Micro-connectomics: Probing the organizational principles of neuronal networks at the cellular scale

Abstract

Defining the organizational principles of neuronal networks at the cellular scale, or *micro-connectomics*, is a key challenge of modern neuroscience. Accelerated by methodological advances, recent experimental studies have generated rich data on anatomical, physiological and genetic factors determining the organization of neuronal networks. In this Review, we will focus on graph theoretical parameters of micro-connectome topology, often informed by economical principles that conceptually originate with Ramón y Cajal's conservation laws. First, we summarize results from experimental studies in intact small organisms and in tissue samples from larger nervous systems. We then evaluate the evidence for an economical trade-off between biological cost and functional value in the organization and development of neuronal networks. In general, the wiring cost of neuronal networks was nearly, but not strictly, minimized by the spatial positioning and connectivity of neurons. Features that reduce the number of synaptic connections between neurons, such as hubs, were more expensive to wire than the theoretical minimum. It seems reasonable to infer from contemporary micro-connectomics that many aspects of intricately detailed neuronal network organization are indeed the outcome of competition between two fundamental selection pressures: low biological cost and high functional value. Future studies will be needed to clarify which aspects of network topology are most functionally valuable and to identify the biological mechanisms controlling expression of cost and topological pressures on the development and evolution of micro-connectomes.

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Box 2: Cell-type specific connectivity: 540 words
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Introduction

Ramón y Cajal proposed in 1899 that neuronal structure is the histological outcome of adaptations to 'laws of conservation' for time, space and material[1]. According to this influential hypothesis, each building block inherent to neuronal networks, ranging from the sub-cellular composition of synaptic vesicles and connectivity between single cells to larger-scale neuronal tracts between brain regions, represents the result of a trade-off between the costs of maintaining it and the evolutionary/functional benefits it provides[2,3]. More than a century later, the quest for general principles underlying nervous system organization and neuronal information processing continues – remaining one of the greatest challenges of modern neuroscience. *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 will 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 in the nematode worm *Caenorhabditis elegans* (*C. 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]. It remains an open question, however, whether principles observed in the small cellular connectomes of invertebrate nervous systems translate to connectivity found in neuronal networks of higher animals. To address this question, we will turn to recent reports that studied the statistics of partial micro-connectomes in the brains of mammals. Results of these pioneering studies indicate that there are parallels between network motifs of small nervous systems and cellular connectivity found in the samples of neuronal tissue of bigger brains. Despite promising progress in recent years and exciting technological advances[8,9], for example in dense electron microscopic (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[10,11]. A key question is also which generative mechanisms give rise to common complex structural properties in neuronal network organization. We will therefore discuss studies that provided insights into the role of neuronal lineage, synaptic plasticity, and neuronal activity for specific patterns in connectivity. To appreciate how these programs are reflected in observed network properties, we will 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 will 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.

Micro-connectome topology in small nervous systems

Out of the many species with small nervous systems that have been studied over the last decades[12–18], two organisms in particular have been utilized to address fundamental questions of micro-connectomic organization: the nervous system of *C. elegans*[5] and the brain of the fruit fly *Drosophila melanogaster*[19]. Both come with the practical advantage that their nervous systems are relatively small, their individual neuronal components have been studied in great detail, and the advent of powerful new imaging approaches allows investigators to link animal behaviour to neuronal network dynamics at cellular resolution (see REF[20] for a review). Both the worm and the fly also have an extensive history as genetic model systems and their genomes include *homologues* of many neurally expressed mammalian genes[21–23]. Despite their small size they 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 the brains of mammals[24]. We will briefly introduce the connectome(s) of each of these organisms, summarize evidence on the statistics of their cellular connectivity, and discuss what can be learnt through the connectomic analysis of these two model systems.

C. elegans. The nervous system of the nematode worm *C. elegans* remains one of only few nervous systems that have been essentially completely mapped at the cellular scale[5]. The adult hermaphrodite *C. elegans* comprises in total 959 somatic cells, of which the nervous system makes up 302 neurons (282 in the somatic nervous system, 20 in the pharynx)[25]. *C. elegans* neurons are structurally simple, possess a highly stereotypical morphology and show only little branching[5]. *C. elegans* neurons have been sub-divided into sensory neurons, motor neurons and interneurons[5,6]. Sensory neurons were classified according to their spatial closeness to known sensory apparatus or functional dissection, motor neurons as cells with neuromuscular junctions, and all remaining cells have been termed interneurons[5,6]. Comprising about 6400 chemical synapses and 900 gap junctions[6], the overall connectivity of the *C. elegans* somatic nervous system is relatively sparse (a connection density of 4%/10% for the binarized/weighted graph; FIG. 1). Only recently did studies begin to systematically map out the neurotransmitter systems in *C. elegans*, revealing the molecular identity of about 90% of neurons[26]; the majority of neurons use as their principal neurotransmitter either acetylcholine or glutamate, but monoaminergic and peptidergic neurotransmitter receptors are also expressed by many neurons. There is now a large body of evidence that demonstrates that both gap junction and synaptic connectomes of *C. elegans* possess complex network properties (BOX 1): The *C. elegans* connectome has a *hierarchical* organization (sensory neurons are more presynaptic, whereas motor neurons are more postsynaptic)[6,10,27], and a *modular* community structure among functionally related neurons[27–32]; its binary synaptic connectivity conforms to a *small-world* organization[6,33], demonstrates a long-tailed degree distribution[6,34], and has a greater-than-random occurrence of some triplet and quadruplet *motifs*[6,35,36]. Interestingly, the *hub* neurons of the *C. elegans* connectome are organized in a *rich-club*[37,38]; this network *core* is mainly

composed of command interneurons of the locomotor circuit[39–41], has high *centrality* (i.e. many shortest path motifs between peripheral neurons are routed through one or more hub neurons of the rich-club)[6,37], comprises a large number of *long-range connections* between distant functional modules[37,42,43], and forms early during connectome development[37].

Studies investigating the wiring *economy* of the *C. elegans* connectome found that it is strongly, but not strictly, minimized for *wiring cost*[43–45]. In other words, although principles to minimize wire can explain several key aspects of the composition of the *C. elegans* connectome, their explanatory power seems 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. This high cost presumably is justified, however, by the high functional value added by integrative topology to overall network performance[2]. For example, the gap junction hub neuron RMG links several important sensory neurons and is of great importance for controlling the global state of the animal (FIG. 2)[46,47]. Connectivity between interneurons in the rich-club provides the anatomical basis for diverse computational tasks, such as coordinated locomotion and foraging[39–41]. Forward and backward movements, for example, are generated in two functionally separate subsets of neurons in this core, potentially coordinated through reciprocal inhibition[6,44,48]. Roberts et al. recently demonstrated that random search behaviour in the worm can be approximated by a connectome-based stochastic model of this circuit[49]. Whereas high-cost network components may provide the base for global integration in the network, specific functional programs access this resource flexibly and in a context-dependent manner[11]. Since many neurons of the worm have *multiplexed* functions[10,50], i.e. 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[51]. High-cost structural features of the connectome may also subserve the functional *degeneracy* in *C. elegans*[52], perhaps contributing to the animals' adaptability. Further progress in the functional interpretation of neuronal topology will come from a better integration of anatomical data with other fundamental layers of neuronal network functioning (BOX 1), such as the molecular identities of neurons[26], neuronal lineage[53], and large-scale recordings of neuronal activity under more naturalistic conditions[54]. 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[53-55]. For example, Bubmarger et al.[56] recently compared the wiring of *C. elegans* and its close relative, the predatory nematode *Pristionchus pacificus,* and found that topological differences in configuration of the pharyngeal ganglion might relate to the very different feeding strategies of both animals.

Drosophila. Four orders of magnitude bigger than the nervous system of the worm, the brain of the fruit fly *Drosophila melanogaster* consists of about 100,000 neurons. The *Drosophila* brain has been anatomically parcellated into distinct neuropil compartments, each providing a domain for functionally specific neuronal computation[57,58]. A whole-brain

connectivity map of *Drosophila* has been constructed using light-microscopy (LM) and multi-color cell labeling techniques, focusing on the projection pathways of about 10% of all neurons between functionally or clonally-defined brain regions[59,60]. Applying graph theory to the *Drosophila* projection map provided insight into its large-scale organizational properties, demonstrating 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 associated with motor and auditory processing[59–61]. While the feasibility of LM to measure finer-grained synaptic connectivity between the densely packed neurons in the fly remains limited[62], electron microscopy (EM) can provide the necessary sub-cellular resolution, and anatomical reconstructions for parts of the fly's optic lobe, such as cartridges of the lamina[19,63], and columns of the medulla have been released[7,64]. Lamina and medulla represent important signal processing pathways in the *Drosophila* visual system: they are both composed of about 750 hexagonally shaped modules and demonstrate a *retinotopical* organization (which *per se* represents a cost-effective organizational solution to minimize wire while preserving the spatiotemporal relationships in visual information[65]). Simulations based on connectome reconstruction of a lamina cartridge demonstrated that the overall configuration and modular organization of its neurons can be well characterized by a combination of wiring minimization and volume exclusion[63]. 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[63]. Analysis of a single EM-reconstructed medulla column (reconstructed volume: 37 × 37 × 50 µm) demonstrated that its connectivity is highly directed, relatively sparse (each presynaptic site contacted on average only about 3-4 postsynaptic sites), can be partitioned into modular processing pathways, and has a long-tailed distribution in connectivity weights (the number of synaptic contacts between a pair of connected neurons)[7]. Follow-up analysis of additionally reconstructed neighbouring medulla columns demonstrated that synaptic wiring patterns in neuronal branching and columnar connectivity are highly stereotypical[64]. Although precise wiring seems intuitively beneficial for accurate transmission of visual information, reported values on wiring accuracy should be interpreted with caution since some of the reconstructed synapses could actually not be traced reliably and a large number of postsynaptic sites were omitted[7,64]. 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 involved in motion detection in the fly[66].

To summarize, graph theoretical analysis of the connectomes of model organisms, such as *C. elegans* and *Drosophila*, has generated strong evidence that even small nervous systems demonstrate hallmarks of a 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, 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 allows flexible switching between different behaviours and integration among functional modules. The parallel pathway architecture of the *Drosophila* optic lobe, retinotopically arranged and parsimoniously wired, seems strongly optimized for robust, high-speed processing of information. Computational benefits through common pathway motifs, such as *convergence* and *divergence* (BOX 1), have been reported for information processing in the *Drosophila* olfactory system (FIG 2)[67], *Drosophila* larva[68], and even mammalian retina[69]. Further clarifying the functional value of anatomically defined network motifs in such small model organisms will require a combination of analysis strategies, such as whole-animal neuro-behavioural mapping[54,70] and selective targeting of individual neuronal components in the network[46,47,54]. 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[8,52,71]; many neurons in small nervous systems are multi-functional, complicating definite mappings between structure and function[15,51]; and a comprehensive integration of connectomic data with maps on the molecular identity of neurons[10,50] and/or functional activity at larger-scales has yet to be established[54].

Micro-connectomics in samples of mammalian brains

Over the last few years, several initiatives have started to systematically map inter-regional connectivity in the rodent brain using trans-synaptic tracers and LM[72]. 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[73,74]. Although retrograde and anterograde tracing techniques clearly represent powerful tools for the study of afferent and efferent connection profiles of brain regions, an analysis of whole-brain connectivity at the scale of individual synapses remains technically challenging[8]. Even a conceptual framework for the quantitative analysis of synaptic connectivity at the whole-brain scale has yet to be established[8,9]. This 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*[75,76], indicating that axo-dendritic proximity alone is not sufficient to predict the existence of a synaptic connection[77–79]. Starting with principles that can already be suggested from analysis of individual cells' morphology, we next outline recent evidence on network motifs in local neuronal 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[80,81]. Since complete EM-based mapping of the many thousands of input synapses onto even a single cortical neuron remains challenging[82], and most EM data sets cannot cover the full extent of all neuronal projections[8], studies have mainly analysed neuronal arbors reconstructed by LM[83–88] (but see REFs[79,89]). Several approaches have been introduced to estimate the branching pattern of neuronal arbors[83] and how neuronal arbors fill the space they are embedded in[90]. The *fractal dimension,* for example, is a metric used to quantify the extent to which arborization of neurons fully occupies the 3D space available and has been linked to the propensity for synaptic connectivity (see REFs [91,92] for reviews; FIG. 3). More recently introduced computational models have greatly expanded the possibilities to quantify neuronal morphology and to probe underlying constraints. Wen et al.[87] 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[87]. Analysis of basal dendrites of pyramidal neurons in the cerebral cortex demonstrated that dendritic arbor radius scales with total dendritic length, as does the pair-wise spatial correlation between dendritic branch segments[86]. A *minimum-spanning-tree* (MST) approach was introduced to study the relationship between neuronal arbor structure and connectivity[93]. Cuntz et al.[84] improved on this conceptual framework and developed an algorithmic procedure, which allows comparison of synthetically grown dendritic trees to LM reconstructed neuronal trees. Their 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[88]. While application of this analysis strategy to a large set of morphological reconstructions from the NeuroMorph.org database[94] 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[88].

Taken together, data on dendritic branching and morphology over 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[88]. These intricate branching patterns are likely established during development through, for example, self-avoidance rules and competition between sibling dendrites[95]. 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[86,96], but remains 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 (see REF[97] for a review). At the level of local circuits, it is well established that synaptic strengths follow a lognormal distribution, i.e. the majority of unitary excitatory postsynaptic potentials (EPSPs) in simultaneous recordings of pre- and postsynaptic neurons are small, with only a small proportion of large EPSPs[98–101] (FIG. 3). 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[102–104]; probing a link between information processing and connection strength experimentally, however, has long remained difficult. Combining *in vivo* optical imaging of principal neurons (PNs) in layer (L) 2/3 of mouse primary visual cortex (V1) with post-hoc whole-cell recordings in slices, a recent study by Cossell et al.[105] demonstrated that a small subset of strong synaptic connections preferentially links PNs with similar *receptive field* structures. These strong connections among PNs have been suggested to provide a mechanism for selective amplification of thalamic input signals in V1[106]; the observed overall distributions in synaptic weights in this region could hence relate to the functional couplings among PNs. 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[107,108]. Studies using calcium imaging in slices of developing hippocampus found that sub-populations of GABAergic interneurons (INs)[109] and early-born PNs[110] display a high degree of functional connectivity. Morphological and physiological characterization of these cells demonstrated that early-born INs 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[111]. Classification of INs according to their arborization into *connector hubs* (e.g. hippocampal INs with long-range axons to the medial septum[112,113]) and *provincial hubs* (e.g. INs that display mainly intra-hippocampal arborization[114]) has been proposed[111]. IN hubs may also be involved in orchestrating synchronous network activity[115,116]. A role for IN 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[117]. Heavy-tailed distributions of functional connectivity were also observed in studies using *transfer entropy* analysis of spontaneous neuronal activity recorded by multi-electrode arrays (MEAs) *in vitro*[118,119] and in silicon-based microprobe recordings *in vivo*[108]. Interestingly, these studies found that highly-connected hub neurons may be organized in rich-clubs[107,108], that neurons comprising these network cores receive significantly more inputs[108], display significantly higher firing rates[108], and may form their connections early during network development[107].

Although there seems to be good evidence for hubs and long-tailed distributions in functional connectivity, patch-clamp studies of structural connectivity between PNs generally did not observe such distributions[100]. 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[120], and modeling work which

demonstrated that neuronal networks with hubs can potentially better explain the distribution of *in vivo* firing rates[121].

Clustered and modular connectivity. Following the seminal work by Watts and Strogatz[33], 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[100], and a series of studies examined this phenomenon in functional connectivity derived from spontaneous activity in cultures, slices, and the living animal[122–129]. The emergence of small-world features was also reported for developing neuronal networks in dissociated neuronal cultures[107,126,130]. Importantly, different forms of clustering have been reported in the literature[98,100,129]: 'topological clustering' has been described for structured connectivity in which neurons form closed triangular motifs irrespective of inter-nodal distances[100,129,131]; in contrast, 'spatial clustering' refers to topological clustering that can be mainly explained by a distance-related drop-off in connectivity, i.e., clustered nodes also tend to be spatially adjacent[132]. Finally, sub-cellular 'synaptic clustering' refers to the spatial arrangement of synapses on the dendritic tree. Synaptic clustering likely plays an important role in synaptic integration[80], and was observed, for example, in synapses with functionally related inputs during spontaneous activity[133], and among synapses of hippocampal neurons whose pre-synaptic neurons shared a similar developmental time window[120]. Numerous modeling studies have tested how clustering-related properties may evolve during neuronal network formation[134], how clustering is maintained by plasticity mechanisms[135,136], and whether it affects synchronization dynamics[117,137]. Whereas evidence in support of the functional role of synaptic clustering in dendritic computation in some areas of the brain has accumulated quite consistently[133,138], testing the functional significance of topological properties, such as a small-worldness in local connectivity, remains difficult[117,139].

Several recent studies have also reported a modular structure in local synaptic connectivity. Combining *in vivo* functional imaging with mono-synaptic retrograde trans-synaptic tracing, Wertz et al.[140] provided an important example of how to determine functional modular organization in mouse V1 across L2/3 and 5. The study inferred the pre-synaptic networks of single PNs in L2/3 and found that there are layer-specific functional modules that could be locked to the direction preference of the post-synaptic cell[140]. Modular connectivity was also reported in two recent EM studies in mice[141,142]. A non-random community structure in the synaptic connectivity of 201 V1 L2/3 PNs was found in a study combining *in vivo* functional imaging with post-hoc EM reconstruction (reconstructed volume: 450 × 450 × 50 µm; FIG. 3)[141]. Importantly, the study also confirmed previous electrophysiological work, which had shown that PNs with similar orientation selectivity are preferentially connected to each other[101]. An EM study of parts of the lateral geniculate nucleus (LGN; reconstructed volume: 400 × 600 × 280 µm), indicated that the organization of synaptic connectivity can be *fuzzy*, i.e. indicative of a strongly overlapping modular affiliation in which network nodes belong to several different sub-networks[142]. Future work will have

to test the functional relevance of such differences in modular structure to rule out potential (slicing) artefacts due to subsampling of the neuronal network[8].

Network motifs. Micro-connectomic organization has also been studied by quantifying the distribution of specific higher-order network motifs[35]. In one of the first experimental studies of these local building blocks, Markram et al.[143] estimated PN connectivity in slices of L5 rat somatosensory cortex using whole-cell patch-clamp recordings and demonstrated that the proportion of bidirectional/reciprocal connections clearly exceeded the number of connections 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 PNs of rat visual cortex[131], ferret prefrontal cortex[149], and rat somatosensory cortex[100]. An above-random occurrence of transitive three-neuron motifs in synaptic IN connectivity, such as feed-forward triplets, and a large overlap between chemical and gap junction connectivity, has also been reported for cerebellar networks[144]. Searching for rules that could potentially explain experimentally observed wiring motifs, Perin et al.[100] reported that the connection probability of PNs is increased when neurons receive common input and that the latter is correlated with the number of common neighbours – the so-called 'common neighbour rule'[100]. Evidence in qualitative agreement with this principle came from functional connectivity studies using transfer entropy analysis of the intrinsic neuronal spontaneous activity in cortical slice[119], and could relate to results from imaging studies on co-activation dynamics among PNs in auditory[145], visual[153] and prefrontal cortex[147]. Despite recent patch-clamp work *in vivo*[148,149], which seems to be largely in line with previous reports on local connectivity *in vitro*, findings on network motifs remain controversial due to potential artefacts caused by sparse local electrophysiological recordings and slicing of neuronal tissue. Some of the studies discussed above have made attempts to take these effects into account, but many questions remain.

Motifs in reciprocal connectivity between cortical PNs, more generally, have been hypothesized to contribute to various computational tasks, such as amplifying inputs[105,141], shaping receptive field properties[101,150], and prolonging activity for computation in higher-order cortical areas[151,152]. Network motifs could also provide a structural backbone for synchronizing functional cell assemblies (often referred to as "cliques")[153,154]. In the retina, wiring motifs, in particular asymmetric connectivity of starbust amacrine cells to direction-selective ganglion cells, have been confirmed as key mechanisms for the computation of motion direction[79]; convergence and divergence pathways are well-studied wiring motifs in circuits underlying chromatic processing[69]. Further studies will be required to connect the quantitative analysis of structural motifs with experiments that test the functional motifs in cell-type and layer-specific connectivity (see REFs[155–157] for reviews; BOX 2). It also remains to be determined how the occurrence of specific synaptic motifs correlates with large-scale neuronal architecture[141,144], whether it differs between brain regions[99,100,131,143,144], and how motifs impact on specific computational needs for information processing. A recent modeling study, for example, suggested that the empirical differences in motif distributions in 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' vs. 'sequences of activity')[158].

Applying graph theory to analysis of local connectivity, studies have revealed a wide range of topological properties. Since most graph theoretical analyses of mammalian micro-connectomes were applied to sparse graphs, for example constructed from patch-clamp recordings in slices acquired over many specimens, it seems likely that these and other pioneering data will have under-sampled the intact mammalian cortex. More specifically, 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. Therefore, graph theoretical results that are based on path lengths between neurons in cortical networks should currently be regarded as provisional. As techniques for cortical tissue mapping continue to improve, and synaptic connectivity can be more completely mapped for several cubic millimetres of cortex, the more integrative aspects of mammalian micro-connectome topology, such as long-distance projections between neuronal modules, are likely to be resolved more clearly.

Development of micro-connectomic architecture

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 both at the sub-cellular and cellular scale (FIG. 4), and excellent reviews on the development of specific synaptic connectivity are available[159–166]. Here, we restrict ourselves to a discussion of recent work that tested how genetic fate may relate to cell-type specific connectivity and we briefly discuss how cell-lineage and plasticity mechanisms may contribute to micro-connectomic organization.

Cell-lineage dependent connectivity. Numerous studies in smaller nervous systems have provided evidence that neuronal lineage is a driver of network topology. In *C. elegans*, for example, neurons that share similar birth dates during development have been linked to long-range connectivity, hubs, and rich-clubs[37,167]. Analysis of neuronal lineages in *Drosophila* provided key insights into the highly modular organization of its inter-regional connectivity[60]. Although there is a large body of evidence on the genetic mechanisms underlying early patterning, arealization and lamination of mammalian cortex, it has not been possible until recently to directly assess how features in local connectivity are linked to embryonic origin and developmental history[163]. 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[168], and later via chemical synapses[169]. Lineage-dependent circuit formation may thereby give rise to 'ontogenetic modules' as precursors of the mature

columnar structure of the neocortex[160,170]. Furthermore, recent studies that combined retroviral fate mapping with *in vivo* imaging have found that sister neurons in visual cortex may share functional features, such as orientation preference[170]. This wiring logic may also link to recent reports of preferential structural connectivity among functionally related PNs in rodent L2/3 visual cortex[101,141]. Developmental time windows may reflect differential synaptic connectivity found in sub-regions of the hippocampus[120,171]. Xu et al.[172] demonstrated that hippocampal PNs from the same clone possess a high probability of receiving common input from nearby INs, which could link them to functional cell assemblies. Similar to the fate of PNs, the position, morphology and physiology of INs are also strongly affected by developmentally regulated genetic programs, and the place and time of birth. INs migrate tangentially through the cortex (see REFs [173,174] for reviews), and seem to organize in a lineage-dependent manner, potentially in spatially distinct topological clusters[175]. Birth-dating studies demonstrated that INs born at different embryonic time points follow sometimes circuitous migration routes to populate different cortical layers[176,177]. Lineage-specific IN clustering has been reported for all major IN classes[178]. 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[114]. IN networks that form early in development may provide anatomical foundations and topological scaffolding for later development of functional connectivity and control of PN dynamics[97].

Taken together, these results suggest that functionally relevant topological patterning between PNs and INs may be established over the course of development with lineage and neuron birth time as important (yet certainly not exclusive) determinants of connectivity. Whether these processes can be formally described by any kind of generative model, e.g., *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, based on the rate[179–181], pattern[182,183] or timing of spikes[184,185], cooperativity among inputs[186]; or a combination of rules[187]. These learning rules act in concert with homeostatic synaptic scaling mechanisms, which contribute to stability of neuronal firing rates[188]. Spike timing-dependent plasticity (STDP) is a synaptic learning rule that has attracted particular attention because of its physiological plausibility and computational appeal[189]. 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[190] and are subject to neuromodulation[191]. Of importance for the 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[184]. 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[192], suggesting that specific synaptic learning rules may be associated with specific network functions, such as the storage and recall of information in the case of the hippocampus[192] (see also REF[159]). A series of modeling studies has used STDP to derive mechanistic explanations for topological findings, such as lognormal distributions in synaptic weights[193], and the refinement of recurrent connectivity in developing V1[194]. Hebbian-like adaptive rewiring rules have been implicated in explaining the development of small-world[195] or rich-club structure[196]; homeostatic structural plasticity has been suggested to be involved in maintaining a topologically efficient global network architecture[197].

In sum, the studies summarized above suggest that the structure of local connectivity is pre-configured by genetic programs and continuously re-modelled by a combination of plasticity mechanisms to optimize its information processing and storage capabilities.

Emerging principles and functional implications

In the previous sections of this Review, we have detailed the topological properties of micro-connectomes, both in structural reconstructions of nearly intact small nervous systems and in partial connectomes derived from small samples of mammalian brains. We have also outlined some of the determining factors that may guide the development and plasticity of micro-connectomes. In the remainder, we return to Ramón y Cajal's seminal concept of conservation laws and review some of the evidence that micro-connectomic organization does indeed represent the expression of a few fundamental selection pressures.

Shared constraints, diverse neuronal morphology. 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 (BOX 1) that underlie neuronal signalling and 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 itself represents 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 likely involves a trade-off between various biological costs, among them cellular material and conduction time delay[84]. Diversity in functionally specialized cells allows 'division of labour' in circuits and likely represents a prerequisite for the optimization of functionality at the global network scale.

Diverse circuits, shared network motifs. Diversity does not stop at the level of neurons, of course, but is a prominent architectural feature that distinguishes functional units/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 sensory-motor, visual, and prefrontal cortices (in slice) all reported significantly above-random frequency of specific reciprocal higher-order network motifs among PNs[100,131,143,152]. Modular structure is another ubiquitous feature of local connectivity, as demonstrated by both structural reconstruction of synaptic connectivity using EM[141,142] and graph theoretical analysis of functional connectivity data[119]. Pathway motifs, such as convergence and divergence, similarly recur in various systems of early sensory processing[66,67,79]. Canonical motifs in PN and IN connectivity, such as feed-back inhibition, feed-forward inhibition, and dis-inhibition 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 significance for storage and processing of information in networks and how they contribute to the economical use of resources in nervous systems[117,158]. Both topological and elementary functional motifs are not only conserved among different brain regions but have homologues across species[198,199]. 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. How specific combinations of these 'computational primitives'[200] give rise to emergent functional states, however, 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[166,201], via maximization of potential connectivity at the scale of dendritic arbors[96], to generative economical growth models for the developing *C. elegans* connectome[202]. Tracking the development of cellular topology in larger brains remains difficult, but a few studies provided at least indirect evidence for molecular cues and lineage as drivers of their organization. A recent study modified the expression levels of cell adhesion molecules (CAMs) in cells of the developing *Drosophila* lamina cartridge and demonstrated that N-Cadherin (CadN)-mediated differential adhesion is involved in neurite positioning, which is a prerequisite for economical wiring[203,204]. Genetic fate mapping demonstrated that some aspects of local connectivity are pre-configured[160,168,205]. While it may be expected that such structure provides guidance for the formation of functional connectivity, the exact degree of genetic regulation and topological pre-configuration is not fully understood. Differences between neuronal subsystems seem likely: whereas connectivity within modules specialized pathways for early sensory processing likely benefit 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 in plasticity could represent an evolutionarily preserved strategy for the economical use of specialized biological resources and contribute to the adaptability of the organism. The link between various forms of plasticity, energy-saving signalling strategies, such as *sparse coding*[206], and network topology needs further investigation, though.

Conclusion

In this Review we summarized recent studies on the organizational principles of neuronal networks at the microscopic scale. We first outlined common themes that emerged from the study of small, invertebrate connectomes, such as *C. elegans,* and partial connectomes in samples of mammalian brains. We then outlined 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. Also, most of the current graph theoretical metrics do not capture all 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 makes it suitable for analysis of big datasets, a feature that will be increasingly important as experimental detailed data on larger neuronal networks at synaptic resolution become available in future.

Box 1 **| Graph theory.** The starting point for all graph theoretical analysis is the definition of *nodes* and *edges;* see REF[207] for an introductory text. For micro-connectomes, a node will typically represent a specific neuron, and an edge a synaptic or gap-junction connection between two neurons. Most commonly used graph theoretical metrics are calculated on *undirected* connectivity. The *degree* (k) simply describes how many edges connect to one node *i*. The *degree distribution P*(k) allows comparison of overall network structure to models, such as *random*, *regular* or *scale-free networks*. Nodes with a high degree are often referred to as *hubs*. A pervasive and well-studied organizational feature is the community structure, or *modularity*, of a network. It describes how well a network can be partitioned into communities and numerous methods and many algorithms for modular partitioning have been developed[208]. A hierarchically nested modular structure across spatial scales has been referred to as the hallmark of complex systems[209]. Connectivity of hub nodes may play 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 inter-connected than the high degree nodes of a comparable random network. The topological distance between a pair of nodes *i* and *j* can be estimated by the *path length*, i.e. the minimal number of synapses that need to be traversed to connect node *i* to *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*. It gained particular prominence in the formalization of the *small-world property*[33]. Expanding on traditional approaches to assess networks, the *multi-layer* network framework includes not just one, but multiple types of relationships among neuronal nodes, e.g. chemical synapses, electrical gap junctions and extra-synaptic neuromodulatory interactions. Furthermore, a better integration of classical *circuit motifs,* involving combinations of both excitatory and inhibitory neurons, will be required to link micro-connectomic topology to physiological function.

Box 2 | **Cell-type specific connectivity.** A large body of evidence suggests that the probability of a connection between two neurons depends on the pre- and postsynaptic *cell type*[77,210–212]. In the cortex, two broad classes of neurons can be distinguished: *projection neurons* (PNs) and local-circuit *interneurons* (INs). PNs comprise about 80% of all cortical neurons; they use the excitatory neurotransmitter glutamate for signaling 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 V1 PN dendrites come from neurons >200 µm apart[213]). PNs also make local connections to their neighbouring cells. The remaining 20% of cortical neurons are INs. Inter-neuronal efferent connectivity is mainly local and most INs release the inhibitory neurotransmitter *GABA*. A common result from patch-clamp recordings is a relatively low local connectivity among PNs. For example, the PN connectivity rate estimated by patch-clamp recordings in slices of L2/3 rat somatosensory and visual cortex decreased as a function of distance from ≈10% for cells in close proximity (<25 µm) to a rate of <1% for PNs more than 200 µm apart[98]. Connectivity in slices of L5 rat somatosensory cortex has been reported to decay from ≈20% for nearby neurons to less than 5% for neurons >200 µm apart[100]. Connectivity of L2 primary somatosensory cortex (S1) PNs was also sparse *in vivo*[214]. Local connectivity in L2/3 in V1 ranged between 10% and 20% in both rat[131] and mouse[101,105]. PN connectivity likely depends on intersomatic orientation of cells[140], on the cortical layer, and from where they receive their input or send their outputs[215]. Evidence indicated that PNs are preferentially connected if they receive common inter- and intra-laminar input[216] and if they share similar receptive fields[101,105]. EM reconstruction showed that PN connectivity in V1 L2/3 is more likely if cells share similar orientation preferences[141]. New transgenic mouse lines that express specific fluorescent genetic markers have made it feasible to differentiate INs broadly into largely non-overlapping subclasses: parvalbumin-expressing (PV^+), somatostatin-expressing (SOM^+) and serotonin receptor 5HT3a -expressing ($5HT3aR^+$) INs. Together, PV^+, SOM^+ and $5HT3aR^+$ INs account for almost 100% of all cortical INs[217]. In combination with knowledge on the innervation sites of INs, predictions about specific computational roles and circuit functions become feasible[81,218]. Combining optogenetic stimulation and whole-cell recordings, studies have started to map IN connectivity rates within and between IN sub-classes and to PNs. In L2/3 and L5 visual cortex[219,220] and L2/3 in S1[221], studies found a high connectivity among PV^+ INs. In contrast, few or no chemical synapses have been observed among SOM^+ INs[219,222]. Similarly, intra-class connectivity among VIP^+ INs, a subgroup of 5HT3aR INs, is sparse. Between-class connectivity has also been examined and a connectivity scheme from VIP^+ to SOM^+ and PV^+ INs to control disinhibition of local PNs has recently gained attention[220,223,224]. Interestingly, the same connectivity scheme was replicated across different cortical regions[225]. High synaptic connectivity has been reported for the inputs from PNs onto PV^+ INs in V1[98,216,226], with more moderate connectivity for the inputs from PNs onto VIP^+ and SOM^+ INs in V1 and S1[220]. *In vivo* patch-clamp recordings in L2/3 barrel cortex[149] have largely confirmed *in vitro* connectivity rates from PNs to PV^+ INs [221] and SOM^+ INs[227].

Figure 1 Complex topological properties of the *C. elegans* connectome. a | The nervous system of the nematode *C. elegans* represents the first whole-animal connectome at cellular resolution. It was reconstructed by serial-section electron microscopy (EM) and post-hoc manual tracing in the 1980s[5]. The panel depicts an updated version of the originally published connectivity matrix between a subset of 279 neurons, including chemical synapses (red) and gap junctions (blue)[6]; the matrix is reordered according to three main functional classes of neurons: sensory neurons, interneurons and motor neurons. **b |** The *C. elegans* connectome obeys Rents' rule[31]; i.e. 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). Rentian scaling was initially introduced to assess the physical embedding of very large-scale integrated (VLSI) computer chips; physical Rentian scaling in connectivity of *C. elegans* has been interpreted as evidence for an economical spatial embedding. **c |** Modeling studies found that the principles of wiring cost minimization are a good predictor of the actual position of neurons in the *C. elegans* connectome[44]. Depicted is the correlation between the actual neuronal positions and the positions predicted by a wiring-cost minimization model. For a perfect match, neurons would fall on the diagonal. As depicted, some neurons clearly deviate from the rule (AVG, PVP and PVQ); in particularly the position of some command interneurons and pioneer neurons cannot be predicted well. **d |** Highly connected interneurons of the locomotor circuit of *C. elegans* form a rich-club[37]. This network core may facilitate integration between different functional modules of the nervous system through topological shortcuts. Experiments demonstrated that neurons in the rich-club are of great functional importance for coordinated movement and flexible switching between different network states[49]. **e |** Distribution of connection distances of the *C. elegans* connectome[37], grouped into three different classes: rich-club, feeder and local edges. Rich-club neurons comprise a very large proportion of the long-range connections (rich-club edges, black); feeder edges (grey), which connect peripheral neurons to rich-club neurons, and local edges (white), which connect only peripheral neurons, demonstrate generally lower connection lengths. Panel **a** is reproduced, with permission, from REF.6; part **b** is adapted and modified, with permission, from REF.31; panel **c** is modified, with permission, from REF.44; part **d** is adapted, with permission, from REF.37; part **e** is modified, with permission, from REF.37.

Figure 2 Linking network structure and functional dynamics. a | Network motifs may facilitate efficient information processing. (i) Using optogenetic stimulation in olfactory receptor neurons (ORNs) and electrophysiological recordings across multiple layers of the *Drosophila* olfactory system, a recent study tested the potential effects of pathway motifs[67]. Schematic depicts the simplified circuitry of a single glomerulus, where optogenetically induced signals from ORNs first diverge and converge onto six postsynaptic projection neurons (PNs); the PNs later reconverge onto specific higher-order lateral horn neurons (LHNs). Results indicate that convergence, divergence, and reconvergence motifs rendered the signal progressively more informative. (ii) Potential synergistic effects through a feed-forward convergence and reconvergence pathway have also been proposed by a study on multimodal action selection in *Drosophila larva*[68]. Depicted is a summary of the reconstructed multilevel multimodal convergence brain pathway for rolling in the larva[68]. Signal from nociceptive (orange) and mechanosensory neurons (green) first converges on first-order multi-sensory interneurons (blue) that integrate the input; these interneurons converge again at later processing stages (second-order (yellow) and third-order (magenta) projection neurons) before the signal reaches the nerve cord[68]. (iii) Hubs represent another topological feature that can provide integration of information across the network. The gap junction hubs of the *C. elegans* 'hub-and-spoke' circuit, RMG (green polygon), are densely connected with various sensory neurons (red triangles) and mediate different behaviours, depending on the neuromodulatory state (arrows indicate synaptic connections; gap junctions are depicted as resistor symbols)[10,46]. **b |** Upper panel (i) depicts the normalized ΔF/F calcium fluorescence time series from 109 head neurons of a freely moving *C. elegans*[54]. After decomposing the data using a principal component analysis (PCA), patterns of network activity could be linked to specific action sequences. The lower panels show two phase plots with the first two principal components (PC1 and PC2) derived from the network activity; either color-coded for six different activity states (ii), or for increases in Ca^{2+} activity of three selected neurons (iii). **c |** Selective optogenetic stimulation of *C. elegans* RMG neurons expressing channelrhodopsin-2 induces rapid changes in behavioural state, such as persistent forward movement[46]. The panel depicts stimulation during two different oxygen conditions (red: 21% oxygen; blue: 7% oxygen); the worm generally tries to avoid environments with high oxygen and therefore demonstrates a higher arousal level at 21% oxygen. Combining connectomics with insight from targeted stimulation at the level of single neurons (Panel c), and whole-brain cellular imaging (Panel b) will enable development of integrative models for how neuronal structure, function and behaviours are linked in the worm. **d |** Schematic of a stochastic neuronal model to simulate random search in *C. elegans*[49]. The model was informed by connectome data on the locomotor circuit of the worm, whose forward (F) and reverse (R) command neuron units are connected through reciprocal inhibition. The activation state of each unit is indicated by a colour combination (pink indicates that the unit is active). If F and R units are conjointly active (or inactive) the animal pauses (X, Y). Figures in panel **a** modified, with permission, from REF.11,67,68; panels in part **b** modified, with permission, from REF.54; panel **c** is reproduced, with permission, from REF.46; panel **d** is modified, with permission, from REF.49.

Figure 3. Fractal neuronal arborization and motifs in mammalian local connectivity. a | Fundamental aspects of neuronal arborization have been linked to principles to conserve cellular cytoplasm and conduction time, reminiscent of Ramón y Cajal's conservation laws for space, time and material[85,88]. The panel illustrates schematically four hypothetical dendritic arbor designs: whereas 'compact' branching (i) balances conduction time cost and dendritic path length, designs depicted in panel ii-iv are suboptimal. Either they demonstrate excessive time costs (ii), or their propensity for synaptic contacts is too low (iii) or high (iv). **b |** Branching patterns can be predicted from the principles of wiring cost minimization. Depicted is the scaling relationship between total dendritic length (L) and the number of dendritic branch points (n) 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 (MST) model [88]. **c |** Synaptic connectivity matrix derived by EM reconstruction of 201 excitatory neurons of mouse L2/3 visual cortex[141]. Connectivity is reordered to represent its modular clustered community structure; connectivity was more likely for excitatory neurons that shared similar orientation preference. **d |** A lognormal distribution of synaptic weights, here quantified as unitary excitatory postsynaptic potentials, has been found in local connectivity of the visual cortex of the rat[131] and the mouse barrel cortex[99]. **e |** Putative monosynaptic connectivity derived from electrophysiological recordings in rat medial prefrontal cortex during a T-maze working memory task[228]. Pyramids depict pyramidal neurons, circles INs, boxes depict neurons that could not been defined; arrows represent excitatory connections, crossbars indicate inhibitory connections. Neuronal firing was behaviour and position-selective; colors 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 (magenta) trajectories. Inferred connectivity is locally clustered; some neurons demonstrate hub-like features. Panel **a** is reproduced and modified, with permission, from REF.87; panel **b** is reproduced, with permission, from REF.88; panel **c** is reproduced, with permission, from REF.141; panel **d** is reproduced, with permission, from REF.97; panel **e** is reproduced, with permission, from REF.228.

Figure 4. Growth and maintenance of micro-connectomes. a | Long-term *in vivo* imaging of adult-born dentate gyrus granule cells (DGCs) in mouse allows an analysis of cell-intrinsic and extrinsic factors on the branching structure of developing dendrites[229]. Depicted are new-born DGCs, which were labelled with a specific GFP-expressing retro-virus and imaged from day post injection (dpi) 15 onwards. Reconstructions demonstrate how dendrites undergo overgrowth and pruning over time (green indicates that a new branch was added; yellow indicates that a branch was pruned; branches that were added and pruned between imaging sessions are coloured purple). **b |** Development of neuronal network topology has been studied by electrophysiological recordings from multi-electrode arrays (MEAs) *in vitro*. MEA recordings can be used to record spontaneous activity of neurons growing in culture and to analyse the emergent spatiotemporal patterns of functional connectivity developing over several weeks or months. Emergence of topological features, such as a small-world organization and a rich-club structure have been reported (in Panel **b** electrodes with red circles depict nodes that have been classified as rich-club nodes; lower panel depicts network development from days *in vitro* (DIV) 14 to 24)[107,126]. The advent of more complex tissue culturing systems, in particular 3D culturing scaffolds, such as cerebral organoids grown from human pluripotent stem cells[230], as well as recording platforms that allow the study of neuronal networks at sub-cellular resolution[231], will provide important complementary insight into network function and principles that drive the development of neuronal topology. **c |** Emergence of complex neuronal network structure has also been modelled using computational growth simulations *in silico*[232]. Depicted are artificial neurons that grow over several days (4, 8, 16) following local rules that approximate some of the more established dynamics in neurite outgrowth and branching (axons and dendrites are depicted in green, respectively blue). Panel in **a** is adapted, with permission, from REF.229; panel **b** is reproduced and modified, with permission, from REF.107, and panel **c** is modified, with permission, from REF232.

Glossary

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

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

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

Core Subset of nodes in the network, which is highly interconnected and contributes to the global integrity of the network.

Cost Used as an umbrella term for biological pressures and expenditures (metabolism, material, etc.) 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.

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

Economy, economical Used here to describe the trade-off between the biological cost and the functional value of topologically complex networks.

Efficiency (topological) Metric of network integration that is calculated as the inverse of the average shortest path of a network.

Fractal dimension A measure of the extent to which a self-similar process, like a dendritic tree, completely occupies the 3D space available: more intricately branching arborization will have higher fractal dimension indicating greater space occupancy.

Fuzzy organization Refers to network components that belong not exclusively to a single module, but are part of multiple, potentially overlapping communities.

Genetic fate mapping Relating the statistics of mature cellular connectivity to neuronal birth dates or embryonic origin.

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

Homology The conservation of organizational pattern between species or across different structures within an organism.

Hub Node with a high degree of connectivity to other nodes in the network and which is of high importance to the integrity of the network.

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

Modularity The property that a network can be decomposed into groups of nodes which are densely inter-connected to each other, but only sparsely connected to nodes in other modules.

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

Motif A pattern of connectivity between a few (typically < 10) nodes. Some motifs are over-represented in connectomes. For example, the closed triangular motif between 3 nodes is typical of highly clustered neuronal networks.

Multiplexity Refers to the existence of more than one type of edge between network nodes. Micro-connectomes are multiplex because there are at least chemical synaptic, gap junction and extra-synaptic neuromodulatory connections between neurons.

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

Preferential attachment rule 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.

Receptive field The domain of a sensory space that can stimulate electrical activity of a neuron.

Retinotopy, retinotopic mapping A common feature found in visual cortical areas, which describes the spatially ordered mapping of visual inputs from the retina to cortical neurons.

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.

Scale-free 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.

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

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

Synaptic clustering The concept that functionally correlated synaptic inputs on dendritic branches are also spatially co-located.

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

Topographic mapping Anatomical co-location of neurons with similar receptive fields.

Transfer entropy An information theoretic measure for the directed interaction between two time series; it measures the information that the past of a variable *I* (source) provides about the current value of another variable *J* (target), in addition to the information provided by the past of *J* alone[233].

Tree A connected graph without cycles or closed loops.

References

1. Ramón y Cajal, S. R. *Histology of the nervous system of man and vertebrates*. **1,** (Oxford University Press, USA, 1995).
2. Bullmore, E. & Sporns, O. The economy of brain network organization. *Nat. Rev. Neurosci.* **13,** 336–349 (2012).
3. Sterling, P. & Laughlin, S. *Principles of Neural Design*. (MIT Press, 2015).
4. Sporns, O. *Discovering the Human Connectome*. (MIT Press, 2012).
5. White, J. G., Southgate, E., Thomson, J. N. & Brenner, S. The structure of the nervous system of the nematode Caenorhabditis elegans. *Philos Trans R Soc Lond B Biol Sci* **314,** 1–340 (1986).
6. Varshney, L. R. *et al.* Structural properties of the Caenorhabditis elegans neuronal network. *PLoS Comput Biol* **7,** e1001066 (2011).

Comprehensive and detailed analysis of the topological properties of the C. elegans connectome. The authors also introduced ways to visualize potential information flow in the worm.

7. Takemura, S. *et al.* A visual motion detection circuit suggested by Drosophila connectomics. *Nature* **500,** 175–181 (2013).
8. Helmstaedter, M. Cellular-resolution connectomics: challenges of dense neural circuit reconstruction. *Nat. Methods* **10,** 501–507 (2013).
9. Lichtman, J. W. & Denk, W. The big and the small: challenges of imaging the brain's circuits. *Science* **334,** 618–623 (2011).
10. Bargmann, C. I. Beyond the connectome: how neuromodulators shape neural circuits. *Bioessays* **34,** 458–465 (2012).
11. Bargmann, C. I. & Marder, E. From the connectome to brain function. *Nat. Methods* **10,** 483–490 (2013).

Excellent review of historical and more recent connectome studies, in particular, the connectivity diagram of the stomatogastric ganglion (STG) of the crab, and *C elegans.* The authors also provide a good overview of the limitations of current connectomic approaches.

12. Menzel, R. The honeybee as a model for understanding the basis of cognition. *Nat. Rev. Neurosci.* **13,** 758–768 (2012).
13. Frazier, W. T., Kandel, E. R., Kupfermann, I., Waziri, R. & Coggeshall, R. E. Morphological and functional properties of identified neurons in the abdominal ganglion of Aplysia californica. *J. Neurophysiol.* **30,** 1288–1351 (1967).
14. Cassenaer, S. & Laurent, G. Conditional modulation of spike-timing-dependent plasticity for olfactory learning. *Nature* **482,** 47–52 (2012).
15. Marder, E. & Bucher, D. Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. *Annu Rev Physiol* **69,** 291–316 (2007).
16. Randel, N. *et al.* Neuronal connectome of a sensory-motor circuit for visual navigation. *Elife* **3,** e02730 (2014).
17. Zucker, R. S., Kennedy, D. & Selverston, A. I. Neuronal circuit mediating escape responses in crayfish. *Science* **173,** 645–650 (1971).
18. Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117,** 500–544 (1952).
19. Meinertzhagen, I. A. & O'Neil, S. D. Synaptic organization of columnar elements in the lamina of the wild type in Drosophila melanogaster. *J. Comp. Neurol.* **305,** 232–263 (1991).

20. Ahrens, M. B. & Engert, F. Large-scale imaging in small brains. *Curr. Opin. Neurobiol.* **32,** 78–86 (2015).
21. Bargmann, C. I. Neurobiology of the Caenorhabditis elegans genome. *Science* **282,** 2028–2033 (1998).
22. Adams, M. D. *et al.* The genome sequence of Drosophila melanogaster. *Science* **287,** 2185–2195 (2000).
23. Howe, K. *et al.* The zebrafish reference genome sequence and its relationship to the human genome. *Nature* **496,** 498–503 (2013).
24. Bullmore, E. & Sporns, O. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci* **10,** 186–198 (2009).
25. WormAtlas, Altun, Z.F., Herndon, L.A., Wolkow, C.A., Crocker, C., Lints, R. and Hall, D.H. (ed.s) 2002-2016. Available at: http://www.wormatlas.org. (Accessed: 25th January 2016)
26. Pereira, L. *et al.* A cellular and regulatory map of the cholinergic nervous system of C. elegans. *Elife* e12432 (2015).
This study provides an important update on the neurotransmitter systems in the worm C. elegans and combines this molecular information with connectomic data in a motif analysis.
27. Chatterjee, N. & Sinha, S. Understanding the mind of a worm: hierarchical network structure underlying nervous system function in C. elegans. *Prog. Brain Res.* **168,** 145–153 (2007).
28. Sohn, Y., Choi, M.-K., Ahn, Y.-Y., Lee, J. & Jeong, J. Topological cluster analysis reveals the systemic organization of the Caenorhabditis elegans connectome. *PLoS Comput Biol* **7,** e1001139 (2011).
29. Pan, R. K., Chatterjee, N. & Sinha, S. Mesoscopic Organization Reveals the Constraints Governing Caenorhabditis elegans Nervous System. *PLoS ONE* **5,** e9240 (2010).
30. Pavlovic, D. M., Vértes, P. E., Bullmore, E. T., Schafer, W. R. & Nichols, T. E. Stochastic blockmodeling of the modules and core of the Caenorhabditis elegans connectome. *PloS One* **9,** e97584 (2014).
31. Bassett, D. S. *et al.* Efficient physical embedding of topologically complex information processing networks in brains and computer circuits. *PLoS Comput Biol* **6,** e1000748 (2010).
32. Jarrell, T. A. *et al.* The connectome of a decision-making neural network. *Science* **337,** 437–444 (2012).
33. Watts, D. J. & Strogatz, S. H. Collective dynamics of small-world networks. *Nature* **393,** 440–442 (1998).
34. Barabasi, A. L. & Albert, R. Emergence of scaling in random networks. *Science* **286,** 509–512 (1999).
35. Milo, R. Network motifs: simple building blocks of complex networks. *Science* **298,** 824–827 (2002).
36. Sporns, O. & Kotter, R. Motifs in brain networks. *PLoS Biol* **2,** 1910–1918 (2004).
37. Towlson, E. K., Vértes, P. E., Ahnert, S. E., Schafer, W. R. & Bullmore, E. T. The rich club of the C. elegans neuronal connectome. *J. Neurosci.* **33,** 6380–6387 (2013).
This study demonstrates the presence of a rich-club in the C. elegans connectome, and shows that this network core is formed early during development.
38. Colizza, V., Flammini, A., Serrano, M. A. & Vespignani, A. Detecting rich-club ordering in complex networks. *Nat. Phys.* **2,** 110–115 (2006).
39. Li, W., Feng, Z., Sternberg, P. W. & Xu, X. S. A C. elegans stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. *Nature* **440,** 684–687 (2006).

40. Tsalik, E. L. & Hobert, O. Functional mapping of neurons that control locomotory behavior in Caenorhabditis elegans. *J. Neurobiol.* **56,** 178–197 (2003).
41. Zhen, M. & Samuel, A. D. C. elegans locomotion: small circuits, complex functions. *Curr. Opin. Neurobiol.* **33,** 117–126 (2015).
42. Latora, V. & Marchiori, M. Efficient behaviour of small-world networks. *Phys Rev Lett* **87,** 198701 (2001).
43. Kaiser, M. & Hilgetag, C. C. Nonoptimal component placement, but short processing paths, due to long-distance projections in neural systems. *PLoS Comput Biol* **2,** e95 (2006).
44. Chen, B. L., Hall, D. H. & Chklovskii, D. B. Wiring optimization can relate neuronal structure and function. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 4723–4728 (2006).
This study demonstrates that wiring minimization principles can be used to predict the placement of many neurons in the C. elegans connectome.
45. Pérez-Escudero, A. & de Polavieja, G. G. Optimally wired subnetwork determines neuroanatomy of Caenorhabditis elegans. *Proc. Natl. Acad. Sci.* **104,** 17180–17185 (2007).
46. Laurent, P. *et al.* Decoding a neural circuit controlling global animal state in C. elegans. *eLife* **4,** e04241 (2015).
47. Macosko, E. Z. *et al.* A hub-and-spoke circuit drives pheromone attraction and social behaviour in C. elegans. *Nature* **458,** 1171–1175 (2009).
48. Kawano, T. *et al.* An imbalancing act: gap junctions reduce the backward motor circuit activity to bias C. elegans for forward locomotion. *Neuron* **72,** 572–586 (2011).
49. Roberts, W. M. *et al.* A stochastic neuronal model predicts random search behaviors at multiple spatial scales in C. elegans. *eLife* **5,** e12572 (2016).
50. Hobert, O. A map of terminal regulators of neuronal identity in Caenorhabditis elegans. *Wiley Interdiscip. Rev. Dev. Biol.* (2016).
51. Briggman, K. L. & Kristan Jr, W. B. Multifunctional pattern-generating circuits. *Annu Rev Neurosci* **31,** 271–294 (2008).
52. Trojanowski, N. F., Padovan-Merhar, O., Raizen, D. M. & Fang-Yen, C. Neural and genetic degeneracy underlies Caenorhabditis elegans feeding behavior. *J. Neurophysiol.* **112,** 951–961 (2014).
53. Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J. N. The embryonic cell lineage of the nematode Caenorhabditis elegans. *Dev. Biol.* **100,** 64–119 (1983).
54. Kato, S. *et al.* Global brain dynamics embed the motor command sequence of Caenorhabditis elegans. *Cell* **163,** 656–669 (2015).
55. Avena-Koenigsberger, A., Goñi, J., Solé, R. & Sporns, O. Network morphospace. *J. R. Soc. Interface* **12,** 20140881 (2015).
56. Bumbarger, D. J., Riebesell, M., Rödelsperger, C. & Sommer, R. J. System-wide rewiring underlies behavioral differences in predatory and bacterial-feeding nematodes. *Cell* **152,** 109–119 (2013).
57. Rein, K., Zöckler, M., Mader, M. T., Grübel, C. & Heisenberg, M. The Drosophila Standard Brain. *Curr. Biol.* **12,** 227–231 (2002).
58. Ito, K. *et al.* A systematic nomenclature for the insect brain. *Neuron* **81,** 755–765 (2014).
59. Chiang, A.-S. *et al.* Three-dimensional reconstruction of brain-wide wiring networks in Drosophila at single-cell resolution. *Curr. Biol.* **21,** 1–11 (2011).
60. Ito, M., Masuda, N., Shinomiya, K., Endo, K. & Ito, K. Systematic analysis of neural projections reveals clonal composition of the Drosophila brain. *Curr. Biol.* **23,** 644–655 (2013).
61. Shih, C.-T. *et al.* Connectomics-based analysis of information flow in the Drosophila

brain. *Curr. Biol.* **25,** 1249–1258 (2015).
62. Meinertzhagen, I. A. & Lee, C.-H. in *Advances in Genetics* (ed. Theodore Friedmann, J. D. and S. G.) **80,** 99–151 (Academic Press, 2012).
63. Rivera-Alba, M. *et al.* Wiring economy and volume exclusion determine neuronal placement in the Drosophila brain. *Curr. Biol.* **21,** 2000–2005 (2011).
64. Takemura, S. *et al.* Synaptic circuits and their variations within different columns in the visual system of Drosophila. *Proc. Natl. Acad. Sci. U. S. A.* **112,** 13711–13716 (2015).
65. Chklovskii, D. B. & Koulakov, A. A. Maps in the brain: what can we learn from them? *Annu Rev Neurosci* **27,** 369–392 (2004).
66. Serbe, E., Meier, M., Leonhardt, A. & Borst, A. Comprehensive characterization of the major presynaptic elements to the Drosophila OFF motion detector. *Neuron* **89,** 829–841 (2016).
67. Jeanne, J. M. & Wilson, R. I. Convergence, divergence, and reconvergence in a feedforward network improves neural speed and accuracy. *Neuron* **88,** 1014–1026 (2015).
68. Ohyama, T. *et al.* A multilevel multimodal circuit enhances action selection in Drosophila. *Nature* **520,** 633–639 (2015).
69. Euler, T., Haverkamp, S., Schubert, T. & Baden, T. Retinal bipolar cells: elementary building blocks of vision. *Nat. Rev. Neurosci.* **15,** 507–519 (2014).
70. Vogelstein, J. T. *et al.* Discovery of brainwide neural-behavioral maps via multiscale unsupervised structure learning. *Science* **344,** 386–392 (2014).
71. Bhatla, N., Droste, R., Sando, S. R., Huang, A. & Horvitz, H. R. Distinct neural circuits control rhythm inhibition and spitting by the myogenic pharynx of C. elegans. *Curr. Biol.* **25,** 2075–2089 (2015).
72. Osten, P. & Margrie, T. W. Mapping brain circuitry with a light microscope. *Nat. Methods* **10,** 515–523 (2013).
73. Oh, S. W. *et al.* A mesoscale connectome of the mouse brain. *Nature* **508,** 207–214 (2014).
74. Rubinov, M., Ypma, R. J., Watson, C. & Bullmore, E. T. Wiring cost and topological participation of the mouse brain connectome. *Proc. Natl. Acad. Sci.* **112,** 10032–10037 (2015).
75. Peters, A. & Feldman, M. L. The projection of the lateral geniculate nucleus to area 17 of the rat cerebral cortex. I. General description. *J. Neurocytol.* **5,** 63–84 (1976).
76. Braitenberg, V. & Schüz, A. Cortex: statistics and geometry of neuronal connectivity. *Springer Berlin* (1998).
77. Kasthuri, N. *et al.* Saturated reconstruction of a volume of neocortex. *Cell* **162,** 648–661 (2015).
78. Mishchenko, Y. *et al.* Ultrastructural analysis of hippocampal neuropil from the connectomics perspective. *Neuron* **67,** 1009–1020 (2010).
79. Briggman, K. L., Helmstaedter, M. & Denk, W. Wiring specificity in the direction-selectivity circuit of the retina. *Nature* **471,** 183–188 (2011).
80. Branco, T. & Häusser, M. The single dendritic branch as a fundamental functional unit in the nervous system. *Curr. Opin. Neurobiol.* **20,** 494–502 (2010).
81. Klausberger, T. & Somogyi, P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* **321,** 53–57 (2008).
82. Boergens, K. M., Berning, M. & Helmstaedter, M. in *Dendrites* (eds. Stuart, G., Spruston, N. & Häusser, M.) 623–638 (Oxford University Press, 2016).
83. Sholl, D. A. Dendritic organization in the neurons of the visual and motor cortices of the cat. *J. Anat.* **87,** 387 (1953).

84. Cuntz, H., Forstner, F., Borst, A. & Häusser, M. One rule to grow them all: A general theory of neuronal branching and its practical application. *PLoS Comput Biol* **6,** e1000877 (2010).
85. Budd, J. M. *et al.* Neocortical axon arbors trade-off material and conduction delay conservation. *PLoS Comput Biol* **6,** e1000711 (2010).
86. Wen, Q., Stepanyants, A., Elston, G. N., Grosberg, A. Y. & Chklovskii, D. B. Maximization of the connectivity repertoire as a statistical principle governing the shapes of dendritic arbors. *Proc. Natl. Acad. Sci.* **106,** 12536–12541 (2009).
87. Wen, Q. & Chklovskii, D. B. A Cost–benefit analysis of neuronal morphology. *J. Neurophysiol.* **99,** 2320–2328 (2008).
88. Cuntz, H., Mathy, A. & Häusser, M. A scaling law derived from optimal dendritic wiring. *Proc. Natl. Acad. Sci.* **109,** 11014–11018 (2012).
This study found that key aspects in the branching structure of dendrites follow scaling laws, which can be predicted by a simple model of wiring cost minimization.
89. Lu, J., Tapia, J. C., White, O. L. & Lichtman, J. W. The interscutularis muscle connectome. *PLoS Biol* **7,** e1000032 (2009).
90. Smith, T. G., Marks, W. B., Lange, G. D., Sheriff, W. H. & Neale, E. A. A fractal analysis of cell images. *J. Neurosci. Methods* **27,** 173–180 (1989).
91. Werner, G. Fractals in the nervous system: Conceptual implications for theoretical neuroscience. *Front. Physiol.* **1,** (2010).
92. Fernández, E. & Jelinek, H. F. Use of fractal theory in neuroscience: Methods, advantages, and potential problems. *Methods* **24,** 309–321 (2001).
93. Mitchison, G. Neuronal branching patterns and the economy of cortical wiring. *Proc. R. Soc. Lond. B Biol. Sci.* **245,** 151–158 (1991).
94. Ascoli, G. A. Mobilizing the base of neuroscience data: the case of neuronal morphologies. *Nat. Rev. Neurosci.* **7,** 318–324 (2006).
95. Lefebvre, J. L., Sanes, J. R. & Kay, J. N. Development of dendritic form and function. *Annu. Rev. Cell Dev. Biol.* **31,** 741–777 (2015).
96. Stepanyants, A. & Chklovskii, D. B. Neurogeometry and potential synaptic connectivity. *Trends Neurosci.* **28,** 387–394 (2005).
97. Buzsáki, G. & Mizuseki, K. The log-dynamic brain: how skewed distributions affect network operations. *Nat. Rev. Neurosci.* **15,** 264–278 (2014).
Comprehensive review discussing a wide range of studies that reported heavy-tailed distributions in key physiological parameters of nervous systems.
98. Holmgren, C., Harkany, T., Svennenfors, B. & Zilberter, Y. Pyramidal cell communication within local networks in layer 2/3 of rat neocortex. *J. Physiol.* **551,** 139–153 (2003).
99. Lefort, S., Tomm, C., Sarria, J.-C. F. & Petersen, C. C. The excitatory neuronal network of the C2 barrel column in mouse primary somatosensory cortex. *Neuron* **61,** 301–316 (2009).
100. Perin, R., Berger, T. K. & Markram, H. A synaptic organizing principle for cortical neuronal groups. *Proc. Natl. Acad. Sci.* **108,** 5419–5424 (2011).
101. Ko, H. *et al.* Functional specificity of local synaptic connections in neocortical networks. *Nature* **473,** 87–91 (2011).
102. Mizuseki, K. & Buzsáki, G. Preconfigured, skewed distribution of firing rates in the hippocampus and entorhinal cortex. *Cell Rep.* **4,** 1010–1021 (2013).
103. Pajevic, S. & Plenz, D. The organization of strong links in complex networks. *Nat. Phys.* **8,** 429–436 (2012).

104. Yassin, L. *et al.* An embedded subnetwork of highly active neurons in the neocortex. *Neuron* **68,** 1043–1050 (2010).
105. Cossell, L. *et al.* Functional organization of excitatory synaptic strength in primary visual cortex. *Nature* (2015).
106. Li, Y., Ibrahim, L. A., Liu, B., Zhang, L. I. & Tao, H. W. Linear transformation of thalamocortical input by intracortical excitation. *Nat. Neurosci.* **16,** 1324–1330 (2013).
107. Emergence of rich-club topology and coordinated dynamics in development of hippocampal functional networks in vitro. *J. Neurosci.* **35,** 5459–5470 (2015).
108. Nigam, S. *et al.* Rich-club organization in effective connectivity among cortical neurons. *J. Neurosci.* **36,** 670–684 (2016).
109. Bonifazi, P. *et al.* GABAergic hub neurons orchestrate synchrony in developing hippocampal networks. *Science* **326,** 1419–1424 (2009).
110. Marissal, T. *et al.* Pioneer glutamatergic cells develop into a morpho-functionally distinct population in the juvenile CA3 hippocampus. *Nat. Commun.* **3,** 1316 (2012).
111. Cossart, R. Operational hub cells: a morpho-physiologically diverse class of GABAergic neurons united by a common function. *Curr. Opin. Neurobiol.* **26,** 51–56 (2014).
112. Melzer, S. *et al.* Long-range–projecting GABAergic neurons modulate inhibition in hippocampus and entorhinal cortex. *Science* **335,** 1506–1510 (2012).
113. Jinno, S. *et al.* Neuronal diversity in GABAergic long-range projections from the hippocampus. *J. Neurosci.* **27,** 8790–8804 (2007).
114. Picardo, M. A. *et al.* Pioneer GABA cells comprise a subpopulation of hub neurons in the developing hippocampus. *Neuron* **71,** 695–709 (2011).
115. Quilichini, P. P. *et al.* Hub GABA neurons mediate gamma-frequency oscillations at ictal-like event onset in the immature hippocampus. *Neuron* **74,** 57–64 (2012).
116. Ellender, T. J., Nissen, W., Colgin, L. L., Mann, E. O. & Paulsen, O. Priming of hippocampal population bursts by individual perisomatic-targeting interneurons. *J. Neurosci.* **30,** 5979–5991 (2010).
117. Buzsáki, G., Geisler, C., Henze, D. A. & Wang, X.-J. Interneuron diversity series: circuit complexity and axon wiring economy of cortical interneurons. *Trends Neurosci.* **27,** 186–193 (2004).
118. Ito, S. *et al.* Large-scale, high-resolution multielectrode-array recording depicts functional network differences of cortical and hippocampal cultures. *PLoS ONE* **9,** e105324 (2014).
119. Shimono, M. & Beggs, J. M. Functional clusters, hubs, and communities in the cortical microconnectome. *Cereb. Cortex* bhu252 (2014). doi:10.1093/cercor/bhu252
This study demonstrates interesting parallels in topological properties of functional connectivity derived from spontaneous activity and previous work that used patch-clamp recordings.
120. Druckmann, S. *et al.* Structured synaptic connectivity between hippocampal regions. *Neuron* **81,** 629–640 (2014).
121. Koulakov, A. A., Hromádka, T. & Zador, A. M. Correlated connectivity and the distribution of firing rates in the neocortex. *J. Neurosci.* **29,** 3685–3694 (2009).
122. Yu, S., Huang, D., Singer, W. & Nikolić, D. A small world of neuronal synchrony. *Cereb. Cortex* **18,** 2891–2901 (2008).
123. Gerhard, F., Pipa, G., Lima, B., Neuenschwander, S. & Gerstner, W. Extraction of network topology from multi-electrode recordings: is there a small-world effect? *Front. Comput. Neurosci.* **5,** 4–4 (2010).
124. Pajevic, S. & Plenz, D. Efficient network reconstruction from dynamical cascades

identifies small-world topology of neuronal avalanches. *PLoS Comput. Biol.* **5,** e1000271 (2009).
125. Bettencourt, L. M., Stephens, G. J., Ham, M. I. & Gross, G. W. Functional structure of cortical neuronal networks grown in vitro. *Phys Rev E Stat Nonlin Soft Matter Phys* **75,** 21915 (2007).
126. Downes, J. H. *et al.* Emergence of a small-world functional network in cultured neurons. *PLoS Comput. Biol.* **8,** e1002522 (2012).
127. Srinivas, K. V., Jain, R., Saurav, S. & Sikdar, S. K. Small-world network topology of hippocampal neuronal network is lost, in an in vivo glutamate injury model of epilepsy. *Eur J Neurosci* **25,** 3276–3286 (2007).
128. Takahashi, N., Sasaki, T., Matsumoto, W., Matsuki, N. & Ikegaya, Y. Circuit topology for synchronizing neurons in spontaneously active networks. *Proc. Natl. Acad. Sci.* **107,** 10244–10249 (2010).
129. Stetter, O., Battaglia, D., Soriano, J. & Geisel, T. Model-free reconstruction of excitatory neuronal connectivity from calcium imaging signals. *PLoS Comput. Biol.* **8,** e1002653 (2012).
130. de Santos-Sierra, D. *et al.* Emergence of small-world anatomical networks in self-organizing clustered neuronal cultures. *PLoS ONE* **9,** e85828 (2014).
131. Song, S., Sjostrom, P. J., Reigl, M., Nelson, S. & Chklovskii, D. B. Highly nonrandom features of synaptic connectivity in local cortical circuits. *PLoS Biol* **3,** e68 (2005).
132. Song, H. F., Kennedy, H. & Wang, X.-J. Spatial embedding of structural similarity in the cerebral cortex. *Proc. Natl. Acad. Sci.* **111,** 16580–16585 (2014).
133. Kleindienst, T., Winnubst, J., Roth-Alpermann, C., Bonhoeffer, T. & Lohmann, C. Activity-dependent clustering of functional synaptic inputs on developing hippocampal dendrites. *Neuron* **72,** 1012–1024 (2011).
134. Gritsun, T. A., le Feber, J. & Rutten, W. L. Growth dynamics explain the development of spatiotemporal burst activity of young cultured neuronal networks in detail. *PLoS ONE* **7,** e43352 (2012).
135. Kube, K., Herzog, A., Michaelis, B., de Lima, A. D. & Voigt, T. Spike-timing-dependent plasticity in small-world networks. *Neurocomputing* **71,** 1694–1704 (2008).
136. Kwok, H. F., Jurica, P., Raffone, A. & van Leeuwen, C. Robust emergence of small-world structure in networks of spiking neurons. *Cogn. Neurodyn.* **1,** 39–51 (2007).
137. Kim, S.-Y. & Lim, W. Effect of small-world connectivity on fast sparsely synchronized cortical rhythms. *Phys. Stat. Mech. Its Appl.* **421,** 109–123 (2015).
138. Gökçe, O., Bonhoeffer, T. & Scheuss, V. Clusters of synaptic inputs on dendrites of layer 5 pyramidal cells in mouse visual cortex. *eLife* **5,** e09222 (2016).
139. Hilgetag, C. C. & Goulas, A. Is the brain really a small-world network? *Brain Struct. Funct.* 1–6 (2015).
140. Wertz, A. *et al.* Single-cell–initiated monosynaptic tracing reveals layer-specific cortical network modules. *Science* **349,** 70–74 (2015).
141. Lee, W.-C. A. *et al.* Anatomy and function of an excitatory network in the visual cortex. *Nature* **532,** 370–374 (2016).
A recent study that reconstructed excitatory synaptic connectivity in the mouse visual cortex and demonstrated that its connectivity is modular with functionally related neurons forming preferential connectivity with each other.
142. Morgan, J. L., Berger, D. R., Wetzel, A. W. & Lichtman, J. W. The fuzzy logic of network connectivity in mouse visual thalamus. *Cell* **165,** 192–206 (2016).
143. Markram, H., Lübke, J., Frotscher, M., Roth, A. & Sakmann, B. Physiology and

anatomy of synaptic connections between thick tufted pyramidal neurones in the developing rat neocortex. *J. Physiol.* **500,** 409–440 (1997).
144. Rieubland, S., Roth, A. & Häusser, M. Structured connectivity in cerebellar inhibitory networks. *Neuron* **81,** 913–929 (2014).
145. Rothschild, G., Nelken, I. & Mizrahi, A. Functional organization and population dynamics in the mouse primary auditory cortex. *Nat. Neurosci.* **13,** 353–360 (2010).
146. Ohki, K., Chung, S., Ch'ng, Y. H., Kara, P. & Reid, R. C. Functional imaging with cellular resolution reveals precise micro-architecture in visual cortex. *Nature* **433,** 597–603 (2005).
147. Otsuka, T. & Kawaguchi, Y. Firing-pattern-dependent specificity of cortical excitatory feed-forward subnetworks. *J. Neurosci.* **28,** 11186–11195 (2008).
148. Jouhanneau, J.-S., Kremkow, J., Dorrn, A. L. & Poulet, J. F. A. In vivo monosynaptic excitatory transmission between layer 2 cortical pyramidal neurons. *Cell Rep.* **13,** 2098–2106 (2015).
149. Pala, A. & Petersen, C. C. In vivo measurement of cell-type-specific synaptic connectivity and synaptic transmission in layer 2/3 mouse barrel cortex. *Neuron* **85,** 68–75 (2015).
150. Li, L., Li, Y., Zhou, M., Tao, H. W. & Zhang, L. I. Intracortical multiplication of thalamocortical signals in mouse auditory cortex. *Nat. Neurosci.* **16,** 1179–1181 (2013).
151. Douglas, R. J., Koch, C., Mahowald, M., Martin, K. A. & Suarez, H. H. Recurrent excitation in neocortical circuits. *Science* **269,** 981–985 (1995).
152. Wang, Y. *et al.* Heterogeneity in the pyramidal network of the medial prefrontal cortex. *Nat. Neurosci.* **9,** 534–542 (2006).
153. Harris, K. D., Csicsvari, J., Hirase, H., Dragoi, G. & Buzsáki, G. Organization of cell assemblies in the hippocampus. *Nature* **424,** 552–556 (2003).
154. Miller, J. K., Ayzenshtat, I., Carrillo-Reid, L. & Yuste, R. Visual stimuli recruit intrinsically generated cortical ensembles. *Proc. Natl. Acad. Sci. U. S. A.* **111,** E4053-4061 (2014).
155. Tremblay, R., Lee, S. & Rudy, B. GABAergic interneurons in the neocortex: From cellular properties to circuits. *Neuron* **91,** 260–292 (2016).
156. Womelsdorf, T., Valiante, T. A., Sahin, N. T., Miller, K. J. & Tiesinga, P. Dynamic circuit motifs underlying rhythmic gain control, gating and integration. *Nat. Neurosci.* **17,** 1031–1039 (2014).
Comprehensive survey of how specific connectivity motifs may relate to synchronous neuronal activity and canonical neuronal computations.
157. Harris, K. D. & Mrsic-Flogel, T. D. Cortical connectivity and sensory coding. *Nature* **503,** 51–58 (2013).
158. Brunel, N. Is cortical connectivity optimized for storing information? *Nat. Neurosci.* (2016).
159. Batista-Brito, R. & Fishell, G. The developmental integration of cortical interneurons into a functional network. *Curr. Top. Dev. Biol.* **87,** 81–118 (2009).
160. Gao, P., Sultan, K. T., Zhang, X.-J. & Shi, S.-H. Lineage-dependent circuit assembly in the neocortex. *Development* **140,** 2645–2655 (2013).
161. Greig, L. C., Woodworth, M. B., Galazo, M. J., Padmanabhan, H. & Macklis, J. D. Molecular logic of neocortical projection neuron specification, development and diversity. *Nat. Rev. Neurosci.* **14,** 755–769 (2013).
162. Kolodkin, A. L. & Tessier-Lavigne, M. Mechanisms and molecules of neuronal wiring: a primer. *Cold Spring Harb. Perspect. Biol.* **3,** a001727 (2011).
163. Rakic, P. Evolution of the neocortex: a perspective from developmental biology. *Nat.*

Rev. Neurosci. **10,** 724–735 (2009).
164. Shen, K. & Scheiffele, P. Genetics and cell biology of building specific synaptic connectivity. *Annu. Rev. Neurosci.* **33,** 473 (2010).
165. Sur, M. & Rubenstein, J. L. Patterning and plasticity of the cerebral cortex. *Science* **310,** 805–810 (2005).
166. Van Ooyen, A. Using theoretical models to analyse neural development. *Nat. Rev. Neurosci.* **12,** 311–326 (2011).
167. Varier, S. & Kaiser, M. Neural development features: Spatio-temporal development of the Caenorhabditis elegans neuronal network. *PLoS Comput Biol* **7,** e1001044 (2011).
168. Yu, Y.-C. *et al.* Preferential electrical coupling regulates neocortical lineage-dependent microcircuit assembly. *Nature* **486,** 113–117 (2012).
169. Yu, Y.-C., Bultje, R. S., Wang, X. & Shi, S.-H. Specific synapses develop preferentially among sister excitatory neurons in the neocortex. *Nature* **458,** 501–504 (2009).
170. Li, Y. *et al.* Clonally related visual cortical neurons show similar stimulus feature selectivity. *Nature* **486,** 118–121 (2012).
171. Deguchi, Y., Donato, F., Galimberti, I., Cabuy, E. & Caroni, P. Temporally matched subpopulations of selectively interconnected principal neurons in the hippocampus. *Nat. Neurosci.* **14,** 495–504 (2011).
172. Xu, H.-T. *et al.* Distinct lineage-dependent structural and functional organization of the hippocampus. *Cell* **157,** 1552–1564 (2014).
173. Marín, O. & Rubenstein, J. L. R. A long, remarkable journey: Tangential migration in the telencephalon. *Nat. Rev. Neurosci.* **2,** 780–790 (2001).
174. Batista-Brito, R. & Fishell, G. in (ed. Biology, B.-C. T. in D.) **87,** 81–118 (Academic Press, 2009).
175. Brown, K. N. *et al.* Clonal production and organization of inhibitory interneurons in the neocortex. *Science* **334,** 480–486 (2011).
176. Valcanis, H. & Tan, S.-S. Layer specification of transplanted interneurons in developing mouse neocortex. *J. Neurosci.* **23,** 5113–5122 (2003).
177. López-Bendito, G. *et al.* Preferential origin and layer destination of GAD65-GFP cortical interneurons. *Cereb. Cortex* **14,** 1122–1133 (2004).
178. Ciceri, G. *et al.* Lineage-specific laminar organization of cortical GABAergic interneurons. *Nat. Neurosci.* **16,** 1199–1210 (2013).
179. Bliss, T. V. P. & Lømo, T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232,** 331–356 (1973).
180. Mulkey, R. M. & Malenka, R. C. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron* **9,** 967–975 (1992).
181. Dudek, S. M. & Bear, M. F. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proc. Natl. Acad. Sci.* **89,** 4363–4367 (1992).
182. Larson, J. & Lynch, G. Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. *Science* **232,** 985–988 (1986).
183. Rodriguez-Moreno, A. *et al.* Presynaptic self-depression at developing neocortical synapses. *Neuron* **77,** 35–42 (2013).
184. Markram, H., Lübke, J., Frotscher, M. & Sakmann, B. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275,** 213–215 (1997).
185. Bi, G. & Poo, M. Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *J. Neurosci.* **18,**

10464–10472 (1998).
186. McNaughton, B. L., Douglas, R. M. & Goddard, G. V. Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. *Brain Res.* **157,** 277–293 (1978).
187. Sjöström, P. J., Turrigiano, G. G. & Nelson, S. B. Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. *Neuron* **32,** 1149–1164 (2001).
188. Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C. & Nelson, S. B. Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* **391,** 892–896 (1998).
189. Abbott, L. F. & Nelson, S. B. Synaptic plasticity: taming the beast. *Nat. Neurosci.* **3,** 1178–1183 (2000).
190. Kullmann, D. M. & Lamsa, K. P. Long-term synaptic plasticity in hippocampal interneurons. *Nat. Rev. Neurosci.* **8,** 687–699 (2007).
191. Seol, G. H. *et al.* Neuromodulators control the polarity of spike-timing-dependent synaptic plasticity. *Neuron* **55,** 919–929 (2007).
192. Mishra, R. K., Kim, S., Guzman, S. J. & Jonas, P. Symmetric spike timing-dependent plasticity at CA3-CA3 synapses optimizes storage and recall in autoassociative networks. *Nat. Commun.* **7,** (2016).
193. Gilson, M. & Fukai, T. Stability versus neuronal specialization for STDP: Long-tail weight distributions solve the dilemma. *PLOS ONE* **6,** e25339 (2011).
194. Ko, H. *et al.* The emergence of functional microcircuits in visual cortex. *Nature* **496,** 96–100 (2013).
195. Kwok, H. F., Jurica, P., Raffone, A. & Leeuwen, C. van. Robust emergence of small-world structure in networks of spiking neurons. *Cogn. Neurodyn.* **1,** 39–51 (2006).
196. Vértes, P. E., Alexander-Bloch, A. & Bullmore, E. T. Generative models of rich clubs in Hebbian neuronal networks and large-scale human brain networks. *Phil Trans R Soc B* **369,** 20130531 (2014).
197. Butz, M., Steenbuck, I. D. & van Ooyen, A. Homeostatic structural plasticity increases the efficiency of small-world networks. *Front. Synaptic Neurosci.* **6,** (2014).
198. van den Heuvel, M. P., Bullmore, E. T. & Sporns, O. Comparative connectomics. *Trends Cogn. Sci.* **20,** 345–361 (2016).
199. Borst, A. & Helmstaedter, M. Common circuit design in fly and mammalian motion vision. *Nat. Neurosci.* **18,** 1067–1076 (2015).
200. Marcus, G., Marblestone, A. & Dean, T. The atoms of neural computation. *Science* **346,** 551–552 (2014).
201. Miller, K. D. Synaptic economics: competition and cooperation in synaptic plasticity. *Neuron* **17,** 371–374 (1996).
202. Nicosia, V., Vértes, P. E., Schafer, W. R., Latora, V. & Bullmore, E. T. Phase transition in the economically modeled growth of a cellular nervous system. *Proc. Natl. Acad. Sci.* **110,** 7880–7885 (2013).
203. Schwabe, T., Borycz, J. A., Meinertzhagen, I. A. & Clandinin, T. R. Differential adhesion determines the organization of synaptic fascicles in the Drosophila visual system. *Curr. Biol.* **24,** 1304–1313 (2014).
204. Kaschula, R. & Salecker, I. Neural circuit assembly: Economically wired by a single cadherin. *Curr. Biol.* **24,** R555–R557 (2014).
205. Li, Y. *et al.* Clonally related visual cortical neurons show similar stimulus feature selectivity. *Nature* **486,** 118–121 (2012).
206. Olshausen, B. A. & Field, D. J. Sparse coding of sensory inputs. *Curr. Opin. Neurobiol.* **14,** 481–487 (2004).
207. Fornito, A., Zalesky, A. & Bullmore, E. *Fundamentals of brain network analysis*.

(Academic Press, 2016).
208. Sporns, O. & Betzel, R. F. Modular brain networks. *Annu. Rev. Psychol.* **67,** 613–640 (2016).
209. Simon, H. A. in *Facets of Systems Science* 457–476 (Springer US, 1991).
210. Thomson, A. M., West, D. C., Wang, Y. & Bannister, A. P. Synaptic connections and small circuits involving excitatory and inhibitory neurons in layers 2–5 of adult rat and cat neocortex: triple intracellular recordings and biocytin labelling in vitro. *Cereb. Cortex* **12,** 936–953 (2002).
211. Thomson, A. M. & Lamy, C. Functional maps of neocortical local circuitry. *Front. Neurosci.* **1,** 19 (2007).
212. Watts, J. & Thomson, A. M. Excitatory and inhibitory connections show selectivity in the neocortex. *J. Physiol.* **562,** 89–97 (2005).
213. Stepanyants, A., Martinez, L. M., Ferecskó, A. S. & Kisvárday, Z. F. The fractions of short-and long-range connections in the visual cortex. *Proc. Natl. Acad. Sci.* **106,** 3555–3560 (2009).
214. Jouhanneau, J.-S., Kremkow, J., Dorrn, A. L. & Poulet, J. F. In vivo monosynaptic excitatory transmission between layer 2 cortical pyramidal neurons. *Cell Rep.* **13**, 2098-2106 (2015).
215. Harris, K. D. & Shepherd, G. M. The neocortical circuit: themes and variations. *Nat. Neurosci.* **18,** 170–181 (2015).
216. Yoshimura, Y. & Callaway, E. M. Fine-scale specificity of cortical networks depends on inhibitory cell type and connectivity. *Nat. Neurosci.* **8,** 1552–1559 (2005).
217. Rudy, B., Fishell, G., Lee, S. & Hjerling-Leffler, J. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Dev. Neurobiol.* **71,** 45–61 (2011).
218. Kepecs, A. & Fishell, G. Interneuron cell types are fit to function. *Nature* **505,** 318–326 (2014).
219. Pfeffer, C. K., Xue, M., He, M., Huang, Z. J. & Scanziani, M. Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. *Nat. Neurosci.* **16,** 1068–1076 (2013).
220. Karnani, M. M. *et al.* Cooperative subnetworks of molecularly similar interneurons in mouse neocortex. *Neuron* **90**, 86-100 (2016).
221. Avermann, M., Tomm, C., Mateo, C., Gerstner, W. & Petersen, C. C. Microcircuits of excitatory and inhibitory neurons in layer 2/3 of mouse barrel cortex. *J. Neurophysiol.* **107,** 3116–3134 (2012).
222. Ma, Y., Hu, H. & Agmon, A. Short-term plasticity of unitary inhibitory-to-inhibitory synapses depends on the presynaptic interneuron subtype. *J. Neurosci.* **32,** 983–988 (2012).
223. Lee, S., Kruglikov, I., Huang, Z. J., Fishell, G. & Rudy, B. A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nat. Neurosci.* **16,** 1662–1670 (2013).
224. Pi, H.-J. *et al.* Cortical interneurons that specialize in disinhibitory control. *Nature* **503,** 521–524 (2013).
225. Hangya, B., Pi, H.-J., Kvitsiani, D., Ranade, S. P. & Kepecs, A. From circuit motifs to computations: mapping the behavioral repertoire of cortical interneurons. *Curr. Opin. Neurobiol.* **26,** 117–124 (2014).
226. Hofer, S. B. *et al.* Differential connectivity and response dynamics of excitatory and inhibitory neurons in visual cortex. *Nat. Neurosci.* **14,** 1045–1052 (2011).
227. Kapfer, C., Glickfeld, L. L., Atallah, B. V. & Scanziani, M. Supralinear increase of recurrent inhibition during sparse activity in the somatosensory cortex. *Nat. Neurosci.* **10,** 743–753 (2007).

228. Fujisawa, S., Amarasingham, A., Harrison, M. T. & Buzsáki, G. Behavior-dependent short-term assembly dynamics in the medial prefrontal cortex. *Nat. Neurosci.* **11,** 823–833 (2008).
229. Gonçalves, J. T. *et al.* In vivo imaging of dendritic pruning in dentate granule cells. *Nat. Neurosci.* **19,** 788–791 (2016).
230. Lancaster, M. A. *et al.* Cerebral organoids model human brain development and microcephaly. *Nature* **501,** 373–379 (2013).
231. Müller, J. *et al.* High-resolution CMOS MEA platform to study neurons at subcellular, cellular, and network levels. *Lab Chip* **15,** 2767–2780 (2015).
232. Koene, R. A. *et al.* NETMORPH: A framework for the stochastic generation of large scale neuronal networks with realistic neuron morphologies. *Neuroinformatics* **7,** 195–210 (2009).
233. Schreiber, T. Measuring information transfer. *Phys. Rev. Lett.* **85,** 461–464 (2000).

Biographies

Manuel Schröter is a postdoctoral fellow at the Behavioural and Clinical Neuroscience Institute (BCNI), University Cambridge, UK. During his Ph.D. studies at the University of Cambridge, and his M.Sc. in Neurosciences at the Ludwig-Maximilian University Munich, he has applied graph theoretical analysis to study neuronal network properties both at the macro- (whole-brain fMRI) and micro-scale (neuronal cultures). His current research focuses in particular on the connectivity analysis of developing neuronal network in vitro. He is recipient of a Medical Research Council (MRC) postdoctoral fellowship.

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Ed Bullmore is a Professor of Psychiatry and Head of the Department of Psychiatry, and Clinical Director of the Behavioural and Clinical Neurosciences Institute, in the University of Cambridge. Since 2005, he has also worked half-time for GlaxoSmithKline, currently focusing on immuno-psychiatry. He trained in medicine and psychiatry before completing a PhD in statistical analysis of MRI data in 1997. He has published about 500 papers including several graph theoretical studies of brain connectivity and networks and his work has been highly cited.

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Key points

- Micro-connectomics provides a promising approach to studying the wiring rules of neuronal network organization at the cellular scale and eventually to develop models of neuronal function.
- Analysis of fully reconstructed nervous systems demonstrates that micro-connectomes are often governed by wiring economy principles, such as to conserve space, time and material.
- Pioneering work from patch-clamp recordings and electron microscopic reconstruction in the mammalian brain indicates that generic motifs in neuronal network organization translate across scales and species.
- Understanding of the specific functional implications of neuronal topology will require a systematic integration of connectomes with behavioural data, functional imaging, and insights into development of cells and connectivity.

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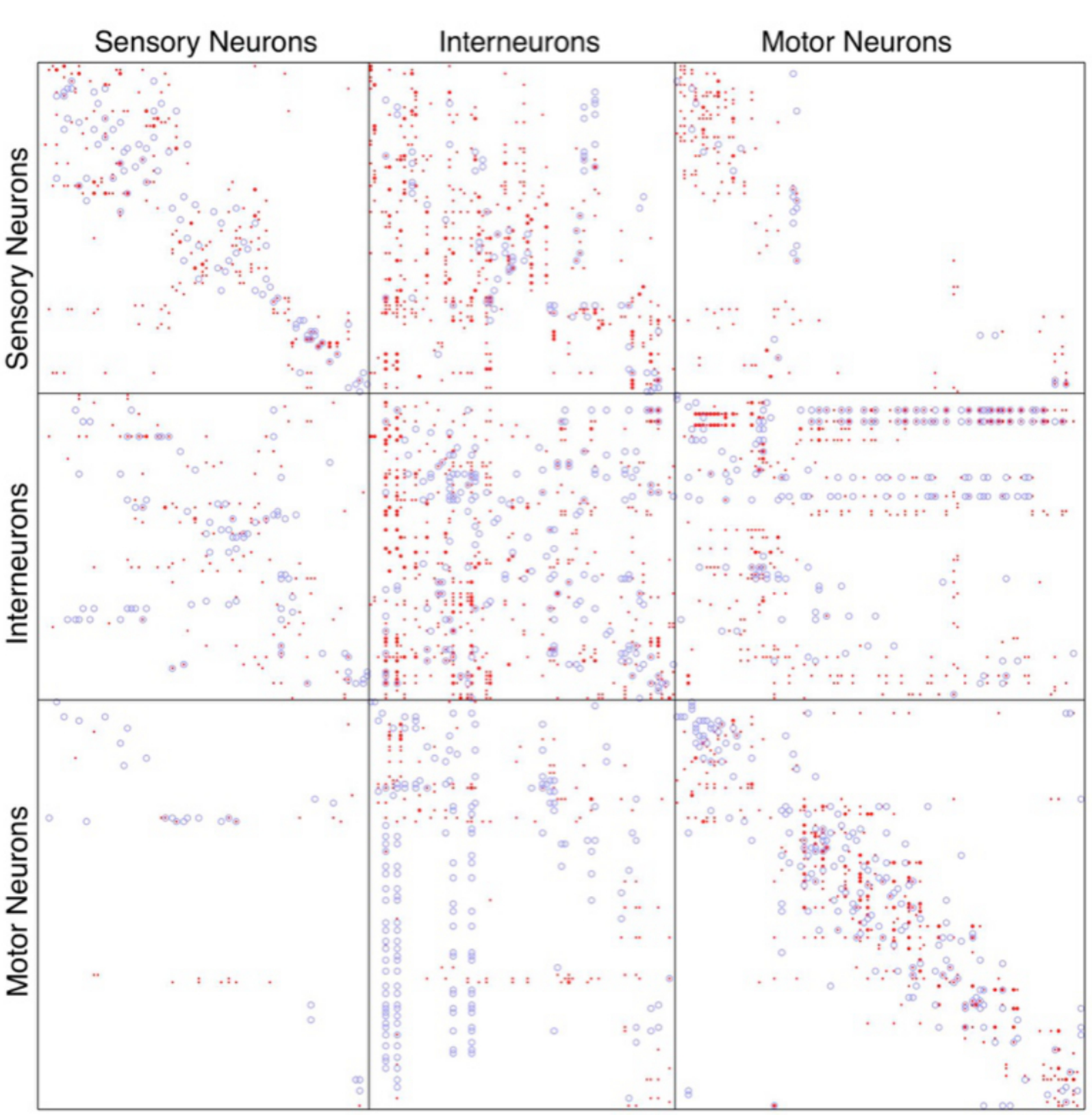
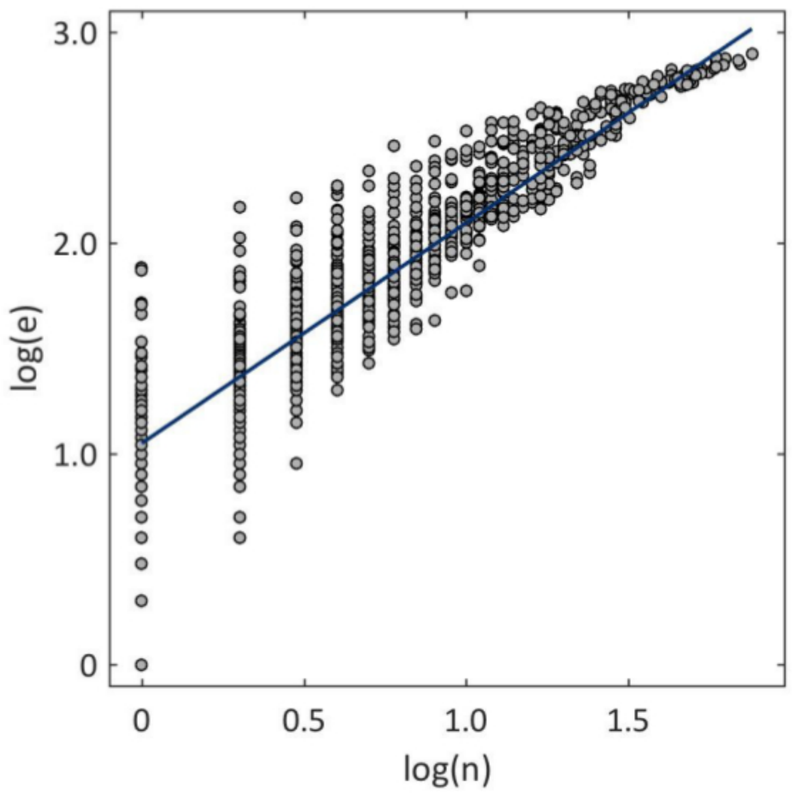
ETB is employed half-time by the University of Cambridge and half-time by GlaxoSmithKline (GSK); he holds stock in GSK.

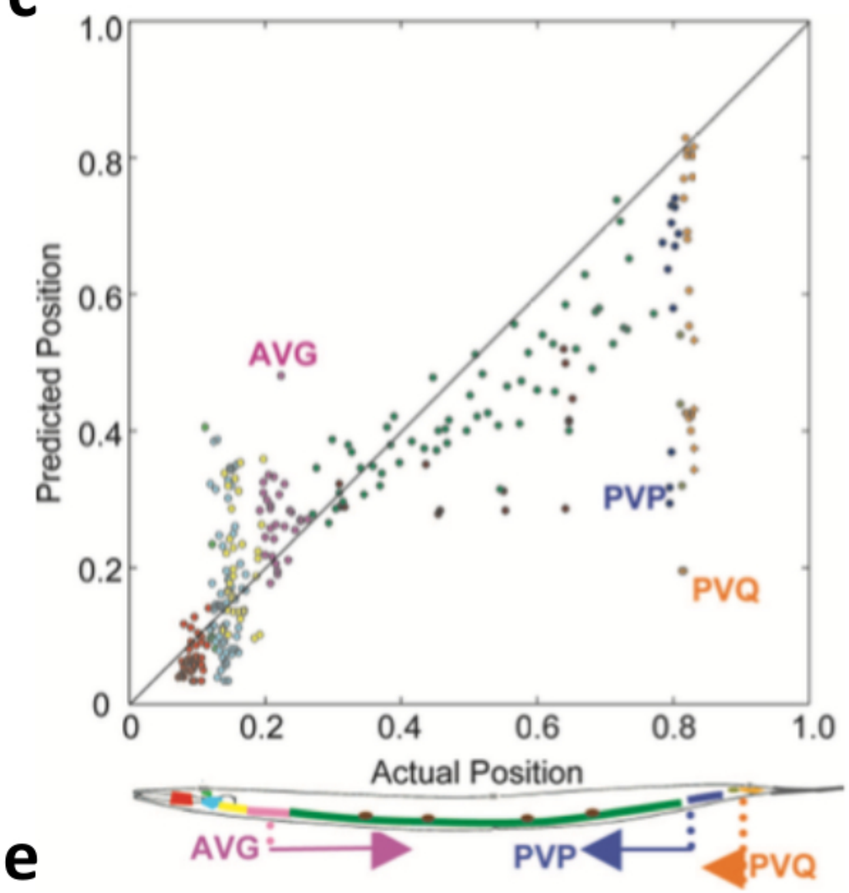
a


Sensory Neurons
Interneurons
Motor Neurons
Sensory Neurons
Interneurons
Motor Neurons

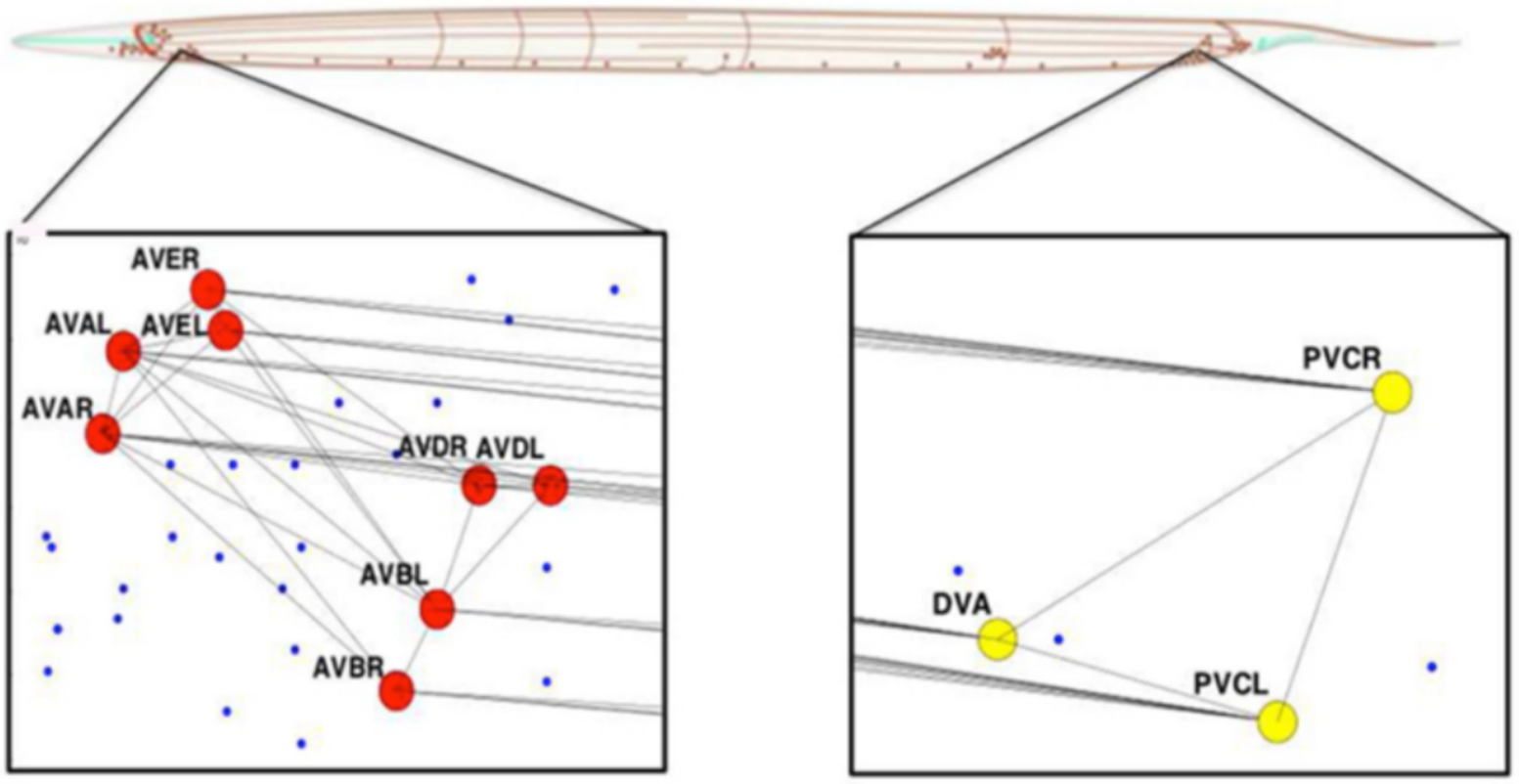
b


log(e)
log(n)
0
1.0
2.0
3.0
0.5
1.5

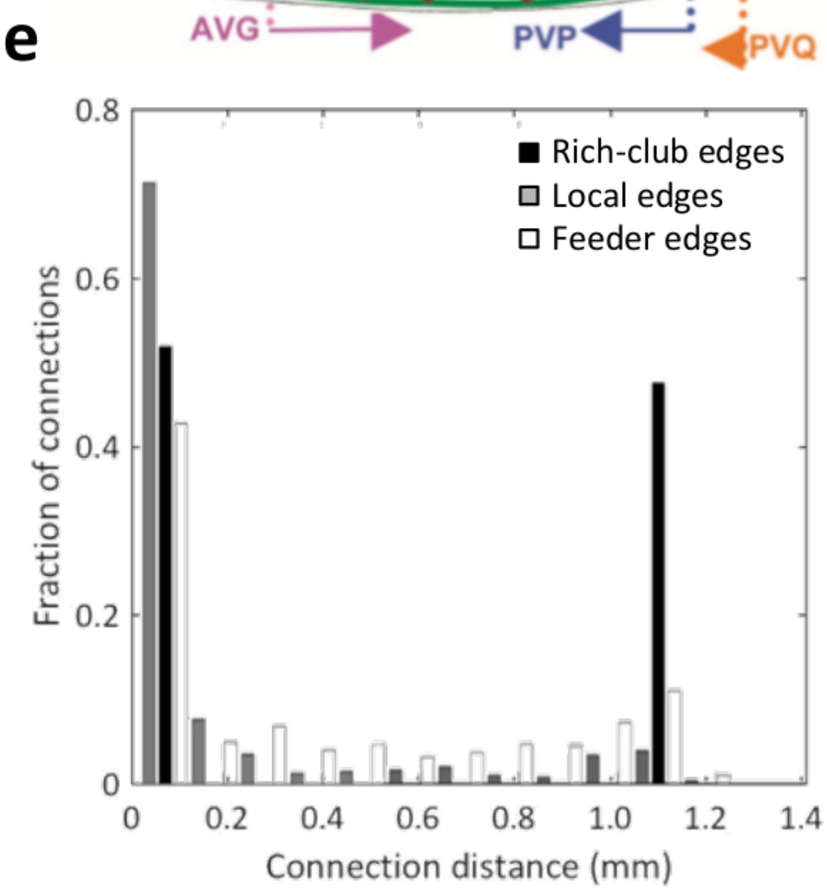
c


Predicted Position
Actual Position
AVG
PVP
PVQ
0
0.2
0.4
0.6
0.8
1.0

d

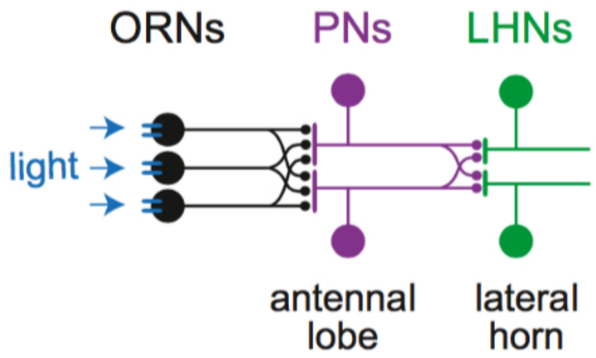

AVER
AVAL
AVEL
AVAR
AVDR AVDL
AVBL
AVBR
PVCR
DVA
PVCL

e


Rich-club edges
Local edges
Feeder edges
Fraction of connections
Connection distance (mm)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4

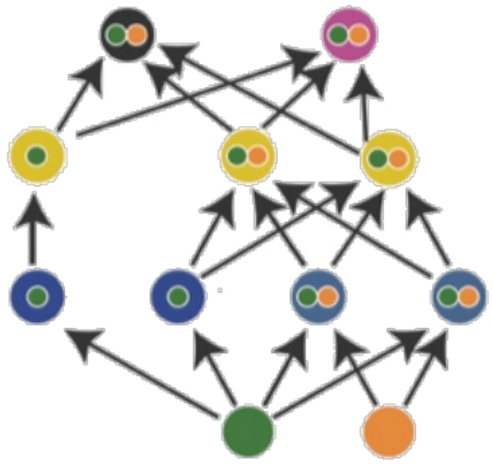
a

i.



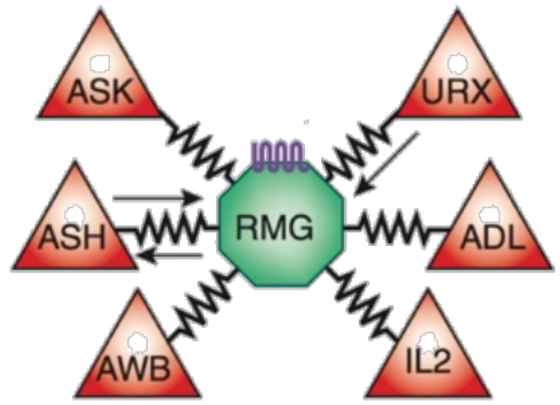
Drosophila

ii.



Drosophila larva

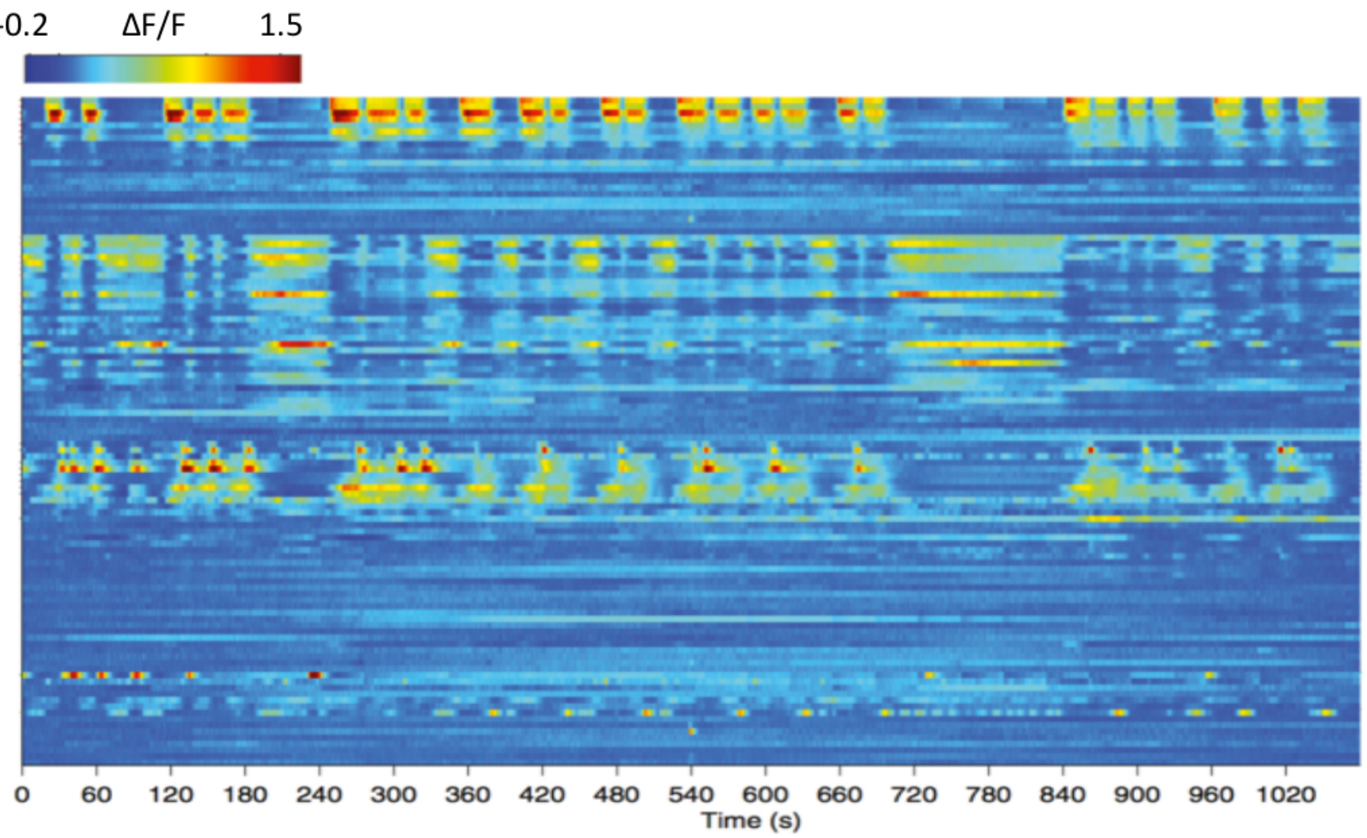
iii.



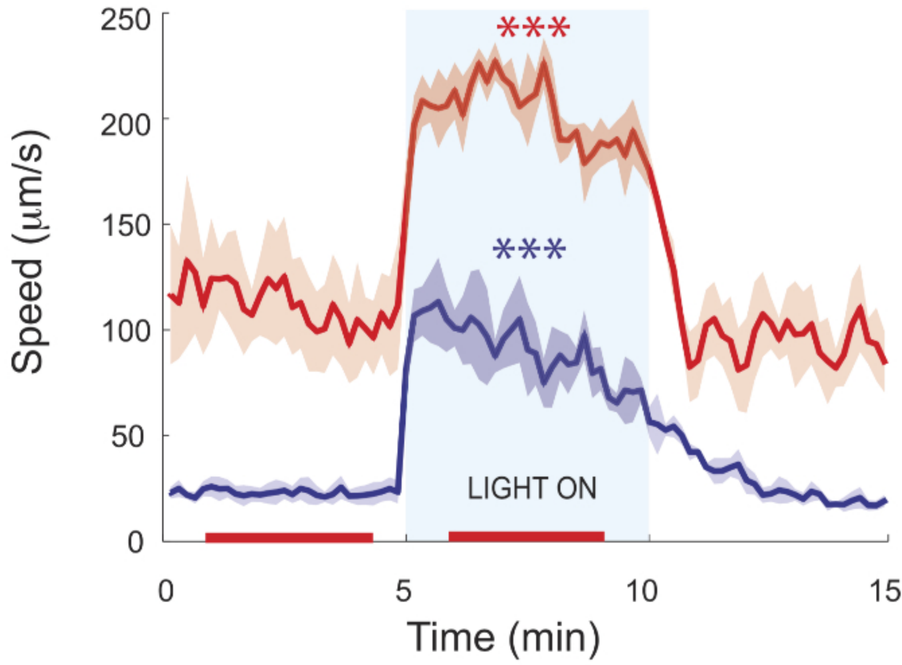
C. elegans

b

i.



c



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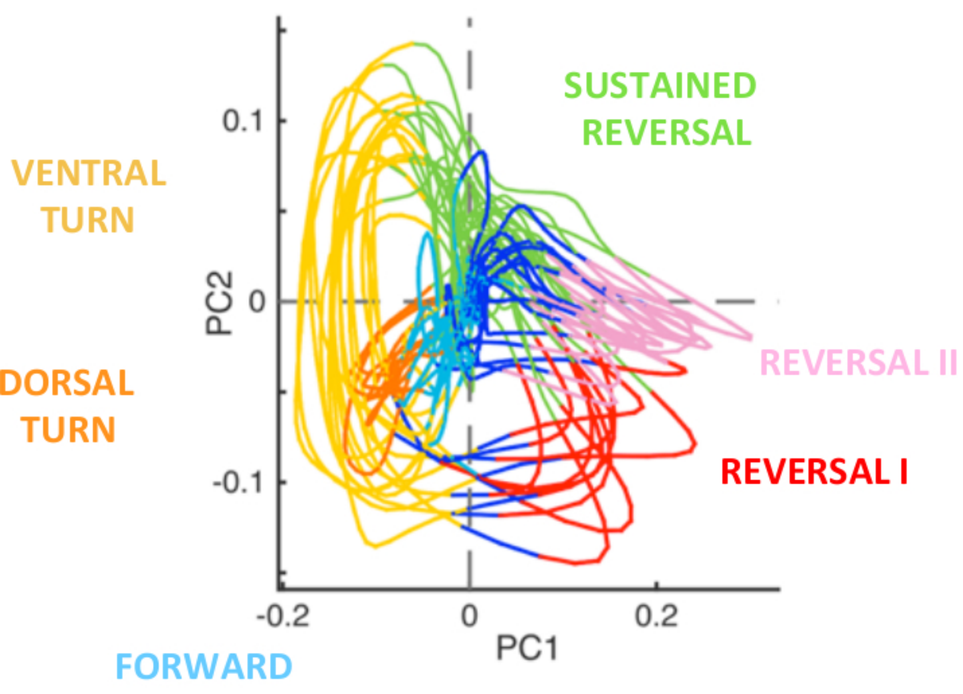

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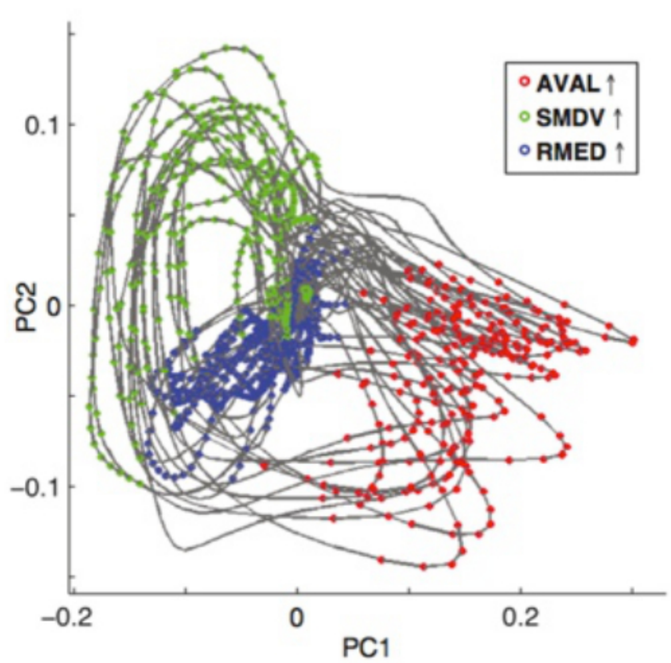
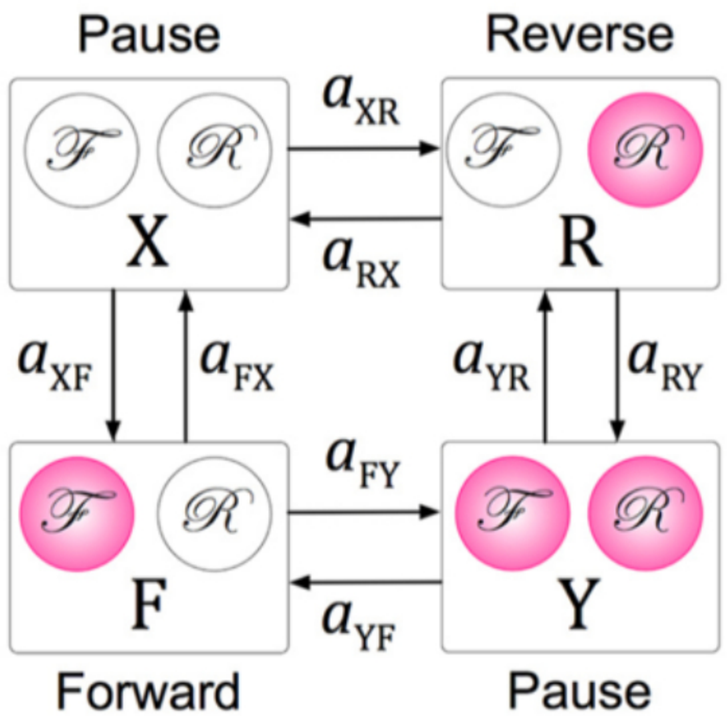


d



a

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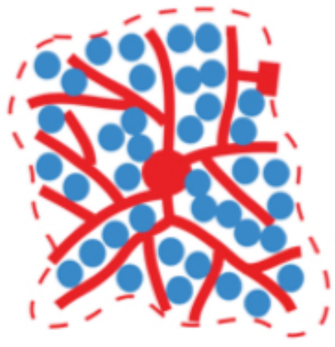
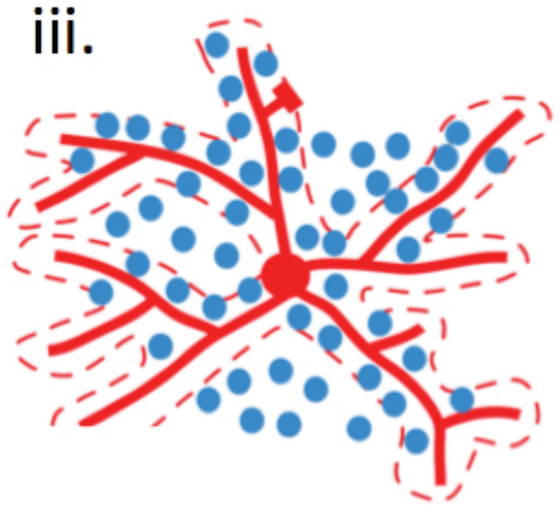


ii.

iii.



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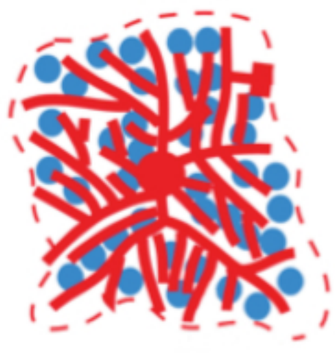
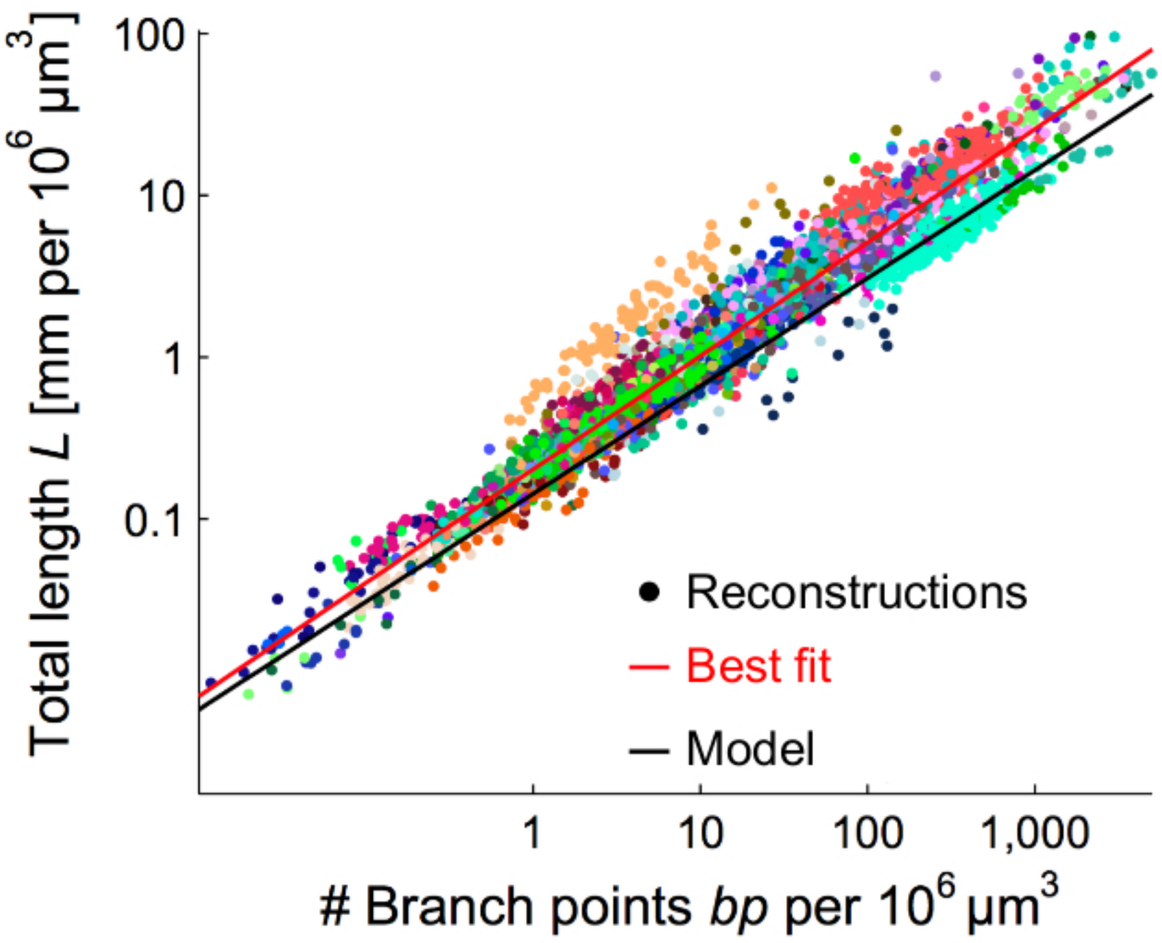


b



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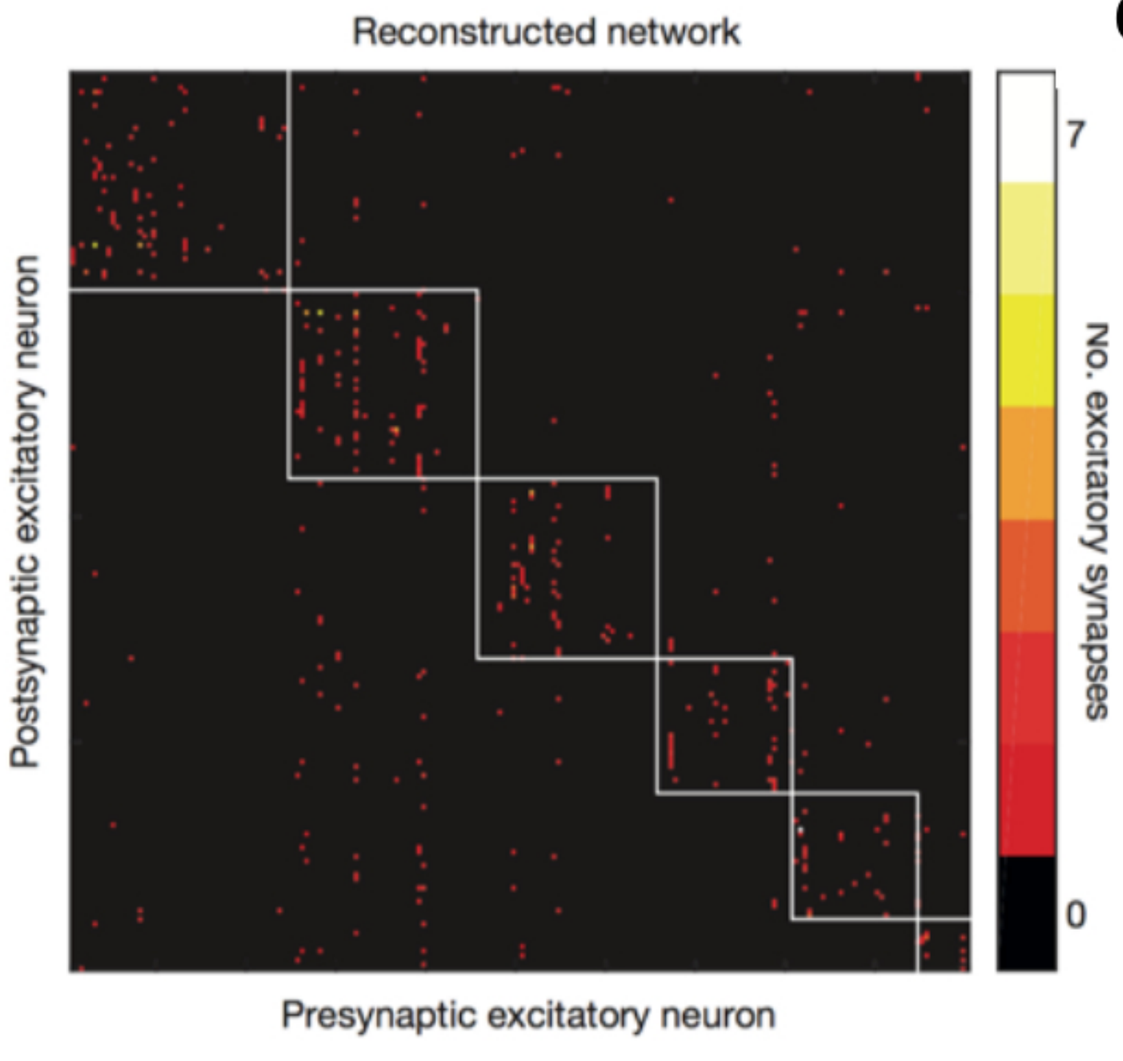

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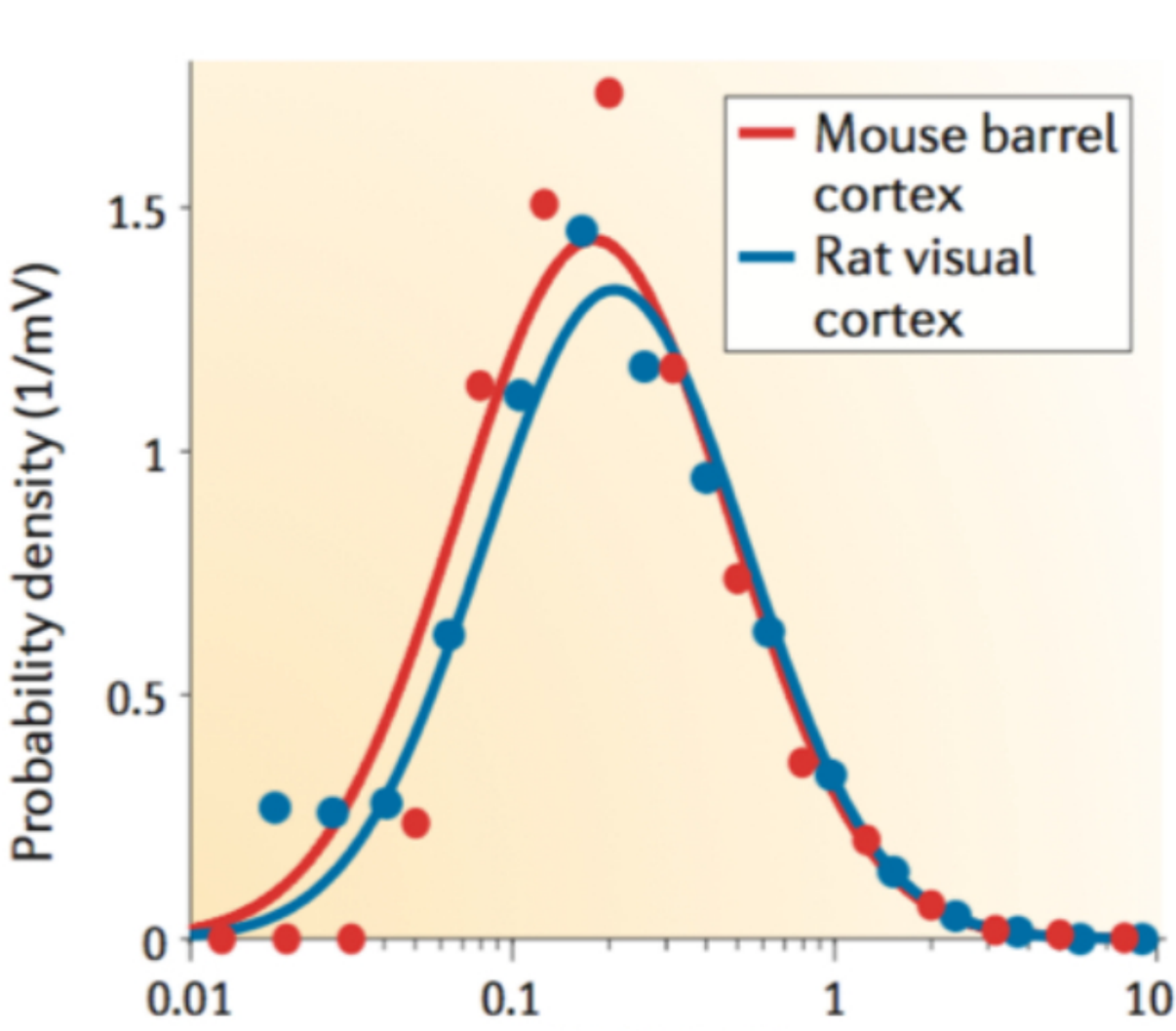
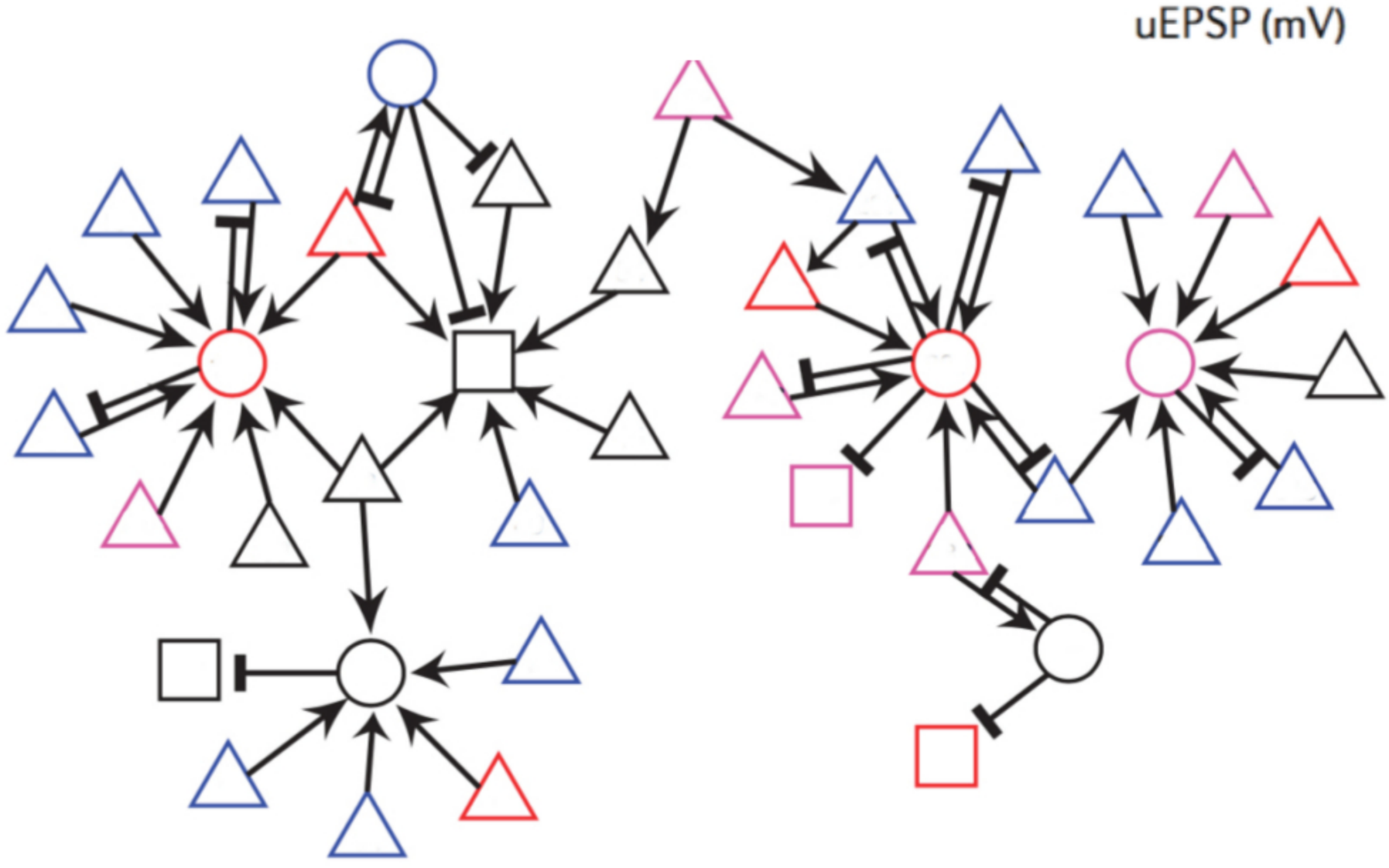
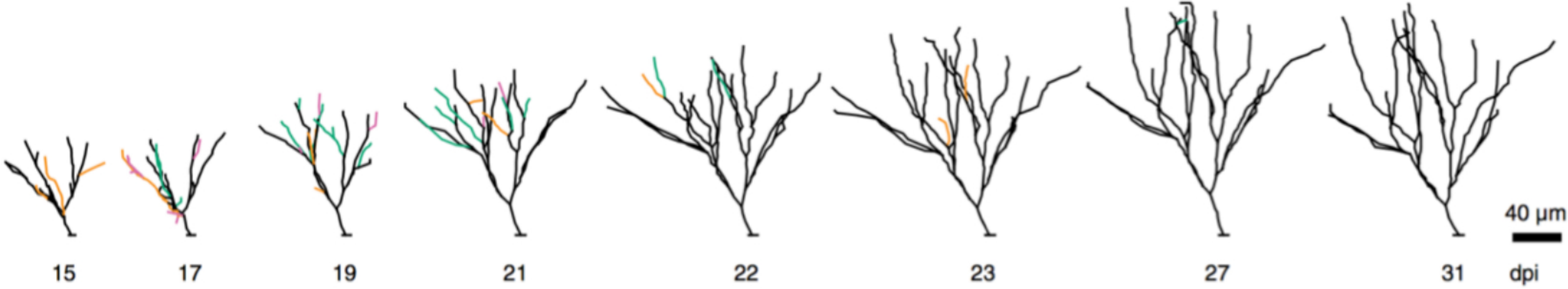


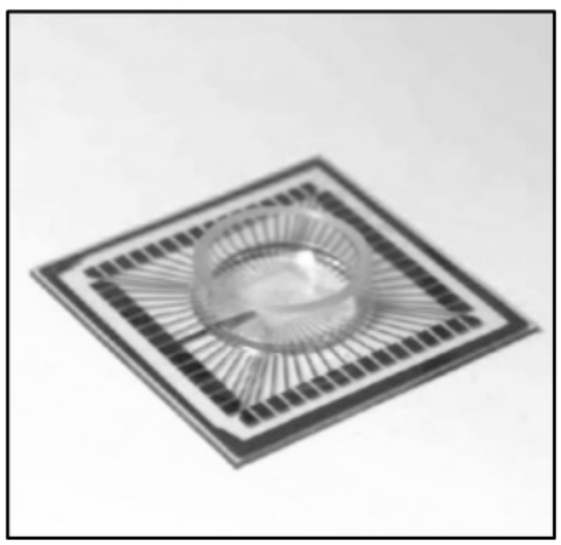
e

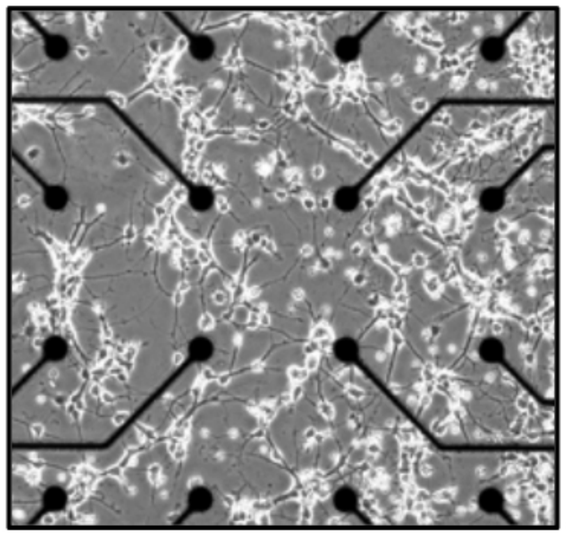


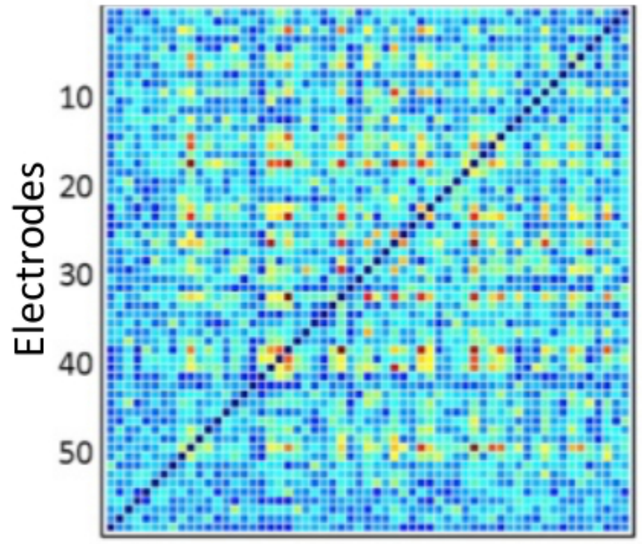
a

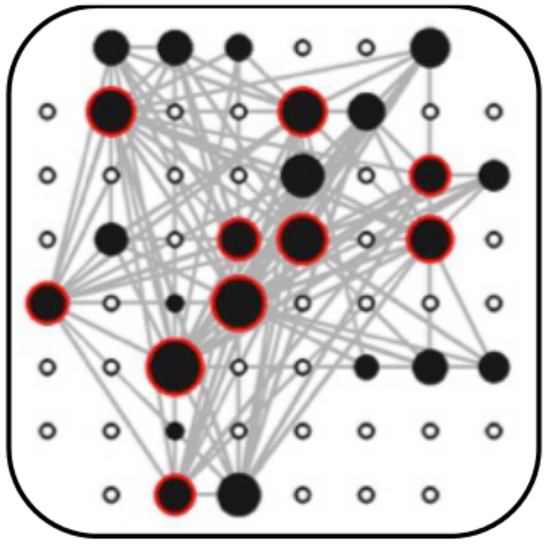

40 µm
15
17
19
21
22
23
27
31
dpi

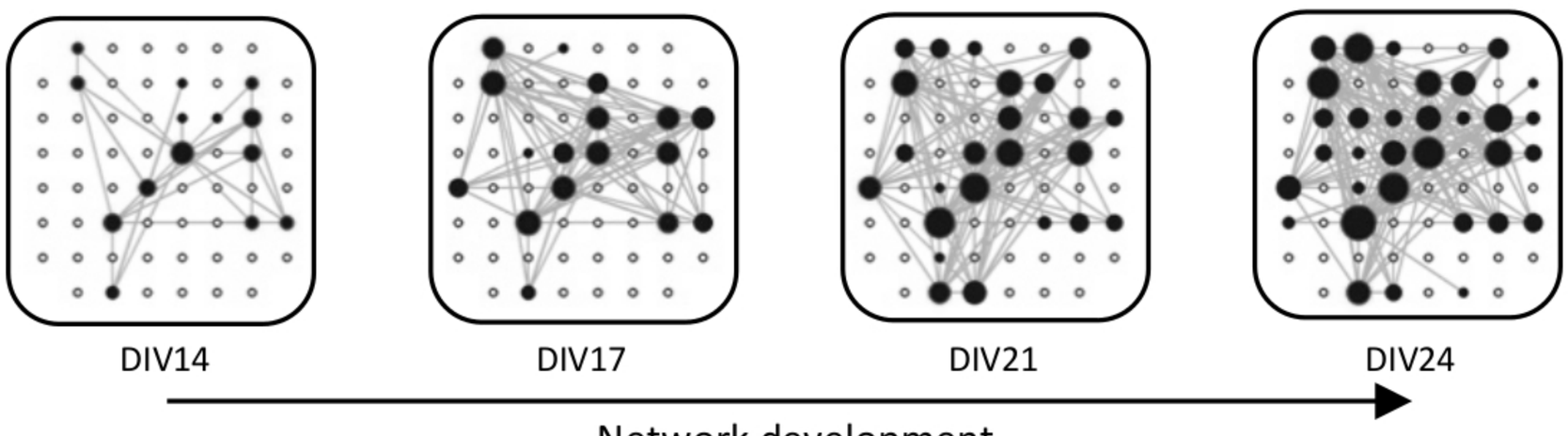
b


Multi-electrode array

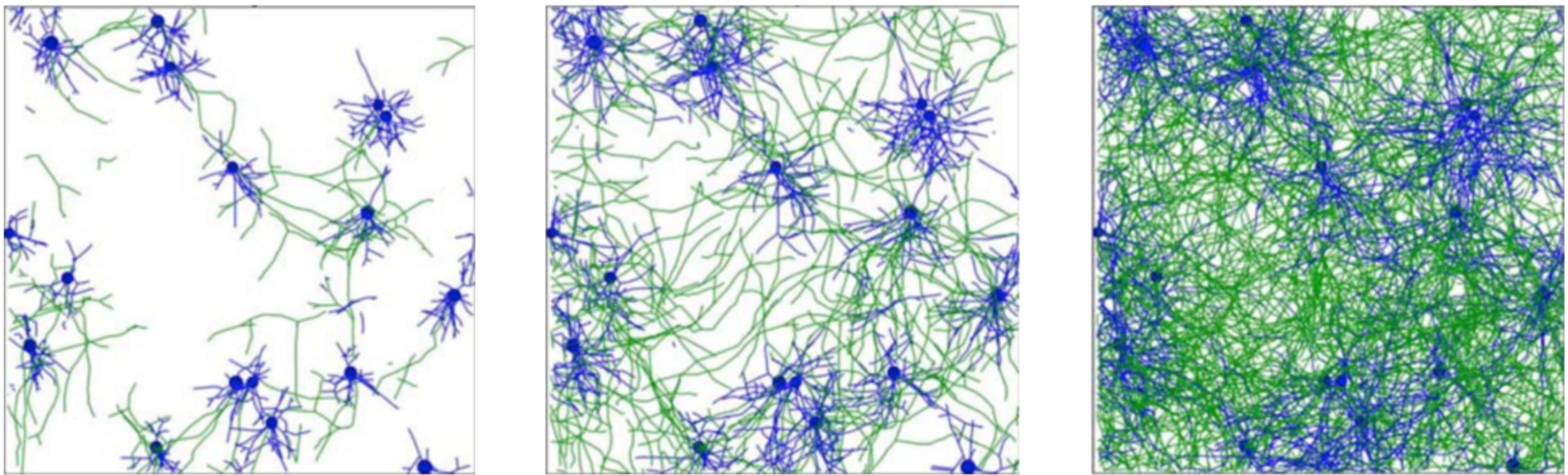

Hippocampal cells *in vitro*


Association matrix
Electrodes
10
20
30
40
50

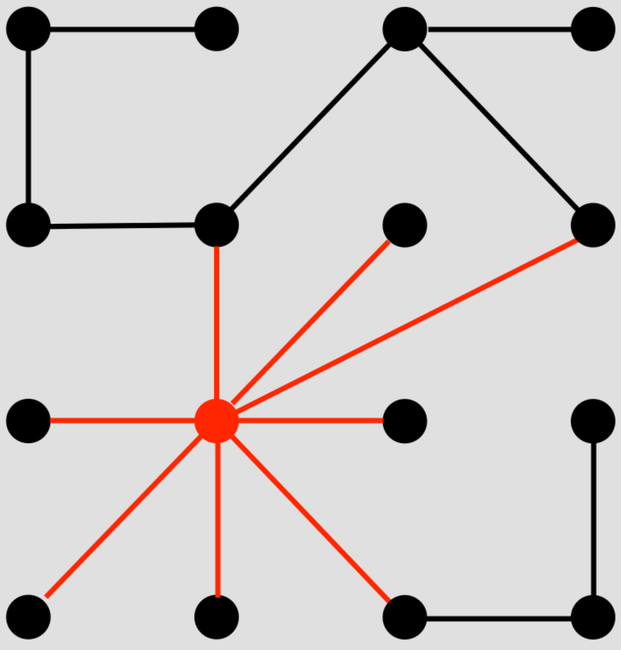

Graph analysis


DIV14
DIV17
DIV21
DIV24
Network development

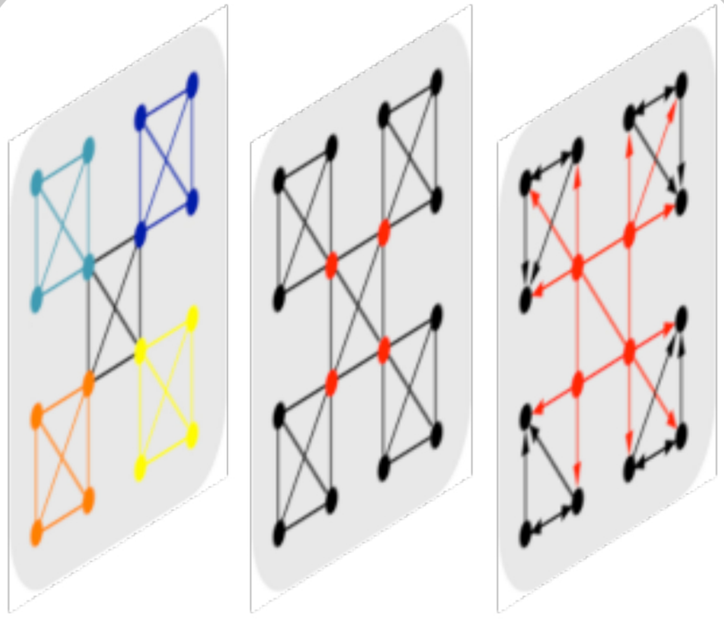
c



Hub nodes



Multi-layer analysis



Node

Edge (undirected)

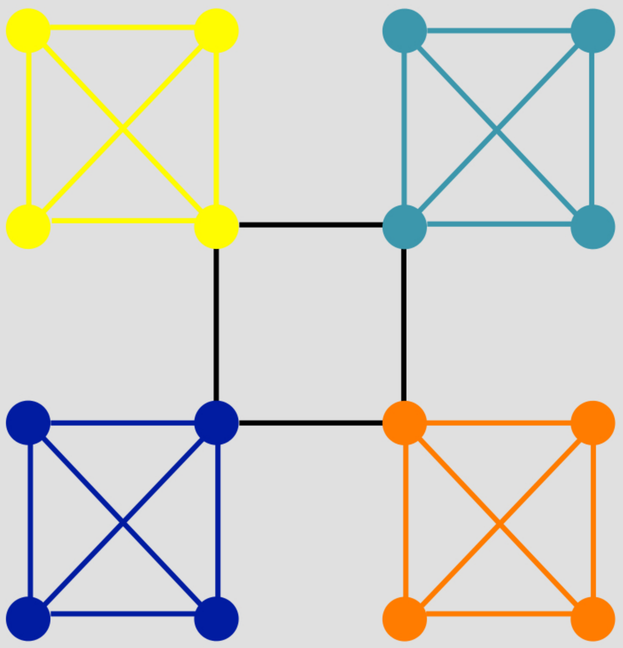
Excitatory effect

Inhibitory effect

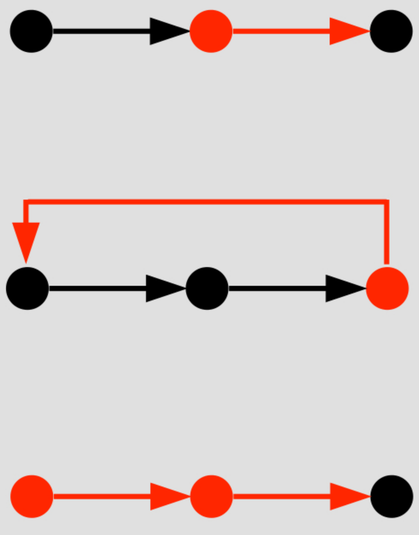
Modules

Network layer

Modularity



Circuit motifs

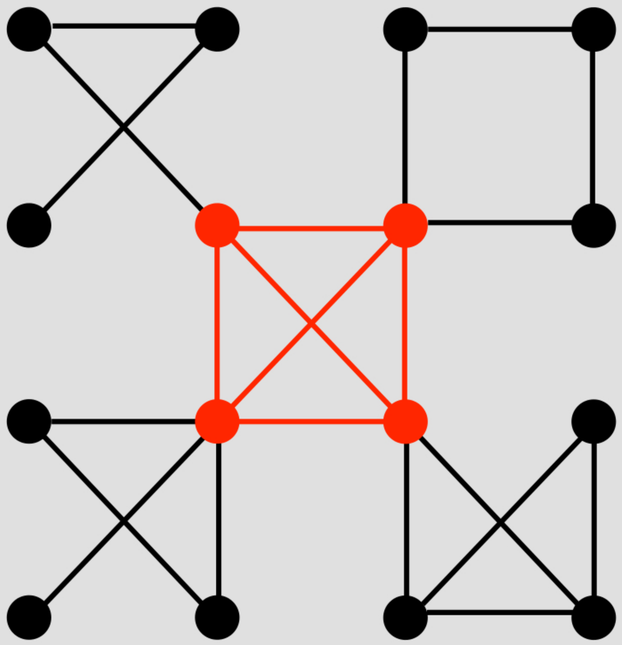


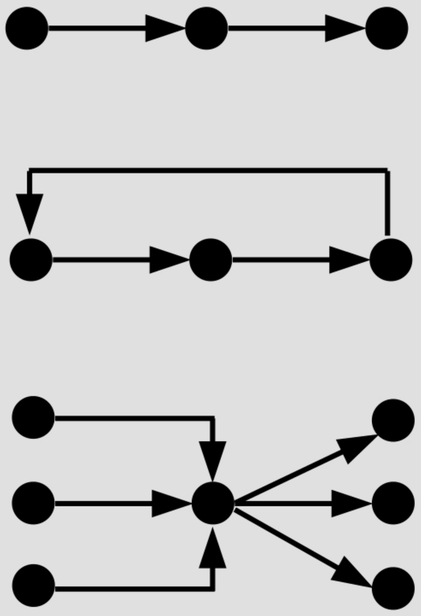
Feed-forward inhibition

Feed-back inhibition

Disinhibition

Rich-club





Feed-forward excitation

Feed-back excitation

Convergence/divergence