High Frequency Functional Brain Networks in Neonates Revealed by Rapid Acquisition Resting State fMRI

Adam P.R. Smith-Collins,^{1,2*} Karen Luyt,¹ Axel Heep,¹ and Risto A. Kauppinen²

¹Neonatal Neuroscience, St Michael's Hospital, School of Clinical Sciences, University of Bristol, Bristol, United Kingdom

²CRIC Bristol and School of Experimental Psychology, University of Bristol, Bristol, United Kingdom

Abstract: Understanding how spatially remote brain regions interact to form functional brain networks, and how these develop during the neonatal period, provides fundamental insights into normal brain development, and how mechanisms of brain disorder and recovery may function in the immature brain. A key imaging tool in characterising functional brain networks is examination of T2*-weighted fMRI signal during rest (resting state fMRI, rs-fMRI). The majority of rs-fMRI studies have concentrated on slow signal fluctuations occurring at <0.1 Hz, even though neuronal rhythms, and haemodynamic responses to these fluctuate more rapidly, and there is emerging evidence for crucial information about functional brain connectivity occurring more rapidly than these limits. The characterisation of higher frequency components has been limited by the sampling frequency achievable with standard T2* echoplanar imaging (EPI) sequences. We describe patterns of neonatal functional brain network connectivity derived using accelerated T2*-weighted EPI MRI. We acquired whole brain rs-fMRI data, at subsecond sampling frequency, from preterm infants at term equivalent age and compared this to rs-fMRI data acquired with standard EPI acquisition protocol. We provide the first evidence that rapid rs-fMRI acquisition in neonates, and adoption of an extended frequency range for analysis, allows identification of a substantial proportion of signal power residing above 0.2 Hz. We thereby describe changes in brain connectivity associated with increasing maturity which are not evident using standard rs-fMRI protocols. Development of optimised neonatal fMRI protocols, including use of high speed acquisition sequences, is crucial for understanding the physiology and pathophysiology of the developing brain. *Hum Brain Mapp* 36:2483–2494, 2015. © 2015 Wiley Periodicals, Inc.

Key words: fMRI; neonatal; multiband magnetic resonance imaging; connectivity

Contract grant sponsor: The David Telling Charitable Trust (to K.L.); Contract grant sponsors: an Academy of Medical Sciences Clinical Lecturer Starter Grant [funded by The Wellcome Trust, The Medical Research Council, The British Heart Foundation, Arthritis Research UK, Prostate Cancer UK (to A.S.C.)].

*Correspondence to: Adam P.R. Smith-Collins, CRIC Bristol, 60 St Michael's Hill, Bristol BS2 8DS, United Kingdom.
E-mail: as13726@bristol.ac.uk

Karen Luyt, Axel Heep, and Risto A. Kauppinen contributed equally to the conception of this manuscript.

Received for publication 16 January 2015; Revised 2 March 2015; Accepted 3 March 2015.

DOI: 10.1002/hbm.22786

Published online 18 March 2015 in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

Neuronal cell migration and synaptogenesis forming connectivity between deep brain structures and cortical layers is crucial for formation and integration of networks in the newborn brain. Understanding the evolution of functional connectivity between disparate brain regions in the immature brain gives insight in normal human brain development, and may address the consequences of brain injury in early life on integrity of functional brain networks. A key method for mapping neural integration non-invasively is analysis of functional T2*-weighted MRI (fMRI) images acquired 'at rest' (rs-fMRI) [Biswal et al., 1995; Fransson, 2005] to infer coordination of neural activity across disparate brain regions ('functional' connectivity). This method provides complementary information to other techniques assessing macroscopic structural brain lesions or the integrity of white matter tracts. There is increasing interest in exploring newborn brain development using rs-fMRI [Fransson et al., 2007; Smyser et al., 2010, 2013], with evidence that network connections are disrupted in both the short [Smyser et al., 2013] and long-term [White et al., 2014] in infants born prematurely.

Most rs-fMRI work has focussed on fluctuations in T2*-weighted, blood oxygenation level dependent (BOLD) fMRI signals occurring at <0.1 Hz, despite the fact that neuronal fluctuations as measured with electrophysiological means occur much more rapidly. Although haemodynamic changes underpinning the T2*-weighted fMRI signals occur more slowly than this due to the inherent nature of neurovascular coupling [Liu, 2013], limiting investigation to those changes occurring at <0.1 Hz leads to potential loss of crucial information about neonatal brain function. There is emerging evidence that meaningful fluctuations in BOLD activity in adult brain networks occur beyond commonly applied frequency filter limits [Boubela et al., 2013; Kalcher et al., 2014; Niazy et al., 2011].

One reason for the concentration on very low frequency components is a limitation on the ability to characterise higher frequency fluctuations without aliasing, dependent upon the speed at which whole brain rs-fMRI acquisition can be accomplished. With typical fMRI protocols, the time taken to acquire each whole brain volume (repetition time, TR) is of the order of 2–3 s, meaning that frequency information from BOLD fluctuations cannot be reliably sampled at frequencies above 0.17–0.25 Hz without contamination by aliasing. A method of improving rs-fMRI sampling frequency is accomplished by exciting multiple brain slices simultaneously and using spatially distributed receiver coils to encode anatomical data, allowing accelerated data reconstruction, the so-called multiband MRI [Larkman et al., 2001; Moeller et al., 2010]. The employment of multiband echoplanar imaging (EPI) and robust rs-fMRI acquisition protocols improves temporal resolution and robustness of resting state networks [Feinberg et al., 2010], and allows effective characterisation of higher

frequency fluctuations than has previously been possible [Boubela et al., 2013; Kalcher et al., 2014; Niazy et al., 2011; Smith et al., 2012].

A consideration with all analysis of spontaneous BOLD fluctuations is signal contamination from non-neuronal influences on BOLD signal, particularly physiological processes including respiratory and cardiac cycles and autonomic changes. Although frequency filtering may remove some of these confounding contributions, many occur within the frequency bands of interest, and others are aliased into them [Gohel and Biswal, 2015], meaning that frequency filtering alone will not obviate against signal contamination with physiological noise. In a subgroup of our infants, we characterised the signal power of these physiological variables, determined their impact on regional BOLD signal fluctuations and estimates of functional connectivity, and attempted to remove any influence from our data using retrospective image correction [RETROICOR; Glover et al., 2000].

We characterised regional BOLD signal acquired from 21 ex-preterm neonates at two different TR values, using multiband and standard EPI sequence. We additionally compared findings when the multiband sequence was used at the same TR as the standard sequence, and compared stability across multiple sessions with the short TR multiband sequence. We describe differences in the derived functional connectivity patterns and explore the characteristics of the rs-fMRI in terms of signal-to-noise-ratio (SNR) and power spectral density, and also explore the impact of different bandpass filters on the multiband data and the influence of physiological parameters on the acquired data in order to demonstrate the potential this approach offers for improved characterisation of infant brain networks.

METHODS

Subjects

Twenty one infants (seven male) born at <32 weeks completed gestation (mean $29 \text{ w} \pm 2 \text{ d}$, range 26 ± 0 – 31 ± 5) were scanned at term corrected age (mean $37 \text{ w} \pm 5 \text{ d}$, range 37 ± 0 – 40 ± 0). Infants with congenital anomalies, known genetic disorders, congenital heart disease or problems requiring surgical intervention were excluded from the study. No infants were receiving positive pressure respiratory support at the time of scanning, although three infants were receiving supplementary oxygen at flow rates $<0.1 \text{ L/min}$. All parents gave informed, written consent. The study has National Research Ethics approval (Ref 10/GO106/10) on behalf of the NHS Health Research Authority, and was performed in accordance with the Declaration of Helsinki.

All infants were scanned in natural sleep, without sedation. Prior to scanning, they were fed and settled to sleep in the preparation room. To minimise acoustic noise, we

utilised mouldable putty (Affinis, Coltene, Switzerland) in the external auditory canal, and covered the ears with ear muffs ('Minimuffs,' Nautus, Seattle) with a noise reduction rating of 7 dB. In the scanning environment, infants were placed in a custom made vacuum moulding cushion, which reduced movement and vibration, and may have made infants feel more secure.

MRI compatible physiological monitoring equipment (Invivo Expression, Gainesville, FL) was used to monitor ECG and oxygen saturation. In a subgroup of 10 infants, additional respiratory monitoring was added and ECG and respiratory data were exported via a Biopac MP150 system and AcqKnowledge Software (Biopac Systems, CA). Infants were continuously monitored during scanning for movement, episodes of desaturation or becoming unsettled or distressed.

MRI Acquisition

MRI images were acquired using a 3T Siemens Magnetom Skyra MRI scanner (Siemens, Erlangen, Germany) with a 32-channel head coil. Infants were held in a vacuum-moulded whole body cushion, with the centre of the head placed as close as possible to the coil isocentre. Functional images were acquired in two runs:

Standard EPI protocol: TR 1,700 ms, TE 30 ms, Flip angle 90°, FOV 160 × 160, 30 slices 2.5 × 2.5 × 2.5 mm³, 0.7 mm gap. Phase encoding A>>P, phase oversampling 0%, partial fourier off, GRAPPA off, bandwidth 1,698 Hz/Px. One hundred and seventy volumes acquired in 295 s.

Short TR multiband protocol: TR 906 ms, TE 30 ms, Flip angle 60°, FOV 160 × 160, 30 slices 2.5 × 2.5 × 2.5 mm³, 0.7mm gap. Acceleration factor 3, interleaved. Phase encoding A>>P, phase oversampling 0%, partial fourier off, GRAPPA off, bandwidth 1,698 Hz/Px. Three hundred volumes acquired in 280 s.

For both protocols, in-plane acceleration methods (GRAPPA) were avoided due to increased motion susceptibility artefact, and partial Fourier was not used due to increased risk of regional signal dropout.

All infants had high resolution T1-weighted and T2-weighted structural images acquired according to institutional neonatal MRI protocol.

In a subgroup of 15 subjects, two additional sequences were acquired. Firstly, a second run of 300 images were acquired with the short TR multiband protocol, identical to that described above. This was subsequently used to determine the stability and reproducibility of estimated network connections. Secondly, to investigate whether differences between the sequences could be ascribed to the method of scan acquisition, rather than the speed of acquisition, a run of 170 images was acquired with a long TR multiband protocol (TR = 1,700 ms), using multiband acquisition but with parameters otherwise identical to the standard EPI protocol.

Image Processing and Analysis

fMRI images were preprocessed using SPM8 (WDIN, UCL, London), implemented in MATLAB (The Mathworks). Images were realigned and unwarped, head motion parameters estimated, normalised to an infant template [Shi et al., 2011] and then transformed into MNI space. They were smoothed with a Gaussian kernel (FWHM 4 × 4 × 4 mm). Signal-to-fluctuation-noise-ratio (SFNR) was determined within a 15 × 15 mm region of interest using the yw_epi toolbox for SPM (PNRC, Cincinnati).

Functional connectivity

The primary functional connectivity analysis was performed using the Conn toolbox [Whitfield-Gabrieli and Nieto-Castanon, 2012] for SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK). Resting state data were extracted from 100 regions of interest (ROIs), comprising the Brodmann areas, key areas of the 'default mode network' and thalami. Regressors of no interest, representing effects of subject movement and effects arising in white matter or CSF (based on maps from the neonatal template) were calculated and removed. In a subgroup of 10 subjects, we determined the potential impact of physiological noise on our data using retrospective image correction [RETROICOR; Glover et al., 2000]. Regressors describing effects attributable to cardiovascular effects (third order), respiratory effects (fourth order), and cardio-respiratory interactions were derived using the PhysIO toolbox for SPM [Kasper et al., 2009]. These regressors were used to clean up our imaging data and were included as additional potential nuisance factors. For the main comparison of connectivity effects detected by the different sequences, T2* data were bandpass filtered between 0.005 and 0.5 Hz. In a subsidiary analysis, the influence of reducing the upper filter limit to 0.2 Hz was investigated (see Results).

Inter-regional functional connectivity was calculated by multiple regression estimating the individual attributable influence of signal variance in each ROI on the signal in each other ROI. Reliability of connections across subjects was determined by *t*-tests, with correction for multiple comparisons with false discovery rate (FDR) control ($\alpha = 0.05$). In the subgroup where multiband sequences were repeated, we compared connectivity estimates determined independently from the first and second acquisition runs. Direct contrasts were made between connectivity parameters acquired using different acquisition protocols.

In a subsidiary analysis, the influence of corrected gestation age at the time of scanning on connectivity parameters were estimated across subjects using ANCOVA, and contrasted between acquisition protocols.

ROI signal extraction and power spectral analysis

Detailed analysis of the distribution of power within different frequencies of the extracted T2* signal was performed

TABLE I. Regions of interest from which detailed time series were analyzed for frequency characteristics

Region	MNI coordinates
Left primary motor cortex	(−39, −30, 51)
Right primary motor cortex	(39, −30, 51)
Left primary auditory cortex	(−57, −31, 7)
Right primary auditory cortex	(57, 31, 7)
Left thalamus	(−15, −22, 5)
Right thalamus	(9, −19, 9)
Dorsal anterior cingulate cortex	(−1, 8, 50)
Frontal pole	(−3, 44, 42)
Medial prefrontal cortex	(−6, 53, −8)
Posterior cingulate cortex	(−6, 48, 33)

Data were extracted from 6-mm radius spheres centred on the given coordinates in the normalised brains, transformed into MNI space. Coordinates derived from Fransson et al. [2013].

by extracting signal from 10 different ROIs (Table I) using the Rex toolbox for SPM (MIT, Cambridge, MA). The ROIs chosen were a subset of those investigated in another recent study examining distribution of the power of T2* in neonatal resting state data [Fransson et al., 2013]. Power spectral density was estimated using the Welch [1967] method, implemented in MATLAB, and calculated for different frequency bins of interest.

Additional T2* data were extracted from two further ROIs, one from left lateral ventricle which contained only CSF, and another containing grey matter from left temporal gyrus in the same slice as the ventricular ROI. Again power spectra were derived, and these were compared to those from respiratory and heart rate data, as detailed below.

Physiological data analysis

In a subgroup of 10 infants, ECG and respiratory cycle data were captured using the Biopac MP150 System and AcqKnowledge software, sampled at 100 Hz. The raw signal was bandpass filtered between 0.01 and 10 Hz. Physiological signals were time-locked to fMRI data acquisition and regressors reflecting cardiac, respiratory, and cardiac-respiratory interaction terms were derived using RETROICOR methods, implemented with the PhysIO toolbox for MATLAB, and were included in subsidiary analysis as described above.

Power spectral density analysis was performed on filtered ECG and respiratory signal using the Welch method, as described above for the T2* signal data.

RESULTS

Image Characteristics and Power-Frequency Distribution

High quality T2*-weighted images were acquired in all 21 neonates during natural sleep. Examples of raw images are presented in Figure 1. We acquired and analysed 72 functional data sets [21 ‘standard EPI’ (TR = 1,700 ms), 36 ‘fast’ multiband (TR = 906 ms), 15 ‘slow’ multiband (TR = 1,700 ms)]. Of these data sets, seven had required reacquisition due to sequences being terminated as a result of infant motion or waking from sleep (two standard, four short TR multiband, one long TR multiband).

SFNRs were similar between standard (mean 124, SD 53.3) and short TR multiband (mean 115.6, SD 45.4) sequences. There was a trend for lower SFNR for the long TR multiband (mean 54.2, SD 5.6), although not significantly lower than with short TR ($t(14) = 1.79$, $P = 0.1$).

Figure 1.

Example raw images from a single subject from T1 structural image (panel 1), long TR ‘standard’ T2* sequence (panel 2), and short TR ‘multiband’ sequence (panel 3).

Figure 2.

Mean power spectral density, averaged across 10 cortical brain regions for data acquired with low TR (906 ms) multiband (solid line), high TR (1,700 ms) multiband (dotted line), and standard (TR 1,700 ms) EPI (dashed line) sequences. Data are normalised to the same area under the curve across sequences.

Analysis of the power spectral density of signal fluctuations from 10 brain regions showed that a substantial proportion of spectral power in the multiband sequence occurred at frequencies that could not be sampled using the slower acquisition (Fig. 2; Table II). Indeed, even with TR = 1,700 ms, some power would be lost by use of typical bandpass filter limits of <0.2 Hz. Critically, use of the rapid acquisition multiband sequence samples events which cannot be directly measured using the slower sequences.

Similar to recent findings in adults [Kalcher et al., 2014], there was evidence that different brain regions had distinct patterns of power distribution (Fig. 3). Most had the majority of their power at low frequencies, but maintained some into higher frequency bands.

Functional Brain Networks

Estimates of brain networks with standard and multiband acquisitions

Functional connectivity analysis revealed a multitude of brain regions with significantly interrelated patterns of T2*

TABLE II. Proportion of power at individual frequency bands for short TR multiband (TR 906 ms), standard EPI (TR 1,700 ms), and long TR multiband (TR 1,700 ms) acquisitions

Frequency band	Proportion of power		
	Short TR multiband	Standard	Long TR multiband
<0.1 Hz	0.33	0.37	0.42
0.1–0.2 Hz	0.19	0.28	0.28
0.2–0.3 Hz	0.16	0.35	0.3
>0.3 Hz	0.32

Figure 3.

Power spectral density of resting-state fMRI data in different brain regions, estimated with the standard (dotted line) and low TR multiband (solid line) sequences. Data are normalised to the same area under the curve across brain regions and sequences.

signal fluctuation, implying functional connectivity. Table III and Figure 4 show the 10 most significant functional connections between disparate brain regions, as derived from the short TR multiband sequence, and the difference in connectivity estimates derived between the short TR multiband (column 1) and standard (column 2) EPI sequences. Of the 10 selected connections identified with the multiband acquisition, 7 were found to be significant with the standard acquisition. The other three connections showed smaller estimated connectivity weights, and did not reach significance

TABLE III. Functional connections revealed during rs-fMRI using the short TR (906 ms) multiband sequence (A), the standard EPI (TR 1,700 ms) sequence (B), the first (C) and second (D) acquisition runs from the subgroup with two multiband acquisition runs

Source	Target	A—Multiband			B—Standard			C—Multiband run 1			D—Multiband run 2		
		Beta	T(19)	p-FDR	Beta	T(19)	p-FDR	Beta	T(14)	p-FDR	Beta	T(14)	p-FDR
L primary somatosensory	L primary motor	0.57	19.2	<0.0001	0.56	9.89	<0.0001	0.54	17.25	<0.0001	0.54	18.03	<0.0001
R primary somatosensory	R primary motor cortex	0.55	18.08	<0.0001	0.59	10.58	<0.0001	0.53	12.52	<0.0001	0.54	16.93	<0.0001
R associative visual cortex	R secondary visual	0.35	15.03	<0.0001	0.53	17.6	<0.0001*	0.37	10.94	<0.0001	0.33	10.6	<0.0001
L ventral ACC	L dorsal ACC	0.22	12.34	<0.0001	0.21	4.47	0.006	0.22	10.05	<0.0001	0.24	12.94	<0.0001
L dorsal ACC	R ventral ACC	0.27	12.01	<0.0001	0.21	2.74	0.32	0.26	14.55	<0.0001	0.28	12.49	<0.0001
R insula	R Primary Auditory	0.4	11.26	<0.0001	0.34	3.68	0.05	0.35	8.64	<0.0001	0.4	10.56	<0.0001
R thalamus	L thalamus	0.28	10.41	<0.0001	0.41	8.3	<0.0001	0.27	8.97	<0.0001	0.22	6.31	<0.0001
L fusiform cortex	L visual association area	0.08	9.12	<0.0001	0.12	4.85	0.01	0.07	5.02	0.0002	0.08	8.1	0.0001
Medial PFC	L dorsal ACC	0.11	8.32	<0.0001	0.06	2.85	0.22	0.13	6.35	0.0006	0.13	6.47	0.0005
L supramarginal gyrus	L primary auditory	−0.38	6.79	<0.0001	−0.31	2.64	0.26	−0.4	4	0.027	−0.36	6.27	0.0007

For all connections, Beta is the standardised coefficient and p-FDR is the FDR corrected probability of significance. R, right; L, left; PFC, prefrontal cortex; ACC, anterior cingulate cortex.

in the standard acquisition data. Across the 10 connections, there was a mean difference of 29% in the connectivity strengths ('beta' values, Table III) derived from the multiband versus standard data. Direct comparison of all brain connectivity parameters between the two sequences identified one significant interconnection where the standard sequence led to a greater connectivity estimate than did the multiband sequence (Table III—right associate visual cortex to secondary visual cortex), although the connection was identified as significant in both data sets. The long TR multiband (TR = 1,700 ms) sequence revealed similar patterns of connectivity to those derived from the other sequences, although with lower reliability, possibly due to both lower SFNR and smaller subject numbers. Four of the 10 strongest connections from the short TR multiband acquisition were significant in the long TR multiband data, although the others showed similar trends. No connections were found using the long TR multiband sequence which were not identified in the other two sequences.

Replicability of networks using multiband acquisition

To test whether the differences observed between multiband and standard acquisitions arose from the enhanced temporal resolution and frequency information available from the multiband sequence, or whether they represented poor replicability of functional connectivity analysis between scans, in a subgroup of 15 infants we compared networks derived from two identical multiband acquisitions acquired at different time points during the scanning protocol. Across all possible connections, there were no signifi-

cant differences in functional brain networks estimated from the two acquisitions. Table III (columns C and D) shows the same 10 most significant connections reported previously, with parameter estimates from the first and second multiband acquisitions. All these connections were successfully identified as significant in both data sets, despite reduced statistical power. There was a small mean difference of 9.6% between the connectivity parameters estimated from the two data sets, and no significant differences were found between the brain networks derived from these two acquisitions, arguing for the robust replicability of data derived using the short TR multiband sequence.

Temporal resolution versus use of expanded frequency spectrum

The differences in estimated connection strength derived from the multiband compared to the standard EPI sequence could be attributable to either increasing the range of frequency information from which parameters were estimated, or merely from improved temporal characterisation of the very low frequency information which has traditionally been interrogated to derive functional connectivity parameters. To investigate these alternatives, we compared parameters derived from the multiband sequence with the upper limit of the bandpass filter set at 0.5 vs. 0.2 Hz. Table IV shows differences between the 10 selected connection parameters when the data were filtered at <0.5 Hz vs. <0.2 Hz. Direct comparison of the connectivity parameters estimated with different bandpass settings did not reveal statistically significant differences, but estimated connections did

Figure 4.

Functional connectivity map showing the 10 most statistically significant inter-regional connections estimated with the low TR multiband sequence. Red lines show positive and blue negative functional interconnections. All $P < 0.0001$, FDR corrected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

tend to be smaller and less reliable with the lower filter limit, and 1 of the 10 analysed connections was not significant when higher frequency information was discarded, despite being derived from the same images.

Detection of subject effects on connectivity parameters

To determine whether pulse sequence choice affected our ability to detect influences of subject characteristics on functional connectivity, we determined changes in functional connectivity according to the corrected gestational age (CGA) at which infants were scanned. Figure 5 shows functional connections which were significantly influenced by CGA, and Table V shows parameters for these connections for multiband and standard acquisition sequences. Whilst most patterns of connectivity change were detected with both sequences, two of the nine functional connections were not found to be significant in the data derived from the standard sequence. No significant connectivity changes were found in the standard EPI scans that were not detected with the multiband acquisitions.

Spectral Characteristics and Influence of Physiological Fluctuations

To assess whether incorporating higher frequency bands for analysis would be significantly influenced by known physiological variables, we calculated power spectra for cardiac and respiratory cycles, recorded during scanning in a subgroup of infants. Figure 6 depicts these spectra together with power spectra from ROIs containing CSF and grey matter within the same single slice. 7.4% of the power of respiratory effects and 0.7% of cardiac effects occurred at <0.5 Hz in our infants. Inclusion of cardiac and respiratory cycle data as nuisance regressors did reveal effects in brain regions containing large blood vessels at the circle of Willis, middle, and anterior cerebral arteries. However, there were no significant effects on estimated functional connectivity in brain ROI, even at a very liberal statistical threshold of $P < 0.5$.

Following estimation of the physiological nuisance regressors, T_2^* data were corrected using RETROICOR. The T_2^* data from CSF and grey matter ROIs did not show any evidence of harmonic fluctuations reflecting aliasing from cardiac or respiratory cycles.

TABLE IV. Comparison of the 10 most statistically significant connections derived from low TR (906 ms) multiband rs-fMRI acquisition (see also Table 2), with data bandpass filtered at 0.005–0.5 Hz vs. 0.005–0.2 Hz

Source	Target	Multiband < 0.5 Hz			Multiband < 0.2 Hz		
		Beta	T(19)	p-FDR	Beta	T(19)	p-FDR
L primary somatosensory	L primary motor	0.57	19.2	<0.0001	0.31	18.3	<0.0001
R primary somatosensory	R primary motor cortex	0.55	18.08	<0.0001	0.29	17.99	<0.0001
R associative visual cortex	R secondary visual	0.35	15.03	<0.0001	0.36	4.17	0.048
L ventral ACC	L dorsal ACC	0.22	12.34	<0.0001	0.28	3.5	0.17
L dorsal ACC	L ventral ACC	0.27	12.01	<0.0001	0.14	9.13	<0.0001
R insula	R primary Auditory	0.4	11.26	<0.0001	0.14	11.68	<0.0001
R thalamus	L thalamus	0.28	10.41	<0.0001	0.16	10.03	<0.0001
L fusiform cortex	L visual association	0.08	9.12	<0.0001	0.07	8.48	<0.0001
medial PFC	L dorsal ACC	0.11	8.32	<0.0001	0.1	9.15	<0.0001
L supramarginal	L primary auditory	−0.38	6.79	<0.0001	−0.07	−6.26	<0.0001

Figure 5.

Functional connectivity map showing the inter-regional connections influenced by CGA of infants at the time of scanning, as estimated using the low TR multiband sequence. Red lines show positive and blue negative changes in functional connectivity with advancing age. All $P < 0.05$, FDR-corrected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

This study demonstrates for the first time that more complete characterisation of functional brain networks in neonates requires rapid data acquisition, allowing interrogation of signal fluctuations across an extended frequency spectrum. We have shown that this goal can be achieved effectively and reproducibly with an accelerated fMRI sequence. Our data show that the intrinsic signal characteristics of the rs-fMRI data imply the need for rapid acquisition to fully characterise the data. Furthermore, we show that there are statistically, and potentially clinically, significant differences in the information about functional brain networks that can be derived using accelerated acquisition methods. This makes the case for recommending future studies of infant functional brain networks to optimise sequence parameters, including subsecond TR, to improve the quality of data and enhance our understanding of functional networks in the developing brain.

In the present study we were able to acquire high quality images at subsecond TR, gaining improved temporal characterisation of T2* signal whilst maintaining SNR similar to that with standard acquisitions. Even greater degrees of accelerated acquisition and shorter TRs are possible, but maintaining signal quality at higher speeds becomes increasingly challenging, and there are increasing risks of signal artefact. A particular limitation will be artefacts from steady state free precession (SSFP) as flip angles decrease and TR approaches the T2 value of the imaged tissues [Bhattacharyya and Lowe, 2004; Lowe et al., 1998]. This is a particular limitation as the neonatal brain has a higher water content, and thus longer T2 relaxation time, than adult brains. Mean T2 values in white matter may be as high as 350 ms in preterm infants, although these do tend to decrease with increasing gestational age [Counsell et al., 2003]. Therefore, while even shorter TR and greater temporal resolution may be achievable in neonatal rs-

TABLE V. Functional connections significantly influenced by corrected gestational age at time of scanning, from multiband versus standard sequences

Source	Target	Multiband			Standard		
		Beta	F	p-FDR	Beta	F	p-FDR
R DLPFC	R primary auditory	-0.16	5.39	0.003	-0.03	4.32	0.037
R Inferior temporal gyrus	R primary somatosensory	0.04	5.42	0.003	0.02	4.94	0.009
R primary somatosensory	R inferior temporal gyrus	0.06	4.32	0.038	0.03	5.07	0.007
R subgenual cortex	R primary somatosensory	-0.03	4.91	0.005	-0.04	4.35	0.017
R subgenual cortex	Posterior cingulate cortex	0.05	4.9	0.005	0.05	4.94	0.009
Posterior cingulate cortex	R subgenual cortex	0.14	4.22	0.047	0.04	5.42	0.003
L subgenual cortex	Posterior cingulate cortex	-0.05	4.87	0.01	-0.04	3.67	0.126
L premotor cortex	R inferior parietal lobule	0.14	4.43	0.029	0.03	4.28	0.04
L dorsal posterior cingulate	R primary auditory cortex	-0.15	4.29	0.04	-0.03	3.67	0.08

For all connections, beta is the standardised coefficient of change per week of corrected gestation, and p-FDR is the FDR corrected probability of significance. L, left; R, right; DLPFC, dorsolateral prefrontal cortex.

Figure 6.

Mean power spectral density of respiratory (solid line) and cardiac (long dashed line) cycles during rs-fMRI acquisition, together with mean power spectral density of T2* signal from cerebrospinal fluid in the left lateral ventricle (short dashed line) and left superior temporal gyrus (dotted line) from the same

brain slice. Power spectral density for T2*, respiratory and cardiac data are normalised to the same area under the curve. Note that frequency scale (x-axis) changes at 1 Hz for ease of visualisation at lower frequencies.

fMRI, it will be increasingly challenging to achieve good SNR with falling TR, and there will be a practical lower limit set by the T2 of brain tissue in these infants.

There is emerging evidence from rs-fMRI studies in adults using short TR multiband acquisition which supports the conclusions of the present study. Boubela et al. [2013], reported that use of high frequency (>0.25 Hz) components of rs-fMRI signal alone could identify commonly identified resting-state networks using temporal independent component analysis. Subsequent studies have confirmed that previously identified resting state networks show connectivity in higher frequency bands than previously thought [Gohel and Biswal, 2015], and indeed that some connectivity ‘hubs’ are only identified in the high-frequency signal components [Liao et al., 2013]. The present study supports this view of meaningful network information occurring in higher frequency bands than can be investigated with commonplace fMRI acquisitions, and extends these findings to neonates. Furthermore, we provide the first evidence that some influences of clinically relevant parameters on brain connectivity parameters may only be detected when using high frequency fluctuation data, meaning that effects may be obfuscated if fMRI acquisition sequences have excessively long TR. Recent findings in adults have shown how the frequency characteristics of different brain regions can be identified using multiband acquisition, and that significant power is contained in higher frequency components of the spectra [Kalcher et al., 2014]. The results of the present study showed similar diversity in spectral distribution across

brain regions, and our power spectra likewise showed significant power distributed beyond 0.1 Hz.

A number of previous studies have examined resting state functional networks in neonates, born either preterm or at term. All of these studies have used acquisitions with relatively long TR (1,500–3,000 ms), and none have investigated fluctuations at frequencies >0.15 Hz. Fransson et al. [2007] were the first to identify patterns of resting-state activity in sedated ex-preterm infants, showing early networks involving somatosensory and motor cortex, temporal and auditory cortex, and postero-lateral parietal cortex. They subsequently showed similar, although more well developed, networks in term born infants scanned during natural sleep. Further studies have identified how these networks show longitudinal changes in preterm infants, from very limited regions of activity at around 28 weeks CGA to more complete networks by term equivalent age [Doria et al., 2010; Smyser et al., 2013]. There is also evidence that more distant areas take longer to develop strong interconnections, and that ex-preterm infants have less mature brain networks at term equivalent age than do infants born at term. Further work has identified how these infantile resting-state networks continue to develop over the first 2 years of life, to a state resembling adult networks [Gao et al., 2009; van den Heuvel et al., 2014]. Our data support the existence of extensive functional networks in ex-preterm infants at term, with the networks identified using the short TR multiband sequence being more extensive than with the standard sequence. Consistent with previous findings, less distant brain regions may form stronger

interconnections early in development. As in the above studies, we identified changes in functional brain connectivity with increasing maturity at the time of scanning. Crucially, comparison of these effects estimated from our rapid multiband and standard acquisitions shows that additional maturational changes were identified with our rapid sequence which were not detected with the standard long-TR scanning protocol. This finding strongly supports the case for using rapid acquisition fMRI methods in studies of maturational or clinical influences on early brain development.

One interesting study of the spectral power characteristics of the neonatal rs-fMRI signal showed a substantial drop off in power with increasing signal frequency (0.01–0.15 Hz), using MRI data acquisition at TR of 2,000 ms [Fransson et al., 2013]. Our power spectra did show most power at low frequencies, but the data from short TR multiband acquisition in particular showed a less steep drop off in power, and contained significant information in the higher frequency part of the spectrum. This implies that determining the signal characteristics of T2* fluctuations in the neonatal brain is dependent on the acquisition parameters used for sampling, as well as intrinsic characteristics of the underlying neuronal networks.

Acquiring rs-fMRI data at higher sampling frequency has three potential advantages which may improve network characterisation—acquisition of more volumes or data points within a given time, interrogation of meaningful signal effects occurring at higher frequencies than could otherwise be sampled and better characterisation of nuisance effects which should be removed from processed data. Any of these elements could have contributed to our improved characterisation of neonatal brain networks.

We cannot eliminate the possibility of non-neuronal noise occurring within our measured T2* signal, but this is an issue for all fMRI studies, and is not limited to multiband or low TR sequences. Possible contributions from ‘vasomotion’ [Mayhew et al., 1996; Razavi et al., 2008] occur ~0.1 Hz, and therefore, will be less heavily weighted in our short TR data, which covers a larger frequency spectrum. As discussed previously, respiratory and cardiac noise may be aliased into lower frequency T2* fluctuations, but our acquisition uses a TR which is still significantly higher than T2 in these infants and is at no greater risk of SSFP artefacts causing aliasing than a longer TR sequence. As the neonatal heart rate is higher than in adults (2–3 Hz), it is difficult to fully capture the influence of this within our T2* data for use with RETROICOR or other data clean up methods. Even shorter TR might help with this, but is practically limited by the long T2 of brain tissue in this population, as discussed above. However, our data should at least be no more susceptible to non-neuronal artefacts than standard acquisitions with longer TR, and contains an extended amount of neurally driven signal change.

We acknowledge that our study population of ex-preterm infants may have differences in the frequency characteristics of fluctuations in T2* signal compared to

healthy term babies, older children, or adults. Such effects might arise due to differences in the frequency of underlying neuronal activity, differences in neurovascular coupling, or differences in the impact of vascular changes on measured T2* signal in these infants. With regard to frequency of underlying neuronal activity, there is evidence that background EEG rhythms in neonates show a higher proportion of power in lower frequency [delta (< 4 Hz) and theta (4–8 Hz)] bands compared to adults, and that the proportion of power in low frequency bands decreases with increasing maturity [Bell et al., 1991]. Ex-preterm neonates investigated with EEG at term corrected age also show a relative increase in the proportion of delta activity compared to term born infants of equivalent CGA [Scher et al., 1994]. This suggests that in other populations, there is potentially even greater advantage in sampling high frequency T2*, if the frequency of this signal change reflects the frequency of the underlying electrical activity [Hiltunen et al., 2014; Neuner et al., 2014; Whitman et al., 2013]. We do note recent findings suggesting a possible dissociation between patterns of functional networks detected with rs-fMRI and scalp EEG [Omidvarnia et al., 2014]. This study showed that connectivity strength estimates from EEG were strongly influenced by signal amplitude, with a bimodal pattern, with strong interregional connectivity shown only between transient high amplitude bursts of spontaneous activity and little connectivity shown between background fluctuations. This contrasts with their rs-fMRI connectivity, which showed no evidence of amplitude modulation, possibly suggesting dissociation between brain networks measured using EEG and rs-fMRI. This is an intriguing finding, and while it is inherent in the nature of the two methods that they have rather different sensitivity and spatio-temporal characteristics, further work is needed to clarify the detailed physiological relationship between neuronal activity and T2* signal changes in this age group. There is evidence that the shape of the ‘haemodynamic response function,’ as assessed by the change in T2* signal measured in somatosensory cortex contralateral to an experimental stimulus, does change with increasing maturity, but that a positive T2* change is seen even in preterm infants [Arichi et al., 2012]. These outstanding questions about the precise nature of neurovascular coupling in this population do not obviate the benefits of improved rs-fMRI acquisition in neonates, but rather emphasises the need to improve characterisation of the rs-fMRI signal, by methods such as those described here, in order to optimise our understanding of neonatal functional brain development.

CONCLUSIONS

Noninvasive characterisation of functional brain networks in infants is crucial to our understanding of human brain development and of neonatal brain injury and repair. In this study, we have described the application of

a multiband EPI protocol for rapid acquisition of whole-brain rs-fMRI data, with high spatial and temporal resolution, in naturally sleeping infants. We have demonstrated that using this approach, as well as adoption of an optimised preprocessing pipeline, both alters the signal characteristics of the derived T2* data and enhances the detection of functional networks within the neonatal brain. These methods improve characterisation and temporal resolution of rs-fMRI signals and allow potential interrogation of neurovascular fluctuations occurring at higher frequencies than have previously been investigated in this population. This approach adds significantly to the robustness of neonatal rs-fMRI, and adoption of subsecond acquisition protocols should be considered for future studies of both resting state and task-related fMRI by investigators in this field.

ACKNOWLEDGMENTS

ASC is supported by a NIHR Clinical Lectureship. KL is supported by a Walport Senior Clinical Lectureship. The authors are grateful to Ms. Aileen Wilson for assistance with MRI scanning, and to all participating infants and their families.

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