Recovery from an acute relapse is associated with changes in motor resting-state connectivity in multiple sclerosis

INTRODUCTION

Resting-state functional MRI (rs-fMRI) of the brain has been successfully used to identify altered functional connectivity in the motor network in multiple sclerosis (MS).[1] In clinically stable patients with MS, we recently demonstrated increased coupling between the basal ganglia and the motor network.[1] Accordingly, rs-fMRI in MS is particularly suited to investigate functional reorganisation of the motor network in the remission phase after a relapse because the resting-state connectivity pattern is not influenced by interindividual differences in motor ability and task performance. In this prospective rs-fMRI study, we mapped acute changes in resting-state motor connectivity in 12 patients with relapsing forms of MS presenting with an acute relapse involving an upper limb paresis. Previous functional MRI (fMRI) studies have shown that the activation of sensorimotor areas was stronger and more widespread in the brain of patients with MS compared to healthy controls and increased proportionally with the extent of MS-related brain damage.[2] We therefore hypothesised that a motor relapse involving paresis of the upper limbs would trigger an acute compensatory increase in motor resting-state connectivity and that the compensatory increase in functional connectivity would decrease over the following days or weeks in proportion to the degree of clinical remission.

SUBJECTS AND METHODS

Participants

We studied 12 patients with MS presenting with acute motor deficits involving paresis of the left (n=5) or right (n=7) arm. MRI and neurological examination including Expanded Disability Status Scale (EDSS) score was performed twice, at inclusion and at follow-up.[3] The relapse was treated with a three-day course of intravenous methylprednisolone 1 g daily which was initiated after the first scan. Written informed consent was obtained from all patients prior to any examination, and all protocols were approved by the local scientific ethical committee (protocol no. KF01–131/03). Clinical characteristics are listed in table 1 (see online supplementary material).

MRI

MRI was performed on a Siemens 3.0 T Magnetom Trio Scanner using echo planar imaging. The first rs-fMRI was performed within 24 h after relapse onset, while the second rs-fMRI was obtained 6 to 21 days later. Please refer to online supplementary material for further details.

Resting-state connectivity analysis

Independent component analysis (ICA) was applied to the fMRI data to separate functional brain networks that show temporally correlated BOLD-signal fluctuations. We performed spatial ICA using the Group-ICA-Toolbox for fMRI (http://icatb.sourceforge.net/) with the number of

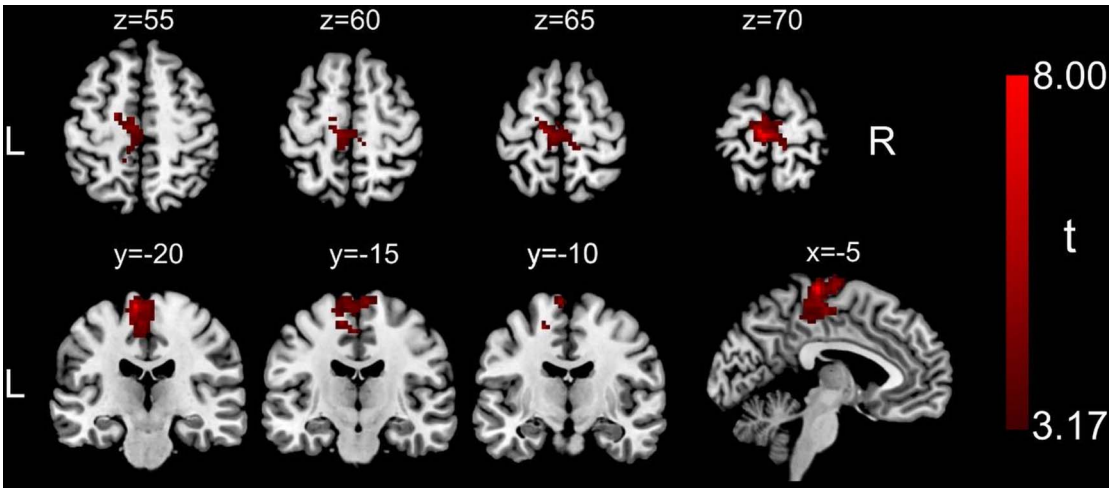

Figure 1 Changes in motor resting-state connectivity during the acute remission phase reflect clinical improvement. The supplementary motor area (SMA) and mesial primary motor cortex (M1) expressed a decrease in coupling strength with the motor resting-state network from the first to second fMRI session which reflected the magnitude of clinical improvement. The stronger motor remission, the more the SMA and mesial M1 reduced its functional connectivity strength with the network. No brain regions showed the opposite relationship, an increase in motor resting-state connectivity with increasing motor remission. No significant change in motor resting-state connectivity was detected over time. The four axial slices are arranged from most caudal to most cranial (z-coordinates, Montreal Neurological Institute (MNI)) and the three coronal slices are arranged from most posterior to most anterior (y-coordinates, MNI). The red overlay indicates t_{10} statistical values as shown in the colour bar to the right.

components fixed at 20. The sensorimotor network and two control networks, the primary visual and the default-mode network, were identified by a template matching procedure.[1] Details are provided in online supplementary material.

Statistical analysis

We aimed at capturing changes in motor resting-state connectivity between onset of the motor relapse and after a remission period of 6–21 days, (ie, between the first and second rs-fMRI session). Specifically, we wished to test which brain regions that showed a reduction in motor resting-state connectivity in proportion to clinical remission by assessing changes in motor resting-state connectivity (time 2—time 1) with improvement in EDSS score as a covariate (time 2—time 1). The same analysis was performed for two non-motor networks, the primary visual and default-mode network. Correction for multiple comparison was performed using cluster-wise maximum permutation statistics with a two-sided entry threshold of $p<0.01$. Clusters surviving a threshold of $p_{FWE}<0.05$ corrected for multiple comparisons over the whole brain were considered statistically significant.

RESULTS

Regression analysis revealed a statistically significant relationship between improvement in EDSS score and reduction in motor resting-state connectivity in the supplementary motor area (SMA) extending into the bilateral mesial primary motor cortex (M1) (figure 1; $p_{FWE}=0.002$; $t_{10}=7.58$; peak at Montreal Neurological Institute coordinates x, y, z=−6, −25, 70) while no brain regions showed the opposite relationship. Further, changes in EDSS score did not correlate with changes in resting-state connectivity in the primary visual or default-mode network.

DISCUSSION

We found that the SMA and mesial M1 reduced its connectivity strength with the motor network in proportion to recovery of motor function after acute relapse. The SMA and M1 are key motor regions. SMA integrates converging input from subcortical motor (basal ganglia, thalamus and cerebellum) and parietal cortex afferents, and sends projections to the M1 and directly to the corticospinal tract.[4] An acute relapse might therefore trigger a compensatory increase in functional resting-state motor connectivity involving the SMA. Our results indirectly support this notion, showing that the strength in motor resting-state connectivity of SMA decrease in proportion to functional recovery of motor function. The reduced coupling of SMA and M1 to the motor network most likely reflects a return to a normal pattern of resting-state motor connectivity paralleling clinical motor improvement.

Neither the visual nor the default-mode network showed regional connectivity changes scaled to the magnitude of motor recovery, suggesting that the reorganisation associated with the acute motor lesion was spatially specific to the motor network. The network-specific pattern of functional reorganisation is reminiscent to a recent rs-fMRI study in a stroke cohort with heterogeneous acute stroke lesions. In that study, changes in functional brain connectivity during recovery were mainly restricted to networks that contained the focal stroke lesion.[5]

EDSS is strongly weighted towards motor function and in this study we used changes in EDSS score as a surrogate measure of clinical motor recovery.[3] It would have been interesting to also include more direct and objective measures of motor performance such as the timed Nine-hole Peg Test and 25 feet Walk test. This would allow us to more specifically relate the dynamic changes in motor resting-state connectivity associated with a motor relapse to dexterity or walking. Another limitation of this study is the relative small sample size and the fact that no rs-fMRI data was obtained before the relapse which would have required a large-scale prospective cohort study.

In summary, this prospective rs-fMRI study identified changes in resting-state functional connectivity in key motor regions in the acute phase of a MS relapse with paresis of an upper limb. Clinical improvement was associated with a weakening of motor resting-state connectivity in SMA extending into mesial M1, presumably indicating a normalisation of the connectivity pattern with recovery of motor function. It is possible that rs-fMRI may be able to distinguish between patients with MS with a favourable and poor motor outcome after an acute relapse based on the reorganisation pattern.

However, the potential of rs-fMRI to predict the individual capacity of spontaneous functional recovery requires studies that contrast the resting-state connectivity pattern in patients with a favourable and poor motor outcome after a relapse in a larger cohort of patients.

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Contributors A-MD contributed to the design of the study, recruitment of subjects, scanning and clinical evaluation of subjects, data analysis, interpretation of results and wrote the paper. MB contributed to the design of the study, recruitment of subjects, discussed the results and commented on the paper. OBP contributed to the design of the study and commented on the paper. FS contributed to the design of the study, recruitment of subjects and commented on the paper. PSS contributed to the design of the study and commented on the paper. HRS contributed to the data analysis, interpretation of results and writing of the paper. KHM contributed to the design of the study, performed the analysis of imaging data, statistical analysis, interpretation of results and wrote the paper.

Competing interests MB reports serving on scientific advisory boards for Biogen Idec, Merck-Serono, Novartis, Sanofi-Aventis and Teva; receiving speaker honoraria from Biogen Idec, Merck-Serono, Bayer-Schering, Novartis, Teva and Sanofi-Aventis; has received consulting honoraria from the Danish Multiple Sclerosis Society, Biogen Idec and Merck-Serono; has received funding for travel from Biogen Idec, Merck-Serono, Sanofi-Aventis, Genzyme and Solvay Pharma. FS has served on scientific advisory boards, been on the steering committees of clinical trials, served as a consultant, received support for congress participation, received speaker honoraria, or received research support for his laboratory from Bayer Schering, Biogen Idec, Genzyme, Lundbeck, Merck Serono, Novo Nordisk, Novartis, Sanofi-Aventis, Schering Plough and Teva. PSS reports having served on scientific advisory boards Biogen Idec, Merck Serono, Novartis, Genmab, TEVA, Elan, GSK; has been on steering committees or independent data monitoring boards in clinical trials sponsored by Merck Serono, Genmab, TEVA, GSK, Bayer Schering, and he has received funding of travel for these activities; has received speaker honoraria from Biogen Idec, Merck Serono, TEVA, Bayer Schering, Sanofi-aventis, Genzyme and Novartis. HRS has received honoraria as speaker from Lundbeck A/S, Valby, Denmark, Biogen Idec, Denmark A/S, Genzyme, Denmark and MerckSerono, Denmark, honoraria as editor from Elsevier Publishers, Amsterdam, the Netherlands and Springer Publishing, Stuttgart, Germany, travel support from MagVenture, Denmark and grant support from Biogen Idec, Denmark A/S.

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REFERENCES

1 Dogonowski A-M, Siebner HR, Sørensen PS, *et al*. Expanded functional coupling of subcortical nuclei with the motor resting-state network in multiple sclerosis. *Mult Scler* 2013;19:559–66.
2 Lee M, Reddy H, Johansen-Berg H, *et al*. The motor cortex shows adaptive functional changes to brain injury from multiple sclerosis. *Ann Neurol* 2000;47:606–13.
3 Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33:1444–52.
4 Dum RP, Strick PL. The origin of corticospinal projections from the premotor areas in the frontal lobe. *J Neurosci* 1991;11:667–89.
5 Ovadia-Caro S, Villringer K, Fiebach J, *et al*. Longitudinal effects of lesions on functional networks after stroke. *J Cereb Blood Flow Metab* 2013;33:1279–85.

Supplementary Material

In the following pages we describe in detail the demographic data of the participants and the functional magnetic resonance imaging (fMRI) analyses. Moreover, a series of post-hoc analyses were performed to qualify the main findings reported in this paper.

Index:

Participants

Patients were recruited from The Danish Multiple Sclerosis Center, Copenhagen, Denmark, and comprised 12 MS patients with an acute motor relapse. All patients fulfilled the revised McDonald criteria.[1] Eleven patients had relapsing-remitting MS and one patient had secondary progressive MS. All patients presented with acute motor deficits including paresis of the left (n=5) or right (n=7) upper limb. Additional symptoms are listed in table 1 along with the clinical characteristics. All patients were right-handed as revealed by the Edinburgh Inventory.[2] Patients were neurologically examined including Expanded Disability Status Scale (EDSS) score by the same

neurologist at inclusion and follow-up.[3] The relapse was treated with a three-day course of intravenous methylprednisolone 1 g daily which was initiated after the first scan. Written informed consent was obtained from all patients prior to scanning, and all protocols were approved by the local scientific ethical committee (protocol no. KF01–131/03). See table 1 for further details on the participants.

Magnetic Resonance Imaging

MRI was performed on a Siemens 3.0 Tesla Magnetom Trio Scanner. The first resting-state fMRI (rs-fMRI) scan was performed within 24 hours after relapse onset, while the second rs-fMRI was obtained 6 to 21 days after relapse onset. Echo planar imaging (EPI) was used for rs-fMRI (TR=2490ms, TE=30ms, 3×3×3mm resolution, FOV=192×192mm, 42 interleaved slices) and 480 brain volumes were acquired over 20 minutes. During rs-fMRI, subjects were instructed to rest with their eyes closed without falling asleep, and refrain from any voluntary cognitive or motor activity. The cardiac cycle was monitored with an infrared pulse oximeter attached to the index finger and respiration was monitored with a pneumatic thoracic belt. Patients continued to take their medication as usual. Smoking and caffeine intake was matched between first and follow-up scans for each subject.

Pre-processing

The fMRI data were pre-processed using statistical parametric mapping software (SPM8, http://www.fil.ion.ucl.ac.uk/spm). The first two brain volumes were excluded from analysis to allow for T1 equilibrium. The EPI images were motion corrected by a six-parameter rigid body realignment to the time series mean using a two-step procedure, and spatially normalised to a

symmetrical version of the Montreal Neurological Institute (MNI) 305 template. The fMRI scans from patients with left upper limb paresis were flipped as if all patients had left cerebral lesions and right upper limb paresis. Further pre-processing steps included spatial smoothing (Gaussian kernel, 6 mm full-width at half maximum) and high-pass filtering (1/128 Hz cut-off frequency). Prior to the statistical analysis, we ensured that the estimated volume to volume translational movement was below 1.5 mm. To further reduce the possibility that effects of motion played a role in the analysis we used a 24-parameter expansion of the estimated motion parameters to explain residual effects of motion.[4] These motion effects and physiological noise caused by the heart beat and respiration was modelled using multiple linear regression.[5] This enabled us to model resting-state signal changes related to physiological noise. Further details regarding image acquisition and pre-processing of the rs-fMRI data are described in a previous study.[6]

Resting-state connectivity analysis

Independent component analysis (ICA) was applied to the fMRI data to separate functional brain networks that show temporally correlated BOLD-signal fluctuations. The spatial ICA was based on the infomax algorithm using the Group-ICA-Toolbox for fMRI (http://icatb.sourceforge.net/) with the number of components fixed at 20. The sensorimotor network and two control networks - the primary visual and the default-mode network were identified by a template matching procedure in the GIFT software. This template-matching was done by estimating a spatial correlation coefficient of the independent components with templates of brain networks based on Brodmann areas (BA) as defined in the WFU PickAtlas.[7] We defined the templates of brain networks combining basic knowledge of the primary brain networks and from Damoiseaux et al. 2006 [8] (default-mode network) as follows: the sensorimotor network as BA 1, 2, 3, 4 and 6; the visual network as BA 17, 18, and 19 and default-mode network as BA 7, 11, 20, 23, 31, 32, and 37.[6]

Statistical analysis

We aimed at capturing acute changes in motor resting-state connectivity occurring in the 6-21 days after motor relapse (i.e. between the first and second rs-fMRI session). Specifically, we wished to test which brain regions showed a reduction in motor resting-state connectivity in proportion to clinical remission using the Randomise tool (http://fsl.fmrib.ox.ac.uk).[9] We used a random permutation test to assess for changes in motor resting-state connectivity (time 2 – time 1) including the improvement in EDSS score as a covariate (time 2 – time 1). The same analysis was performed for two non-motor networks, the primary visual and default-mode network. Correction for multiple comparison was done using cluster-wise maximum permutation statistics with a two-sided entry threshold of $p<0.01$. Clusters surviving a threshold of $p_{FWE}<0.05$ corrected for multiple comparisons over the whole brain were considered statistically significant.

Effects of motion

Due to a concern that the correlations observed were caused by spurious correlations arising from motion we reanalysed the data following the procedure outlined in Power et al. 2012.[10] We calculated frame-wise displacement from estimated motion parameters assuming a 20 cm diameter sphere, and excluded volumes (and the following volumes due to spin history effects) whenever the FD exceeded 0.5 mm. We also calculated the volume to volume root mean squared change in the BOLD-signal over the entire volume (DVARS) and likewise excluded volumes (and following volumes) exhibiting a signal change of 0.5% or above. Removal of these volumes were applied in the temporal filtering procedure (by regressing out the volume) to ensure that the temporal filtering was still valid. All other pre-processing, ICA and analysis steps remained unchanged. The results of

this analysis remained unchanged with the only difference that the significant cluster in the motor area was slightly reduced in size (Figure S1).

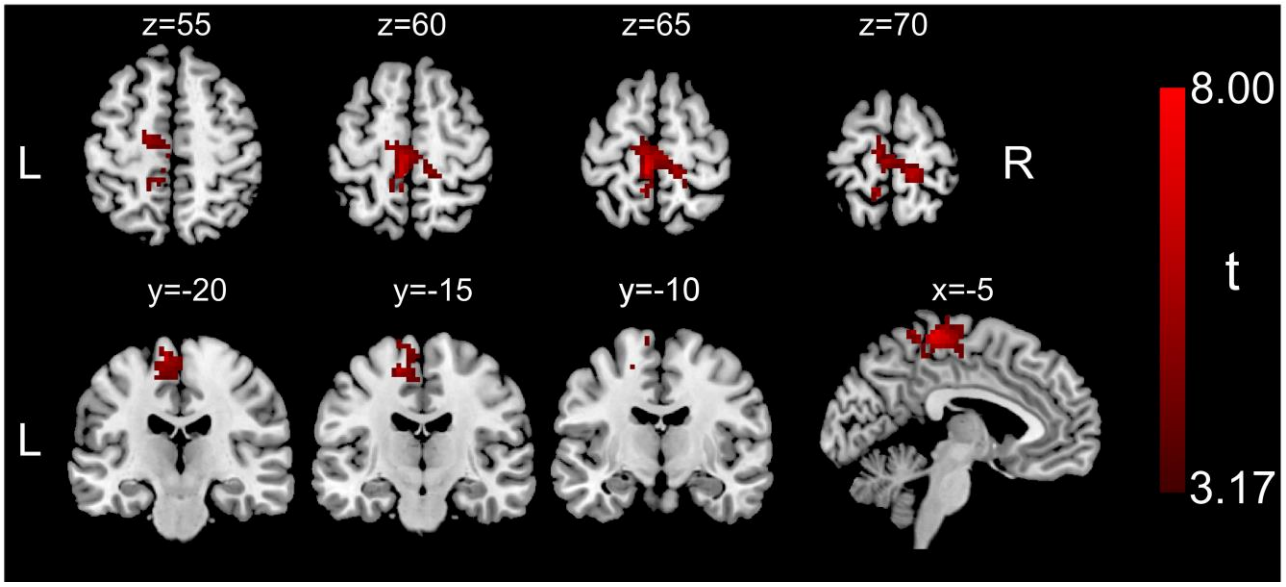

Fig S1: **Changes in motor resting-state connectivity with exclusion of volumes due to motion. Post-hoc analyses.** The figure corresponds to figure 1 in the main manuscript but with preprocessing and analysis done with volumes excluded. The volume of the activated area was reduced from 339 voxels (9.2 mm^3) to 329 voxels (8.9 mm^3) when excluding volumes affected by motion as defined by the criteria mentioned above.

Post-hoc we investigated if there were any correlation between disease duration, gender, and age and the change in EDSS score. This analysis did not reveal any significant correlations. The results for correlation with disease duration were (one sample t-test): p=0.39, t=0.90, df=10, whereas the result for correlation with gender was p=0.12, t=-1,73, df=10 and finally age: p=67, t=0.44, df=10. However, the limited sample size in our study does not allow us to make definite claims or analyses concerning the effect of disease duration, gender or age.

Comments on the MS lesion location

We were interested in identifying a common reorganisation pattern independent of the location of the MS lesion. Therefore, we performed neither contrast-enhanced nor spinal cord MRI to localise acute MS lesions. Most likely plaques in cervical spinal or subcortical brain white matter or brain stem were responsible for the arm paresis in the majority of patients. The cortico-subcortical output pathways of the cortico-spinal tract are affected by both cerebral or spinal cord lesions and it can therefore be argued that they share the same pattern of intracerebral motor reorganisation. Indeed, the results demonstrate that rs-fMRI is capable of linking very early changes in motor resting-state connectivity to functional remission after an acute motor relapse regardless of the location of the acute MS lesion.

Table 1: Clinical characteristics of patients

Patient #	Age,yr/ Sex	Days between scans	Affected upper limb, R/L	Additional relapse symptoms*	Clinical Remission	EDSS Scan1	EDSS Scan2	Disease duration	Disease-modifying Treatment
1	46/M	10	R	Paresis of R leg	+	3.0	1.0	1	-
2	46/M	9	L	Paresis and ↓position sense in L leg, Dizziness, Double vision	+	6.0	3.5	6	GA
3	28/M	11	R	Hypoesthesia R hand Paraparesis	+	5.0	4.0	3	-
4	35/F	8	L	Paresis of L leg	+	4.0	2.5	6	IFN-β
5	36/F	9	R	Paresis of R leg	+ Fatigue of R arm with use	3.5	2.5	13	NTZ
6	45/M	8	L	Paresis of L leg and paraesthesia in legs ↓Attention, ↓Concentration	+	4.0	2.5	7	NTZ
7	32/F	7	R	Paresis of R leg. Hypoesthesia R arm, Paraesthesia in R leg, Dysaesthesia face R	+	3.5	2.0	0	-
8	59/F	11	R	Paresis of R leg	+	6.5	6.5	16	
9	35/F	21	L	Paresis of L leg Paraesthesia in legs	+	3.5	3.5	1	IFN-β
10	25/F	10	L		+	4.0	2.5	6	NTZ
11	45/M	7	R	Paresis of R leg	+ Partial recovery	6.0	5.0	4	-
12	55/F	6	R	Paresis of R leg	+	4.5	3.5	24	NTZ
Median (range) Nb	41 (25-59) 7F/5M	9 (6-21)	7R/5L			4.0 (3.0-6.5)	3.0 (1.0-6.5)	6 (0-24)	

EDSS = Expanded Disability Status Scale; F = female; GA = Glatiramer acetate; IFN-β = Interferon beta; L = left; M = male; NTZ = Natalizumab; R = right; *Additional relapse symptoms associated with the upper limb paresis

References

[1] Polman CH, Reingold SC, Edan G, *et al*. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol* 2005;**58**:840–46.

[2] Oldfield RC. Assessment and analysis of handedness - edinburgh inventory. *Neuropsychologia* 1971;**9**:97–113.

[3] Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;**33**:1444–52.

[4] Friston KJ, Williams S, Howard R, *et al*. Movement-related effects in fMRI time-series. *Magn Reson Med* 1996;**35**:346–55.

[5] Glover GH, Li TQ, Ress D. Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. *Magn Reson Med* 2000;**44**:162–67.

[6] Dogonowski A-M, Siebner HR, Sørensen PS, *et al*. Expanded functional coupling of subcortical nuclei with the motor resting-state network in multiple sclerosis. *Mult Scler* 2013;**19**:559–66.

[7] Maldjian JA, Laurienti PJ, Kraft RA, *et al*. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage* 2003;**19**:1233–39.

[8] Damoiseaux JS, Rombouts SARB, Barkhof F, *et al*. Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A* 2006;**103**:13848–53.

[9] Winkler AM, Ridgway GR, Webster MA, *et al*. Permutation inference for the general linear model. *Neuroimage* 2014;**92**:381–97.

[10] Power JD, Barnes KA, Snyder AZ, *et al*. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage* 2012;**59**:2142–54.