



Trinity College Dublin

Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Conscious information processing during and after recovery
from deep anaesthesia

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Submitted in fulfilment of the requirements for the Master of Science degree

November 2021

Funded by the Irish Research Council
Government of Ireland Postgraduate Scholarship



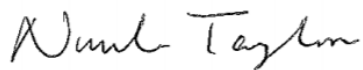
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Nicola Taylor

30/09/2020

Acknowledgements

Firstly, I would like to thank my supervisor Lorina, without whom this work could not have taken shape. You have been hugely supportive and flexible in the face of my considerable and fluctuating needs, and have provided invaluable guidance as well as a multitude of opportunities for me to develop over the last two years. Your kindness and clarity of thought have been inspiring to me, and I will carry forward many valuable lessons from you into various aspects of my life in the future.

I would like to thank the Irish Research Council for part-funding my degree, with Lorina providing funds for the first year. I would also like to thank Lorina and her colleagues at Western University for collecting the fMRI part of the dataset I used in my thesis.

I would like to thank all members of the Naci lab in TCD past and present for providing much needed support, companionship and laughs throughout. I'm excited to see where everyone ends up in the future. I would especially like to thank Remy McMeekin for completing the data collection for this project with such professionalism and enthusiasm. I would also like to thank Feng in particular – I'm so glad that we started this journey together and I have learned so much from you. I'm sorry we didn't finish together as we planned, but look forward to supporting you until the end the way you have done for me – it'll be grand!
😊

Finally, I would like to thank my family and friends who have been a constant source of advice, comfort and encouragement throughout this process. In particular, Mum, Dad, Andrew and James, for putting up with me and being there in the best and worst of times, as well as for their help in the final stages. And Peter, for everything, always.

Summary

This thesis aimed to answer two important questions in anaesthesia research. Firstly, despite an abundance of studies examining brain changes upon abolition of consciousness by anaesthesia, very few have focused on how these brain features reconstitute upon the recovery of consciousness. I addressed this question in Chapter 2. Secondly, there is very limited knowledge on brain mechanisms that might enable the preservation of conscious awareness, in rare cases, under general anaesthesia. I addressed this question in Chapter 3. Naturalistic paradigms provide an opportunity to examine the neural dynamics underlying changes in higher-order cognitive processes during and after anaesthesia. This thesis used a unique dataset where consciousness was abolished with deep propofol anaesthesia in a healthy group of 16 participants inside the 3T MRI scanner, while participants were exposed to a complex, plot-driven narrative (from the movie *Taken*) as well as during the resting state. Scans took place while participants were awake, under deep anaesthesia, and after they had recovered behavioural responsiveness.

In Chapter 2, I calculated functional connectivity (FC) within and between seven major networks across the brain. I found that during *Taken*, in contrast to the resting state, the FC increased significantly under deep anaesthesia and remained elevated during recovery relative to the awake state. Furthermore, widespread inter-subject correlation during the story, a measure of stimulus-driven information processing, did not restore back to awake levels in the recovery state. My results suggest that, immediately upon recovery, complex information processing is impaired relative to typical wakefulness, and both measurements of brain activity in the resting state and clinical behavioural scales fail to detect this effect.

In Chapter 3, an independent group of participants (N=25) listened to the same story outside of the scanner and rated its suspense at two-second intervals. I first established that these suspense ratings were highly correlated across individuals and tracked plot developments that required ongoing higher-order cognitive processes, such as sustained attention and working memory, to follow. Therefore, I could use the average suspense rating as a psychological descriptor of narrative understanding over time. In wakeful participants, suspense ratings predicted robust activation, at the group and individual level, of a set of brain areas, including regions of the auditory attention and language processing network (the left/ right superior temporal gyrus/ sulcus, supramarginal gyrus) and the salience network (the

left/right anterior insular gyrus, cingulate sulcus, brainstem areas, and the thalamus). At the group level, this activation was abolished during deep sedation. However, 1/16 participants (6%) during deep anaesthesia demonstrated the complete activity patterns associated with high-order auditory attention and cognitive and emotional appraisal of the story in wakeful participants, suggesting that this individual processed and understood the story similarly to awake participants. Traditional methods of detecting awareness under anaesthesia put its incidence at 0.1-0.2%, however they may underestimate its true incidence due to their reliance on patient recall. The isolated forearm technique (IFT) does not require explicit recall and produces higher estimates of up to 4.6%, however a positive result on the IFT requires several capacities in addition to conscious awareness which may be impacted by anaesthetic agents. The higher rate reported in my study may reflect the lower task demands of understanding an auditory story compared to the command-following required for IFT. My findings provide a novel marker for measuring awareness under general anaesthesia and underscore the importance of developing methods of assessing consciousness that do not require behavioural response or explicit recall. Future research should aim to develop methods that translate the current approach to cheaper, more portable technologies.

Keywords: Neuroimaging, Naturalistic Paradigm, Anaesthesia, Recovery, Covert Awareness

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List of abbreviations

A1 = Primary auditory cortex
AAGA = Accidental awareness under general anaesthesia
AAN = American academy of neurology
AN = Auditory network
BIS = Bispectral index
BN = Between-network
BOLD = Blood-oxygen-level-dependent
DAN = Dorsal attention network
DMN = Default mode network
DoC = Disorders of consciousness
ECN = Executive control network
EEG = Electroencephalography
FDR = False discovery rate
fMRI = Functional magnetic resonance imaging
FWE = Family wise error
GABA = Gamma-aminobutyric acid
GLM = General linear model
GSR = Galvanic skin response
HRF = Haemodynamic response function
IFT = Isolated forearm technique
IPS = Intraparietal sulcus
ISC = Inter-subject correlation
MT = Middle temporal area
NAP5 = 5th National Audit Project on AAGA in Ireland and the United Kingdom
PFC = Prefrontal cortex
PET = Positron emission tomography
PTSD = Post-traumatic stress disorder
RSS = Ramsay sedation scale
SM = Sensory-motor network
SN = Saliience network
SR = Suspense rating
TMS = Transcranial magnetic stimulation
V1 = Primary visual cortex
VN = Visual network
WN = Within-network

Chapter 1: General introduction

Anaesthesia provides an invaluable tool for understanding how brain systems give rise to consciousness and cognition, but its complex and multifaceted effects make study in this area challenging. Since the advent of its use in the 19th century (Kelz & Mashour, 2019), general anaesthesia has been associated with alterations, and particularly abolishment, of consciousness. Clinically, in an anaesthetic context, patients are considered to have lost consciousness when they fail to respond to loud noises or shaking, and show physiological changes such as irregular respiration, loss of muscle tone and altered blood pressure (Brown, Lydic, & Schiff, 2010). Its clinical aim is to allow patients to undergo medical interventions, such as major surgery, that would otherwise be intolerably painful and unpleasant, by removing all conscious sensation. In a research context, general anaesthesia has been used to study the neural basis of consciousness, by allowing its temporary and reversible abolition. If some neural characteristic is fundamental and specific to consciousness, then removal of consciousness by general anaesthesia will result in its abolition. Therefore, many studies have investigated what features of the brain reduce or disappear upon administration of general anaesthetic. Very few studies have investigated how these brain features reconstitute upon recovery of consciousness. Furthermore, there is very limited knowledge on what neural mechanisms might enable the preservation of conscious awareness, in rare cases, under general anaesthesia. This relates to a central challenge in anaesthesia research: understanding the relationship between brain changes and concomitant changes in cognitive function. Therefore, my thesis aims to use a naturalistic neuroimaging paradigm, designed to assess stimulus-driven neuro-cognitive changes in the brain during anaesthesia, to investigate i) the neurocognitive changes associated with recovery of consciousness following anaesthesia, and ii) neural markers of preserved consciousness during deep anaesthesia.

Behavioural responsiveness and consciousness

In a clinical anaesthesia context, conscious awareness is measured using standardised scales (e.g. the Ramsay Sedation Scale; Ramsay, Savage, Simpson, & Goodwin, 1974) in which a person's behavioural response to verbal commands or sensory stimulation is assessed by a trained clinician. Patients are considered to have lost consciousness when they no longer respond to loud noises or shaking, and become behaviourally non-responsive. A different population who, similarly to individuals under deep/general anaesthesia, become

behaviourally non-responsive are patients exhibiting disorders of consciousness (DoC) after severe brain injury. Patients with DoC, including those in coma and persistent vegetative state (Jennett & Plum, 1972), are incapable of overt behavioural responses and are thought to lack awareness of both themselves and their environment (Laureys, Owen, & Schiff, 2004). A brief discussion of DoC research is relevant to questions of consciousness in anaesthesia, as in both cases, an absence of behavioural response is assumed to reflect the absence of conscious awareness. However, research over the last 15 years on severely brain-injured patients has clearly documented cases in which behavioural responsivity diverges from conscious awareness (Sanders, Tononi, Laureys, & Sleigh, 2012). In a seminal study, Owen and colleagues (2006) reported that a brain-injured patient with a diagnosis of vegetative state, indicating the presence of wakefulness but a complete lack of conscious awareness, in fact showed neural activation patterns demonstrating that she understood and responded to commands to imagine navigating rooms in a house or playing tennis. This finding has been replicated many times since (Cruse et al., 2011; Bardin et al., 2011; Goldfine et al., 2013; Fernández-Espejo & Owen, 2013; Naci & Owen, 2013; Gibson et al., 2016; Huang et al., 2018), with cohort studies establishing that at least 14-19% of behaviourally non-responsive brain-injured patients show neural evidence suggestive of preserved conscious awareness (Monti et al., 2010; Kondziella et al., 2016). Where the severity of brain-injured patients are concerned, it remains unclear whether the remaining 86-81% are genuinely unconscious, or whether a lack of response on these mental command-following tasks could be due to myriad other reasons, including an inability to understand the task, impaired attentional abilities, a lack of willingness or motivation to engage with the task, or an inability to perform the desired mental behaviour (DiPerri, Stender, Laureys & Gosseries, 2014). Therefore, findings of covert awareness in patients with DoC clearly motivate investigation of conscious processes in patients who are behaviourally non-responsive due to general anaesthesia. Indeed, a small proportion of patients (0.1%-4.6%; Mashour et al., 2012; Sanders et al., 2017) under general anaesthesia do experience unintended intraoperative awareness, or accidental awareness under anaesthesia, when they are presumed to be unconscious.

The brain under anaesthesia

Traditionally in cognitive neuroscience, conscious cognition is studied using laboratory-based behavioural tasks which attempt to isolate a particular cognitive function, for example memory, attention or executive function, and relate it to some measure of neural

functioning (Poldrack and Yarkoni, 2016). These behavioural tasks typically require participants to respond to repeated presentation of discrete, simple stimuli. However, this class of technique is plainly not appropriate for studying cognitive changes in behaviourally non-responsive states, such as during anaesthesia, in which both eye-opening and behavioural response is impaired. For this reason, many studies have used a resting state paradigm to investigate anaesthesia-related brain changes (Heine et al., 2012; Mortazavi et al., 2018). In resting state studies, participants' intrinsic neural activity is monitored, while they are lying down quietly with no sensory or cognitive stimulation. Brain activity can be monitored directly using electroencephalography (EEG), or indirectly using positron emission tomography (PET) which records changes in metabolism or, most commonly, functional magnetic resonance imaging (fMRI), which tracks fluctuations in the brain's magnetic field assumed to reflect local haemodynamic changes in brain tissue (Aine, 1995). fMRI studies in healthy humans have consistently identified sensory and higher-order resting state networks, which comprise brain areas that are spatially distinct but demonstrate temporally synchronous functional activity, and are therefore functionally related (De Luca et al., 2006; Damoiseaux et al., 2006; Raichle, 2011; Smitha et al., 2017). Higher-order networks include: the executive control network (ECN), which links dorsolateral prefrontal and posterior parietal cortices and is involved in behavioural response planning and monitoring during complex intellectual activities (Kroger et al., 2002; Seeley et al., 2007); the dorsal attention network (DAN), a dorsal frontoparietal system, which exerts top-down control on attentional processes (Corbetta & Shulman, 2002); the salience network (SN), anchored in anterior cingulate and orbital frontoinsula cortices and involved in identifying the most relevant internal and external stimuli (Menon & Uddin, 2010; Seeley et al., 2007); and the default mode network (DMN), which is distributed across the thalamus, posterior cingulate cortex, precuneus, frontal and parietal cortices, and is key to internally-oriented processes, such as introspection and spontaneous thought (D'Argemba et al., 2005; Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010). Recent studies have also linked the DMN to monitoring shifts in the external environment (Buckner, Andrews-Hanna, & Schacter, 2008), for example playing a role in encoding, updating and retaining information in working memory (Vatansever, Manktelow, Sahakian, Menon, & Stamatakis, 2017). Primary networks include those involved in visual (VN), auditory (AN) and sensorimotor (SM) processing. Resting state studies have produced some consistent results showing the impact of various levels of anaesthesia on these networks.

The majority of previous resting state studies have suggested that anaesthesia is associated with reduced connectivity across the brain (Alkire, Hudetz, & Tononi, 2008). Propofol is the most commonly used anaesthetic agent in clinical interventions and is thought to produce its effects by enhancing transmission of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (Irifune et al., 1999; Alkire & Haier, 2001; Brown et al., 2010). Accordingly, early PET studies of cerebral metabolism reported dose-dependent decreases across all brain regions following propofol anaesthesia (Alkire et al., 1995; Kaisti et al., 2002), with some areas, such as the thalamus, frontoparietal, cingulate and precuneus, more depressed than others (Fiset et al., 1999). Similarly, fMRI studies have found that the detectability of resting state networks reduces from 81% in awake individuals to 71% under mild sedation, and drops dramatically to 46% for deep anaesthesia (Guldenmund et al., 2016). This suppression of brain connectivity under anaesthesia is reportedly not equal across brain regions – reviews consistently report a particular reduction in connectivity *within* higher-order networks, alongside preservation of connectivity within sensory processing networks such as the AN and VN (Bonhomme, Boveroux, Brichant, Laureys, & Boly, 2012; Hudetz, 2012; MacDonald, Naci, MacDonald & Owen, 2015; Mortazavi et al., 2018). This effect has been documented in the DMN (Greicius et al., 2008; Boveroux et al., 2010; Jordan et al., 2013; Huang et al., 2014), the ECN (Boveroux et al., 2010; Boly et al., 2012) and the SN (Guldenmund et al., 2013; Palanca et al., 2015). EEG studies shedding light on the direction of connectivity suggest that the impact of anaesthesia in these areas is driven by selective inhibition of fronto-parietal feedback loops, while feed forward connectivity remains relatively unaffected (Ku, Lee, Noh, Jun, & Mashour., 2011; Boly et al., 2012; Lee et al., 2013; Ranft et al., 2016).

Finally, recent studies have used novel techniques to assess how the complexity of neural signals changes with disruptions of consciousness. When the cortex is perturbed by transcranial magnetic stimulation (TMS), responses across the brain in deep anaesthesia become undifferentiated and synchronous, showing global, stereotypical patterns (Sarasso et al., 2015) thought to reflect a breakdown of causal interactions between brain areas (Massimini, Ferrarelli, Sarasso, & Tononi, 2012; Casaratto et al., 2016). The brain under anaesthesia shows a reduced number of discriminable brain states (Stamatakis, Adapa, Absalom, & Menon, 2010; Barttfeld et al., 2015). Loss of consciousness under propofol has been linked to a reduction in complexity using other EEG measures of complexity (Schartner et al., 2015; Pal et al., 2018), as well as network and information-theory based analyses of

fMRI data (Pappas et al., 2019; Luppi et al., 2019; Varley et al., 2020; Vatansever et al., 2020). Despite developing our understanding of anaesthesia-induced brain changes, resting state studies have a significant limitation. As participants are not presented with any stimuli, resting state data cannot shed light on how observed neural changes relate to cognitive changes, or how information processing of external sensory stimuli breaks down during loss of consciousness (Naci et al., 2018).

Several anaesthesia studies have presented participants with simple stimuli which do not require eye-opening or behavioural response, thus providing insight into how well-circumscribed sensory-specific processes are altered by anaesthesia. The general pattern of reported results suggests that sedation induces dose-dependent impairment of sensory information processing, sequentially from more to less complex. Activations in association cortices reflecting higher-order processing declines first upon administration of anaesthesia, while primary sensory processing activations are preserved until higher doses (Bonhomme et al., 2012; MacDonald et al., 2015; Mortazavi et al., 2018). Many studies to date have focused on the auditory system. Temporal lobe activations characteristic of sound and speech perception during auditory presentation of sentences are preserved in both light and deep propofol sedation, while inferior frontal and posterior temporal activations associated with higher-level speech comprehension are disrupted at light sedation (Davis et al., 2007) and deep anaesthesia (Plourde et al., 2006). In propofol-induced sedation, fMRI activation of higher-order regions related to auditory processing of musical stimuli showed a negative correlation to increasing drug concentration, while activation in basic auditory areas was preserved even in deep anaesthesia (Veselis et al., 2004; Dueck et al., 2005). Heinke and colleagues (2004) reported that the widely distributed frontotemporal network activated in wakeful processing of sentences was sequentially affected by propofol, with activity in frontal areas abolished directly after participants became unresponsive, while temporal activation persisted until being abolished at a higher dosage of propofol. A similar pattern of dose-dependent suppression of activation, with an initial and primary effect on higher-order brain regions, has been reported for other brain functions, including tactile stimulation (Antognini, Buonocore, Disbrow, & Carstens, 1997; Bonhomme et al., 2001), visual processing (Martin et al., 2000; Heinke & Schwarzbauer, 2001; Ramani, Qiu, & Constable, 2007;) and memory (Pryor et al., 2015). However, the sole use of this task-based approach has been criticised for providing a limited view of brain changes that are relevant only to the relatively narrow and curtailed processes the tasks are designed to elicit. This examination of

highly circumscribed brain regions during presentation of simple, discrete stimuli is not necessarily indicative of how cognitive processing take place in the real world, where the meaning of stimuli is heavily influenced by their dynamic context, which evolves and builds over time.

Naturalistic neuroimaging paradigms

To address the limitations of both resting state and traditional cognitive tasks, a novel approach has been developed in recent years, where individuals freely listen to or view complex and naturalistic stimuli that evolve over time, in the form of real life media such as film clips, television advertisements or spoken narratives. These naturalistic paradigms are reportedly more effective at detecting differences in brain activity than resting state fMRI for several reasons. Firstly, media that have been directed and produced with the specific aim of entertaining or communicating to people are by their nature extremely engaging. This not only improves participant compliance, but also improves the quality and quantity of neuroimaging data that can be acquired, especially in the context of fMRI (Eickhoff, Milham & Vanderwal, 2020). By decreasing participants' level of boredom, use of engaging naturalistic stimuli increases the amount of time participants can tolerate being scanned, decreases head motion (Centeno et al., 2016) and reduces the amount of time asleep in the scanner (Vanderwal, Kelly, Eilbott, Mayes, & Castellanos, 2015). These factors lead to increased reliability and signal to noise ratio, compared to resting state paradigms (Wang et al., 2017). Secondly, naturalistic paradigms are effective in detecting the functional roles of neural networks. An intrinsic challenge of neuroimaging research is distinguishing cognition-related signal from confounding noise from other sources such as cardiac/ respiratory cycles and blood pressure (Murphy, Birn, & Bandettini, 2013), and the unconstrained nature of resting state paradigms makes doing so very difficult. In contrast, naturalistic paradigms drive distinct activation patterns across brain networks, capturing their functionally distinct roles by engagement of multiple higher-order cognitive functions, such as attention, comprehension and integration of information across multiple time-scales (Sonkusare, Breakspear, & Guo, 2019). Finally, there is also evidence that biologically relevant signal variability that is missed in resting state studies of cognition may be captured by naturalistic paradigms (Cantlon and Li, 2013; Vanderwal, Eilbott, & Castellanos., 2019). Compared to traditional task-based neuroimaging studies, naturalistic stimuli provide an optimal way of studying complex, higher-order cognition in a way that closely mimics how information is

processed in a real-world context. For this reason, there is growing recognition of the value of using naturalistic stimuli to study cognitive processes and understand the neural basis of real-world functioning and clinical dysfunction (Sonkusare et al, 2019; Eickhoff et al., 2020).

One such naturalistic paradigm, introduced by Naci and colleagues (2014), has been used to link conscious cognition, and its abolition due to anaesthesia or brain injury, to features of brain dynamics during viewing of plot-driven movies or listening to auditory excerpts of audio-visual movies. Previous to this work, studies had shown that individual healthy participants demonstrate widespread synchronous neural activity, known as inter-subject correlation (ISC), when viewing engaging movies inside the MRI scanner (Hasson et al., 2004; Hasson et al., 2008; Hasson, Malach, & Heeger 2010). In a series of studies, Naci and colleagues explored the functional localization and cognitive specificity of ISC from different brain networks, to investigate whether they could be linked with conscious information processing, and in turn be used to investigate conscious awareness and its breakdown in behaviourally non-responsive populations such as severely brain injured patients or individuals undergoing deep anaesthesia. Below, I summarize the key studies (Naci, Cusack, Anello, & Owen 2014; Naci, Graham, Owen, & Weijer, 2017; Naci et al., 2018; Deng, et al., 2020) that pertain to the questions and methodological approach that I take in this thesis.

In an initial study, Naci et al. (2014) sought to develop a neural index of executive function, a process that requires conscious processing, in healthy individuals, as they viewed a brief and highly engaging movie clip by Alfred Hitchcock, in the MRI scanner. Well-directed movies and audio narratives are designed to evoke a common experience in viewers, and the neural synchronisation observed across viewers' brains is thought to reflect similar engagement of their cognitive processing, especially during highly emotional and engaging parts of the plot (Kober et al., 2008; Nummenmaa et al., 2012; Nummenmaa et al., 2014). However, previous to this work it remained unknown which part of this brain activity reflected high-order cognitive processes related to plot understanding, such as executive function – a high-order cognitive function that requires conscious awareness.

Naci et al. (2014) demonstrated that the timing of ISC in the fronto-parietal network during the movie was predicted by independently measured indices of executive function across the movie's timeline. One group of participants performed a dual task - i.e. watching

the Hitchcock movie (outside of the scanner) while simultaneously performing a sustained attention task - thus, providing a moment-to-moment measure of the movie's executive demands. A second group independently rated how suspenseful the plot was every two seconds of the movie. The authors reasoned that an understanding of the narrative's plot requires recruitment of higher-order processes, as the viewer must continuously attend to and integrate information from various points in the story, as well as from stored knowledge, and therefore, suspense ratings provided a qualitative measure of the engagement of these complex cognitive processes. The study showed that, similarly to the executive demands, suspense ratings predicted synchronised activity in frontoparietal regions, and that the most attentionally-demanding points corresponded to the peaks in suspense ratings. Importantly, this synchronous frontoparietal activity could be detected in each individual participant, using a set of leave-one-out analyses. Based on these findings, the authors concluded that the synchronized activity in front-parietal regions represented the common executive processing and conscious understanding of the movie, which could be investigated as a single individual level.

This enabled the authors to investigate the presence of covert conscious processing during the same movie in two behaviourally non-responsive patients with DoC diagnoses, who were thought to lack consciousness. One patient, who had remained behaviourally non-responsive for 16 years, demonstrated a similar frontoparietal brain response, time-locked to the story's evolving plot, to the group of healthy participants, thus allowing the authors to infer that he understood the movie in a similar way to conscious healthy individuals. This was the first study to show that naturalistic paradigms could be used to investigate the presence of conscious awareness in populations where behavioural responsiveness is lacking, such as in some severely brain injured patients or individuals undergoing deep anaesthesia.

Subsequently, the authors developed an auditory-only version of this paradigm (Naci et al., 2017), to investigate the presence of consciousness in individuals who have their eyes closed, such as those under deep anaesthesia, and cannot process audio-visual stimuli. By using a short auditory excerpt from the movie *Taken* (Morel, 2008), that depicts a dramatic narrative arc, the study showed high ISC in the fronto-parietal regions of healthy participants, above and beyond the ISC produced by a scrambled baseline stimulus which was circumscribed to the auditory regions in the temporal lobe (Naci et al. 2017). Similar to the findings in Naci et al. (2014), frontoparietal activity could be reliably predicted from the rest

of the group, in 93% of participants (14/15). These studies demonstrated the suitability of this stereotypical story-evoked brain activity as a neural signature of conscious higher-order processing in individual participants that can be investigated in behaviourally non-responsive populations who have impaired vision or eye-opening, such as anaesthetised individuals.

Subsequently, Naci and colleagues (2018) used this paradigm to investigate how information processing breaks down under anaesthesia. Healthy individuals (N=16) completed fMRI scans during both the resting state and while they were presented with the Taken audio story, during wakefulness and deep propofol anaesthesia. During propofol-induced unconsciousness, by using regional ISCs as a measure of regional story-driven information processing, they found abolition of information processing in higher order fronto-parietal regions, which stood in contrast to some preservation of information processing in auditory regions. By measuring brain network-specific functional connectivity, they found that during deep anaesthesia, as participants listened to the Taken story, there was a significant increase in connectivity *between* brain networks across the brain. By contrast, during the resting state, they found the more commonly reported decrease in *within-network* connectivity. The authors interpreted this result as a reduction in how differentiated the functional activity of brain networks became during deep propofol-induced unconsciousness. They suggested that the loss of external information processing in frontoparietal regions under deep anaesthesia may be due to an erosion of their differentiation from lower-order sensory networks. This finding was in line with previous resting state TMS studies reporting that, under anaesthesia, neural responses become undifferentiated and synchronous, suggesting a breakdown of causal interactions between brain areas (Sarasso et al., 2015; Massimini et al., 2012; Casaratto et al., 2016).

This proposal was further supported by the finding that a group of DoC patients (N=5) who showed no behavioural or neuroimaging evidence of conscious awareness also showed reduced differentiation of higher-order and sensory networks during the story. This stood in stark contrast to findings from a second group of DoC patients (N=6), who had demonstrated covert consciousness via independent neuroimaging assessments, and in turn showed the story-driven functional differentiation, similarly to healthy controls (Naci et al., 2018).

Finally, the study found that healthy individuals, who showed higher differentiation between the frontoparietal and sensory brain networks during the audio story, performed better on an independent, behavioural, language acuity test. Taken together, findings from these three studies included in Naci et al. (2018) supported the proposal that functional differentiation of brain networks is important for specific cognitive functions that require conscious awareness. Crucially, use of the naturalistic paradigm in the study elucidated a potential neural mechanism which was not accessible via resting state, as it allowed examination of how the brain responded in the presence of external stimulation.

A more recent study by the same group (Deng et al., 2020) utilized the engagement of neurocognition during this paradigm to investigate the basis of inter-individual responsiveness variability in moderate propofol anaesthesia. fMRI scans and a simple auditory target detection task were completed when participants were awake, and under moderate sedation, i.e. absent spontaneous conversation, sluggish speech but preserved responses to questions and commands (level 3 on the Ramsay Sedation Scale; Ramsay et al., 1974). They found that brain connectivity during the Taken audio story predicted the effect of moderate doses of propofol on individual behavioural responsiveness better than data collected during the resting state. The inherent functional connectivity within the ECN prior to sedation, while listening to the audio story, differentiated individuals who were dramatically slowed down during moderate anaesthesia versus others that showed no significant change in reaction times relative to their awake state on the detection task. Faster responders had stronger ECN connectivity than slower responders. No such differentiation could be made using resting state data.

Together, these two recent studies (Naci et al., 2018; Deng et al., 2020) clearly delineate the value of using the naturalistic audio-story based fMRI paradigm to investigate changes in brain dynamics and cognition as consciousness is impaired by anaesthesia. As well as being brief, easy to administer and suitable for participants with impaired eye-opening or closed eyes, this paradigm recruits neurocognitive responses to complex stimulation, and thus allows characterisation of activity within and across brain networks as consciousness fades. As shown Naci et al. (2018), the paradigm also provides the opportunity to test whether insights gained from anaesthesia-based studies apply to different populations, from healthy cognition in awake participants to impairments of consciousness and cognition in individuals with severe brain injury. Additionally, it provided a platform for investigating

common neural mechanisms that underlie loss of consciousness across vastly different conditions, such as under deep anaesthesia and after severe brain injury.

The current thesis

In my thesis, I build on this previous work. By utilizing this unique paradigm that enables direct examination of the neural dynamics underlying alterations of cognition and consciousness under anaesthesia, I address two important gaps. The first gap my thesis will address is the relative lack of studies examining the transition back into consciousness following anaesthesia. My aim is to address the question of whether and to what extent the brain dynamics disrupted by propofol anaesthesia reconstitute upon recovery of consciousness. A full understanding of how the brain recovers consciousness following anaesthesia is important for both scientific and clinical reasons. As well as adding to our knowledge of the brain mechanisms of consciousness and cognition, it may aid in developing prognostic markers or potential therapeutic targets for patients with DoC. Understanding the state of the brain as patients emerge from anaesthesia is also crucial given evidence from animal (Culley, Baxter, Yukhananov, & Crosby, 2004; Lin & Zuo, 2011; Callaway, Jones & Royse, 2012; Zurek et al., 2014; Jiang, Miao, & Chen, 2017) and human studies (Rasmussen et al., 2003; Hanning, 2005; Newman, Stygall, Hirani, Shaefi, & Maze, 2007; Krenk, Rasmussen, & Kehlet, 2010; Evered et al., 2018) that cognition can remain impaired after behavioural signs of consciousness have returned.

The research that does exist in this area presents inconsistencies. Some fMRI resting state studies suggest that brain dynamics return to baseline awake levels upon restoration of behavioural responsiveness (Boveroux et al., 2010; Guldenmund et al., 2016; Mashour et al., 2020). By contrast, research that employs other research methods, such as animal studies, PET or resting state measures of information processing in the brain, indicates that neural activity remains altered upon recovery (Långsjö et al., 2012; Monti et al., 2013; Liu et al., 2019; Pal et al., 2020;). As discussed above, Naci et al. (2018) demonstrated that use of the audio story paradigm produced strikingly different results to that of the resting state during deep propofol anaesthesia, potentially identifying a mechanism underlying the loss of external information processing as consciousness fades. However, their analysis probed only the transition from wakefulness to deep anaesthesia and did not examine the recovery of consciousness. Therefore, in Chapter 2, I will use the same naturalistic paradigm as in Naci et

al. (2018) to characterise neurocognitive changes associated with recovery of consciousness relative to wakefulness, as well as deep anaesthesia.

The studies I have summarized above emphasise the value of naturalistic paradigms in examining group-level differences in brain dynamics during anaesthesia-induced loss of consciousness, however little is known about the inter-individual variability of brain responses, including preservation of awareness, under deep anaesthesia. The second aim of my thesis, explored in Chapter 3, will fill this gap. This is important as accidental awareness under general anaesthesia has been associated with poor psychological outcomes, including post-traumatic stress disorder (Aceto et al., 2013), acute and long-term distress (Cook et al., 2014) and flashbacks (Bruchas, Kent, Wilson, & Domino, 2011).

As previously established (Naci et al., 2017; 2018) the auditory naturalistic paradigm provides an effective way of examining neural evidence of residual cognition and consciousness in states of behavioural non-responsiveness, be it due to severe brain-injury or due to deep/general anaesthesia. Similarly to behaviourally non-responsive patients with DoC, covert consciousness can occur in patients under deep anaesthesia. A high inter-individual variability has frequently been reported in the behavioural and cognitive effects of moderate anaesthesia (Chennu, O'Connor, Adapa, Menon, & Bekinschtein, 2016; Bola, Orłowski, Płomecka, & Marchewka, 2019; Deng et al., 2020), but the observation of covert consciousness in deep anaesthesia is less frequent. At deep anaesthesia dosages, accidental awareness under general anaesthesia is rare, however, estimates of incidence vary from 0.1% to 4.6% depending on method of detection used (Myles et al., 2004; Avidan et al., 2008; Avidan et al., 2011; Zhang et al., 2011; Mashour et al., 2012; Sanders et al., 2017). Detecting rates of conscious awareness during anaesthesia is highly challenging, but the naturalistic paradigm discussed herein may be particularly suited to this task (Graham, Weijer, & Naci, 2018). Given the known possibility of dissociation between behavioural responsiveness and conscious awareness (Sanders et al., 2012), careful monitoring for behavioural signs of consciousness will not suffice. Furthermore, the amnestic effects of anaesthetic agents (Bonhomme et al., 2012; Brown et al., 2010) mean that asking participants to retrospectively recall whether they were aware, or not, will not provide an accurate incidence rate.

The examination of neural activity for markers of consciousness is a promising solution. However current depth-of-anaesthesia monitors, which use EEG to do so, suffer

from severe limitations. Chief amongst them are poor sensitivity in identifying those already known to be aware (Schneider et al., 2002; Schuller, Newell, Strickland, & Barry, 2015), and a lack of clarity on how the global EEG signal relates to conscious awareness (Boly, Sanders, Mashour, & Laureys, 2013). A naturalistic fMRI paradigm such as in Naci and colleagues (2014; 2017), in contrast, aims to elicit distinctive patterns of brain activity in specific areas that have been previously linked to higher-order cognitive processes underlying conscious awareness, providing substantially stronger evidence for the presence of consciousness (Graham et al., 2018). Naturalistic paradigms thus provide a powerful tool for seeking to address the limitations of current neuroimaging methods used to detect awareness under anaesthesia. Therefore, in Chapter 3 I will use the audio story paradigm to investigate whether neural signatures of conscious awareness can be detected in individual participants under deep anaesthesia.

The data analysed in Chapter 2 and Study 2 in Chapter 3 were previously collected by my supervisor and colleagues at Western University, Canada. The total dataset comprises structural and functional MRI, behavioural and demographic data from 19 participants at four timepoints: baseline wakefulness, under moderate sedation via propofol, under deep anaesthesia via propofol, and at recovery of consciousness following cessation of propofol. The dataset has two important features that make it valuable in the present anaesthesia research. Firstly, the depth of sedation in the ‘deep anaesthesia’ condition, in which participants become behaviourally unresponsive, is unusual for research studies in healthy participants. The depth of sedation in most similar research neuroimaging studies in healthy participants corresponds more closely to the ‘moderate sedation’ condition in the current dataset, i.e. drowsy and sluggish but still responsive to verbal commands. This is because attainment of deep anaesthesia without participant intubation in a research setting is technically challenging and requires close collaboration of advanced neuroimaging and clinical anaesthesia expertise, which was uniquely available in this study. Secondly, the dataset includes functional MRI data from both resting state and a naturalistic paradigm, enabling comparison of the impact of anaesthesia on the brain during the absence and presence of stimulation. Parts of the dataset have previously been used in published studies and preprints, to investigate questions relating to brain dynamics and disruptions of consciousness (Naci et al., 2018; Luppi et al., 2019; Varley et al., 2020; Deng et al., 2020).

Chapter 2: Perturbations of brain dynamics during loss and recovery of consciousness

Introduction

General anaesthesia has long been used to study the neural basis of consciousness as it allows temporary, reversible abolition of consciousness. If some neural characteristic is fundamental and specific to consciousness, then removal of consciousness by general anaesthesia will result in this characteristic's abolition. Therefore, many studies have investigated what characteristics of the brain reduce or disappear upon administration of general anaesthetic. However, there is a dearth of studies investigating the brain changes that are observed as a person recovers consciousness. The aim of this chapter is to investigate recovery from propofol-induced anaesthesia, by comparing measures of brain dynamics at the point of recovery to those during states of wakefulness and deep anaesthesia.

A comprehensive understanding of how the brain recovers following loss of consciousness is important for several reasons. From a scientific perspective, studying how particular neural systems are restored to baseline functioning after loss of consciousness will add to our knowledge of the brain mechanisms of consciousness and cognition. In the clinic, the presence of consciousness is assessed by the presence or absence of behavioural responses to simple commands or stimuli (Brown et al., 2010), rather than an assessment of more complex cognitive processing. Therefore, it is valuable to know whether there is widespread restoration to baseline levels of functioning across the brain immediately at recovery, or whether particular networks continue to show alterations. Clinically, understanding the pattern of neural recovery may potentially aid in developing therapeutics or prognostic markers for patients with disorders of consciousness. Finally, it is relevant for patients emerging into consciousness after general anaesthesia. This is particularly important given previous research in animals suggesting that cognition can be impaired in the immediate aftermath of recovery from general anaesthesia (Culley et al., 2004; Lin & Zuo, 2011; Callaway et al., 2012; Zurek et al., 2014; Jiang et al., 2017), and in humans, showing postoperative cognitive dysfunction, especially in vulnerable groups such as infants and the elderly (Rasmussen et al., 2003; Hanning, 2005; Newman et al., 2007; Krenk et al., 2010; Evered et al., 2018).

To contextualise findings about recovery of consciousness, it is first useful to consider the more comprehensively studied transition from wakefulness to loss of consciousness. Several reviews have summarised the literature to date on cortical brain changes associated with anaesthesia-induced unconsciousness (Hudetz, 2012; Bonhomme et al., 2012; Uhrig, Dehaene & Jarraya, 2014; Kelz & Mashour, 2019), with some key findings consistently reported across studies. General anaesthesia is broadly associated with reduced cortico-cortical connectivity in higher order brain networks, such as the DMN, ECN and SN, alongside relative preservation of connectivity of the low-level sensory cortices (Bonhomme et al., 2012). A review by MacDonald et al. (2015) concluded that anaesthetic-induced sedation impairs sensory information processing in a dose-dependent manner, with activation in higher-order areas involved in more complex processing being disrupted first. They suggested that in deep sedation, processing of simple sensory stimuli appears to be preserved, by contrast to higher order processing which is abolished. One important limitation of comparing brain states of wakeful individuals to those under anaesthesia is the difficulty in dissociating changes that are related to loss of consciousness, from those that are not specific to consciousness but rather other drug effects.

With respect to recovery, several recent studies, using diverse methodologies, have reported inconsistent findings which question the restoration of baseline connectivity of higher order networks, such as the DMN, ECN and SN. Boveroux et al. (2010) reported that the reductions in fMRI-measured functional connectivity within fronto-parietal networks seen in deep propofol anaesthesia showed the opposite pattern upon recovery of consciousness. Similarly, a recent preprint reported that EEG measures of frontal and posterior dynamics decreased during propofol anaesthesia and returned to baseline levels just before recovery of consciousness (Mashour et al., 2020). In a study looking at detection of resting state networks using fMRI, rates of detection were similar before and after recovery from propofol anaesthesia, having almost halved during deep sedation (Guldenmund et al., 2016). A study looking at brain connectivity using graph theoretical measures derived from resting state fMRI, however, reported a divergent result to the above. The clustering coefficient, a measure of how closely regions of interest clustered together, increased in propofol-induced unconsciousness, indicating an increase in localised processing and a decrease in integration of information across the brain (Monti et al., 2013). Crucially, this measure remained elevated after participants recovered consciousness. This result was mirrored in a recent resting state fMRI study which used a novel approach to quantify regional information

content by measuring entropy, or complexity, of the blood oxygen-dependent imaging (BOLD) signals (Liu et al., 2019). Recovery from propofol-induced deep anaesthesia was associated with a return to awake levels in sensory but not high-order (i.e. dorsal and medial prefrontal, cingulate, parietotemporal cortices and hippocampal) areas. This suggested that, despite recovery of behavioural responsiveness, neural activity in higher-order brain regions remained impaired.

A recent animal study aimed to dissociate the drug- and state-related effects of anaesthesia by using a model in which rats could be wakened from sevoflurane-induced unconsciousness by injection of carbachol into the prefrontal cortex (Pal et al., 2020). They examined EEG measures of cortical connectivity and dynamics. In line with previous literature, they found a reduction in these measures during sevoflurane-induced unconsciousness, however, contrary to expectations, these measures were not restored to wakeful levels upon return of consciousness. These results demonstrated that, at least in rat models, wakefulness can return following anaesthetic-induced unconsciousness without restoration of cortical connectivity, and suggest that impaired connectivity might instead be more closely linked to the presence and lingering effect of anaesthesia on the system. Similarly, a PET study in human participants, which examined cerebral blood flow changes associated with loss and return of consciousness after dexmedetomidine infusion, showed that although functional connectivity between frontal and parietal networks reduced with loss of consciousness, neocortical activity did not restore to baseline levels upon recovery of consciousness (Långsjö et al., 2012). Recovery of consciousness in this study was defined as an eye-opening response to tactile or verbal stimulation, which requires much more limited cognitive functioning than rich processing of a complex stimulus. The authors suggested that recovery of consciousness occurs in a bottom-up manner, with emergence into a conscious state preceding recovery of the full repertoire of cortical processing necessary for rich conscious experiences.

These inconsistencies regarding the state of cortical activity and dynamics restoration upon recovery from anaesthesia may be due to the fact that, until recently, fMRI studies of anaesthesia have almost exclusively investigated its impact on brain network connectivity during the resting state (Mortazavi et al., 2018). Collecting data from participants during the resting state under various degrees of sedation is easy, because for several reasons (e.g. impaired awareness, instruction comprehension or motor ability) individuals in these states

may not be able to partake in traditional fMRI cognitive tasks (Fox & Greicius. 2010). However, it has been recognised that, while the resting state paradigm may shed light on the minimal characteristics needed for conscious cognitive processes, the lack of external stimulation presented to participants precludes full engagement of brain networks in their cognitive roles (Heine et al., 2012).

In addition to natural engagement of attention, listening to plot-driven narratives requires minimal behavioural input from participants, and is therefore highly suitable for testing complex information processing independently of behavioural output or eye opening (Naci et al., 2017; 2018), both of which are abolished under deep anaesthesia. Importantly, plot-driven movies or audio stories by masterful directors, which have been designed specifically to capture and hold attention similarly across people (Hasson et al., 2008), are particularly useful in understanding cognitive processing in cases of impaired consciousness, such as in patients with disorders of consciousness (Naci et al, 2014; Naci et al, 2016; Haugg et al., 2018; Naci et al., 2018), or those under general anaesthesia (Naci et al., 2018; Graham et al., 2018).

Examining brain dynamics while participants are presented with a complex naturalistic stimulus allows characterisation of consciousness in terms of higher-order cognitive processes, such as emotion, theory of mind, attention and executive function (Naci et al., 2016). Therefore, examining brain dynamics in healthy participants who are transitioning out of and back into consciousness, when they are presented with such stimuli, provides crucial information about the cognitive processes underlying the neural changes observed during anaesthesia-induced unconsciousness and recovery, which cannot be offered by resting state data. For example, Naci et al. (2018) reported a striking difference in the functional connectivity changes associated with loss of consciousness depending on whether participants were in the resting state or exposed to an engaging audio story, inside the MRI scanner. While during the resting state in anaesthesia-induced loss of consciousness, participants showed a *decrease in within-network* connectivity (relative to wakefulness), during the audio story condition, loss of consciousness was instead associated with *increased between-network* connectivity. However, only the transition between awake state and deep anaesthesia was investigated in the Naci et al. (2018) study. An investigation of recovery of consciousness using such a naturalistic paradigm may shed light on how and in what order

cognitive processes, or the brain networks associated with them, are reconstituted following anaesthesia.

The current study will build on Naci et al. (2018)'s findings to investigate what functional connectivity changes are associated with loss and recovery of consciousness using a naturalistic audio story paradigm, and relate the two to gain insights on features of large-scale networks' functional connectivity that support conscious awareness. As no previous work apart from Naci et al. (2018) has used naturalistic paradigms to explore these questions, I will take an exploratory approach, and examine large-scale changes across the brain, in seven networks, rather than focusing on areas previously found to be important by resting state studies.

Based on results previously reported in Naci et al. (2018), I hypothesise that the impact of deep anaesthesia and recovery of consciousness on brain functional connectivity will differ depending on whether participants are in the resting state or exposed to the audio story. In the resting state, I expect to find a reduction in connectivity upon loss of consciousness, especially *within* higher-order networks, which will return to baseline levels once participants have recovered consciousness. By contrast, in the audio story condition, I expect to see the opposite pattern, i.e. an increase in connectivity during deep anaesthesia, particularly in *between-network* connectivity, that will return to baseline levels during recovery.

Method

Participants

Ethical approval was obtained from the Health Sciences Research Ethics Board and Psychology Research Ethics board of Western University, Canada. All experiments were performed in accordance with the relevant guidelines and regulations set out by the research ethics board. All participants were right-handed, native English speakers with no history of neurological disorders. Nineteen healthy adults (18-40 years of age; 6 female) participated. They signed informed consent before participating and were remunerated for their time. Three participants were excluded from analyses due to headphone malfunction or physiological impediments to reaching deep anaesthesia.

Materials

Participants were presented with an audio clip from the movie *Taken* (Morel, 2008). The clip (*Taken*) is a five-minute long excerpt from an engaging, suspenseful part of the movie depicting a phone call between a father and teenage daughter. Over the course of the phone call, the daughter witnesses kidnappers breaking into her apartment and abducting her friend. She attempts to hide from them before being discovered and abducted. The father then speaks to the kidnappers, threatening to find and kill them, if they do not return his daughter. The dialogue, background music and sound effects build suspense throughout the clip that climaxes with the daughter's abduction. The audio story was presented to participants via noise cancellation headphones (Sensimetrics, S14; www.sense.com), at a volume level that was adjusted to be comfortable for each participant and kept constant for the entire scan.

The level of participants' responsiveness before, during and after propofol administration was measured by the Ramsay Sedation Scale (RSS), a clinical scale from one to six in which a person's response to a voice or physical stimulation is assessed subjectively (Ramsay et al., 1974). Participants in the current study were considered to be deeply anaesthetised when they were assigned an RSS score of five, i.e. when they could not engage in conversation, stopped responding to verbal commands and could only be roused by physical stimulation. Participants were deemed to have recovered consciousness when they

received an RSS score of two, indicating that they were oriented, calm and capable of engaging in conversation, as assessed by clinical anaesthesiologists.

Level of participants' behavioural responsivity was also measured using an auditory target detection task. Participants were presented with a short auditory stimulus ("beep") via headphones and were instructed to press a button on a response box, which was taped to the participant's hand to avoid it being dropped during anaesthesia, when they heard this beep. Reaction time (i.e. the time of button press minus the time of the beep presentation) was recorded as an objective measure of behavioural responsiveness.

Additionally, prior to scanning for each of the three sessions, volunteers were asked to perform a basic verbal recall memory test, which further assessed each individual's wakefulness/ anaesthesia level independently of the anaesthesia team. This was an adapted form of the brief recall test from the Mini Mental State Exam (Folstein, Folstein, & McHugh, 1975). Participants were asked to remember a list of three unrelated objects named by a researcher (e.g. Ball-Flag-Tree, Flower-Egg-Rope), in order to repeat them a short while afterwards. After 10 minutes, they were asked to recall the words. The use of three different lists for the awake, deep, and recovery sessions prevented familiarity effects, and their order of presentation was counterbalanced across participants to offset any order effects.

Procedure

Design. The study used a repeated-measures design. Participants laid down in the MRI scanner and completed the measures listed below at three time points: before propofol administration (awake state), during propofol administration (deep anaesthesia state) and after propofol administration (recovery state).

Measures completed:

- 1) *Clinical rating of responsiveness.* Participants' level of responsiveness were rated by three independent assessors using the RSS.
- 2) *Objective measure of behavioural responsiveness.* Participants completed the auditory target detection task described above.
- 3) *Memory test.* Participants completed the verbal recall task described above.

- 4) *Functional Resting state scan.* For the eight-minute resting state scan, protocol followed that of well-established research initiatives across the world (e.g. Biswal et al., 2010; Alivisatos et al., 2012; Devor et al., 2013). Participants were asked to lay still, relax and close their eyes but not fall asleep.
- 5) *Functional Taken scan.* Participants were presented with *Taken* story (five minutes) and asked to listen to the clip with their eyes closed.

Additionally, a structural MRI scan (duration five minutes) was obtained at the start of the data collection period. The total scanning time (including the structural and two functional scans in the awake session, and two functional scans in each of the deep and recovery sessions) was 44 minutes.

Sedation Procedure.

Awake. Before entering the scanner, a 20G i.v. cannula was inserted into a vein on the dorsum of the non-dominant hand of the participants. The propofol infusion system was connected to the cannula before the first scanning session began. In this first session no propofol was administered to the participants. Also prior to scanning in the awake state, three independent assessors (two anaesthesiologists, one anaesthesia nurse) evaluated participants' level of sedation by communicating with them in person in the scanner room. Participants were fully awake, alert and communicated appropriately. The use of the RSS was not required in this session, as it is intended for use in critical care session or for patients requiring sedation. All participants in the awake state successfully recalled 3/3 items in the verbal recall task.

Deep anaesthesia. Intravenous propofol was administered with a Baxter AS 50 (Singapore). An effect-site/ plasma steering algorithm was combined with the computer-controlled infusion pump to incrementally control the sedative effect of the propofol. The infusion pump was manually adjusted to produce the desired level of sedation, according to targeted concentrations of propofol as predicted by the TIVA Trainer (the European Society for Intravenous Anaesthesia; eurovisa.eu). The pharmacokinetic model provided target-controlled infusion by adjusting infusion rates of propofol over time to achieve and maintain the target blood concentrations as specified by the Marsh 3 (Marsh, White, Morton, & Kenny, 1991) compartment algorithm for each participant, as incorporated in the TIVA

Trainer software. At the beginning of the deep anaesthesia scanning session, propofol infusion began with a target effect-site concentration of 0.6µg/ml and oxygen was titrated to maintain SpO₂ above 96%. Responsiveness was assessed using the RSS at this point, and if the level was less than five the concentration was increased incrementally by 0.3µg/ml. Participants were considered to have reached RSS level five when three independent assessors (two anaesthesiologists, one anaesthesia nurse) deemed that they had stopped responding to verbal commands, could not engage in conversation and were rousable by physical stimulation only. Repeated assessments of responsiveness were carried out between increments until an RSS score of five was achieved, at which point the propofol concentration was kept stable for the total scanning session. During deep anaesthesia, the mean estimated effect-site Propofol concentration was 2.48 (1.82-3.14)µg/ml and the mean estimated plasma Propofol concentration was 2.68 (1.92-3.44)µg/ml. Mean total mass of propofol administered was 486.58 (373.30–599.86) mg. The variability of these concentrations and doses is typical for studies of the pharmacokinetics and pharmacodynamics of propofol. Participants were monitored inside the scanner room throughout the deep anaesthesia session by an anaesthesia nurse and two anaesthesiologists. Researchers also monitored participants' facial expressions and movements from outside the scanner room using an infrared camera. Scanning commenced only once the agreement among the three anaesthesia assessors on the RSS score of five was consistent with the lack of response in both verbal and computerized behavioural tests. No movement, variation in responsiveness or any other state change was observed during the deep anaesthesia scan for any participant included in analysis.

Recovery. After the deep anaesthesia scanning session was complete, propofol administration was terminated. Repeated assessments of responsiveness were carried out until an RSS score of two (i.e. co-operative/ oriented/ tranquil) was obtained, at which point the participant was deemed to have recovered consciousness and the recovery state scanning session took place. All participants in the recovery state successfully recalled 3/3 items in the verbal recall task.

fMRI Data Acquisition. A 3 Tesla Siemens Prisma system, with a 32-channel head coil, was used to acquire structural and functional images. An anatomical volume was obtained using a T1-weighted 3D MPRAGE sequence (voxel size: 1 x 1 x 1 mm, TA=5 minutes 38 seconds, echo time [TE]=4.25ms, matrix size = 240 x 256 x 192, flip angle [FA]

= 9 degrees). Functional echo-planar images (EPI) during Resting State (256 scans) and Taken (155 scans) conditions were acquired using a T2*-weighted EPI sequence (33 slices, voxel size = 3 x 3 x 3 mm, inter-slice gap of 25%, repetition time [TR] = 2000ms, TE = 30ms, matrix size = 64x64, FA=75 degrees).

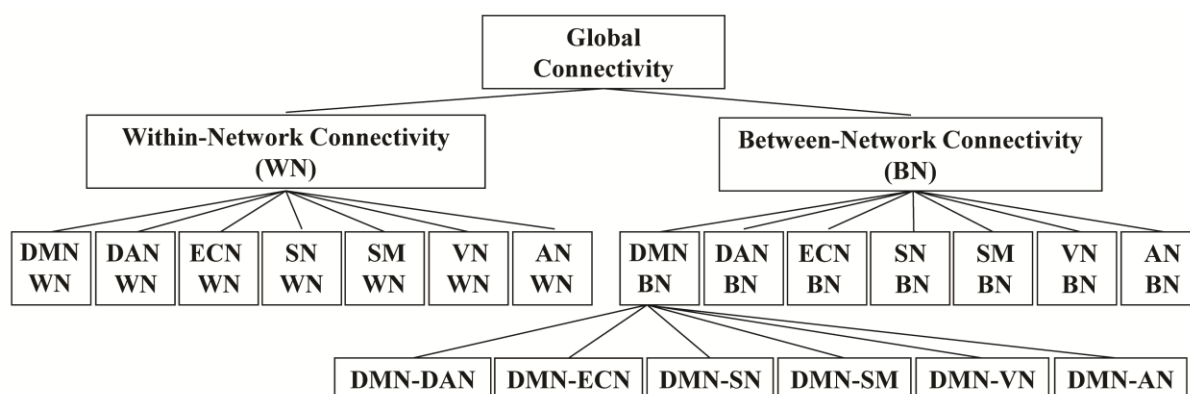
Analysis

SPM Preprocessing. Prior to analyses, the first five scans of each scanning run were discarded in order to achieve T1 equilibrium and allow participants to adjust to the scanner noise. Data were analysed using SPM8 (Wellcome Institute of Cognitive Neurology, <http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) and the Automatic Analysis pipeline software (AA; Cusack et al., 2014), implemented in MATLAB version R2017b. The following preprocessing steps were applied: slice timing correction, motion correction, registration to structural images, normalisation to a template brain and smoothing (using a Gaussian smoothing kernel of 10mm full width at half maximum; Peigneux et al., 2006). Spatial normalisation was performed using SPM8's segment-and-normalise procedure, whereby the T1 structural was segmented into grey and white matter and normalised to a segmented MNI-152 template. These normalisation parameters were then applied to all EPIs. The time series in each voxel was high pass filtered with a cutoff of 1/128 Hz to remove low frequency noise, and scaled to a grand mean of 100 across voxels and scans in each session.

Functional Connectivity. To avoid creating artificial anti-correlations, as has been reported in previous studies (Anderson et al., 2011; Murphy, Birn, Handwerker, Jones, & Bandettini, 2009), global signal regression was not performed on the current data. Functional connectivity was computed using Pearson correlation, which quantifies the similarity of fMRI blood-oxygen-level-dependent (BOLD) signals over time. The following long-range brain networks were investigated: the default mode network (DMN), the dorsal attention network (DAN), the executive control network (ECN), the salience network (SN), the sensorimotor network (SM), the visual network (VN) and the auditory network (AN). These networks have been well established in previous studies using seed-based analyses of resting state data (Raichle, 2011). See Table 1 for the regions of interest included in each network. *Within-network connectivity* (i.e. the Pearson correlation coefficient [r] between regions of interest within a particular network), *between-network connectivity* (i.e. the r between a particular network and all other networks) and *global connectivity* (i.e. averaged within- and

between-network connectivity values) were calculated for each condition, as illustrated in Figure 1.

Figure 1.
Overview of Functional Connectivity Metrics.



Note. WN = Within-network, BN = Between-network. DMN = Default Mode Network, DAN = Dorsal Attention Network, ECN = Executive Control Network, SN = Saliience Network, SM = Sensorimotor Network, VN = Visual Network, AN = Auditory Network.

To represent connectivity as participants transitioned out of and back into consciousness, I additionally calculated difference scores. For each participant, I subtracted connectivity values in the awake state from those in the deep anaesthesia state to represent the change when participants lost consciousness (awake to deep). Similarly, I calculated scores representing the change after recovery of consciousness by subtracting connectivity values in the deep anaesthesia state from those in the recovery state (deep to recovery).

Repeated-measures ANOVAs and paired-samples permutation t-tests were used for comparisons of interest, with false discovery rate (FDR) correction for multiple comparisons. Permutation tests assess the null hypothesis by comparing the distribution of observed values to that of a null set created by arbitrarily assigning the data-points to different labels (Nichols & Holmes, 2002). Each time a permutation test is run each data-point is assigned a new label, therefore the precise p-value generated by the test varies each time it is run. In this way weak

effects may present as ‘unstable’ results which vary between falling below and above the threshold for statistical significance. In the results section below I report where this is the case. As Pearson correlation values are non-normally distributed (Fisher, 1915), all correlations were transformed to z-values using Fisher’s r-to-z transformation before statistical analyses. However, all graphical representations show the original, non-z-transformed correlation values.

Table 1.

MNI Co-ordinates for Selected Regions of Interest for Seven Functional Networks.

Network	Label in Fig. 2	ROI	MNI coordinates		
Default Mode Network	1	Posterior cingulate/precuneus	0	-52	27
	2	Medial prefrontal	-1	54	27
	3	Left lateral parietal	-46	-66	30
	4	Right lateral parietal	49	-63	33
	5	Left inferior temporal	-61	-24	-9
	6	Right inferior temporal	58	-24	-9
Dorsal Attention Network	7	Left frontal eye field	-29	-9	54
	8	Right frontal eye field	29	-9	54
	9	Left posterior IPS	-26	-66	48
	10	Right posterior IPS	26	-66	48
	11	Left anterior IPS	-44	-39	45
	12	Right anterior IPS	41	-39	45
	13	Left MT	-50	-66	-6
	14	Right MT	53	-63	-6
Executive Control Network	15	Dorsal medial PFC	0	24	46
	16	Left anterior PFC	-44	45	0
	17	Right anterior PFC	44	45	0
	18	Left superior parietal	-50	-51	45
	19	Right superior parietal	50	-51	45
Salience Network	20	Dorsal anterior cingulate	0	21	36
	21	Left anterior PFC	-35	45	30
	22	Right anterior PFC	32	45	30
	23	Left insula	-41	3	6
	24	Right insula	41	3	6
	25	Left lateral parietal	-62	-45	30
	26	Right lateral parietal	62	-45	30
Sensorimotor Network	27	Left motor cortex	-39	-26	51
	28	Right motor cortex	38	-26	48

	29	Supplementary motor area	0	-21	48
Visual Network	30	Left V1	-7	-83	2
	31	Right V1	7	-83	2
Auditory Network	32	Left A1	-62	-30	12
	33	Right A1	59	-27	15

Note. From Raichle (2011). ROI: Region of interest, IPS: Intraparietal sulcus, MT: Middle temporal area, PFC: Prefrontal cortex, V1: Primary visual cortex, A1: Primary auditory cortex.

Inter-Subject Correlation. To examine information processing during the Taken audio story, I calculated whole-brain inter-subject correlation (ISC) of neural activity across participants. Group-level correlational analyses measured, for each voxel, the correlation of each participant's time-course with the mean time-course of all other subjects. This was calculated for awake, deep anaesthesia and recovery states. I formally compared ISC in the awake and recovery states using a repeated measures t-test. Significant clusters/voxels survived the $p < 0.001$ threshold, corrected for multiple comparisons with the family wise error (FWE).

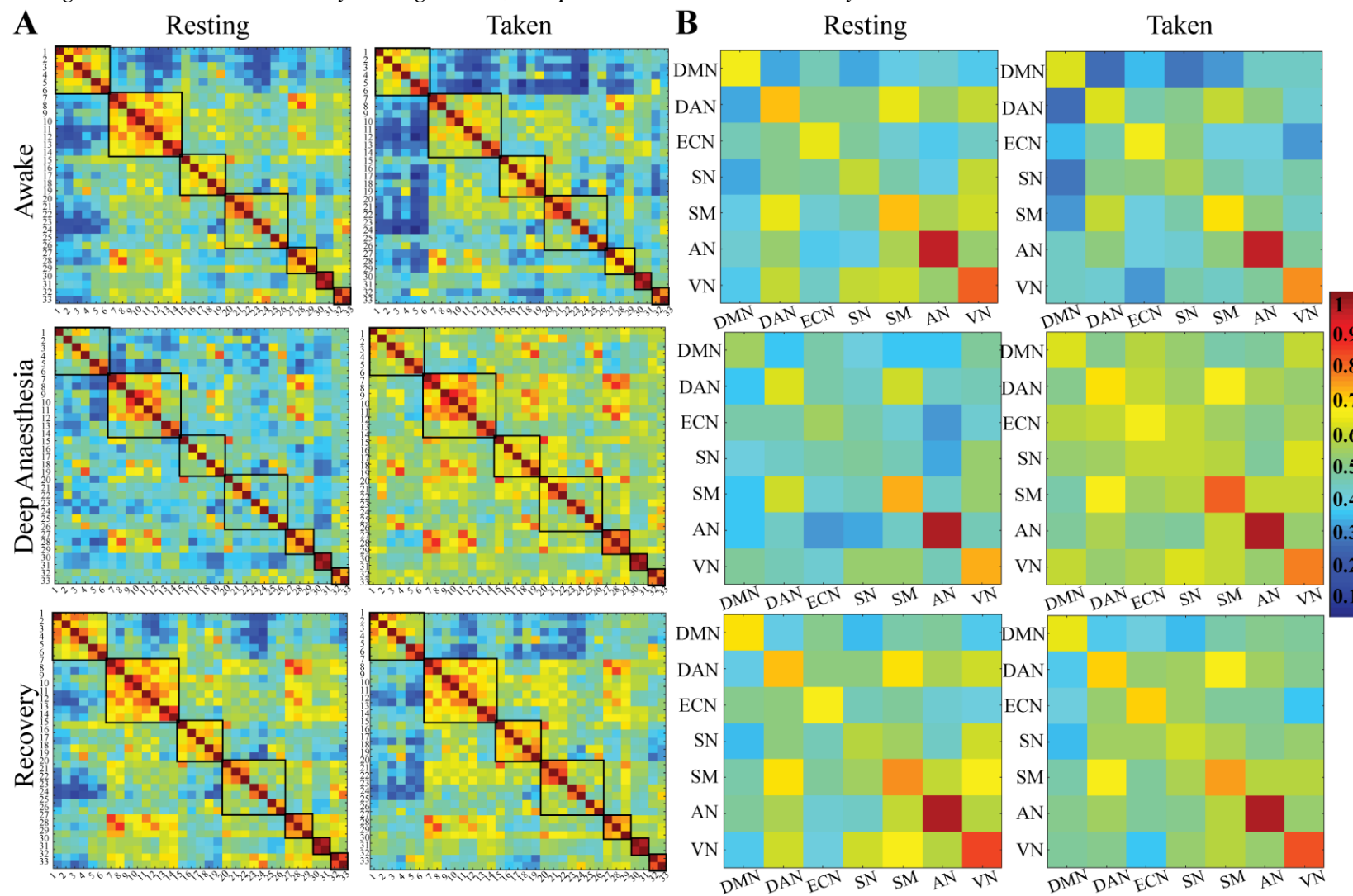
Results

I investigated the functional connectivity values that characterised the awake, deep anaesthesia and recovery states, during both resting state and Taken conditions at a global brain level, a sensory vs higher-order network level, and at the level of individual networks. Below, I discuss each of these in turn.

First, I investigated how patterns of global connectivity varied between the resting state and the Taken conditions, as participants transitioned out of and back into consciousness (Figure 2).

Figure 2.

Average Functional Connectivity during Awake, Deep Anaesthesia and Recovery States.



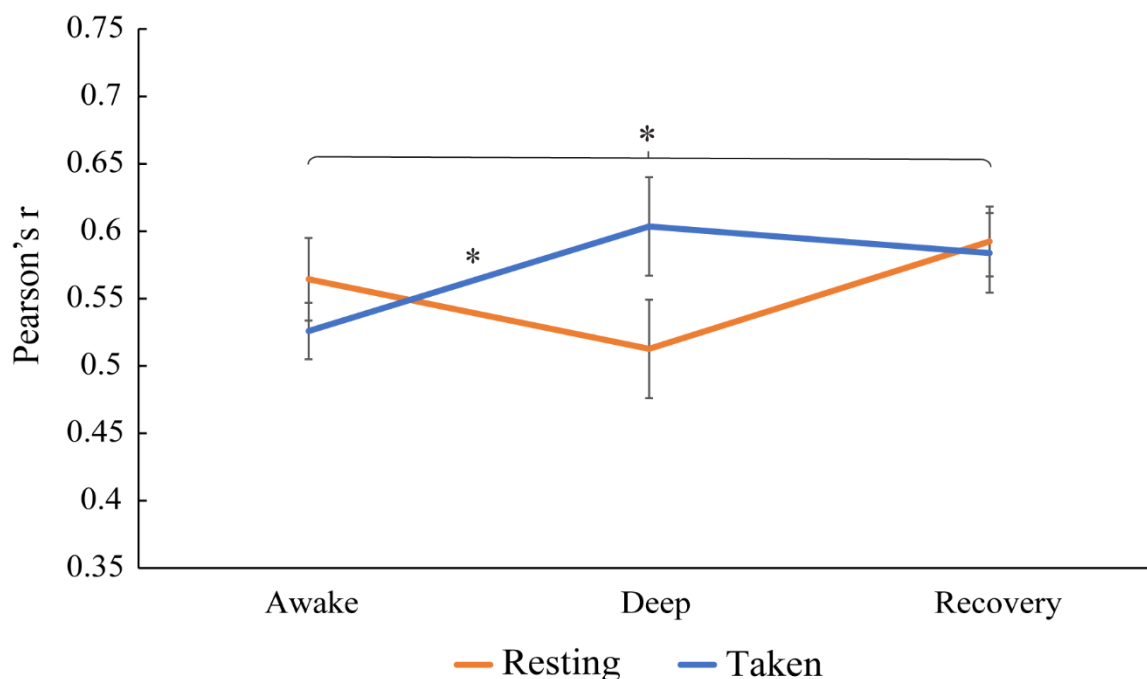
CONSCIOUS PROCESSING DURING AND AFTER ANAESTHESIA

Note. Average functional connectivity during (from top to bottom) awake, deep anaesthesia and recovery states in both resting state and Task conditions. Panel A: group-averaged Pearson correlations between regions of interest (ROI), labelled numerically as identified in Table 1. Each square represents the Pearson correlation between one ROI and itself (middle diagonal) or with each other ROI. Panel B: network-level averages. DMN: Default Mode Network, DAN: Dorsal Attention Network, ECN: Executive Control Network, SN: Salience Network, SM: Sensorimotor Network, VN: Visual Network, AN: Auditory Network.

Using a two-way repeated measures ANOVA with factors *state* (awake, deep anaesthesia, recovery) and *condition* (resting state, Taken), a significant interaction effect, $F(2,30)=5.003$, $p<0.05$, indicated that the effect of *state* on global connectivity varied depending on whether participants were in the resting state or presented with the Taken audio story. Permutation tests revealed that during the Taken condition, global connectivity increased in both deep and recovery states relative to awake ($p<0.05$; FDR corrected). By contrast, during the resting state, connectivity showed no significant differences across the three states of consciousness (Figure 3).

Figure 3.

Global Connectivity across States of Consciousness.



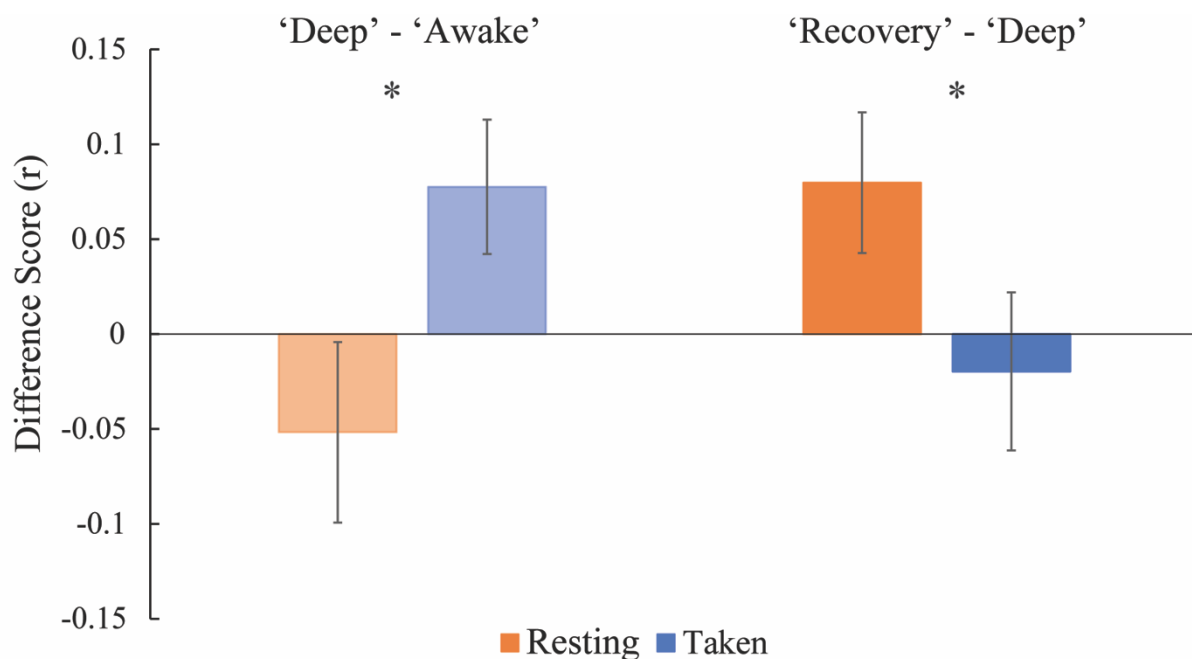
Note. Patterns of global connectivity across awake, deep and recovery states, for both resting state and Taken conditions. * indicates $p<0.05$.

To directly investigate whether the global patterns of connectivity differed significantly across conditions, I calculated the difference values representing each transition

of consciousness (i.e., ‘deep’ – ‘awake’; ‘recovery’ – ‘deep’). These difference scores were then directly compared across the resting state and Taken conditions using a two-way repeated measures ANOVA with factors *transition* (‘deep’ – ‘awake’, ‘recovery’ – ‘deep’) and *condition* (resting state, Taken) (Figure 4). A weak interaction effect, $F(1,15)=4.486$, $p=0.051$, indicated that the two conditions showed different trends as participants transitioned out of and back into consciousness. In the transition from awake to deep anaesthesia, the Taken condition showed a significantly bigger increase in connectivity than resting state ($p<0.05$; FDR corrected). The opposite pattern was observed in the transition back to consciousness, i.e. from deep anaesthesia to recovery, connectivity during the resting state showed a significantly bigger ($p<0.05$; FDR corrected) increase than that in the Taken condition.

Figure 4.

Functional Connectivity Difference Scores in Transitions of Consciousness.



Note. ‘Deep’ – ‘Awake’ represents the change in global connectivity when participants lost consciousness. ‘Recovery’ – ‘Deep’ represents the change in global connectivity when consciousness was restored.

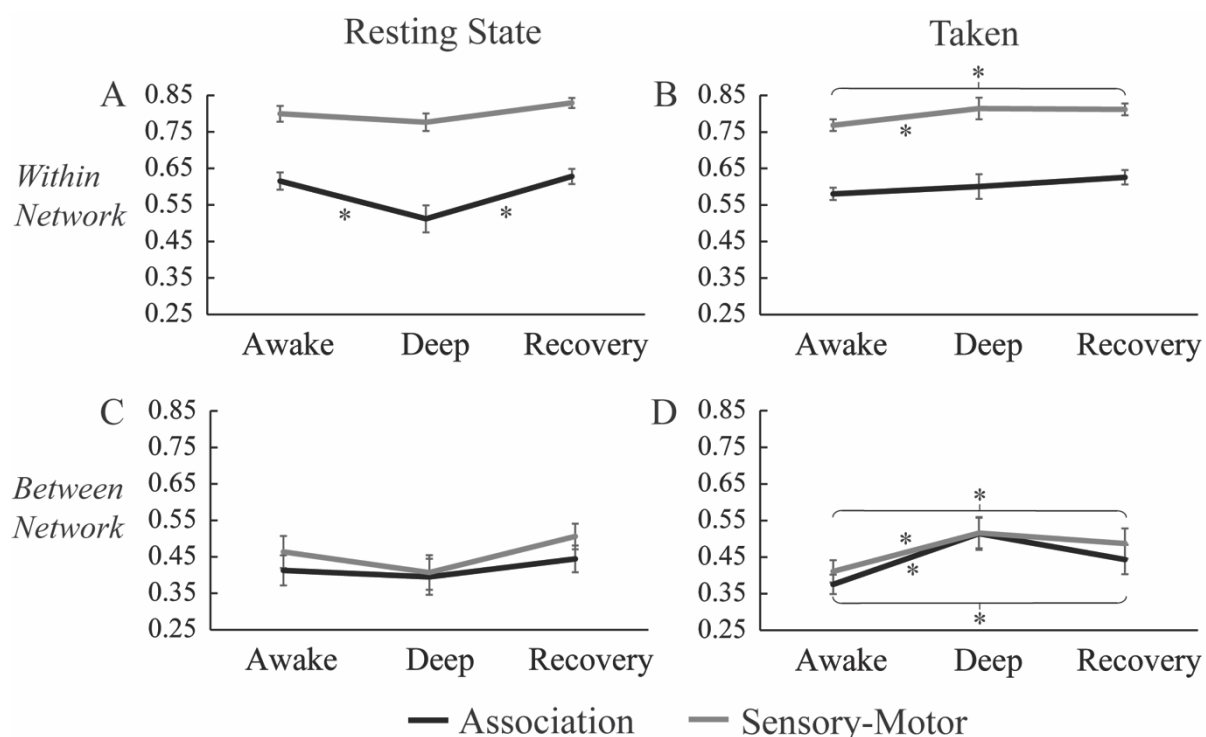
My results from the awake to deep anaesthesia transition were consistent with those from Naci et al. (2018). Additionally, for the first time, I demonstrated that recovery after deep anaesthesia is associated with opposite effects, depending whether the participant is in the resting state or presented with complex naturalistic stimulation. Specifically, during loss of consciousness participants showed increased connectivity in the Taken condition and no change in the resting state, while recovery of consciousness was accompanied by increased connectivity in the resting state and no change in the Taken condition. This provided strong support for my first hypothesis, namely, that the impact of deep anaesthesia and recovery of consciousness on functional connectivity differs depending on whether participants are in the resting state or exposed to complex naturalistic stimulation.

Secondly, I investigated the relative contribution of higher-order (DMN, DAN, ECN, SN) versus sensory (SM, VN, AN) networks in transitions of consciousness, during each condition. I carried out two-way repeated measures ANOVAs with factors *state* (awake, deep anaesthesia, recovery) and *network type* (association, sensory-motor), separately for the within- (WN) and between-network (BN) connectivity values for each condition (Figure 5). This decision was based on previous studies showing a differential effect of condition during anaesthesia manipulations for within- and between-network connectivity (Boveroux et al., 2010; Naci et al., 2018; Liu et al., 2019). A significant main effect of *network type* was reported for resting state WN, $F(1,15)=399.315$, $p<0.0001$. Significant *state*network type* interaction effects were reported for the following ANOVAs: resting state BN, $F(2,30)=15.199$, $p<0.001$; Taken WN, $F(2,30)=4.401$, $p<0.05$, Taken BN, $F(2,30)=7.229$, $p<0.01$.

During the resting state, the transition from awake to deep anaesthesia was characterised by a decrease in *within-network* connectivity in association, but not sensory networks (Figure 5A). However, the statistical significance of this decrease was somewhat unstable when FDR correction for multiple comparisons was applied (i.e. $p<0.05$ in 6/10 cases), indicating a weak result. The transition from deep anaesthesia to recovery similarly showed a significant ($p<0.05$; FDR corrected) increase of *within-network* connectivity in the association, but not sensory networks (Figure 5A). There were no significant differences in *between-network* connectivity in either transition (Figure 5C).

Figure 5.

Within- and Between-Network Connectivity in Sensory and Association Networks.



Note. Changes in within- and between-network connectivity for the sensory and higher-order networks in the awake, deep and recovery states, in each condition.

During the Taken condition, on the other hand, the pattern seen in global connectivity changes – i.e., increased connectivity from awake to deep anaesthesia, and no difference between deep anaesthesia and recovery – was driven by more widespread effects. In the transition from awake to deep anaesthesia, connectivity *within* sensory-motor, but not association networks, increased ($p < 0.05$; FDR corrected), while there was no significant difference in *within-network* connectivity between deep anaesthesia and recovery states (Figure 5B). In *between-network* connectivity, both sensory-motor and higher-level networks increased from awake to deep anaesthesia states ($p < 0.05$; FDR corrected) with no significant difference in either measure *between* deep anaesthesia and recovery states (Figure 5D).

In summary, during loss of consciousness, in the resting state, functional connectivity changes were driven by decreased connectivity *within* higher-order association networks. By contrast, in the Taken condition, I observed much more widespread changes in the opposite direction. Functional connectivity changes were driven by increased *within- and between-*

network connectivity in sensory-motor networks, as well as increased *between-network* connectivity in higher-order networks. Results from loss of consciousness were consistent with my hypotheses. During recovery of consciousness, the resting state showed the expected increased connectivity *within* higher-order association networks. By contrast, in the Taken condition there was no significant change in connectivity during recovery relative to deep anaesthesia, contrary to my prediction.

Thirdly, to investigate whether specific individual networks contributed predominantly to the changes seen in sensory and higher-order groups, I examined changes in connectivity measures in each of the seven networks (Figure 6). To this end, I carried out separate two-way repeated measures ANOVAs with factors *state* (awake, deep anaesthesia; or deep anaesthesia, recovery; or awake, recovery) and *network* (DMN, DAN, ECN, SN, SM, VN, AN), for the within- and between-network connectivity values, for each condition. I then used permutation tests to investigate what differences drove these effects.

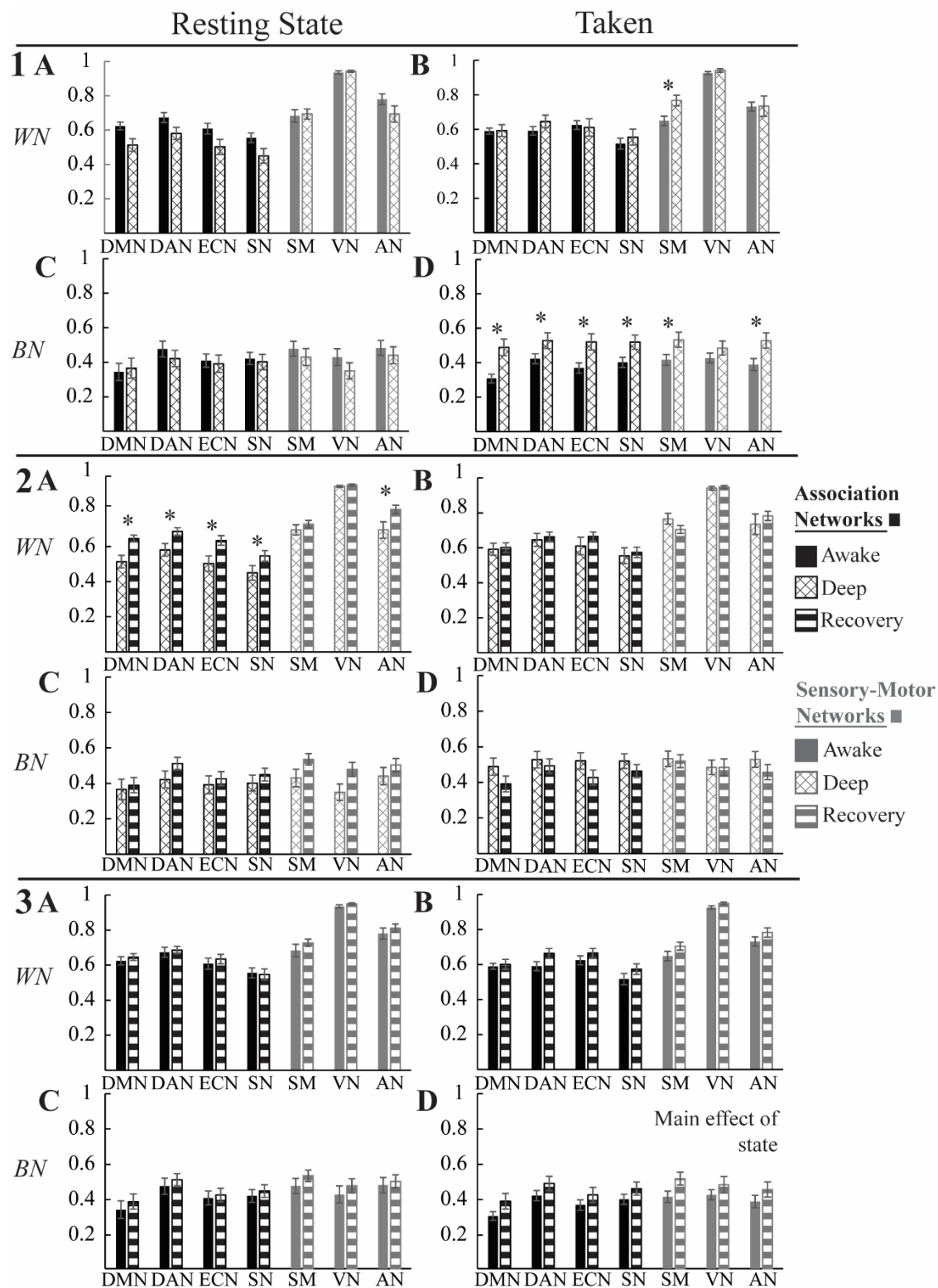
Changes from awake to deep anaesthesia. Significant *state*network* interaction effects were reported for all four ANOVAs (Figure 6.1) (resting state BN: $F(6,90)=6.365$, $p<0.001$; resting state WN: $F(6,90)=3.134$, $p<0.01$; Taken BN: $F(6,90)=6.191$, $p<0.001$; Taken WN: $F(6,90)=3.839$, $p<0.01$). Loss of consciousness during the resting state was not associated with significant differences in the *within-* (Figure 6.1A), or *between-network* connectivity of individual networks (Figure 6.1C) connectivity. By contrast, during the Taken condition, loss of consciousness was associated with increased *within-network* connectivity in the SM network ($p<0.05$; FDR corrected) (Figure 6.1B), and increased *between-network* connectivity in 6/7 networks ($p<0.05$; FDR corrected), with no change in VN (Figure 6.1D).

Changes from deep anaesthesia to recovery. Significant *state*network* interaction effects were reported for all four ANOVAs (Figure 6.2) (resting state BN: $F(6,90)=8.194$, $p<0.001$; resting state WN: $F(6,90)=2.248$, $p<0.05$; Taken BN: $F(6,90)=6.954$, $p<0.001$; Taken WN: $F(6,90)=2.541$, $p<0.05$). Return of consciousness during the resting state was associated with increased *within-network* connectivity in DMN ($p<0.05$; FDR corrected), and I observed similar, but weaker, effects for the DAN, ECN and AN ($p<0.05$ in 5/10, 2/10, 2/10 tests respectively) (Figure 6.2A). There were no significant differences in *between-network* connectivity (Figure 6.2C). By contrast, upon return of consciousness during the Taken

condition there were no significant differences in either *within-* (Figure 6.2B) or *between-network* (Figure 6.2D) connectivity.

Figure 6.

Within- and Between-Network Connectivity in Seven Brain Networks.



Note. WN: within-network. BN: between-network. Panel A: awake to deep anaesthesia transition. Panel B: deep anaesthesia to recovery transition. Panel C: differences between awake and recovery states.

To summarise, in loss of consciousness during the resting state, I observed no significant changes at the level of individual networks. By contrast, in the Taken condition there was an increase in *within-network* connectivity in 1/7 networks (SM), and *between-network* connectivity in 6/7 networks (DMN, DAN, ECN, SN, SM, AN; not VN). In recovery of consciousness during the resting state, I observed increased *within-network* connectivity in 4/6 networks (DMN, DAN, ECN, AN; not SN, SM, VN). By contrast, during the Taken condition, there were no significant changes in connectivity upon recovery of consciousness.

How can these differences in connectivity changes across the resting state and Taken conditions be interpreted? As has been previously suggested (Naci et al., 2018), it may be that participants in the Taken condition fail to show the expected reduction of functional connectivity (and indeed show a change in the opposite direction) due to the nature of the task, which requires engagement of higher cognitive functions. Perhaps the systems necessary to fully engage the cognitive processes involved in understanding and following an audio story have not fully recovered in the recovery state. Indeed the study by Långsjö et al. (2012) suggested that recovery of consciousness occurs in a bottom-up manner, with an initial emergence into a conscious state that precedes the recovery of the full repertoire of cortical processing necessary for rich conscious experiences.

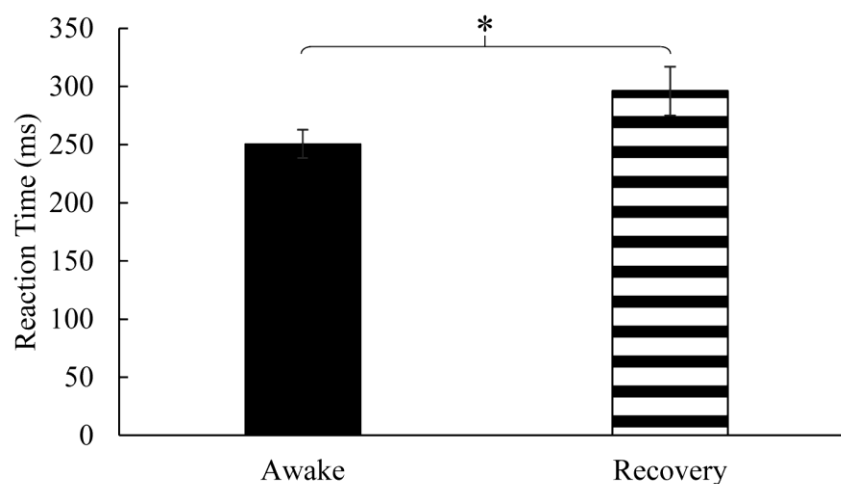
To investigate further whether certain brain networks do not restore back to baseline awake levels upon recovery of consciousness, I carried out comparisons of connectivity values across the awake and recovery states. During the Taken condition, unlike the resting state, global connectivity did not restore to the wakefulness baseline — it remained elevated in recovery relative to the awake state ($p < 0.05$; FDR corrected) (Figure 3). Similarly, there was increased connectivity *within* sensory-motor networks (Figure 5B), and increased *between-network* connectivity in both association and sensory-motor networks ($p < 0.05$; FDR corrected) (Figure 5D), while the resting state condition showed no significant differences in any awake versus recovery state comparisons (Figures 5A, 5C). Finally, I carried out separate two-way repeated measures ANOVAs with factors *state* (awake, recovery) and *network* (DMN, DAN, ECN, SN, SM, VN, AN), for the *within-* (WN) and *between-network* (BN) connectivity values, for each condition (Figure 6.3). In the Taken condition, there was an increase in *between-network* connectivity across all networks in recovery relative to the awake state (Figure 6.3D), as indicated by a main effect of *state*, $F(1,15)=9.56$, $p < 0.01$. This increase relative to the awake state was not driven by any particular networks. *Within-*

network connectivity showed no (Figure 6.3B) significant differences between awake and recovery states. Consistent with previously summarized results, and by contrast to the Taken condition, no differences between awake and recovery states were seen in the resting state condition at the level of individual networks, in either *within-* (Figure 6.3A) or *between-network* connectivity (Figure 6.3C).

To summarise, upon recovery of consciousness, in the resting state all brain networks showed full restoration to baseline awake state levels of connectivity. By contrast, in the Taken condition, between-network connectivity values remained elevated. These results suggest that processing of the Taken story immediately upon recovery is not the same as during wakefulness. Indeed, the functional connectivity values suggest that story-related cognition during recovery is more similar to deep anaesthesia, than wakefulness, which suggest that cognitive processes are still impacted by anaesthesia immediately upon recovery of consciousness. This interpretation was supported by the results of auditory detection task, which showed that the reaction time in the recovery state ($m=296\text{ms}$, $SE=21\text{ms}$) was significantly slower than in the awake state ($m=251\text{ms}$, $SE=12.18\text{ms}$), $t(15)=-2.163$, $p<0.05$ (Figure 7).

Figure 7.

Auditory Detection Task Reaction Times, in Awake and Recovery States.

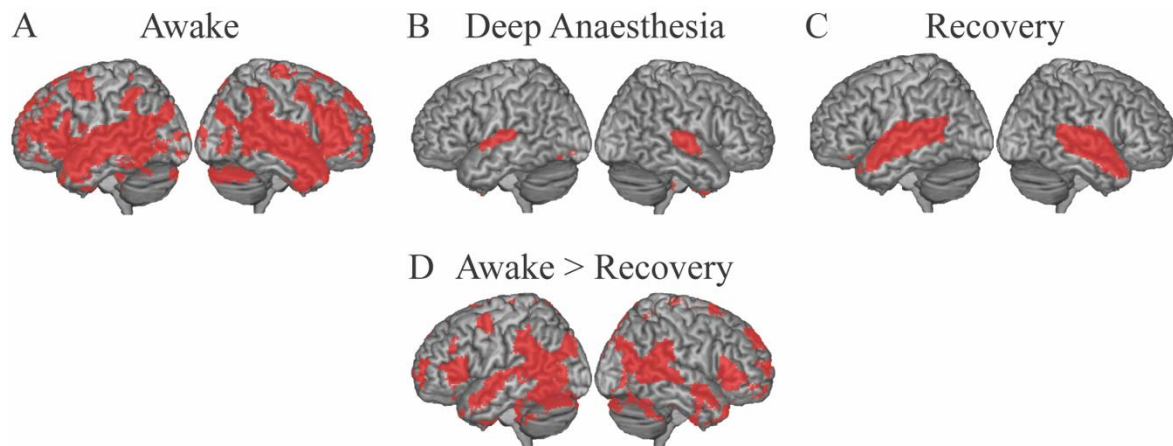


Note: Auditory detection task reaction times., in awake and recovery states.

To more directly examine any differences in information processing during the Taken condition in the awake and recovery states, I investigated the whole brain inter-subject correlation (ISC) of neural activity while participants were presented with the audio story. ISC during movie-viewing in awake, healthy individuals is well-established (Hasson et al., 2004; 2008; 2010) and is linked to the cognitive processes underlying a common understanding and experience of the story's plot (Naci et al., 2014; Naci et al., 2016; Naci, Sinai, & Owen, 2017). In the awake state, I found widespread synchronisation of neural activity ($p < 0.001$; FWE corrected) across participants, as they were presented with the 'Taken' audio story (Figure 8A). A wide range of brain regions, including temporal regions associated with auditory processing, frontal and parietal areas associated with higher order cognition and the salience network associated with cognitive and emotional appraisal, showed significantly correlated time-courses of brain activation across participants, consistent with previous studies (Naci et al., 2014; 2017). In the deep anaesthesia state, as I expected, this synchronisation was dramatically reduced and limited to primary auditory processing regions with the exception of two small clusters in the caudate nucleus and the anterior cingulate cortex (Figure 8B). Surprisingly, in the recovery state, ISC was not restored back to levels similar to the awake state, but, rather, presented a level that was intermediate between those observed in the awake and the deep anaesthesia state. In recovery, the ISC was widespread within the temporal cortex bilaterally, including primary auditory, secondary auditory processing regions and the temporal pole (Figure 8C), but did not extend to networks outside the temporal lobes, suggested limited extra-modal information processing immediately upon recovery. Indeed, the ISC in the awake state was significantly ($p < 0.005$, FWE corrected) stronger relative to recovery in a set of widespread cortical regions, including frontal and parietal regions and parts of the salience network (Figure 8D). This result adds further support to the interpretation that, at the point of recovery of consciousness following anaesthesia, the neural systems underlying the ability to understand complex and naturalistic information that evolves over time remain functionally altered relative to baseline wakefulness.

Figure 8.

Brain-wide Inter-subject Correlation of Neural Activity during the Audio Story.



Note. A - C: $p < 0.001$ (FWE corrected) in awake, deep anaesthesia and recovery states. D: In the awake state, participants showed significantly more ($p < 0.005$) inter-subject correlation than in the recovery state.

Although I did not predict this result, it is fitting with previous studies such as the one by Långsjö et al. (2012), which suggest that recovery of the full repertoire of cortical processing necessary for rich conscious experiences does not take place immediately following recovery from anaesthesia.

Discussion

In this chapter, I described an experiment that aimed to characterise the changes in brain dynamics associated with loss and recovery of consciousness induced by propofol anaesthesia. By comparing patterns of functional connectivity change seen when participants were in the resting state to those when they were presented with an engaging narrative stimulus, I hoped to gain additional insights into the impact of anaesthesia on networks involved in higher order cognition, particularly in relation to recovery of consciousness.

In transitioning both out of and back into consciousness, the direction and magnitude of global connectivity changes were significantly different between the resting state and the complex narrative condition. Examining each condition individually further clarified these differences. During loss of consciousness, in the resting state, there was no change in functional connectivity, whereas by contrast, in the Taken condition, connectivity increased significantly. During recovery of consciousness following deep anaesthesia, there was a significant increase in global connectivity of the resting state, while global changes of connectivity in the Taken condition did not reach significance. These findings are similar to those from Naci et al. (2018), who showed that resting state and Taken conditions reveal opposite effects of anaesthesia on the brain. I extend this previous study here, by demonstrating, for the first time, that these conditions show opposite effects on the brain as participants recover consciousness after deep anaesthesia. Therefore, the current study confirms the value of examining functional connectivity changes in anaesthesia using a naturalistic stimulation, and demonstrates insights beyond those offered by resting state paradigms.

During loss of consciousness in the resting state I found no significant difference in global connectivity relative to the awake states. This contrasts to previous research showing a reduction in connectivity upon anaesthesia-induced loss of consciousness in the resting state, particularly among higher order networks such as the DMN and ECN (Boveroux et al., 2010; Schrouff et al., 2011; Bonhomme et al., 2012; Ranft et al., 2016; Kelz & Mashour, 2019). The diverging results may be accounted for by methodological differences. First, an important difference to note between my study and previous work is that the networks I investigated were based on predefined regions of interest from previous literature (Raichle, 2011) rather than using a data-driven approach such as independent component analysis. A

second difference is the global analysis of connectivity conducted in the current study, which captured functional connectivity across both between and within-network connectivity from seven major brain networks. This brain-wide network approach, employed due to the novelty of the naturalistic audio story paradigm, contrasted with that of most previous studies, which examined a smaller number of networks or regions of interest in a hypothesis-driven manner. Indeed, at a more specific level of analysis, i.e., at a network-group level, in particular for the within-network connectivity of the association networks, I observed the expected connectivity reduction consistent with previous results (Boveroux et al., 2010; Huang et al., 2014; Naci et al., 2018).

The effects of consciousness loss during the resting state, when participants are lying quietly in the scanner with no external stimuli presented to them, are most prominently seen in reduced within-network connectivity. This has previously been interpreted as a breakdown in the temporal aspect of the functional brain architecture, which prevents the communication within and between areas of brain networks that is necessary for external perception (Boveroux et al., 2010), and represents a decreased or abolished neural capacity to integrate information (Schrouff et al., 2011; Kelz & Mashour, 2019). Upon recovery of consciousness, in the resting state, I found the expected increase in connectivity that has been reported in previous resting state studies (Boveroux et al., 2010; Mashour et al., 2020; Guldenmund et al., 2016). Association networks showed increased within-network connectivity in recovery relative to deep anaesthesia. At the individual network level, I observed significant connectivity increases within three association networks, the DMN, DAN, ECN, and one sensory network, the AN. Thus my results reinforce previous findings that anaesthesia-induced unconsciousness during the resting state is characterised primarily by a reduction of functional connectivity between brain areas that is restored immediately at the recovery of behavioural responsiveness (Mortazavi et al., 2019).

By contrast, loss of consciousness during complex naturalistic stimulation does not follow this pattern. At an individual network level, connectivity between six out of seven networks (DMN, DAN, ECN, SN, SM, AN) increased in deep anaesthesia relative to awake, in line with results from Naci et al. (2018). There was also an increase in connectivity within the sensory-motor networks as a group. These results suggest that loss of consciousness during complex stimulation leads to loss of functional differentiation between brain networks, rather than a reduction of connectivity within or between them. This is consistent

with previous transcranial magnetic stimulation studies, which have directly perturbed the unconscious brain, and demonstrated that responses in different areas of the brain become undifferentiated from each other (Casali et al., 2013; Massimini et al., 2012). Furthermore, these results suggest that when the unconscious brain is exposed to complex naturalistic stimulation, the feed-forward processing cascade evoked by the stimulus echoes in this undifferentiated manner throughout the brain (Naci et al., 2018). In this way, the inhibitory effect of propofol on neural connectivity that has been previously reported in resting state studies (Brown et al., 2010), may be overpowered.

The novel contribution of my study was the finding that during the Taken condition there was no significant connectivity change as consciousness recovered after deep anaesthesia. Functional connectivity in the recovery state remained significantly higher than the awake baseline. This effect was seen across sensory-motor and association networks, suggesting a widespread impact of anaesthesia on the brain that persists immediately following recovery of behavioural responsiveness. This was an unexpected result, as I had predicted a return of connectivity to baseline levels after deep anaesthesia, in line with previous resting state studies (Boveroux et al., 2012; Mashour et al., 2020). The finding that brain connectivity was still significantly altered suggested that the processing of complex information may remain impaired at the initial point of consciousness recovery from a clinical perspective. This interpretation was further supported by my whole-brain synchronisation analyses, which provide a proxy for neural information processing (Hasson et al., 2004; 2008, 2010; Naci et al., 2014; 2017). The widespread synchronisation present in participants as they listened to the audio story while awake was abolished under deep anaesthesia, except in primary auditory processing regions. Crucially, wakeful synchronisation patterns were not restored during the recovery state, and instead remained limited to temporal auditory processing regions. These neuroimaging findings were consistent with the results of the behavioural vigilance task, which showed that reaction times in recovery were slower than in the awake state, further suggesting a continued impact of anaesthesia on the brain.

This novel finding highlights the limitations of clinical bedside scales that measure behavioural responsiveness as a proxy for awareness. In measuring simple behavioural responses they cannot shed light on the extent of information processing in the brain. This dissociation between the results of clinical behavioural scales of awareness and neuroimaging

evidence of information processing in the brain has been previously well documented in patients with disorders of consciousness (Owen et al., 2006; Bardin et al., 2011; Cruse et al., 2011; Naci & Owen, 2013; Fernández -Espejo & Owen, 2013; Kondziella et al., 2016; Gibson et al., 2016; Naci et al., 2017; Naci et al., 2018), where, similarly to individuals under the effect of anaesthesia, behaviour cannot comprehensively inform about the level of information processing in the brain (Sanders et al., 2012).

Although unexpected based on most resting state studies using propofol, my result is consistent with a limited number of studies that have used analysis methods that aim to capture information processing in the brain. Monti et al. (2013) used graph theoretical metrics to examine information processing during the resting state. They reported persistent elevation, relative to the awake state, of the clustering coefficient, i.e. a measure of how closely regions of interest cluster together, in participants who had recovered following propofol-induced anaesthesia. This finding suggested that the recovery state was characterised by more localised information processing, and less integration of information across various brain regions (Monti et al., 2013). Liu and colleagues (2019) measured entropy of regional BOLD signals during graded propofol sedation, aiming to examine changes in the available repertoire of intrinsic brain states during anaesthesia, in different regions. They reported a reduction in entropy of both sensory and high-order regions during deep anaesthesia, relative to the wakeful state, which, upon recovery, showed a return to baseline levels only in sensory regions. This suggests that, after full behavioural recovery, higher-order brain regions still showed the impact of anaesthesia and were characterised by reduced complexity.

My result is also in line with other anaesthesia studies that have used different anaesthetic agents in animals, or alternative neuroimaging technologies in humans. For example, in a recent animal study, Pal et al (2019) reported that rats, who recovered responsivity following sevoflurane-induced unconsciousness, did not show restoration of EEG-based cortical connectivity measures. Levels of consciousness were thus dissociated from large-scale cortical connectivity, prompting the authors to call for a re-evaluation of the role of such measures in distinguishing conscious and unconscious states. Långsjö et al. (2012) found that participants, after induction of deep anaesthesia using the anaesthetic agent dexmedetomidine, could recover conscious responsivity without seeing a corresponding return of neocortical reactivity, as measured by PET imaging. These authors suggested that

recovery of consciousness occurs in a bottom-up manner, with emergence into a more basic conscious state preceding recovery of higher-order elements of cortical processing that are necessary for rich conscious experiences. The lack of restoration of functional connectivity to baseline levels shown in my study is consistent with this suggested hierarchy of recovery.

Two limitations should be considered. First, propofol was used in the current study due to its wide prominence in clinical interventions. Therefore, although, as noted above, there is some consistency with previous studies using other anaesthetic agents, such as dexmedetomidine, further research is needed investigate the current paradigm across different anaesthetic agents in order to determine the generalisability of this study's findings across the various anaesthetic agents. Second, functional connectivity and ISC in recovery was assessed in this study immediately after the recovery of behavioural responsiveness. Therefore, I cannot draw any conclusions regarding the length of time post cessation of propofol administration that would be required for the neurocognitive processes involved in information processing to return to baseline, pre-anaesthesia levels.

Chapter 3: Developing a neural marker of narrative understanding and investigating covert awareness under deep anaesthesia

In Chapter 2 I used a naturalistic paradigm to investigate which functional connectivity changes characterise recovery from propofol-induced deep anaesthesia. The current chapter uses the same paradigm to address another gap in anaesthesia research – the lack of a sensitive neuroimaging-based marker of conscious awareness during general anaesthesia. Anaesthesia has been used for over 150 years to reversibly abolish consciousness in clinical medicine, but its effects on individuals can vary substantially. At moderate doses, suppression of behavioural responsiveness varies from individual to individual (Chennu et al., 2016; Bola et al., 2019). At deep anaesthesia doses, in rare cases, patients who have been given a dosage appropriate for clinical general anaesthesia, and appear non-responsive, nevertheless retain accidental awareness under general anaesthesia (AAGA). Under current estimates, 20,000 to 40,000 patients experience AAGA yearly in the USA alone (Avidan et al., 2008). AAGA has been associated with poor psychological outcomes, although evidence is somewhat mixed (e.g. see Whitlock et al., 2015). A review of diverse studies reported that between 0-71% of patients who experienced AAGA subsequently developed post-traumatic stress disorder (PTSD; Aceto et al., 2013). Distress felt by postsurgical patients who have experienced AAGA relates to feelings such as helplessness, abandonment and an inability to communicate (Osterman, Hopper, Heran, Keane, & van der Kolk, 2001). In the 5th National Audit Project on AAGA in Ireland and the United Kingdom (NAP5), 51% of patients who reported AAGA experienced distress, while 41% suffered from long-term negative outcomes (Cook et al., 2014). A narrative review of the literature concluded that psychological consequences of AAGA included anxiety and flashbacks, in addition to PTSD, and called for greater recognition of the phenomenon within the psychological/ counselling community (Bruchas et al., 2011). Therefore, AAGA can be a major concern for both patients (Klafta & Roizen, 1996; Myles et al., 2000) and anaesthesiologists (Pandit et al., 2014).

Because its risk factors are not yet fully understood, it is difficult to estimate the precise risk of AAGA for individual patients. Additionally, the lack of sensitive depth-of-anaesthesia monitoring devices makes prevention and detection of AAGA extremely challenging. The typical estimates of the incidence of AAGA are very low. However, these figures are based on techniques which require patients to recall their experience. The lowest figures are reported by studies that require patients to spontaneously recall being aware post-

operatively. For example, NAP5, which reported an average AAGA incidence rate of 0.005%, only included self-reports submitted by patients who had specific memories of being aware (Pandit et al., 2014). The use of structured post-operative interviews, such as the Brice interview (Brice, Hetherington & Uting, 1970), results in a higher incidence rate of 0.1-0.2%, which is the most widely accepted and typically cited figure in anaesthesia literature (Liu, Thorp, Graham & Aitkenhead, 1991; Sandin Enlund, Samuelsson, & Lennmarken, 2000; Sebel et al., 2004). Several large randomised controlled trials have reported incidence rates varying between 0.1% and 0.5% (Myles et al., 2004; Avidan et al., 2008; Avidan et al., 2011; Zhang et al., 2011; Mashour et al., 2012).

In line with these traditional methods of detection, it has been stated that “AAGA is a test of memory” (Cook & Pandit, 2015, p. 471). However, reliance on patients remembering and explicitly reporting their experience of AAGA may be problematic when searching for a true reflection of its incidence. Anaesthetic drugs do not selectively target a person’s consciousness, but also induce amnesia, or the inability to form memories (Perousansky & Pearce, 2011; Brown et al., 2010). It is possible therefore that some proportion of patients may experience AAGA in the moment, but cannot recall so after the fact. One line of evidence that lends support to this idea is the finding that methods not relying on patient recall produce higher incidence rates. The Isolated Forearm Technique (IFT) is one such method, in which a cuff is inflated around a patient’s arm to prevent neuromuscular blockades causing paralysis in that arm (Turnstall, 1977). Clinicians can then ask patients to move their hand to indicate preserved awareness. A recent multi-centre international study involving 260 patients found that 4.6% of patients were positive responders, demonstrating their awareness under general anaesthesia (Sanders et al., 2017). Furthermore, none of those who demonstrated awareness via IFT later recalled having this awareness in postoperative structured interviews. This consideration is also relevant for the discussion of psychological outcomes of AAGA as outlined above. If memory formation is disrupted by anaesthetic agents, it is possible that some patients have negative experiences in the moment that are subsequently forgotten and never reported, but nevertheless potentially lead to adverse long-term psychological effects (Glannon, 2014). Therefore, detecting and eradicating AAGA with or without explicit recall may be important for safeguarding patient safety (Sanders Raz, Banks, Boly, & Tononi, 2016).

The IFT currently provides the most sensitive measure of real-time detection of AAGA, and as a behavioural report method meets the standard for consciousness research. However, it requires command-following to take place. Patients must hear and understand the instruction to squeeze their hand, and then have the capacity to complete the action. Successful completion of the hand squeeze requires several capacities in addition to conscious awareness, including motor responsiveness, and motivation and intention to act. As noted by previous authors (Sanders et al., 2012; Sanders et al., 2017), it may be that some patients retain some level of awareness but, due to an impairment of some other capacity caused by the anaesthetic agent, do not communicate it via IFT. Indeed, we have recently shown that due to an inherent vulnerability in structural and functional features of the frontal and parietal brain regions, a significant proportion of healthy individuals (17%) become unable to make basic behavioural responses (e.g., button press) according to commands under anaesthesia, even when there are consciously aware at moderate dosages (Deng et al., 2020). Therefore, while a positive result on the IFT test demonstrates awareness, a negative result cannot be taken to confirm a lack of awareness.

One solution is to directly examine neural activity that is sufficient to indicate the presence of conscious processing. However, the methods for detecting conscious awareness from brain activity in this population are lagging behind. The Bispectral Index (BIS) monitor uses an EEG signal to monitor frontal neural activity, which is taken as a quantitative index of a patient's level of consciousness (Avidan et al., 2008). However, the BIS, the most ubiquitous brain-based depth-of-anaesthesia monitor in clinical settings, has severe limitations. Foremost, the precise relationship between the frontal EEG signal detected by BIS and conscious awareness remains unknown (Boly et al., 2013). Furthermore, the BIS has well-established poor sensitivity – it does not reliably distinguish between positive/ negative IFT responders (Schneider et al., 2002) – even in awake individuals who have not undergone general anaesthesia (Schuller et al., 2015). Huang et al. (2018) recently used an fMRI mental imagery paradigm to demonstrate that a healthy participant under deep propofol anaesthesia showed modulation of neural activity indicative of mental command following. However, this paradigm suffers from the same limitations as the IFT, in that a positive result requires successful performance of a multitude of functions that may be dissociated from conscious awareness (Naci et al., 2014; Naci et al., 2017; Deng et al., 2020). Therefore, methods for detecting consciousness via brain activity that have been investigated to date do not provide a

sensitive means of interrogating consciousness-specific activity, in a way that requires minimal rule compliance from people under anaesthesia.

To fill this gap, here I examine whether a recent approach developed by Naci and colleagues (Naci et al., 2014; Naci et al., 2017; Naci et al., 2018) to detect conscious processing in a different patient population – those with DoC who, similarly to AAGA patients, cannot express any conscious awareness behaviourally – may be used to detect accidental awareness under deep anaesthesia. The current study builds on this previous work to ask whether we can use an engaging auditory narrative to detect covert awareness in deep anaesthesia.

In this chapter, I examine whether sustained attention and emotional processing throughout the audio narrative can be detected, based on the neural activity of healthy participants who listened to the audio story in an fMRI scanner, after induction of deep anaesthesia via propofol injection. To investigate recruitment of sustained attention and emotional processing, I will use a previously established qualitative measure of the listeners' cognitive and emotional processing throughout the narrative, i.e. the perception of suspense on a moment-by-moment basis (Naci et al., 2014). The suspense of a dramatic narrative, such as *Taken*, arises not only from basic physical properties captured in the sound envelope, such as the amplitude and tone of the musical soundtrack. The perception of suspense is crucially driven by ongoing sustained attention, as the current features of the movie (e.g., a young girl is alone in a foreign country, far away from her family) are compared to stored knowledge of the world (e.g., human traffickers prey on vulnerable targets), what has happened previously in the story (e.g., the girl's friend has been kidnapped), and what may happen in future (e.g., she may be kidnapped).

Therefore, in experiment 1, I quantify the perception of suspense throughout the story in an independent behavioural group of healthy awake participants (N=25), who listened to it in the laboratory and rated how suspenseful it was every two seconds, on a 1-9 Likert scale, from least to most suspenseful. The objectives of experiment 1 are as follows. First, I will test whether the ratings of suspense are similar across individuals, thus suggesting a similar experience of the story in different participants. Based on previous work with similarly engaging and suspenseful audio-visual narratives (Naci et al., 2014), I hypothesize that the ratings of suspense would be similar across different individuals. Second, I will test whether

the suspense ratings provide a measure of participants' understanding of the story's narrative over time, and thus, can be linked to conscious processing of the story's plot. I hypothesize that the ratings of suspense will closely follow the plot developments and, therefore, provide a proxy measure for narrative understanding over time.

In experiment 2, I will investigate the neural basis of the sustained attention and emotional processes concomitant with conscious understanding of the story's narrative in an independent group of participants, who listened to the audio story inside an MRI scanner. Based on previous studies using similarly engaging and suspenseful audio-visual and auditory narratives (Naci et al., 2014; 2017), I hypothesize that, in the awake individuals' group, the suspense rating will reliably predict activity in higher-order brain regions linked to sustained attention and emotional processing, including regions that underlie high-order attention and information integration, such as those in frontal and parietal regions. I will also test whether the neural correlates of sustained attention and emotional processes can be estimated reliably in individual awake participants. Again, based on previous studies using this naturalistic approach, I predict that neurocognitive processes underlying narrative understanding can be reliably detected at the single awake participant level. If this is indeed the case, it will provide a neural marker of the perception of suspense that I can apply to deeply anaesthetized individuals who are behaviourally non-responsive. Secondly, in this case, I will carry out the same analysis on data from the deep anaesthesia condition, to investigate whether any deeply anaesthetized participants show neural evidence of following the audio story's narrative, and thus of covert awareness, despite their clinical status of unconsciousness based on the RSS (Ramsay et al., 1974). Given the most widely accepted low (0.1-0.2%) incidence rates of accidental awareness under general anaesthesia (Liu, Thorp, Graham & Aitkenhead, 1991; Sebel et al., 2004; Sandin et al., 2000), and my study's relatively small sample size, I remain agnostic as to whether I will observe evidence of conscious processing under deep anaesthesia in any of study participants.

Method

Experiment 1

Participants. Ethical approval was obtained from Trinity College Dublin's School of Psychology Research Ethics board. 25 healthy adults (seven male) between 18 and 34 years of age ($M=20.44$, $SD=3.62$) took part. All participants were native English speakers, had no current diagnosis of neurological or psychiatric illness and were not taking psychotropic drugs at the time of the study. Informed consent was obtained for each participant prior to the experiment.

Design & Procedure. Participants, independent from those who underwent scanning and anaesthetisation via propofol as described in Chapter 2, completed a ratings task while listening to the Taken audio story in order to develop a qualitative measure of the higher-order cognitive processes necessary for following and understanding the story's narrative.

Data collection took place in a testing room in the Global Brain Health Institute in Trinity College Dublin. Participants sat at a computer and were presented with the same Taken audio story described in Chapter 2, via over-ear headphones. The audio story was broken down into clips that were two seconds long (156 clips in total) and played sequentially. After each clip finished playing, participants rated it on a scale of one (least) to nine (most) suspenseful. They had up to three seconds to make a response. Participants recorded their ratings by pressing the appropriate number on the computer keyboard using their right index finger. Once a response was made, the next clip would begin immediately. Alternatively, if no response was made, the next clip would begin after three seconds. While the audio clips were playing, the computer screen was black with a white fixation cross in the centre. Participants were prompted to record their rating by the word 'Respond' appearing on screen, and received feedback (i.e. displaying of the words 'Missed Response') if they failed to record any response for a particular clip within the three second time limit. After completing the suspense ratings task, participants completed a 14-item multiple-choice questionnaire testing their knowledge of the content of the Taken audio story. All participants achieved over 78% accuracy, with 20/25 participants answering all questions correctly, suggesting that they successfully processed the story as a whole. Participants indicated via a

feedback questionnaire that the interruptions did not disrupt the coherence of the story's plot and their perception of suspense throughout.

Analysis. I averaged suspense ratings for each two second clip across participants to create a group-averaged score of how suspense ratings varied across the timecourse of the audio story. To determine how similar suspense ratings were across the group, I then compared each participant's rating to the average rating of the rest of the group in a leave-one-out set of Pearson correlation analyses. To calculate an average correlation value, I normalised each correlation coefficient using Fisher's r-to-z transformation, computed the average of these values and then back-transformed them into a correlation coefficient.

Experiment 2

Participants. Participants for this experiment were described in Chapter 2.

Procedure.

Design. As described in Chapter 2, functional scans were obtained as participants were presented with the Taken audio clip in the MRI scanner both in the awake state and during deep propofol anaesthesia. Details of propofol administration are described in Chapter 2.

fMRI data acquisition. fMRI acquisition was described in Chapter 2.

fMRI preprocessing. fMRI preprocessing was described in Chapter 2.

Analysis – Inter-Subject Correlation. Brainwide ISC was calculated as described in Chapter 2, for neural activity in the awake and deep anaesthesia states.

Analysis – fMRI regression.

Sound envelope. To investigate whether activation patterns were associated with perceptual processing I extracted the sound envelope of the auditory narrative via the Matlab MIRtoolbox

(<http://www.jyu.fi/hum/laitokset/musiikki/en/research/coe/materials/mirtoolbox>). The sound

envelope describes how sounds in a stimulus change over time, allowing characterisation of the basic physical properties of the audio clip, such as amplitude, frequency and pitch. I built a generalised linear model (GLM), with the sound envelope as a regressor, by using statistical parametric mapping (SPM) to derive auditory characteristic-related brain activation at a group level and for each individual, while participants were in awake and deep anaesthesia states.

Suspense ratings. I used the behavioural results from experiment 1 above to estimate brain activation patterns associated with information processing during the narrative. The group-averaged suspense ratings were transformed into z-scores and used as a parametric regressor in a GLM of the group fMRI data in both awake and deep anaesthesia states. Both sound envelope and suspense regressors were generated by convolving boxcar functions with a canonical haemodynamic response function (HRF). I also included in all general linear models 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. Fixed-effect analyses were performed in each subject, corrected for temporal auto-correlation using an AR (1)+white noise model. Linear contrasts were used to obtain subject-specific estimates for each effect of interest. Linear contrast coefficients for each participant were entered into a second level random-effects analysis. Clusters or voxels that survived at $p < 0.001$ threshold, corrected for multiple comparisons with family wise error (FWE) were considered statistically significant.

To test whether the suspense ratings could reliably predict activity in individual participants in similar brain regions as observed in the group analyses, I also entered the z-scored average ratings as a regressor in the GLM for each individual participant. Clusters or voxels that survived at $p < 0.05$ threshold, corrected for multiple comparisons with family wise error (FWE) were considered statistically significant.

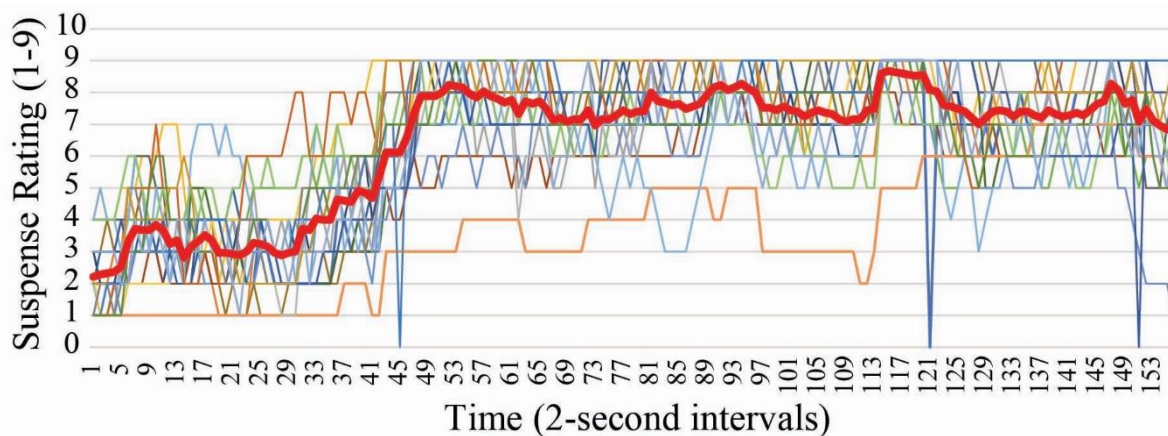
Results

Experiment 1

Ratings of suspense across the audio story's timeline were highly (Pearson's correlation $r=0.90$; $SE=0.07$) significantly ($t(24)=20.56$, $p=9.6e-17$) correlated between individuals (Figure 9). This suggested that different participants experienced the story very similarly to one another, on the moment-to-moment basis.

Figure 9.

Participants' Suspense Ratings Across Audio Story.



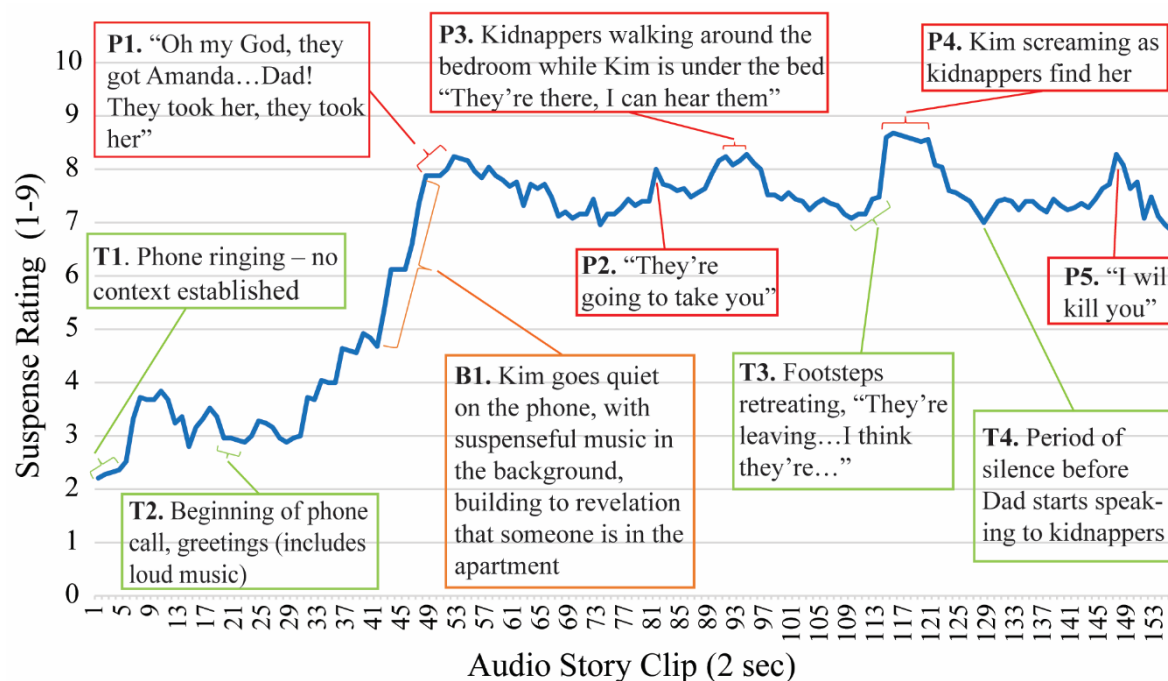
Note. Participants' suspense ratings, from least (1) to most (9) suspenseful, plotted against the audio story's timeline. Thin coloured lines represent each participant's suspense ratings over time, while the thick red line depicts the group average.

To investigate what might be driving participants' suspense ratings, I examined the pattern of the average suspense ratings in the context of the story's narrative over the time. Figure 10 presents the average suspense ratings, with description of the narrative at some of the peaks and relative troughs. Ratings are lowest at the beginning of the audio story, when there is little context and a relatively ordinary conversation between Kim and her father takes place (T1, T2; suspense rating [SR] of 2.3, 2.9 respectively). However, over a 16-second period (clip 41-49) there is a building of suspense (B1; $SR = 4.7-7.8$) where Kim stops

speaking. As silence is punctuated by suspenseful music and her father's questions, Kim eventually reveals there is someone in the house. This is an important turning point in the narrative, after which suspense ratings never fall below 6.9. This building period is immediately followed by the first major peak (P1; SR = 8), where it is established that kidnappers have entered the apartment and have taken Amanda (Kim's friend) – a fact that is important for interpretation of all events following on from it. The remaining clips consist of peaks (P2-P5; SR = 8, 8.19, 8.48, 8.28) and relative troughs (T3, T4; SR = 7.1, 7), which only make sense in the context of the evolving narrative. For example, P3 (SR = 8.48) and T3 (SR = 7.1) consist of similar non-speech occurrences - in both sets of clips, the audience can hear footsteps and creaking of doors/ floorboards, alongside suspenseful background music, while Kim hides under the bed. However, to a listener following the narrative they are importantly differentiated as points of relatively high and low suspense respectively, by their order in the storyline and Kim and her father's commentary on the phone ("They're there" vs "They're leaving.").

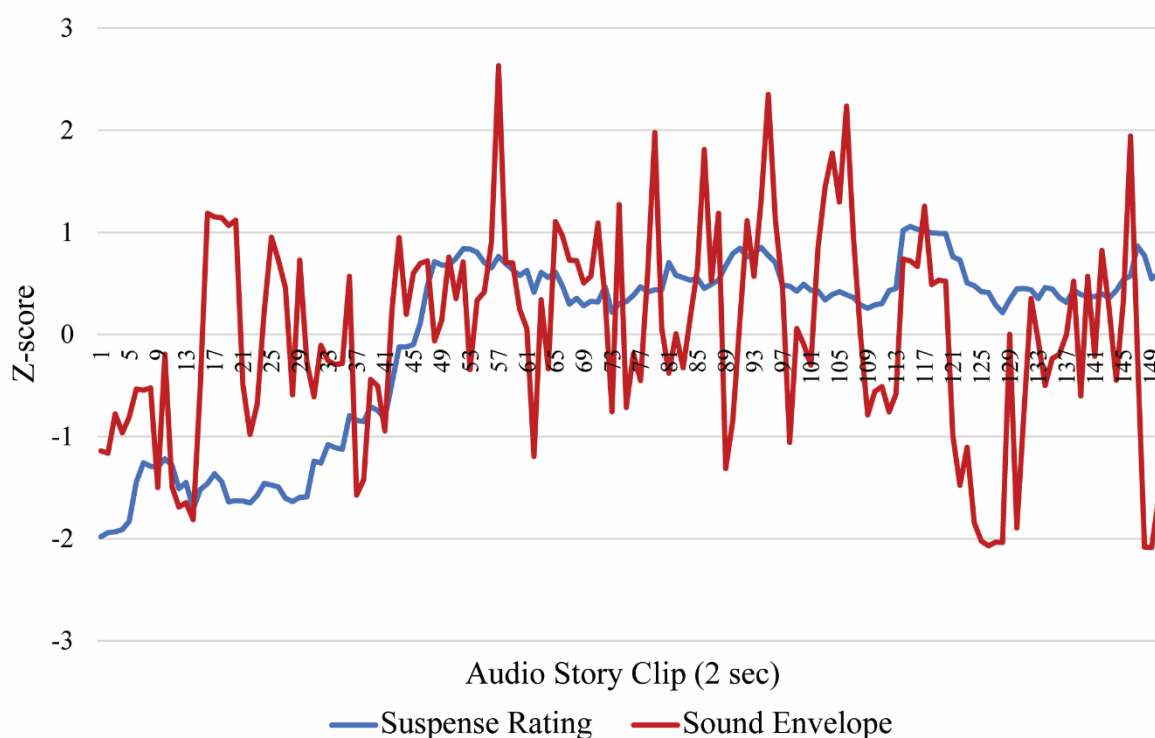
Figure 10.

Audio Story Plot Description at Key Moments.



Note. Group-averaged suspense ratings across the Taken audio story. P1-P5 (red boxes): peaks. T1-T4 (green boxes): relative troughs. B1 (orange box): phase of suspense building.

All clips identified by participants as the most suspenseful in the audio story contain speech, non-speech human sounds, music and non-musical sounds (e.g. footsteps, door creaking etc). To confirm that the average suspense ratings were not driven purely by basic physical properties of the sounds, I plotted the sound envelope values, or the evolution over time of the sound energy in the story, every two seconds and compared them to the suspense ratings (Figure 11). The two sets of values follow distinct patterns. While average suspense ratings start low, steeply incline and remain high in a way that tracks the narrative arc as described above, sound envelope values show frequent peaks and troughs that are evenly distributed and do not follow the story's time course. Therefore, although basic sound properties, especially those of the musical soundtrack, likely play an important role in creating an atmosphere of suspense, they cannot fully account for the participants' suspense ratings.

Figure 11.*Suspense Ratings vs Sound Envelope.*

Note. Group-average suspense rating (blue line) compared to sound envelope values (red line), over the time course of the audio story. Both values are z-scored for visualisation purposes.

My first hypothesis, that suspense ratings would be similar across different individuals, was strongly supported. The results also provide support for my second hypothesis, that the suspense ratings would closely follow the narrative's plot developments over time. Together, my results indicate that participants experienced the audio clip in a very similar way to each other, and that this common experience was driven not only by basic physical properties of the sound, but primarily by an understanding of the story's narrative that requires ongoing higher-order cognitive processes, such as sustained attention and working memory. Therefore, the average suspense rating can be used as a psychological descriptor reflecting, albeit indirectly, the ongoing changes in high-order information processing throughout the audio story.

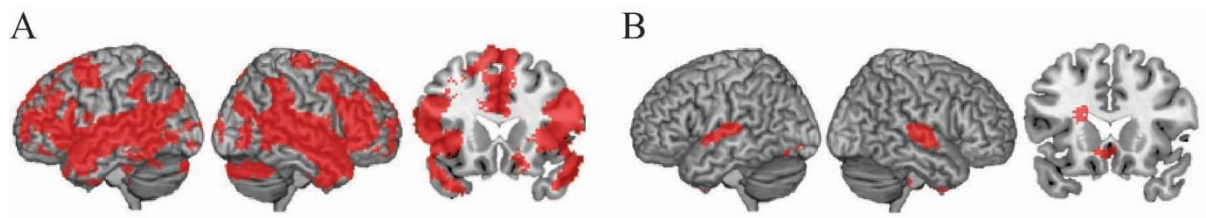
Experiment 2

I therefore used this psychological descriptor to investigate the neural basis of the sustained attention and emotional processes that underlie the narrative understanding in wakeful individuals, and whether neural markers of narrative understanding would be present in deeply anaesthetized participants.

First, I characterized how global information processing during the auditory narrative changes from the wakeful to the deeply anaesthetized state. To this end, I used a previously established method (Naci et al., 2014; 2017; 2018), where it was demonstrated that the extent of stimulus-driven cross-subject correlation across different individuals provides a measure of regional stimulus-driven information processing. In the awake state, participants showed widespread synchronisation of neural activity ($p < 0.001$; FWE corrected) as they listened to the Taken audio story (Figure 12A). A wide range of brain regions, including temporal regions associated with auditory processing, frontal and parietal areas associated with sustained attention and elements of the salience network, showed correlated time-courses of activation across participants (Figure 12A). In the deep anaesthesia state, this synchronisation was dramatically reduced and limited to primary auditory processing regions, with the exception of two small clusters in the caudate nucleus and the anterior cingulate cortex (Figure 12B). This result was consistent with previous studies (Naci et al., 2014; 2017; 2018), and confirmed that at the group level, information processing is dramatically reduced in the deeply anaesthetised individuals, with abolition in high-order regions (i.e., fronto-parietal cortex) and narrow preservation in sensory specific regions (i.e., auditory cortex).

Figure 12.

Inter-Subject Correlation during Taken, in Awake and Deep Anaesthesia States.



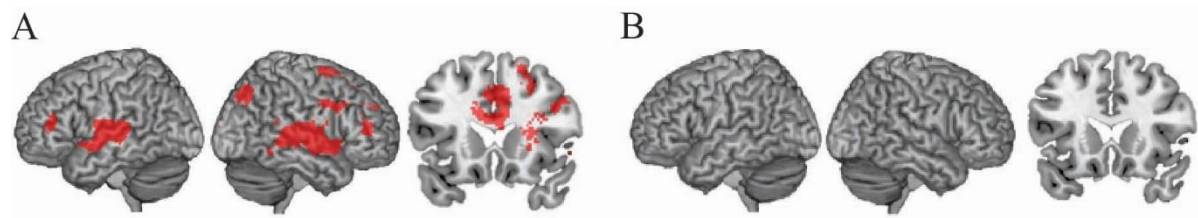
Note. Whole brain intersubject correlation ($p < 0.001$, FWE corrected) during the ‘Taken’ audio story. A: participants in the awake state. B: participants in the deep anaesthesia state.

Next, I investigated specifically which aspects of this synchronized widespread brain activity were driven by the sensory, auditory features of the audio story, and which were driven by the higher-order attention and emotional processing of the story narrative.

I used SPM to model the relationship between the story’s sound envelope and changes in brain activity over time. At the group level, during wakefulness, the sound envelope predicted ($p < 0.001$; FWE corrected) brain activity in bilateral primary and secondary auditory cortex, as well as left inferior frontal sulcus (Figure 13A). Table 2 provides the full list of brain regions. By contrast, this activation was abolished in deep anaesthesia, in which the sound envelope did not predict any significant clusters at the group level (Figure 13B).

Figure 13.

Neural Activity Predicted by Sound Envelope, in Awake and Deep Anaesthesia States.



Note. Areas of group-level activity that were significantly estimated ($p < 0.001$, FWE corrected) by a GLM model with the sound envelope of the audio story entered as a regressor. A: participants in the awake state. B: participants in the deep anaesthesia state.

Table 2.

Activation clusters produced by the story's sound envelope at the group level, in the awake state.

Cluster Peak Location	Peak MNI Coordinates			Cluster-wise p-value	Associated Cognitive Function
	x	y	z		
Superior Temporal Gyrus [L]	-66	-18	6	$p < 0.000$	Auditory perception
Superior Temporal Gyrus [R]	56	-4	-2	$p < 0.000$	Auditory perception
Inferior Frontal Sulcus	-44	36	16	$p < 0.000$	Auditory attention
Hippocampal Gyrus/ Parietooccipital Sulcus [R]	16	-34	4	$p < 0.000$	Visual processing
Mid Occipital Gyrus	36	-74	42	$p < 0.000$	Visual processing
Parietooccipital Sulcus / Hippocampal Gyrus [L]	-12	-40	7	$p < 0.000$	Visual processing
Calcarine Gyrus	8	-96	8	$p < 0.000$	Visual processing

In individual awake participants, the sound envelope predicted significant activations ($p < 0.05$, FWE corrected) in auditory regions shown by the group-level analyses in the majority of participants (left hemisphere: 11/16; right hemisphere: 14/16) (Table 3).

Table 3.

Presence of group-level activation peaks predicted by the sound envelope in individual participants during the awake state.

Peak Cluster Location	Participants																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Superior Temporal Gyrus [L]	+	+	+		+		+	+		+	+			+	+	+	11/16
Superior Temporal Gyrus [R]	+	+	+		+	+	+	+		+	+	+	+	+	+	+	14/16
Inferior Frontal Sulcus					+		+	+			+			+	+	+	7/16
Hippocampal Gyrus/ Parietooccipital Sulcus [R]							+	+		+	+						4/16
Mid Occipital Gyrus Parietooccipital Sulcus / Hippocampal Gyrus [L]	+				+			+		+	+		+				6/16
Calcarine Gyrus					+			+		+	+				+		5/16

During the deeply anaesthetized state, despite the lack of group-level activation, individual level GLMs showed activation ($p < 0.05$, FWE corrected) in auditory regions of 37.5% of the participants (6/16) (Table 4).

Table 4.

Presence of awake group-level activation peaks predicted by the sound envelope in individual participants, during the deep anaesthesia state.

Peak Cluster Location	Participants																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Superior Temporal Gyrus [L]	+							+		+		+		+			6/16
Superior Temporal Gyrus [R]	+							+		+		+		+			6/16
Inferior Frontal Sulcus										+		+		+			4/16
Hippocampal Gyrus/ Parietooccipital Sulcus [R]								+	+					+			4/16
Mid Occipital Gyrus Parietooccipital Sulcus /	+						+	+						+			5/16
Hippocampal Gyrus [L]									+	+				+			3/16
Calcarine Gyrus									+	+				+			3/16

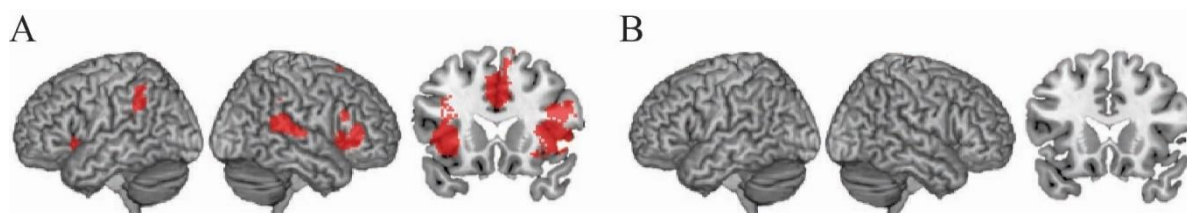
In summary, the analyses of the story's auditory properties showed robust activation of auditory regions in the wakeful state, at the group and individual level, as well as robust auditory activation in some individual deeply anaesthetized participants, despite the absence of group-level activations in this state. This result suggests a high inter-subject variability of neural responsivity to sensory stimulus properties in deeply anaesthetized individuals. This is consistent with reports of high inter-subject effect variability of anaesthesia on the brain (Chennu et al., 2016; Bola et al., 2019).

Subsequently, I used the independently-derived suspense ratings to investigate the neural basis of the sustained attention and emotional processes that underlie narrative understanding in wakeful individuals, and whether neural markers of narrative understanding would be present in deeply anaesthetized participants. First, I used the group-averaged z-

scored suspense ratings calculated in experiment 1 as a regressor in the group-level analysis of the wakeful state. The group-averaged suspense ratings significantly ($p < 0.001$; FWE corrected), predicted activity in a set of brain areas, including regions of the auditory attention and language processing network (the left/ right superior temporal gyrus/ sulcus, supramarginal gyrus) (Naci et al., 2013; Michalka, Kong, Rosen, Shinn-Cunningham, & Somers, 2015; Tobyne, Osher, Michalka, & Somers, 2018) and the salience network (the left/right anterior insular gyrus, cingulate sulcus, brainstem areas, and the thalamus) (Menon 2015; Seeley 2019), with clips rated as ‘highly suspenseful’ predicting stronger activity in these regions (Figure 14A). Table 5 shows the full list of the brain areas in which activation was predicted by the suspense ratings at the awake group-level. As expected, in contrast to the awake state, the brain activity underlying the perception of suspense was abolished during deep anaesthesia, at the group-level (Figure 14B).

Figure 14.

Neural Activity Predicted by Suspense Ratings, in Awake and Deep Anaesthesia States.



Note. Areas of activity, at the group-level, significantly estimated ($p < 0.001$, FWE corrected) by a GLM model with the story’s suspense ratings as regressor. Panel A: participants in the awake state. Panel B: participants in the deep anaesthesia state.

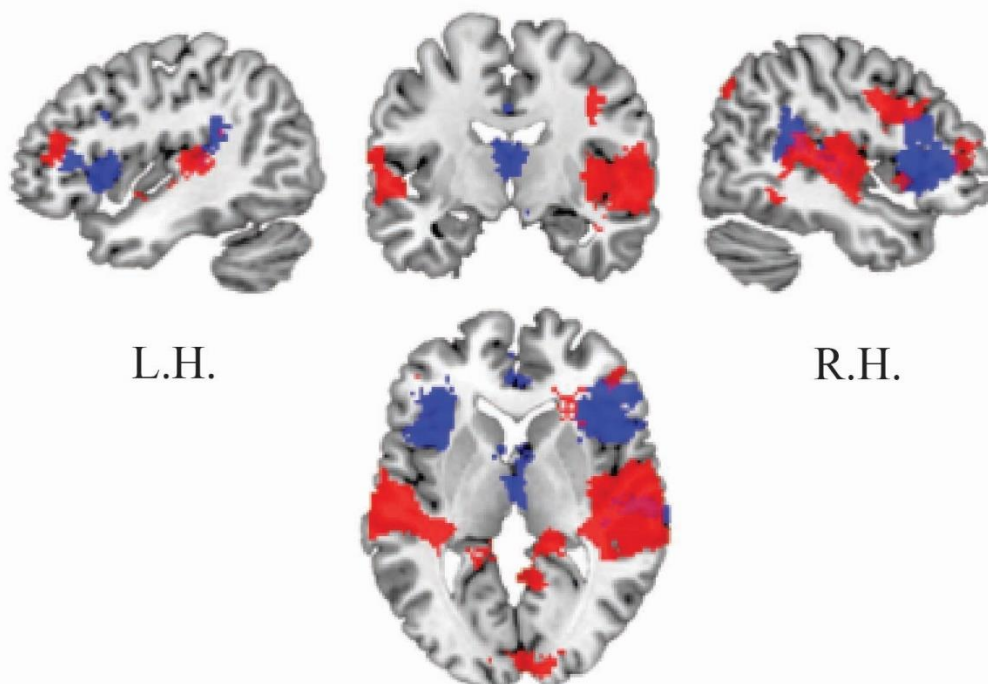
Table 5.*Activation clusters produced by the suspense ratings at the group level, in the awake state.*

Cluster Peak Location	Peak MNI Coordinates			Cluster p-value	Associated Cognitive Function
	x	y	z		
Superior Temporal Sulcus/ Gyrus (R)	54	-18	14	p<0.000	Auditory attention
Superior Temporal Gyrus/ Sulcus (L)	-52	-42	22	p<0.000	Auditory attention
Supramarginal Gyrus (L)	-62	-44	38	p<0.000	Language understanding
Anterior Insular Gyrus (R)	38	30	10	p<0.000	Saliency
Cingulate Sulcus	2	20	34	p<0.000	Saliency
Thalamus Nucleus (R)	6	-12	16	p<0.000	Saliency
Anterior Insular Gyrus (L)	-36	24	6	p<0.000	Saliency
Brainstem	-4	-28	-22	p<0.000	Saliency

I compared the significant activity predicted by suspense ratings to that estimated by the sound envelope, at the group level. Despite some areas of overlap in areas associated with auditory processing, it is clear that the two models predict distinct patterns of activation (Figure 15). In particular, the group-averaged suspense regressor, *not* the sound envelope regressor, predicts activity in the left supramarginal gyrus, which has been associated with higher-order auditory functions such as phonological (Celsis et al., 1999) and short-term memory processing (Becker, MacAndrew & Fiez, 1999). Additionally, it predicts activity in the saliency network including the anterior insular gyrus, cingulate sulcus, thalamus and brainstem regions.

Figure 15.

Neural Activity Predicted by Sound Envelope vs Suspense Ratings, in Awake State.



Note. Areas of group-level activity significantly estimated ($p < 0.001$, FWE corrected) by the sound envelope (in red) and the group-averaged suspense ratings (in blue) of the audio story, while participants were in the awake state. R.H = right hemisphere; L.H = left hemisphere.

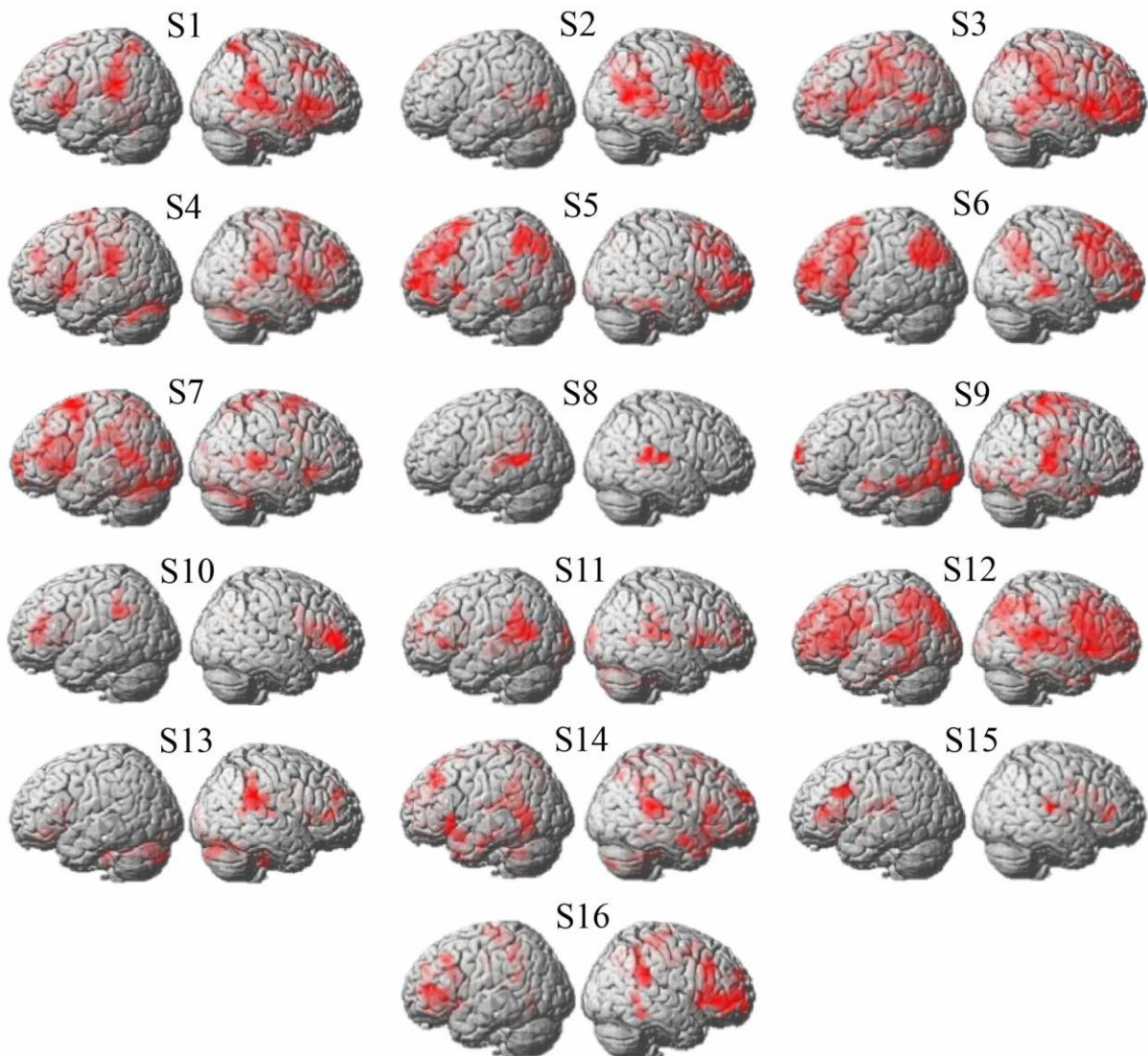
As previously discussed, in this narrative, suspense is an emotion that is elicited through ongoing attention to, and cognitive and emotional appraisal of, the story's plot. Hence, is it fitting that the perception of suspense during the story recruits not only regions in the auditory attention network, but also key nodes of the salience network, which is involved in attention and emotional processing (Seeley et al., 2019; Menon, 2015). In summary, results at the awake group level were consistent with my hypothesis, and showed that the neural basis of suspense processing during the movie are dissociable from those underpinning the processing of basic sensory (auditory) features of the narrative. Rather, the processing of suspense is underpinned by regions involved in high-order auditory attention and cognitive and emotional appraisal, further suggesting that the processing of suspense is an adequate proxy for the processing of the story's high-level features, leading to understanding of its narrative over time.

Subsequently, I investigated the neural basis of suspense processing in single wakeful individuals, to test whether a robust marker of narrative processing could be found at the single-subject level. As aforementioned, in the behavioural group from experiment 1, the suspense ratings throughout the narrative showed a very robust ($r=0.90$; $SE=0.07$) and significant inter-subject correlation ($t(24)=20.56$, $p=9.6e-17$), and reflected the evolution of the story's plotline over time, confirming a common understanding of the story across different individuals listening to this narrative (Fig. 9). Therefore, the similarity of the story's suspense ratings enabled model-based predictions of the underlying brain activity that could be applied to individual participants.

For each participant, I carried out single-subject GLMs using the z-scored average suspense scores as a regressor. All participants in the awake state showed significant activation ($p<0.05$, FWE corrected) of the areas listed in Table 5. In 12/16 cases (75%), at least 5/8 of these areas showed significant activation (Figure 16; Table 6). 4/16 individuals (S3, S4, S12, S14) showed activation of all areas predicted at the group level. In summary, at least 5/8 group-level activation clusters could be reliably estimated in the majority (75%) of the individual participants, with the remaining minority 25% showing 4/8 or 3/8 group-level activations. This result suggested that processing of suspense provided a neural marker of narrative understanding that could be reliably predicted at the single individual basis.

Figure 16.

Activity Predicted by Suspense Ratings in Individual Participants in the Awake State.



Note. Significant activity ($p < 0.05$, FWE corrected) estimated by the story's suspense ratings from independent behavioural group, in wakeful participants during the Taken audio story. S1-S16: Participants 1-16.

Table 6.

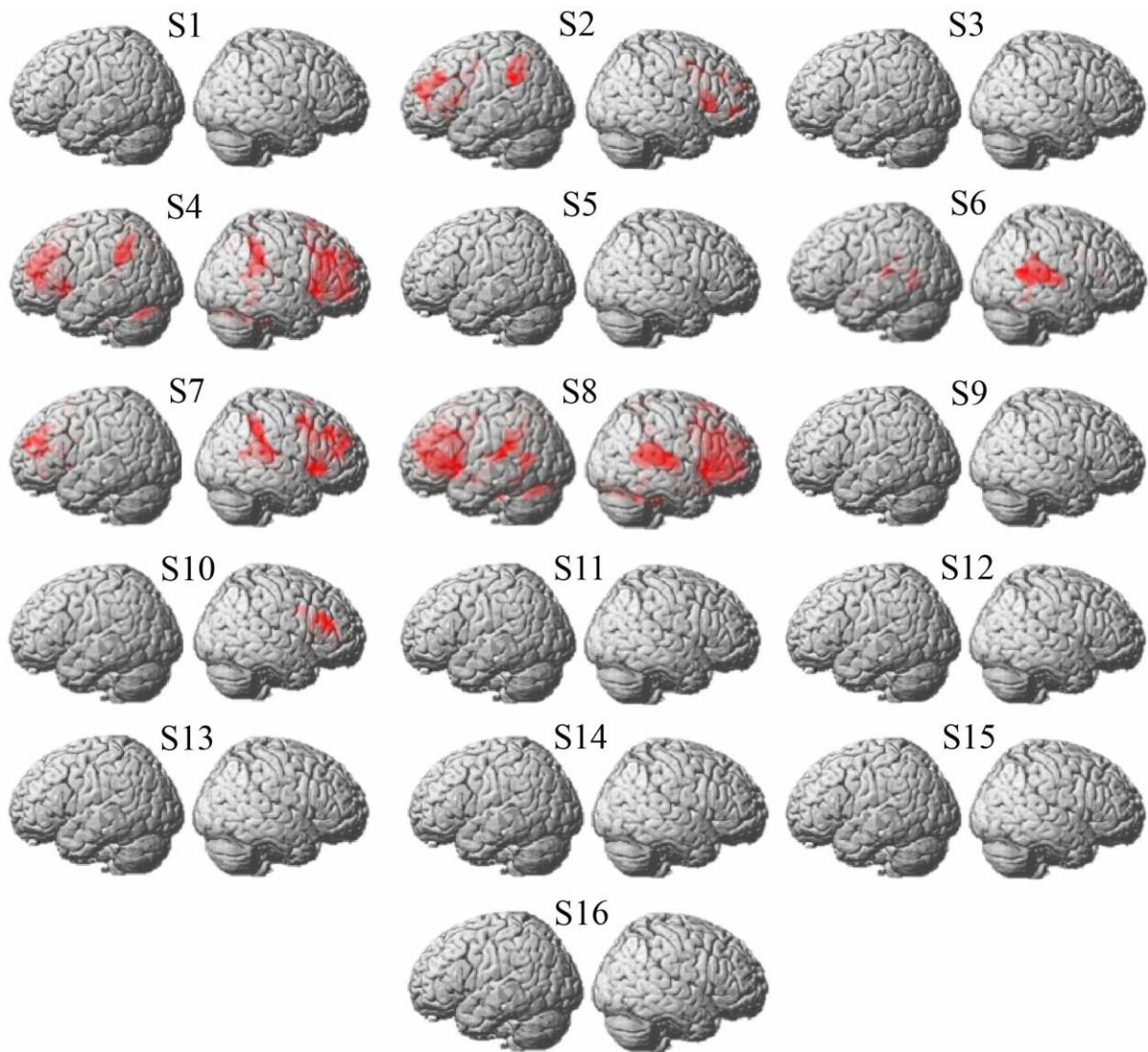
Presence of awake state group-level activation peaks predicted by the suspense ratings in individual participants during the awake state.

Cluster Peak Location	Participants																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Superior Temporal Sulcus/ Gyrus (R)	+	+	+	+		+	+	+			+	+	+	+	+	+	13/16
Superior Temporal Gyrus/ Sulcus (L)	+	+	+	+	+		+	+			+	+		+		+	11/16
Supramarginal Gyrus (L)	+		+	+	+	+				+	+	+		+		+	10/16
Anterior Insular Gyrus (R)	+	+	+	+	+		+		+	+		+	+	+	+	+	13/16
Cingulate Sulcus	+		+	+	+	+				+		+	+	+		+	10/16
Thalamus Nucleus	+		+	+	+		+	+	+	+	+	+	+	+		+	13/16
Anterior Insular Gyrus (L)	+	+	+	+	+	+	+			+	+	+	+	+	+	+	14/16
Brainstem		+	+	+			+	+	+			+	+	+			9/16

Based on these results, I investigated the processing of suspense in individual deeply anaesthetized individuals. Despite a lack of activation shown at group-level analysis, six deeply anaesthetized participants (S2, S4, S6, S7, S8, S10) showed significant activation ($p < 0.05$, FWE corrected) in at least some (i.e. between 1/8 and 8/8) of the suspense areas seen in the awake group (Figure 17; Table 7).

Figure 17.

Activity Predicted by Suspense Ratings in Individual Participants under Deep Anaesthesia.



Note. Significant activity ($p < 0.05$, FWE corrected) estimated by the story's suspense ratings in deeply anaesthetized participants during the Taken audio story. S1-S16: Participants 1-16.

Table 7.

Presence of awake group-level activation peaks predicted by the suspense ratings in individual participants, during the deep anaesthesia state.

Peak Cluster Location	Participants																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Superior Temporal Sulcus/ Gyrus (R)				+		+	+	+									4/16
Superior Temporal Gyrus (L)				+		+		+									3/16
Supramarginal Gyrus (L)		+		+				+									3/16
Anterior Insular Gyrus (R)		+		+		+	+	+		+							6/16
Cingulate Sulcus				+			+	+									3/16
Thalamus Nucleus (R)				+		+	+	+									4/16
Anterior Insular Gyrus (L)		+		+			+	+									4/16
Brainstem				+		+		+									3/16

Two deeply anaesthetized participants (S4, S8) demonstrated activity in *all* high-order auditory attention and salience network areas identified in the wakeful participant group. However, in the awake state, S8 showed suspense-related activation in only a subset (i.e. 4/8) of these areas (left/ right superior temporal sulcus/ gyrus, brainstem and thalamus; Table 6), which makes activation of the additional suspense-related areas in this individual under deep anaesthesia difficult to interpret. By contrast, S4 activated all 8 of the suspense-related areas in the wakeful state, and again showed activation in all 8 regions in the deeply anaesthetized state.

Therefore, we can be sure that at least one of the 16 participants (6%) demonstrated the complete neural activity patterns associated with high-order auditory attention and cognitive and emotional appraisal of the story during deep anaesthesia. This striking result suggested that this individual processed and understood the story similarly to awake participants, when they were considered to be clinically unconscious.

Discussion

In this chapter I described two experiments that aimed to use a novel naturalistic method to detect conscious information processing in participants under deep anaesthesia. Using a conservative, hypothesis-driven method previously established to detect awareness in unresponsive DoC patients (Naci et al., 2014), I examined conscious processing of a suspenseful audio story at the group and individual level in participants, while they were awake and subsequently under deep propofol-induced anaesthesia.

Results from experiment 1 replicated previous findings (Naci et al., 2014) that behavioural ratings of suspense in suspenseful plot-driven narratives are highly correlated among individuals, and closely follow plot developments. On top of basic physical properties which can be captured in the sound envelope, an individual's experience of how suspenseful a narrative, such as the one used in this study, is arises through an understanding of the relevance of specific items intrinsic to the plot (e.g., human traffickers, large sums of money), their potential uses (e.g., abduct people, to pay for a ransom), the circumstances of the protagonists (e.g., a girl is hiding under a bed when her abductors enter the room, her father is far away), and their own 'state of mind' (e.g., feeling powerless, deceived), which the listener has to attend to on an ongoing basis, in order to feel suspense as the plot develops. My analysis demonstrated that an understanding of the plot is necessary for participants to rate these particular clips as more or less suspenseful than others, and thus my results suggested that different participants had a very similar understanding of this engaging auditory story. Therefore, I concluded that the suspense ratings were appropriate as a proxy for narrative understanding and could be used to investigate its presence in an independent group of deeply sedated individuals.

Experiment 2 examined the neural basis of the story's suspense ratings in wakeful participants, and investigated whether its neural correlates could reliably be predicted in individual participants, in order to develop a neural marker of conscious awareness, that could be used to investigate its presence in deeply anaesthetized individuals. In the awake state, at a group-level I found wide-spread synchronisation, including in sensory and higher-order brain regions, such as temporal regions associated with auditory attention, frontal and parietal areas associated with supra-modal attention and executive function, and the salience network, associated with cognitive and emotional appraisal. This was consistent with

previous evidence showing synchronised brain activation across participants during engaging, plot-driven naturalistic narratives (Hasson et al., 2010; Naci et al., 2014; Naci et al., 2018; Eickhoff et al., 2020), especially when the narrative evokes strong emotions (Kober et al., 2008; Nummenmaa et al., 2012; Nummenmaa et al., 2014).

To understand whether aspects of this brain activity could be linked to conscious processing, I first investigated whether it was merely driven by the processing of low-level acoustic properties of the audio story, or driven further by processing of the higher-order features that lead to narrative understanding. I found that the sound envelope of the audio story predicted activity primarily in regions associated with primary sensory (auditory) processing (Kaas & Hackett, 1999), which suggested that much of the synchronized brain activity during the story was not driven by the low-level auditory features. I then investigated the relationship between the suspense ratings, which tracked higher-order processes including sustained attention and emotional processing, and the synchronized brain activity across participants. Suspense ratings predicted activity in a largely distinct set of brain regions to those predicted by the sound envelope, including bilateral superior temporal gyrus/sulcus, supramarginal gyrus, bilateral anterior insular gyrus, cingulate sulcus, thalamus and brainstem areas. The superior temporal sulcus and gyrus have been implicated in auditory top-down attention (Michalka et al., 2015; Tobyne et al., 2017). The supramarginal gyrus has been associated with language-based higher-order auditory functions, such as phonological (Celsis et al., 1999) and short-term memory processing (Becker et al., 1999). The involvement of these areas in processing the auditory story is appropriate, as language processing, memory and auditory attention are prerequisites, to being able to follow the narrative plot and, thus, experience the rise and fall of suspense over time.

The other regions activated by suspense ratings (bilateral anterior insular gyrus, cingulate sulcus, thalamus and brainstem areas) are key nodes of the salience network (Menon, 2015; Seeley 2019), which detects and co-ordinates the response to salient stimulus in the environment, in the service of cognitive and emotional appraisal. Insular areas in particular have been suggested to provide a link between stimulus-driven processing and awareness of interoceptive and subjective feelings (Craig & Craig, 2009). The involvement of neural regions that are key to both cognitive and affective processing (Menon & Uddin, 2010) is in line with this processing of suspense, which requires attention to both external information during the narrative, and implicit assessment of internal cues that signal the

valence and arousal levels the individual experiences. These results were consistent with a previous study which found that participants' ratings of the valence and arousal of audio narratives correlated with activity in the insula and the thalamus (Nummenmaa et al., 2014), two key areas of the salience network. Thus, the results of experiment 2 in this chapter were consistent with those of experiment 1 in suggesting that the suspense ratings were an appropriate behavioural proxy of high-order information processing and integration over time, which require conscious awareness. Furthermore, the finding that the majority of this distributed network of brain activations (i.e. at least 5/8 areas) elicited by the suspense ratings could be reliably observed in 75% (12/16) single wakeful individuals, suggested that it could provide a neural marker of narrative understanding, for testing its the presence in single deeply anaesthetized individuals. Therefore, I could use these suspense ratings as a regressor to examine the brain activity of individual deeply anaesthetised participants.

At the group-level, both sound envelope- and suspense rating-related activation was abolished under deep anaesthesia, in line with previous evidence of severe reduction in global cerebral metabolism (Kaisti et al., 2002) and disconnection across brain areas (Alkire & Miller, 2005). As the individual level, consistent with findings of preserved processing of simple sensory stimuli under deep anaesthesia (MacDonald et al., 2015), some (6/16) individuals showed brain activity related to the low level acoustic sound properties, as established in wakeful individuals. Critically, suspense ratings predicted significant brain activity in one individual under deep anaesthesia in all eight brain areas associated with suspense ratings in the awake group, and with processing of suspense in this individual in the wakeful state. Therefore, this result provided very strong evidence that they followed the plot and experienced suspense on a moment-to-moment basis, in the same way as awake individuals, despite their designation of unconsciousness based on the clinical behavioural Ramsay scale (Ramsay et al., 1974) from three independent assessors. The designation of unconsciousness (RSS score of five) was determined as participants became behaviourally non-responsive, could not engage in conversation, stopped responding to verbal commands, and did not produce any spontaneous responses. Therefore, for the first time, these results demonstrate that it is possible to detect, by using a naturalistic paradigm, neural activity that is meaningfully linked to proxy measurements of consciousness understanding, in a participant who is deeply anaesthetised.

These results mirror those from the body of literature describing cases in which evidence gathered with behavioural assessments of consciousness diverges from brain-based evidence of conscious awareness (Sanders et al., 2012). A growing body of evidence demonstrates that a proportion of patients with a clinical diagnosis of unconsciousness, such as patients deemed to be in a vegetative state (Jennett & Plum, 1972), demonstrate conscious awareness in functional neuroimaging paradigms (Owen et al., 2006; Bardin et al., 2011; Cruse et al., 2011; Fernández -Espejo & Owen, 2013; Naci & Owen, 2013; Gibson et al., 2016; Kondziella et al., 2016; Naci et al., 2017; Naci et al., 2018). This advance in the state-of-the-art understanding of brain-based measures of consciousness drove the recent update in the guidelines of the American Academy of Neurology (AAN) (Giacino et al., 2018). The AAN currently recommends that functional neuroimaging may be used in cases where behavioural results are ambiguous or absent, to assess the presence of consciousness in severely brain-injured patients. Similarly, in rare cases, patients under general anaesthesia might temporarily resemble behavioural non-responsive patients with DoC, in manifesting no behavioural signs consciousness despite retaining conscious mental activity (Graham et al., 2018). This inability to communicate consciousness behaviourally may be, at least in part, driven by impaired motor and cognitive function that prevents behavioural responses, both in patients with DoC and under anaesthesia. Indeed, we recently showed that a significant proportion of healthy individuals (17%) became unable to make basic behavioural responses (e.g., button press) according to commands, under anaesthesia even when they were consciously aware at moderate dosages (Deng et al., 2020).

These results are highly relevant for the monitoring depth-of-anaesthesia for the detection of AAGA. They highlight novel markers that, if replicated in a clinical context, may help to improve the accuracy of awareness monitoring during clinical anaesthesia. The 6% incidence rate of covert awareness during deep anaesthesia I found in this study is considerably higher than those produced by methods that require explicit recall (0.1-0.5%; Sebel et al., 2004; Myles et al., 2004), which suffer from several limitations, and this higher rate may indeed be due to the absence of such a requirement. None of the study participants who underwent anaesthesia had explicit recall of the events during the deep anaesthesia session upon recovery. Similarly, the IFT, an established method that does not rely on explicit recall, produces higher incidence rates (4.6%; Sanders et al., 2017) than traditional methods that do. Therefore, my results further emphasise the importance of developing techniques for awareness-monitoring that do not require explicit recall. The higher rate found

in my study relative to IFT may be explained by the lower task demands of listening to an engaging story compared to responding behaviourally to rule-based commands while under the effect of anaesthesia. Listening to plot-driven narratives is naturally engaging, requires no behavioural collaboration from volunteers, and therefore, is highly suitable for testing covert consciousness independently of behavioural output or eye opening (Naci et al., 2017; 2018).

The method used in my study has an important advantage over current state-of-the-art brain-based monitors that are used to assess depth-of-anaesthesia in the clinic. BIS, the most widely used EEG-based clinical system, does not directly evaluate brain activity resulting from mental processes, but instead records and compares regionally non-specific spontaneous signals before and during anaesthesia. By contrast, engaging plot-driven narratives elicit distinctive patterns of brain activity in specific brain areas that can be linked to conscious cognitive function, such as executive function or memory. Evidence of covert cognition in accordance with a-priori predictions based in behavioural and fMRI manipulations provides considerably stronger evidence for the presence of consciousness than BIS or similar EEG-based systems (Graham et al., 2018). However, the well-acknowledged limitations of fMRI (i.e. its prohibitive cost and the need for patients to lie still in the scanner) make it an unrealistic solution for bedside detection of AAGA in surgical settings. Therefore, future integration of the auditory story paradigm described here with cheaper and more portable technologies, such as EEG or galvanic skin response (GSR), is key to its clinical translation. Naturalistic paradigms have previously been combined with EEG assessments to examine attentional processes in healthy awake participants (Ki et al., 2016). Future research should therefore extend such work to participants under anaesthesia to examine the paradigm's potential applicability as a depth-of-anaesthesia monitor in a clinical context.

Finally, it is worth considering several limitations. First, it is important to note that some participants, while awake, did not show suspense-related activation in the full set of areas activated at the group level. This made it difficult to interpret the presence of such activity under deep anaesthesia, such as in the case of participant S8. However, this type of result is not unique to this study, but rather, reflects the inherent high variability observed in single-subject brain activation maps throughout fMRI studies. Additionally, this result underscores another fact well-documented in other studies seeking to establish neural markers of conscious awareness in behaviourally non-responsive populations. This is that a negative result cannot be interpreted as a definitive lack of awareness, as absent or impartial

activations can be observed in healthy awake participants due to several factors, including falling asleep, becoming drowsy in the scanner, or being a weak activator (i.e. showing low neural activations across tasks and conditions). Second, propofol was used in the current study, due to its wide prominence in clinical interventions. It is therefore impossible to determine based on current data whether these results generalize to other anaesthetic agents. Future research could investigate the same paradigm across different anaesthetic agents to determine its generalisability across anaesthetic agents. Third, replication in a larger sample is required to generalise the 6% incidence rate to the wider population.

Chapter 4: General discussion

Thesis objectives

This thesis aimed to answer two important questions in anaesthesia research. Firstly, despite an abundance of studies examining brain changes upon abolition of consciousness by anaesthesia, very few have focused on how these brain features reconstitute upon the recovery of consciousness. I addressed this question in Chapter 2. Secondly, there is very limited knowledge on brain mechanisms that might enable the preservation of conscious awareness, in rare cases, under general anaesthesia. I addressed this question in Chapter 3. Both questions relate to a central challenge in anaesthesia research: understanding the relationship between brain changes and concomitant changes in cognitive function. Naturalistic paradigms provide an opportunity to examine the neural dynamics underlying changes in higher-order cognitive processes during and after anaesthesia. This thesis used a unique dataset where consciousness was abolished with deep propofol anaesthesia in a healthy group of participants inside a 3T MRI scanner, while participants were exposed to a complex, plot-driven narrative. This gave me the opportunity to address these two gaps in the field.

Perturbations of brain dynamics during loss and recovery of consciousness

In the study described in Chapter 2, 16 healthy adult participants underwent fMRI scans at three states of consciousness: awake (before propofol administration), propofol-induced deep anaesthesia (unresponsive), and recovery (return of consciousness following cessation of propofol). Behavioural responsiveness for clinical status designation was assessed by three independent assessors (two anaesthesiologists, one anaesthesia nurse) using the RSS (Ramsay et al., 1974), along with a memory and target detection task. fMRI data was collected in each state under two conditions: while participants were lying quietly in the scanner with no external stimulation, i.e. the resting state, and while they were presented with a five-minute engaging audio story from the movie *Taken* (Morel, 2008). I measured functional connectivity within and between seven brain networks, and compared it across the three states of consciousness and the two conditions.

Changes in global connectivity across the states of consciousness showed different trends in the naturalistic compared to the resting state paradigm. During the resting state there were no differences between awake, deep anaesthesia and recovery states. The lack of significant differences across transitions of consciousness during the resting state contrasts with previous literature showing a reduction in connectivity upon anaesthesia-induced loss of unconsciousness in the resting state, particularly within higher order networks (Boveroux et al., 2010; Schrouff et al., 2011; Bonhomme et al., 2012; Ranft et al., 2016; Kelz & Mashour, 2019). Methodological differences may account for this variation in results. In particular, I used predefined regions of interest (Raichle et al., 2011) rather than a data-driven approach such as independent component analysis used in these previous studies. Additionally, I used a brain-wide, broad measure of global connectivity that averaged across within- and between-network connectivity over seven diverse regions. This may have obscured more subtle differences in the dataset. Indeed, the expected differences in resting state data emerged in my study when I carried out comparisons at the network-type and individual-network levels (see below). By contrast, during Taken, connectivity increased significantly between the awake state and deep anaesthesia, as previously reported Naci et al. (2018), and critically, showed no difference between deep anaesthesia and recovery. The diverging effects during the resting state compared to the Taken narrative provided strong support for my first hypothesis, namely, that the impact of deep anaesthesia and recovery of consciousness on functional connectivity differs depending on whether participants are in the resting state or exposed to complex naturalistic stimulation.

The opposite effects of deep anaesthesia on connectivity have previously been interpreted (Naci et al., 2018) with reference to the different neural processes recruited by resting state and naturalistic paradigms. In the former, participants lay quietly in the scanner with no external stimulation and, thus, are engaged in spontaneous cognition. In the latter, participants are presented with an external stimulus, and thus recruit stimulus-driven cognition. Given that, in this study, I used Pearson correlation as the measure of functional connectivity, higher functional connectivity directly reflects higher similarity of regional time-courses and consequently of functional stimulus-driven responses in different regions. Therefore, increased connectivity during the narrative condition suggests that the stimulus-evoked feed-forward processing cascade triggered by the stimulus, does not undergo increasingly more complex and different transformations along the sensory hierarchy, but rather is echoed undifferentiated throughout the brain, thus overcoming the inhibitory effect

of propofol on neural connectivity that has been reported in resting state studies (Brown et al., 2010). This is consistent with previous TMS studies, which have directly perturbed the unconscious brain, and demonstrated that responses in different areas of the brain become undifferentiated from each other (Massimini et al., 2012; Casali et al., 2013).

The novelty of this study related to findings from the recovery state. For the first time, I found that during the processing of a complex naturalistic stimulus, by contrast to the resting state, functional connectivity in the recovery state was not significantly different to the deep anaesthesia state. This is striking, as it suggests that the functional responses triggered by complex naturalistic stimuli immediately on recovery of behavioural responsivity are altered and similar to deep anaesthesia, i.e. undifferentiated throughout the brain. As previously mentioned, the global connectivity measure I calculated was broad and averaged across seven networks across the brain. Therefore, to further understand what drove the effects I found at the global level, I investigated functional connectivity the network-type level and across the individual networks.

To find out whether higher-order and sensory networks make differential contributions to brain dynamics in these transitions of consciousness, I investigated connectivity changes at the sensory vs higher-order network level. I calculated an average within- and between-network connectivity value for the four higher-order networks (DMN, DAN, ECN, SN) and the three sensory networks (SM, VN, AN), at each state of consciousness in both resting state and Taken conditions. During loss of consciousness, in the resting state, functional connectivity changes were driven by decreased connectivity *within* higher-order association networks. By contrast, in the Taken condition there were more widespread changes in the opposite direction. Functional connectivity changes were driven by increased *within-* and *between-network* connectivity in sensory-motor networks, as well as increased *between-network* connectivity in higher-order networks. During recovery of consciousness, the resting state showed the expected increased connectivity *within* higher-order association networks. By contrast, in the Taken condition there was no significant change in connectivity during recovery relative to loss of consciousness, contrary to my prediction.

I then investigated whether specific individual networks contributed predominantly to the changes seen in sensory and higher-order groups, by examining changes in connectivity

measures in each of the seven networks. In loss of consciousness, during the resting state, there were no significant changes at the level of individual networks. By contrast, in the Taken condition there was an increase in *within-network* connectivity in 1/7 networks (SM), and in *between-network* connectivity in 6/7 networks (DMN, DAN, ECN, SN, SM, AN; not VN). In recovery of consciousness, during the resting state, there was increased *within-network* connectivity in 4/6 networks (DMN, DAN, ECN, AN; not SN, SM, VN). By contrast, during the Taken condition, there were no significant changes in connectivity upon recovery of consciousness. Despite the lack of significant differences in the resting state between awake and deep anaesthesia, the transition from deep anaesthesia to the recovery state was marked by increases in within-network connectivity in higher-order networks. The lack of difference between deep anaesthesia and recovery during Taken, on the other hand, manifested across both sensory and higher-order networks. My results, therefore, reinforce previous findings that anaesthesia-induced unconsciousness during the resting state is characterised primarily by a breakdown of connectivity within higher-order brain networks that is restored immediately at the recovery of behavioural responsiveness (Mortazavi et al., 2019). My findings demonstrate for the first time that, during presentation of a complex narrative stimulus under deep anaesthesia, there is a widespread impact on connectivity across both sensory and higher-order networks that persists for a time into upon the recovery of consciousness.

To investigate alterations of functional connectivity upon recovery of consciousness, I carried out comparisons of connectivity values between the awake and recovery states. Upon recovery of consciousness, in the resting state, all brain networks showed full restoration to baseline awake state levels of connectivity. By contrast, in the Taken condition, between-network connectivity remained significantly elevated relative to wakefulness. Functional connectivity values suggest that stimulus-driven cognition during recovery is more similar to deep anaesthesia than wakefulness, which suggests that cognitive processes may still be impacted by anaesthesia immediately upon recovery of consciousness. This interpretation was consistent with results of auditory detection task, which showed that the reaction time in the recovery state was significantly slower than in the awake state.

Furthermore, direct examination of global information processing during the Taken condition, measured via whole brain ISC analyses, supported the functional connectivity findings. The widespread ISC, across temporal, frontal, parietal and salience network regions,

that was present during wakefulness, was dramatically reduced and limited to primary auditory regions under deep anaesthesia, as expected from previous results (Naci et al., 2018). However, in recovery, the ISC remained limited to within the temporal cortex bilaterally, including primary and secondary auditory processing regions, but did not extend to networks outside the temporal lobes, suggesting limited extra-modal processes remain impacted immediately upon recovery. This result further suggests that at the point of recovery of consciousness following anaesthesia, the neural systems underlying complex cognition remain functionally altered relative to baseline wakefulness.

In summary, these results suggested that the use of a naturalistic paradigm offers important insights beyond those produced by both clinical behavioural assessment of conscious awareness and resting state fMRI. Converging results from the narrative condition suggested that immediately upon recovery complex cognition is impaired relative to full wakefulness. Both resting state neuroimaging and behavioural scales used for determining recovery from anaesthesia fail to capture these impairments at recovery.

My results are consistent with a limited number of previous studies showing that some measures of brain dynamics may not restore to baseline levels upon regaining of consciousness. In a recent animal study, Pal et al. (2019) showed that rats who were awakened from sevoflurane-induced unconsciousness did not show a return to baseline wakeful EEG measures of cortical connectivity and dynamics. However, these results were difficult to generalise to humans, as the measure of wakefulness used, i.e. the presence of the righting reflex, cannot be linked to measures of conscious cognition that we use as proxy for consciousness in humans. Indeed, the authors acknowledge that their observations may speak only to changes in level of arousal, rather than phenomenological consciousness or cognitive processing. Another study, in humans, examined graph theoretical measures of resting state fMRI data before, during and after propofol-induced unconsciousness (Monti et al., 2013). The clustering coefficient, a measure of how closely regions of interest cluster together, was reduced in deep anaesthesia, as expected, but remained reduced in wakeful recovery. This suggested that the recovery state was characterised by more localised information processing, and less integration of information across various brain regions. A more recent resting state study examined entropy, or brain state complexity, during anaesthesia and reported that, during recovery, entropy in high-order regions failed to restore to awake levels (Liu et al., 2019). However, as these two studies examined the resting state only, their effects could not

be specifically linked to cognitive processing. Långsjö et al (2012) induced a state of deep anaesthesia via the anaesthetic agent dexmedetomidine, from which some participants could be awakened by tactile stimulation despite continuous dosing of the drug. They found that individuals could recover conscious responsiveness without producing a corresponding return of neocortical reactivity, as measured by PET imaging. The authors suggested that recovery of consciousness occurs in a bottom-up manner, with emergence into a more basic conscious state preceding recovery of higher-order elements of cortical processing that are necessary for rich conscious experiences. The lack of restoration of functional connectivity to baseline levels shown in my study is consistent with this suggested hierarchy of recovery. However, similarly to Monti et al. (2013) and Liu et al. (2019), Långsjö et al. (2012)'s study could not directly measure cognitive processes or conscious contents because these are intractable during spontaneous cognition in the resting state. By contrast, my study, for the first time, used a direct measure of stimulus-driven information processing to test whether conscious cognition at recovery was impaired relative to awake baseline. To my best knowledge, this is the first study that shows impaired neurocognition at recovery from anaesthesia with direct assessment of stimulus-driven information processing.

My findings add to our knowledge about how brain systems recover following anaesthesia, and have several implications. Firstly, they inform how we should expect the brain to function in the immediate aftermath of anaesthesia. This knowledge is key to coma studies where brain reactivity at the point of recovery from sedation is assessed to test for the presence of covert awareness (e.g. Duclos et al., 2020). In such studies, brain reactivity to external stimuli is examined soon after sedation has ceased, to determine a patient's level or likelihood of recovery in coma. However, in order to accurately judge how closely an individual's brain activity resembles that of a healthy person, it must first be established what stimulus-driven reactivity upon recovery from anaesthesia looks like in healthy individuals.

Secondly, my findings have implications for our understanding of conscious awareness and how states of conscious processing should be understood. It has been proposed that the traditional representation of the continuum of 'levels' of consciousness, e.g. awake > sedated/sleeping > coma, is not appropriate. Bayne and colleague (2016) have argued that it is unclear how various states of consciousness (e.g. mild anaesthesia vs dreaming) can be coherently and accurately ordered relative to each other. Rather, they suggest that a more appropriate method of modelling consciousness is to do so in a

multidimensional space (Bayne et al., 2016), where populations with altered consciousness can be rated on multiple dimensions, including conscious content and functionality, that could be measured in terms of both behavioural and neural evidence (Bayne et al., 2017). This provides an interesting framework for interpreting my results, as participants in the immediate aftermath of anaesthesia exhibit restored levels of response to command and intrinsic spontaneous brain activity, but altered stimulus detection speed and stimulus-evoked cognition. Thus, my results underscore the benefits of characterising states of consciousness in terms of multiple dimensions, as it allows a more nuanced and comprehensive understanding of altered states of consciousness.

Finally, it is important to consider that conscious cognition in my study was measured only at the point of immediate recovery. Future should investigate the time schedule for the reconstitution of cognitive processes by measurements at repeated time-points after recovery, to determine when cognition is fully restored following loss of consciousness. This will help to derive evidence-driven clinical guidance regarding the time of full recovery from anaesthesia for healthy individuals. It should be noted that this may differ for patients who undergo general anaesthesia for medical reasons, who may have underlying comorbidities that will likely affect time of recovery.

Developing a neural marker of auditory narrative understanding and investigating covert awareness under deep anaesthesia

The aim of chapter 3 was to use the same naturalistic paradigm to investigate whether neural signatures of narrative understanding, and therefore conscious awareness, could be detected in any participants who were, according to their clinical behavioural designation, unconscious under deep anaesthesia. In experiment 1, 25 participants listened to the Taken narrative and rated how suspenseful the plot was, from ‘least’ to ‘most’, at two second intervals. I investigated how similar ratings of different individuals were to each other, and established how closely the average ratings followed narrative plot developments, to examine whether suspense ratings could serve as a qualitative proxy of narrative understanding. In experiment 2, I examined the fMRI data from the Taken condition of the experiment outlined in Chapter 2. Using the suspense ratings as a proxy for narrative understanding, I investigated, in the awake state, the neural basis of the sustained attention and emotional processes concomitant with conscious understanding of the story’s narrative. I examined this

at both the group and single-subject level to establish whether the neural markers of narrative understanding could be reliably detected at the individual level. I then carried out the same analysis on data from participants under deep anaesthesia, to investigate whether these neural markers could be detected in any deeply anaesthetized individuals.

Results from experiment 1 strongly supported my first two hypotheses, i.e. that that suspense ratings would be similar across different individuals, and would closely follow the narrative's plot developments over time. Suspense ratings were highly significantly correlated between individuals ($r=0.90$). My analysis of the peaks and troughs of the average ratings demonstrated that participants' experience was driven not only by basic physical properties of the sound, but, primarily, by an understanding of the story's narrative that requires ongoing higher-order cognitive processes. Therefore, it was appropriate to use the average suspense rating as a psychological descriptor reflecting, albeit indirectly, the sustained attention and emotional processes necessary for conscious understanding of the story's narrative.

In experiment 2, I examined the neural basis of suspense processing in participants undergoing fMRI. In the awake state, complex cognition during the narrative was related to activity in a set of brain areas, with clips rated as 'highly suspenseful' predicting stronger activity in these regions. These included regions of the auditory attention and language processing network (Naci et al., 2013; Michalka et al., 2015; Tobyne et al., 2018), in particular the supramarginal gyrus, which has been associated with higher-order auditory functions, such as phonological (Celsis et al., 1999) and short-term memory processing (Becker et al., 1999). Additionally, they included nodes of the salience network, i.e., the left/right anterior insular gyrus, cingulate sulcus, brainstem areas, and the thalamus (Menon et al., 2015; Seeley et al., 2019). In plot-driven narratives, suspense is an emotion elicited through ongoing attention to and cognitive appraisal of the story's plot. Hence, it is fitting that the perception of suspense during the Taken narrative was subserved not only by regions of the auditory attention network, but also by key nodes of the salience network, which are involved in supramodal attention and emotion processing (Menon et al., 2015; Seeley et al., 2019). Activation patterns due to processing of suspense were dissociable from those due to processing of low-level auditory features (i.e., the sound envelope), which activated regions of the primary and secondary auditory cortex.

Importantly, suspense ratings activated a highly similar subset of regions at the individual-level, with 12/16 participants (75%) exhibiting activity in most (i.e. at least 5/8) of the areas observed at the group level, and the remaining 25% of participants showing either 3/8 or 4/8 clusters. This result suggested that the neural signature of narrative understanding could be reliably observed in individual participants. Therefore, I could use it as a predictor to test for the presence of conscious narrative understanding in individual deeply sedated participants.

In deep sedation, at the group level, narrative-related brain activity was abolished under deep anaesthesia, as expected. This was consistent with a previous study using the same dataset (Naci et al., 2018), which showed that overall sensory-driven information processing was extremely reduced, and primarily limited to small clusters of activation in the primary auditory cortex during deep anaesthesia. However, by contrast to the group finding, during the Taken story, one deeply anaesthetised participant showed activation in all eight areas associated with processing of suspense in the awake group, and the same set of regions related to their own processing of suspense in the awake state. These findings suggest that this individual could follow the plot and feel suspense on a moment-to-moment basis in a similar way to healthy individuals, and therefore, may have been consciously aware despite the clinical designation of ‘unconscious’ during deep anaesthesia. Extrapolated to the whole sample, this result suggests that 1/17 (6%) individuals were aware during deep anaesthesia.

To the best of my knowledge, this is the first time that specific time-locked neural activations have been linked to a behavioural proxy for narrative understanding in an audio-only narrative, and therefore could be used to interrogate higher-order conscious processing in individual participants under deep anaesthesia. While one previous study examined the processing of suspense as a proxy for narrative understanding during an audio-visual movie (Naci et al., 2014), previous studies using the Taken audio story (Naci et al., 2017; 2018) have not directly examined the neural markers related to the understanding of its narrative. Naci et al. (2014) found that ratings of suspense during a brief movie by Alfred Hitchcock reliably predicted activity in the fronto-parietal network, traditionally linked to attention and executive function, in an independent group of individual healthy participants. Differently to this previous study, suspense ratings in my study predicted activity in nodes of the auditory attention and salience network rather than fronto-parietal areas. The reason for this difference may be explained by the different modalities of the stimuli used in the two studies, i.e. an

audio-visual narrative in Naci et al. (2014) compared to an auditory-only narrative here. Previous studies have demonstrated differences in the regions involved in higher-order processing of stimuli depending on their sensory modality. While visual-based attentional tasks have been found to activate a set of fronto-parietal regions, including frontal eye-fields, the superior parietal lobule and the intraparietal sulcus, auditory attention tasks are associated with activity in temporal regions, such as the superior and medial temporal gyri (Braga, Wilson, Sharp, Wise, & Leech., 2013; Michalka et al., 2015; Tobyne et al., 2017).

Results from experiment 2 provide a novel marker for measuring accidental awareness under general anaesthesia (AAGA). AAGA refers to rare instances in which patients who have been given an anaesthetic dose appropriate for clinical general anaesthesia and appear non-responsive according to standard behavioural scales (e.g. Ramsay et al., 1974) nevertheless retain conscious awareness. While the most commonly cited incidence rates estimate that 0.1-0.2% of patients experience AAGA (Liu, Thorp, Graham & Aitkenhead, 1991; Sandin et al., 2000; Sebel et al., 2004), the traditional methods of detection may underestimate its true incidence. These methods require patients to retrospectively recall their experience; however, anaesthetic agents induce amnesia, which impairs recall of experiences under anaesthesia (Brown, Lydic & Schiff, 2010; Perousansky & Pearce, 2011). Furthermore, the isolated forearm technique (IFT; Turnstall, 1977), which does not require recall of experience and instead measures command-following in the moment, produces a substantially higher rate of 4.6% (Sanders et al., 2017). A positive result on the IFT requires several capacities in addition to conscious awareness, including motor responsiveness, and motivation and intention to act – any of which may be impaired by anaesthetic agents. Indeed, we have recently shown that a significant proportion of healthy individuals (17%) become unable to make basic behavioural responses (e.g., button press) according to commands under anaesthesia, even when they are consciously aware at moderate dosages (Deng et al., 2020). Therefore, while a positive result on the IFT test demonstrates awareness, a negative result cannot be taken as evidence for lack of awareness. My results suggest that the true incidence may be more in line with rates indicated by the IFT than rates produced by measures that require explicit post-anaesthesia recall. The higher rate reported in my study, compared to IFT, may reflect the lower task demands of understanding an auditory story compared to the command-following required for IFT. However, it is important to note that replication in a larger sample is required to generalise the 6% incidence rate to the wider population.

Nevertheless, my findings suggest that there is a clear advantage to using a neuroimaging paradigm that monitors brain activity specifically linked to higher-order cognitive processes in comparison to command-following protocols, such as the IFT. They also suggest an advantage over current state-of-the-art EEG-based anaesthesia monitors, such as the BIS (Avidan et al., 2008), that only capture global signals that are not specifically related to cognitive processes and yield poor specificity (Schneider et al., 2002). My results, therefore, emphasise the importance of developing techniques that i) do not require explicit recall, ii) are easily engaged with/ do not require compliance with task demands and iii) examine distinctive brain signals that are specifically linked to conscious awareness.

This novel finding mirrors those from patients with DoC, which demonstrate that a lack of behavioural responsiveness does not always indicate a lack of awareness (Monti et al., 2010; Naci et al., 2014; Kondziella et al., 2016; Naci et al., 2018). It highlights limitations of bedside scales for measuring behavioural responsiveness as a proxy of awareness, as these behavioural scales cannot shed light on covert cognition. This is further emphasised by my results from Chapter 2, which suggest that behavioural scales used for determining recovery from anaesthesia fail to capture impairments in information processing-related neural activity that persist after recovery of overt behavioural response.

It should be noted that the detection of covert awareness described here took place in a research context, where anaesthesia was administered to healthy volunteers. My findings require replication in a clinical sample to determine if the rate generalises to patients undergoing presurgical general anaesthesia. In light of the limited applicability of fMRI at the bedside, due to its cost and the requirement to lay in a scanner, future research should investigate this marker with portable, cheaper technologies such as EEG and GSR, to examine their applicability as a depth-of-anaesthesia monitor in a clinical context.

Conclusion

In this thesis, I carried out two studies to address important gaps in anaesthesia research. In my first study, I used an auditory-only naturalistic paradigm to investigate the neurocognitive changes associated with recovery of consciousness in the immediate aftermath of propofol-induced deep anaesthesia. I demonstrated that, unlike the resting state, during the processing of the auditory narrative at recovery, functional connectivity between

widespread regions remained significantly elevated relative to the awake state, and was indistinguishable from the unconscious state. In the second study, I used the same naturalistic paradigm to investigate whether neural markers of preserved conscious awareness could be detected in individual deeply anaesthetised participants. One out of 16 participants demonstrated cognition-specific neural activity time-locked to narrative plot changes, suggesting they followed the story on a moment-to-moment basis similarly to awake participants. My findings underscore the limitations of traditional methods, such as behavioural scales and resting state neuroimaging paradigms, in capturing information related to conscious processing of stimuli. Paradigms that present individuals with complex and plot-driven naturalistic stimuli provide a valuable additional approach to interrogating cognitive processing and how it is impacted by loss and recovery of consciousness during anaesthesia.

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