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SCIENTIFIC COMMENTARIES

Big news from small world networks after stroke

How does the brain accomplish any of its tasks? Each 'bit' of the brain receives a piece of information, performs a specific calculation on it, and forwards the processed information on to the next bit. Communication is crucial and needs to take place between different bits of the brain located near and far. Somehow, from local processing and functioning interconnections a thought, sensation or motor command emerges. The brain is a complex network comprising multiple 'nodes' and 'links', and the notion that only one place in the brain is responsible for anything amounts to phrenology. Nodes (also termed 'vertices') in large-scale neuronal networks usually represent anatomical regions. Links (also termed 'edges') represent functional or effective connections. The brain requires an optimal balance between regional segregation and inter-regional, global integration of neuronal activity. Measures are now available to give summary descriptions of the network structure.

In order to understand pathological states of the brain therefore, it seems critical to determine what happens to brain network structure and function. In this issue of *Brain,* Wang *et al.* (2010) present an interesting set of data on reorganization of the motor executive network in patients suffering from subcortical stroke. This study is particular in that the authors focused on changes of network dynamics during the recovery process, rather than describing local activation phenomena only. Wang and colleagues used a longitudinal approach with follow-up testing at 1 week, 2 weeks, 1 month, 3 months and 1 year after stroke. The main finding is that the network does change, and the reorganized network gradually deviates more and more from what might be considered optimal network architecture. In the process of recovery, the global motor executive network with 21 predefined regions of interest becomes less 'clustered' and shows less functional segregation overall. However, within this less clustered network, the ipsilesional primary motor cortex and the right cerebellum (dentate nucleus) become gradually more 'important'. That is, the more patients recover, the more connectivity is augmented from and towards these two areas. Topographically, functional connectivity increases between ipsilesional primary motor cortex and contralesional motor areas like primary motor cortex, dorsal and ventral premotor cortex and posterior cingulate gyrus, as well as between ipsilesional dorsal premotor cortex, contralesional superior parietal lobe and contralesional cerebellum. This shift towards a network spreading out to the contralesional hemisphere confirms and extends earlier findings based largely on movement-related EEG coherence analyses (Strens *et al.*, 2004; Gerloff *et al.*, 2006).

While it seems apparent that studying brain function and functional reorganization requires the analysis of network-like activity, it is far less clear which is the best way of doing this. Advances in signal processing have allowed more sophisticated analysis of both magnetic resonance images and EEG and magnetoencephalography (MEG) signals. In particular, techniques such as correlation, coherence analysis, partial coherence analysis, directed transfer functions, partial directed transfer functions and mutual information permit measurement of single connections between pairs of regions. These approaches have been used in several studies but have also been criticized (Schoffelen *et al.*, 2008). Recently, new techniques such as that employed by Wang *et al.* allow more advanced analyses including global measures. Their approach is called 'graph theory' and looks at networks as a set of 'vertices' (nodes) with 'edges' (links) between them. This fits the brain well because the nodes can be considered the small bits resembling a collection of a few cortical columns, and the edges can be considered the connections between them. For MRI, the nodes are voxels and for EEG/MEG the nodes are electrodes/sensors or derivatives, and techniques such as correlation can calculate the connections.

In graphs, the connections can be regular or random, or something in between. An in-between type graph that shows both good local connections and some distant connections can be called a 'small world network'. This concept dates back to earlier ideas, not in brain research but in sociology, in which scientists like Milgram and Travers became interested in the social interconnections within a modern population (Milgram, 1967; Travers and Milgram, 1969). It was not the widely recognized 'Milgram experiment' on obedience to authorities which he started in the early 1960s. In fact, it was later work in which Stanley Milgram wanted to determine the probability that two randomly selected individuals would know each other. This relationship can be analysed as a graph. A population can be seen as a social network with a defined average path length between any two nodes (individuals). Milgram used information packages sent by mail to measure these path lengths. He developed a procedure to count the number of ties between any two people. Upon arrival of a package at its destination, the researchers could count the number of times it had been forwarded from person to person. The average path length was around six, suggesting that people in the

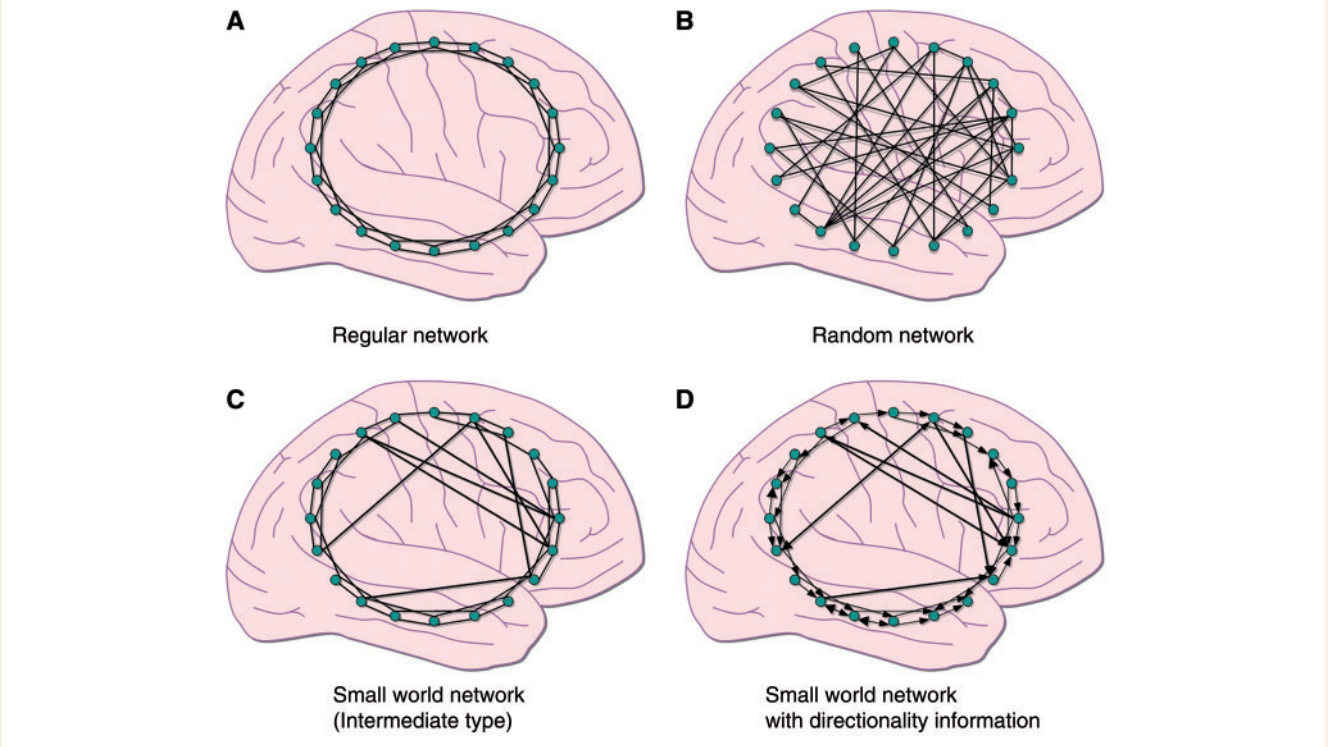

Figure 1 Examples of networks. (**A**) Regular network in which each node has connections only with 2 nearest neighbours on each side, links are assumed to be reciprocal, no directional information is given (high clustering, no 'random' links with longer path lengths). (**B**) Random network in which all nodes are randomly linked to other nodes of the network (low clustering, high path lengths). (**C**) In-between type network with small world properties (= small world network) (high clustering, low path lengths), and (**D**) additional use of advanced correlation approaches like directed transfer function (directed transfer functions) providing directionality information within small world networks. Green dots = nodes (vertices); black lines = links (edges); black arrows = directed links.

United States are separated by about six people on average—a small world!

Transferring this to brain physiology, path length or the normalized weighted version of this parameter, *lambda*, describes the average minimum number of connections that link any nodes of the network. *Lambda* can be interpreted as a parameter denoting the ability of parallel information propagation. With respect to regional information processing, the so-called clustering coefficient or its weighted normalized version *gamma* is of interest in small world networks. *Gamma* quantifies the extent of local efficiency of information transfer in a network. The current view is that small world networks have relatively high *gamma* and low *lambda*, i.e. they tend to process information within regional clusters and avoid excessive connections between clusters (Watts and Strogatz, 1998; Latora and Marchiori, 2001). When *gamma* decreases and/or *lambda* increases, then a network shifts toward a random network which, when excessive, is considered non-optimal (Fig. 1A–C). This is exactly what Wang *et al.* have observed in their longitudinal study on stroke patients. This is a novel finding and puts network reorganization after a focal brain lesion into a different perspective, away from descriptive anatomy and towards measures of network efficiency. The work also raises many conceptual questions. First, why does the increase in randomness not come about immediately after the ischaemic stroke when the network is acutely disrupted? Wang *et al.* do not see the first significant change towards a random network until 10–14 days after the ischaemia. One measure of a network is its robustness to 'lesion', and it appears that the brain is 'robust' in this regard.

Second, does it make sense that changing a network towards a 'random state' is nature's response to a lesion? At this point, we do not know the molecular, histological or even the exact functional substrates of 'network randomization'. Non-optimal axonal outgrowth may be one factor, but cannot explain the data completely. Modulation of synaptic gains in pre-existing networks, unmasking of salient connections and disordered or re-adjusted timing of information transfer are only some of the alternative explanations that come to mind. One intriguing possibility is that, while restructuring of the network is necessary for recovery, it is not possible to maintain optimal network organization. These questions will have to be addressed in future studies.

The recent data on small-world network changes in pathological brain states are stimulating and prompt the reasonable working hypothesis that 'network randomization' could be a common final pathway of how the brain reacts to lesions or neurodegenerative processes (Bartolomei *et al.*, 2006; Ponten *et al.*, 2007; Stam *et al.*, 2007; Rubinov *et al.*, 2009). Whether this really corresponds to striving for and finally creating new effective connections remains to be determined.

Under which conditions should network activity be measured? Of note, the study of Wang and co-workers was done at rest. Earlier connectivity analyses in stroke patients were performed in

the context of motor tasks (Strens *et al*., 2004; Gerloff *et al*., 2006; Grefkes *et al*., 2008). Resting-state connectivity, on the other hand, has gained growing interest in recent years. In patients with neurological deficits including hemiparesis or neuropsychological sequelae, there is an obvious advantage to resting state measures. They are not contaminated by differences in task performance when the results are compared with normal controls. But this also has a downside. How modality specific can resting-state connectivity be in the absence of a task? Each motor task may use specific nodes and links of the motor network to different degrees. When is it more appropriate to focus on task-related changes of network activity rather than looking at only the resting state? How far does resting-state functional connectivity go beyond modern non-invasive measures of structural connectivity (Pannek *et al*., 2009)? While the results of resting-state analysis are intriguing, it will be necessary to delineate its precise functional relevance further.

Another 'area to watch' is temporal resolution. The blood oxygenation level-dependent (BOLD) signal, as used in the study by Wang *et al.*, is a relatively indirect measure of neuro-electric activity. Because of the haemodynamic response, temporal resolution of the BOLD signal is quite limited and there is a substantial delay between real-time modulations of neuronal activity and subsequent changes of the BOLD signal. Subtle adjustments of neuronal timing are likely to escape our attention unless they mediate large metabolic effects. How precisely can the function of neuronal networks be characterized at the time scale of haemodynamic responses? It might be necessary to apply small-world mathematics to faster, e.g. oscillatory signals and phase information in and across different frequencies as obtained with EEG or MEG, even though there is an inherent trade-off with reduced spatial resolution in the latter technologies. This compromise, however, may become less problematic as more advanced algorithms for inverse problem solutions and computations in the source space become available (Palva *et al*., 2010). Similar improvements can also be achieved by using complex spatial filtering approaches rather than iterative dipole fitting procedures. These algorithms and the use of special correlative measures like phase coherence allow for a more reliable detection of where EEG or MEG signals actually come from. Because of the inverse problem, these solutions, even if they are based on spatial filtering rather than on dipole modelling, are always somewhat ambiguous. Finally, there is the issue of directionality. Many anatomical regions may have bidirectional functional connections so that it might be appropriate to model them as 'binary' links without directional information of signal propagation. However, ultimately we want to know which bit of the brain sends and which one receives specific information at any step during cognitive processing (Fig. 1D) and there are mathematical approaches such as directed transfer functions that allow for generating this information. These approaches provide insight into causal relations and model time-dependent flow patterns (Giannakakis and Nikita, 2008).

In summary, understanding how the brain adapts to lesions like stroke is of major interest both clinically and from a basic neuroscience perspective. Several studies have demonstrated altered regional activation patterns likely to represent adaptive reorganization of local processing (Weiller *et al*., 1992; Ward *et al*., 2003; Lotze *et al*., 2006) but not much information is available on dynamic network changes in the course of functional recovery. The study of Wang *et al.* published in this issue adds valuable information and, at the same time, raises many stimulating but as yet unanswered questions. In a great variety of investigations of brain function, aspects of small world networks can be seen. Small world networks have many descriptors that can indicate the brain's features. These could contribute in the future to an overall sense of how the brain is functioning. The analysis of multi-site communication in the brain is a rapidly growing and most interesting field in systems neuroscience. For the time being, the concept that 'network randomization' might be a general response of the brain to lesions is stimulating and ripe for further enquiry.

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Angiogenesis in glioblastoma: just another moving target?

Inhibition of angiogenesis as a concept experienced a renaissance in neuro-oncology in 2009. Based on encouraging phase II data suggesting increased response rates and improved quality of life, including a corticosteroid-sparing effect (Friedman *et al*., 2009; Kreisl *et al*., 2009), bevacizumab—an antibody that targets vascular endothelial-derived growth factor (VEGF)—was approved for the treatment of recurrent glioblastoma in the US and, for example, in Switzerland; but not throughout the European Union (Weller and Stupp, 2009). Further, the results of a 2:1:2 randomized trial comparing the VEGF receptor antagonist, cediranib (Batchelor *et al*., 2007), with the alkylating agent lomustine, and the combination of cediranib and lomustine in patients with recurrent glioblastoma are to be presented at the American Society of Clinical Oncology meeting in June 2010. Finally, not only bevacizumab (and probably soon cediranib, too), but also another anti-angiogenic agent, the integrin antagonist cilengitide, are currently being evaluated in registration trials for patients with newly diagnosed glioblastoma (Stupp *et al*., 2010).

Theoretical support for the therapeutic approach of angiogenesis inhibition in glioblastoma stems from the idea that the endothelial cell is the only stable, reliable element in an increasingly heterogenous and chaotic tumour microenvironment. Genetic instability of glioma cells might drive rapid selection processes resulting in the generation of multiple and diverse resistant tumour cell clones. In contrast, it has commonly been assumed that (true) endothelial cells, which are non-neoplastic host cells recruited by the growing tumours, would be resistant to the development of resistance, because of their stable genetic phenotype. Yet, clinical experience has already taught us that such views are over-simplified.

First, not all vessel formation in glioma depends on VEGF. This is apparent from the rate of responding patients defined by classical neuroradiological outcome criteria of 30–50% (Batchelor *et al*., 2007; Friedman *et al*., 2009; Kreisl *et al*., 2009). Whilst these data should be welcomed as promising, it must not escape our notice that at least half of glioblastomas do quite well in the presence of bevacizumab or cediranib, indicating that not all glioblastoma-related angiogenesis is strictly dependent on VEGF. Second, the responses to anti-angiogenic agents targeting VEGF are commonly transient, suggesting that there are effective escape mechanisms for blood vessel formation, contradicting the wishful thinking of endothelial resistance to the development of resistance to targeted anti-angiogenic therapy. In fact, numerous other molecules, including other VEGF-family members as well as placenta-derived, hepatocyte and fibroblast growth factor, are implicated in the primary or acquired resistance to VEGF-antagonistic treatments.

Against this background, in the current issue of *Brain*, El Hallani *et al*. (2010) address a largely neglected mechanism by which gliomas maintain vascular perfusion—the formation of vessel-like structures by the tumour cells themselves, referred to as 'vasculogenic mimicry of the tubular type'. They identify a subset of glioblastomas characterized by 'blood vessels' that are lined by non-endothelial cells. This interpretation is based on the presence of collagen IV, a marker of blood vessel basement membranes, in the absence of the expression of CD34, a universal marker for endothelial cells. To support the idea that the vessel-lining cells are tumour cells, the authors demonstrate the amplification of epidermal growth factor receptor (EGFR) in these cells, in a tumour known to harbour the molecular phenotype of EGFR amplification. Some of the tumour cells express smooth muscle actin, indicating that these have trans-differentiated into vascular smooth muscle-like cells. Intriguingly, the authors provide one example of a tissue section where there appears to be an anastomosis between an endothelially lined and a tumour-derived 'vessel'. Finally, the authors analyse the subpopulation of glioma-initiating cells by CD133 sorting from two tumours, one with and one without putative tumour-derived blood vessels. CD133+ cells from the former tumour generate vessel-like structures in tube formation assays, whereas the latter do not. An expression of endothelium-associated genes was observed in both populations of glioma-initiating cells. These observations lead the authors to propose that the subpopulation of glioma-initiating cells may even possess the plasticity to form blood vessels. Taken as a whole, this study indicates that some glioblastomas may grow in