Micro-connectomics: probing the organization of neuronal networks at the cellular scale

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Abstract | Defining the organizational principles of neuronal networks at the cellular scale, or micro-connectomics, is a key challenge of modern neuroscience. In this Review, we focus on graph theoretical parameters of micro-connectome topology, often informed by economical principles that conceptually originated with Ramón y Cajal's conservation laws. First, we summarize results from studies in intact small organisms and in samples from larger nervous systems. We then evaluate the evidence for an economical trade-off between biological cost and functional value in the organization of neuronal networks. Various results suggest that many aspects of neuronal network organization are indeed the outcome of competition between these two fundamental selection pressures.

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In 1899, Ramón y Cajal proposed that neuronal structure is the outcome of adaptations to 'laws of conservation' for time, space and material[1]. According to this hypothesis, each building block of neuronal networks — from the subcellular composition of synaptic vesicles and connectivity between single cells to the larger-scale axonal tracts between brain regions — is the result of a trade-off between the cost of maintaining it and the evolutionary or functional benefits that it provides[2,3]. More than a century later, the quest to determine general principles underlying nervous system organization and neuronal information processing continues. Micro-connectomics, the graph theoretical analysis of organizational principles in neuronal connectivity at the cellular scale, has become a fruitful conceptual framework in this endeavour[4].

In this Review, we survey recent experimental evidence on topological themes that emerged from the connectomic study of small neuronal networks, some of which were reconstructed at the level of individual synapses and gap junctions. Considering the anatomical structure of these nervous systems, such as the connectome of the nematode *Caenorhabditis elegans*[5], classic studies have provided evidence that neuronal networks express organizational motifs that may underlie elementary units of neuronal information processing and provide a structural architecture for flexible adaptation to environmental constraints[6–8]. However, it remains a largely open question whether principles observed in the small cellular connectomes of invertebrate nervous systems translate to the connectivity that is found in neuronal networks of higher animals[9].

To address this question, we turn to recent studies that examined the statistics of partial micro-connectomes in the brains of mammals. The results of these pioneering studies indicate that there are parallels between the network motifs of small nervous systems and the cellular connectivity that is found in neuronal tissue from bigger brains. Despite promising progress in recent years and exciting technological advances[10,11] — for example, in dense electron microscopy (EM) reconstruction — micro-connectomics in the mammalian brain is still in its infancy. Further empirical validation and conceptual work are required to establish a more comprehensive and mechanistic understanding of the links between neuronal topology, computation and, ultimately, behaviour[12,13].

A key question is: which generative mechanisms give rise to common complex structural properties in neuronal network organization? We therefore discuss studies that provided insights into the role of neuronal lineage, synaptic plasticity and neuronal activity for specific patterns of connectivity. To appreciate how these programs are reflected in the observed network properties, we summarize experimental work that has used genetic fate mapping and retroviral tracing to relate the statistics of mature cellular connectivity to neuronal birth dates or embryonic origin. These studies provide exciting new insights into how lineage and development contribute to specific topological features. Finally, we try to extract some shared principles that have emerged from connectomic studies at the cellular scale, and discuss commonalities that indicate a possible evolutionary selection of common network phenotypes across different neuronal systems and species.

Cost
Used as an umbrella term for biological pressures and expenditures (that is, metabolism, material, and so on) that are incurred during development and maintenance of neuronal networks. Long-range connections, for example, are costly because their myelination requires a lot of cellular material.

Connectome
An abstract network representation of the connections between neurons in the whole nervous system or parts of a nervous system.

Motifs
Patterns of connectivity between a few (typically fewer than 10) nodes. Some motifs are over-represented in connectomes. For example, the closed triangular motif between three nodes is typical of highly clustered neuronal networks.

Topology
Mathematics of the pattern of connections between elements, regardless of their organization in physical space.

Genetic fate mapping
An approach in which the statistics of mature cellular connectivity are related to neuronal birth dates or embryonic origin.

Small-world organization
A metric of global network complexity. Compared with random graphs, small-world networks have high clustering but approximately equivalent path length.

Rich club
A topologically integrative network feature that comprises greater-than-random connectivity between a relatively small number of high-degree hubs. A rich club is linked by feeder connections to a large number of more-peripheral and sparsely connected nodes.

Core
A subset of nodes in the network that are highly interconnected and contribute to the global integrity of the network.

Small nervous systems

Of the many species with small nervous systems that have been studied[14–20], two organisms in particular have been utilized to address fundamental questions of micro-connectomic organization, *C. elegans* and the fruitfly *Drosophila melanogaster*. The practical advantages of these species are that their nervous systems are relatively small, their individual neuronal components have been studied in great detail and, given the advent of powerful new imaging approaches, their behaviour can be linked to neuronal network dynamics at cellular resolution (reviewed in REF. 21). Both *C. elegans* and *D. melanogaster* have also been extensively used as genetic model systems, and their genomes include homologues of many neurally expressed mammalian genes[22,23]. Despite their small size, their nervous systems represent standard examples of complex biological networks, demonstrating organizational properties that have also been reported to exist at other scales: for example, in inter-regional connectivity of mammalian brains[24]. Below, we briefly introduce the connectomes of these two organisms, summarize evidence on the statistics of their cellular connectivity and discuss what can be learned through the connectomic analysis of these systems.

Caenorhabditis elegans. The nervous system of *C. elegans* remains one of only a few nervous systems that have been essentially completely mapped at the cellular scale. The adult hermaphrodite *C. elegans* comprises 959 somatic cells, of which 302 are neurons (282 in the somatic nervous system and 20 in the pharynx)[5]. *C. elegans* neurons are structurally simple, possess a highly stereotypical morphology and have been subdivided into sensory neurons, motor neurons and interneurons[5]. Sensory neurons were classified according to morphological and positional features that may support receptive function, whereas motor neurons were classified as cells with neuromuscular junctions; all remaining cells have been termed interneurons. Comprising about 6,400 chemical synapses and 900 gap junctions[6], the overall connectivity of the *C. elegans* somatic nervous system is relatively sparse (the network has a connection density of about 10%; FIG. 1). Studies have only begun to systematically map out the neurotransmitter systems in *C. elegans*, revealing the molecular identity of about 90% of the neurons[25]; most neurons use acetylcholine or glutamate as their principal neurotransmitter, but monoaminergic and peptidergic neurotransmitter receptors are also expressed by many neurons.

A large body of evidence now exists demonstrating that both the gap junction and synaptic connectomes of *C. elegans* possess complex network properties (BOX 1; FIG. 1). The *C. elegans* connectome has a hierarchical organization (sensory neurons are more presynaptic, whereas motor neurons are more postsynaptic)[6,26] and a modular community structure among functionally related neurons[27–30]. Moreover, its binary synaptic connectivity conforms to a small-world organization[31], demonstrates a long-tailed degree distribution[32] and has a greater-than-random occurrence of some triplet and quadruplet motifs[8,33]. Interestingly, the hub neurons of the *C. elegans* connectome are organized in a rich club[34,35] (BOX 1); this network core is mainly composed of command interneurons of the locomotor circuit[36,37], has high centrality (that is, many of the shortest path motifs between peripheral neurons are routed through one or more hub neurons of the rich club)[6], comprises a large number of long-range connections between distant functional modules[38,39] and forms early during connectome development[34].

Studies investigating the wiring economy of the *C. elegans* connectome found that it is strongly, but not strictly, minimized for wiring cost[39–41]. In other words, although wiring minimization principles can explain several key aspects of the composition of the *C. elegans* connectome, their explanatory power seems to be less convincing when it comes to topological properties such as hubs and rich clubs. These and other topologically integrative components of the *C. elegans* connectome are expensively and not minimally wired. However, their high cost presumably is justified by the functional value that is added by integrative topology to the overall performance of the network[2]. For example, the gap junction hub neuron RMG links several important sensory neurons and is of great importance in controlling the global state of the animal[42,43] (FIG. 2). The connectivity between interneurons in the rich club provides the anatomical basis for vital neuronal computation of behaviours, such as coordinated locomotion and foraging[36,37]. Forward and backward movements, for example, are generated in two functionally separate subsets of neurons in this core, potentially coordinated through reciprocal inhibition[37,44]. Moreover, a study recently demonstrated that random search behaviour in the worm can be approximated by a connectome-based stochastic model of this circuit[45]. Whereas high-cost network features may provide the propensity for global integration among different modules of the network, specific functional programs use connectivity components flexibly and in a context-dependent manner[12,13]. As many neurons of *C. elegans* have multiplexed functions[12,46] — that is, they contribute to more than one behaviour — network features that maximize the use of the limited number of neurons in the worm are clearly of great value[47]. The high-cost structural features of the connectome may also subserve the functional degeneracy in *C. elegans*[48], perhaps contributing to the animals' adaptability.

Further progress in the interpretation of neuronal topology in *C. elegans* will come from a better integration of anatomical data with other fundamental functional aspects of the neurons in the networks, such as their molecular identity[25], their neuronal lineage[49] and their activity under more naturalistic conditions[50] (FIG. 2). Finally, comparative analyses provide a promising approach to gain insight into how ecological niches and environmental demands relate to differences in the network morphospace of small nervous systems[51]. For example, a study[52] recently compared the wiring of *C. elegans* and that of a close relative, the predatory nematode *Pristionchus pacificus,* and found that topological

differences in configuration of the pharyngeal ganglion might relate to the different feeding strategies of the two species.

Drosophila melanogaster. Three orders of magnitude bigger than the nervous system of the worm, the brain of *D. melanogaster* consists of about 100,000 neurons. The fly brain has been anatomically parcellated into distinct neuropil compartments, each of which provides a domain for functionally specific neuronal computation[53,54]. A whole-brain connectivity map of *D. melanogaster* has been generated using light microscopy and multicolour cell labelling techniques, reconstructing neuronal projections between functionally or clonally

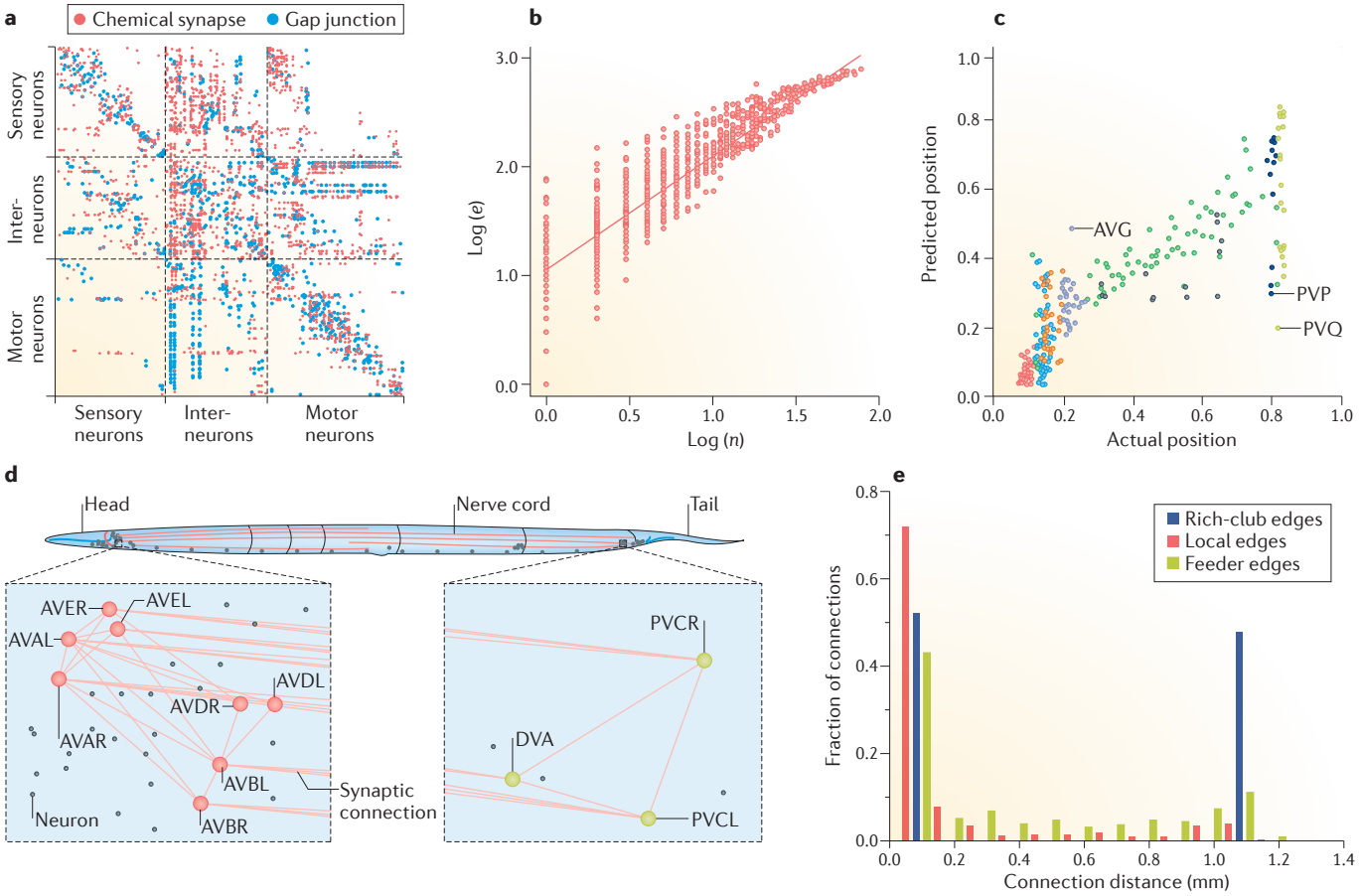
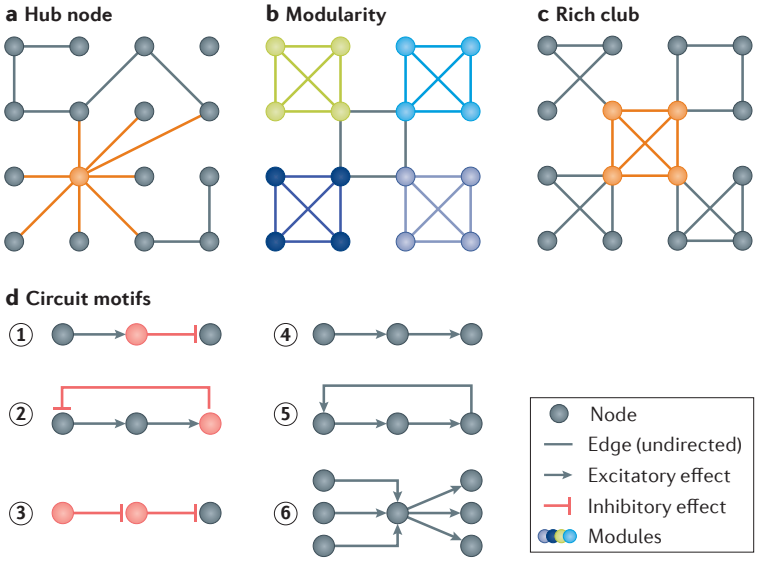


Figure 1 | **Complex topological properties of the *Caenorhabditis elegans* connectome. a** | The nervous system of the nematode *Caenorhabditis elegans* represents the first whole-animal connectome to be deciphered at cellular resolution. It was reconstructed by serial-section electron microscopy and post hoc manual tracing in the 1980s[5]. This part depicts an updated version of the originally published connectivity matrix between a subset of 279 neurons[6], including chemical synapses (red) and gap junctions (blue); the matrix is reordered according to three main functional classes of neurons: sensory neurons, interneurons and motor neurons. **b** | The *C. elegans* connectome obeys Rent's rule[30]; that is, the network demonstrates a fractal scaling relationship between the number of connections (*e*) to a set of neurons and the number of neurons in the set (*n*). This physical Rentian scaling has been interpreted as evidence for a cost-efficient embedding of the *C. elegans* connectome in physical space. **c** | Modelling studies found that the principles of wiring cost minimization are a good predictor of the actual position of neurons in the *C. elegans* connectome[40]. This part shows the correlation between the actual neuronal positions and the positions predicted by a wiring cost minimization model (each neuron is depicted as a circle; neuronal positions were normalized by the length of the worm; circle colours indicate the affiliation of neurons to specific ganglia of the worm). For a perfect match, neurons would fall on the diagonal. As depicted, some neurons clearly deviate from the rule. For example, the positions of the interneurons AVG, PVP and PVQ were not predicted well. **d** | Highly connected interneurons of the *C. elegans* connectome form a rich club[34]. This network core has been suggested to facilitate integration between different functional modules of the connectome through topological shortcuts. Experiments demonstrated that neurons in the rich club are important for coordinated movement and switching between behavioural states[36,37]. The upper panel shows a schematic of the body of the whole worm; the two boxes below show the physical position of rich-club neurons and their connectivity in the head (red circles) and tail (green circles); grey circles indicate neurons that are not part of the rich club. **e** | This part shows the distribution of connection distances of the *C. elegans* connectome[34], grouped into three different classes: rich-club, feeder and local edges. Rich-club neurons comprise a very large proportion of the long-range connections; feeder edges, which connect peripheral neurons to rich-club neurons, and local edges, which connect only peripheral neurons, demonstrate generally shorter connection distances. Part **a** is adapted with permission from REF. 6. Part **b** is adapted with permission from REF. 30. Part **c** is adapted with permission from REF. 40, Copyright (2006) National Academy of Sciences, USA. Part **d** and part **e** are republished with permission of Society of Neuroscience, from: The rich club of the *C. elegans* neuronal connectome. Towlson, E. K., Vértes, P. E., Ahnert, S. E., Schafer, W. R. & Bullmore, E. T., *J. Neurosci.* 33, 6380–6387 (2013); permission conveyed through Copyright Clearance Center, Inc.

Box 1 | **Graph theory**

The starting point for all graph theoretical analysis is the definition of nodes and edges (for an introductory text, see REF. 200). For micro-connectomes, a node typically represents a specific neuron, and an edge usually represents a synaptic or gap junction connection between two neurons (see the figure). Most commonly used graph theoretical metrics are calculated on undirected connectivity. The degree (k) describes how many edges connect to one node *i*. The degree distribution *P*(k) allows the overall network structure to be compared to null models, such as random, regular or scale-free networks. Nodes with a high degree are often referred to as hubs (see the figure, part **a**; the hub node is highlighted in orange). A pervasive and well-studied organizational feature is the community structure, or modularity, of a network (see the figure, part **b**). It describes how well a network can be partitioned into communities, and numerous methods and algorithms for modular partitioning have been developed[201]. A hierarchically nested modular structure across spatial scales has been referred to as the hallmark of complex systems[202]. The connectivity of hub nodes may have a role in integrating between different communities of a network. Hubs may also be organized in a rich club — a network core component that is significantly more interconnected than the high-degree nodes of a comparable random network (see the figure, part **c**; the rich club is highlighted in orange). The topological distance between a pair of nodes *i* and *j* can be estimated by the path length; that is, the minimal number of synapses that need to be traversed to connect node *i* to node *j*. The inverse of the average shortest path length of nodes has been proposed as a measure of the topological efficiency of a network. A measure of the local efficiency of a node is the clustering coefficient, which gained particular prominence in the formalization of the small-world property[31]. To characterize the neurophysiological functions inherent to micro-connectomes more thoroughly, it will be necessary to expand on traditional graph theoretical concepts. Graph theoretical analysis of micro-connectomes may include, for example, connection weights — that is, the number and strength of synaptic contacts between two neurons. Moreover, mechanistic explanations of neuronal network functioning may require the inclusion of classic inhibitory circuit motifs, feedforward inhibition (1), feedback inhibition (2) and disinhibition (3), and excitatory circuit motifs, feedforward excitation (4), feedback excitation (5) and pathway convergence and divergence (6) (see the figure, part **d**).



Centrality
A general term for the topological importance of a node in a network. Centrality can be quantified in many ways including the degree and closeness of each neuron.

Economy
Here, this term describes the trade-off between the biological cost and the functional value of topologically complex networks.

Degeneracy
This term describes the property of a system in which different structural components can give rise to very similar functions.

Morphospace
The low-dimensional space of network phenotypes observed in natural populations and simulated by generative models of network development and evolution.

defined brain regions of about 10% of all neurons[55,56]. Applying graph theory to the fly projection map has provided insight into its large-scale organizational properties, demonstrating that it has a heavy-tailed distribution of neuronal projection strengths, a hierarchical modular structure among its sensory processing units, small-world characteristics and a rich-club structure comprising regions that are associated with motor and auditory processing[55–57].

The feasibility of measuring finer-grained synaptic connectivity between the densely packed neurons in the fly by light microscopy remains limited[10,11,58]; however, EM can provide the necessary subcellular resolution for this task, and anatomical reconstructions of parts of the fly's optic lobe, such as cartridges of the lamina[59,60] and columns of the medulla[61,62], have been released. The lamina and medulla represent important signal-processing circuits in the fly visual system: they both comprise about 750 hexagonally shaped modules and have a retinotopical organization (which per se represents a cost-effective organizational solution to minimize wiring while preserving the spatiotemporal relationships in visual information[3,63]). Simulations based on connectome reconstruction of a lamina cartridge showed that the overall configuration and modular organization of its neurons can be well characterized by a combination of wiring minimization and volume exclusion[60]. In the 'optimally wired' lamina, neurons that make the most synaptic connections are located close together and centrally, whereas cells with fewer synapses are placed in the periphery[60]. EM reconstruction of single columns of the fly medulla (reconstructed volume: 37 × 37 × 50 µm) revealed that its connectivity is highly directed, relatively sparse (each presynaptic site contacted, on average, only about 3 to 8 postsynaptic sites), can be partitioned into modular processing pathways and has a long-tailed distribution in connectivity weights[61]. Follow-up analysis of wiring variation in the medulla showed that synaptic connectivity patterns are highly stereotypical among neighbouring medulla columns[62]. Although precise wiring seems to be intuitively beneficial for the accurate transmission of visual information, reported values on wiring accuracy should be interpreted with caution as some of the reconstructed synapses could actually not be traced reliably and a large number of postsynaptic sites were omitted[61,62]. Despite these limitations, connectomic insight into the architecture underlying connectivity between lamina and lobula targets has provided important clues for a more-detailed functional characterization and validation of historical models of neuronal circuitry that is involved in motion detection in the fly[9,61,64].

To summarize, graph theoretical analysis of the connectomes of model organisms, such as *C. elegans* and *D. melanogaster*, has generated strong evidence that even small nervous systems demonstrate hallmarks of a

Scale-free networks
A class of complex networks defined by a heavy-tailed degree distribution that can be approximated by a power-law. High-degree hubs have a higher probability in scale-free networks than in comparable random graphs.

Topological efficiency
A metric of network integration that is calculated as the inverse of the average shortest path length of a network.

Clustering coefficient
The clustering coefficient of a node is calculated as the fraction of triangular connections between the nearest neighbours of a node divided by the maximal possible number of such connected triangles.

Graph theory
The mathematical analysis of graphs comprising nodes and edges. Graphs can have directed or undirected, weighted or non-weighted edges.

Retinotopical organization
A common feature that is found in visual cortical areas, which describes the spatially ordered mapping of visual inputs from the retina to cortical neurons.

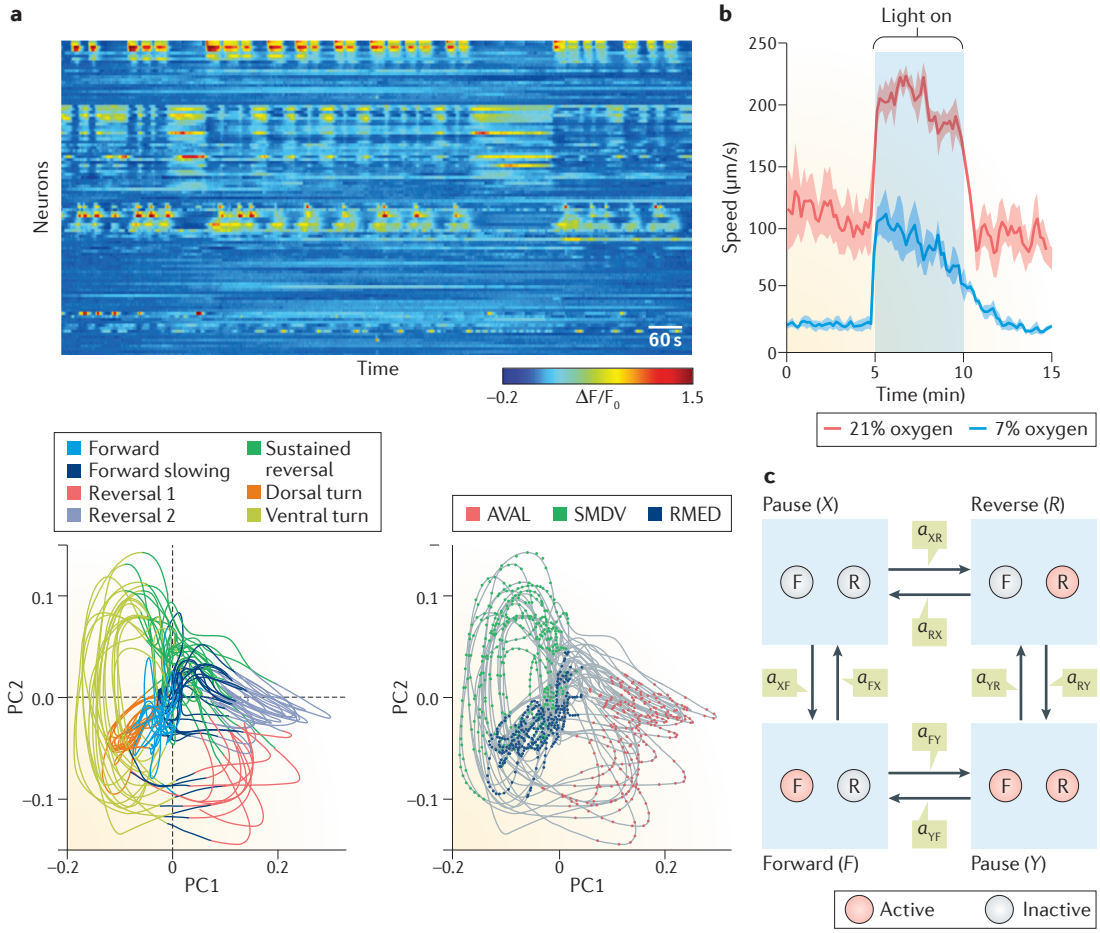


Figure 2 | **Linking structure and function in the *Caenorhabditis elegans* connectome.** Combining connectomics with large-scale functional imaging and targeted stimulation at the level of single neurons will provide a better understanding of how neuronal structure, function and behaviour are inter-related in the worm. **a** | This part shows key results of a study that recorded neuronal dynamics at cellular resolution in the freely moving worm and then correlated changes in neuronal population activity with locomotor states. The upper panel depicts the normalized $\Delta F/F_0$ calcium fluorescence time series from 109 head neurons over an 18 min recording session[50]. A principal component (PC) analysis of the covariance structure of the inferred neuronal time series was used to estimate how activity changes in neuronal subpopulations correspond to simultaneously recorded locomotion. The study then derived a temporal integral for each PC and traced these temporal PCs over time so that PC-derived trajectories of neuronal population activity could be related to observed changes in locomotion. The lower left panel shows the neuronal state space of the first two PCs for the recording displayed above. The state space evolved cyclically; that is, similar dynamical states were visited repeatedly. Moreover, it was possible to link specific action sequences to subregions of the neuronal state space. Subregions of the neuronal state space could also be linked to calcium activity increases in specific neurons (indicated for three example neurons in the lower right panel). **b** | Targeted ablation and stimulation of *Caenorhabditis elegans* neurons have provided important insights into circuit function. This part shows the effect of optogenetic stimulation of the gap junction hub neurons, known as RMG, expressing channelrhodopsin 2 on *C. elegans* movement during two different oxygen conditions (21% oxygen and 7% oxygen)[42]. Selective stimulation of RMG neurons allowed switching of the worm's behavioural state and induction of persistent forward movement. The worms generally avoid environments with high oxygen, and, accordingly, a higher arousal level is observed at 21% oxygen (data are averaged over 27 animals; the mean ± the standard error of the mean is depicted). **c** | A schematic of a Markov model to simulate random search in *C. elegans* is shown[45]. The model was informed by connectome data on the locomotor circuit of the worm, whose forward (F) and reverse (R) command neurons are connected through reciprocal inhibition. If F and R units are conjointly active (or inactive), the animal pauses (X, Y). The arrows indicate the transition paths of units from one state to another ('*a*' indicates the rate constant). Part **a** is adapted with permission from REF. 50, Elsevier. Part **b** is adapted with permission from REF. 42. Part **c** is adapted with permission from REF. 45.

complex topology. Because the connectomes of both species share fundamental topological features in their large-scale organization, it seems reasonable to hypothesize that these commonalities represent evolutionarily preserved network phenotypes for neuronal computation, perhaps representing the outcome of an economical trade-off between biological costs and functionally adaptive value. Additional research is required to reveal the functional

implications of topological motifs (FIG. 3), in particular those that are more costly to wire. Hubs and rich clubs of the *C. elegans* connectome, for example, seem to provide a topological scaffold that enables flexible switching between different behaviours and integration among functional modules. The parallel pathway architecture of the fly optic lobe, retinotopically arranged and parsimoniously wired, seems to be strongly optimized for robust, high-speed processing of information. Pathway motifs, such as convergence and divergence (BOX 1), have been shown to support effective information transmission and integration in the fly olfactory system[65], in action selection in the *D. melanogaster* larva[66] (FIG. 3) and, even, in the mammalian retina[67]. Further clarification of the functional value of anatomically defined network motifs in such small model organisms will require a combination of analysis strategies, such as whole-animal neurobehavioural mapping[68] and selective targeting of

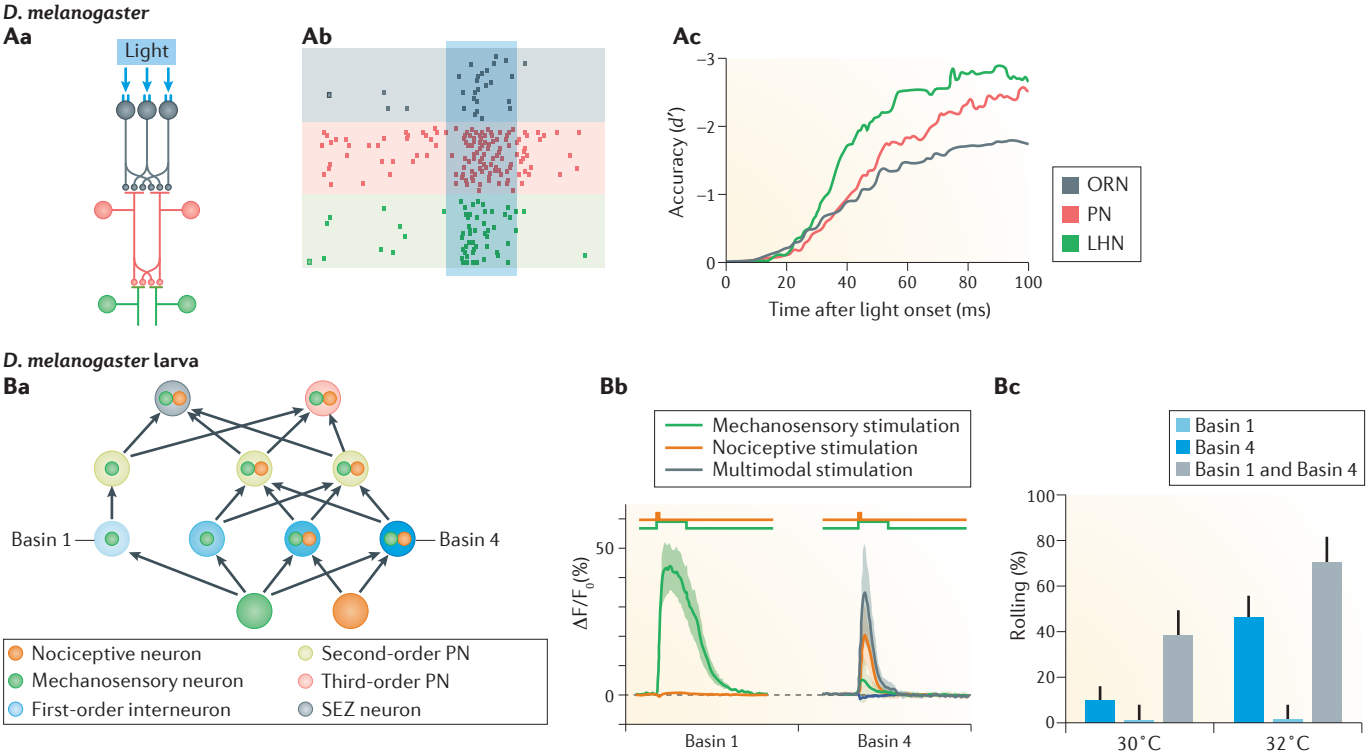


Figure 3 | **Network motifs support information processing in *Drosophila melanogaster*.** Recent advances in dense structural reconstruction of cellular networks and targeted optogenetic stimulation of specific cell types have opened new avenues to investigate the potential contribution of network motifs to neuronal information processing. **A** | Using optogenetic stimulation in olfactory receptor neurons (ORNs) and electrophysiological recordings across three layers of the *Drosophila melanogaster* olfactory system, a study investigated the potential role of pathway motifs for sensory information processing[65]. In the olfactory glomerulus circuit (part **Aa**), the axon of each ORN diverges to form connections with all postsynaptic projection neurons (PNs) in the glomerulus; that is, each PN gets input from all ORNs. In turn, the axons of PNs reconverge onto higher-order lateral horn neurons (LHNs). The raster plot (part **Ab**) reveals the spike activity recorded from an ORN, a PN and a LHN during repeated optogenetic stimulation (100 ms light flashes on ORNs expressing channelrhodopsin 2). To probe whether the described circuit structure does improve information processing, the study investigated the stimulus detection accuracy (*d′*) at each layer (part **Ac**). The metric *d′* quantifies how well the distribution of spontaneous and evoked firing rates can be separated. Results show that *d′* increases markedly from early to late stages of the processing pathway (values represent median *d′* over 58 ORNs, 44 PNs and 25 LHNs; *d′* was calculated in 80 ms sliding windows starting at stimulus onset). **B** | Enhanced action selection through a multilevel multimodal convergence pathway motif in the *D. melanogaster* larva is shown[66]. Part **Ba** depicts a schematic of the electron microscopy-reconstructed feedforward circuit involved in the selection of the fastest escape behaviour in the larva (rolling). At the first level, neurons sensitive to nociceptive and mechanosensory cues form contacts with first-order interneurons, so-called Basins 1–4. The pathway then ascends to second-order PNs, which further integrate multimodal information and form connections with third-order brain PNs, as well as with feedback and descending neurons from the suboesophageal zone (SEZ). First-order Basin interneurons respond differentially to sensory stimulation (part **Bb**): Basin 1 neurons show elevated calcium transients only to mechanosensory stimulation (lines represent mean ± standard error of the mean); Basin 4 neurons respond to both mechanosensory and nociceptive cues. Moreover, results show that Basin 4 neurons integrate multimodal cues synergistically, that is, the recorded response to multimodal stimulation is markedly higher than the summed response to the individual unimodal sensory cues. Using thermogenetic activation to selectively stimulate Basin 1 and Basin 4 neurons, the study also revealed that rolling could be triggered already at this first level of the pathway (part **Bc**). Activation of first-order interneurons triggered rolling in a dose-dependent manner. Moreover, the probability of triggering rolling was higher for Basin 4 neuron stimulation than for Basin 1 neuron stimulation and, notably, increased when both types of interneurons were stimulated simultaneously (error bars represent 95% CIs). The results suggest that action selection for rolling is computed early in the sensory processing hierarchy and that the described convergence circuit motif facilitates the integration of temporally correlated multimodal sensory information. Part **A** is adapted with permission from REF. 65, Elsevier. Part **B** is adapted with permission from REF. 66, Macmillan Publishers Limited.

Box 2 | **Cell type-specific connectivity**

A large body of evidence suggests that the probability of a connection between two neurons depends on the presynaptic and postsynaptic cell type[203–205]. In the cortex, two broad classes of neurons can be distinguished: principal (projection) cells and local-circuit interneurons. Principal cells comprise about 80% of all cortical neurons; they use the excitatory neurotransmitter glutamate for signalling and make up to several thousands of synaptic connections to other neurons of their class. Many of the axonal projections to other projection neurons are long distance (up to 80% of synapses onto primary visual cortex (V1) principal-cell dendrites come from neurons more than 200 μm away[206]). Principal cells also make local connections to their neighbouring cells. The remaining 20% of cortical neurons are interneurons. Interneuronal efferent connectivity is mainly local, and most interneurons release the inhibitory neurotransmitter GABA. Patch-clamp recordings often reveal that there is relatively low local connectivity among principal cells. For example, the principal-cell connectivity rate estimated by patch-clamp recordings in slices of layer 2/3 (L2/3) rat somatosensory and visual cortices decreased as a function of distance from about 10% for cells in close proximity (<25 μm) to a rate of less than 1% for principal cells more than 200 μm apart[96]. Connectivity in slices of L5 rat somatosensory cortex has been reported to decay from about 20% for nearby neurons to less than 5% for neurons more than 200 μm apart[98]. A study revealed that the connectivity of principal cells in L2 primary somatosensory cortex (S1) is also sparse *in vivo*[146]. The local connectivity in L2/3 in V1 ranged between 10% and 20% in both rats[128] and mice[99,103]. Principal-cell connectivity probably depends on intersomatic orientation of cells, on the cortical layer in which neurons are located and on where they receive their input from or send their outputs to (reviewed in REFS 154,207). Evidence indicates that projection neurons are preferentially connected if they receive common inter- and intra-laminar inputs[208] and if they share similar receptive fields[99,103]. An electron microscopy reconstruction showed that principal-cell connectivity in V1 L2/3 is more likely if cells share similar orientation preferences[138]. New transgenic mouse lines that express specific fluorescent genetic markers have made it feasible to differentiate interneurons broadly into largely non-overlapping subclasses: parvalbumin-expressing (PV^+), somatostatin-expressing (SOM^+) and 5-hydroxytryptamine (serotonin) receptor 3A-expressing ($5\text{-}HT_{3A}R^+$) interneurons. Together, PV^+, SOM^+ and $5\text{-}HT_{3A}R^+$ interneurons account for almost 100% of all cortical interneurons[209]. In combination with knowledge on the innervation sites of interneurons, predictions about specific computational roles and circuit functions become feasible[79,210]. Combining optogenetic stimulation and whole-cell recordings, studies have started to map interneuron connectivity rates within and between interneuron subclasses and to principal cells. In L2/3 and L5 of the visual cortex[211,212] and in L2/3 of the mouse barrel cortex[213], studies found a high connectivity among PV^+ interneurons. By contrast, few or no chemical synapses have been observed among SOM^+ interneurons[212,214]. Similarly, intra-class connectivity among vasoactive intestinal peptide-expressing (VIP^+) interneurons, a subgroup of $5\text{-}HT_{3A}R^+$ interneurons, is sparse[212]. Between-class connectivity has also been examined, and, in particular, a connectivity scheme from VIP^+ to SOM^+ and PV^+ interneurons to control disinhibition of local principal cells has recently gained a lot of attention[212,214–216]. Interestingly, this connectivity scheme was found in different cortical regions[217]. High synaptic connectivity has been reported for the inputs from principal cells onto PV^+ interneurons in V1 (REFS 96,218), with more moderate connectivity for the inputs from principal cells to VIP^+ and SOM^+ interneurons in L2/3 V1 and S1 (REF. 212). *In vivo* patch-clamp recordings in L2/3 of the barrel cortex[147] have largely confirmed *in vitro* connectivity rates from rodent principal cells to PV^+ interneurons[213] and SOM^+ interneurons[219].

individual neuronal components in the network[36,42,50] (FIG. 2). Despite recent progress in the description of connectivity in small nervous systems and first glimpses of how specific network motifs may impart information processing capabilities, current connectome reconstructions are still far from perfect. Even small mistakes in annotating synaptic connections can have major implications for the functional interpretation of network topology[10,48,69]; many neurons in small nervous systems are multifunctional, complicating definite mappings between structure and function[17,47]; and a comprehensive integration of connectomic data with maps on the molecular identity of neurons[12,25,46] and functional activity at larger-scales[50] has yet to be established.

The mammalian brain

Over the past few years, several initiatives have started to systematically map inter-regional connectivity in the rodent brain using trans-synaptic tracers and light microscopy[70]. These studies have set the stage for comprehensive graph theoretical analysis of the complex topological organization of larger-scale neuronal networks comprising all or part of the mammalian cerebral cortex[71,72]. Although retrograde and anterograde tracing techniques clearly represent powerful tools for the study of afferent and efferent connection profiles of brain regions, analysis of whole-brain connectivity at the scale of individual synapses remains technically challenging[10,11]. Even a conceptual framework for the quantitative analysis of synaptic connectivity at the whole-brain scale has yet to be established. The latter has become increasingly clear as a number of recent EM studies have highlighted fundamental limitations in influential theoretical proxies for the study of cellular networks, such as Peters's rule[73,74], indicating that axodendritic proximity alone is often not sufficient to predict the existence of a synaptic connection[75–77]. Starting with principles that can already be suggested from the morphological analysis of individual cells, we outline recent evidence for network motifs in local connectivity of mammalian brains (BOX 2).

Neuronal arborization and wiring optimization. Characterizing the organizational patterns of neuronal arbors, as well as the type, location and distribution of synaptic inputs and outputs, provides important insights into how morphology links to neuronal computation and connectivity[78,79]. As complete EM-based mapping of the many thousands of input synapses onto even a

Peters's rule
The assumption that synaptic connectivity can be inferred from the spatial overlap of axons and dendrites.

Fractal dimension
A measure of the extent to which a self-similar process, like a dendritic tree, completely occupies the Euclidean dimensions of space in which it is embedded; more intricately branching arborization will have higher fractal dimension indicating greater space occupancy.

Minimum-spanning-tree
An undirected graph that connects all nodes in the network with the minimum number of connections.

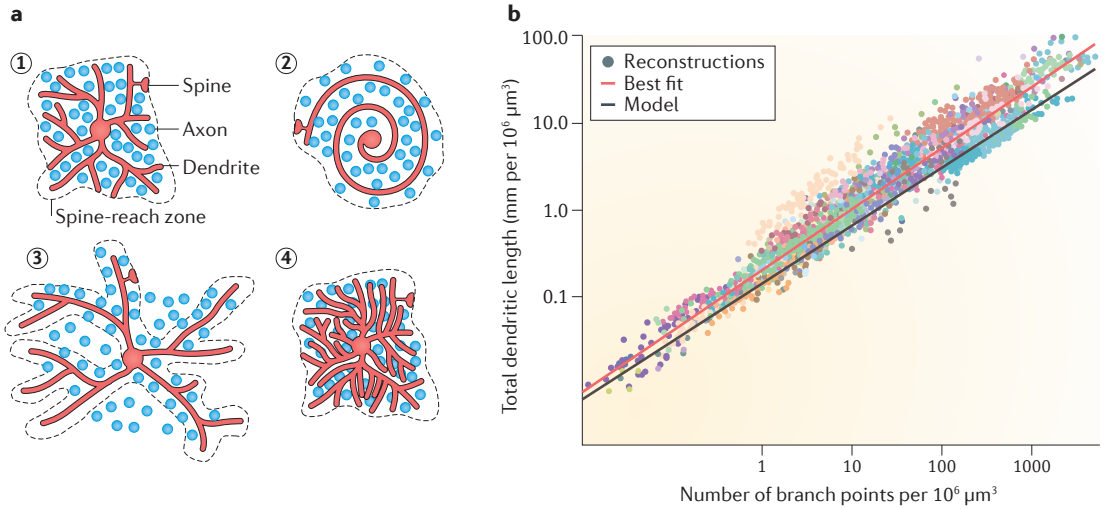


Figure 4 | **Dendritic arborization and optimal wiring. a** | Fundamental aspects of neuronal arborization have been linked to principles of conserving cellular cytoplasm and conduction time, reminiscent of Ramón y Cajal's conservation laws for space, time and material[1,82–86]. To receive inputs from other neurons, dendrites and axons have to establish synaptic connections. It has been suggested that axons can only form synapses if they pass dendritic arbors within a so-called spine-reach zone. To explain how dendritic branching structure affects the propensity to establish synaptic connectivity and how branching topology may correspond to dendritic costs, four hypothetical dendritic branching designs are shown[85]. The 'compact' branching design (1) demonstrates a good balance between total dendritic length and dendritic path length, that is, the distance that input signals have to travel from a synapse to reach the soma. For this design, dendritic arbors can establish, on average, one potential connection with each passing axon. The other design types (2–4) are less optimal: the 'compact non-branching' design (2) incurs excessive dendritic path lengths, the 'sparse' branching design (3) has a lower propensity for synaptic contacts, and the 'dense' branching design (4) is more costly than the compact design and incurs a higher total dendritic length. **b** | Branching patterns can be predicted from the principles of wiring cost minimization. The chart shows the scaling relationship between total dendritic length and the number of dendritic branch points of a large collection of morphologically reconstructed neuronal arbors (each dot represents one neuron; colours represent different neuron types), as well as the relationship among these parameters predicted by a minimum-spanning-tree model[86]. Part **a** is adapted with permission from: A cost–benefit analysis of neuronal morphology. Wen, Q. & Chklovskii, D. B., *J. Neurophysiol.* 99, 2008, 2320–2328. Part **b** is adapted with permission from REF. 86, Proceedings of the National Academy of Sciences (PNAS).

single cortical neuron remains challenging and most EM data sets cannot cover the full extent of all neuronal projections[10,11,80], studies have mainly analysed neuronal arbors reconstructed by light microscopy[81–86] (but see REFS 77,87). Several approaches have been introduced to estimate the branching pattern of neuronal arbors[81] and how neuronal arbors fill the space they are embedded in[88]. The fractal dimension, for example, is a metric that is used to quantify the extent to which arborization of neurons fully occupies the three-dimensional space available and has been linked to the propensity for synaptic connectivity (reviewed in REFS 89,90). More recently, computational models have greatly expanded our ability to quantify neuronal morphology and to probe underlying constraints. One study[85] tested how dendritic branching structure links to principles of wiring cost minimization by applying evolutionary optimization algorithms and found that the dendritic arbor structure of Purkinje cells in the cerebellum is largely consistent with rules to minimize total wire length or to avoid overlap of spine-reach zones (FIG. 4). Moreover, analysis of basal dendrites of pyramidal neurons in the cerebral cortex showed that dendritic arbor radius scales with total dendritic length, as does the pairwise spatial correlation between dendritic branch segments[84].

A minimum-spanning-tree approach was introduced to study the relationship between neuronal arbor structure and connectivity[91]. One study[82] improved on this conceptual framework and developed an algorithmic procedure that enables comparison of synthetically grown dendritic trees to light-microscopy-reconstructed neuronal trees. This analysis provided a series of interesting new insights into how constraints for economical resource allocation, such as the balance between the cost of biological material and the cost of conduction time, may govern dendritic architecture[86]. Although application of this analysis strategy to a large set of morphological reconstructions from the NeuroMorpho.org database[92] demonstrated that dendrites of the various cell classes balance wiring costs differently, the relationships between key features in their morphology followed scaling laws that could be predicted by models based on wiring minimization principles[86] (FIG. 4).

Taken together, data on dendritic branching and morphology from a wide range of cells fit well with principles to conserve cellular cytoplasm and conduction time — reminiscent of Ramón y Cajal's postulate that many details of neuronal morphology can be explained by general conservation laws for space, time and material[1,82–86]. These intricate branching patterns are probably

Transfer entropy
An information theoretic measure for the directed interaction between two time series; it measures the information that the past of a source variable provides about the current value of a target variable, beyond the information provided by the past of the target variable alone.

established during development through, for example, self-avoidance rules and competition between sibling dendrites[93]. How organizational principles at the scale of axonal and dendritic arbors relate functionally to optimal information processing and storage capacities at the network level has been addressed by a series of theoretical studies[84,94], but these relationships remain to be tested experimentally.

Long-tailed synaptic connectivity. As documented for many biological and social networks, key neurophysiological parameters in neuronal networks follow long-tailed distributions (reviewed in REF. 95). At the level of local circuits, synaptic strengths follow a lognormal distribution; that is, most unitary excitatory postsynaptic potentials (EPSPs) in simultaneous recordings of presynaptic and postsynaptic neurons are small, with only a small proportion of large EPSPs[96–99] (FIG. 5). Preferentially connected subgroups of neurons, comprising a skeleton of a few strong connections, have been suggested to provide a means of effective information processing and stimulus representation in local networks[100–102]. However, probing a link between information processing and connection strength has long remained difficult to do experimentally. By combining *in vivo* optical imaging of principal cells in layer 2/3 (L2/3) of mouse primary visual cortex (V1) with post hoc whole-cell recordings in slices, a recent study[103] showed that a small subset of strong synaptic connections preferentially links principal cells with similar receptive fields. These strong connections have been suggested to provide a mechanism for selective amplification of thalamic input signals in V1 (REF. 104); the observed distribution of synaptic weights in this region could hence relate to the functional couplings among principal cells.

The presence of cells, or groups of cells, with high functional connectivity could also indicate network components with high topological centrality, such as hubs and rich clubs[105,106]. Studies using calcium imaging in slices of developing hippocampus found that subpopulations of GABAergic interneurons[107] and early-born principal cells[108] display a high degree of functional connectivity. Morphological and physiological characterization of these cells demonstrated that early-born interneurons in particular show features in their axonal arborization and effects on local network activity that may determine their fate as functional hubs in the network[107,109]. Classification of interneurons according to their arborization into connector hubs (for example, hippocampal interneurons with long-range axons to the medial septum and to other areas[110,111]) and provincial hubs (for example, interneurons that display mainly intra-hippocampal arborization[112]) has been proposed[109]. Interneuron hubs may also be involved in orchestrating synchronous network activity[107,113,114]. A role for interneuron networks in connecting distinct target regions in the brain with long-range axonal projections has been suggested as a means of keeping the average path length of neuronal networks low[115]. Heavy-tailed distributions of functional connectivity were also observed in studies using transfer entropy analysis of spontaneous neuronal activity recorded by multi-electrode arrays *in vitro*[116] and in silicon-based microprobe recordings *in vivo*[106]. Interestingly, these and other studies found that highly connected hub neurons

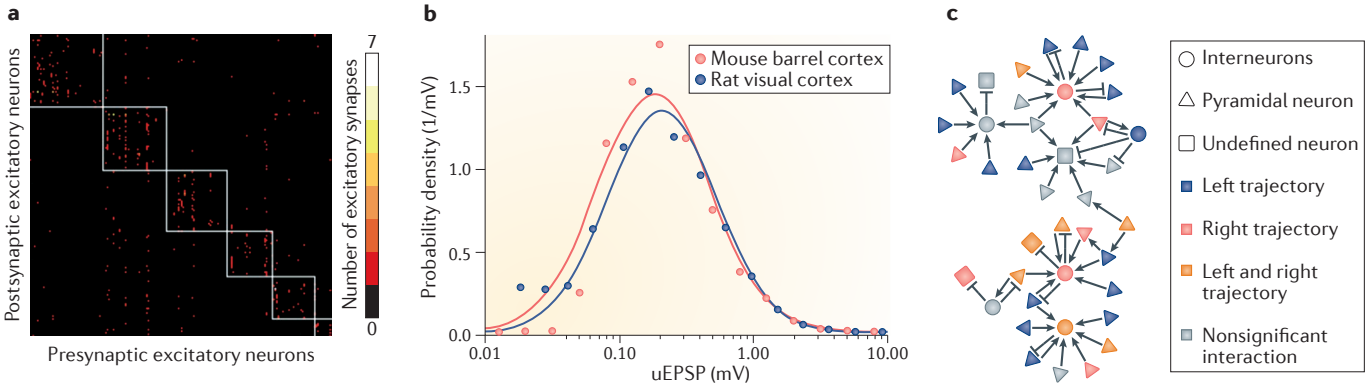


Figure 5 | **Complex topological features in mammalian local connectivity. a** | A non-random modular structure was found in synaptic connectivity derived by electron microscopy reconstruction of excitatory neurons of mouse layer 2/3 (L2/3) visual cortex[138]. This part shows connectivity among 201 excitatory neurons reordered according to their community affiliation (in the figure, denoted by the white boxes). Moreover, combining *in vivo* calcium imaging of excitatory neuronal activity during a visual-stimulation task with post hoc electron microscopy reconstruction of their synaptic connectivity demonstrated that L2/3 excitatory neurons are more preferentially connected if they share similar orientation selectivity[138]. **b** | An approximately lognormal distribution of synaptic weights, quantified as unitary excitatory postsynaptic potentials (uEPSPs), has been reported by electrophysiological studies across different areas of the mammalian brain[95]. Heavy-tailed distributions may indicate preferentially connected functional groups of neurons and strong connections that could facilitate effective and reliable information processing in local networks. **c** | Putative monosynaptic connectivity derived from electrophysiological recordings in the rat medial prefrontal cortex during a T-maze working-memory task is shown[220]. Circles depict interneurons, pyramids depict pyramidal neurons, squares depict neurons that could not be defined; the arrows represent excitatory connections, crossbars indicate inhibitory connections. Neuronal firing was behaviour and position selective; the colours indicate whether firing rates of individual neurons could be linked to specific T-maze positions on either left (blue), right (red) or left and right (orange) trajectories. The inferred monosynaptic connectivity is locally clustered, with some neurons demonstrating hub-like features. Part **a** is adapted with permission from REF. 138, Macmillan Publishers Limited. Part **b** is adapted with permission from REF. 95, Macmillan Publishers Limited. Part **c** is adapted with permission from REF. 220, Macmillan Publishers Limited.

may be organized in rich clubs and that neurons comprising these network cores receive more inputs, display higher firing rates and may form their connections early during network development[105,106].

Although there seems to be good evidence for hubs and long-tailed distributions in functional connectivity, patch-clamp studies of structural connectivity between principal cells generally did not observe such distributions[98]. Also, to our knowledge, no EM study of cortical tissue has yet found hub neurons defined by anatomical connectivity alone. Some indirect support for heavy-tailed distribution of synaptic contacts at the cellular level comes from new synaptic labelling methods, such as mGRASP (mammalian green fluorescence protein reconstitution across synaptic partners)[117], and modelling work that demonstrated that neuronal networks with hubs can potentially better explain the distribution of *in vivo* firing rates[118].

Clustered and modular connectivity. Following the seminal work by Watts and Strogatz[31], which demonstrated the presence of a small-world organization in the *C. elegans* connectome, numerous studies searched for above-random clustering in neuronal connectivity at the cellular scale. Features indicating a small-world topological organization were reported for synaptic connectivity of patch-clamp recordings in L5 rat somatosensory cortex[98], and a series of studies examined this phenomenon in functional connectivity derived from spontaneous activity in cultures, slices and living animals[119–126]. The emergence of small-world features was also reported for developing neuronal networks in dissociated neuronal cultures[123,127]. Importantly, different forms of clustering have been reported in the literature: 'topological clustering' has been described for structured connectivity in which neurons form closed triangular motifs irrespective of internodal distances[126,128]. By contrast, 'spatial clustering' refers to nodal clustering that can be mainly explained by a distance-related drop-off in connectivity (that is, clustered nodes also tend to be spatially adjacent)[129]. Finally, subcellular 'synaptic clustering' refers to the spatial arrangement of synapses on the dendritic tree. This type of clustering probably has an important role in synaptic integration[78] and has been observed, for example, in synapses with functionally related inputs during spontaneous activity[130] and among hippocampal synapses with presynaptic neurons that share a similar developmental time window[117]. Numerous modelling studies have tested how clustering-related properties may evolve during neuronal network formation[131,132], how clustering is maintained by plasticity mechanisms[133] and whether it affects synchronization dynamics[115,134]. Although there has been a steady accumulation of evidence for a functional role of synaptic clustering in dendritic computation in some areas of the brain[130,135,136], the functional importance of topological properties, such as a small-worldness in local connectivity, remains difficult to test.

Several recent studies have also reported a modular structure in local synaptic connectivity. Through a combination of *in vivo* functional imaging and monosynaptic retrograde trans-synaptic tracing, one study[137] inferred the presynaptic networks of single L2/3 principal cells in V1 and found that there are layer-specific functional modules that could be locked to the direction preference of the postsynaptic cell. Two recent EM studies in mice also provided evidence of modular connectivity[138,139]. One of these studies[138] combined *in vivo* functional imaging with post hoc EM reconstruction (reconstructed volume: $450 \times 450 \times 50\,\mu m$) and reported a non-random community structure in the synaptic connectivity of 201 V1 L2/3 principal cells (FIG. 5). Importantly, this study confirmed previous electrophysiological work[99] that had shown that principal cells with similar orientation selectivity are preferentially connected to each other. The other EM study[139] examined parts of the lateral geniculate nucleus (reconstructed volume: $400 \times 600 \times 280\,\mu m$) and found that the organization of synaptic connectivity can be 'fuzzy'; that is, it can be indicative of a strongly overlapping modular affiliation in which network nodes belong to several different subnetworks. Future work will have to test the functional relevance of such differences in modular structure to rule out potential artefacts due to subsampling of the neuronal network or preparation of tissue samples.

Network motifs. Micro-connectomic organization has also been studied by quantifying the distribution of specific higher-order network motifs. One of the first experimental studies of these local building blocks[140] estimated principal-cell connectivity of L5 rat somatosensory cortex using whole-cell patch-clamp recordings in slices and showed that the proportion of bidirectional connections clearly exceeded the number of connections that one would expect in a random network. Using similar electrophysiological methods, follow-up studies reported evidence for an over-representation of network motifs in the local connectivity among principal cells in the rat visual[128] and somatosensory cortex[98], as well as in the ferret prefrontal cortex[141]. An above-random occurrence of three-neuron motifs in synaptic interneuron connectivity, such as feedforward triplets, and a large overlap between chemical and gap junction connectivity were also reported for cerebellar networks[142]. A study searching for rules that could explain experimentally observed wiring motifs[98] reported that the connection probability of principal cells in L5 somatosensory cortex is increased when neurons have a common neighbour and that the probability of finding a connection among principal cells scales approximately linearly with the number of common neighbours — the so-called common neighbour rule. A comparable effect was subsequently reported for functional connectivity inferred from spontaneous neuronal activity in cortical slice cultures recorded on multi-electrode arrays[116]. How such principles in synaptic organization link to the computational functionality of neuronal 'cliques', such as those found in the local connectivity of auditory[143], visual[144] and frontal cortices[145], remains to be tested. Despite data from recent patch-clamp work *in vivo*[146,147], which seem to be largely in line with previously reported data on local connectivity *in vitro*, the findings on network motifs remain controversial owing to potential artefacts that are caused by sparse local electrophysiological recordings and the slicing of neuronal tissue.

Motifs in reciprocal connectivity between cortical principal cells, more generally, have been hypothesized to contribute to various computational tasks, such as amplifying inputs[104,138,148], shaping receptive field properties[99] and prolonging activity for computation in higher-order cortical areas[149]. Network motifs could also provide a structural backbone for synchronizing functional cell assemblies[138,150,151]. In the retina, wiring motifs, in particular asymmetric connectivity of starburst amacrine cells to direction-selective ganglion cells, are very likely to contribute to the computation of motion direction[77]; convergence and divergence is also a well-studied wiring motif in connectivity between bipolar cells and W3 retinal ganglion cells[67]. Further studies will be required to connect the quantitative analysis of structural motifs with experiments that test the functional motifs in cell type-specific and layer-specific connectivity (reviewed in REFS 152–154; BOX 2). It also remains to be determined how the occurrence of specific synaptic motifs relates to features of neuronal network architecture at the mesoscopic scale[63,138], whether it differs between brain regions[97,98,128,141,142] and how motifs affect specific computational needs for information processing. A recent modelling study, for example, suggested that the empirical differences in motif distributions across different cortical regions may indicate that neuronal network architectures are optimized for the storage of different forms of information (information stored in the form of specific 'attractor states' versus 'sequences of activity')[155].

In applying graph theory to the analysis of local connectivity, studies have started to reveal the fine-scale topological properties of cellular networks in the mammalian brain. However, most graph theoretical analyses so far have characterized partial mammalian micro-connectomes that have been inferred from sparse electrophysiological recordings in slices (acquired over many specimens) or small volumes of EM-reconstructed nervous tissue. How significantly these pioneering data sets have under-sampled the intact neuronal networks and therefore biased the estimation of connectivity statistics will have to be tested systematically. As it remains difficult to fully map the axonal arborization and dendritic connectivity of neurons with extensive axonal projections up to several millimetres from the neuronal soma, graph theoretical results that are based on path lengths between neurons in cortical networks should currently be regarded as provisional.

Micro-connectome development

Which mechanisms and developmental programs give rise to complex micro-connectomic topology? Which aspects are genetically determined, and which aspects develop postnatally in response to environmental contingencies? Much experimental evidence has been produced to address these questions at both subcellular and cellular scales (for excellent reviews on the development of specific synaptic connectivity, see REFS 156–163). Here, we briefly discuss how cell-lineage and plasticity mechanisms may contribute to micro-connectomic organization.

Cell lineage-dependent connectivity. Numerous studies in small nervous systems have provided evidence that neuronal lineage and birth time are important drivers of spatial and topological features of neuronal networks. In *C. elegans*, for example, most neurons that share early birth dates are connected by long-range connections, become hub nodes and are organized in a rich club[34,164].

Although there is a large body of evidence for genetic mechanisms underlying early patterning, arealization and lamination of the mammalian cortex, it was not possible until recently to directly assess how features in local connectivity are linked to embryonic origin and developmental history. Using genetic fate mapping and retroviral labelling in radial glial cells, studies have now demonstrated that vertically aligned cortical sister (principal) neurons preferentially form connections with each other, first via transient electrical connections[165] and later via chemical synapses[166]. Lineage-dependent circuit formation may therefore give rise to 'ontogenetic modules' as precursors of the mature columnar structure of the neocortex[157]. Furthermore, recent studies that combined retroviral fate mapping with *in vivo* imaging have found that sister neurons in the visual cortex may share functional features, such as orientation preference[167]. This wiring logic may also link to recent reports of preferential structural connectivity among functionally related principal cells in L2/3 of the rodent visual cortex[99,138].

A link between differential synaptic connectivity and developmental time windows was also reported for subregions of the hippocampus[117,168]. One study[169] showed that hippocampal principal cells from the same clone possess a high probability of receiving common input from nearby interneurons, which could link them to functional cell assemblies. Similar to the fate of principal cells, the position, morphology and physiology of interneurons are also strongly affected by developmentally regulated genetic programs, and by their place and time of birth. Interneurons migrate tangentially through the cortex (reviewed in REFS 156,170) and seem to organize in a lineage-dependent manner, potentially in spatially distinct topological clusters[171]. Birth-dating studies revealed that interneurons born at different embryonic time points follow sometimes circuitous migration routes to populate different cortical layers[172,173]. Lineage-specific interneuron clustering has been reported for all major interneuron classes[174]. From a topological point of view, it is also interesting that cells that may be destined to become (GABAergic) hub neurons in the developing hippocampus share an early birth date[112]. Interneuron networks that form early in development may provide anatomical foundations and topological scaffolding for later development of functional connectivity and control of principal-cell dynamics[95,109].

Taken together, these results suggest that functionally relevant topological patterning between principal cells and interneurons may be established over the course of development with lineage and neuron birth time as important (but not exclusive) determinants of connectivity. Whether these processes can be formally described by a generative model (for example, preferential attachment) remains to be tested.

Activity-dependent plasticity. Activity-dependent plasticity is important in shaping the network architecture during development and also maintains the malleability of mature networks to enable adaptation to new functional demands. Most of the plasticity takes place at the level of synaptic connections, and multiple synaptic learning rules have been identified, on the basis of the rate[175–177], pattern[178,179] or timing of spikes[180,181], the cooperativity among inputs[182], or a combination of rules[183]. These learning rules act in concert with homeostatic synaptic scaling mechanisms, which contribute to stability of neuronal firing rates[184]. Spike timing-dependent plasticity (STDP) is a synaptic learning rule that has attracted particular attention because of its physiological plausibility and computational appeal[185]. In STDP, the order and precise timing of presynaptic and postsynaptic spikes determine the outcome of correlated presynaptic and postsynaptic activity. However, because little is known about the actual spike trains that drive synaptic plasticity during development, we do not know which of these rules, or combination of rules, prevail. Interestingly, synaptic learning rules are often both source cell and target cell specific[186], and are subject to neuromodulation[187]. Of importance in functional network connectivity, most of these synaptic learning rules are directional. In fact, STDP was discovered in bidirectionally connected pairs of cortical neurons, with the connection one way strengthening in response to one spike order and the reciprocal connection weakening for the complementary spike order[180]. However, recently it was reported in hippocampal CA3 recurrent connections that the plasticity in this system is symmetrical, with both positive and negative spike pairings inducing potentiation[188], suggesting that specific synaptic learning rules are associated with specific network functions, such as the storage and recall of information in the case of the hippocampus[188]. A series of modelling studies has used STDP to derive mechanistic explanations for topological findings, such as lognormal distributions in synaptic weights[189], and the refinement of recurrent connectivity in the developing V1 (REF. 190). Hebbian-like adaptive rewiring rules have been implicated in explaining the development of small-world[132] or rich-club structure[191]; homeostatic structural plasticity has been suggested to be involved in maintaining a topologically efficient global network architecture[192].

In summary, the findings discussed above suggest that the structure of local connectivity is preconfigured by genetic programs and continuously remodelled by a combination of plasticity mechanisms to optimize its information processing and storage capabilities.

Emerging organizational principles

In the remainder of this Review, we return to Ramón y Cajal's seminal concept of conservation laws and examine some of the evidence that micro-connectomic organization does indeed represent the expression of a few fundamental selection pressures.

Shared constraints and diverse neuronal morphology. In searching for the wiring rules in micro-connectomic topology, it helps to realize that evolution had several million years to optimize the various functional layers that underlie neuronal signalling and the storage of information. It is therefore reasonable to assume that these evolutionary pressures have also optimized the mechanisms that determine cellular network topology. But what exactly is neuronal topology optimized for? Which constraints have to be overcome?

Ramón y Cajal famously inferred a few general conservation laws, specifically for space, time and material. Translating these laws to the language of connectomics, conservation of space means that networks are wired to minimize the amount of intracranial volume that connectivity consumes; conservation of material means that networks are wired to minimize the amount of biological resources that connectivity consumes; and conservation of time means that networks are wired to minimize the conduction delay in transmitting an electrical signal between neurons. Arguably, these categories are still rather broad, but they provide a good starting point for quantifying how biophysical constraints, such as the electrical resistance of cytoplasm[3], shape micro-connectomic topology and to look for motifs in micro-connectomic structure that are advantageous for the computation of information. Importantly, any discussion on the optimality of network layouts must not forget that cellular diversity and specialization by themselves represent the result of an optimization process. Although all neuronal components may share fundamental constraints, the experimentally observed diversity in neuronal types and morphology is a salutary reminder that there are different ways to optimize neuronal structure to fit computational needs. As demonstrated in theoretical studies, this optimization at the level of neuronal arborization is likely to involve a trade-off between various biological costs, among them cellular material and conduction time delay[82–86]. Diversity in functionally specialized cells allows 'division of labour' in circuits and is likely to represent a prerequisite for the optimization of functionality at the global network scale.

Diverse circuits and shared network motifs. Diversity does not stop at the level of neurons, of course, but is a prominent architectural feature that distinguishes functional units and circuits across the brain. The characteristic composition of cells and their inter-connectedness is key to an understanding of how different neuronal networks confer specific functions and computations. Interestingly, however, there are several motifs in the topological organization of micro-connectomes that are shared across functional circuits and across different species. For example, studies in sensorimotor, visual and prefrontal cortices (in slices) have all reported above-random frequency of specific reciprocal higher-order network motifs among principal cells[98,128,141]. Modular structure is another ubiquitous feature of local connectivity, as demonstrated by structural reconstruction of synaptic connectivity using EM[138,139] and graph theoretical analysis of functional connectivity data[116]. Pathway motifs, such as convergence and divergence, similarly recur in various systems of early sensory processing[65–67]. Canonical motifs in principal-cell and interneuron connectivity (BOX 1), such as feedback

Preferential attachment
A generative model or growth rule for the formation of scale-free networks. During development, new nodes are more likely to connect to hub nodes that already have high degree and many connections to other nodes. It is often referred to as the 'rich-get-richer' rule.

Sparse coding
A parsimonious neuronal signalling strategy that requires only a small set of active neurons to encode an item.

inhibition, feedforward inhibition and disinhibition, coexist across the cortex and are a prerequisite for the generation of neuronal dynamics. A more-detailed analysis of the regionally specific quantity and composition of these motifs will provide a more comprehensive understanding of their operational importance in the storage and processing of information in networks and in how they contribute to the economical use of resources in nervous systems[115,155]. Both topological and elementary functional motifs are not only conserved among different brain regions but also have homologues across species[2,9,193]. One possible reason for the ubiquity of these topological motifs is that they might represent economical solutions to a trade-off between biological costs and recurring computational needs. However, how specific combinations of these 'computational primitives' (REF. 194) give rise to emergent functional states remains a largely open question.

Economical growth and plasticity. Studying micro-connectome development and plasticity has provided important insights into the processes that give rise to its intricate topological organization. A large body of work has implicated various economical principles in the formation of neuronal networks, ranging from intra- and inter-axonal competition for growth factor signals[163,195], via maximization of potential connectivity at the scale of dendritic arbors[84], to generative economical growth models for the developing *C. elegans* connectome[196]. Tracking the development of cellular topology in larger brains remains difficult, but a few studies have provided at least indirect evidence for molecular cues and cell lineage as drivers of their organization. Indeed, a recent study modified the expression levels of cell adhesion molecules in cells of the developing *D. melanogaster* lamina cartridge and demonstrated that N-cadherin-mediated differential adhesion is involved in neurite positioning, which is a prerequisite for economical wiring[197] (for a discussion, see REF. 198). Moreover, genetic fate mapping demonstrated that some aspects of local connectivity are preconfigured[157,165–167]. Although it may be expected that such structure provides guidance for the formation of functional connectivity, the exact degree of genetic regulation and topological preconfiguration is not fully understood. There are probably differences in plasticity across neuronal subsystems: whereas the connectivity of specialized pathways for early sensory processing probably benefits from precise wiring imposed by strong genetic regulation, local connectivity of higher cortical areas may benefit from topological scaffolds that allow more activity-dependent fine-tuning. Such subsystem-specific differences could represent an evolutionarily preserved strategy for the economical use of specialized biological resources and contribute to the adaptability of the organism. However, the link between various forms of plasticity, energy-saving signalling strategies such as sparse coding[199] and network topology needs further investigation.

Conclusion

In this Review, we examined recent studies on the organizational principles of neuronal networks at the microscopic scale. We first outlined common themes that have emerged from the study of small, invertebrate connectomes, such as *C. elegans,* and partial connectomes in samples of mammalian brains. We then examined evidence for a complex topological organization of micro-connectomes that is consistent with seminal concepts of conservation laws now quantitatively explicable by a competition or economical trade-off between the cost of wiring and topological integration. However, there is still a large gap in our understanding of how micro-connectomic topology and neuronal computation are linked. In addition, most current graph theoretical metrics do not capture everything that there is to say about the structure and function of neural systems. In particular, the distinct functional roles of different types of neurons and the directionality of information flow in neuronal networks are often not considered. Nevertheless, multiple lines of evidence suggest that the parameters emerging from micro-connectomic analysis describe some essential features of the organization of neural networks. The numerical tractability and generalizability of graph theoretical analysis make it suitable for analysis of big data sets, a feature that will be increasingly important as detailed experimental data on larger neuronal networks at synaptic resolution become available in the future.

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Competing interests statement

The authors declare competing interests: see Web version for details.