exclusively from a stable population of non-dividing postmitotic neurons.

The challenge now lies in evaluating whether the AAVhu32 vector has the potential to be effective in the even larger and more complex human brain. A logical step forward would be testing it in non-human primates. Information is also required on the prevalence of pre-existing neutralizing antibodies against AAVh32 in paediatric and adult human populations. The normal serum chemistry and lack of any measurable adverse effects in the cats treated in the current study is encouraging, particularly as high doses of intravenously administered AAV vectors in SMA (Mendell *et al.*, 2017) and haemophilia B (Nathwani *et al.*, 2011) clinical trials have resulted in elevated serum liver enzymes, which required transient corticosteroid treatment to normalize.

The promise of gene therapy lies in the potential to cure genetic diseases from a single administration. To realize this for global brain disorders, the hunt for more effective and efficient vectors must go on. The study by Young Yoon *et al.* will therefore be welcomed, particularly by scientists and clinicians working in the rare paediatric neurodegenerative arena where the prognosis for patients is bleak. No effective treatments are available but novel approaches such as gene therapy could prove to be a light at the end of a dark tunnel.

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Competing interests

The authors report no competing interests.

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Indirect connectome-based prediction of post-stroke deficits: prospects and limitations

This scientific commentary refers to 'Post-stroke deficit prediction from lesion and indirect structural and functional disconnection', by Salvalaggio *et al.* (doi:10.1093/brain/awaa156).

The past few decades have seen an evolution of our understanding of the brain as a multidimensional network. Behavioural deficits in neurological disease are now increasingly ascribed to alterations in structural and functional brain networks. This also holds true for stroke, where a focal lesion may be accompanied by widespread structural and functional network alterations (Umarova *et al.*, 2016; Cheng *et al.*, 2020). Remote

dysfunction resulting from a focal lesion was originally labelled 'diaschisis', a term introduced by von Monakow in 1906. Advanced neuroimaging methods have subsequently expanded the 'diaschisis' concept to include altered network connectivity (for review see Catani and Ffytche, 2005; Carrera and Tononi, 2014). Several methods can be used to determine the extent of (remote) network alterations. Direct approaches measure connectivity and its disruption directly, using neuroimaging techniques such as structural or functional MRI in stroke patients. Indirect methods make use of datasets of functional or structural connections (the 'connectome') of healthy subjects derived from resting state functional MRI and diffusion tensor imaging data, respectively. By projecting a patient's lesion onto such datasets, it is possible to calculate the structural and functional connectivity of the lesioned area in the healthy connectome and thereby estimate the disconnection resulting from the lesion (Fig. 1). These indirect methods avoid the need to perform complex structural or functional imaging in individual patients. A small number of studies have applied indirect connectivity analysis to stroke patients, and the analyses have yielded plausible patterns of disconnectivity. However, the predictive value of the various methods of disconnection mapping with respect to stroke deficits remains unclear. In this issue of *Brain*, Salvalaggio and co-workers compare direct and indirect methods of estimating connectivity and lesion-symptom mapping in their ability to predict stroke deficits across several domains (Salvalaggio *et al.*, 2020). Specifically, the aim of the study was to compare the accuracy of four neuroimaging methods in predicting deficits: lesion localization, indirect estimates of structural and functional disconnections, and direct functional connectivity analysis (Fig. 2).

The authors studied an existing cohort of patients with first-ever stroke, who underwent neuropsychological and behavioural assessment as well as MRI approximately 2 weeks post-stroke (Corbetta *et al.*, 2015). The broad behavioural test battery covered multiple functional domains including focal deficits, i.e. motor and visual impairment, and several cognitive domains, i.e. language, verbal memory, spatial memory, and contralesional visual bias (spatial attention). For the imaging analysis, stroke lesions were delineated in all patients on anatomical MRI. Structural disconnection resulting from the stroke lesion was indirectly estimated using a software toolkit (Foulon *et al.*, 2018). The lesions were transferred into standard space, and diffusion-weighted imaging data for 176 young healthy subjects from the Human Connectome Project were used to identify all fibre tracks passing through each lesion. By averaging the results from these 176 maps for each patient, the authors generated structural disconnection maps that showed, for each brain voxel, the probability of structural disconnection owing to a given stroke lesion (Fig. 2). Indirect estimation of functional disconnection was performed via lesion-network mapping (Boes *et al.*, 2015). To this end, the authors used the resting state functional MRI data of the same 176 young healthy control subjects, and calculated voxel-wise correlations between the time course of the blood oxygen level-dependent (BOLD) signal for an individual stroke lesion and the rest of brain. For each patient, the results were averaged across all 176 maps to produce a functional disconnection map. In addition to these indirect measures of structural and functional disconnection, functional connectivity was also estimated directly for a subgroup of patients in whom resting state functional MRI data were available (Siegel *et al.*, 2016). Principal component analysis was applied to reduce the dimensionality of lesion, indirect structural and functional disconnection maps, and of direct functional disconnection images, and the resulting features were entered into multivariate analysis using a ridge regression model trained to predict patients' behavioural outcomes (Corbetta *et al.*, 2015). Finally, the authors calculated the proportion of the overall variance of each behavioural domain explained by the different imaging methods.

The study by Salvalaggio *et al.* is commendable for several reasons. It is the first direct comparison of established and standardized methods of disconnection mapping after stroke. The authors sought to validate the predictive power of the various methods for estimating connectivity across multiple behavioural domains with different degrees of network distribution and lateralization. As well as providing insights into functional impairment resulting from stroke—which has rarely been studied by analysis of structural or functional disconnection—this approach also provides useful information on the strengths and limitations of the methods, especially the easily accessible methods of indirect disconnection analysis.

The key results can be summarized as follows: (i) simple lesion maps consistently performed best in predicting behaviour across all domains; (ii) indirect structural disconnection maps were at best comparable to simple lesion maps, but did not improve prediction if added to lesion maps; (iii) performance of indirect functional disconnection maps for prediction of behaviour was very poor and sometimes little better than chance; and (iv) direct measurement of functional disconnection was significantly better than indirect functional disconnection mapping in predicting patients' behaviour (Fig. 2).

In the subgroup of patients ($n = 88$) with resting state functional MRI data available, Salvalaggio *et al.* compared direct analysis of functional disconnection to the indirect methods. Direct functional connectivity analysis predicted 42% of variance in language performance, which was comparable to the performance of indirect structural disconnection maps (47%) and better than that of indirect functional disconnection maps (16%). For several other domains (i.e. left motor, right motor, verbal memory, spatial memory), direct functional disconnection explained a small proportion of the variance, while the indirect predictive

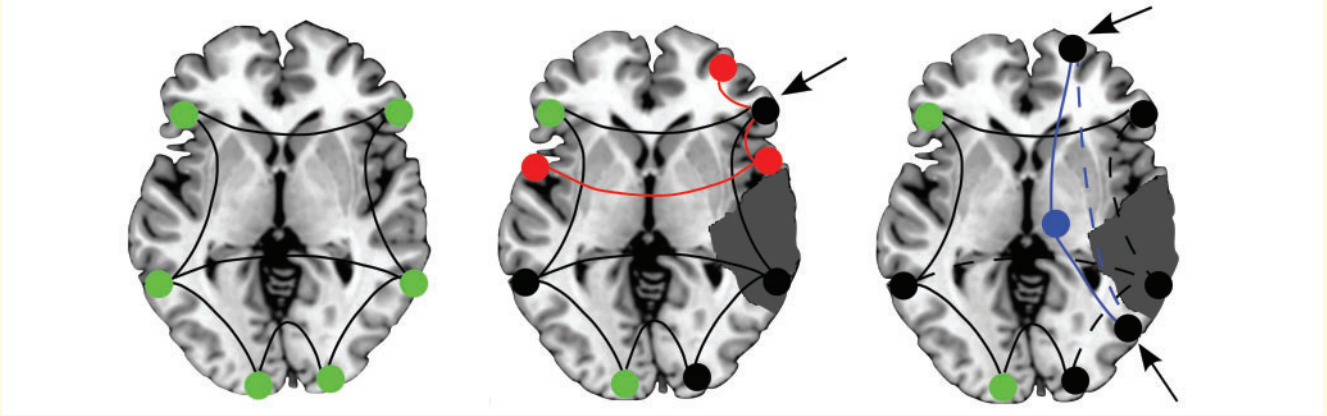
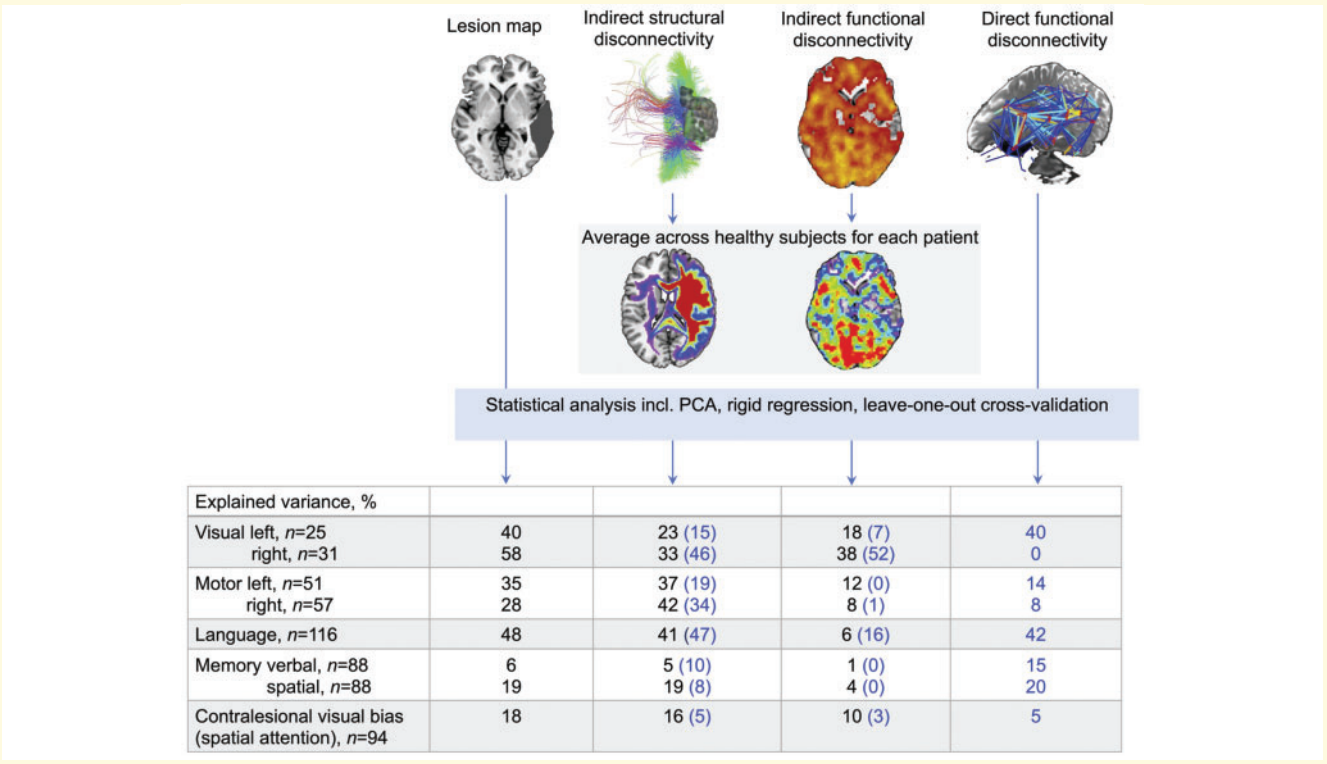


Figure 1 Schematic and simplified illustration of connectivity in a healthy network of interest (*left*), and following functional (*middle*) and structural (*right*) disconnection owing to a focal brain lesion. *Left*: Healthy large-scale network of interest with functional nodes and structural inter-connectivity. *Middle*: The indirect functional disconnection approach assesses abnormal functional network by projecting a lesion onto the healthy brain and evaluating the BOLD signal correlations between the lesion and the rest of the brain. The functional network with activity synchronized to a lesion site (black nodes) is considered to be functionally damaged. If a given functional centre (shown with arrow) is also part of another network (shown in red), its dysfunction will be context-specific. *Right*: The structural disconnection approach dissects fibre tracks disrupted by a lesion (black dashed lines). It also considers 'disconnected' regions (shown with arrows) that are connected by alternative pathways (blue network).



Explained variance, %				
Visual left, *n*=25 right, *n*=31	40 58	23 (15) 33 (46)	18 (7) 38 (52)	40 0
Motor left, *n*=51 right, *n*=57	35 28	37 (19) 42 (34)	12 (0) 8 (1)	14 8
Language, *n*=116	48	41 (47)	6 (16)	42
Memory verbal, *n*=88 spatial, *n*=88	6 19	5 (10) 19 (8)	1 (0) 4 (0)	15 20
Contralesional visual bias (spatial attention), *n*=94	18	16 (5)	10 (3)	5

Figure 2 Schematic illustration of study methods and results (Salvalaggio *et al.*, 2020). The results of the additional analysis in the subgroup of patients (*n* = 88) with resting state functional MRI data available are shown in blue in parentheses.

model based on functional disconnection was not significantly better than chance.

The results raise important questions, both in terms of the behavioural impact of brain network changes following stroke, and also the methods used to study these changes. Given the promising preliminary results obtained with indirect techniques for estimating structural and functional brain connectivity, it appears rather disappointing that simple lesion measurement was comparable or even better at predicting behavioural deficits after stroke

Glossary

Connectome: The structural or functional connection matrix of the human brain.

Functional connectivity analysis: Method for evaluating synchronization between different brain regions. Regions with correlating activity are considered to comprise functional networks. In functional MRI, BOLD signal oscillations and their correlations across brain regions are assessed.

Functional disconnection: The loss of synchronization—and thus functional coupling—between network nodes.

Structural disconnection = dis-connectome: Estimation of fibre tracks disrupted by a lesion. Usually evaluated by extracting all structural connections passing through a lesion projected onto the structural connectome of healthy subjects.

than indirect structural disconnection mapping, and was far better than indirect functional disconnection mapping. A number of methodological issues may have influenced the results. First, lesion localization was not restricted to one specific territory, and the study included patients with brainstem and cerebellar lesions. While methods of disconnection mapping are well established for supratentorial lesions, they may be less valid for lesions in infratentorial regions. Second, the methodology may not yet have been optimized, which may make the results susceptible to even slight changes in parameter settings. For example, for both of the indirect methods of disconnection mapping, the results of the subgroup analyses differed from those of the whole sample: e.g. for structural disconnectivity for spatial memory, R^2 was equal to 0.08 and 0.19, respectively. The large variability in results for the same focal deficit depending on the affected hemisphere also points in this direction. In a previous study by the same group, direct functional connectivity mapping yielded higher predictive scores than in the present study, even though the same overall method was applied to the same patient cohort (Siegel *et al.*, 2016).

There are also more general concerns related to the use of indirect methods of brain connectivity measurement in stroke patients. In addition to focal lesions, stroke patients often show cerebrovascular disease or atrophy. For these patients, the assumption that individual brain regions have the same structural connectivity across patients and as compared to healthy young controls like the participants in the Human Connectome Project, may not hold true. Use of direct structural connectivity maps might have yielded different results, but was not part of the current study.

The comparison of indirect functional lesion network mapping to direct measurement of functional connectivity showed that direct estimation of functional disconnection was better at predicting behaviour. At the same time, the predictive ability of functional disconnection overall was very limited. It may well be that the examination of resting state functional connectivity, though informative in neuropsychiatric or neurodegenerative disorders such as Alzheimer's disease, is not as useful for explaining behavioural impairment after stroke. Widespread network alterations undoubtedly occur following stroke, and represent a mixture of disconnection and adaptive reorganization (Carter *et al.*, 2010). But while resting state connectivity is easy to study, analysis of task-related alterations in brain networks may be more appropriate for understanding functional reorganization after stroke and for predicting behavioural impairment, which usually occurs in a task-related brain state. In other words, resting state functional disconnection mapping assesses brain networks in a context-unspecific way. This contradicts the concept of dynamic 'diaschisis', which postulates that remote dysfunction of a given functional centre is context-specific (Carrera and Tononi, 2014, Fig. 1). The limited explanatory power of the indirect disconnection maps also raises questions as to the validity of the underlying concept. It casts a degree of doubt over whether the disruption of structural connections between any given cortical region and a stroke lesion will result in a behaviour-relevant functional and/or structural disconnection of the lesioned area, given that the lesioned area may well be connected via alternative pathways and functional in other networks beyond the one that is damaged (Fig. 1). Furthermore, connectome-based indirect analyses provide a static result that is independent of time post-stroke. However, time is of critical importance for stroke patients, as large-scale network reorganization takes place soon after stroke and shows high levels of inter-individual variability (Umarova *et al.*, 2016). Notably, the methods were able to explain less of the variance in deficits in cognitive domains with widely distributed underlying networks—memory and spatial attention. As their architecture is sculpted across the lifespan, these networks may demonstrate greater interindividual variability in anatomy, capacity and lesion compensation than networks underlying focal deficits.

The work of Salvalaggio *et al.* lays the foundations for future research, by demonstrating the importance of face-to-face comparison of methods. It also shows that the reliability, validity and clinical impact of neurocomputational and neuroimaging approaches should be evaluated not only based on the plausibility of visual results or on a comparison of 'target' versus 'control' syndromes, but also in terms of the amount of behavioural variation each is able to explain. Third, the study confirms that the comprehensive analysis of lesion maps provides crucial information and remains a critical method for predicting stroke deficits.

And finally, it shows that new methods are required for the study of networks, as brain network analysis comprises much more than simple disconnection mapping.

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Making neurogenetics a global endeavour

This scientific commentary refers to 'The role of genetics in Parkinson's disease: a large cohort study in Chinese mainland population', by Zhao *et al.* (doi:10.1093/brain/awaa167).

Genetic research in neurological disease has overwhelmingly focused on European populations. This has contributed to inequity in our understanding of diseases that affect diverse populations around the world, and represents a failure to engage with data that will be key to interpreting our rapidly expanding clinical-genetic resources. In this issue of *Brain*, Zhao and co-workers report the results of a comprehensive study of single gene/Mendelian causes of Parkinson's disease in a large patient population in China (Zhao *et al.*, 2020).

The study was based at Xiangya Hospital in Changsha, Hunan Province, and also involved the 'Parkinson's Disease and Movement Disorders Multicenter Database and Collaborative Network in China' (PD-MDCNC). Hunan province has a population of 67 million people, and China a population of 1.4 billion. The study recruited 1676 participants with Parkinson's disease, including sporadic early onset disease (EOPD) ($n = 1242$), as well as autosomal recessive (ARPD) ($n = 192$) and autosomal dominant disease (ADPD) ($n = 242$). DNA from each participant was sequenced using whole exome sequencing, and analysed for copy number variants using multiplex ligation probe-dependent amplification (MLPA). This revealed that 34% of the patients with ARPD, 4% of those with ADPD and 5% of those with EOPD carried pathogenic mutations in known Parkinson's disease genes. These data will be a tremendous resource for the interpretation of variants in known risk genes, as well as for the discovery of new genes in the future.

As in existing series of European patients, *PRKN* was the most common gene causing ARPD. However, the current study also identified mutations that have not been seen in the very large numbers of European ARPD/EOPD patients in whom this gene has been analysed. For example, 2% of ARPD families in the Chinese study carried a homozygous exon 6 skipping mutation in *PRKN* (c.619-1G>C) that segregates with the disease and leads to an abnormal truncated form of parkin. This mutation has previously been reported in another Chinese patient with Parkinson's disease (Shi *et al.*, 2018) but has not been reported in ARPD or EOPD patients of European origin (Klein *et al.*, 2018; Landrum *et al.*, 2018).