of light microscopy called stochastic optical reconstruction microscopy (STORM)[9]. The authors used 3D STORM to study synapses between *in-vitro* cultured mouse neurons derived from the brain's hippocampus region, which is involved in learning and memory. The synapses under observation release the neurotransmitter glutamate. This analysis revealed that RIM is confined to protein nanoclusters of around 80 nanometres in diameter that lie close to the active zone. By contrast, other scaffold proteins and fusion factors, such as MUNC-13 and Bassoon, showed a more uniform distribution.

Is the position of these RIM-rich nanoclusters related to vesicle-fusion sites? The researchers monitored fusion events at the presynaptic membrane using a protein-based sensor that fluoresces following vesicle–membrane fusion. Mathematical modelling of the fluorescence patterns revealed that fusion sites are restricted to particular regions of the membrane. Moreover, a different form of super-resolution light microscopy called photoactivated localization microscopy that allows live imaging, confirmed that RIM density increased within 40 nm of these fusion sites.

The authors next investigated whether the RIM-rich fusion sites might be coordinated with the position of the postsynaptic apparatus dedicated to receiving the neurotransmitter signal. A sophisticated scaffold resides close to the postsynaptic membrane — a part of which is the multi-domain protein PSD-95, which is involved in the clustering of AMPA- and NMDA-type glutamate receptor proteins[10,11]. Precise measurement of RIM and PSD-95 densities revealed a clear spatial correlation between the components. Tang *et al.* therefore concluded that a nanoscale columnar structure spans the synaptic cleft, bringing RIM-enriched sites of synaptic-vesicle fusion face-to-face with postsynaptic PSD-95 nanodomains (Fig. 1).

Finally, Tang and colleagues asked if the nanocolumns could be a stable architectural motif or whether they are involved in the regulatory changes in synaptic strength that are crucial for cognitive functions. The authors pharmacologically activated NMDA receptors to depress synaptic strength. Although there was no immediate change in the architecture of the nanocolumn, after 25 minutes a subset of RIM nanoclusters suddenly grew larger — notably only those lying opposite PSD-95 nanodomains and residing in nanocolumns. Thus, retrograde signals that mediate the upregulation of presynaptic release in response to postsynaptic changes might specifically target the scaffold proteins and release machinery located opposite the postsynaptic glutamate receptors to modulate synaptic strengthening. As such, the nanocolumn could provide an important regulatory platform.

This study generates pressing questions. For instance, to understand the physical nature of the nanocolumns, it would be interesting to determine what regulates their formation. Trans-synaptic pairs of cell-adhesion membrane proteins are obvious candidates for mediating nanocolumn formation. Perhaps such adhesion molecules ultimately control the positioning and recruitment of RIM.

Alternatively, diffusible signals might cross the cleft and specifically trigger assembly of nanocolumns on the scale of a few tens of nanometres. In addition, RIM itself could be involved in nanocolumn formation — RIM contains a central domain that binds to the intracellular part of calcium channels[12], which ultimately trigger synaptic-vesicle fusion.

In the future, the nanocolumn concept should be validated and extended by investigating more proteins, including synaptic cell-adhesion proteins and other cytoplasmic scaffold proteins, and by combining imaging with genetic manipulation. Although the details of trans-synaptic coordination and the proteins involved might turn out to vary between synapse types and organisms, the nanocolumnar architectural motif could be a fundamental and generic building principle for synapses. ■

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SYSTEMS NEUROSCIENCE

A modern map of the human cerebral cortex

An authoritative map of the modules that make up the cerebral cortex of the human brain promises to act as a springboard for greater understanding of brain function and disease. SEE ARTICLE P.171

B. T. THOMAS YEO & SIMON B. EICKHOFF

The human brain's cerebral cortex is crucial for sensory and motor processing, as well as for mental functions such as interpreting language and logical reasoning, the complexity of which distinguishes us from other animals. On page 171, Glasser *et al.*[1] describe an updated map of the human cerebral cortex. This long-awaited advance provides a reference atlas that will allow those researching brain structure, function and connectivity to work within a common, systems-neuroscience framework.

Regional differentiation within the cerebral cortex has long prompted attempts to identify the cortex's distinct compartments, from classical neuroanatomical studies at the beginning of the twentieth century[2] to modern non-invasive, *in vivo* methods based on magnetic resonance imaging (MRI). Such endeavours are complicated by the fact that every location in the brain can be described by an almost infinite set of features, including density of receptor proteins for various neurotransmitter molecules, long-range connections to other parts of the brain, and specialization for neural computations that support specific functions. Almost all previous studies have attempted to delineate cortical compartments using a single feature (Fig. 1). By contrast, Glasser and colleagues capitalize on the unprecedented quality and breadth of MRI data gathered by the Human Connectome Project, the aim of which is to elucidate the neural pathways that underlie brain function and behaviour using cutting-edge brain-imaging methods[3].

MRI provides unparalleled access to the living brain. A single MRI machine can take many different measurements (known as modalities) — from establishing the relative density of neuron-insulating myelin sheaths to determining the thickness of the cortex, both of which can vary sharply between

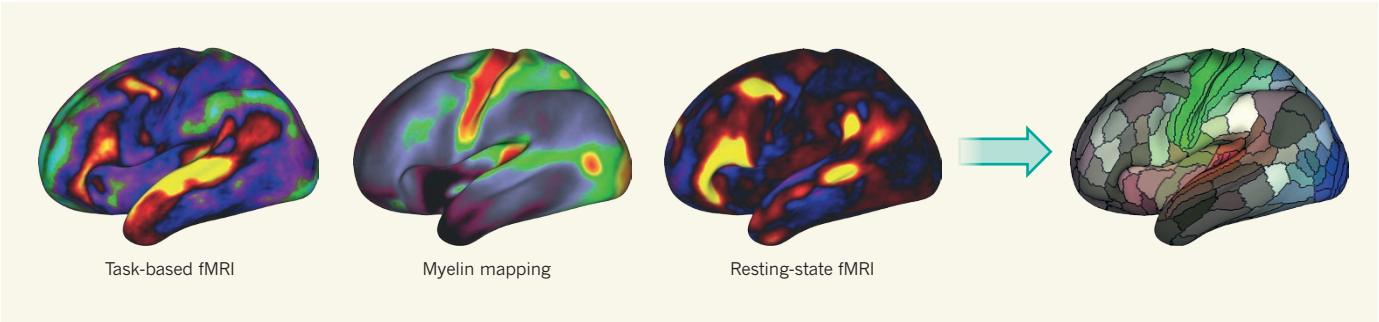

Figure 1 | Mapping function in the brain. Glasser *et al.*[1] defined distinct regions in the human cerebral cortex using a combination of brain-mapping techniques that have previously been used only separately, including: task-based functional magnetic resonance imaging (fMRI), which informs on the functions of different regions; relative density of the neuron-sheathing substance myelin, which provides information about cortical architecture; and resting-state fMRI, which details neural connectivity within and between different regions. In each of these three panels, colours provide a heat map of the measurements. The result is a map that delineates 360 distinct cortical areas. Different colours represent how connected each area is to sensory inputs (hearing, red; touch, green; vision, blue) and to systems involved in cognition (light and dark). Mixed colours show areas in which functional systems overlap. (Images taken from ref. 1.)

cortical areas. Furthermore, functional MRI (fMRI) can measure the changes in blood flow that accompany mental tasks, as well as whole-brain activity in resting states, providing information about regional neural activity that accompanies different brain states. The authors' integration of information from several MRI modalities not only moves this work closer than previous attempts to the classical definition of a cortical area, but also has several key advantages over other investigations.

First, some modalities reveal borders not clearly reflected in others. For instance, the border between areas 3a and 3b of the somatosensory cortex (which processes information about touch and pain) is easily delineated by myelin mapping, but not by resting-state fMRI. As another example, Glasser *et al.* developed a resting-state fMRI technique that maps topographic neural connectivity within the visual cortex. The sharp transition between levels of topographic connectivity across area boundaries allows much clearer delineation of discrete areas involved in early stages of visual processing than do myelin maps or conventional resting-state fMRI approaches[4,5].

Second, convergence across different MRI modalities reduces the likelihood of misdefining borders as a result of feature-specific noise or bias. This is important, given the indirect nature of most modalities — for example, fMRI measures the blood-flow changes that accompany neuronal activity as a proxy for neuronal activity itself. Consequently, complex computational pre-processing is often necessary to differentiate signal from noise. Agreement across modalities increases confidence that borders reflect biological reality rather than measurement biases.

Finally, an integrative approach better equips researchers to describe the properties of each area, as exemplified by Glasser and colleagues' comprehensive supplementary material. The authors find, for instance, that a cortical area characterized in the 1950s by its low myelin content[6] seems to be involved in language processing as measured by task-based fMRI — a finding consistent with a recent meta-analysis of more than 10,000 imaging experiments across 83 behavioural tasks[7]. Therefore, Glasser and colleagues' map represents the convergence of decades of classical neuroanatomical studies with modern non-invasive studies.

In contrast to the burgeoning field of resting-state fMRI mapping, which has largely focused on fully automatic approaches to divide the brain into parcels that have homogeneous connectivity patterns[8], Glasser and colleagues used a semi-automatic approach that explicitly incorporates prior knowledge from neuroanatomical studies to define the borders in their map. This inclusion represents a crucial and long overdue advance over agnostic, exclusively computational approaches. However, using prior knowledge to choose which modalities to trust in cases of conflicting evidence entails the danger of introducing confirmatory biases. Moreover, it could result in differential mapping quality between areas in which there is relevant, well-known information — such as the somatosensory and visual cortices — and those for which less knowledge exists, such as the prefrontal and parietal cortices. The latter pair is of particular interest to many neuroscientists, because these areas compute most functions that are specific to humans. Indeed, given that the authors explicitly ignore certain modality information for their data set that is functionally meaningful but fractionates classical cortical areas, further investigation will be crucial to understand how borders that are strongly demarcated in only one modality can be differentiated from modality-specific noise.

On a related theme, although Glasser *et al.* have delineated 360 cortical areas, these regions could potentially be subdivided into smaller, more-uniform units that are less distinct from each other. For example, different portions of the somatosensory cortex that represent distinct body parts might be considered as distinct computational units. Furthermore, examples of new areas being defined with the advent of more-sensitive or complementary methods are commonplace[9]. As such, it remains unclear what the 'optimal' number of areas to be defined is — let alone the 'correct' number. We suspect that the optimal number might be application-dependent. The authors' work, although seminal, will therefore probably not be the final word on this topic.

A key innovation in the current study is an automatic algorithm that seeks to delineate cortical areas in individual human subjects, a much more complex task than producing a map of the average brain. Previous work has attempted to estimate, in individual subjects, 10–20 functional networks (for example, see ref. 10), but Glasser and colleagues' goal of delineating 360 areas is more ambitious. Capturing inter-individual biological variability and differentiating such variability from measurement noise is essential to understand the relationship between brain organization and individual differences in behaviour, as well as for clinical applications.

The authors' validation of this algorithm focused on only a small portion of the cortex, so further investigation will be crucial. Nevertheless, their work represents a major step towards individual-specific 'biomarkers' of brain dysfunction, because individual-specific quantities of each area, such as grey-matter volume or connectional strength to other areas, can now be computed, and could be strongly predictive of individual differences in behaviour or disease.

Glasser and co-workers' atlas is the first multimodal map targeted at defining cortical areas, and therefore represents a major advance in human brain mapping. It is now up to researchers to use the anatomical framework provided, compare it with alternative approaches to mapping the human brain, and populate the defined areas with functional

and disease-related information. By doing so, we can begin to integrate multimodal data to understand how individual differences in brain organization can explain differences in function, behaviour and disorder. ■

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CANCER

Endothelial-cell killing promotes metastasis

To migrate into the lungs, cancer cells in the bloodstream must cross the lung's endothelial-cell barrier. A study shows that cancer cells can achieve this feat by signalling to induce endothelial-cell death. SEE LETTER P.215

CLAUDIO R. ALARCÓN & SOHAIL F. TAVAZOIE

Cancer cells often migrate from where the cancer initially formed, to colonize other parts of the body in a process called metastasis, which is associated with poor clinical prognosis. On page 215, Strilic *et al.*[1] uncover a surprising mechanism that migrating cancer cells in the bloodstream use to cross the lung's barrier of endothelial cells. The authors show that cancer cells send a signal that makes endothelial cells undergo a type of cell-death program called necroptosis (also known as programmed necrosis). Once in the lung, the cancer cells form lethal metastatic colonies.

The past three decades have provided increasing evidence that supports the key roles of endothelial cells in the formation and progression of tumours to a malignant state that has a poor prognosis for the patient[2–4]. Tumour cells rely heavily on the endothelial cells of blood vessels to enable continued tumour growth, because tumours need blood vessels to obtain oxygen and nutrients and expel metabolic waste.

Tumour cells exploit and manipulate endothelial cells by using intricate signalling mechanisms, such as those involving protein factors, secreted by tumours, that attract and remodel endothelial cells. Remodelling of blood vessels by tumour-derived proteins can enable cancer cells that reside in the primary site of tumour growth to enter the blood circulation, providing an escape route for the cells to reach distant organs[5,6]. After entering the bloodstream, cancer cells must cross the endothelial barriers that prevent them from entering other organs (Fig. 1a). In certain tissues, such as the lung or brain, the interface between the tissue and the bloodstream is relatively impenetrable to tumour cells[6].

Strilic and colleagues' work began with an observation made when tumour cells and endothelial cells were cultured together *in vitro*. The researchers noted that such co-culture leads to an increase in endothelial-cell death. However, rather than exhibiting the typical cell-shape changes and molecular features of apoptosis, the most common form of programmed cell death, the dying endothelial cells exhibited features associated with another cell-death program called necroptosis. For example, the dying cells exhibited compromised cell-membrane integrity, as monitored by dye uptake.

To confirm the observed cell-death mechanism, the authors inhibited proteins that mediate necroptosis[7], and found that this inhibited tumour-induced endothelial-cell death, whereas perturbing apoptotic signalling did not. They found that this necroptotic cell-death program was activated in both human and mouse endothelial cells exposed to a wide variety of cancer cell lines. Moreover, intravenous injection of mouse melanoma skin-cancer or lung-cancer cells into mice caused lung endothelial cells to undergo necroptotic death.

What advantage does killing endothelial cells afford tumour cells? Strilic and colleagues carried out *in vitro* experiments in which they inhibited necroptosis and observed reduced tumour-cell migration across an endothelial-cell monolayer, leading the authors to propose

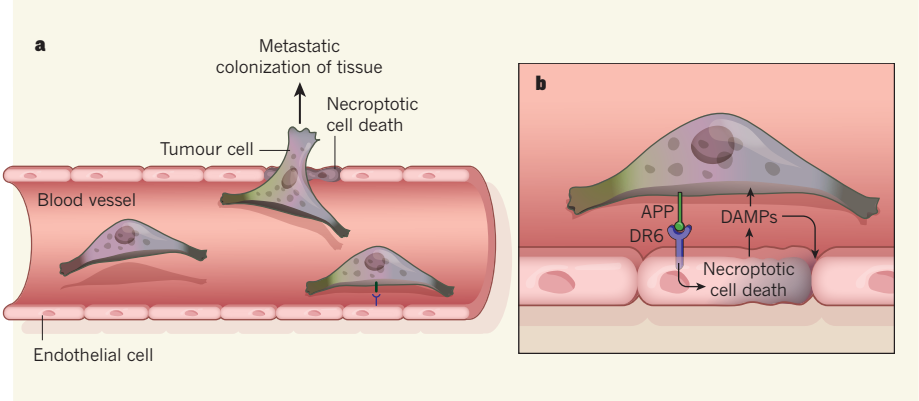

Figure 1 | Tumour cells migrate into tissues by killing cells that block their entry. **a**, Strilic *et al.*[1] show that tumour cells can induce necroptotic cell death of blood-vessel endothelial cells, which enables migrating (metastatic) cancer cells in the bloodstream to cross the endothelial-cell barrier and enter the adjacent tissue to colonize a new tumour site. **b**, Necroptotic endothelial-cell death is induced by amyloid precursor protein (APP) on the tumour surface, which interacts with the death receptor 6 protein (DR6) on endothelial cells. Tumour-cell migration from the bloodstream into the adjacent tissue may be enhanced either directly, as a consequence of endothelial-cell death and the resulting disruption of the endothelial barrier, or indirectly because of the release of damage-associated molecular pattern molecules (DAMPs) from dying endothelial cells that could open the endothelial barrier between cells or enhance tumour migration properties.