Mapping of whole-cerebrum resting-state networks using ultra-high resolution acquisition protocols

Seong Dae Yun\textsuperscript{1} | Patricia Pais-Roldán\textsuperscript{1} | Nicola Palomero-Gallagher\textsuperscript{2,3,4} | N. Jon Shah\textsuperscript{1,5,6,7}

\textsuperscript{1}Institute of Neuroscience and Medicine–4, Medical Imaging Physics, Forschungszentrum Jülich, Jülich, Germany
\textsuperscript{2}Institute of Neuroscience and Medicine–1, Structural and Functional Organisation of the Brain, Forschungszentrum Jülich, Jülich, Germany
\textsuperscript{3}C. & O. Vogt Institute for Brain Research, Heinrich-Heine-University, Düsseldorf, Germany
\textsuperscript{4}Department of Psychiatry, Psychotherapy and Psychosomastics, Medical Faculty, RWTH Aachen, Aachen, Germany
\textsuperscript{5}Institute of Neuroscience and Medicine–11, Molecular Neuroscience and Neuroimaging, JARA, Forschungszentrum Jülich, Jülich, Germany
\textsuperscript{6}JARA – BRAIN – Translational Medicine, Aachen, Germany
\textsuperscript{7}Department of Neurology, RWTH Aachen University, Aachen, Germany

Correspondence
N. Jon Shah, Institute of Neuroscience and Medicine–4, Forschungszentrum Jülich, 52425 Jülich, Germany.
Email: n.j.shah@fz-juelich.de

Abstract
Resting-state functional magnetic resonance imaging (fMRI) has been used in numerous studies to map networks in the brain that employ spatially disparate regions. However, attempts to map networks with high spatial resolution have been hampered by conflicting technical demands and associated problems. Results from recent fMRI studies have shown that spatial resolution remains around 0.7 mm\(^3\), with only partial brain coverage. Therefore, this work aims to present a novel fMRI technique that was developed based on echo-planar-imaging with keyhole (EPIK) combined with repetition-time-external (TR-external) EPI phase correction. Each technique has been previously shown to be effective in enhancing the spatial resolution of fMRI, and in this work, the combination of the two techniques into TR-external EPIK provided a nominal spatial resolution of 0.51 mm\(^3\) (0.26 mm\(^3\) voxel) with whole-cerebrum coverage. Here, the feasibility of using half-millimetre in-plane TR-external EPIK for resting-state fMRI was validated using 13 healthy subjects and the corresponding reproducible mapping of resting-state networks was demonstrated. Furthermore, TR-external EPIK enabled the identification of various resting-state networks distributed throughout the brain from a single fMRI session, with mapping fidelity onto the grey matter at 7T. The high-resolution functional image further revealed mesoscale anatomical structures, such as small cerebral vessels and the internal granular layer of the cortex within the postcentral gyrus.

KEYWORDS
EPIK, half-mm in-plane resolution, resting-state fMRI, TR-external phase correction, whole-cerebrum

1 | INTRODUCTION

Since the first demonstration of blood-oxygenated-level-dependent (BOLD) contrast using MRI (Ogawa et al., 1990), fMRI, as it has become known, has been widely used to explore brain function under either resting-state or evoked by a stimulus-driven paradigm. Task-evoked fMRI detects functional signals involved with a specific given task, which reflect only a small fraction of the brain's overall activity...
In contrast, resting-state fMRI (rs-fMRI) focuses on spontaneous neuronal activity fluctuating at very low frequencies (<0.1 Hz), by which correlated brain areas in disparate regions, termed ‘resting-state network (RSN)’, can be identified (Biswal et al., 1995, 1997; Smitha et al., 2017; van den Heuvel & Hulshoff Pol, 2010). Thus, rs-fMRI has been employed by many groups for the investigation of overall brain function and its underlying connectivity (Smitha et al., 2017). In addition, the task-free acquisition of rs-fMRI allows various neurological issues to be studied in patients (e.g., cognitive dysfunction, psychiatric disorders, consciousness, etc.) or young children (e.g., neonates, infants, etc.), who either have difficulties or simply cannot comply with designed paradigms (Adhikari et al., 2019; Heine et al., 2012; Zhang et al., 2019).

Recent advances in fMRI techniques enable the depiction of neuronal activation with a submillimetre voxel size. Previously, several fMRI studies have employed high-resolution imaging techniques to measure the fMRI signal with a cortical depth-dependence (referred to as ‘laminar fMRI’; e.g., Huber et al., 2017; Yu et al., 2014). However, most of these high-resolution laminar fMRI investigations of activation profiles through the cortex have only targeted functional activation evoked by a task paradigm in a particular brain region (Chai et al., 2020; Guidi et al., 2016; Huber et al., 2015; Kashyap et al., 2018; van Mourik et al., 2019), leaving the laminar dynamics underlying the resting-state largely unexplored. This is mainly due to the technical limitations of the current fMRI techniques, which are only able to offer submillimetre voxel size with limited brain coverage.

There have been numerous attempts to improve the spatiotemporal resolution of fMRI, including the use of a reduced field-of-view (FOV) (Heidemann et al., 2012; Kemper et al., 2018; Zimmermann et al., 2011) or the use of non-Cartesian and random sampling trajectories (Fang et al., 2016; Jiang et al., 2018; Kasper et al., 2019). Although a reduced FOV can usually achieve a higher spatial resolution than full-FOV schemes, the restricted FOV has a critical limiting factor in brain coverage. The non-Cartesian approaches improve spatial resolution by employing a more efficient k-space sampling scheme, such as radial or spiral trajectories (Jiang et al., 2018; Kasper et al., 2019); however, off-resonance artefacts, typically resulting from these trajectories, render a robust localisation of functional activity difficult without the application of an additional correction method (Kasper et al., 2019). Although the random sampling method also offers more robust image reconstruction, leading to a highly accelerated acquisition, its complex image reconstruction can introduce distortions to functional time-series data, resulting in the reduction or slight sinusoidal variations of BOLD amplitudes (Fang et al., 2016; Zong et al., 2014); it is noted, however, that the key HRF characteristics in the aforementioned study by Fang et al. are well preserved.

For the reasons described above, full-FOV Cartesian sampling methods are still widely used, and of these, echo-planar imaging (EPI) (Mansfield, 1977) is the most commonly implemented method for submillimetre-resolution fMRI studies (Kay et al., 2019; Koizumi et al., 2019; Kok et al., 2016; Sharoh et al., 2019; van Dijk et al., 2020). However, the spatial resolution achieved in several recent works has remained around $0.7 \times 0.7 \times 0.7$ mm$^3$, and most methods are only able to provide limited brain coverage.

This work presents a novel fMRI methodology, providing a half-millimetre in-plane resolution with whole-cerebrum coverage. The imaging method was developed based on the combination of ‘TR-external EPI phase correction’ (Wielopolski et al., 1998; Yun & Shah, 2020) with ‘EPI with keyhole’ (EPIK) (Caldeira et al., 2019; Shah, 2015; Shah et al., 2019; Shah & Zilles, 2003, 2004; Yun et al., 2013, Yun & Shah, 2019, Yun & Weidner, 2019, 2020; Yun & Shah, 2017, 2019; Zaitsev et al., 2001, 2005), both of which have been shown to be effective in improving the spatial resolution and brain coverage while maintaining comparable BOLD detection performance when compared to a standard EPI method (Caldeira et al., 2019; Shah et al., 2019; Yun et al., 2013, Yun & Shah, 2017, 2019, 2020). The developed imaging method is termed ‘TR-external EPIK’ (Yun et al., 2020; Yun & Shah, 2019).

This work focuses on the evaluation of a half-millimetre protocol implemented using TR-external EPIK at 7T. A qualitative inspection of the achieved spatial resolution was performed by identifying mesoscale anatomical structures in reconstructed images. The protocol was employed in rs-fMRI to demonstrate high-resolution mapping of activated voxels in a number of RSNs distributed widely over the brain. Furthermore, rs-fMRI data were acquired from a group of subjects to verify the robust detection of functional signals using TR-external EPIK. The imaging performance of TR-external EPIK was evaluated by comparison with the imaging parameters of previously published submillimetre-resolution fMRI studies.

2 | MATERIALS AND METHODS

2.1 | TR-external EPIK

Figure 1a shows a sequence diagram of the proposed TR-external EPIK method. The entire TR loop consists of two excitation sub-loops. The first excitation sub-loop $[\text{TR}_{\text{E1}}]$ acquires three navigator echoes for the correction of $N/2$ ghost artefacts, whereas the second excitation sub-loop $[\text{TR}_{\text{Main}}]$ performs echo-planar readout according to the EPIK scheme (Figure 1b). The lack of navigator echoes in the second sub-loop facilitates a decreased minimum TE, enabling a larger imaging matrix size for a higher spatial resolution at any given TE. As demonstrated previously, the flip angles for the first and second sub-loops were determined as 90° and 90° (Yun & Shah, 2020).

Figure 1b illustrates a schematic representation of three-shot EPIK with respect to the temporal indices. For each measurement, the central $k$-space is fully sampled with a Nyquist rate ($\Delta k_n = 1/\text{FOV}$), which ensures an optimum signal-to-noise ratio (SNR) and contrast-to-noise ratio for every single time frame subsequently reconstructed. However, the peripheral $k$-space is sparsely sampled, as in multi-shot EPI ($\Delta k/n = 3/\text{FOV}$). Crucially, the peripheral $k$-space is continually updated every three shots according to a sliding-window reconstruction scheme (Noll et al., 1991). This acquisition scheme can achieve a higher apparent temporal resolution than the community-standard...
method, EPI. This work employs 48 central k-space lines for the keyhole region (Shah et al., 2019; Yun et al., 2013, 2020; Yun & Shah, 2017, 2019; Zaitsev et al., 2001). A more detailed description of the features of EPIK is given in Supporting Information (see 5.1. with Figure S1).

The above TR-external EPIK scheme enabled a half-millimetre protocol with the following parameters: TR = 3500 (i.e., 15.7 ms for TRPC + 3484.3 ms for TRmain), TE = 22 ms, FOV = 210 x 210 mm², matrix = 408 x 408 x 108 slices (0.51 x 0.51 x 1.0 mm³), partial Fourier = 5/8, three-fold in-plane/three-fold inter-plane (GRAPPA/multiband) acceleration (Griswold et al., 2002; Larkman et al., 2001; Setsompop et al., 2012), bandwidth = 721 Hz/Px and αPC/αMain = 9°/90°. Here, an isovoxel protocol (0.63 x 0.63 x 0.63 mm³) was also configured with the same TR, TE, FOV and acceleration conditions as above, but with a different matrix size: 336 x 336 x 123 slices.

A 3D anatomical image was acquired using a T1-weighted magnetisation-prepared, rapid gradient echo (MP2RAGE) pulse sequence with the following parameters: matrix size = 376 x 400 with 256 sagittal slices (0.6 mm isotropic), TR/TE/T1/T2/TE = 4440/840/2370/2.08 ms. The above imaging configuration was employed on a Siemens Magnetom Terra 7T scanner with a single-channel Tx/32-channel Rx Nova medical coil supplied by the manufacturer.

2.2 | In vivo measurements

All in vivo measurements in this work were performed on healthy volunteers screened, in addition, for neurological or psychiatric illnesses. After a complete description of the study, written informed consent was obtained before scanning. The local institutional review board (RWTH Aachen University, Germany) approved the study protocol (EK 346/17), screening questionnaires and consent forms. Eighteen healthy volunteers (14 males, 4 females; mean age, 30.22 years; range, 23–47 years) were recruited for the study. In order to control for the effect of physiological noise, respiratory and cardiac signals were recorded using a pneumatic belt positioned around the subject’s chest and a pulse-oximetre placed on the second, third or fourth finger of the left hand.

2.3 | Resting-state fMRI and data analysis

The data acquisition consisted of four dummy scans, to reach a steady-state, and 172 scans to acquire 10-min of rs-fMRI data. Functional data were pre-processed using MATLAB (MathWorks, Inc.) and AFNI (Analysis of Functional NeuroImages, NIH, Bethesda, MD) to
perform slice-timing correction, realignment, regression of cardio-
respiratory signals, regression of the mean white-matter and mean
CSF signal, and temporal filtering (0.005–0.12 Hz band-pass) with
regression of motion parameters. In addition, the effect of large pial
vessels on functional activation was reduced using a previously
described method (Curtis et al., 2014; Menon, 2002), whereby the
maximum likelihood estimator is initially derived from a fit of the
phase image to the magnitude image using the chi-squared function
minimisation (Press et al., 1992), and the estimator is then subtracted
from the magnitude image to remove signal components presumably
attributed to field inhomogeneities from large veins. The structural
data were co-registered to the functional images using FreeView with
a linear transformation (FsTutorial, MultiModalRegistration, https://
surfer.nmr.mgh.harvard.edu). Data sets from five subjects were
excluded for the following reasons: one subject’s data set showed
very high motion displacement (i.e., mean displacement >0.5 mm),
which possibly led to the poor functional activation of the studied
networks, two subjects’ data sets had very noisy signals in the
recorded physiological data (i.e., respiratory and cardiac signals) lead-
ing to unreliable data pre-processing, and the other two subjects’ data
sets had only partially recorded physiological data due to systematic
difficulties.

Independent component analysis (ICA) with Melodic (FSL, FMRIB
Software Library, Oxford, UK) was individually performed for each
subject’s functional data, that is, without concatenation of all subjects’
data sets. Based on the standard routine of FSL, the optimal number
of ICA components was determined as being 74 and 76 for the data
sets from the 13 subjects. This analysis quantified the probability of
the voxel belonging to a given network. From all the automatically
detected components for each subject, the best matching compo-
ents to present RSNs were manually selected. The activated voxels
were obtained with a statistical threshold (probability ≥0.5).

2.4 Group analysis: Reproducibility

The quality of the functional scans was assessed by inspecting the
image SNR. The mean signal value was calculated by extracting the
brain using the standard SPM segmentation routine (Wellcome
Department of Imaging Neuroscience, UCL, London, UK) and
obtaining its mean value. The standard deviation of noise was
obtained from regions of interest (ROIs) located at the four corners of
the reconstructed images. The noise ROI at each corner was deter-
mained with a sufficiently large matrix size (40 × 60 voxels) so as to
include enough noise sources from the background while avoiding the
inclusion of structural noise (e.g., N/2 ghosts). The calculation was
performed for each temporal frame of each subject (i.e., 172 temporal
frames per subject), and the mean ± SD SNR across the temporal
dimension was obtained.

In order to check the reproducibility of the mapping of the RSNs,
five representative subjects showing the same RSN were selected,
and their activated voxels were individually compared in the space of
their subject-specific mean functional scans. Furthermore, the
prevalence of each RSN in the data was quantified as the number of
subjects in which the RSN was identified. Furthermore, a second-level
analysis was carried out to inspect a group-wise network-specific acti-
vation which was obtained by first averaging unthresholded RSN maps
from each subject and then thresholding the averaged maps with
mean probability. This involved spatial normalisation to a com-
mon template in MNI (Montreal Neurological Institute) space. Here,
the spatial correlation between the RSNs from our data and those
from reference templates (Shirer et al., 2012) was also computed.

2.5 Evaluation of imaging performance

In order to provide insight into the spatial resolution and brain cover-
age provided by TR-external EPIK, its imaging performance was evalu-
ated by directly comparing the imaging parameters employed in
several previous submillimetre-resolution fMRI studies (Berman
et al., 2021; Fracasso et al., 2018; Guidi et al., 2020; Heidemann
et al., 2012; Huber et al., 2020; Kasper et al., 2019; Kay et al., 2019;
Kemper et al., 2018; Koizumi et al., 2019; Kok et al., 2016; Sharoh
et al., 2019; van Dijk et al., 2020). The parameters compared were:
(1) in-plane voxel size (mm), (2) normalised in-plane FOV (mm), calcu-
lated by √FOVx FOVy, (3) slice thickness (mm), (4) normalised slice
throughput, calculated by the number of slices provided per 3.5 s of
TR, (5) volume of a single voxel (mm³), (6) number of voxels per tem-
poral volume (M), calculated by the normalised slice throughput × in-
plane matrix sizes, (7) TE and (8) volumetric TR.

3 RESULTS

3.1 Functional scan with a half-millimetre in-
plane resolution

Figure 2 shows reconstructed images obtained from the half-
millimetre protocol. Here, the surface of the grey matter (GM) and the
white matter (WM) was extracted from all the acquired slices and ren-
dered in 3D (Figure 2a), effectively demonstrating the distinct con-
trasts between the GM and WM with near whole-brain coverage
provided by TR-external EPIK. The 3D rendered surface of GM and
WM was obtained using a 3D visualisation tool, ParaView (www.
paraview.org).

For more detailed visual inspection, sectional slices (axial, coronal
and sagittal) were taken from a single-volume functional scan, as
shown in Figure 2b,d,e. Figure 2b shows four of the 108 axial slices;
the entire reconstructed slices can be found in Supporting Information
(see Figure S2). From the axial slices, specific brain regions were chosen
(marked by white rectangles: r1–r4) in which the following meso-
scale anatomical structures can be observed (Figure 2c): small cerebral
vessels (red arrows in r2) or the internal granular layer of the cortex
(yellow arrows) located on the anterior wall of the postcentral gyrus
(r3), on the Heschl gyrus (r2) or within the calcarine sulcus (r4). Here,
the position of layer IV, highlighted with a white line, can be found in
the Supporting Information Section 5.3 (see Figure S3), in which the resolution performance of TR-external EPIK was further verified with a high-resolution phantom (see Figure S4). The complete extent of the brain covered by TR-external EPIK can be verified from the coronal and sagittal images (Figure 2d,e). It is important to note that the cortical ribbon can even be seen clearly in these resliced images. In addition, these depictions also show that all slices were reconstructed without any significant inter-slice artefacts, which can sometimes occur as a result of the multi-band reconstruction (McNabb et al., 2020). The signal behaviour along the direction in which multi-band acceleration was applied (i.e., superior to inferior direction) is very continuous and shows no evidence of abrupt signal changes.

3.2 | Mapping of RSNs

Figure 3 shows identified RSNs for a representative subject. Since there is no gold-standard set of networks to study rs-fMRI data, the
The following five networks were selected here: default mode, sensorimotor (LH), sensorimotor (RH), fronto-parietal (executive) and the visual network, all of which were relatively common in our subjects. The activated voxels were overlaid on the 3D rendered outer cortical surface, which was generated by applying the standard SPM segmentation routine to the mean image of the re-aligned functional scans.

Figure 3 shows the results presented in three different sectional views (axial, coronal and sagittal). The anatomical scan showing the same representation of the activated voxels can be seen in Figure 3c. Figure 3 clearly demonstrates that the extensive brain coverage provided by TR-external EPIK enabled the five RSNs to be simultaneously determined from a single fMRI session. In addition, the half-millimetre protocol (0.26 mm$^3$ voxels) enabled the identification of functional voxels very locally along the cortical ribbon.
For a more detailed examination, three RSNs (default mode, sensorimotor (RH) and visual) are displayed separately in their representative axial, coronal and sagittal slices (Figure 4a); directly overlaid on the mean image of the re-aligned functional scans. For each network, an ROI was selected (marked by the green rectangles) and is displayed in a magnified view in Figure 4b. The size of the selected ROI is 40 x 40 voxels (i.e., 20 x 20 mm²), and the GM regions obtained from the segmentation of the co-registered anatomical scan using FreeSurfer are depicted in blue. Here, in order to investigate the behaviour of the functional profiles around the grey matter, 20 lines, starting from ‘P₁’ and ending at ‘P₂’ and crossing the cortical ribbon, were manually defined from each enlarged depiction. The network-specific probability profiles along the lines were examined, and their mean ± SD profile is shown in Figure 4c (see the solid black line). The length of each examined line is 10 voxels (5.1 mm), and 100 points were sampled along its length. In the same figure, the signal intensity of the background image is also plotted with a black dotted line, and a gradual decrease in signal intensity can be observed from the cerebrospinal fluid (CSF) to the WM region (i.e., T₂/T₂* contrast). The GM region obtained from the anatomical scan is also delineated here with a dashed line, which verifies that the functional activation from the three networks is mostly confined to the GM regions. The peak that is found around the pial surface in Figure 4c likely corresponds to the effect of the large-vessel contribution in the gradient-echo EPI sequences.

3.3 | RSNs from a group of subjects: reproducibility

Table 1 shows the image SNR of the acquired functional time-series data from each subject. The standard deviation of the SNR observed from each subject is shown to be very small, that is, on average, 4.29/2.07 (entire brain/GM), demonstrating that no significant SNR variation along the temporal dimension accrued during the fMRI session. Moreover, the averaged image SNR from all subjects for all
temporal scans was 251.63/231.37 (entire brain/GM), and its standard deviation across subjects was 26.64/35.57 (entire brain/GM). Notwithstanding the fact that the SNR varies between subjects, the relatively small standard deviation across subjects indicates that robust fMRI acquisition was performed throughout.

Figure 5 summarises the RSNs obtained from the 13 subjects for five well-known RSNs: auditory, default mode, sensorimotor, fronto-parietal and visual. The plot shows that although the detection pattern was different between subjects, overall, the RSNs were reliably detected, which demonstrates the robust performance of the half-millimetre protocol in the detection of RSNs.

Figure 6 shows individually displayed, activated voxels at their respective slice location for five representative subjects in the same RSN (e.g., subjects 3, 4, 5, 8 and 10 for the auditory network). The figure reveals that the identified networks had a similar activation pattern across different subjects, which demonstrates the reproducibility of mapping RSNs using TR-external EPIK. In addition, the localisation of the activated voxels along the cortical ribbon can also be verified from each subject.

The reproducibility of the detection of RSNs was further verified with the results of the second level analysis (see Figure 7). For each network, the mean group-wise activated voxels are depicted on the averaged anatomical scan. Here, the number of subjects used for the group analysis is shown in each image panel (e.g., n = 7 for auditory). Although the background image is blurry due to averaging, it can be seen that the RSNs were reliably detected from a group of subjects. The identified RSNs were also shown to have a good agreement with those from the reference template RSNs defined in the MNI space; that is, the spatial correlation of each RSN was highest with the same RSN from the template and lower for different RSNs (see the correlation matrix at bottom-right of Figure 7).

### 3.4 Imaging performance of the half-millimetre protocol

Figure 8 shows the imaging parameters employed in our present work in comparison to those in previous submillimetre-resolution fMRI studies. As shown in Figure 8a, TR-external EPIK has the highest in-

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**TABLE 1** Image SNR of the half-millimetre protocol

| Subject | Entire brain/GM          |
|---------|--------------------------|
| 1       | 272.88 ± 4.35/240.47 ± 4.01 |
| 2       | 233.83 ± 4.51/199.93 ± 1.77 |
| 3       | 289.65 ± 6.62/274.92 ± 3.67 |
| 4       | 244.25 ± 3.99/207.37 ± 2.58 |
| 5       | 287.95 ± 4.81/268.34 ± 1.38 |
| 6       | 279.82 ± 3.24/276.62 ± 1.34 |
| 7       | 261.40 ± 2.89/261.63 ± 1.75 |
| 8       | 256.55 ± 2.71/240.94 ± 1.69 |
| 9       | 256.70 ± 9.07/245.27 ± 1.81 |
| 10      | 218.67 ± 3.81/211.71 ± 2.09 |
| 11      | 239.45 ± 3.30/229.98 ± 1.78 |
| 12      | 209.99 ± 2.87/161.90 ± 1.13 |
| 13      | 209.00 ± 3.65/188.76 ± 1.95 |
| Average | 251.63 ± 4.29/231.37 ± 2.07 |

Note: For each subject, the mean ± SD across the temporal dimension (i.e., in total, 172 temporal volumes) is shown, calculated over the entire brain as well as the GM region only. The last row shows the average of the computed SNR values from all subjects. The standard deviation of SNR across the 13 subjects was computed as 26.64 and 35.57 for the entire brain and the GM, respectively.
plane spatial resolution (0.51 mm) compared to prior publications. Importantly, the spatial resolution was achieved with a sufficiently large in-plane FOV (210 mm), which can cover an entire normal, adult-sized brain sliced in any orientation (see Figure 8b). In comparison, the normalised in-plane FOV provided in most prior studies was smaller than 150 mm, indicating that only a particular slice location and rotation would allow the brain to be entirely encompassed within the in-plane FOV. To facilitate a robust SNR for a group study, the employed slice thickness in TR-external EPIK (1 mm) was larger than its in-plane voxel size. However, Figure 8c shows that there are also submillimetre-resolution fMRI studies performed with a non-isovoxel size where the slice thickness reaches as high as 1.8 mm. The results of normalised slice throughput showed that it was smaller than 50 for most prior cases (see Figure 8d). TR-external EPIK gave the second largest normalised slice throughput, with the method proposed by Kay et al. giving the largest. However, the comparatively smaller slice throughput in TR-external EPIK can be explained as being due to the significantly bigger in-plane matrix size of TR-external EPIK (408 × 408) when compared to that of Kay et al. (2019) (200 × 162). Figure 8e,f also shows that TR-external EPIK gives the third minimum voxel volume (0.26 mm³) and the largest number of voxels per temporal volume (17.98 M). In TR-external EPIK, the spatial resolution and the slice throughput were achieved under a typical TE and volumetric TR setting. That is, the values from our study (22 ms/3500 ms) are shown to be within the range of values provided in other literature, and even longer TEs and TRs can be found depending on the application (see Figure 8g,h). More detailed imaging parameters of the prior studies and the technical
The application of TR-external EPIK for rs-fMRI was also demonstrated with an isovoxel protocol (0.63 × 0.63 × 0.63 mm³) for the same group of subjects as employed in the half-millimetre protocol. Figure 9 shows results for the same five RSNs as depicted in the half-millimetre protocol case. The same second-level analysis and spatial correlation computation were also carried out (see Figure 10). These results also show a high mapping fidelity onto the cortical ribbon and robustness in detecting the same RSNs as the half-millimetre case. Verification of TR-external EPIK for other submillimetre isovoxel sizes is given in Supporting Information (see Figure S6).

4 | DISCUSSION

4.1 | TR-external EPIK for high-resolution fMRI

This work demonstrates the ability of the TR-external EPIK mapping technique to achieve a half-millimetre in-plane pixel size and enable the acquisition of whole-cerebrum, resting-state functional signals at 7T. The technique was developed based on EPIK and a TR-external EPI phase correction.

The feasibility of using EPIK for fMRI has been verified in several previous publications (Yun et al., 2013; Yun, Shah, et al., 2019; Yun & Shah, 2017). The features of EPIK and the rationale for its use in the fMRI conducted in these works are described in detail in the Supporting Information (see Section 5.1). An explanation is provided for the following five topics: (1) Comparison of fMRI results to the community-standard method, EPI, (2) Temporal stability and the importance of keyhole-region sampling, (3) Spatial resolution and point spread...
FIGURE 8 Imaging performance of TR-external EPIK in comparison to other submillimetre-resolution studies. The eight bar charts above show a comparison of the imaging parameters used in the current study and 12 previous submillimetre fMRI studies performed at 7T. The parameters compared were: (a) in-plane voxel size (mm), (b) normalised in-plane FOV (mm), (c) slice thickness (mm), (d) normalised slice throughput (slice/3.5 s), (e) volume of a single voxel (mm³), (f) number of voxels per temporal volume (M), (g) TE (ms) and (h) TR/volume (ms).
FIGURE 9   Results of resting-state networks (RSNs) obtained with the isovoxel protocol. The five identified RSNs (default mode, sensorimotor (LH), sensorimotor (RH), fronto-parietal and visual) are shown, overlaid on (a) their representative axial slice locations, (b) three sections slices (axial, coronal and sagittal) and (c) the anatomical scan (i.e., MP2RAGE)
function (PSF), (4) Temporal detectability of functional signals and 5) Limitations of EPIK. The TR-external implementation of the navigator echoes shortens the minimum possible TE in the main imaging sub-loop enabling higher resolution fMRI (Yun & Shah, 2020) compared to the TR-internal implementation scheme (Heid, 1997; Wong, 1992). In this work, this benefit, in combination with EPIK, allowed an in-plane matrix size of $408 \times 408 \ (0.51 \times 0.51 \text{ mm}^2)$ with a TE of 22 ms and a FOV of $210 \times 210 \text{ mm}^2$. Importantly, this in-plane FOV combined with the acquisition of 108 slices (1 mm thick) is large enough to cover an entire typical human cerebrum sliced in any direction.

Although there have been numerous submillimetre-resolution fMRI studies, the voxel size provided in most previous methods was achieved using a considerably smaller matrix size ($<200 \times 200$) and a smaller FOV ($<200 \times 200 \text{ mm}^2$) than TR-external EPIK, meaning that only a part of the brain was imaged. Moreover, the slice throughput determining the brain coverage was also substantially smaller in the previous works. Due to the above technical limitations, rs-fMRI has not previously been performed with the spatial resolution and brain coverage demonstrated here. In this work, a relatively long TR (3.5 s) was employed to record the rs-fMRI signals. However, since rs-fMRI focuses on low-frequency fluctuations (<0.1 Hz), conventional data analyses require a TR of 5 s to cope with the Nyquist sampling of the upper limit (Berman et al., 2021). Accordingly, the use of a long TR for rs-fMRI has also been demonstrated in previous publications: TR of 3960, 5000 and 5856 ms (Barry et al., 2021; Berman et al., 2021; Sharoh et al., 2019). Depending on the method selected from the literature, it may be possible to achieve an increased spatial resolution or larger slice coverage by a protocol adjustment. Despite the difficulty of performing a fair comparison between different methods under the given imaging conditions, the comparison results show that the TR-external method offers clear improvements.

### 4.2 Spatial resolution of TR-external EPIK

The relatively long readout in EPI-based sequences can deleteriously affect the spatial resolution of the reconstructed image. That notwithstanding,
the PSF analysis performed both in our prior (Yun et al., 2013; Yun & Shah, 2017; Yun, Shah, et al., 2019) and current work (Figure S5b) shows that EPIK has a sharper PSF shape and better spatial resolution than EPI when all other parameters are held constant. This work employed a relatively large partial Fourier acceleration factor (PF of 5/8) for the half-millimetre protocol. However, results from the PSF simulation (Figure S5d) showed that the spatial resolution is determined not only by the partial Fourier factor, but also by the imaging matrix size, that is, the number of actually sampled points = partial Fourier factor × imaging matrix size. Therefore, despite the high partial Fourier factor, the relatively large matrix size (408 × 408) in our work substantially increases the number of sampled points, subsequently contributing to enhanced spatial resolution. It is noted that several previous submillimetre-resolution fMRI studies (Feinberg et al., 2018; Kashyap et al., 2021; Maass et al., 2015) employing a PF of 5/8 also used a relatively large matrix size (e.g., 213 × 213 and 256 × 256) which was, however, much smaller than that of the TR-external EPIK sequence used here.

The spatial resolution of TR-external EPIK was demonstrated for the identification of mesoscale anatomical structures such as small cerebral vessels or a dark stripe, which is located at approximately 50% of the cortical depth in discrete regions throughout the brain. Within the calcarine sulcus, this stripe resembles the stria of Gennari, which is a heavily myelinated tangential band at 60% of the cortical depth (Vogt & Vogt, 1919) and is only visible in high-resolution structural MRI (Clare & Bridge, 2005; Eickhoff et al., 2005; Walters et al., 2007). Although the stria of Gennari is a feature unique to the primary visual cortex (Zilles et al., 2015), we also detected comparable stripes in the anterior wall of the postcentral gyrus (primary somatosensory area 3), on the Heschl gyrus (primary auditory cortex) and in the parietal operculum (secondary somatosensory cortex). Furthermore, these areas, only the primary somatosensory cortex is characterised by the presence of two clearly identifiable Baillarger stripes (myeloarchitectonic layer 4 or 5b), of which, the inner one is more densely myelinated than the outer one (Zilles et al., 2015). A common feature of all areas containing a dark stripe is the presence of a prominent and densely packed inner granular layer. Therefore, we have interpreted the stripe as being cytoarchitectonic layer IV and not myeloarchitectonic layer 4 or 5b. It is important to note that these features were visible in fMRI images, which are primarily intended to depict function and not to demonstrate anatomical features.

This work employed a relatively small acceleration combination for parallel imaging and multi-band techniques (i.e., 3 × 3), which ensured reliable image reconstruction without significant intra-slice aliasing or inter-slice leakage artefacts (McNabb et al., 2020). However, further improvements in spatiotemporal resolution may also be possible in TR-external EPIK by means of a higher acceleration combination, such as 4 × 4 or 2 × 5, as demonstrated in previous works (Moeller et al., 2010; Vu et al., 2017).

4.4 | Non-BOLD contrasts

In order to overcome the relatively low spatial specificity to neuronal activation in gradient-echo (GE) BOLD, several non-BOLD contrasts have previously been demonstrated using cerebral blood flow (e.g., ASL), cerebral blood volume (e.g., VASO) or cerebral metabolic rate of oxygen (e.g., calibrated BOLD) (Borogovac & Asllani, 2012; Huber et al., 2017 and 2019; Germuska & Wise, 2019). Although the non-BOLD contrasts offer improved spatial specificity and functional quantification, they have relatively low sensitivity compared to GE-BOLD contrast (10%–20% for ASL, 40%–60% for VASO and 5%–15% for calibrated BOLD) (Huber et al., 2019), making their use in submillimetre-resolution fMRI challenging. Furthermore, the underlying mechanisms of each contrast (e.g., labelling, blood nulling or reach of functional steady-state) usually requires a much longer TR than the GE-BOLD method, which may provide limited brain coverage for a given TR. For these reasons, fMRI methods with GE-BOLD contrasts are still widely used in submillimetre-resolution fMRI. Several approaches have been used to reduce the large-vessel BOLD effect in GE-BOLD by applying a correction method or replacing it with spin-echo BOLD. In this work, the correction method (Curtis et al., 2014; Menon, 2002) was applied to reduce the large-vessel effect. However, as our earlier work verified the use of spin-echo EPIK for detecting haemodynamic signals in a perfusion study (Shah et al., 2019), it is expected that spin-echo TR-external EPIK can also be systematically configured to give the same high-resolution advantage as well as increased spatial specificity.

4.5 | Functional resolution

One of the main challenges of interpreting high-resolution fMRI relates to the complexity of the neural hemodynamic responses and
4.6 | Potential use of TR-external EPIK

In high-resolution fMRI studies, an errorless co-registration between functional and anatomical scans is often required to aid a better depiction of functional signals. A recent work has presented a method that effectively reduces the co-registration errors stemming from the use of different imaging sequences for functional and anatomical scans by using the same base sequence for both scans (van der Zwaag et al., 2010). In particular, the heterogeneously distributed cerebral veins and venules (large pial veins running tangentially to the cortex and ascending venules draining blood from deep cortical layers towards the surface) introduce a bias in the fMRI signal and is especially prevalent in GE-studies where the contribution of the macrovasculature is considerably higher. Moreover, the varying capillary density across the cortical depth further conditions the actual spatial resolution of functional responses. Simulation studies have demonstrated an enhanced specificity of the fMRI signal towards the microvasculature at higher fields using short TEs (Pflugfelder et al., 2011).

Additionally, several works have estimated the PSF of the hemodynamic responses linked to neuronal activity, which would represent the minimum space unit that could be resolved with fMRI contrast. This ultimate resolution was estimated to be 0.1 mm in an optical study of the cerebral blood volume in mice (Vazquez et al., 2014), 0.86 mm for spin-echo BOLD fMRI and 0.99 mm for GE-BOLD fMRI in a human study of ocular dominance columns (Chaimow et al., 2018). Taking into account the unavoidable presence of physiological motion and the resultant blurring in human fMRI, the true haemodynamic PSF is probably narrower than the PSF in the presence of physiological motion, which in turn results in a higher putative functional resolution. Notwithstanding the controversial definition of the ultimate functional spatial resolution, the fact that published, experimental submillimetre fMRI studies have detected distinct signals originating in highly segregated cortical units, such as cytoarchitectonically-defined layers (e.g., cortical depth-specific activation upon engagement of a finger in a sensory or a motor task [Huber et al., 2017]), strongly supports the ambition of acquiring and analysing functional data with submillimetre-resolution as high as provided here by TR-external EPIK (0.51 × 0.51 × 1.0 mm³; 0.63 × 0.63 × 0.63 mm³).

activation will be the focus of future studies. Furthermore, the spin-echo configuration of TR-external EPIK will also be explored, and a similar high-resolution advantage and spatial specificity are expected. A comparison of SE-BOLD TR-external EPIK with non-BOLD methods will also be investigated in potential future work.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The original in vivo data from 13 subjects can be shared by submitting a request to the corresponding author (N. Jon Shah: n.j.shah@fz-juelich.de) under a formal data-sharing agreement. The general protection of personal data and privacy policy, declared by the Council of Europe (https://www.coe.int/en/web/personal-data-protection-and-privacy), applies to health-related data (CM/Rec(2019)2). The protection of in vivo data or metadata derived from the original data is further described by the corresponding ethics/internal administering documents. The sharing is based on the consent of the subject whose data are to be shared; these subjects will be informed beforehand.

ORCID

Seong Dae Yun https://orcid.org/0000-0001-7398-1899
Patricia Pais-Roldán https://orcid.org/0000-0002-9381-3048
Nicola Palomero-Gallagher https://orcid.org/0000-0003-4463-8578
N. Jon Shah https://orcid.org/0000-0002-8151-6169

REFERENCES

Adhikari, B. M., Hong, L. E., Sampath, H., Chiappelli, J., Jahanshad, N., Thompson, P. M., Rowland, L. M., Calhoun, V. D., Du, X., Chen, S., & Kochunov, P. (2019). Functional network connectivity impairments and core cognitive deficits in schizophrenia. Human Brain Mapping, 40(16), 4593–4605. https://doi.org/10.1002/hbm.24723
Barry, R. L., Babu, S., Anteraper, S. A., Triantafyllou, C., Keil, B., Rowe, O. E., & Atassi, N. (2021). Ultra-high field (7T) functional magnetic resonance imaging in amyotrophic lateral sclerosis: a pilot study. NeuroImage: Clinical, 30, 102648. https://doi.org/10.1016/j.nicl.2021.102648
Berman, A. J. L., Grissom, W. A., Witzel, T., Nasr, S., Park, D. J., Setsompop, K., & Polimeni, J. R. (2021). Ultra-high spatial resolution BOLD fMRI in humans using combined segmented-accelerated VFA-FLEET with a recursive RF pulse design. Magnetic Resonance in Medicine, 85(1), 120–139. https://doi.org/10.1002/mrm.28415
Biswal, B., Hudetz, A. G., Yetkin, F. Z., Haughton, V. M., & Hyde, J. S. (1997). Hypercapnia reversibly suppresses low-frequency fluctuations in the human motor cortex during rest using echo-planar MRI. Journal of Cerebral Blood Flow and Metabolism, 17(3), 301–308. https://doi.org/10.1097/00004647-199703000-00007
Yun, S. D., & Shah, N. J. (2017). Whole-brain high in-plane resolution fMRI using accelerated EPIK for enhanced characterisation of functional areas at 3T. *PLoS One*, 12(9), e0184759. https://doi.org/10.1371/journal.pone.0184759

Yun, S. D., & Shah, N. J. (2020). Analysis of EPI phase correction with low flip-angle excitation to reduce the required minimum TE: Application to whole-brain, submillimeter-resolution fMRI at 3 T. *Magnetic Resonance in Medicine*, 84, 1416–1429. https://doi.org/10.1002/mrm.28218

Yun, S. D., Weidner, R., Weiss, P. H., & Shah, N. J. (2019). Evaluating the utility of EPIK in a finger tapping fMRI experiment using BOLD detection and effective connectivity. *Scientific Reports*, 9(1), 10978. https://doi.org/10.1038/s41598-019-47341-y

Zaitsev, M., D’Arcy, J., Collins, D. J., Leach, M. O., Zilles, K., & Shah, N. J. (2005). Dual-contrast echo planar imaging with keyhole: Application to dynamic contrast-enhanced perfusion studies. *Physics in Medicine and Biology*, 50(19), 4491–4505. https://doi.org/10.1088/0031-9155/50/19/005

Zaitsev, M., Zilles, K., & Shah, N. J. (2001). Shared k-space echo planar imaging with keyhole. *Magnetic Resonance in Medicine*, 45(1), 109–117. https://doi.org/10.1002/1522-2994(200101)45:1<109::aid-mrm1015>3.0.co;2-x

Zhang, H., Shen, D. G., & Lin, W. L. (2019). Resting-state functional MRI studies on infant brains: A decade of gap-filling efforts. *NeuroImage*, 185, 664–684. https://doi.org/10.1016/j.neuroimage.2018.07.004

Zilles, K., Palomero-Gallagher, N., & Amunts, K. (2015). Myeloarchitecture and maps of the cerebral cortex. In *Brain mapping: An encyclopedic reference* (pp. 137–156). Elsevier Academic Press.

Zimmermann, J., Goebel, R., De Martino, F., van de Moortele, P. F., Feinberg, D., Adriany, G., Chaimow, D., Shmuel, A., Ugurbil, K., & Yacoub, E. (2011). Mapping the organization of axis of motion selective features in human area MT using high-field fMRI. *PLoS One*, 6(12), e28716. https://doi.org/10.1371/journal.pone.0028716

Zong, X., Lee, J., John Poplawsky, A., Kim, S. G., & Ye, J. C. (2014). Compressed sensing fMRI using gradient-recalled echo and EPI sequences. *NeuroImage*, 92, 312–321. https://doi.org/10.1016/j.neuroimage.2014.01.045

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