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Influence of epoch length on measurement of dynamic functional connectivity in wakefulness and behavioural validation in sleep

Rebecca S. Wilson [a], Stephen D. Mayhew [a], David T. Rollings [a,b], Aimee Goldstone [a], Izabela Przezdzik [a], Theodoros N. Arvanitis [c], Andrew P. Bagshaw [a,*]

[a] Birmingham University Imaging Centre School of Psychology, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom
[b] Department of Neurophysiology, Queen Elizabeth Hospital, Birmingham, B15 2TH, United Kingdom
[c] Warwick Manufacturing Group, University of Warwick, Coventry, CV4 7AL, United Kingdom

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ABSTRACT

Conventional functional connectivity (FC) analysis of fMRI data derives a single measurement from the entire scan, generally several minutes in duration, which neglects the brain's dynamic behaviour and potentially loses important temporal information. Short-interval dynamic FC is an attractive proposition if methodological issues can be resolved and the approach validated. This was addressed in two ways; firstly we assessed FC of the posterior cingulate cortex (PCC) node of the default mode network (DMN) using differing temporal intervals (8 s to 5 min) in the waking-resting state. We found that 30-second intervals and longer produce spatially similar correlation topography compared to 15-minute static FC measurements, while providing increased temporal information about changes in FC that were consistent across interval lengths. Secondly, we used NREM sleep as a behavioural validation for the use of 30-second temporal intervals due to the known fMRI FC changes with sleep stage that have been observed in previous studies using intervals of several minutes. We found significant decreases in DMN FC with sleep depth which were most pronounced during stage N2 and N3. Additionally, both the proportion of time with strong PCC-DMN connectivity and the variability in dynamic FC decreased with sleep. We therefore show that dynamic FC with epochs as short as tens of seconds is a viable method for characterising intrinsic brain activity.

Introduction

Functional connectivity (FC) can be measured using fMRI as the temporal correlation of low-frequency (<0.1 Hz) blood-oxygenation-level-dependent (BOLD) signal fluctuations from separate brain regions (Friston, 2011). These brain regions collectively form intrinsically connected networks (ICNs), which are believed to underpin cognitive function (Hellyer et al., 2014; Stevens and Spreng, 2014). As such, FC has increasingly been used to characterise ICNs in the healthy and pathological brain (Fox and Greicius, 2010; Van Dijk et al., 2010). Data is typically acquired during 'rest' when an experimental subject is not engaged in a task, and the analysis of resting data generally proceeds by calculating the FC over several minutes. Although this static measure of FC enables a useful insight into the average strength of the functional relationship between brain regions, the brain is a dynamic system that clearly alters its state on much shorter timescales. In recent years there has been considerable interest in using fMRI to quantify dynamic changes in FC within and between ICNs (Buckner et al., 2013; Hutchison et al., 2012), which reflect alterations in neuronal and behavioural states with greater temporal precision.

There is evidence that FC fluctuates over an fMRI scan lasting several minutes (Allen et al., 2014; Brodbeck et al., 2012; Chang and Glover, 2010; Gonzalez-Castillo et al., 2014; Hutchison et al., 2012; Tagliazucchi et al., 2012b; Zalesky et al., 2014), and that these fluctuations have a neurophysiological underpinning (Brookes et al., 2011; Jerbi et al., 2010; Miller et al., 2009; Tagliazucchi et al., 2012b; Thompson et al., 2013). The quantification of dynamic FC opens up a new dimension for analysis, offering an alternative and complementary methodology to understand the complexities of brain function. For example, interactions between ongoing brain activity and stimulus evoked responses have been reported (Mayhew et al., 2013; Sadaghiani et al., 2010), and a dynamic FC approach would allow for a more complete characterisation of inter-individual and inter-trial variability in the brain's response to individual stimuli. Sleep is another dynamic behaviour which would benefit from improved characterisation using dynamic FC. Sleep is characterised clinically using 30-second EEG epochs, based on robust electrophysiological signatures which represent different

Abbreviations: FC, Functional connectivity; PCC, Posterior cingulate cortex; NREM, Non-rapid eye movement sleep; ICN, Intrinsically connected networks; mPFC, Medial prefrontal cortex; IPL, Inferior parietal lobe; MTL, Medial temporal lobe; PH, Parahippocampal gyrus

* Corresponding author at: School of Psychology, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom.
E-mail address: a.p.bagshaw@bham.ac.uk (A.P. Bagshaw).

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sleep stages (AASM, 2007). Understanding what these sleep stages represent in terms of alterations to brain function and specifically the modification of large scale brain networks is challenging with EEG alone, although EEG remains the 'gold standard' technique to define the sleep stages themselves. As such, investigating sleep using fMRI requires simultaneous EEG-fMRI acquisition (Duyn, 2012). Harnessing the ability of fMRI to characterise brain networks will enable further understanding of the brain processes occurring within a stable sleep stage, but also the transitions between stages, the processes underlying which remain poorly understood.

These questions require the ability to quantify FC at timescales much smaller than those generally used. However, there has been an understandable reluctance to compute fMRI FC using temporal intervals shorter than several minutes due to the risk of introducing spurious correlations (Van Dijk et al., 2010). Electrophysiological recordings clearly demonstrate that averaging several minutes worth of data loses the dynamic interplay between brain regions which is central to the brain's function, thus short-interval dynamic FC is an attractive proposition if methodological issues can be resolved. While some previous work has investigated the appropriate temporal interval for FC calculation for various applications (Leonardi and Van De Ville, 2014; Sakoğlu et al., 2010; Whitlow et al., 2011), a clear consensus remains to be found. One major difficulty is validating the results in the absence of a gold standard measurement of FC or behavioural correlate of FC.

In this study, we addressed this question by comparing static FC to the mean dynamic FC calculated by averaging the correlation values from each short epoch, using a range of epoch lengths. Our rationale was that if FC values from short epochs are dominated by noise, their average will tend to zero rather than converging on the static value of FC over the same period. To address the question of validation further, we identified a behavioural situation where FC changes are relatively well characterised and a clear hypothesis of expected results can be formed. We studied changes in FC that have previously been observed in the descent into sleep, with a focus on the default mode network (DMN) as the network that has received the most attention and has the clearest changes (Horovitz et al., 2008, 2009; Larson-Prior et al., 2009; Sämann et al., 2010; Spoormaker et al., 2010).

The DMN is the most widely studied ICN within the brain. It comprises of the posterior cingulate cortex (PCC), medial prefrontal cortex (mPFC), inferior parietal lobe (IPL), medial temporal lobe (MTL) and the parahippocampal gyrus (PH) (Buckner et al., 2008). The PCC is generally recognised as the central region of the DMN (Fransson and Marrelec, 2008) and is one of the most globally connected areas of the brain (Cole et al., 2010). A number of different behaviours have been linked with the DMN, including self-referential thought, emotional processing (Gusnard and Raichle, 2001) and disengagement from external stimuli (Fransson, 2005). This has resulted in the DMN becoming a focus of research for many psychiatric disorders (Broyd et al., 2009). In addition, FC of the PCC is believed to play an important role in maintenance of consciousness (Bagshaw and Cavanna, 2013; Cavanna and Trimble, 2006; Heine et al., 2012; Leech et al., 2012; Uehara et al., 2013; Vanhaudenhuyse et al., 2010). As such, FC has been investigated in several studies of the most common alteration of consciousness, non-rapid eye movement sleep (NREM) (Horovitz et al., 2008, 2009; Larson-Prior et al., 2009; Sämann et al., 2010; Spoormaker et al., 2010) and rapid eye movement (REM) sleep (Koike et al., 2011).

It has been shown that during light sleep (non-REM stage 1: N1), DMN FC is maintained (Horovitz et al., 2008; Larson-Prior et al., 2009), although as sleep progresses there appears to be a PCC–mPFC 'decoupling' with reduced FC strength between these brain regions (Horovitz et al., 2009; Sämann et al., 2010). This suggests a possible breakdown in long-range FC (Massimini et al., 2005) and a large-scale change in the functional configuration of the brain during sleep (Spoormaker et al., 2010). However, this issue has only been addressed using epoch lengths of several minutes to determine FC strength. The aim of this study is to show that using shorter epoch lengths can replicate these results, which would provide support for using dynamic measures of FC, presenting a much richer and more behaviourally relevant temporal FC profile. We therefore restricted our analysis to the PCC as a seed region and the other DMN nodes as targets in order to focus on providing a detailed investigation of the effect of epoch length on FC within the DMN during wake and sleep.

The current study investigated dynamic FC during wakeful rest and sleep, using sleep not only as a behavioural validation of our methodology but also as a behaviour with temporal variation, as seen on EEG over seconds not minutes, that would benefit from the approach of dynamic rather than static FC. We focussed on the DMN as one of the most well studied ICNs which, in the PCC node, contains one of the brain regions previously linked with the changes in consciousness experienced during sleep (Sämann et al., 2011). We aimed to investigate how FC strength calculated during the awake resting-state varied with decreasing length of data epochs used in a seed-based analysis. Once validated, we applied this short-window approach to EEG-fMRI recordings during sleep to investigate how the temporal dynamics of DMN FC varied between different sleep stages.

Materials and methods

Experimental design

Eight healthy adult volunteers (5 male, age 32 ± 6 years) participated in the study. The participants were pre-screened for any neurological, medical or sleep disorders. Participants gave written informed consent and the study was approved by the Research Ethics Board of the University of Birmingham. Following a normal night of sleep participants attended a single 15 min, resting-state fMRI session during the daytime. Participants returned on a later date at their usual bedtime (between 22:00 and 00:00), in order to facilitate natural sleep, and underwent simultaneous EEG-fMRI.

Waking RS fMRI acquisition

During the daytime session participants were instructed to keep their eyes open and not think of anything in particular throughout a 15-min fMRI scan. A 3T Philips Achieva MRI scanner with an eight-channel head coil was used to acquire T1-weighted anatomical images (1 mm isotropic voxels) and T2*-weighted fMRI data with whole brain coverage (3 × 3 × 4 mm voxels, TR = 2000 ms, TE = 35 ms, 450 volumes, flip angle 80°, SENSE factor = 2). Subject's respiratory and cardiac cycles were measured using a pneumatic bellows and pulse oximeter. Participants wore earplugs and headphones to minimise acoustic noise and their head was supported with foam padding to reduce motion artefact.

Sleep EEG-fMRI acquisition

The subjects returned for the second session in which EEG data were recorded from 62 Ag/AgC1 MR-compatible EEG electrodes (EasyCap), with additional electrodes placed below the left clavical to record the electrocardiogram (ECG), and below the left eye to record the electrooculogram (EOG). The reference electrode was positioned at FCz, and the ground electrode at AFz. EEG data were acquired at a sampling rate of 5 kHz, with hardware filters 0.016–250Hz, using MR-compatible BrainAmp MR-plus EEG amplifiers (Brain Products, Germany). Impedance at all recording electrodes was maintained below 20 kΩ. The EEG clock was synchronised to the MR scanner clock to ensure consistent sampling of the gradient artefact (Mandelkow et al., 2006). The participants were instructed to sleep if they could and to signal to terminate the session once when they were no longer able to sleep. Whole brain T2*-weighted fMRI data (3 × 3 × 4 mm voxels, TR = 2000 ms, TE = 35 ms, 450 volumes, flip angle 80°, SENSE factor = 2) were acquired in consecutive 15-min scans. Subject's respiratory and cardiac