory-motor cortices responsible for external awareness (Mason et al, 2007). If this hypothesis holds true, it should be absent in cases where conscious mental activity does not occur, such as coma, vegetative state, and anesthesia. However, in this study I have shown that the DMN was present in two comatose patients who eventually recovered consciousness, while it was absent in those who did not recover consciousness. Its presence in comatose survivors of cardiac arrest suggests that the DMN cannot be exclusively responsible for conscious awareness, as these patients were comatose at the time of scanning. The presence of DMN connectivity in reversible coma is supported by the current literature finding preserved DMN in some case studies of VS, deep anesthesia in the monkey, and light sedation in the healthy individual (Boly et al., 2009; Vincent et al., 2007; Grecius et al., 2008). The DMN, therefore, may represent a more fundamental or intrinsic property of functional brain organization (Boly et al., 2008; Vincent et al., 2007). It could be suggested that the DMN is needed for consciousness to occur but cannot be exclusively responsible for conscious awareness. The DMN may be necessary for consciousness to occur as the two patients who regained consciousness had DMN connectivity and those who did not regain consciousness had absent DMN connectivity. However, the DMN cannot be sufficient to produce awareness, as both patients who regained consciousness were comatose at the time of the resting-state scan.

Metabolic Considerations in the DMN of Comatose Patients

The absence of DMN connectivity in patients with irreversible coma is thought to be the result of ischemic insult from the cardiac arrest to areas within the network that are considered "watershed areas" which are border zone regions to major cerebral arteries

(Torvik, 1984). These areas include the part of the cortex and adjacent subcortical white matter that is located at the border zone of anterior cerebral artery and middle cerebral artery which is inclusive of the medial prefrontal cortex and the posterior cingulate cortex. These mesial structures lie between the middle cerebral artery and posterior cerebral artery (Derdeyn et al., 2001). In addition, these vulnerable frontoparietal areas of the brain also have a high metabolic demand and an abundance of excitatory NMDA neurotransmitter receptors. When blood flow ceases during cardiac arrest (no-flow period) and subsequently during the post-resuscitation period (low-flow) areas within the DMN including the mPFC and PCC/precuneus are highly susceptible to injury. This is supported by reported dysfunction of cortical connectivity in areas of the DMN in VS (Laureys et al., 1999). A common pattern of impaired regional cerebral glucose metabolism was found in a heterogeneous case series of four VS patients when compared to the controls. The reduction in glucose metabolism was seen in cortical areas including the prefrontal, temporoparietal association areas, and posterior cingulate cortex/precuneus, i.e. areas known to be implicated in the DMN. In addition, VS patients had a significant difference in effective connectivity between the left prefrontal and premotor cortex and the posterior cingulate in comparison to controls (Laureys et al., 1999). The dysfunction of the DMN following traumatic brain injury is thought to be a result of the high rate of metabolism within these cortical regions. The 'metabolism hypothesis' theory of the DMN has also been used to describe the progression of Alzheimer's disease which shows alterations in the DMN connectivity at rest. Buckner et al. (2005) suggest that cerebral metabolism associated with default mode network activity

during the resting state over one's lifetime predisposes these cerebral cortical regions to AD-related changes, including amyloid deposition, metabolic disruption, and atrophy.

The high metabolic demand of cortical areas in the DMN in comparison to the rest of the cortex predisposes this area to a greater degree of atrophy following a reduction in blood flow. Most recently, Horstmann et al. (2010) examined brain atrophy in twelve patients who regained consciousness after coma due to cardiac arrest and a common neuroanatomical pattern of brain tissue loss in areas implicated in the default mode network including: frontal medial regions along the midline, lateral and medial parietal regions, and medial temporal lobe structures.

Structural Rewiring in Recovery

In the current study I demonstrated functional connectivity in DMN in survivors of coma due to cardiac arrest but its absence in non-survivors. I hypothesize that the presence of the DMN in the survivors results from intact long-range corticocortical and corticothalamic connectivity as the anoxic-ischemic insult during coma following cardiac arrest is not as severe in comparison to those patients who do not survive. Cortical areas in the DMN of survivors presumably do not have the degree of damage as non-survivors and still maintain functional connectivity as observed through the BOLD signal. The emergence of a patient from reversible vegetative state has been subject to several studies. In these patients, cortical areas where metabolism was most impaired during VS returned to near-normal values after recovery (Laureys et al., 2006). The precuneus demonstrated the largest recovery- related metabolic change followed by the association cortices of the frontoparietal regions of the DMN. Laureys et al. (2006) hypothesize that the altered state of consciousness in VS patients is due to a functional "disconnection

syndrome". Disconnections in long-range corticocortical (between anterior and posterior regions of the DMN) and corticothalamic (between midline-posterior cortices and nonspecific thalamic nuclei) were identified in 60 VS patients, and partial functional restoration of these connections occurred upon reversible VS. The idea of the long-distance rewiring in these posterior medial cortices is also supported by a diffusion tensor MRI in a case study of recovery from a minimally conscious state, which showed increased fractional anisotropy, assumed to reflect myelinated fiber density in the cuneus and precuneus (Voss et al., 2006). It is suggested that this white matter reorganization might reflect axonal sprouting or neurite outgrowth, or potentially neurogenesis (which is known to occur in associative cortices in normal primates) (Gould et al, 1999). In addition, changes in the metabolism of the thalamus have been reported after recovery from VS (Laureys et al, 2000). Increased thalamocortical connectivity has also been observed between the intralaminar regions of the thalamus and the prefrontal cortices following recovery. Taking into account the reduction in metabolism in both the PCC/precuneus and thalamus Laureys et al. (2000) suggest that the PCC/precuneus is the primary driver of information processing in critical parts of the neural network correlates of consciousness (NNCC). The critical linkage between the thalamic and PCC/precuneus from brainstem arousal systems has been proposed which is thought to link between the two aspects of consciousness (arousal/wakefulness and awareness). The cholingeric mesopontine projections (from the lateral tegmental reticular formation, parabrachial and laterodorsal tegmental nuclei) to the anteroventral thalamic nucleus are involved in cortical arousal (Vogt & Laureys, 2005). The anteroventral nucleus has prominent excitatory projections to the PCC/precuneus which is thought to mediate the high level of

glucose metabolism (Vogt & Laureys, 2005). It is has been proposed that the PCC is the key player in the NNCC, a critical junction where both arousal and awareness link.

Functional Neuroimaging Predictive Values and Limitations

Functional neuroimaging is a relatively new tool for measuring cognitive function in cases of impaired consciousness. It is superior and complementary to the bedside assessment of cognitive function. The currently used clinical tools in the ICU such as the GCS rely on voluntary movements which may be very small, easily exhausted, and inconsistent in severely brain-injured patients (Laureys et al., 2002). Functional neuroimaging studies have shown markers of emergence from VS before current clinical tools in various case studies of individual patients (Owen et al., 2006). In this thesis I have shown that functional neuroimaging can have prognostic value. A review paper by Di et al. (2008) examined cerebral activations to various stimuli to determine if neuroimaging holds any predictive power for clinical recovery in altered states of consciousness. In a cumulative 15 PET fMRI studies with a total of 48 VS patients (all with various etiologies of impairment) they found that higher level associative cortical activation was superior to absent or low-level primary activation for predicting recovery of consciousness with a 93% specificity and 63% sensitivity ($p<0.001$). Nine of their 11 patients with atypical 'higher order' associative cortical activation patterns recovered consciousness while 21 of their 25 patients with typical primary cortical activation patterns and 4 patients without any cortical activation failed to recover. This critical review of a relatively small sample size suggests that functional neuroimaging can provide important prognostic information beyond that available from bedside

examination alone. In another recent study, Coleman et al. (2009) found neuroimaging data were superior to the behavioral assessment of 2 patients with impaired consciousness at the time of scanning. These patients, while deemed to be in VS, had clear signs of speech comprehension using fMRI. More importantly, across the whole group of 41 patients, the level of auditory processing revealed by fMRI correlated significantly (r_s = 0.81, $P < 0.001$) with subsequent behavioral recovery, six months after the scan.

While functional neuroimaging is a promising tool in the determination of functional recovery and awareness in patients with altered states of consciousness it has some notable limitations. There are challenges in the acquisition, analysis, and interpretation of the fMRI data in this patient population (for review Giacino et al., 2006). In this special patient population, it is not a surprise that there are only sparse heterogeneous case series' or case studies in this area. While achieving a large homogenous group of participants is difficult, a large multi-centered study would aid in understanding conscious processes and the ability to regain awareness in patients with severe brain injuries. Large group studies are difficult to undertake because of the heterogeneous pathology of the injury. Pathology and extent of brain injuries including such things as large lesions in cortex, skull defects, and hydrocephalus pose challenges in processing functional neuroimaging data in this patient population or to normalize their group data and compare them to a healthy control group.

Motion artefacts caused by spontaneous movements such as gag reflex and biting on the ventilator tube can contaminate the data and pose further difficulties in the data analysis. This can be eliminated through sedation and use of paralytic agents but the effect of sedation on the BOLD signal has not been fully investigated. In our study,

propofol, fentanyl, or midazolam was administered to the patients by the treating physicians not involved in the study in order to keep the patients calm and comfortable as per routine clinical practice. The effect of sedation on the DMN is thought to be minimal. Propofol was the preferred agent that was used in these patients. It is thought to have the least effect on cerebral perfusion (Wallerstedt et al., 1999). Propofol has been shown to attenuate the BOLD response of the auditory cortex to acoustic stimulation in a dose-dependent manner but it did not completely abolish primary cortical responses to acoustic stimulation (Dueck et al., 2005). Isoflurane an inhalational anaesthesia, is known to induce a profound impairment of consciousness. However, when it was used on monkeys the resting state fluctuations in BOLD signals including DMN connectivity were still preserved (Vincent et al., 2007). Similar to our findings, these studies support that coma affects BOLD above and beyond the effects of anesthetics.

In comatose patients following cardiac arrest, the coupling of neuronal activity to hemodynamics, the measurement used in fMRI, may also be different from normal healthy controls or other disease states. It has been shown that evoked cerebral blood oxygenation changes in the damaged brain due to ischemia and brain tumors measured by near infrared spectroscopy differ from those in the normal brain (Sakatani et al., 2003). In some patients limited activation areas were seen in BOLD-fMRI during simple motor task in comparison to healthy controls where robust activations were seen (Sakatani et al., 2003). Rossini et al.(2004) reported that stimulus-locked electromagnetic brain activity, which elicits an identifiable fMRI BOLD activation in healthy individuals, frequently fails to evoke similar activation in patients who have suffered a previous stroke. These studies demonstrate the neurovascular uncoupling may reflect altered vasomotor

reactivity. While it is possible that the anoxic-ischemic event following cardiac arrest in our patients had subsequent effects on the cerebral vascular response we found BOLD fMRI was sensitive enough to predict outcome. This is in line with a previous study from our group that found fMRI was sensitive to detect differences in BOLD due to somatosensory stimulation between population groups (Gofton et al., 2009). In addition, it is worth noting that systemic blood oxygenation was completely restored in all patients in this study with a ventilator. The BOLD functional connectivity may also not be as altered when looking at resting state connectivity patterns such as the DMN in comparison to activation studies that have time locked events (whether block design or event related paradigms).

Advantages and limitations of resting state fMRI

The acquisition of resting-state fMRI data to determine DMN connectivity is straightforward and simple to perform than the standard fMRI paradigms which make it an attractive test to study disorders of consciousness. Using sophisticated paradigms in patients who are unable to communicate with the external environment can be challenging. Since these patients are unable to cooperate with tasks, false negative results can occur if testing for conscious awareness (Owen et al., 2007). Resting state fMRI, however, overcomes this issue as it can examine functional connectivity in the absence of any stimulation. However, spontaneous BOLD signal fluctuations in the resting state are more prone to artifacts than evoked data. Both physiological (such as respiratory and cardiopulmonary) and non-physiological (such as low frequency scanner drift, and higher frequency 60 Hz) noise contaminates resting state data. Using a bandpass frequency filter

which filters out low and frequency noise aids in minimizing artefacts. ICA is able to separate out the relevant signal from the artefacts present in the data. ICA is able to separate different sources of signals that are linearly mixed together in the fMRI data without a priori information (Calhoun et al., 2001). This data-driven blind source separation has been shown to effectively separate spatially distributed brain networks, including the DMN, from other types of signal and noise (Bluhm et al.2008; Espositio et al., 2006; Grecius & Menon, 2004). In addition, ICA was used to identify the DMN in comatose patients as it may identify network nodes missed by conventional ROI-based connectivity analysis. A biased selection of a ROI in patients with altered states of consciousness based on other fMRI studies may inaccurately represent the network connectivity if the selection of a particular region, on which subsequent correlations of connectivity were based, was impaired. As DMN connectivity has not been previously investigated in a group level analysis of coma it was unknown if one region within the DMN would display a decrease in connectivity in comparison to the rest of the network. ICA was used to overcome this problem. However, there are some limitations in using ICA. The number of independent components to be extracted from the resting state data has to be defined a priori and the results are dependent on that number. In this study we used MDL criteria to mathematical estimate the number of components in the data in an attempt to avoid under or over estimating the sources of signal in the data. We chose 20 independent components which was the upper limit of the range found in control subjects and are approximately the number of components used in other resting state data studies (Bluhm et al., 2008, Grecius et al., 2008). ICA requires the selection of the component of interest which could still result in bias in interpreting the data. In this study a template

matching procedure was employed to automatically select the component with highest correlation to a spatial DMN template that included all areas known to encompass the network in healthy control subjects. Thus, this method might bias the selection towards the healthy control group.

Conclusions

This is the first study to examine altered connectivity of the DMN in a large cohort of comatose patients after cardiac arrest. It is also the first study to examine DMN connectivity in relation to functional recovery in altered states of consciousness. I have demonstrated that DMN functional connectivity persists in reversible coma following cardiac arrest but the DMN is absent in patients with irreversible coma. DMN cannot be exclusively related to conscious awareness as it was present in two cases of reversible coma. Instead, DMN may be needed for consciousness to occur but not sufficient on its own to elicit awareness. DMN may be a more intrinsic property of brain function that is lost when anoxic-ischemic insult to the brain is severe enough to cause irreversible coma.

References

American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care (2005) Circulation 112.

Al Thenayan E, Savard M, Sharpe M, Norton L, Young GB (2008) Predictors of poor neurologic outcome after induced mild hypothermia following cardiac arrest. Neurology 71:1535-1537.

Auer DP (2008) Spontaneous low-frequency blood oxygenation level-dependent fluctuations and functional connectivity analysis of the 'resting' brain. Magnetic Resonance Imaging 26:1055–1064.

Baars BJ (2005) Global workspace theory of consciousness: toward a cognitive neuroscience of human experience. Progress in Brain Research 150:45-53.

Baars BJ, Ramsoy TZ, Laureys S (2003) Brain, conscious experience and the observing self. Trends in Neuroscience 26:671-675.

Bassetti C, Bromio F, Mathis J, Hess CW (1996) Early prognosis in coma after cardiac arrest: a prospective clinical, electrophysiological and biochemical study of 60 patients. J Neurol Neurosurg Psychiatry 61:610–615.

Bekinschtein T, Niklison J, Sigman L, Manes F, Leiguarda R, Armony J, Owen A, Carpintiero S, Olmos L (2004) Emotion processing in the minimally conscious state. J. Neurol. Neurosurg. Psychiatry 75:788.

Biswal B, Yetkin FZ, Haughton VM, Hyde JS (1995) Functional connectivity in the motor cortex of the resting human using echo-planar mri. Magnetic Resonance in Medicine 34:537-541.

Boly M, Phillips C, Tshibanda L, Vanhaudenhyse A, Scharbus M, Dang-Vu TT, Moonen G, Hustinx R, Maquet P, Laureys S (2008) Intrinsic brain activity in altered states of consciousness: How conscious is the default mode of brain function? Ann NY Acad Sci 1129:119-29.

Boly M, Tshibanda L, Vanhaudenhuyse A, Noirhomme Q, Schnakers C, Ledoux D, Boveroux P, Garweg C, Lambermont B, Phillips C, Luxen A, Moonen G, Bassetti C, Maquet P, Laureys S (2009) Functional connectivity in the default network during resting state is preserved in a vegetative but not in a brain dead patient. Human Brain Mapping 30:2393–2400.

Boveroux P, Bonhomme V, Boly M, Vanhaudenhuyse A, Maquet P, Laureys S (2008) Brain function in physiologically, pharmacologically, and pathologically altered states of consciousness. International Anesthesiology Clinics 46:131-146.

Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE, Mathis CA, Morris JC, Mintun MA (2005) Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci. 25:7709-17.

Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The brain's default network. Anatomy, function, and relevance to disease. Ann. N.Y. Acad. Sci. 1124:1-38.

Buechler CM, Blostein PA, Koestner A, Hurt K, Schaars M, McKernan J (1998) Variation among trauma centers' calculation of Glasgow Coma Scale score: results of a national survey. Journal of Trauma: Injury, Infection, and Critical Care 45: 429-432.

Bluhm R, Osuch E, Lanius R, Boksman K, Neufeld R, Theberge J, Williamson, P (2008) Default mode network connectivity: effects of age, sex, and analytic approach. Neuroreport 19:887-891.

Calhoun V, Adali T, Pearlson G, Pekar J (2001) A Method for Making Group Inferences From Functional MRI Data Using Independent Component Analysis. Human Brain Mapping 14:140-151.

Calhoun VD, Adali T, Hansen LK, Larsen J, Pekar (2003) ICA of functional MRI data: an overview. Fourth International Symposium on Independent Component Analysis and Blind Source Separation 281-288.

Callans, DJ (2004) Out-of-Hospital Cardiac Arrest — The Solution Is Shocking. New Eng J Med 351:632-634.

Castelli F, Happe F, Frith U, Frith C (2000) Movement and mind: a functional imaging study of perception and interpretation of complex intentional movement patterns. Neuroimage 12:314-325.

Cauda F, Micon BM, Sacco K, Duca S, D'Agata F, Geminiani G, Canavero S (2009) Disrupted intrinsic functional connectivity in the vegetative state. J Neurol. Neurosurg. Psychiatry 80:429-431.

Chausson N, Wassouf A, Pegado F, Willer JC, Naccache L (2008) Electrophysiology: mismatch negativity and prognosis of coma. Rev Neurol 164 Spec No 1:F34-F35.

Coleman MR, Davis MH, Rodd JM, Robson T, Ali A, Owen AM, Pickard JD (2009) Towards the routine use of brain imaging to aid the clinical diagnosis of disorders of consciousness. Brain 132: 2541-2552.

Cordes D, Haughton VM, Arfanakis K, Carew JD, Turski PA, Moritz CH, Quigley MA, Meyerand ME (2001) Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. American Journal of Neuroradiology 22:1326-1333.

Crossman J, Bankes M, Bhan A, Crockard HA (1998) The Glasgow Coma Score: Reliable evidence? Injury 29:435–437.

Damoiseaux JS, Rombouts SARB, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann, CF (2006) Consistent resting-state networks across healthy subjects. PNAS 103:13848-13853.

De Luca M, Beckmann C, De Stefano N, Matthews P, Smith S (2005). fMRI resting state networks define distinct modes of long-distance interactions in the human brain. NeuroImage 29:1359-1367

Derdeyn CP, Khosla A, Videen TO, Fritsch SM, Carpenter DL, Grubb RL, Powers WJ (2001) Severe hemodynamic impairment and border zone-region infarction. Neuroradiology 220: 195-201.

Di H, Boly M, Weng X, Ledoux D, Laureys S (2008) Neuroimaging activation studies in the vegetative state: predictors of recovery? Clinical Medicine 8:502-507.

Di HB, Yu SM, Weng XC, Laureys S, Yu D, Li QJ, Qin PM, Zhu YH, Zhang SZ, Chen YZ (2007) Cerebral response to patient's own name in the vegetative and minimally conscious states. Neurology 68:895-899.

Dueck MH, Petzke F, Gerbershagen HJ, Paul M, Hesselmann V, Girnus R, Krug B, Sorger B, Goebel R, Lehrke R, Sturm V, Boerner U (2005) Propofol attenuates responses of the auditory cortex to acoustic stimulation in a dose-dependent manner: a FMRI study. Acta Anaesthesiol Scand 49:784-91.

Esposito, F, Bertolino A, Scarabino T, Latorre V, Blasi G, Popolizio T, Tedeschi G, Cirillo S, Goebel R, Di Salle F (2006) Independent component model of the default-mode brain function: Assessing the impact of active thinking. Brain Research Bulletin 70: 263-269.

Fox MD, Raichle ME (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neuroscience 8:700-711.

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks. PNAS 102:9673-9678.

Franco R, Pritchard A, Calhoun VD, Mayer AD (2009) Inter-rater and inter-method reliability for selecting the default mode network during data-driven analyses. Human Brain Mapping 30: 2293-2303.

Fransson P (2005) Spontaneous low-frequency BOLD signal fluctuations: an fMRI investigation of the resting-state default mode of brain function hypothesis. Human Brain Mapping 26:15-29.

Fransson P (2006) How default is the default mode of brain function? Further evidence from intrinsic BOLD signal fluctuations. Neuropsychologia 44: 2836-2845.

Gardiner MJ, Leather R, Teo K (1999) The Prevention of Sudden Death from Ventricular Arrythmia. Chapter 1, Epidemiology, Canadian Cardiovascular Society.

Garrity AG, Pearlson GD, McKiernan K, Lloyd D, Kiehl KA, Calhoun VD (2007) Aberrant "Default Mode" Functional Connectivity in Schizophrenia. Am J Psychiatry 164:450-457.

Geocadin R (2010) Advances in the care of brain injury after resuscitation from cardiac arrest. American Academy of Neurology Conference Education Program Syllabus, Toronto 2010.

Giacino J, Hirsch J, Schiff N (2006) Functional neuroimaging applications for assessment and rehabilitation planning in patients with disorders of consciousness. Arch Phys Med Rehabil 87:67-76.

Gofton T, Chouinard P, Young GB, Bihari F, Nicolle M, Lee D, Sharpe M, Yen Y, Takahashi A, Mirsattari SM (2009) Functional MRI study of the primary somatosensory cortex in comatose survivors of cardiac arrest. Experimental Neurology 217:320-327.

Gould E, Reeves AJ, Graziano MS, Gross CG (1999) Neurogenesis in the neocortex of adult primates. Science. 286:548–552.

Greene JD, Sommerville RB, Nystrom LE, Darley JM, Cohen JD (2001) An fMRI investigation of emotional engagement in moral judgment. Science 293:2105-2108.

Greicius MD, Kiviniemi V, Tervonen O, Vainionpaa V, Alahuhta S, Reiss AL, Menon V (2008) Persistent default-mode network connectivity during light sedation. Hum Brain Mapp 29:839-47.

Greicius MD, Menon V (2004) Default-mode activity during a passive sensory task: uncoupled from deactivation but impacting activation. J Cogn Neur 16:1484-1492.

Greicius MD, Srivastava G, Reiss AL, Menon V (2004) Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci USA 101:4637-42.

Greicius MD, Krasnow B, Reiss AL, Menon V (2003) Functional connectivity in the resting brain: A network analysis of the default mode hypothesis. Proc Natl Acad Sci USA 100:253-8.

Gusnard DA, Raichle M (2001) Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci 2: 685-694.

Hahn RB, Ross TJ, Stein EA (2007) Cingulate activation increases dynamically with response speed under stimulus unpredictability. Cereb. Cortex 17:1664-1671.

Horovitz SG, Fukunaga M, de Zwart JA, van Gelderen P, Fulton SC, Balkin TJ, Duyn JH (2007) Low frequency BOLD fluctuations during resting wakefulness and light sleep: a simultaneous EEG-fMRI study. Hum. Brain Mapp 29:671-82.

Horovitz SG, Braun AR, Carr WS, Picchioni D, Balkin TJ, Fukunaga M, Duyn JH (2009) Decoupling of the brain's default mode network during deep sleep. PNAS 27:11376-11381.

Horstmann A, Frisch S, Jentzsch RT, Muller K, Villringer A, Schroeter ML (2010) Resuscitating the heart by losing the brain. Brain atrophy in the aftermath of cardiac arrest. Neurology 74:306-312.

Houlden DA, Li C, Schwartz ML, Katic M (1990) Median nerve somatosensory evoked potentials and the Glasgow Coma Scare as predictors of outcome in comatose patients with head injuries. Neurosurgery 27:701-707.

Huettel SA, Song AW, McCarthy G (2009) Functional magnetic resonance imaging. Sinauer Associates.

Hutchison RM, Mirsattari SM, Jones CK, Gati JS, Leung LS (2010) Functional networks in the anesthetized rat brain revealed by independent component analysis of resting-state fMRI. J Neurophysiol (online ahead of print).

Hypothermia After Cardiac Arrest Study Group (2002) Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. N Engl J Med 346:549-556.

Jasper HH (1958). The ten-twenty electrode system of the International Federation. Electroenceph. Clin. Neurophysiol. 10: 371-375.

Jennet B, Bond M (1975) Assessment of outcome after severe brain damage. Lancet 1:480-484.

Keenan JP, Nelson A, Oconnor M (2001) Self recognition and the right hemisphere. Nature 409:305.

Laureys S, Boly M, Maquet P (2006) Tracking the recovery of consciousness from coma. J. Clin. Invest. 116:1823–1825.

Laureys S, Faymonville ME, Luxen A, Lamy M, Franck G, Maquet P (2000) Restoration of thalamocortical connectivity after recovery from persistent vegetative state. Lancet 355:1790-1791.

Laureys S, Faymonville ME, Peigneux P, Damas P, Lambermont B, Del Fiore G, Degueldre C, Aerts J, Luxen A, Franck G, Lamy M, Moonen G, Maquet P (2002) Cortical processing of noxious somatosensory stimuli in the persistent vegetative state. Neuroimage 17:732-741.

Laureys S, Lemaire C, Maquet P, Franck G (1999) Cerebral metabolism during vegetative state and after recovery to consciousness. Journal of Neurology Neurosurgery and Psychiatry 67: 121.

Laureys S, Owen A, Schiff N (2004) Brain function in coma, vegetative state, and related disorders. Lancet 3:537-546.

Lee YC, Phan TG, Jolley DJ, Castley HC, Ingram DA, Reutens DC (2010) Accuracy of clinical signs, SEP, and EEG in predicting outcome of hypoxic coma: a meta-analysis. Neurology 74: 572-580.

Maddock JR (1999) The retrosplenial cortex and emotion: new insights from the functional neuroimaging of the human brain. Trends Neurosci. 22:310-316.

Mason MF, Norton MI (2007) Wandering minds: the default network and stimulus-independent thought. Science 315:393-395.

Mirsattari SM, Lee DH, Jones D, Bihari F, Ives JR (2004) MRI compatible EEG electrode system for routine use in the epilepsy monitoring unit and intensive care unit. Clin Neurophysiol. 115:2175-80.

Mirsattari SM, Wang Z, Ives JR, Bihari F, Leung LS, Bartha R, Menon RS (2006) Linear aspects of transformation from interictal epileptic discharges to BOLD fMRI signals in an animal model of occipital epilepsy. Neuroimage 30:1133-48.

Mirsattari SM, Davies-Schinkel C, Young GB, Sharpe MD, Ives JR, Lee DH (2009) Usefulness of a 1.5 T MRI-compatible EEG electrode system for routine use in the intensive care unit of a tertiary care hospital. Epilepsy Res. 84:28-32.

Menon DK, Owen AM, Williams EJ, Minhas PS, Allen CMC, Boniface, SJ, Pickard JD (1998) Cortical processing in persistent vegetative state. Lancet 352:200.

Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE (1997) Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. Ann. Neurol. 42:85-94.

Mortiz CH, Rowley HA, Haughton VM, Swartz KR, Jones J, Badie B (2001) Functional MR imaging assessment of a non-responsive brain injured patient. Magnetic Resonance Imaging 19:1129-1132.

Owen AM, Coleman MR (2007) Functional MRI in disorders of consciousness: advantages and limitations. Curr Opin Neurol 20:632-637.

Owen AM, Coleman MR, Boly M, Davis MH, Laureys S, Pickard JD (2006) Detecting awareness in the vegetative state. Science 313:1402.

Owen AM, Menon DK, Johnsrude IS, Bor D, Scott SK, Manly T, Williams EJ, Mummery C, Pickard JD (2002) Detecting residual cognitive function in persistent vegetative state. Neurocase 8:394-403.

Patel A, Alotaibi F, Blume WT, Mirsattari SM (2009) Independent component analysis of subdurally recorded occipital seizures. Clin Neurophysiol. 119:2437-46.

Peltier SJ, Kerssens C, Hamann SB, Sebel PS, Byas-Smith M, Hu X (2005) Functional connectivity changes with concentration of sevoflurane anesthesia. NeuroReport 16:285-288.

Posner JB, Saper CB, Schiff N, Plum F (2007) Plum and Posner's Diagnosis of Stupor and Coma. Oxford University Press 4th edition.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001) A Default Mode of Brain Function. Proc Natl Acad Sci USA 98:676-82.

Raichle ME, Mintun MA (2006) Brain work and brain imaging. Ann Rev Neuroscience 29:449-476.

Raichle ME, Snyder A (2007) A default mode of brain function: A brief history of an evolving idea. NeuroImaging 37:1083-1090.

Rossetti AO, Oddo M, Logroscino G, Kaplan P (2010) Prognostication after cardiac arrest and hypothermia: A prospective study. Annals of Neurology 67:301-307.

Rossini PM, Altamura C, Ferretti A, Vernieri F, Zappasodi F, Caulo M, Pizzella V, Del Gratta C, Romani GL, Tecchio F (2004) Does cerebrovascular disease affect the coupling between neuronal activity and local haemodynamics? Brain 127:99-110.

Sakatani K, Murata Y, Fukaya C (2003) BOLD functional MRI may overlook activation areas in the damaged brain. Acta Neurochir Suppl 87:59-62.

Saxe R, Kanwisher N (2003) People thinking about other people: the role of the temporo-parietal junction in "theory of mind". Neuroimage 19:1835-1842.

Schiff ND, Ribary U, Moreno DR, Beattie B, Kronberg E, Blasberg R, Giacina J, McCagg C, Fins JJ, Llinas R, Plum F (2002). Residual cerebral activity and behavioral fragments can remain in the persistently vegetative brain. Brain 125:1210-1234.

Schacter DL, Addis DR, Buckner RL (2007). Remembering the past to imagine the future: the prospective brain. Nat. Rev. Neurosci. 8:657-661.

Serafetinides EA (1995) Cerebral laterality and consciousness. Arch Neurol 52:337–8.

Shulman GL, Fiez JA, Corbetta M, Buckner RL, Miezin F, Raichle ME, Petersen SE (1997) Common Blood Flow Changes across Visual Tasks: II. Decreases in Cerebral Cortex. J. Cognit. Neurosci. 9:648-663.

Spreng RN, Grady CL (2010) Patterns of brain activity supporting autobiographical memory, prospection, and theory of mind, and their relationship to the default mode network. J Cogn Neurosci. 22:1112-23.

Svoboda E, McKinnon M, Levine B (2006)The functional neuroanatomy of autobiographical memory: A meta-analysis. Neuropsychologia 44:2189-2208.

Teasdale G, Jennett B (1974) Assessment of coma and impaired consciousness. A practical scale. Lancet 2:81-84.

Torvik A (1984) The pathogenesis of watershed infarcts in the brain. Stroke 15:221-223.

Tshibanda L, Vanhaudenhuyse A, Boly M, Soddu A, Bruno M-A, Moonen G, Laureys S, Noirhomme Q (2010) Neuroimaging after coma. Neuroradiology 52:15-24.
Vaillancourt C, Stiell IG (2004) Cardiac arrest care and emergency medical services in Canada. Can J Cardiol 20:1081-1090.

Vanhaudenhuyse A, Noirhomme Q, Tshibanda L, Bruno M-A, Boveroux P, Schnakers C, Soddu A, Perlbarg V, Ledoux D, Brichant J-F, Moonen G, Maquet P, Greicius M, Laureys S, Boly M (2010) Default network connectivity reflects the level of consciousness in non-communicative brain damaged patients. Brain 133: 161–171.

Vincent JL, Patel GH, Fox MD, Snyder AZ, Baker JT, Van Essen DC, Zempel JM, Synder LH, Corbetta M, Raichle ME (2007) Intrinsic function architecture in the anesthetized monkey brain. Nature 447:83-8.

Vogt BA, Laureys S (2005) Posterior cingulate, precuneal and retrosplenial cortices: cytology and components of the neural network correlates of consciousness. Progress in Brain Research 150: 205-217.

Voss HU, Aziz M, Uluç JP, Dyke RW, Kobylarz EJ, McCandliss BD, Heier LA, Beattie BJ, Hamacher KA, Vallabhajosula S, Goldsmith SJ, Ballon D, Giacino JT, Schiff ND

(2006) Possible axonal regrowth in late recovery from the minimally conscious state. J. Clin. Invest. 116:2005–2011.

Wallerstedt SM, Reinstrup P, Uski T, Bodelsson M (1999) Effects of propofol on isolated human pial arteries. Acta. Anaesthesiol. Scand. 43: 1065-1068.

Wijdicks EF, Hijdra A, Young GB, Bassetti CL, Wiebe S (2006) Practice parameter: prediction of outcome in comatose survivors after cardiopulmonary resuscitation (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 67:203-210.

Wijdicks EF (2008) Neurologic complications of critical illness (3rd edition). Oxford University Press: New York.

Wijdicks EF (2005) Clinical scales for comatose patients: the Glasgow Coma Scale in historical context and the new FOUR Score. Rev Neurol Dis. 3:109-17.

Wijnen VJ, van Boxtel GJ, Eilander HJ, de Gelder B (2007) Mismatch negativity predicts recovery from the vegetative state. Clin Neurophysiol 118:597-605.

Young GB (2009a) Neurologic prognosis after cardiac arrest. N Engl J Med 361:605-611.

Young GB (2009b) Coma. Disorders of Consciousness: Ann. N.Y. Acad. Sci. 1157: 32-47.

Young GB, Ropper AH, Bolton CF (1998) Coma and impaired consciousness: a clinical perspective. McGraw-Hill Companies, Inc.

Young GB, McLachlan RS, Kreeft JH, Demelo JD (1997) An electroencephalographic classification for coma. Can J Neurol Sci. 24:320-5.

Young GB (2000) The EEG in coma. J Clin Neurophysiol. 17:473-85.

Zandbergen EGJ, Hijdra A, Koelman HTM, Hart AAM, Vos PE, Verbeek MM, de Haan RJ (2006) Prediction of poor outcome within the first 3 days of postanoxic coma. Neurology 66:62-68.

Zeman A (2001) What in the world is consciousness? Progress in Brain Research 150:1-10.