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Naturalistic paradigms for neuroimaging and bedside measures of conscious awareness

Leah J. Sinai
The University of Western Ontario

Supervisor
Dr. Adrian Owen
The University of Western Ontario

Graduate Program in Psychology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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NATURALISTIC PARADIGMS FOR NEUROIMAGING AND BEDSIDE
MEASURES OF CONSCIOUS AWARENESS

(Thesis format: Monograph)

by

Leah J. <u>Sinai</u>

Graduate Program in Department of Psychology

A thesis submitted in partial fulfillment
of the requirements for the degree of
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The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

Abstract

Complex, naturalistic stimuli can test for covert awareness in behaviourally non-responsive patients. For patients with poor visual function, this thesis aimed to identify an auditory-only stimulus that could evaluate executive function. Also, it assessed if Galvanic Skin Response could be a suitable bedside testing method. Healthy individuals listened to 4 auditory stimuli in the fMRI scanner. During Galvanic Skin Response recording, an independent group of controls listened to an audio narrative and watched a movie. Behaviourally non-responsive patients were also tested during movie viewing. Using fMRI, an audio narrative was identified that produced widespread brain synchronization between healthy participants, critically in the frontoparietal network. Healthy controls showed highly similar GSR to a suspenseful movie. A locked-in syndrome patient had a similar GSR to controls during movie viewing. This narrative can be used for future patient testing, and GSR can be used to test for consciousness at the bedside.

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I would next like to thank Adrian Owen for his guidance, and for providing me with this incredible opportunity. I have a high respect for his ability to manage and consistently support the many members of his lab.

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Finally, thank you to everyone who has encouraged me throughout this project and made this a truly memorable experience. This includes all my wonderful labmates, friends and family who have believed in me every step of the way.

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Chapter 1: Introduction

Disorders of Consciousness

Disorders of consciousness, arising from a variety of etiologies, can render a person incapable of overt behavioural responses, and these patients may also be unaware of themselves and the outside world (Laureys et al., 2004). An accurate diagnosis and understanding of a patient's current state are critical for not only the patient's comfort and care, but also for their family and medical care team. This thesis will address novel neuroimaging and bedside methods to assess a patient's consciousness and residual cognition after brain injury.

Following severe, acute brain injury, neurological disorders that cause impairments to a patient's awareness of the self and surroundings are termed disorders of consciousness (DoC). Coma, vegetative state (VS), minimally conscious state (MCS) are all considered disorders of consciousness (Owen, 2008). After injury, patients can progress through various DoC states before recovering consciousness, or may maintain a DoC diagnosis permanently (Figure 1).
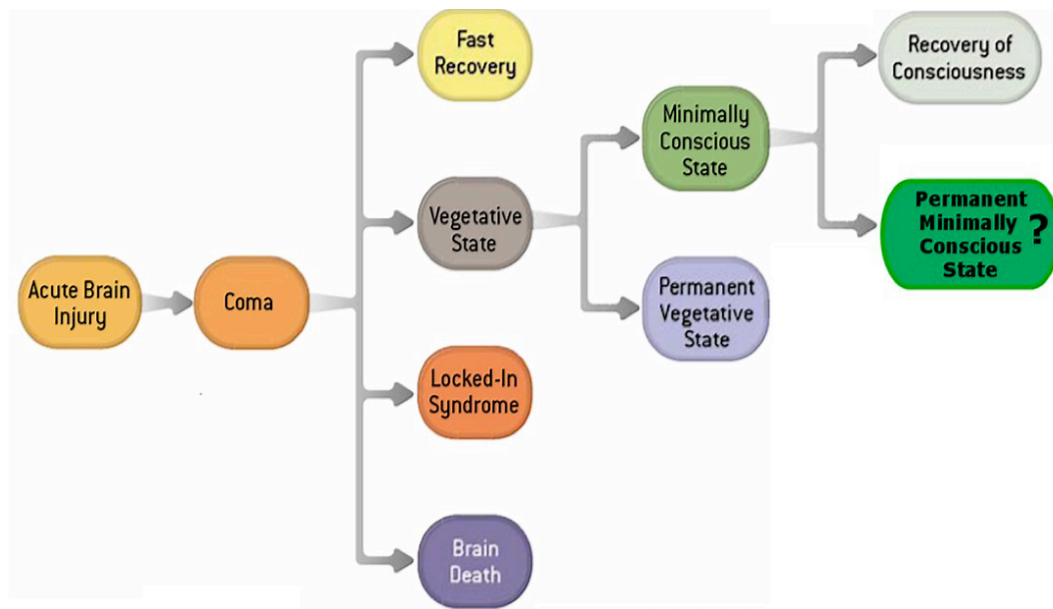
Figure 1: Clinical pathways after acute brain injury (from Laureys, 2007).

Two dimensions are important when discussing disorders of consciousness, each of them existing on a continuum (Laureys et al., 2009). The first is arousal (also known as wakefulness), which refers to a person's sleep-wake cycles and the ability to open one's eyes. A person is considered to have wakefulness if they can open their eyes and possess sleep-wake cycles. Wakefulness requires brain stem function; specifically through actions of the reticular activating system (Parvizi & Damasio, 2001). The second dimension, awareness, refers to one's awareness of the self and surroundings. It also concerns conscious perception, cognition and memories of past experiences. Awareness, also known as consciousness, exists on a continuum, meaning it is not an all-or-nothing concept (Laureys et al., 2002). Clinically, awareness is tested through command following at the bedside (Gosseries et al., 2011). DoC is used to describe a state in which there is a disruption in the normal relationship between awareness and arousal, such that one can exist without the other. Individuals can exist at different levels on each

dimension, and certain combinations of awareness and wakefulness are characteristic of different conditions and clinical diagnoses (Figure 2).
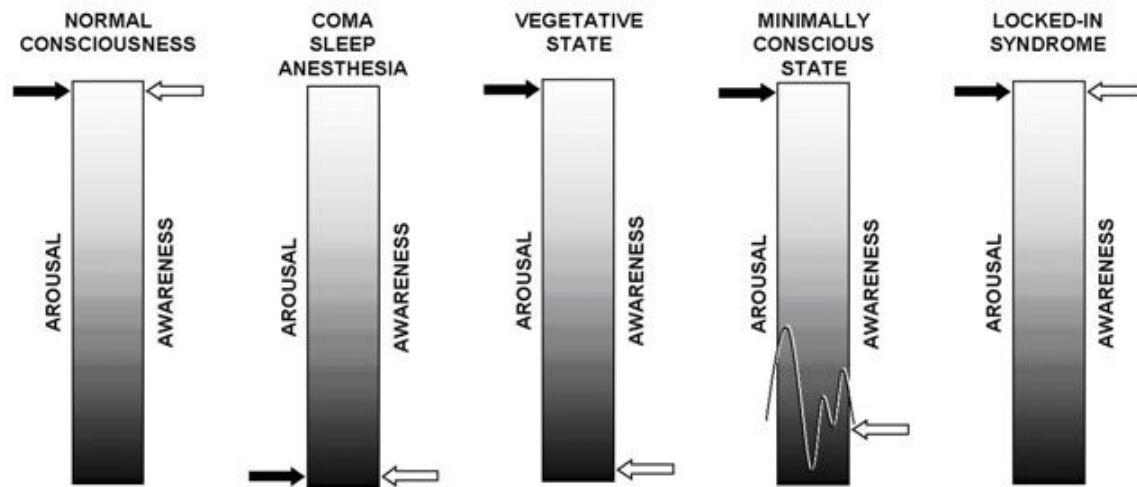
Figure 2: Awareness and arousal levels for disorders of consciousness (From Laureys, Owen & Schiff, 2004).

Coma

After traumatic (i.e. motor vehicle accident or blunt trauma) or non-traumatic (i.e. hypoxia from a cardiac arrest or stroke) acute brain injury, patients may initially be diagnosed as being in a coma. A coma is defined as having low arousal (eyes are closed) and low awareness (Laureys et al., 2009). From the comatose state, patients may transition to a variety of outcomes, including recovery. However, some patients do not regain full awareness and are later diagnosed as being in a vegetative state, minimally conscious state, or having locked-in syndrome.

Vegetative State (VS)

The progression from coma to vegetative state occurs when a patient opens their eyes, indicating they have regained arousal, yet this is not accompanied by regained awareness (Laureys et al., 2002). VS patients have wakefulness, but are unaware of themselves and their surroundings, are unable to respond to external stimuli and are unable to communicate. However, they are able to breathe on their own without the aid of a mechanical ventilator and also exhibit circadian sleep-wake cycles (Laureys et al., 2002). The presence of arousal without awareness is thought to be due to damage to the nuclei of the thalamus and their connections, damage to the cerebral cortex and/or its connections to the thalamus, but without damage to the reticular system (Bernat, 2006). The term "permanent vegetative state" is assigned to individuals who have maintained this condition for one year in the case of traumatic injury, or six months for non-traumatic injury (Owen, 2008).

Minimally Conscious State (MCS)

Compared to the vegetative state, the minimally conscious state is characterized by higher levels of awareness, meaning limited and inconsistent signs of consciousness. It should be noted that the level of awareness in MCS patients has not reached that of a healthy individual. Patients with a MCS diagnosis show reproducible command following, non-reflexive purposeful movement, and/or verbal or gestural yes/no responses (Owen, 2008). Recently, subdivisions of the MCS, MCS PLUS (MCS+) and MCS MINUS (MCS-), have been proposed for this diagnosis due to the heterogeneity of abilities within this category (Bruno, 2011). MCS- patients have the ability to perform non-reflexive movement, while MCS+ patients can perform command following and

language functions in addition to non-reflexive movement. Often, the minimally conscious state is an intermediate condition that is part of the transition from vegetative state to fully regained consciousness. However, in some cases it remains a permanent state. Emergence from the minimally conscious state occurs when a patient can functionally communicate or perform functional use of objects (Giacino et al., 2014).

Locked-In Syndrome (LIS)

Patients who are totally paralyzed, but still maintain the ability to use vertical eye movement or blinking to communicate, are diagnosed with locked-in syndrome (LIS) (Schnakers et al., 2008). LIS is not considered a disorder of consciousness because patients are known to be fully conscious. This condition is generally caused by brainstem lesions, but can also be the result of traumatic brain injury. These patients are difficult to diagnose due to the similarity between LIS and VS/MCS, and LIS patients may have fluctuating levels of arousal in the acute state of their condition, leading to signs of consciousness to be missed during assessment. Most patients diagnosed with LIS as a result of an acute brainstem lesion maintain normal cognitive abilities and full awareness, but cannot perform motor movements.

Diagnosing Disorders of Consciousness

Standardized behavioural scales are used to objectively evaluate awareness at the bedside. A behavioural scale commonly used for DoC patients is the JFK Coma Recovery Scale- Revised (JFK CRS-R) (Kalmar & Giacino, 2005). This scale is composed of six subscales evaluating auditory, visual, motor and verbal functions, as well as communication and arousal (eye opening) (Schnakers et al., 2009). Each subscale contains items of increasing complexity, with lower items receiving lower scores. The

lowest items test reflexive activity and higher items test cognitively mediated behaviours. Patients receive a score up to the maximum value of 23, with lower scores indicating poor awareness, or lack there of. The CRS-R can provide a diagnosis of vegetative state, minimally conscious state or emergence from minimally conscious state depending on the score obtained at the bedside.

However, the inability of a patient to overtly communicate and follow behavioural commands, as measured by the CRS-R, does not always imply absence of consciousness. Since the CRS-R behavioural scale relies on overt behavior and motor movement, it may not detect awareness in some DoC patients if they have motor impairments (Schiff, 2006). Up to 43% of patients have been shown to be misdiagnosed as VS when they do in fact show signs of awareness (Schnakers et al., 2009). Some patients are diagnosed as VS after routine bedside assessment, but after more thorough bedside testing, inconsistent but reproducible behavioral signs of awareness are observed. These inconsistent signs of awareness would reclassify a patient as MCS after an initial misdiagnosis of VS. Neuroimaging can provide a more sensitive measure of consciousness in DoC patients (Cruse et al., 2011; Fernandez-Espejo & Owen, 2013; Naci & Owen, 2013). In these tests, brain activity is used as a proxy for behaviour (Monti et al., 2010). The results from neuroimaging tests can challenge the initial diagnosis given to a patient and may aid in further understanding their abilities and disabilities.

Neuroimaging-based command following paradigms for testing awareness in DoC patients

One category of tasks used for testing covert awareness in DoC patients is termed command following. A command following task requires a patient to actively comply

with the task instructions and follow commands provided to them (Bruno et al., 2010). In an example of a command following task, Owen et al. (2006) tested if DoC patients could perform mental imagery in the fMRI scanner. They designed a task that involved healthy controls and DoC patients performing mental imagery, such as imaging playing tennis or navigating around the rooms in a house. The mental imagery tasks ("tennis" and "house") produced distinct activation patterns in controls. Further, a VS patient produced the same activation patterns when imagining the same scenarios. The patient's ability to follow mental imagery instructions in this task provided strong evidence that she was conscious.

Monti et al. (2010) extended the mental imagery paradigm to communicate with a non-responsive patient. The patient used mental imagery to accurately answer binary questions in the scanner, imagining "tennis" to answer "yes" and "house" to answer "no". This patient was believed to lack awareness following bedside assessment, but neuroimaging results from this command following task indicated that she was indeed aware and could communicate by modulating her brain activity.

In another example of a command following task, researchers used fMRI to measure brain activity during a selective auditory attention task to determine if this could serve as a communication tool for DoC patients (Naci et al., 2013; Naci & Owen, 2013). Healthy controls and 3 behaviourally non-responsive DoC patients selectively attended to the presentation of a target word while ignoring a non-target word, and then used this method for binary communication. The target word was either "yes" or "no", depending on the answer to the question. Healthy controls showed significant activation in the attention network during attending sessions, and used selective attention to accurately

answer binary questions by only paying attention to the word corresponding to the correct answer. Additionally, 2 DoC patients (1 VS and 1 MCS) used selective attention during fMRI scanning to correctly answer the presented questions. This established another command following task that could be used for testing covert awareness in DoC patients and strengthens the argument that some behaviourally non-responsive patients retain awareness after injury.

Cohort studies show that only about 20% of vegetative patients respond to mental tasks (Cruse et al., 2011; Monti et al., 2010). Beyond a genuine lack of awareness in the remaining 80% of the patients, the lack of responses may be due to a variety of reasons, including the patient's inability to understand the task, unwillingness to comply with the task, fluctuating levels of consciousness, or inability to act on the commands, even if they are understood (Di Perri et al., 2014). Brain damaged patients have difficulty sustaining attention for long periods of time (McDowell et al., 1997), and command following tasks require sustained attention during mentally demanding tasks. A paradigm that captures attention easily and requires less mental effort could be beneficial for those patients who may not be able to perform command following tasks.

Naturalistic audiovisual stimulus for DoC patient testing

A novel neuroimaging paradigm has therefore been developed (Naci et al., 2014) to test for covert awareness in patients who may not be able to command follow. This paradigm fits into a category termed naturalistic paradigms, which are characterized by the presentation of real-life stimuli and absence of task instructions. One approach to analyzing fMRI data during naturalistic stimulus presentation is to look at the inter-subject synchronization. This provides information regarding if different brains are

responding in similar ways to a stimulus, and if they are "synchronized" to each other during stimulus duration. In the first study of this kind, Hasson et al. (2004) acquired fMRI data while healthy participants viewed a movie ("*The Good, The Bad and The Ugly*") in the scanner. Researchers then looked at the inter-subject brain synchronization across participants during stimulus viewing by comparing all individual's fMRI time courses. Results showed significant inter-subject correlation in both sensory (auditory and visual) regions and higher-order object areas (occiptotemporal and intraparietal). Most importantly, since synchronization was not present when participants were at rest, they concluded that the movie stimulus induced this inter-subject synchronization. This study indicated that when exposed to the same stimulus, different brains could respond in a similar manner.

By applying the inter-subject synchronization approach, Naci et al. (2014) used a naturalistic paradigm to test for awareness in DoC patients. They aimed to use executive function as a means of quantifying human conscious experience. However, prior to this work, laboratory tests of executive function had not been related to the open-ended nature of conscious experiences. As a solution to this problem, they investigated executive function during movie viewing. By their very nature, engaging movies are designed to give viewers a shared conscious experience driven, in part, by the recruitment of similar executive processes, as each viewer continuously integrates their observations, analyses and predictions, leading to an ongoing involvement in the movie's plot. Although Hasson et al. (2004) observed synchronized brain activity across different individuals watching the same movie, prior to work by Naci et al. (2014), it is not known whether

any of these synchronized activity fluctuations reflect similar executive function across different individuals in response to the evolving executive demands of the movie plot.

Naci et al. (2014) assessed the similarity of movie viewing experiences between different individuals, and further used this viewing to probe for executive function. Healthy individuals watched the suspenseful Alfred Hitchcock movie "*Bang! You're Dead*" in the fMRI scanner. Consistent with previous studies (Hasson et al., 2010), authors found significant widespread inter-subject correlation across the brain. They focused in particular on inter-subject synchronization in frontoparietal regions, known to support executive function (Duncan, 2010; Barbey et al., 2012). A network involving the frontal and posterior parietal cortices, known as the frontoparietal network, contains the executive control network (Niendam et al., 2012). This executive control network is involved in complex cognitions, goal directed behavior, external awareness, attention and planning. To establish that the activity of the frontoparietal region truly reflected executive function during movie viewing, they assessed the movie's executive demands in two additional independent experiments.

One experiment used a dual-task experiment to further investigate the function of the frontoparietal network during movie viewing in healthy volunteers. During the dual-task, executive function resources (including attention) were divided between a key-pressing task, known as the Sustained Attention to Response Task (SART), and movie viewing. The SART required participants to perform a key press every time they heard a "go" digit (one-seven, nine), while withholding their response to a particular "no-go" digit (eight). Participants therefore had to pay attention to the digits they heard, and depending on the digit, make the correct response (key-press or withheld response).

Since executive function is a finite resource, at instances where the movie required more executive function resources, a decrease in performance on the SART occurred. This helped to quantify executive function throughout the movie. Over the course of the movie, the use of executive function resources (as determined by the dual-task) fluctuated in a similar manner compared to the fMRI brain activation time course of the frontoparietal region. This dual-task helped to connect activity in the frontoparietal network with executive function and thereby quantified the use of executive function over the course of the movie. It is therefore reasonable to assume that synchronization of the frontoparietal networks between individuals during movie viewing can be associated with a similar recruitment of executive function.

A stimulus that produces reliable inter-subject synchronization in healthy individuals could be used as a benchmark for assessing the brain function of DoC patients. In other words, if healthy individuals produce a similar fMRI response to each other during a stimulus, a comparison could then be made between the healthy response and the patient's response. The frontoparietal network is described as a "higher-order" network, since it is involved in stimulus processing beyond sensory processing, thus being involved in higher-order cognition. It is required for the conscious perception of external stimuli (Boly et al., 2011). Executive function of the frontoparietal network is critical for experience of the outside world, and this experience of the outside world is a component of conscious awareness. Therefore, looking specifically at the frontoparietal region, which has been shown to support executive function, would help us make inferences regarding if patients are conscious and aware.

Naci et al (2014) used the aforementioned naturalistic audiovisual stimulus (Hitchcock movie) to test for covert awareness in two DoC patients. For both patients, their diagnoses have fluctuated between VS and MCS after repeated CRS-R bedside assessments during their time of non-responsiveness. These patients freely viewed the movie "*Bang You're Dead*" in the fMRI scanner. Researchers then compared each patient's brain activity during movie viewing to the responses of the healthy control group. They used the fMRI time courses of three functional networks (auditory, visual and frontoparietal), obtained from healthy controls as baseline measures. Patient 1, who was given diagnosis of MCS on the day of testing, showed synchronization with healthy subjects for the auditory network, but showed no significant synchronization for the visual and frontoparietal networks. The lack of visual function in this patient may underlie the absence of observable frontoparietal activity, since the stimulus has an important visual component that is required for full understanding of the film. It should be noted that negative findings such as these must be interpreted with caution, since they cannot be used as evidence for lack of awareness. Indeed, negative findings in neuroimaging studies can sometimes occur even in healthy volunteers (Owen & Coleman, 2007).

The auditory and visual networks of Patient 2, who had an MCS diagnosis on the day of testing, had significant synchronization with the auditory and visual networks of healthy individuals, thus indicating a similar sensory experience to controls. Additionally, the frontoparietal network of Patient 2 had significant correlation with that of healthy participants. The patient's frontoparietal activity could be predicted by the executive demands of the movie, which were previously measured in healthy participants

in the dual-task. Therefore, the patient showed similar executive processing of the events in the movie compared to healthy controls. Overall, these results indicated a similar time-locked movie viewing experience, involving both sensory and higher-order regions, for Patient 2 as compared to healthy individuals. This suggested that the patient had a similar conscious cognitive experience compared to healthy individuals when viewing the same movie. Patient 2 showed very few signs of awareness during many years of behavioural assessment: he did not move to commands, communicate, or interact with people or objects in his environment. Despite these behavioural results, this neuroimaging paradigm was able to indicate that he was conscious. In summary, this study showed that executive function activity during naturalistic neuroimaging paradigms can be used as a window into quantifying and understanding the conscious experience of a behaviourally non-responsive patient.

In contrast to command following tasks, naturalistic paradigms look at active cognitive processes, such as executive function during complex stimuli, in the absence of structured instructions. By engaging attention naturally and mimicking real life experiences, this type of task is less strenuous for the patient (Bruno et al., 2010).

fMRI naturalistic paradigms are therefore highly suited for testing covert awareness in DoC patients. However, due to many patients' difficulty in processing visual stimuli and inability of coma patients to open their eyes, audiovisual stimuli may not be effective in a substantial number of patients. To address this problem, I will develop an auditory-only paradigm that could be used to test conscious cognition in DoC patients (see chapter 2). I will determine an effective stimulus that produces highly similar responses across individuals.

Bedside Testing for DoC patients

Although fMRI has proved to be beneficial in assessing disorders of consciousness patients, two limitations of fMRI are that it is expensive and non-portable, leading to restricted usage with the DoC patient population. In addition, patients with metal implants are not able to enter the fMRI scanner. In chapter 3, I argue that Galvanic Skin Response (GSR), a portable and inexpensive technique, may be able to test for covert awareness at the bedside.

Galvanic skin response (GSR), also known as electrodermal activity (EDA), is a psychophysiological technique that measures changes in the electrical conductivity of the skin as a result of increases and decreases in sweat. The autonomic nervous system (and more specifically, the sympathetic nervous system) controls sweat production, and therefore GSR is used as a measure of sympathetic nervous system activity (Critchley, 2002).

The sympathetic nervous system is responsible for the "fight or flight" response to threatening stimuli, and consequently, frightening or threatening stimuli elicit an increase in sweat production (Critchley, 2002). The limbic system also has a major influence on the electrodermal response (Boucsein, 2012). Importantly, skin conductance increases with emotional arousal, attention and cognitive effort, which are important components for testing consciousness (Critchley et al., 2000). Higher-order brain regions that influence GSR include the ventromedial prefrontal cortex, parietal lobe, amygdala, and dorsolateral prefrontal cortex (Critchley, 2002). Therefore, GSR may be used as an indirect behavioural measure of higher-order brain activity driven by emotional and cognitive processes.

In Chapter 3, I will assess whether naturalistic paradigms can be translated to a portable technology, such as GSR, that could be used at the patient's bedside. To this end, I will measure Galvanic Skin Response in both healthy participants and behaviourally non-responsive patients during naturalistic audio and audiovisual stimuli presentation.

Thesis Objectives

1. Develop an auditory-only, naturalistic paradigm by first determining a stimulus that produces highly similar responses across healthy individuals.

2. Determine the suitability of GSR as a bedside testing method for DoC patients. Naturalistic stimuli will first be tested in healthy individuals to find a stimulus that produces highly similar responses. This stimulus will then be used to test for covert awareness in DoC patients at the bedside.

Chapter 2: Developing an auditory-only naturalistic paradigm using fMRI

Introduction

The aim of this chapter is to develop a naturalistic auditory-only paradigm that could be used for testing covert awareness in DoC patients. Previous neuroimaging methods have made important advances towards assessing consciousness in DoC patients (Owen, 2006; Naci & Owen, 2013). However, patient cohort studies show that 4 out of 5 patients do not respond to neuroimaging tests that attempt to elicit command-following brain-based responses in patients (Monti et al., 2010; Cruse et al., 2011). These paradigms require compliance with arbitrary task instructions, and consequently are very effortful for patients who, due to the effects of brain injury, fail to comply with structured instructions (McDowell et al., 1997).

As discussed in the Introduction chapter, naturalistic paradigms for testing DoC patients may provide a solution to this limitation of command following tasks. As they engage attention naturally, they may be more effective for patients who do not have sufficient cognitive resources to modulate their brain activity according to commands. Naci et al. (2014) developed an audiovisual naturalistic paradigm that successfully demonstrated conscious awareness in a patient who had been behaviorally non-responsive for 16 years. However, some chronic DoC patients have impaired visual function, and acute coma patients lack arousal, meaning they have their eyes closed. To test these patients for covert awareness, an auditory-only stimulus is needed. In this chapter, I will test several naturalistic stimuli and validate one auditory-only narrative in

healthy individuals, which may be used for testing covert awareness in DoC patients in future studies.

The first task was to select adequate naturalistic auditory-only stimuli to be tested with fMRI, as high inter-subject synchronization is not seen for all naturalistic stimuli. Hasson et al. (2010) tested four audiovisual stimuli for their ability to produce similar neural responses across different individuals. They showed that a live stream of daily activity in Washington Square Park elicited very little inter-subject synchronization, while clips from two Hollywood films produced moderate inter-subject synchronization. Finally, a well-directed movie by Alfred Hitchcock ("*Bang! You're Dead"*) produced the greatest extent of inter-subject synchronization. This indicates that certain characteristics of the naturalistic stimulus must have an impact on how well subjects are able to time lock to the stimulus. In particular, the movie "*Bang! You're Dead"* produced inter-subject synchronization in the prefrontal cortex, along with sensory (visual and auditory) regions. For inter-subject synchronization to be observed, it is not enough for participants to have the same thoughts. They also must have the same thoughts at the same time, proving the importance of an engaging stimulus that is going to lead viewers through a similar experience.

Potential audio narratives to be used for patient testing could include both words and music, or just words. Previous pilot work in our lab measured behavioural responses to word-only audio narratives, and found they did not elicit robust inter-subject synchronization. That is, individual participants showed variable responses to the word-only narratives. For individual patient testing, we need a stimulus that produces highly similar responses across individual healthy participants to establish a baseline measure.

Therefore, in this study I chose two audio narratives that contain spoken dialogue as well as background music and sound effects, which might engage different listeners in a more similar way than words-only narratives. Furthermore, following the work of Naci et al. (2014) who used a suspenseful movie, I chose suspenseful audio narratives. Conversely, to investigate whether inter-subject synchronization to the narrative depends on comprehension of the narrative or if it can be driven by a suspenseful soundtrack alone, I also tested two suspenseful pieces of music. Thus, I acquired fMRI data in healthy participants during the presentation of 2 audio narratives and 2 music pieces, and compared the inter-subject synchronization elicited by each of these stimuli to determine a stimulus that produced highly similar responses across participants. Critically, I examined the inter-subject synchronization in the frontoparietal network, which has previously been shown to support executive function (Naci et al., 2014, Barbey et al., 2012, Duncan 2010). In this way, I sought to determine whether each stimulus elicited similar executive function demands across different listeners. This would enable us to isolate a stimulus that could be used to test executive function, and therefore conscious awareness, in individual DoC patients.

Method

Participants

Ethics approval was obtained from Western University's Health Sciences Research Ethics Board. All volunteers were right-handed, native English speakers and had no history of neurological disorders. They signed informed consent before participating and were monetarily compensated for their time. 19 volunteers (10 females; 18-31 years) participated in the experiment. Data from 4 participants were excluded due

to interference in the headphones and excessive movement. All analyses were conducted with data from the remaining 15 participants.

Materials

  Stimuli. 4 auditory-only stimuli were tested: 2 auditory narratives ("stories") and 2 musical pieces without lyrics ("songs"). "Taken*"* (5 min 12 sec) is a narrative that is an excerpt from the movie "*Taken"* (2008). Specifically, the clip is taken from a scene when a teenage girl is kidnapped, and her father speaks to the kidnappers over the phone. It contains spoken words as well as background music and sound effects. The narrative "Humanity" (3 min 48 sec) is the final speech delivered by Charlie Chaplin in "*The Great Dictator"* (1940) and is comprised of speech and background music. The instrumental piece "Game of Thrones" (4 min 53 sec) is the theme song from the popular television show of the same name. "Dark Knight" (4 min 28 sec) is the instrumental theme song from "*Dark Knight Rises"* (2012). Finally, the Taken story was used to create an auditory baseline stimulus, referred to as "Noise" (5 min 2 sec). The frequencies in the sound clip were spectrally rotated so that many of the spectro-temporal characteristics of natural speech were maintained, but the clip's meaning is removed.

Procedure

  Design. Auditory stimuli were presented to participants through in-ear headphones. Participants were instructed to keep their eyes closed and pay attention to the sounds. The noise stimulus was presented first to all participants, to avoid any potential priming effects from the "Taken" stimulus. The remaining four clips were counterbalanced. A volume deemed comfortable by the individual was used for the duration of the experiment.

fMRI Data Acquisition. Scanning was performed using a 3 Tesla Siemens Tim Trio system with a 32-channel head coil, at the Robarts Research Institute in London, Ontario, Canada. Participants lay supine in the scanner. Noise cancellation headphones (Sensimetric, S14; www.sens.com) were used for sound delivery. Function echo-planar images (EPI) were acquired (33 slices, voxel size: 3 x 3 x 3mm; inter-slice gap of 25%, TR=2000ms, TE=30ms, matrix size=64x64, FA=75 degrees). An anatomical volume was obtained using a T1-weighted 3D MPRAGE sequence (32 channel coil, voxel size: 1 x 1 x 1mm, TA= 5 min, TE=4.25ms, matrix size= 240x256, FA=9 degrees).

SPM preprocessing. The imaging data were analyzed using SPM8 (Wellcome Institute of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) and the AA pipeline software (Cusack et al., 2015). The processing steps were as follows: correction for timing of slice acquisition, motion correction, normalization to a template brain, and smoothing. The data were smoothed with a Gaussian smoothing kernel of 10mm FWHM (Peigneux et al., 2006). Spatial normalization was performed using SPM8's segment-and-normalize procedure, whereby the T1 structural was segmented into grey and white matter and normalized to a segmented MNI-152 template. These normalization parameters were then applied to all EPIs. The time series in each voxel was high pass-filtered with a cutoff of 1/128Hz to remove low-frequency noise, and scaled to a grand mean of 100 across voxels and scans in each session.

SPM Modeling. The preprocessed data were analyzed in SPM8 using the general linear model. Prior to analyses, the first five scans of each session were discarded to achieve T1 equilibrium and to allow participants to adjust to the noise of the scanner. Fixed-effect analyses were performed in each subject, corrected for temporal auto-

correlation using AR (1)+ white noise model. The regressors were generated by convolving boxcar functions with the canonical hemodynamic response function. Also included in the general linear model were nuisance variables, comprising the movement parameters in the three directions of motion and three degrees of rotation, as well as the mean of each session. Linear contrasts were used to obtain subject-specific estimates for each effect of interest. Linear contrast coefficients for each participant were entered into the second level random-effects analysis. The second-level effects of the intact and baseline conditions were directly compared with a two-sample t-test. Only clusters or voxels that survived at $p < 0.05$ threshold, corrected for multiple comparisons with the family wise error (FWE) (Worsley et al., 1996) were reported.

Cross-subject correlation analysis. Correlation analyses were performed in SPM8. Group-level analyses explored, for each voxel, the cross-subject synchronization in brain activity, by measuring the correlation of each subject's time-course with the mean time-course of all other subjects (Hasson et al., 2004). To correct for multiple comparisons across voxels, only clusters or voxels that survived at $p < 0.05$ family wise error (FWE) threshold (Worsley et al., 1996) were reported.

Group ICA. Independent component analysis (ICA) was employed to determine the brain networks involved in processing naturalistic stimulus. ICA is a beneficial technique due to its data-drive approach to extracting functional networks from fMRI data (Huettel et al., 2009). In other words, ICA does not require any prior assumptions or a model. Instead, it identifies a set of voxels that have similar activations over time and are maximally distinct from each other. In this way, voxels that are part of the same functional network can be identified based on similar responses over time. Each

independent component has a unique time course and a certain spatial location in the brain. Tensor ICA was performed (Beckmann & Smith, 2005) for each stimulus. The MELODIC software was used to perform the ICA, and a 20-component cut-off was implemented for group data (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC). The majority of components originate from neuronal sources, but non-neuronal components can be driven by artifacts such as breathing, movement, and vascular pulsation. Components were classified as non-neuronal based on frequency distribution (majority of high frequency signals) and spatial location (signal originating outside the head). Neuronal components were identified as their respective functional network through visual inspection.

Individual ICA. ICA was performed for each individual, for each stimulus. In addition, ICA was performed in a leave-one-out fashion for the group minus each individual. From all the components extracted, functional networks were identified for each individual and for each group-minus-individual based on visual inspection. From here, the time course of each network was correlated between the individual and group minus that individual for that specific component. By doing so, the individual time course was compared to the rest of the group's. The individual of interest has the time course excluded from the group average to avoid introducing any bias from that individual. This correlation procedure was performed for each individual for each network. An average inter-subject correlation value was obtained for each of the five functional networks.

Prediction SPM. To be eligible for patient testing, a stimulus must be reliable and robust at a single-subject level. To assess this, I investigated whether each participant's

activity could be predicted from the time course from the rest of the group. Single-subject analyses were focused on two main networks, the auditory and frontoparietal networks, which were functionally critical for sensory perception and higher-order cognition during the audio narrative. The time course of the network in question, obtained from the leave-one-out ICA analysis, was used as a regressor in the SPM data model of the participant not included in the ICA analysis. This was performed 15 times, once for each individual. This analysis was performed for the auditory and frontoparietal networks.

Results

I first looked at the extent of inter-subject correlation for the two audio narratives (Figure 3). Figure 3A shows significant brain synchronization of neural activity ($p <$ 0.001, FWE) for Humanity in the auditory and visual cortices. Figure 3B shows the synchronization during noise baseline, with significant synchronization only in the auditory cortex and a small cluster in the right inferior prefrontal cortex. Formal comparison between the intact and baseline stimuli showed significant synchronization in small clusters in the superior/middle temporal gyri, temporo-occipital junction, and occipital cortex (Figure 3C). Figure 3D shows significant synchronization of neural activity for Taken, with widespread brain synchronization being seen across the brain. This includes significant synchronization in the frontoparietal regions, known to support executive function (Naci et al., 2014, Barbey et al., 2012, Duncan 2010). Formal comparison between the intact and baseline stimuli revealed that the intact story elicited significantly more cross-subject synchronization than the baseline bilaterally, in parietal, temporal, motor, and dorsal/ventral frontal/prefrontal cortices (Figure 3E). Since Taken

displayed widespread brain synchronization, most importantly in the frontoparietal network, I performed more in-depth analyses for this stimulus.
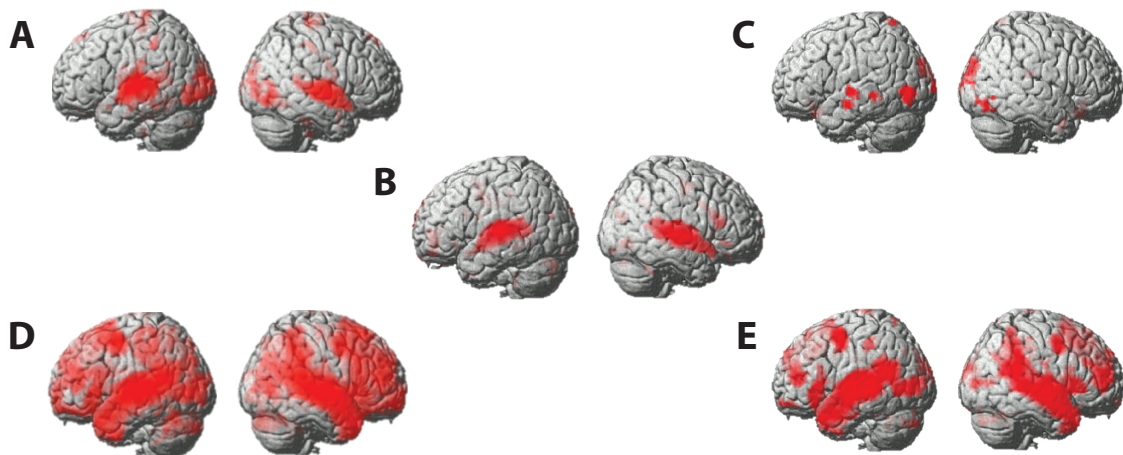
Figure 3: Whole brain correlation for audio-narratives (p < 0.001, FWE). (A) Humanity (B) Noise (C) Humanity - Noise (D) Taken (E) Taken - Noise

In previous work, inter-subject synchronization in the frontoparietal network has been shown to underlie the common understanding of a naturalistic audiovisual narrative across different individuals (Naci et al., 2014). The audio narratives tested here contain both background music and spoken dialogue. Therefore, I also tested 2 music pieces to determine whether the inter-subject synchronization observed in the frontoparietal network for Taken truly depended on comprehending the plot, or whether it could be elicited by suspenseful background music alone. Because the dialogue in the Taken piece was critical for understanding its narrative, I could not test this hypothesis directly with the soundtrack of the Taken piece, but rather addressed this by turning to two independent music pieces. Figure 4A shows significant brain synchronization of neural

activity ($p < 0.001$, FWE) for Dark Knight in the auditory cortex. Figure 4B shows the synchronization during noise baseline (same as Figure 3B). Formal comparison between the baseline and intact stimuli displayed significant synchronization in the superior and inferior temporal gyri, and a small cluster in the motor cortex (Figure 4C). Figure 4D shows brain significant synchronization for Game of Thrones in the auditory cortex. Figure 4E displays the formal comparison between the baseline and intact stimuli revealing small significant clusters in the occipital lobe, temporoparietal junction, and the motor and inferior frontal cortices. In summary, as Figure 4 demonstrates, neither music pieces showed significant synchronization in the frontoparietal network, suggesting that significant synchronization in the frontoparietal network in the Taken stimulus is driven by common understanding of its narrative across the different participants, and not driven solely from a suspenseful soundtrack.

Figure 4: Whole brain correlation for music stimuli ($p < 0.001$, FWE). (A) Dark Knight (B) Noise baseline (C) Dark Knight - Noise (D) Game of Thrones (E) Game of Thrones - Noise

Since Taken displayed widespread inter-subject synchronization, importantly in the frontoparietal network, I performed mo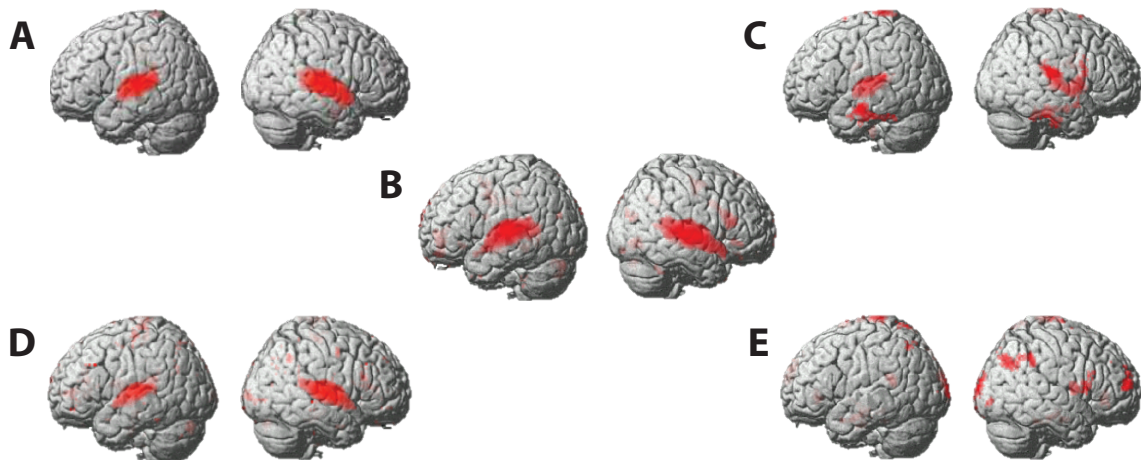re in-depth analyses for this stimulus to determine its suitability for patient testing in future studies. In order to relate the synchronized neural activity across subjects to different and specific aspects of the narrative Taken, I used Tensor Independent Component Analysis (ICA) (Beckmann & Smith, 2005). This divided the fMRI response into independent spatiotemporal elements. Group ICA (n=15) revealed 5 common functional networks in sensory specific (i.e. visual, auditory and motor) and higher-order (medial and frontoparietal) regions. Group ICA networks are shown in colour coded lateral, medial and superior views (Figure 5). To determine the extent of synchronization between individuals in each network, I compared each individual's fMRI response to the rest of the group. For this, I performed individual ICA for each individual's fMRI data, in which I identified the same 5 networks as above. In addition, I performed a leave-one-out group ICA by leaving out one individual's data and performing ICA with the remaining group. For each network, I then correlated the individual's time course with the time course of the leave-one-out group minus that individual. This showed the extent to which each individual's response was similar to the rest of the group. For each network, I averaged these correlations to obtain averaged group inter-subject correlations (Figure 5). The auditory and frontoparietal networks showed significant inter-subject correlation, $t(14) = 16.94$, $p < 0.001$; $t(14) = 11.82$, $p < 0.001$, respectively. In contrast, the visual, medial and motor networks did not show significant inter-subject correlation.
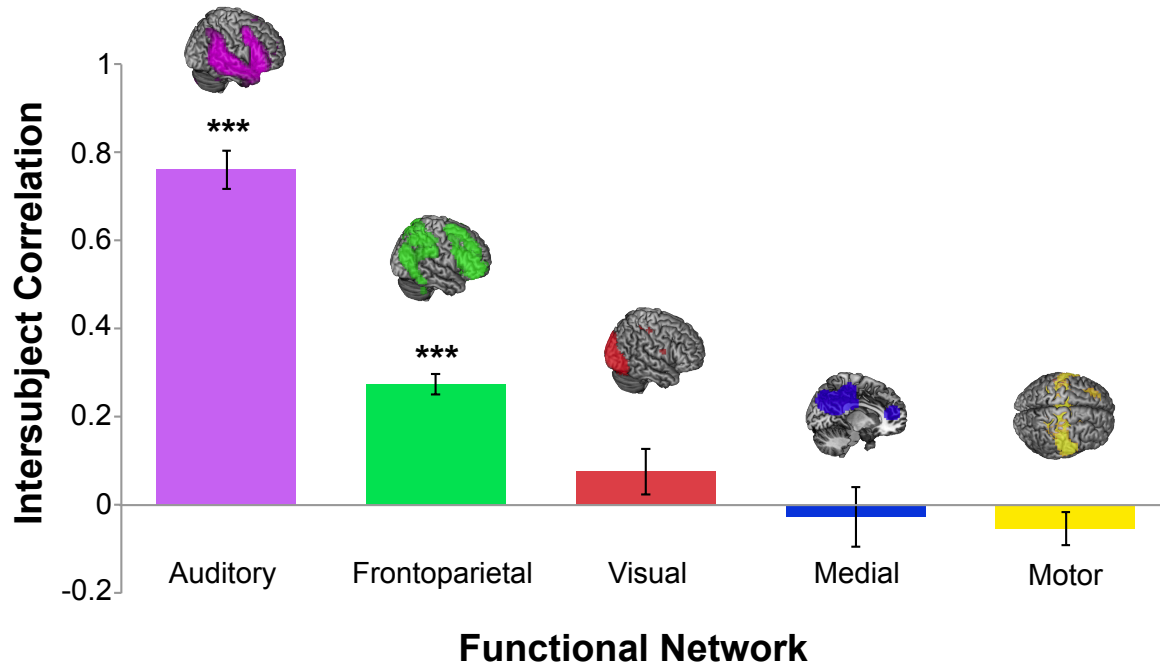
Figure 5: Group averaged inter-subject correlation for each functional network in response to Taken audio-narrative. Colour coded brains represent group ICA networks.

To confidently use this stimulus for patient testing, it must afford reliable prediction of brain activity at the individual subject level. Therefore, I next looked at whether group-level activity could predict brain activity at the single subject level. I focused on the auditory and frontoparietal networks, which were both significantly correlated between participants. The auditory network is critical for perceptual processing of the auditory stimulus. The frontoparietal network, as a "higher-order" network, is important for comprehending the details of the story and integrating them together to form an overall understanding of the plot. For each network, I took the time course of the corresponding independent component from the leave-out-out analysis, and used that as a predictor of the SPM model of the individual who was not included in the group. Figure 6 shows the activity in the auditory and frontoparietal regions could be predicted by the time course of the rest of the group ($p < 0.05$, FWE corr).
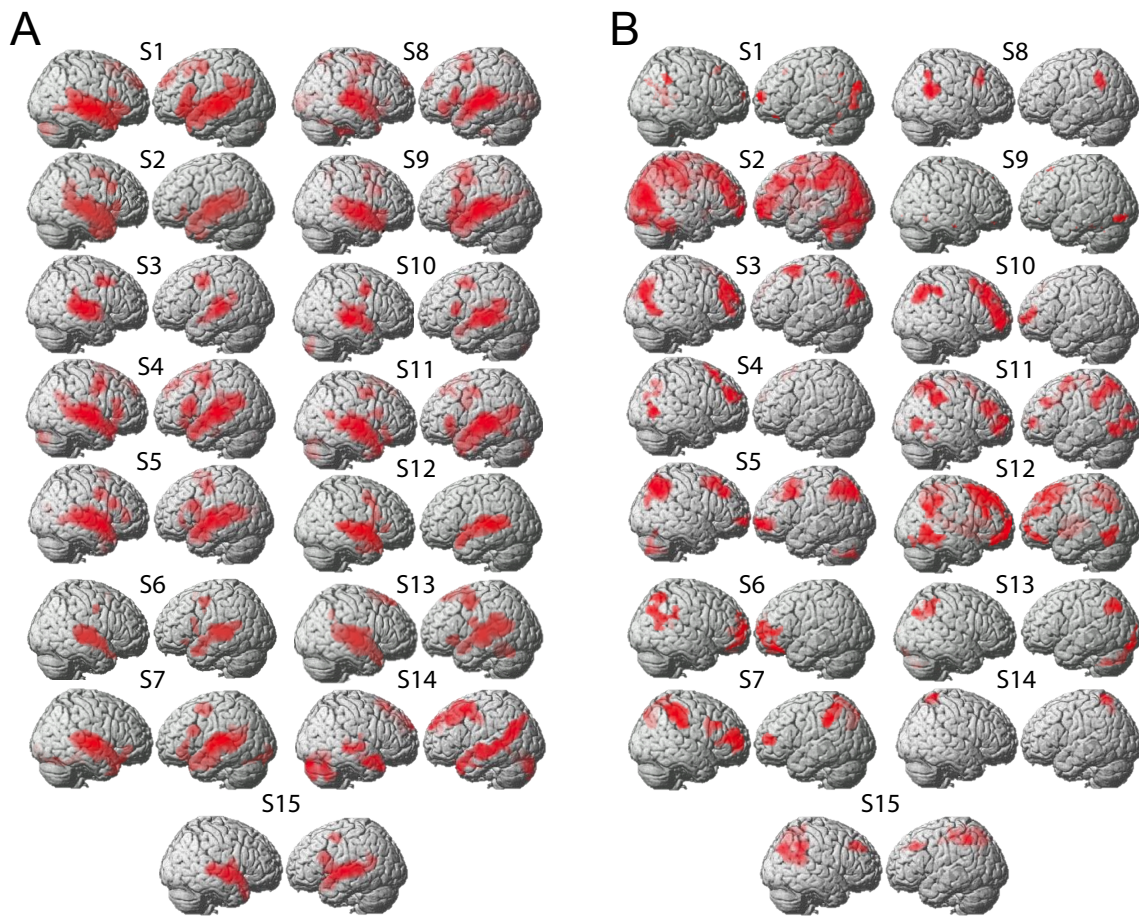
Figure 6: Prediction of each individual's processing of "Taken" using the leave-one-out group time course ($p < 0.05$, FWE corr).
A: Auditory network
B: Frontoparietal network

Discussion

Out of the two auditory narratives tested, the stimulus Taken produced widespread synchronization in healthy participants. Most importantly, this stimulus produced significant inter-subject synchronization in the frontoparietal network, suggesting that it could be used to test executive function in individual patients. By contrast, Humanity showed synchronization only in sensory networks. While both

contained dialogue as well as background music and sound effects, I would argue that the difference between the two clips is that Taken contains more of a "story" than Humanity. This difference could have led to the contrasting results seen for the two narratives. In Humanity, Charlie Chaplin is giving a continuous speech and the background music becomes more intense over time, but the stimulus does not have a plot that is easily understood by listeners, especially without viewing the rest of the movie. The lack of frontoparietal synchronization in our study is comparable to the lack of synchronization in prefrontal, frontal and parietal cortices when individuals viewed a short clip from the full-feature movie "*The Good, The Bad and The Ugly*" (Hasson et al., 2004). Researchers hypothesized this was due to participants' lack of engagement and inability to follow the plot of the movie, since the clip was shown without knowledge of previous scenes and viewers did not understand the context of the clip shown (Jaaskelainen et al., 2008). If listeners do not have a full understanding of the context for a stand-alone clip, this may lead to low inter-subject synchronization in higher-order networks since they cannot think about the clip in a similar way. The stimulus Taken, however, includes a storyline that listeners can comprehend, even without seeing the previous movie scenes. The suspense of the clip comes from understanding what is happening to the characters, in particular that the daughter is alone in Europe and about to get kidnapped. In summary, by contrast to the Humanity stimulus, the story in the Taken stimulus has a beginning, middle and end that participants can time-lock to and think about in a similar manner.

Naci and colleagues (2014) demonstrated that inter-subject synchronization in the frontoparietal network during the movie "*Bang! You're Dead*" was driven by a similar

perception of suspense across different participants. They determined that understanding the details of the film and integrating them together required higher-order executive function, and thus required activity in the frontoparietal network. The perception of suspense is a direct result of this integration. The two music clips tested in the current study did not induce significant inter-subject synchronization in the frontoparietal network, suggesting that synchronized activity during Taken must be driven by the perception of suspense after understanding the narrative, and cannot be driven only by suspenseful background music.

These results suggest that the Taken stimulus is appropriate for testing executive function in behaviourally non-responsive patients. Importantly, to be eligible for patient testing, a stimulus must be reliable on a single subject level. I have demonstrated that the sensory (auditory) and higher-order (frontoparietal) brain responses to the story can be robustly predicted at the single subject level. The reliable responses in the auditory cortex allow us to investigate the similarity of perceptual processing of the stimulus in controls and patients, whereas the reliable responses in the frontoparietal network are critical for testing higher-order cognitive function in patients. The activity in the frontoparietal network has been linked to executive function, and as a mental faculty that is integral to our conscious experience of the world, executive function can serve as a marker of conscious awareness in non-responsive patients (Naci et al., 2014). In other words, if a patient displayed similar activity in the frontoparietal network during the audio narrative, this would be used as an indication of similar executive function, and therefore as an indicator of awareness. It should be noted that this work does not link frontoparietal activity directly to the specific executive demands of the audio narrative.

Future studies will investigate directly how the executive demands of the Taken stimulus drive the frontoparietal activity in healthy controls (using methods similar to those used in Naci et al., 2014), thereby providing a concrete measure of executive function in response to this stimulus. This would allow future studies to establish strong evidence for the presence of covert awareness in any given behaviourally non-responsive patient who responds to the specific executive demands of the auditory story in the same way as healthy controls.

In summary, developing testing methods that involve naturalistic stimuli could aid in testing DoC patients who may not be able to perform command following, and thus is a worthwhile avenue to pursue. The results presented in this chapter suggest that the audio narrative Taken elicits robust perceptual and higher-order function in individual participants that can be predicted from the rest of the group. Therefore, it can be used in a naturalistic auditory-only paradigm for testing chronic DoC patients with impaired visual function, or those in coma, who cannot open their eyes. In addition to determining the specific executive demands of the Taken story that drive frontoparietal activation, an appropriate next step for this project would be to test DoC patients in the fMRI scanner with this stimulus and compare their brain response to that of healthy individuals. However, using fMRI for patient testing has practical disadvantages such as high cost and limited accessibility for patients. Thus, the next chapter will discuss using naturalistic stimuli with a Galvanic Skin Response, an inexpensive technique that can be brought to the patient's bedside.

Chapter 3: Developing a bedside testing method using Galvanic Skin Response

Introduction

In the previous chapter, I presented the development of a naturalistic auditory stimulus that could be used for fMRI patient testing. However, fMRI is both expensive and non-portable, which decreases the ability to use it for DoC patient testing. Developing a testing method that is cheap, portable and would allow patients increased accessibility to testing at the bedside would be beneficial. I will argue in this chapter that Galvanic Skin Response (GSR) during naturalistic stimulus presentation is a possible method for this task.

GSR has been found to reflect perception and cognition (Critchely et al., 2000). Lang et al. (1993) examined if there is a relationship between skin conductance and arousal ratings of discrete stimuli. Individuals rated emotional photographs on a scale of calm to arousing while having their skin conductance measured. There was a significant positive correlation between GSR magnitude after photograph presentation and arousal ratings of the photographs. Therefore, the perception of emotional stimuli influenced skin conductance, and the magnitude of the GSR signal change was dependent on how arousing the stimulus was.

Dawson et al. (1989) investigated the allocation of attention and its relationship to skin conductance using a dual-task paradigm. College students performed an auditory task and a visual task simultaneously; they needed to listen to tones of varying frequencies while reacting as quickly as possible when presented with a light stimulus.

Slower reaction time to the light in specific trials indicated higher attention allocation to the auditory task. Greater attention to the auditory task was significantly positively correlated with increased skin conductance response. These findings suggested that increased attentional allocation is associated with higher GSR.

The two aforementioned studies used discrete stimuli, in which GSR at a specific time point is associated with a discrete stimulus event (i.e. the presentation of a photograph or startling sound). GSR can also be measured during a continuous stimulus, such as an ongoing task, sound clip or movie. By using a continuous stimulus, the ebb and flow of GSR signal change can be observed while events are unfolding in the stimulus. Golland et al. (2014) observed the similarity of autonomic responses between participants during emotional movie viewing. Authors presented individuals with emotional movies, during which they also measured GSR. They observed significant correlation of GSR across individuals during the movie. Thus, inter-subject synchronization of autonomic responses can be seen during continuous stimulus presentation.

In addition to studying healthy individuals, GSR has also been used to study disorders of consciousness patients. Currently there are very few studies that use GSR to test this patient group (Keller et al., 2007; Daltrozzo et al., 2010). For example, Keller et al. (2007) tested if permanent vegetative state patients showed physiological responses to tactile and auditory stimulation. In different sessions, patients received an arm massage, listened to white noise and listened to voices of relatives during GSR recording. Tactile stimulation produced a significant increase in GSR. Hearing white noise also produced a

significant increase in GSR while hearing voices of relatives did not produce any effect. Authors suggested that GSR may indicate levels of arousal in vegetative state patients.

Researchers investigated if GSR to emotional stimuli correlated with scores of responsivity in coma patients (Daltrozzo et al., 2010). They presented healthy controls and coma patients with emotional and neutral auditory stimuli. Results showed that healthy participants had greater GSR to emotional compared to neutral stimuli. Authors then calculated the GSR emotional effect by subtracting the neutral GSR from the emotional GSR for both patients and controls. They found delayed GSR in patients compared to healthy participants in the emotional minus neutral GSR signal. Additionally, the size of the emotional effect in patients had a significant positive correlation with the level of responsivity as measured by the Glasgow Coma Scale (GCS). Therefore, researchers related the skin conductance response to the responsivity levels of these patients. They also concluded some patients with higher responsivity may retain some level of emotional processing.

In summary, GSR provides a sensitive index of sympathetic activity, which has been linked to cognition in healthy individuals. It has been used to test DoC patients at the bedside in a limited number of studies. However, it is unknown if GSR can be used to detect awareness in DoC patients. I first tested healthy individuals during movie and audio story presentation to determine if one or both stimuli elicited similar between-subject GSR at the single subject level. The similarity across healthy individuals will allow us to make reliable inferences regarding covert awareness in DoC patients when these patients are tested using GSR.

Method

Participants

Ethics approval was obtained from Western University's Health Sciences Research

Ethics Board for healthy volunteers and patients (see Appendix A). All volunteers were

right handed and native English speakers. They provided informed consent before

participating and were monetarily compensated for their time. None had previous

experience with the experiment. 20 participants (17 females, 18-25 years) were tested,

and data from 3 were removed due to noise and excess movement. 7 patients (3 females,

20-48 years) were tested. Surrogate decision makers provided informed consent on

behalf of the patient. The Coma Recovery Scale- Revised (CRS-R) was used for

behavioural assessment (Kalmar & Giacino, 2005) at the time of testing. Demographic

and clinical data for the sample of patients tested is included in Table 1.

Patient number	Sex	Age (y)	Interval since Ictus (y)	Etiology	Diagnosis	CRS-R Score on test day
1	F	43	4.6	Non-traumatic	VS	n/a[1]
2	F	25	5.7	Traumatic	MCS	8
3	F	44	20.4	Traumatic	VS	4
4	M	20	0.8	Non-traumatic	Recovery	n/a[2]
5	M	35	16.9	Non-traumatic	VS	6
6	M	48	2.9	Non-traumatic	LIS	15
7	M	32	4.1	Traumatic	e-MCS	22

Table 1: Patient demographic and clinical assessment data.
Abbreviations: CRS-R, Coma Recovery Scale- Revised (Kalmar & Giacino, 2005); VS, Vegetative State; MCS, Minimally Conscious State; LIS, Locked-In Syndrome; e-MCS, emergence from Minimally Conscious State. [1] CRS-R not obtained on day of testing [2] CRS-R is not applicable for recovered patients

Materials

    Stimuli. *"Audio story"* (5 min 12 sec) is an auditory narrative that is an excerpt

from the movie "*Taken"* (2008). Specifically the clip is taken from the scene when the

daughter is kidnapped, and her father speaks to the kidnappers on the phone. It contains

spoken words as well as background music and sound effects, and is the same stimulus

from chapter 2. "Noise" (5 min 2 sec) is an auditory stimulus that was created from the

Taken piece and is used as a baseline stimulus. The frequencies in the sound clip were

spectrally rotated so that many of the spectro-temporal characteristics of natural speech

were maintained, but the clip's meaning is removed. "Movie" (7 min 59 sec) is an audiovisual stimulus that is a shortened version of the Alfred Hitchcock TV episode "*Bang You're Dead*" (1961). All participants had seen the movie Taken before to avoid any familiarity confounds and all were unfamiliar with the movie. The same 3 stimuli were administered to both healthy controls and patients.

Procedure

Stimuli presentation. During audio stimuli, participants had their eyes open but stared at a fixed point directly in front of them. During the audiovisual stimulus, participants had free viewing of the screen placed in their line of sight. All stimuli were presented to healthy controls using over-the-ear headphones and displayed on a computer screen (13" MacBook Air). Participants were instructed to remain awake and pay attention to stimuli. In-ear headphones were used for stimuli presentation for patients and the same computer screen as above was used.

Eyes closed condition was recorded to obtain a physiological GSR baseline during which no stimulus was presented. This condition was measured first for healthy participants, and remaining stimuli were counterbalanced. A physiological baseline was not obtained for patients due to time limitations.

GSR Signal. GSR measures changes in electrical conductance of the skin due to increases and decreases in sweat (Critchley, 2002). The Galvanic Skin Response (GSR) is measured in the unit micro-Siemens (μS). GSR is composed of two components: a background tonic level of sweat and rapid, phasic changes in sweat amounts. The tonic level of sweat is indicative of the overall degree of arousal, and is also called the skin conductance level (SCL) (Nagai et al. 2004). The rapid, phasic changes in skin conductance most often are the direct result of stimulus presentation and this is called

electrodermal response (EDR). The presentation of a continuous stimulus will elicit short-term increases and decreases in sweat response on top of an individual's tonic level that can be visualized with the GSR waveform.

Data Acquisition. Galvanic Skin Response (GSR) data were measured using disposable Ag/AgCl isotonic electrodes on the distal phalanges of the index and middle fingers of the non-dominant hand. The non-dominant hand was chosen since the fingertips are generally less affected by abrasions, and also to avoid motion artifacts. Biopac MP150 acquisition system and AcqKnowledge software (version 4.3) were used for acquisition of GSR data at a sampling rate of 2000 Hz. Participants rested their non-dominant hand in a comfortable position on the table in front of them. Participants were simply instructed to pay attention to the stimulus. Prior to the start of each stimulus, 1 minute of rest was implemented to allow for stabilization of the GSR and for the participant to relax. After 1 minute, stimulus presentation began. During patient testing, GSR electrodes were similarly placed on the distal phalanges of the index and middle finger of the hand that had the least movement and/or had the easiest access to the fingertips. Patients were instructed to pay attention to the stimulus being presented to them.

Preprocessing. The 1st minute of recording prior to stimulus commencement was removed. Each participant's GSR data was downsampled from 2000 Hz to 0.5 Hz (1 point for every 2 seconds) for each condition. Next, data were detrended to remove linear trends caused by gradual increases or decreases in conductivity by subtracting the first-order polynomial fit. GSR data were normalized by calculating Z-scores to adjust for differences in baseline conductivity levels between individuals.

Analysis. For each healthy participant, their normalized GSR was correlated with the average GSR of the group minus that participant over stimulus/condition duration. This leave-one-out procedure enabled each individual's response to be compared to the rest of the group. All individual leave-one-out correlation values were averaged to obtain an overall group inter-subject correlation value for each condition. T-tests were used to determine if the average group correlation differed significantly from 0.

Patient data analysis followed the same preprocessing procedure as the healthy participants mentioned above. The correlation between the average control GSR and each patient's GSR was obtained only for the movie.

Results

Galvanic skin response was used to measure the autonomic response of healthy participants to the auditory and audiovisual stimuli, as well as during the baseline conditions. Figure 7 shows the group averaged inter-subject correlation for each condition. The higher the average GSR group correlation, the more synchronized the individual responses were to each other. The movie and auditory story had significant correlation, $r = .72$, $t(16) = 22.41$, $p < 0.001$, $r = 15$, $t(16) = 2.83$, $p = .012$, respectively. The noise stimulus and eyes closed baseline did not show significant correlation.
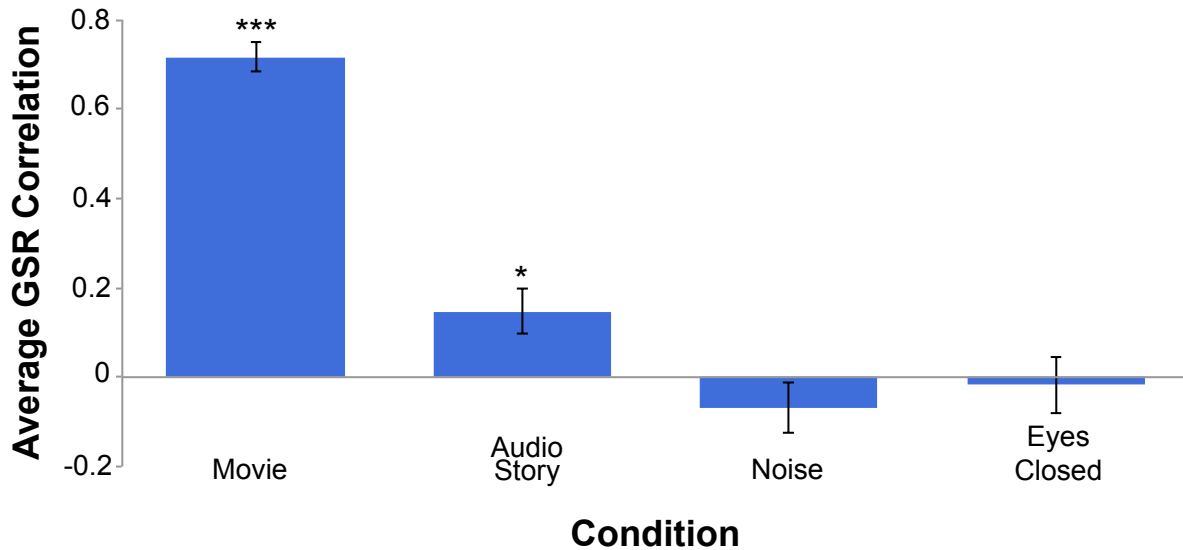
Figure 7: Group averaged inter-subject correlation for movie, audio story, noise and eyes closed conditions. Error bars represent standard error.

The movie showed high inter-subject correlation that was consistent across participants, as demonstrated by the small error bar (representing standard error). By contrast, the audio story had a lower group averaged inter-subject correlation and a high degree of variability across participants. The individual versus group correlation results can further demonstrate this (Figure 8). The individual versus group correlation is indicative of how similar the response was for each individual compared to the rest of the group. For the audio story (Figure 8A), 5/17 participants showed negative correlation values when compared with the rest of the group. 5/17 showed positive correlations below $r = .15$ and the remaining 7 individuals had positive correlations that were almost double this value. The variable individual responses during the audio story can be contrasted with the reliable responses to the movie (Figure 8B), in which all individuals (17/17) have a correlation above $r = .40$.
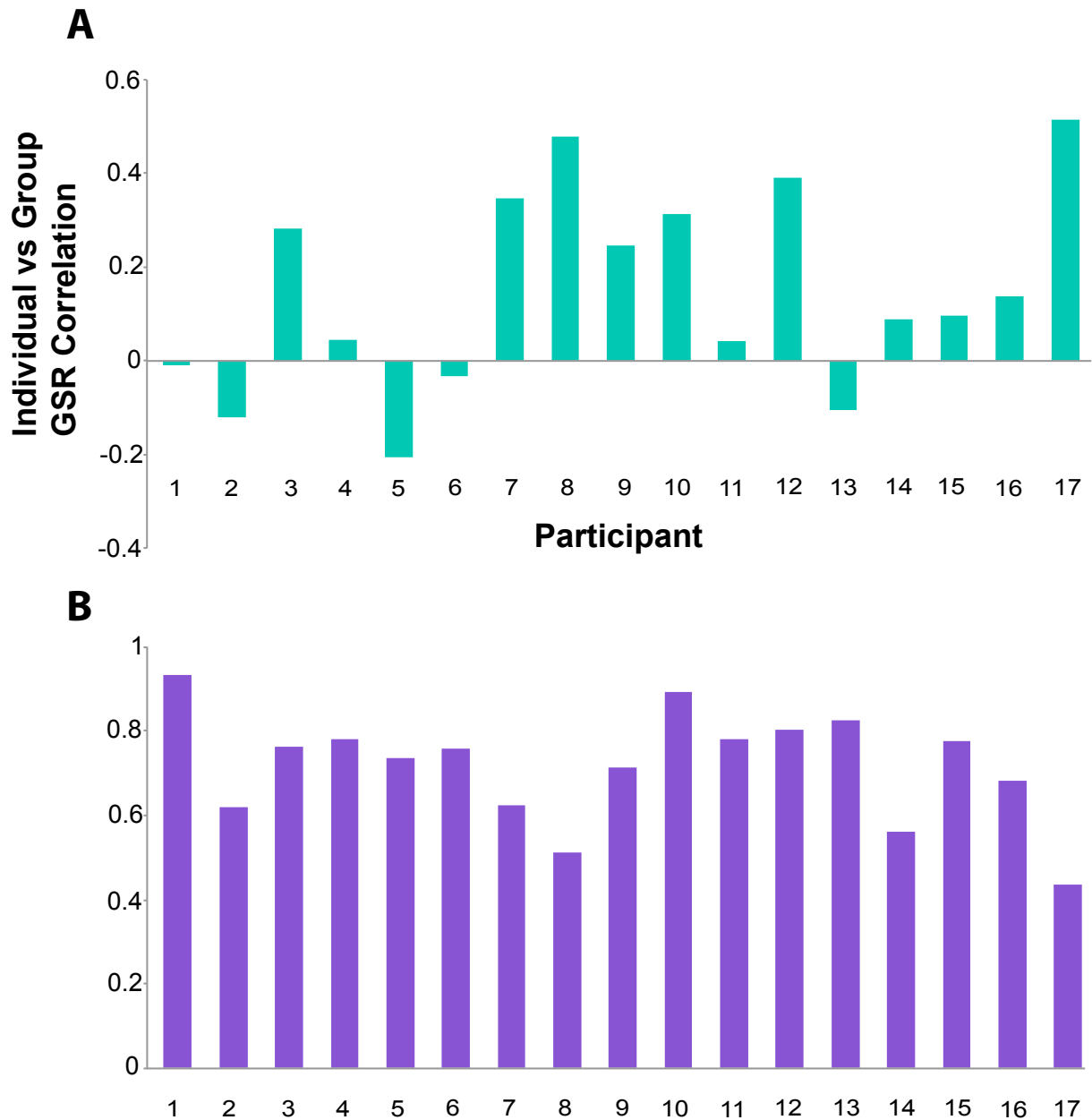
Figure 8: Individual versus group correlations were obtained through a leave-one-out procedure for each healthy control participant.
A: In response to audio story
B: In response to movie

To confidently assess awareness in disorders of consciousness (DoC) patients, a stimulus that elicits a reliable effect at a single subject level is necessary. Because of the high inter-subject variability, the audio story would not be an effective stimulus for patient testing. In contrast, the movie produced highly similar responses across healthy participants, and this high inter-subject synchronization suggested that it is reliable for patient testing.

I measured GSR at the bedside of DoC patients while they viewed the movie stimulus. Each DoC patient was assessed individually by comparing their GSR over the entire course of the stimulus to the GSR from the control group during movie viewing. Figure 9 shows each patient's correlation with the control average. 4 patients (P1, P2, P5, P7) showed a negative correlation with the average control GSR, while 3 patients showed a positive correlation (P3, P4, P6). In this study, a positive correlation indicates a similar response between patient and controls, and is deemed to be a positive result. By contrast, a negative correlation is indicative of a dissimilar response between patient and controls, and is considered a negative result. Negative correlations are not interpreted because negative findings can sometimes be found even in healthy controls (Owen & Coleman, 2007). These negative findings may be due to falling asleep during testing, or physical factors such as moving or coughing, and do not necessarily indicate the lack of cognitive capacity to respond. An example of the effect physical movement and coughing can have on the GSR signal can be seen in Figure 10. Figure 10 shows the GSR time course from P4 and the control group. P4 had excessive movement and coughing, and the timing of the coughs correspond to the peaks in the GSR signal. Thus,

as this patient demonstrates, GSR can be affected by physical confounds such as coughing and subsequently affects the comparison with healthy controls.
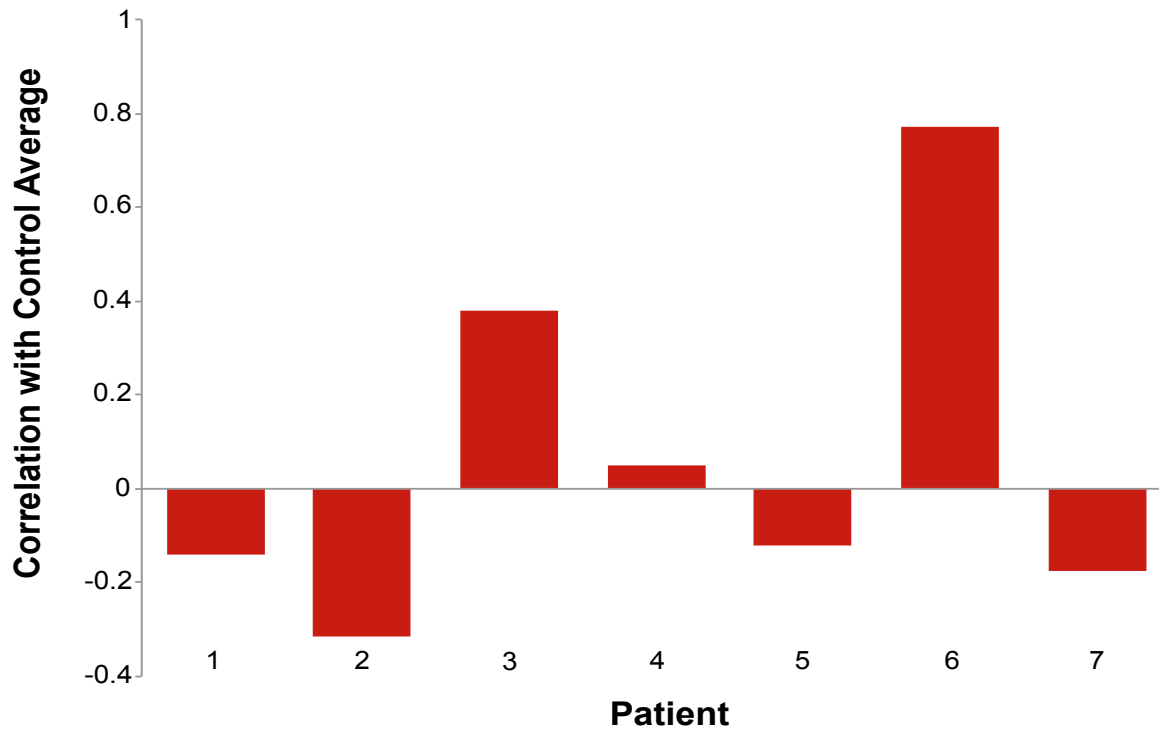

Figure 9: Correlation of individual patient GSR with the control group average in response to the movie stimulus.
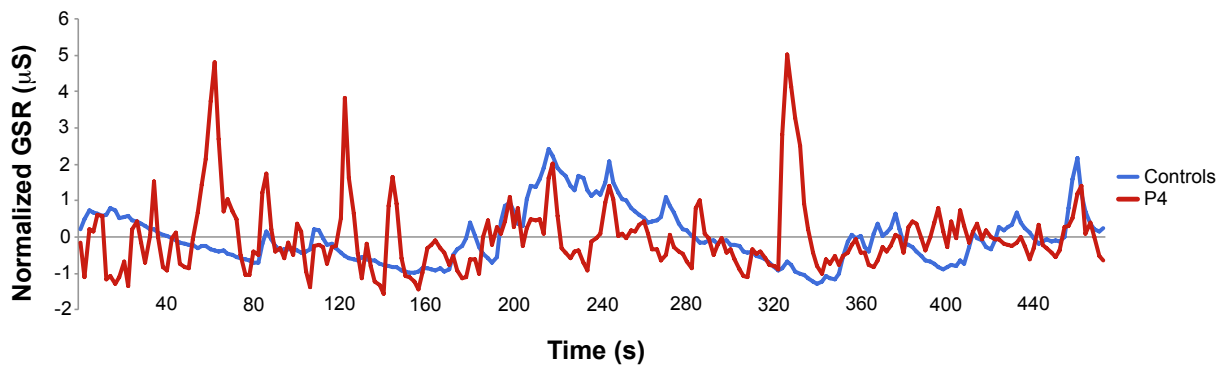
Figure 10: Normalized GSR time course over the duration of movie stimulus for P4 and controls.

For those patients that showed positive correlations, I next tested whether the correlation fell within the range obtained by healthy individuals. Figure 11 displays the correlation to the control group of individual healthy participants and patients. P3 and P4 fell outside of the range obtained from healthy controls, but P6 had a correlation within the range of healthy participants. Therefore P6 was looked at in more detail.
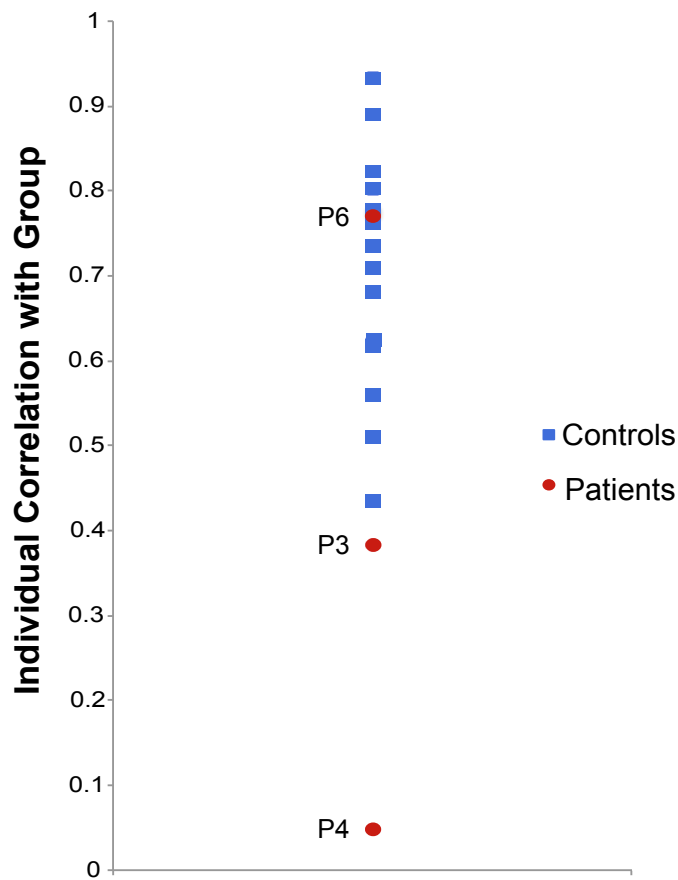
Figure 11: For healthy controls (blue) each value shows the correlation of that individual with the rest of the group. For patients (red), values represent patient correlation with control average.

Figure 12 shows the strong correlation ($r = .77$) between the average normalized control GSR versus the normalized GSR for P6 while viewing the movie. Each data point represents the normalized GSR at one time point during stimulus presentation.
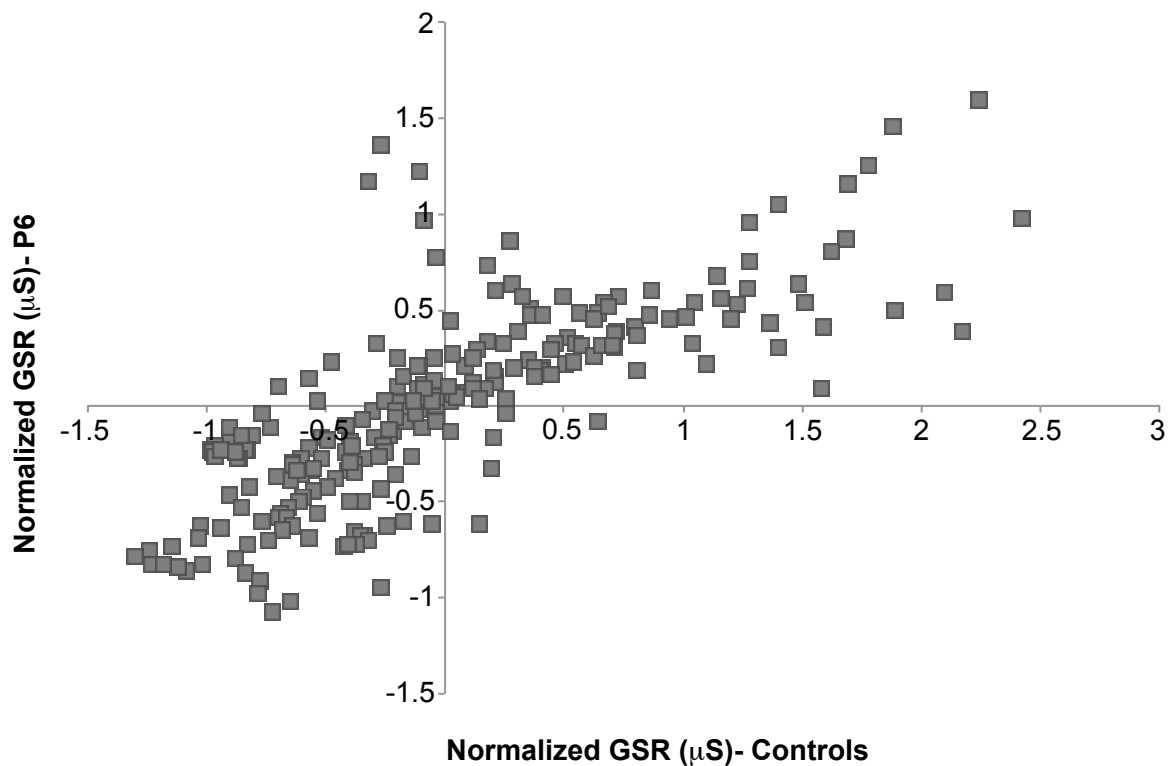
Figure 12: Normalized GSR for control group (X axis) versus normalized GSR for P6 (Y axis) while viewing the movie.

The strong correlation for P6 can also be visualized by the GSR time course, which shows the normalized GSR values over stimulus duration. Figure 13 shows the normalized GSR values for the control group and P6 over the time of movie viewing. The patient's GSR time course shows similar peaks and troughs during the progression of the movie compared to that of controls.
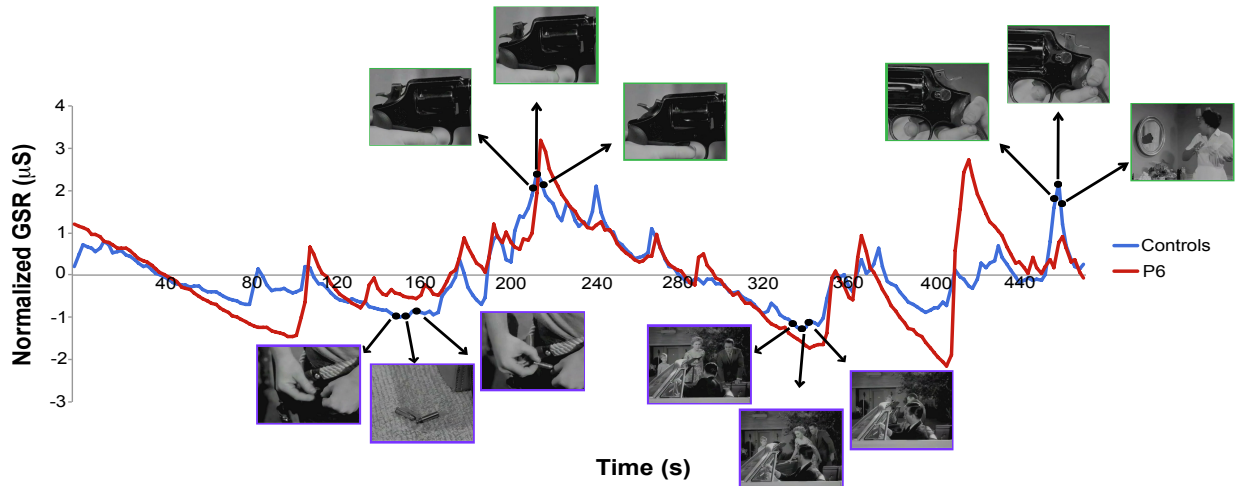
Figure 13: Normalized GSR time course over the duration of movie stimulus for P6 and controls. Still frames from the movie denote the 6 highest suspense ratings (outlined in green) and 6 of the lowest suspense ratings (outlined in purple), obtained from healthy controls (Naci et al., 2014).

To interpret the similarity in GSR between P6 and the control group, these peaks and troughs can be examined further. In an independent experiment, healthy individuals rated movie still-frames from least to most suspenseful (Naci et al., 2014). The ratings were used here to see if perception of suspense is driving the GSR. The 6 frames that were rated the most suspenseful corresponded to 6 of the highest points in the GSR signal (distributed in 2 main peaks) for healthy controls. Moreover, these peaks also correspond to 6 of the highest GSR for the patient. Similarly, 6 of the lowest rated frames are shown, and they correspond to the lowest points in the control GSR signal. These still frames are shown in Figure 13 at the corresponding time point in the film.

Discussion

In this study, I sought to identify a naturalistic stimulus that elicits similar Galvanic Skin Responses across healthy participants that could later be used for testing

individual DoC patients. GSR during audio story and movie stimuli presentation showed contrasting results in healthy participants. Individuals showed highly variable responses to the audio story compared to one another, leading to low group averaged inter-subject correlation. In contrast, the movie elicited highly similar responses from all healthy participants, as indicated by the high inter-subject correlation.

The difference in response reliability when comparing the two stimuli may be due to the differences in cross-modal versus unimodal stimulus processing. The movie, which has both auditory and visual components, likely creates a more absorbing experience for the viewer that leads different viewers to experience the stimulus in more similar ways as compared to an auditory-only stimulus. The concept of multisensory integration could explain the higher inter-subject synchronization for the movie compared to the audio story. If the processing of cross-modal stimuli is enhanced compared to unimodal stimuli, this could lead to more similar responses across participants for the movie as measured by GSR. Visual cues have been shown to modulate processing of auditory stimuli (Kayser et al., 2008). Moreover, anatomical and electrophysiological evidence suggest cross-modal integration occurs in higher-level associative cortical areas, including temporal, parietal and frontal lobes. Having multiple sensory modalities to integrate would affect the activity in these areas, and if these higher-order regions are driving the GSR signal, the GSR signal will be influenced by cross-modal stimuli processing.

Finally, the perception of how emotional a stimulus is may influence the inter-subject correlation for GSR. Work by Golland et al. (2014), discussed previously, demonstrated that scenes of the movie rated most emotional by participants were also the

segments of the movie where the highest GSR correlation was observed. Therefore, perhaps the "*Bang! You're Dead*" movie was more emotionally arousing than the Taken audio-story, and this is why higher inter-subject correlation was observed for the movie compared to the audio-story. Since strong emotions are known to influence GSR, the emotions of the movie may be driving the similar autonomic response in individuals.

A major influence driving the GSR during the movie is the perception of suspense. The 6 still-frames of the movie that were rated as most suspenseful by a group of healthy individuals (Naci et al., 2014) corresponded to 6 of the highest GSR points in this experiment, as measured in a separate group of controls. A loaded gun is shown in the first cluster of suspenseful still frames (210 seconds), but it should be noted that in the movie, the trigger is pulled but the gun does not fire. Therefore, the high GSR at this point is not a startle response to a gunshot but instead is a result of the perception of suspense. This perception of suspense requires previous knowledge of the real-world consequences of a gunshot and understanding of the movie plot, as well as integration of this knowledge. Alfred Hitchcock, the director of this film, has been named the "master of suspense" due to his ability to produce fearful films without having to rely on elements such as violent action scenes. Instead, he uses suspense and the slow addition of new pieces of information to create frightening movies. This suspense also keeps individuals engaged with the movie and leads all viewers along a similar viewing experience. Thus, healthy individuals are responding to specific elements in the film in the same way and integrating the information presented over time in order to understand the plot.

Viewers must also appreciate that the young boy in the film does not understand that he has a real gun. In fact, he believes it is a toy. This is related to theory of mind,

where the viewer must be able to understand another person's mental states, even if they are different from their own (Premack & Woodruff, 1978). Viewers should comprehend that the boy has a real gun, but should also be able to understand the boy has a different view; that is, he believes his gun is not real.

I would argue that both the understanding of suspenseful details of the film and understanding the character's mental states, as well as integrating information together require higher-order cognitive processing, and at least some degree of awareness of the self and surrounding environment. This is why it is an effective stimulus for making inferences regarding awareness in non-responsive DoC patients.

Since the movie produced reliable responses in healthy participants, we can use it for DoC patient testing. A patient who had been diagnosed with Locked-In Syndrome (P6) showed a strong GSR correlation with the healthy control group. In other words, the patient's GSR shows a remarkably similar time course compared to that of healthy controls. In fact, as seen in Figure 11, the correlation of the patient's GSR with the control group is well within the range obtained by healthy participants. Along with global similarity to the control group, the patient GSR also showed local similarities to the healthy GSR. This patient showed peak GSR at the same time points as the healthy control group. Moreover, these are the time points rated most suspenseful, indicating P6 is tracking the suspense of the film like a healthy individual would. This would suggest that the patient understands the plot of the movie and able to combine this knowledge with real-world experiences (i.e. the consequences of a gun shot).

The patient's clinical behavioural profile showed that he could perform visual pursuit and answer questions using up and down eye movement. He was diagnosed with

Locked-In Syndrome, and it was understood that he was aware and conscious. Thus, his behavioural profile and bedside assessment provided evidence that he was aware, and this was confirmed by the GSR experiment. Therefore, this study acts as a proof of principle that GSR can detect awareness in behaviourally non-responsive patients. Additionally, it shows that brain damaged patients who are very impaired can still have preserved and normal GSR, which enables us to use this method to test cognition and awareness in patients.

In the future we would like to use GSR as a measure for detecting covert awareness in behaviourally non-responsive patients, such as those diagnosed to be in a vegetative state. Studies show that despite the lack of behavioural responses, a significant minority of DoC patients are conscious (Owen et al., 2006; Cruse et al., 2011; Naci & Owen, 2013). GSR has several advantages that would enable us to test for covert awareness at the bedside. The portability of the GSR machine means we do not have to transport patients to the fMRI machine, an advantage for patients who are too unstable to transport or live far distances from a scanner. Another advantage of GSR compared to fMRI is that metal implants in the patient's body would not prevent testing. Finally, it is cost-efficient, since the initial cost of the machine is the major cost associated with this method.

Although GSR has potential for patient testing at the bedside, this method has limitations. One disadvantage is the effect physical movement can have on the GSR signal. Any hand or arm movement, as well as coughing or deep breathing, can affect the response (Boucsein, 2012). This is demonstrated by P4, who had excessive movement and coughing during testing. I recorded the timing of his coughs, and the GSR increased

sharply at these time points. His physical movement therefore influenced the GSR time course, and subsequently affected the correlation obtained when I compared his time course with the healthy control time course. It should be noted that P4 was previously in a vegetative state, but had recovered and was aware at the time of testing. Thus, as discussed above, negative results can be observed with GSR even in those who are aware. That being said, false negative findings can be observed with other testing methods (i.e. EEG and fMRI), for not only patients but also for healthy controls (Owen & Coleman, 2007). But, with GSR, both the GSR signal changes from a specific stimulus and behaviour of the individual are observable and able to be timed (Dawson et al., 2007). Consequently, carefully monitoring of the signal and patient behaviour can help with the interpretation of the data.

Another limitation of our current GSR method is the inability to interpret weak positive correlations. For example, P3 showed a moderate positive correlation ($r = .38$) with the control group. This is difficult to interpret since the patient GSR time course indicates some similarity compared to healthy individuals, but the time course did not perfectly track the healthy curve. In the current analysis, the global similarity of the patient's GSR time course to the healthy control time course is used to interpret the patient's response. Future studies should also look at key points during the stimulus and analyze local similarities in the time course. In addition, we should develop more precise methods to determine to what aspects of the film they are responding to at these key points.

Finally, some individuals may not be capable of producing a GSR signal, such as those with spinal cord damage, multiple sclerosis (MS), or Amyotrophic Lateral Sclerosis

(ALS). Patients with spinal cord injury showed impaired GSR to emotional stimuli compared to healthy controls (Nicotra et al., 2006). DoC patients, especially those who may have damaged their spinal cord during trauma, should have their spinal cord activity assessed to rule out this confound. MS and ALS patients also show abnormal GSR compared to healthy individuals (Lensch et al., 2011; Dettmers et al., 1993). Pharmacological drugs that DoC patients receive could also cause an absent GSR signal. Reiterating what was mentioned above, an absent GSR in patients should not be used as evidence of unawareness. In fact, a patient may be aware and conscious, but other factors are causing them to not produce a normal GSR.

The disadvantages of audiovisual stimuli that were discussed in the fMRI chapter are also applicable here. That is, some chronic DoC patients have poor visual function and acute patients, such as those in coma, have their eyes closed. For these patients, an auditory stimulus is needed to test covert cognition and awareness. Future work with GSR will identify an auditory only stimulus that produces highly similar responses across different individuals that could subsequently be used to test patients.

In this chapter, I presented the development of a method that uses Galvanic Skin Response and a naturalistic stimulus to test behaviourally non-responsive patients at the bedside. The movie stimulus produced reliable GSR in healthy individuals and was therefore deemed suitable for patient testing. A locked-in syndrome patient showed a highly similar response to that of a healthy individual during movie viewing, suggesting he understood the movie and that he was aware. This awareness was also confirmed by the patient's behavioural profile. As I discussed in this chapter, GSR holds promise for detecting consciousness at the bedside in DoC patients. Future studies will develop more

sophisticated measures for using GSR to test specific aspects of cognition in this patient

group.

Chapter 4: General Discussion

Using fMRI to identify and validate an auditory-only naturalistic stimulus

Naturalistic paradigms may provide opportunities for testing covert awareness in disorders of consciousness patients who cannot perform command following tasks. A critical step in developing a naturalistic paradigm is to find a suitable stimulus. We require a stimulus that is reliable on the single-subject level and can evaluate higher-order cognitive processes, such as executive function. In chapter 2, I used fMRI to identify an auditory-only narrative (Taken) that produced a similar neural response across healthy participants. Significantly, this inter-subject synchronization was found in the frontoparietal network, which will allow us to make inferences regarding executive functions during future testing with DoC patients.

fMRI for testing chronic and acute DoC patients

Naturalistic paradigms are brief, easy to administer, and require less effort from patients compared to command following tasks. Naci et al. (2014) determined an audiovisual stimulus for testing covert awareness in DoC patients, but since some patients have poor visual function, I worked to find an auditory-only stimulus. Chronic vegetative state and minimally conscious patients can be tested using the auditory-only stimulus in future studies. Testing for residual cognition and consciousness in VS and MCS patients is significant since the diagnosis given to the patient has both medical and legal implications. Treatment and standard of care may differ between the two groups depending on the diagnosis. 96% of surveyed health care professionals believed those in a MCS perceive pain, where only 56% believed VS patients perceive pain (Demertzi et

al., 2009). Therefore, the administration of pain medication a may differ between MCS and VS patients. In addition, surrogate decision makers may be required to choose whether or not to withdraw nutrition and hydration therapy for a chronic patient. This choice would be influenced by the diagnosis and subsequent prognosis provided. In a European survey, 66% of health-care professionals agreed to withdrawing treatment for permanent VS patients compared to only 28% for minimally conscious patients (Demertzi et al, 2009). From a legal point of view, some countries allow the withdrawing of life-sustaining therapies for only VS patients and not MCS patients (Gosseries et al., 2014). Therefore, the diagnosis provided to DoC patients can influence their quality of life, and this is why we are concerned with tests of awareness and consciousness.

Moreover, the auditory-only nature of the fMRI paradigm would allow for comatose patients to be tested for residual cognition and consciousness, and may provide some prognostic value. Some coma patients make a good recovery while others transition to the vegetative or minimally conscious states. Any information that can be provided to health care workers and families regarding the prognosis of the patient can be beneficial for making difficult decisions, especially decisions involving the removal of life-sustaining therapies. To date, no comatose patient has been able to demonstrate covert awareness through command following tasks. This is likely due to command following tasks being too difficult for patients who are highly impaired after their injury. In contrast to command following tasks, naturalistic paradigms require less effort from the patient, and so may be more effective for identifying patients who are aware and have preserved cognition.

GSR as method for testing covert awareness at the bedside

In chapter 3, I evaluated the suitability of using GSR for testing DoC patients at the bedside. A suspenseful movie elicited similar behavioural responses in healthy participants. I then used this GSR study for testing DoC patients. A Locked-In Syndrome patient showed a highly similar GSR compared to healthy controls while viewing this movie, which provided strong evidence that the patient was conscious and aware.

Brain regions influencing fMRI and GSR

As discussed previously, activity in the frontoparietal network has been linked to executive functions (Duncan, 2011; Barbey et al., 2012; Naci et al., 2014), and we are interested in executive function of DoC patients. This is because assessing executive function in DoC patients can provide evidence regarding their conscious experience. The connection between the frontoparietal network and executive function was made using fMRI. However, to use GSR for measuring covert awareness at the bedside, it should be measuring similar executive processes.

During "*Bang You're Dead"* movie viewing, the GSR time course was correlated with fMRI activity in the frontoparietal network (Naci et al., in prep). Additionally, there are overlapping regions known to be involved in influencing GSR and areas of the frontoparietal network. The dorsolateral prefrontal cortex is part of the frontoparietal network, and previously shown to be involved in goal directed aspects of attentional control (Vincent et al., 2008). The dorsolateral prefrontal cortex is also a higher-order region of the cortex known to control GSR (Critchley, 2002). Since the frontoparietal region is responsible for executive function, and GSR correlates with frontoparietal activity, this suggests we can use GSR to measure executive function in response to

complex stimulus. Therefore, this indicates that GSR is a suitable measure for testing

covert awareness in DoC patients at the bedside.

Differences in stimuli eliciting synchronization in fMRI and GSR measures

Taken elicited significant inter-subject correlation when healthy participants

listened to the audio narrative in the fMRI scanner. Specifically, the auditory and

frontoparietal networks displayed significant inter-subject correlation. However, this

audio narrative did not show significant inter-subject synchronization when tested using

GSR. That is, I observed variable responses between healthy controls. For the movie,

Naci et al. (2014) showed significant inter-subject synchronization during fMRI scanning

in the auditory, visual and frontoparietal networks, and the movie also had highly similar

responses across participants when tested using GSR. What would explain the

differences seen between stimuli and measures?

When comparing the two stimuli, the movie showed stronger inter-subject

synchronization in the frontoparietal region compared to Taken as measured by fMRI.

The correlation in the frontoparietal region for the movie was $r = .58$ (Naci et al., 2014),

as opposed to the correlation in the frontoparietal region for Taken was $r = .27$. Since the

frontoparietal region likely is a component driving the GSR, the lower inter-subject

synchronization in the frontoparietal region for Taken, as measured by fMRI, could

explain why there were variable behavioural responses. In addition, as discussed in

chapter 3, cross-modal stimulus integration is stronger than unimodal stimulus

integration.

Conclusion

In this project, I assessed the suitability of using naturalistic paradigms for testing covert awareness in behaviourally nonresponsive patients. In my first study, the audio narrative Taken produced significant inter-subject synchronization in healthy individuals. Moreover, this synchronization was present in the frontoparietal network, shown to support executive function, so this stimulus can be used for future patient testing. The second study examined Galvanic Skin Response as a possible bedside testing method. A suspenseful movie elicited highly similar GSR in healthy controls, indicating it is reliable on a single subject level. I then tested this method in disorder of consciousness patients during movie stimulus viewing. I identified a Locked-In Syndrome patient who showed a highly similar viewing experience compared to the healthy control group for both global and local time scales. Overall, this project has identified novel neuroimaging and bedside methods for testing awareness in disorders of consciousness patients using complex, naturalistic stimuli.

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Appendix A- Ethics

Western Research

Western University Health Science Research Ethics Board
NMREB Delegated Initial Approval Notice

Principal Investigator: Dr. Adrian Owen
Department & Institution: Social Science\Psychology, Western University

NMREB File Number: 105242
Study Title: Mechanisms of perceptual awareness and executive control
Sponsor: Canadian Excellence Research Chair

NMREB Initial Approval Date: May 16, 2014
NMREB Expiry Date: May 31, 2016

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Advertisement	Flyer for Research Credit	2014/04/10
Advertisement	Flyer for Monetary Compensation	2014/04/10
Western University Protocol	Revised Protocol- PDF clean version	2014/04/23
Other	Debriefing Form PDF: Clean copy	2014/04/23
Other	Edinburgh Handedness Questionnaire - Clean copy PDF	2014/04/23
Letter of Information & Consent	Updated LOI+ Consent form	2014/05/14

The Western University Non-Medical Research Ethics Board (NMREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

NMREB approval for this study remains valid until the NMREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University NMREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the Ontario Personal Health Information Protection Act (PHIPA, 2004), and the applicable laws and regulations of Ontario.

Members of the NMREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The NMREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000941.

Ethics Officer, on behalf of Riley Hinson, NMREB Chair

Ethics Officer to Contact for Further Information

Erika Basile ebasile@uwo.ca	Grace Kelly grace.kelly@uwo.ca	Mina Mekhail mmekhail@uwo.ca)	Vikki Tran vikki.tran@uwo.ca

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Curriculum Vitae

LEAH J. SINAI

EDUCATION

Western University, London, Ontario, Canada   2013-present
M.Sc. Candidate, Psychology
Thesis: Naturalistic paradigms for neuroimaging and bedside measures of conscious
awareness
Supervisor: Dr. Adrian Owen

Queen's University, Kingston, Ontario, Canada   2009-2013
B.Sc. (Honours), Biochemistry
Thesis: Role of a conserved Phe in Multidrug Resistance Protein-4 (MRP4) plasma
membrane trafficking and MRP4 interaction regulation with Na^+/H^+ Exchanger
Regulatory Factor 1 (NHERF1) adaptor protein
Supervisor: Dr. Susan Cole

PUBLICATIONS AND CONTRIBUTIONS
Naci, L., Sinai, L., Owen, A. M. (2015). Detecting and interpreting conscious experiences
in behaviourally non-responsive patients. Submitted.

Sinai, L., Cusack, R., Fiacconi, C., Gonzalez-Lara, L., Wild, C., Owen, A. M., Naci, L.
Naturalistic paradigms for neuroimaging and bedside measures of conscious awareness.
Poster presentation at A.S.S.C conference, 2015, Paris, France

Sinai, L., Cusack, R., Fiacconi, C., Gonzalez-Lara, L., Owen, A.M., Naci, L. Probing
conscious experience of rich naturalistic stimuli. Poster presentation at L.O.V.E.
Conference, 2015, Niagara Falls, Canada

Fu, J., Kwok, S., Sinai, L., Abdel-Razek, O., Babula, J., Chen, D., Farago, E.,
Fernandopulle, N., Leith, S., Loyzer, M., Lu, C., Malkani, N., Morris, N., Schmidt, M.,
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mutations. Poster presentation at Vascular Biology Group Conference

TEACHING EXPERIENCE

PSYC 2800: Research Methods   2013-2015
Teaching Assistant.

Princeton Review MCAT Instructor   Summer 2014
General Chemistry Instructor

ACADEMIC AND VOLUNTEER EXPERIENCE

Psychology Graduate Student Association   2014-present
Executive Committee Member

Research Assistant to Dr. Adrian Owen   Summer 2012
Brain and Mind Institute, Western University

Research Assistant to Dr. Robert Hegele   Summer 2011
Robarts Research Institute, Western University