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# Reproducibility of regional structural and functional MRI networks in cerebral small vessel disease compared to age matched and stroke-free controls

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ABSTRACT

Abnormalities in structural and functional MRI connectivity measures have been reported in cerebral small vessel disease (SVD). Previous research has shown that whole-brain structural connectivity was highly reproducible in SVD patients, while whole-brain functional connectivity showed low reproducibility. It remains unclear whether the lower reproducibility of functional networks reported in SVD is due to selective disruption of reproducibility in specific networks or is generalised in patients with SVD.

In this case-control study 15 SVD and 10 age-matched control participants were imaged twice with diffusion tensor imaging and resting state fMRI. Structural and functional connectivity matrices were constructed from this data and the default mode, fronto-parietal, limbic, salience, somatomotor and visual networks were extracted and the average connectivity between connections calculated and used to determine their reproducibility.

Regional structural networks were more reproducible than functional networks, all structural networks showed ICC values $\geq$0.64 (except the salience network in SVD). The functional networks showed greater reproducibility in the controls compared to SVD with ICC values >0.7 for control participants and <=0.5 for the SVD group. The default mode network showed the greatest reproducibility for both control and SVD groups.

Reproducibility of functional networks was affected by disease status with lower reproducibility in SVD compared with controls.

## 1. Introduction

Cerebral small vessel disease (SVD) causes lacunar stroke and is the most common pathology underlying vascular cognitive impairment and dementia [1]. Increasing evidence suggests that cognitive impairment in SVD results from damage to white matter tracts leading to disruption of the complex brain networks mediating cognitive functions, such as executive function and information processing speed [2]. Evidence for this comes from MRI studies which have shown disruption of both structural and functional networks [3,4]. Structural connectivity can be assessed using diffusion tensor tractography MRI and provides a measure of the state of networks dependant on white matter tract integrity [5]. Functional connectivity networks are derived from temporal correlations between the signal from brain regions measured from resting-state Blood Oxygenation Level-dependant (BOLD) functional MRI [6].

The extent of structural network disruption in SVD has been shown to both associate with cognitive impairment [2,7], and predict the future risk of dementia [8]. The degree of structural network disruption was found to mediate the effect of a number of different SVD pathologies, including T2 white matter hyperintensities (WMH), lacunar infarcts, and cerebral microbleeds, on cognitive function, suggesting brain network disruption is a core feature of how different pathologies cause cognitive impairments [2]. Similarly, impaired functional connectivity has been reported in SVD. Many studies have found decreased connectivity between the prefrontal cortex and the more posterior parts of the default mode network [9,10], with an association with reaction times in the Stroop test [11]. In addition, other functional networks have also been reported to show changed connectivity in SVD [12,13].

This has led to the suggestion that network analysis may be a useful method to quantify the degree of SVD pathology, and could perhaps be

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used as a surrogate marker in phase 2 clinical trials of patients with SVD. However, to fulfil this use it needs to be demonstrated that it has a high level of reproducibility on repeat measurements. A previous study has shown that whole brain structural network connectivity measures such as global efficiency are highly reproducible in both SVD and controls [14]. In contrast whole brain functional connectivity, while moderately reproducible in controls, showed low reproducibility in patients with SVD. This parallels previous research in multiple sclerosis which reported poor reproducibility of functional connectivity across the entire brain network, however, higher reproducibility was found in localised regions of interest [15]. In contrast another recent paper in SVD reported reasonable reproducibility in the global efficiency for both the whole brain functional networks and individual subnetworks, such as the default mode network [16]. A recent review [17] of the literature has shown that, in general, reproducibility of functional connectivity from individual connections is, on average, 'poor' with worse reproducibility in disease populations [18,19].

Measurement of functional networks depends on the BOLD signal, which links oxygenation changes to alteration in perfusion, and any diffuse vascular process affecting the small vessels could possibly alter the integrity of this response. An associated variable is the resting state fluctuation amplitude (RSFA), this is a measure of the amplitude of the signal variation seen across the time course for a given voxel/region. The RSFA differs between individuals and disease groups and reflects the level of signal change caused by the physiological behaviour of the vessels over time, the rs-fMRI response. It is possible that the reproducibility of functional connectivity networks may be related to the RFSA seen as a lower RFSA will amplify the effects of noise in the time course resulting in reduced, or more variable, correlations between time courses and, thus, increased variability in the measurements. A reduced RSFA in SVD participants may result in reduced reproducibility in their networks.

It remains unclear whether the lower reproducibility of functional networks reported in SVD is due to a selective disruption of reproducibility in specific localised networks, or a generalised issue relating to the use of functional networks in patients with SVD. In order to investigate this question, we determined the test-retest reliability, which we characterize here as reproducibility, of specific localised brain networks in the brain of patients with SVD and controls. We compared the reproducibility of structural and functional localised networks and determined whether the degree of reproducibility of functional networks depended on their structural integrity. We also investigated whether the reproducibility of the functional networks was related to the RSFA.

## 2. Materials and methods

### 2.1. Participants

Fifteen patients with symptomatic SVD and 10 stroke-free controls who had repeat MRI scans, recruited to a previous study [14], were included in the analysis. Inclusion criteria for the SVD cases were: 1) history of clinical lacunar stroke [20] with MRI evidence of an anatomically appropriate lacunar infarct, 2) presence of confluent White Matter Hyperintensities (Fazekas scale $\geq 2$) [21]. Exclusion criteria were any cause of stroke other than small vessel disease (i.e. embolic stroke, cortical infarction, or large artery disease) and any major central nervous system disease other than SVD. All participants gave written informed consent, the study was approved by East of England - Cambridge East research ethics committee (reference: 14/EE/0014). The corresponding author had full access to the data and takes responsibility for its integrity and data analysis. We have reported this study according to the STROBE reporting guidelines. Researchers were aware of the participants group during analysis.

### 2.2. MRI data acquisition

Details about the MRI data acquisition and data pre-processing have been previously reported [14]. In brief, MRI data were acquired on a 3T Verio MRI system (Siemens AG, Erlangen, Germany) at baseline and a follow-up scan (around two months after the baseline scan).

Data acquisition included:

- 1 mm volumetric T1-weighted MPRAGE
- T2-weighted FLAIR
- T2*-weighted gradient echo
- Axial single shot T2-weighted EPI sequence with diffusion-weighting ($b = 1000$ s·mm$^{-2}$) acquired in 63 non-collinear directions on the whole sphere. Eight non-diffusion weighted images ($b = 0$ s/mm$^{-2}$) were acquired. TE/TR: 106/11,700 ms, GRAPPA: 2, acquisition matrix 128 × 128, FOV: 256 × 256 mm, 63 contiguous 2 mm slices. Acquisition time 14.5 min. Additionally imaged were acquired to allow field mapping. An eleven-minute axial multi-echo EPI resting state sequence during which participants were instructed to attend to a fixation cross was also acquired, TR: 2430 ms, TE1/2/3: 13/31/48 ms, Flip angle: 90°, GRAPPA: 2, acquisition matrix: 64 × 64, FOV: 240 × 240 mm, 34 slices of 3.8 mm thickness, 10% slice gap. Reconstructed voxel dimensions: 3.75 × 3.75 × 4.18 mm. 269 volumes were acquired.

### 2.3. MRI data processing

#### 2.3.1. Diffusion analysis

Full methods are shown in [14], in brief diffusion data were pre-processed using the FSL suite of tools [22], In addition to correction for eddy currents and motion, fieldmaps were prepared and EPI distortion correction was performed. The diffusion data was then skull stripped and the tensor calculated to produce mean diffusivity and fractional anisotropy maps.

Deterministic tractography was then performed, streamlines were seeded within voxels on an evenly-spaced super-resolution grid (0.5 mm$^3$) and were propagated in steps of 0.5 mm using the Euler delta crossings method (EuDX) [23] in dipy. As in [14] streamlines were terminated where tensor fractional anisotropy was less than 0.2, or the angle of propagation >30° By then locating the start and end points of the streamlines to the atlas regions (see 2.4) the connectivity throughout the brain is built up. The number of streamlines is weighted by dividing by the length of streamline due to the multiple seed points along the streamline to determine the connectivity between pairs of atlas regions.

The structural network measure used in this analysis is the average connectivity between the nodes involved in the network in question (see 2.4), for example for the nodes in the default mode network (pars orbitalis, superior frontal, rostral anterior cingulate, isthmus cingulate inferior parietal, precuneus, middle temporal, and the banks of the superior temporal sulcus). The connectivity between each pair of nodes (the number of streamlines after correction) is calculated and then the sum over all pairs divided to create the average connectivity.

#### 2.3.2. rs-fMRI processing

Resting state fMRI data were analysed in SPM [24]. Cortical reconstruction and volumetric segmentation of the T1-weighted images was performed using the Freesurfer suite (http:// surfer.nmr.mgh.harvard.edu; version 5.3 [25]. Details of the data pre-processing have been previously described [14].

The functional network measure used in this analysis is the average correlation coefficient between the nodes involved in the network in question (see 2.4). Again using the default mode network as an example the correlation coefficient between each pair of nodes in the network is calculated then averaged to provide the measure of connectivity for the network.

### 2.4. Network construction

Network nodes were defined from the Desikan-Killiany parcellation [26] and the atlas was created using a procedure previously described [27,28]. For each network node the overlap between each ROI in the parcellation and the maps of the six Yeo canonical resting state networks was calculated [6]. Each ROI was assigned to the subnetwork with the greatest overlap. Using these node assignments 6 localized networks were reconstructed from the structural and functional adjacency matrices, as follows: default mode network, fronto-parietal network, limbic network, salience network, somatomotor network, and the visual network. The default mode network consisted of pars orbitalis, superior frontal, rostral anterior cingulate, isthmus cingulate inferior parietal, precuneus, middle temporal, and the banks of the superior temporal sulcus. The somatomotor network consisted of the precentral, paracentral, postcentral, superior temporal and transverse temporal. The visual network consisted of the cuneus, pericalcarine, lateral occipital, lingual, fusiform, and parahippocampal gyri. The salience network consisted of the caudal anterior cingulate, the posterior cingulate, the supramarginal gyrus and the insula. The frontal network consisted of the pars triangularis, pars opercularis, rostral middle frontal caudal middle frontal gyri. The limbic network consisted of lateral orbitofrontal, frontal pole, medial orbito frontal, entorhinal, temporal pole, and inferior temporal gyri.

### 2.5. Resting state fluctuation amplitude

The resting state fluctuation amplitude (RSFA) was measured for each subject as the standard deviation of the signal time course divided by the mean signal and derived from the whole grey matter (GM), and also from the individual GM regions involved in each of the networks, defined above, and averaged across all regions within each network.

### 2.6. Statistical data analysis

We assessed test-retest reproducibility for average connectivity in the structural and functional networks using Intraclass correlation coefficient ICC(2,1) according to the definition of Shrout & Fleiss [29]. The model used (2) is appropriate where the same rater analyses all the images and is considered representative of all possible raters, as opposed to model 1 where different raters analyse different images and model 3 where the same rater(s) analyse all images and are the only raters of interest. The form (second number, 1) is appropriate as there is only one rater, so only one (pair of) measurement(s) for comparison. Previous work has defined classified the ICC such that values below 0.5 indicate poor reliability, from this to 0.75 moderate reliability, between 0.75 and 0.9 good reliability, and above 0.9 excellent reliability [30]. Intraclass correlations over time were calculated using the statistical analysis package Pingouin in python [31].

We determined if there was a significant correlation between the intraclass correlation coefficients in the structural and functional networks at the network level for the control and SVD groups separately. We also tested if the network strength in the structural default mode network was associated with the pairwise intraclass correlation in the functional network for each pair of nodes. Both tests were performed using Spearman's correction coefficient. To determine whether the reproducibility of the networks was associated with the level of connectivity we also correlated the ICC with the average connectivity for each network from the first scan for both control and SVD groups.

We compared the RSFA between the controls and SVD participants using the Mann-Whitney U-test in both the whole GM and the specific networks and then measured the reproducibility of the RSFA.

The potential confound of difference in time of day of the two scans was compared between the two groups using the Student's unpaired *t*-test.

## 3. Results

### 3.1. Subject characteristics

The SVD group contained ten males and five females with a mean age of 66 (±standard deviation 13) years, and the control group consisted of nine males and one female with a mean age of 68 ± 3 years [14]. The mean time between the two scans was 11.7 ± 4.4 weeks for the control group and 4.9 ± 10.0 weeks for the SVD group; significantly longer for the control group ($p = 0.03$). The difference in time of day between the two scans was 177 (SD ± 84) minutes for controls and 128 (±109) for the SVD group, this difference was not significant ($p = 0.21$). The volume of white matter hyperintensities in the control group was 2471 ± 3609 $mm^3$ and for the SVD group was 27,743 ± 21,681 $mm^3$.Other demographic information can be seen in Table 1, including the score from the Mini-Mental state exam.

### 3.2. Reproducibility of structural networks

In the control group, the limbic, salience, somatomotor and visual structural networks showed good reproducibility (ICC > 0.75). In the SVD group, the default mode, fronto-parietal, limbic and visual networks showed good reproducibility (ICC > 0.75, see Table 2 for detailed statistics).

### 3.3. Reproducibility of functional networks

In the control group, the default mode, fronto-parietal, limbic, somatomotor and visual networks showed good reproducibility (ICC ≥ 0.75). Reproducibility of the salience network in the control group was close to good (ICC = 0.71). By contrast in the SVD group no network showed good reproducibility. The Default Mode Network in the SVD showed the best reproducibility with moderate reproducibility (ICC = 0.50) (see Table 3). Reproducibility was lower in the SVD group when compared with the control group for all networks. Although the confidence intervals for the control participants included the SVD group value for each network suggesting the values are not significantly different.

**Table 1**
Demographic data for the two groups, age, Mini Mental State Exam, years of education and body mass index are tested using the unpaired *t*-test, others are compared using the $chi^2$ test.

| | Controls ($n = 10$) | SVD ($n = 15$) | Group test |
|---|---|---|---|
| Age – years (SD) | 67.67 (2.92) | 65.33 (13.22) | $p = 0.5$ |
| Male sex (%) | 9 (90.00%) | 10 (66.67%) | $p = 0.4$ |
| ethnicity | 8 – white british, 2 – white any other background | 10 – white british, 4 – white any other background, 1 black caribbean | |
| Hypercholesterolaemia (%) | 5 (50.00%) | 8 (53.33%) | $p = 1$ |
| Diabetes mellitus (%) | 0 (0.00%) | 2 (13.33%) | $p = 0.7$ |
| Years of education (SD) | 12.5 (3.5) | 12.0 (3.0) | $p = 0.64$ |
| Mini Mental State Exam (SD) | 29.3 (0.8) | 27.6 (3.0) | $p = 0.098$ |
| Hypertension (%) | 3 (30.00%) | 11 (73.33%) | $p = 0.084$ |
| Body mass index - $kg/m^2$ (SD) | 25.30 (2.74) | 28.61 (4.14) | $p = 0.057$ |
| Smoking: Current (%) | 1 (10.00%) | 3 (20.00%) | $p = 0.7$ |
| Smoking: Ex (%) | 3 (30.00%) | 5 (33.33%) | – |
| Modified Rankin scale (%) 0 | 10 (100.00%) | 5 (33.33%) | $p = 0.011$ |
| 1 | 0 (0.00%) | 5 (33.33%) | – |
| 2 | 0 (0.00%) | 1 (6.67%) | – |
| 3 | 0 (0.00%) | 4 (26.67%) | – |

**Table 2**
Intraclass correlations between baseline and follow-up average connectivity in localized structural networks. The ICC model used is the single random rater model (ICC2). (DMN: default mode network, FPN: frontoparietal network).

| Network | Control ICC | Control ICC CI95% | Control ICC p-value | SVD ICC | SVD ICC CI95% | SVD ICC p-value |
|---|---|---|---|---|---|---|
| DMN | 0.64 | [0.06–0.9] | 0.019 | 0.86 | [0.65–0.95] | $6 \times 10^{-6}$ |
| FPN | 0.66 | [0.11–0.9] | 0.015 | 0.89 | [0.71–0.96] | $1 \times 10^{-6}$ |
| Limbic | 0.94 | [0.5–0.99] | $6 \times 10^{-7}$ | 0.76 | [0.44–0.91] | $3 \times 10^{-4}$ |
| Salience | 0.90 | [0.66–0.97] | $1 \times 10^{-4}$ | 0.47 | [−0.03–0.78] | 0.033 |
| Somatomotor | 0.82 | [0.42–0.95] | 0.001 | 0.67 | [0.27–0.87] | 0.002 |
| Visual | 0.88 | [0.6–0.97] | $1 \times 10^{-4}$ | 0.85 | [0.61–0.95] | $3 \times 10^{-6}$ |

**Table 3**
Intraclass correlations between baseline and follow-up average connectivity in localized functional networks. The ICC model used is the single random rater model (ICC2,1). (DMN: default mode network, FPN: frontoparietal network).

| Network | Control ICC | Control ICC CI95% | Control ICC p-value | SVD ICC | SVD ICC CI95% | SVD ICC p-value |
|---|---|---|---|---|---|---|
| DMN | 0.83 | [0.49–0.96] | $5 \times 10^{-4}$ | 0.50 | [−0.02–0.80] | 0.030 |
| FPN | 0.78 | [0.36–0.94] | 0.002 | −0.06 | [−0.56–0.46] | 0.586 |
| Limbic | 0.76 | [0.26–0.93] | 0.005 | 0.15 | [−0.66–0.40] | 0.702 |
| Salience | 0.71 | [0.15–0.92] | 0.003 | 0.30 | [−0.24–0.70] | 0.132 |
| Somatomotor | 0.76 | [0.29–0.94] | 0.001 | 0.33 | [−0.23–0.72] | 0.114 |
| Visual | 0.75 | [0.31–0.93] | 0.004 | 0.20 | [−0.37–0.64] | 0.238 |

### *3.4. Correlation between intraclass correlation coefficients from functional and structural network at the network level*

No significant correlations were found between the intraclass correlation coefficient of the edge density in the structural network and the intraclass correlation of the average connectivity in the functional network for either the control or the SVD group when correlating over networks (Spearman Rho = 0.089; $p = 0.87$ in the control group, Spearman Rho = −0.6; $p = 0.21$ in the SVD group, Fig. 1). This finding suggests that the reproducibility of structural networks is independent of the reproducibility of functional networks.

### *3.5. Pairwise intraclass correlation coefficient in the default mode network*

We next tested if the reproducibility of the functional networks could be explained by the level of connectivity in the structural network. To assess this we determined if the pairwise reproducibility, i.e. the intraclass correlation calculated node-by-node in the functional default mode network correlated with the edge density derived from the diffusion tensor imaging. We tested this in the default mode network, as the most reproducible functional network. We found no correlation between the edge density in the structural default mode network and the reproducibility of the node-pair intraclass correlation coefficient in the functional network (Spearman Rho = −0.18, $p = 0.63$ in the control group, Spearman Rho = 0.09, $p = 0.75$ in the SVD group, Fig. 2). This finding suggests that the greater reproducibility seen in the control group in the default mode functional network is not driven by the level of structural connectivity in the network.

### *3.6. Correlation between intraclass correlation coefficient and average connectivity*

There was no significant correlation between the ICC and the average connectivity from the networks for either subject group ($p > 0.32$), except for the functional networks in the SVD group where the correlation approached significance ($r = 0.77$, $p = 0.08$).

### *3.7. RSFA variation*

Comparison of the RSFA from the whole GM in the control and SVD groups showed a lower RFSA in the SVD group (2.5 ± 3.0% vs. 1.6 ± 1.2%); however this difference was not significant ($p = 0.34$), both the mean signal and standard deviation used to calculate the RFSA were reduced in the SVD group, but again these differences were not significant ($p = 0.59$ and $p = 0.54$ for mean signal and standard deviation respectively). There was also no difference in the reproducibility of the RFSA between the two groups ($p = 0.99$).

When looking at the individual networks the RSFA was reduced in the SVD group for all 6 networks (DMN: 1.1 ± 1.8% vs. 0.6 ± 0.3%, FPN:

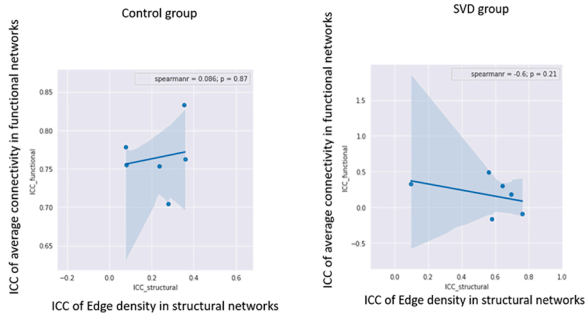


**Fig. 1.** Correlation between intraclass correlation coefficient of the edge density in structural networks and the intraclass correlation of the average functional connectivity in functional networks. (Data points correspond to individual networks). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

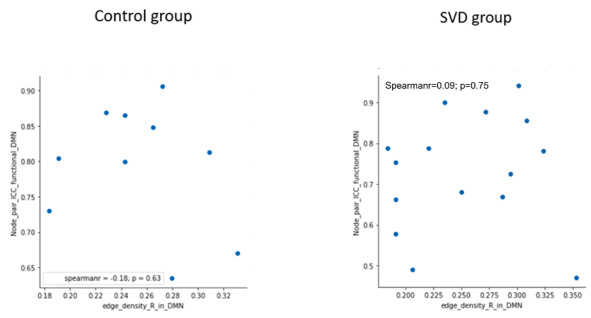


**Fig. 2.** Spearman correlation between intraclass correlation from pair of nodes in the functional default mode and structural network density of the default mode network in the control and the SVD group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

1.2 ± 1.7% vs. 0.7 ± 0.6%, Limbic: 1.0 ± 1.6% vs. 0.5 ± 0.3%, Salience: 1.0 ± 1.8% vs. 0.5 ± 0.2%, Somatomotor: 1.1 ± 1.8% vs. 0.5 ± 0.3%, Visual: 1.1 ± 1.8% vs. 0.6 ± 0.4%). However, this did not reach significance in any case (all $p>0.29$ or less significant). The same is also true of the reproducibility of the RFSA where the visual network showed an increased coefficient of variation in the SVD group with a p value of 0.06 and all other networks were $p>0.11$.

## Discussion

In this study we determined the effects of SVD on reproducibility of both structural and functional canonical brain networks. We have previously shown that global (whole brain) network metrics such as global efficiency have high reproducibility in both SVD and age-matched stroke free controls [14]. In contrast functional global network measures had moderate reproducibility in age matched controls, but low reproducibility in SVD. In this study we looked at reproducibility in individual subnetworks.

Structural subnetworks showed a good level of reproducibility in both SVD cases and controls, with all subnetworks showing moderate or good reproducibility. Highest reproducibility was shown in the control group for the limbic, salience, somatomotor, and visual networks and in the SVD group for the default, fronto-parietal, limbic, and visual networks. In contrast for the functional networks, while the control group showed moderate to good reproducibility for all subnetworks, in the SVD group no networks showed good reproducibility, and reproducibility for each individual network was considerably lower than in the control group. Although this difference should be treated with caution due to the large confidence intervals seen for the reproducibility estimates. The only network which showed moderate reproducibility was the default mode network. The default mode network is perhaps the best-defined functional network and supposedly related to 'background' mental activity i.e., not related to a specific task or function. The higher level of reproducibility seen in the default mode network in SVD compared to other networks may suggest that it is in some way more resilient to the damage caused by SVD or is somewhat independent of it. One possible explanation for this may be that the default mode network is the largest network in terms of brain tissue covered so it is more resilient to tissue damage as there is still a significant amount of 'healthy' tissue remaining, although further work would be needed to investigate this.

It may be that increased variability in SVD is in itself a useful biomarker of disease progression. It is plausible that diseased brains would show a greater variability in connectivity as the disease progresses, however this would need much longer follow-up with multiple scans to investigate.

In further analyses we found no correlation between the reproducibility of structural and functional networks. To further explore factors affecting the reproducibility of the functional networks we determined whether there was a correlation between connectivity in the structural network and reproducibility of functional networks. We tested this in the default network as this was the most reproducible functional network in the SVD cases. We found no correlation, suggesting that the extent of structural connections did not underlie reproducibility of the functional networks. We also investigated whether the reproducibility was linked to the strength of the connections in the network in the first scan although there was no significant correlation the relationship between the initial connectivity and ICC approached significance, which might suggest that the stronger the connectivity in the network is the more reproducible it is, although the data we have is not strong enough to conclude this.

We also investigated whether the size of the RSFA, a measure of the signal change seen in GM over the course of the experiment, could explain the differences seen in the reproducibility between the control and SVD groups. A lower RSFA in SVD participants could result in greater variability of the functional connectivity seen due to the increased effect of noise in the measurements. We found that this was not the case which suggests that the lack of reproducibility in the SVD group is due to genuine changes in the functional connectivity rather than due to a lack of sensitivity due to smaller changes in signal, caused by lower levels of oxygenation change, and by extension brain activation. This is further evidenced by the lack of difference in behaviour in the DMN compared to the other brain networks. As the DMN shows the highest reproducibility in the SVD group it might have been expected that this might be reflected in increased RFSA in this network, but this was not the case suggesting that the reproducibility is not governed by the RSFA in this group. Both the mean signal and standard deviation of the time course was lower in the SVD group. These differences were both not significant and although the reduction in the standard deviation was greater than the reduction in the mean signal it is not possible to draw any conclusions about whether this is meaningful from the current data.

This study used atlas-based definitions for the networks studied, rather than data driven approaches derived from independent component analysis. The main advantage of using pre-defined atlases is that the regions (supposedly) have a biological basis and as such can be directly related to the function of these regions, whereas the independent component analysis approach can produce regions made up of multiple brain areas. It is also true that results using these atlases are reproducible across studies, whereas the data driven network definitions are specific to the dataset in question. Of course, diseased brains are abnormal and it is not clear whether brain atlases are as relevant in participants with disease compared to those without so the true brain networks may be misrepresented when using an atlas. It is possible that independent component analysis derived networks may show different results if the SVD group networks are misplaced or reorganised, however the canonical networks provide information on the state of the brain in SVD.

Our results have important implications for the use of resting-state functional MRI, and the study of subnetworks, in patients with SVD. The low reproducibility of all functional networks implies that resting state networks in patients with SVD are affected by the disease, causing increased variability in the connectivity seen between regions. This is important because resting state MRI is being increasingly used to evaluate interventions in SVD. It is also important because SVD is a highly prevalent condition with increasing age, and many elderly, apparently asymptomatic, individuals have significant SVD on MRI which could interfere with the reliability of functional MRI responses.

Our results are broadly similar to those from a recent study in patients with similar sporadic SVD [16]. Test-retest reliability was assessed for both global efficiency and the default mode network, fronto-parietal network, somatosensory network and visual network and the ICC was in all cases below or marginally above 0.4 indicating poor to fair reliability. In comparison free water measurements derived from DTI showed excellent reliability with an ICC of 0.988. The cause of reduced reproducibility in resting state networks in SVD patients is uncertain. However, the BOLD response is dependent on vaso-neuronal coupling which itself is dependent on the integrity of the neurovascular bundle [32]. Abnormalities of the neurovascular bundle, and particularly involvement of the matrisome and extracellular matrix, have been suggested to be cardinal features of SVD. Therefore, the SVD itself may interfere with the integrity of the BOLD response and its reproducibility via increased variability in an impaired system. Furthermore, the BOLD response is dependent on perfusion, and abnormalities of cerebral blood flow have been demonstrated in SVD [33,34]. Another explanation could be that SVD is associated with functional disconnection resulting in cognitive impairment [35]. These functional alterations can be detected in resting-state networks rather than using diffusion MRI, hence the lower reproducibility. It is odd that the SVD group showed better structural connectivity reproducibility than the control group for some, but not all networks, there is no obvious reason for this result which would need further investigation.

It is also possible that the other differences between the groups may contribute to the results seem. Table 1 shows that the SVD group had

increased incidence of health conditions, although only hypertension approached significance, an increased proportion of women, lower mini-mental state exam score, and 50% more subjects. Although many of these differences were non-significant, they may still have an effect on the results. In particular increased blood pressure and use of anti-hypertensive drugs may have an impact on the blood flow upon which the fMRI signal is based and how this changes over time. Larger studies would be needed to investigate whether this variability explains any of the variability in connectivity seen.

The reproducibility of functional connectivity differences between groups has also been investigated in other neurological disease. Four independent autism vs. control populations were studied and the group differences from the different populations showed low reproducibility [36]. Similarly, in Parkinson's disease, disease related functional connectivity changes were not reproducible between datasets or between random split of a single dataset [37]. Although, these studies are addressing a different question related to the specificity of disease effects they do suggest that functional connectivity is inherently a less reproducible technique than structural connectivity.

The statistical results presented here are not corrected for multiple comparisons, given the number of tests performed multiple comparison correction would have an effect on some of the significant results seen. However, the tests are not independent so the stricter corrections would be inappropriate. Although this lack of correction should be taken into account when considering the results, the main results seen relating to the lack of reproducibility in the functional networks would be unaffected by multiple comparisons. It should also be noted that the difference in reproducibility between the participant groups has not been statistically tested so the effect of multiple comparison correction is unknown, but that the functional connectivity in the control group was statistically reproducible (i.e. the ICC seen was statistically significantly different to zero), while the SVD group's was not and that these results would be unaffected by multiple comparisons.

Although the education status of the study participants is slightly higher than the general population, they do reflect the characteristics of the controls and SVD populations so suggest that the results should be generalizable to the wider sporadic SVD population for comparison with control subjects, however further studies should be carried out for monogenic forms of the disease such as CADASIL or other related conditions such as stoke patients.

Our study does have limitations. Firstly, it was in a relatively small sample size. Secondly acquisition times for DTI were longer than those for functional networks. It has been shown [38] in data from the human connectome project, that increased population sizes and more time-points results in increased reproducibility of functional connectivity measures; however, this would not account wholly for the reduced reproducibility seen compared to the structural networks or explain the differences seen between the control and SVD groups. Thirdly, the gap between scans was significantly longer for the control group, however it is more likely that if this were to have an impact on the reproducibility it would cause a reduction in the control group which is not what is seen. A further limitation is that the group status of the participants was known to investigators, while most of the analysis for the structural and functional connectivity is automated it is possible that this may cause bias in the results.

Further research is needed to justify the use of functional networks as surrogate markers in phase 2 clinical trials of patients with SVD.

In conclusion our results demonstrate good reproducibility for structural network measures, both global and of subnetworks, in both SVD and age-matched controls. In contrast while reproducibility of functional networks measures, both global and those of subnetworks, were moderate to good in controls, albeit with wide confidence intervals, they were poor in patients with SVD in whom only the default network achieved moderate reproducibility. These results have important implications for the use of functional networks in SVD, and caution should be exerted in using functional networks to assess interventions in patients with SVD.

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## Declaration of Competing Interest

The authors report no disclosures.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cccb.2023.100167.

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