

An Introduction to Brain Networks

It is often said that the brain is the most complex network known to man. A human brain comprises about 100 billion (10^{11}) neurons connected by about 100 trillion (10^{14}) synapses, which are anatomically organized over multiple scales of space and functionally interactive over multiple scales of time. This vast system is the biological hardware from which all our thoughts, feelings, and behavior emerge. Clinical disorders of human brain networks, like dementia and schizophrenia, are among the most disabling and therapeutically intractable global health problems. It is therefore unsurprising that understanding brain network connectivity has long been a central goal of neuroscience, and has recently catalyzed an unprecedented era of large-scale initiatives and collaborative projects to map brain networks more comprehensively and in greater detail than ever before (Bohland et al., 2009; Kandel et al., 2013; Van Essen and Ugurbil, 2012). As we will see, one of the implications of modern brain network science is that the human brain may not, in fact, be a uniquely complex system. However, it is certainly timely, challenging and important to understand its organization more clearly.

Central to current thinking about brain networks is the concept of the **connectome**. This word was first coined in 2005 by Olaf Sporns, Giulio Tononi, and Rolf Kötter (2005) and independently in a PhD dissertation by Patric Hagmann (2005) to define a **matrix** representing all possible pairwise anatomical connections between neural elements of the brain (Figure 1.1). The term connectome, in the first and strictest sense of the word, thus stands for an ideal or canonical state of knowledge about the cellular wiring diagram of a brain. The truly exponential growth of research in this area in the last 10 years has led to investigations of a more general concept of the connectome that includes the matrix of anatomical connections between large-scale brain areas as well as between individual neurons; and the matrix of functional interactions that is revealed by the analysis of physiological processes unfolding as slowly as the fluctuations of cerebral blood oxygenation measured with **functional magnetic resonance imaging** (MRI; spanning frequencies below 0.1 Hz), or as fast as the high-frequency neuronal oscillations detectable with invasive and noninvasive electrophysiology (over 500 Hz; see also Chapter 2). A consistent conceptual focus on quantifying, visualizing, and understanding brain network organization

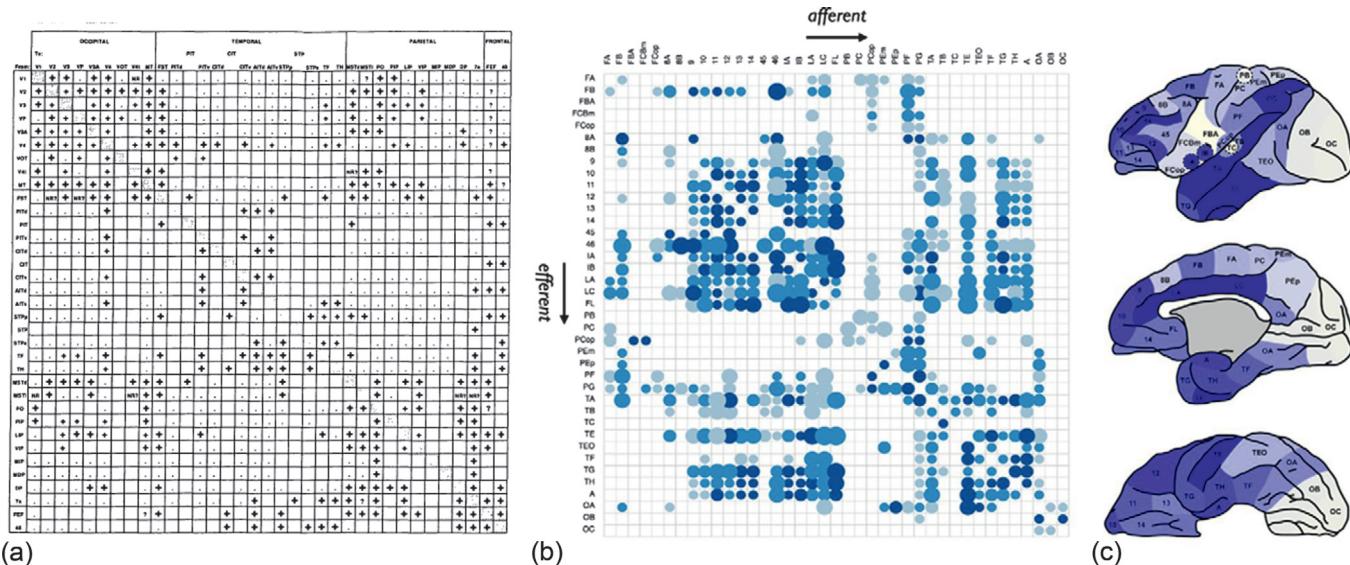


FIGURE 1.1 The connectome as a matrix. (a) One of the first efforts to systematically generate a connectivity matrix for the brain (Felleman and Van Essen, 1991). This matrix represents the connectivity of 32 neocortical areas involved in visual function in the macaque monkey, constructed by collating the results of a large number of published **tract-tracing** studies in this animal. In this matrix, a black cross indicates an outgoing projection from the region listed in the row to the region listed in the column. (b) An updated connectivity matrix of the macaque comprising 39 cortical areas, as reconstructed from an online database of tract-tracing studies. This matrix is organized such that colored elements represent a projection from the region listed in the column to the region listed in the row (see Chapter 3). The size of the dots in each matrix element is proportional to the projection distance and darker colors indicate stronger average reported connectivity strength. (c) The anatomical locations of the areas listed in the matrix in (b). Darker colors identify regions with higher total connectivity to the rest of the network. (a) Reproduced from Felleman and Van Essen (1991) and (b, c) from Scholtens et al. (2014) with permission.

across multiple scales of space and time is a fundamental characteristic of the burgeoning field of **connectomics** (Bullmore and Sporns, 2009).

The relatively recent birth of connectomics should not be interpreted as evidence of a prior lack of neuroscientific interest in brain networks. In fact, many nineteenth and twentieth century neuroscientists—like Ramón y Cajal, Golgi, Meynert, Wernicke, Flechsig, and Brodmann—were well aware of the importance of connectivity and networks in understanding nervous systems. These and other foundational neuroscientists made seminal discoveries and wrote down enduring conceptual insights that have since underpinned the way that we think about nervous systems.

So, what's new? Why do we need new words to label a neuroscientific program that is arguably as old as neuroscience itself? Why now for the connectome and connectomics? Is it just a passing fad, a fashionable blip in professional jargon? Or are there more fundamental factors that can explain why the connectome has exploded as a distinctive focus for neuroscience in the twenty-first century?

In our view, there are two convergent factors driving the scientific ascendancy of connectomics. First, recent years have witnessed rapid growth in the science of networks in general. Since the 1980s there have been major conceptual developments in the statistical physics of complex networks and ever-wider applications of network science to the analysis and modeling of big data. New ways have been found of quantifying the topological complexity of large systems of interacting agents, and striking commonalities have been observed in the organizational properties of a broad array of real-life networks, including, but not limited to, air transportation networks, microchip circuits, the internet, and brains.

The second factor driving the growth of connectomics is the technological evolution of methods to measure and visualize brain organization, across multiple scales of resolution. Since the 1990s there has been significant progress in human neuroimaging science, especially using MRI to map whole brain anatomical and functional networks at macroscopic scale ($\sim 1\text{-}10 \text{ mm}^3$, order of 10^{-2} m) in healthy volunteers and patients with neurological and psychiatric disorders (Bullmore and Sporns, 2009; Fornito et al., 2015). In the last 10 years, there have also been spectacular methodological developments in **tract tracing**, optical microscopy, optogenetics, multielectrode recording, histological gene expression, and many other neuroscience techniques that can now be used to map brain systems at mesoscopic ($\sim 10^{-4} \text{ m}$) and microscopic scales ($\sim 10^{-6} \text{ m}$), under more controlled experimental conditions, and in a wider range of species (Kennedy et al., 2013; Oh et al., 2014; Chung and Deisseroth, 2013; Fenno et al., 2011).

The convergence of these two powerful trends—(1) the mathematical and conceptual developments in complex network science; and (2) the evolution of

technologies for measuring nervous systems—is the crux of what motivates and is distinctively characteristic of the new field of connectomics. This book is about how we can apply the science of complex networks to understand brain connectivity. In particular, we focus on the use of **graph theory** to model, estimate and simulate the **topology** and dynamics of brain networks. Graph theory is a branch of mathematics concerned with understanding systems of interacting elements. A graph is used to model such systems simply as a set of **nodes** linked by **edges**. This representation is remarkably flexible and, despite its formal simplicity, can be used to investigate many aspects of brain organization in diverse kinds of data.

In this introductory chapter, we provide a motivation for why graph theory is useful for understanding brain networks, and offer a brief historical overview of how brain graphs have become a key tool in systems neuroscience. This background provides context for the subsequent chapters, which concentrate in more technical detail on specific aspects of graph theory and their application to connectomic analysis of neuroscientific data.

1.1 GRAPHS AS MODELS FOR COMPLEX SYSTEMS

Complex systems have properties that are neither completely random nor completely regular, instead showing nontrivial characteristics that are indicative of a more elaborate, or complex, organization. Such systems are all around us, and range from societies, economies, and ecosystems, to infrastructural systems, information processing networks, and molecular interactions occurring within biological organisms (Barabási, 2002). These are all big systems—often comprising millions of agents interacting with each other—and they are represented by very diverse kinds of data. It is only in the last 20 years or so that it has become mathematically tractable and scientifically interesting to quantify this daunting complexity.

As methods have been developed to deal with such data, and as these methods have been applied more widely in different domains or fields, it has become clear that superficially different systems—such as friendship networks, metabolic interaction pathways, and very large-scale integrated computer circuits—can express remarkably general properties in terms of their network organization (Albert and Barabási, 2002; Newman, 2003a). From these developments, an interdisciplinary field of network science has formed around the use of general analytic methods to model complex networks, and to explore the scope of common or near-universal principles of network organization, function, growth, and evolution. Principal among these general methods is graph theory.

1.1.1 A Brief History of Graph Theory

The first use of a graph to understand a real-world system is widely credited to the Swiss mathematician Leonhard Euler (1707-1783). In 1735, Euler lived in the Prussian town of Königsberg (now the Russian city of Kaliningrad), which was built around seven bridges across the river Pregel, linking the two main riverbanks and two islands in the middle of the river (Figure 1.2a). An unresolved problem at that time was whether it was possible to walk around the town via a route that crossed each bridge once and only once. Euler solved this problem by representing the four land masses divided by the river as nodes, and the seven bridges as interconnecting edges (Figure 1.2b). From this prototypical graph, he was able to show that no more than two nodes (the start and end points of the walk) should have an odd number of edges connecting them to the rest of the graph for such a walk to be possible. In fact, all four nodes in the Königsberg graph had an odd number of edges, meaning that it was impossible to find any route around the city that crossed each and every bridge only once. In this way, Euler proved once and for all that the system of bridges and islands that comprised the city was organized such that the “Königsberg walk” was topologically prohibited.

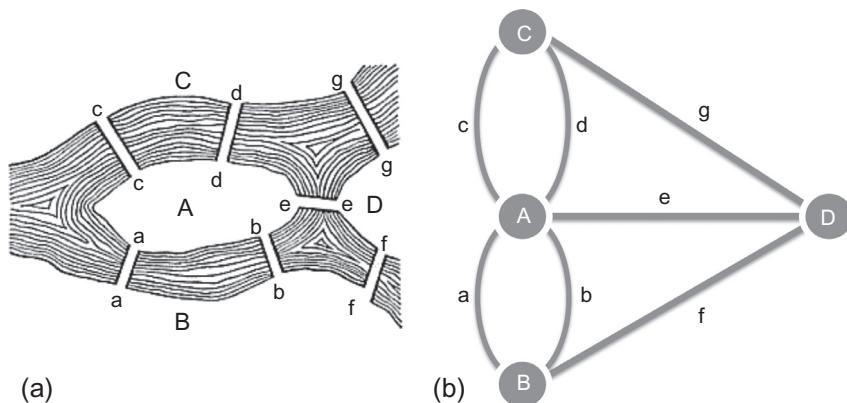


FIGURE 1.2 The origins of graph theory: the first topological analysis by Euler. (a) Simplified geographic map of the Prussian town of Königsberg, which comprised four landmasses (marked A–D) linked by seven bridges (marked a–f). The specific problem that Euler solved was whether it was possible to find a **path** that crossed each bridge once and only once. The geographical map lavishes detail on features like the shape of the islands, and the currents in the river, that are completely irrelevant to solving this problem. (b) Graphical representation of the problem. Euler’s topological analysis successfully simplified the system as a **binary graph**—with nodes for landmasses and edges for bridges—and focused on the **degree**, or number of edges, that connected each node to the graph. (a) Reproduced from Kraitchik (1942) with permission.

The importance of Euler's analysis is *not* in the details of the geography of eighteenth century Königsberg; rather it is important precisely because it successfully ignored so many of those details and focused attention on what later became known as the topology of the problem. The topology of a graph defines how the **links** between system elements are organized. Indeed, this is exactly what Euler's graph focuses on; the network of bridges connecting islands and riverbanks. It is not informed by any other physical aspect of Königsberg, such as the physical distance (length) of the bridges, the distances between them, and so on. More generally, the results of this and any other topological analysis will be invariant under any continuous spatial transformation of the system. To see this, imagine taking the physical map of Königsberg depicted in [Figure 1.2a](#) and increasing or decreasing its physical scale, or rotating it, or reflecting it, or stretching it. None of these (or any other) continuous spatial transformations will have any effect on the number of bridges connecting any particular island to the rest of the town.

Topology developed strongly as a field of mathematics from the late nineteenth century, preceding important developments in the statistical analysis of graphs in the twentieth century. In the 1950s, this work was spearheaded by Paul Erdős and Alfred Rényi, who introduced an influential statistical model for generating random graphs and for predicting some of their topological properties ([Erdős and Rényi, 1959](#); see [Bollobás, 1998](#) for overview). In an Erdős-Rényi graph, there are N nodes and a uniform probability p of each possible edge between them. If p is close to one, the graph is densely connected and if p is close to zero, the graph is sparsely connected. Erdős and Rényi showed that many important properties of these graphs, such as the mean number of connections attached to any single node (also called the mean degree of the graph), and whether the graph is a single **connected component** or contains isolated nodes (which are not connected to other nodes), could be predicted analytically from their **generative model** (Chapters 4, 6, and 10).

Both the Königsberg graph invented by Euler and the random graphs generated by the Erdős-Rényi model are examples of the simplest class of graphs: binary undirected graphs. They are binary graphs because the edges are either absent or present or, equivalently, the edge weight is either zero or one. They are **undirected graphs** because the edges connect nodes symmetrically; no distinction is made between the source and target of a connection. The principles of topological analysis have since been extended to more sophisticated graphs that include both weighted and directed connectivity ([Chapter 3](#)). As we will see in later chapters, these extensions are particularly important for characterizing certain kinds of brain network data.

A critical step from the mathematics of random graph theory to the physics of complex networks was taken by Duncan Watts and Steven Strogatz ([Watts and Strogatz, 1998](#); [Figure 1.3](#)). Like Erdős and Rényi, they defined a generative

model for graphs; but they began their analysis with a simple regular **lattice** of N nodes, each connected directly to an arbitrary number of other nodes. The **Watts-Strogatz model** then randomly selects an edge connecting nodes i and j in the lattice and incrementally rewrites the graph so that this edge now connects node i to another randomly selected node, h , such that $h \neq j$. This generative process of random mutation of connectivity can be applied to each edge with an arbitrary probability of rewiring p_{WS} , so that when $p_{WS} = 1$, all the edges have been randomly rewired and the lattice has been topologically transformed to an Erdős-Rényi random graph (further details of these models are considered in [Chapter 10](#)).

[Watts and Strogatz \(1998\)](#) focused on two key properties of their network model: the **clustering coefficient** and the **characteristic path length**. The clustering coefficient provides an index of the cliquishness or clustering of connectivity in a graph, and is the probability that two nodes each directly connected to a third node will also be directly linked to each other ([Chapter 8](#)). The characteristic path length is commonly used to index the integrative capacity of a network and is a measure of the topological distance between nodes, computed as the minimum number of edges required to link any two nodes in the network, on average ([Chapter 7](#)). The intuition is that a shorter average **path length** results in more rapid and efficient integration across the network ([Latora and Marchiori, 2001](#)). Random graphs have a short characteristic path length and low clustering. On the other hand, the regular lattice analyzed by Watts and Strogatz has high clustering and long characteristic path length ([Figure 1.3](#)).

The first critical discovery revealed by computer simulations of the Watts-Strogatz model was that the rate of change in path length was much faster than the rate of change in clustering, as the probability of rewiring an edge in the lattice was progressively increased from zero towards one. Specifically, changing just a few edges in the lattice dramatically decreased the characteristic path length of the graph, but did not greatly reduce the high average clustering that characterized the lattice. In other words, there was a range of rewiring probabilities that generated graphs with a hybrid combination of topological properties: short path length, like a random graph, and high clustering, like a lattice. By analogy to the qualitatively similar properties of social networks, first explored by [Milgram \(1967\)](#), these nearly-regular and nearly-random graphs were called **small-world** networks. The second main discovery reported by Watts and Strogatz was based on empirical analysis. They measured the path length and clustering of graphs representing three real-life systems and found that the small-world combination of greater-than-random clustering with nearly-random path length was characteristic of all three: a social network (costarring movie actors), an infrastructural network (an electrical power grid), and the neuronal network of the nematode worm, *Caenorhabditis elegans*.

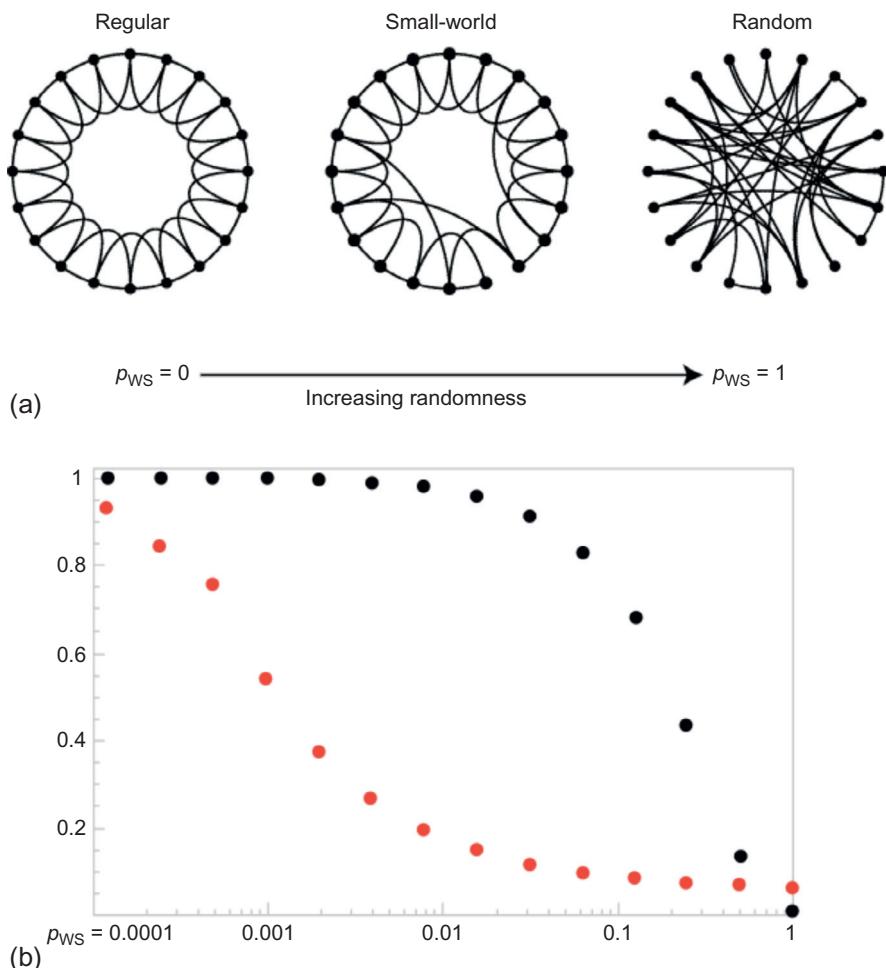


FIGURE 1.3 Small-world networks. (a) The seminal work of Watts and Strogatz (1998) identified a continuum of network topologies ranging from completely regular and lattice-like (left) to completely random (right). Interposed between these extremes is a class of networks with a so-called small-world topology, which can be generated by randomly rewiring (with probability, p_{WS}) an arbitrary proportion of edges in a regular network. (b) Watts and Strogatz found that rewiring just a few edges (small p_{WS}) led to a dramatic reduction in path length (red line), whereas high clustering was more resilient to random rewiring (black line), resulting in a regime of rewiring probability in which the network showed high clustering, like a lattice, and low path length, like a random network. The red and black lines correspond to the average clustering coefficient and average path length, respectively, of the graph at a given p_{WS} , divided by the corresponding value computed in a comparable lattice. The combination of high clustering and short path length is the defining characteristic of small-world networks and has since been described in many real-world systems. *Images reproduced from Watts and Strogatz (1998) with permission.*

At about the same time, [Barabási and Albert \(1999\)](#) introduced another generative model that built a complex graph by adding nodes incrementally ([Chapter 10](#)). In this model, as each new node i is added to the graph, the probability that it will form a connection or edge to any other node, j , is proportional to the number of connections, or degree, of node j . In other words, new nodes connect preferentially to existing nodes that already have a large number of connections and thus represent putative network **hubs**. By this generative process of **preferential attachment**, the “rich get richer,” or nodes that have high degree initially tend to have even higher degree as the graph grows by iterative addition of new nodes. As a result, the distribution of degree across network nodes is not the unimodal Poisson-like distribution that is characteristic of the Erdős-Rényi model; instead it is characteristically fat-tailed, conforming to what is called a **scale-free** or power-law distribution. A scale-free **degree distribution** means that the probability of finding a very high degree hub node in the graph is greater than would be expected if the degree distribution was unimodal, like a Poisson or Gaussian function. More simply, it is likely that a scale-free network will contain at least a few highly connected hub nodes ([Chapters 4 and 5](#)). Barabási and Albert also found evidence of power-law degree distributions in several empirically observed complex systems.

Like the apparent ubiquity of small-worldness, the fact that so many substantively different systems share the property of scale-free degree distributions suggested that some key topological principles might be nearly universal for a large class of complex networks ([Barabási and Albert, 1999](#)). However, it is important to appreciate that the empirical ubiquity of small-worldness or scale-free degree distributions does not by itself mean that random edge rewiring or preferential attachment is the generative mechanism that built all these systems in real life. Systems with complex topological properties, like small-worlds and hubs, can be generated by many different growth rules.

A third major development in the application of graph theory to real-world systems has been the discovery that such networks are modular—they can be nearly decomposed into subsets of nodes that are more densely connected to each other than to nodes in any other **modules** ([Simon, 1962](#)). Following work by Mark Newman, Michelle Girvan, others ([Girvan and Newman, 2002; Newman and Girvan, 2004; Newman, 2004c; 2006](#); for a detailed review, see [Fortunato, 2010](#)), the quantification of network **modularity** has become a large and rapidly developing area of complex network science ([Chapter 9](#)). For example, the global airline network, where nodes are airports and edges are direct flights between them, has a hierarchical modular structure, in which modules can be further decomposed into submodules and so on ([Guimerà et al., 2005; Figure 1.4](#)). Each topological module of the airline network corresponds approximately to a geographical continent or political territory, like the

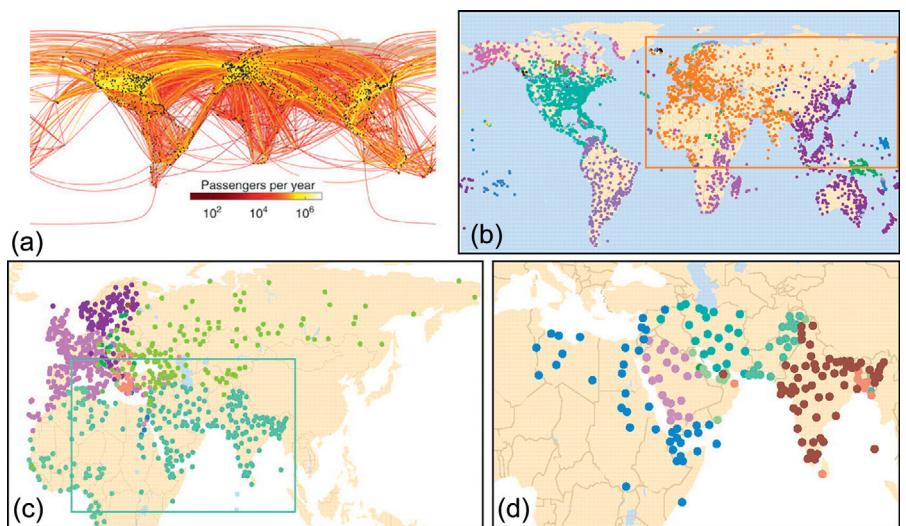


FIGURE 1.4 Hierarchical modular organization of the global air transportation network. (a) A geographical representation of the worldwide airline network. Black dots represent airports (nodes), and colored lines represent passenger traffic between airports (edges). (b) This network has a hierarchical modular structure, such that modules can be further divided into submodules and so on. Shown here is the highest level of the module hierarchy, plotted on a geographic map of the world. Colors correspond to different modules. The modular organization is strongly dominated by geographic location, such that airports in the same continent are in the same module. This is consistent with the fact that most flights link airports in the same country or continent. (c) The next level in the hierarchy, focusing on the orange Eurasian module (box) in (b). This module now splits into different submodules, such as Scandinavia, central Europe, Western Russia, the Middle East, and North Africa. (d) The next level in the hierarchy, focusing on the Middle-Eastern submodule in (c). Again, we see a tendency for airports to segregate into sub-submodules (like India) according to their geographic locations and political affiliations. (a) Reproduced from Grady et al. (2012) with permission. (b-d) reproduced from Sales-Pardo et al. (2007), Copyright (2007) National Academy of Sciences, U.S.A., with permission.

United States or the European Union. This represents the familiar experience that most flights from a US or EU airport are to other airports in the same territory or continental land mass; only a few big airports, corresponding to high degree hubs, such as New York JFK and London Heathrow, have many intercontinental flights. In a network with high topological modularity, the density of intramodular connections is much greater than the density of intermodular connections. Typically most of the intermodular communications are mediated by a few so-called connector hubs that link different modules (Guimerà et al., 2005). It turns out that many real-life systems share this topological property of modularity, again suggesting that it represents a near universal characteristic of complex networks (Simon, 1962).

These three key concepts of graph topology—small-worldness, degree distribution, and modularity—are the tip of an iceberg of complex network science. Additionally, there has been growing interest in the dyadic subdivision of a network into a relatively small core or **rich club** of highly interconnected high degree hubs and a larger periphery of sparsely interconnected lower degree nodes (Colizza et al., 2006; Chapter 6). There has also been important work to identify the topological **motifs** of a network: basic building blocks of connection profiles between small sets of three or four nodes that recur in a network with a frequency that is greater than expected by chance (Chapter 8). Graphs further provide a powerful approach for simulating the effects of damage to a network by studying how global topological properties, such as network connectedness or characteristic path length, are affected as the nodes or edges of a graph are computationally deleted (Chapter 6). Most complex networks are fairly resilient to random attack on their nodes, but are much more vulnerable to a targeted attack that prioritizes the highest degree hub nodes (Albert et al., 2000). For example, if the global airline network was attacked one airport at a time, but the choice of which node to attack next was made at random, a very large number of airports would need to be disabled before intercontinental traffic was affected. This is because only a small number of airports service long-haul flights. However, if the attacks were targeted on those few major hub airports, like JFK or London Heathrow, this would be equivalent to removing most of the intermodular flights between the US and EU modules. The result would be a dramatic increase in the number of flights required to link two cities on different continents (i.e., increased path length) and potentially a **fragmentation** of the network into two or more completely isolated modules. In this way, hubs can increase the vulnerability of many complex networks to targeted attack (Chapter 6).

1.1.2 Space, Time, and Topology

As is hopefully becoming clear, topology is an important aspect of how many networks are organized; but other dimensions must also be considered in the analysis of most, if not all, types of networks. Some complex networks, like the World Wide Web or the semantic web of Shakespeare's sonnets (Motter et al., 2002), are quite purely topological: they don't really exist in space (the web), or space and time (sonnets). There are, however, many other complex networks, particularly biological networks such as the brain, that are embedded in spatial dimensions and are dynamically active over time. For brain network analysis, the familiar three-plus-one dimensions of space and time must thus be incorporated with the more novel fifth dimension of topology.

For spatial networks generally there will be inevitable constraints on how the topological plan can actually be built in the three dimensions of the world (Barthélemy, 2011). For example, to build a high-performance computer chip,

each processing node or logic gate must be physically wired to a number of other nodes according to a topologically complex design for high performance. It is also important that the total amount of wiring used should be as small as possible, because wiring is expensive and generates thermal noise. Furthermore, it is usually mandated that the topology must be embedded in only two dimensions, on the surface of a silicon chip. Empirical analysis and generative modeling of computer circuits and other spatial networks has indicated that conservation of wiring cost is an important factor in network formation that often drives the physical location and connectivity of nodes, such that spatially proximal nodes have a higher probability of connectivity than spatially distant nodes (Christie and Stroobandt, 2000). However, minimization of wiring cost is clearly not the only selection pressure, otherwise all spatial networks would be low-dimensional lattices with topologically clustered nodes embedded as close to each other as possible in physical space. Accordingly, generative models that posit a trade-off or competition between cost minimization and some other topological factor that provides functional benefits, have been more successful in simulating the organization of spatial networks (Vértes et al., 2012). Such economical principles of competition between physical cost and topological value may be generally influential in the formation of networks embedded in space (Latora and Marchiori, 2001; Achard and Bullmore, 2007; Chapter 8).

Most networks will also be active over time with dynamics that are related to the functional performance of the system. Perhaps unsurprisingly, the structural topology of a network has an important influence on the functional dynamics that emerge from interactions between nodes over time. Networks with complex topology have complex dynamics, broadly speaking. For example, networks displaying high dynamical complexity—that is, dynamics which are neither fully segregated nor fully integrated—show a complex, small-world topology (Sporns et al., 2000; see Chapter 8).

It has also been shown that small-world and scale-free network topologies are associated with the emergence of so-called critical dynamics (Chialvo, 2010). Self-organized critical dynamics are often inferred when functional interactions between nodes exist at all scales of space and time encompassed by the system and are statistically distributed as power laws (Chapter 4). Topologically complex networks have been linked to the emergence of such scale-invariant network dynamics, which are consistent with the system being in a self-organized critical state that is interposed between completely ordered and disordered dynamics (Bak et al., 1987). Critical dynamics have been shown computationally to have advantages for information processing and memory, and experimentally they have been inferred from power-law scaling of dynamics in many nervous systems, from cultured cellular networks to whole brain electrophysiological and haemodynamic recordings (Linkenkaer-Hansen et al., 2001; Beggs and Plenz, 2003; Kitzbichler et al., 2009). In general, network topology

plays an important role in constraining system dynamics; and, reciprocally, system dynamics can often drive the evolution or development of network topology.

1.2 GRAPH THEORY AND THE BRAIN

As we have seen, graph theory has played an integral role in recent efforts to understand the structure and function of complex systems. Nervous systems are undoubtedly complex, so it is natural to assume that graph theory may also prove useful for neuroscience. Importantly, graph-based representations of brain networks—brain graphs—can easily be constructed from neural connectivity matrices, such as the ones depicted in [Figure 1.1](#). Each row or column representing a different brain region in the matrix is drawn as a node in the graph, and the values in each matrix element are drawn as edges. In fact, as we will see throughout this book, matrix and graph representations of a network are formally equivalent, and much of the mathematics of graph theory is applied through the analysis of matrices. In this section, we consider how graph theory has been applied to understand brain networks and how it has emerged as a powerful analytic tool for connectomics.

1.2.1 The Neuron Theory and Connectivity at the Microscale

When did the ideas of graph theory and network science first begin to permeate neuroscience? Formally, the first applications of graph theory to neuroscientific data were not published until the end of the twentieth century ([Felleman and Van Essen, 1991](#); [Young, 1992](#); [Watts and Strogatz, 1998](#)). However, the seminal neuron theory, established by Ramón y Cajal's brilliant microscopic studies and theoretical thinking in the late nineteenth and early twentieth century ([Ramón y Cajal, 1995](#)), set the scene for graph theory to later make sense as a model of nervous systems ([Figure 1.5](#)). Ramón y Cajal and some others, using the then-revolutionary technique of silver impregnation to visualize the complex branching processes of individual neurons, claimed that neurons were discrete cells that contacted each other very closely by synaptic junctions. This model contradicted the principal alternative paradigm, the reticular theory advocated by Camille Golgi, who invented the neuronal staining method used by Ramón y Cajal. According to Golgi, there was a continuous syncytial connection between the cell bodies of the nervous system. Both men shared the Nobel Prize in 1906, but it was not until the 1950s when **electron microscopy** finally resolved the theoretical question in favor of Ramón y Cajal ([DeRobertis and Bennett, 1955](#)).

It is now accepted beyond doubt that synaptic junctions are generally points of close contiguity, but not continuity, between connected neurons. Ramón y Cajal's model of discrete neurons interconnected by synapses is naturally suited

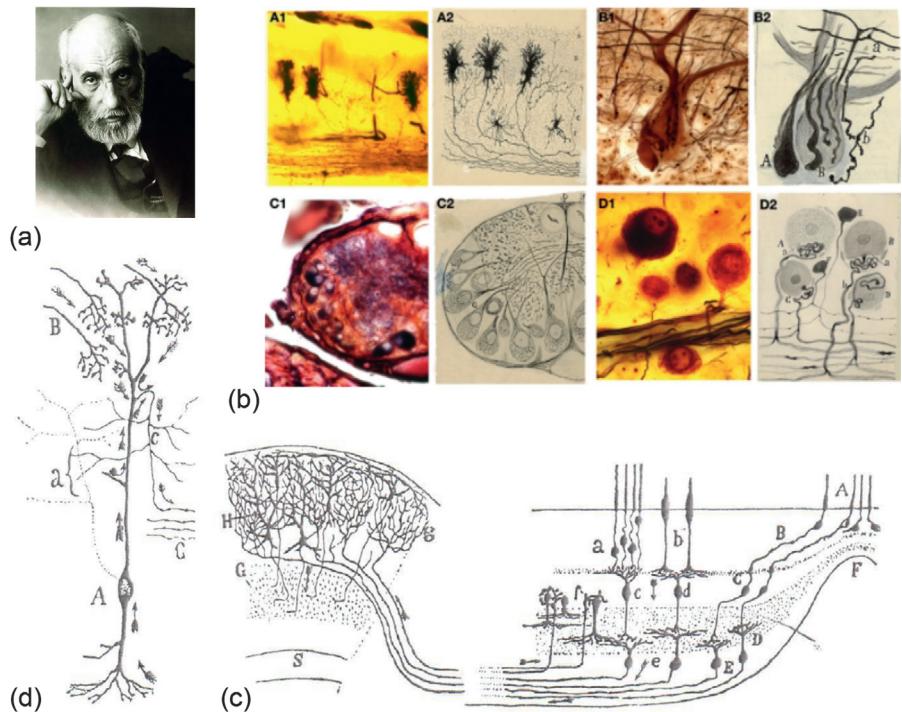


FIGURE 1.5 The pioneering work of Ramón y Cajal (1852-1934). (a) Santiago Ramón y Cajal made countless microscopic slide preparations of nervous tissue (colored slides in (b)), and recorded the data for publication by pen and ink drawings (monochrome panels in (b)). More than a century after the publication of his work, it is easy to recognize many examples of close correspondence between stained cells and drawn cells. (c) From these observations, Ramón y Cajal evolved neuron theory. For example, he showed how activity in retinal cone cells (marked A in the drawing) passed via synaptic junctions to foveal bipolar and ganglion cells (C) and was then projected to axonal arborizations (g) terminating in close proximity to neurons in the superior colliculus (H). Ramón y Cajal also formulated laws of conservation of space, time, and material to explain the morphological adaptations of individual neurons. (d) The so-called shepherd's crook cell of the reptilian optic lobe, for example, had the unusual characteristic that the axon (marked C on the drawing) did not emerge close to the cell body (A). Ramón y Cajal argued that this apparently odd axonal location was mandated by the conservation of material or cytoplasm (in modern parlance, minimization of wiring cost). Assuming correctly that electrical activity must flow from all other parts of the neuron towards the axon and its collateral ramifications (marked by arrows), he reasoned that all other possible locations of the axonal hillock would be associated with “waste of material” or “unnecessary lengthening” of the axonal projection. (b) Reproduced from *Garcia-Lopez et al. (2010)* and (c, d) from *Ramón y Cajal (1995)* with permission.

to a graph theoretic representation, whereby neurons are represented by nodes and axonal projections or synaptic junctions are represented by edges. In this way, one might argue, Ramón y Cajal was the giant on whose shoulders graph theoretic analysis of neural systems formally emerged some 100 years later.

One other theoretical contribution by Ramón y Cajal that remains influential in connectomics is his proposal of a few apparently simple general laws to govern most, if not all, aspects of nervous system anatomy. He summarized these rules, also called Cajal's conservation laws, in the following words:

Doubt for us is unacceptable, and all of the morphological features displayed by neurons appear to obey precise rules that are accompanied by useful consequences. What are these rules and consequences? We have searched in vain for them over the course of many years... Finally however we realized that all of the various conformations of the neuron... are simply morphological adaptations governed by laws of conservation for time, space and material... which must be considered the final cause of all variations in the shape of neurons, [and] should in our view be immediately obvious to anyone thinking about or trying to verify them.

Ramón y Cajal (1995), p. 116, Volume I.

In more modern language, Ramón y Cajal anticipated that many aspects of brain network organization would be driven both by minimization of axonal wiring cost, which conserves cellular material and space; and by minimization of conduction delay in the transmission of information between neurons, which conserves time (Figure 1.5; see also Chapter 8).

It turns out that many aspects of brain network organization do indeed seem to have been selected to minimize wiring cost and/or to minimize metabolic expenditure (Niven and Laughlin, 2008). Topological features like modules and clusters are often anatomically colocalized, which conserves material. Other aspects of the connectome that promote the efficient integration of information across the network, such as short characteristic path length, may have been selected to minimize conduction delay, thus increasing the speed at which information can be exchanged between neurons or conserving time. Connectomics has thus begun to restate and refine Ramón y Cajal's conservation laws in terms of a competition between minimization of wiring cost and maximization of integrative topology (Bullmore and Sporns, 2012; Budd and Kisvárdy, 2012).

1.2.2 Clinicopathological Correlations and Connectivity at the Macroscale

The first attempts to understand macroscopic networks of interconnected cortical areas roughly paralleled Ramón y Cajal's seminal work on microscopic neuronal connectivity. Network diagrams were drawn by clinical pioneers like

Theodor Meynert, Carl Wernicke, and Ludwig Lichtheim to summarize white matter connections between cortical areas, and to explain how the symptoms of brain disorder could be related to pathological lesions. The Wernicke-Lichtheim model of language remains the most successful of these early models of macroscale brain network organization, linking a language production area in the frontal cortex to a language comprehension area in the temporal cortex (as well as a more vaguely located association area). Some aspects of this model were able to account convincingly for the generation of specific symptoms: in particular, a lesion of the arcuate fasciculus linking frontal and temporal language areas was predicted and shown to cause an inability to repeat heard words despite otherwise apparently normal language, so-called conduction aphasia (Lichtheim, 1885; Figure 1.6a). Wernicke generalized these ideas as an associative theory of brain function, in which higher-order cognitive abilities (and their disorders, such as psychosis) were thought to emerge from the integration (or pathological disintegration) of anatomically distributed yet connected cortical areas (Wernicke, 1906). The nineteenth century diagrams of large-scale brain network organization drawn by these pioneers, comprising a few spatially circumscribed areas (nodes) interconnected by white matter tracts (edges), set the scene for graph theoretical analysis of nervous systems at the macroscopic scale, just as the neuron theory laid the foundation for graph-based models at microscopic (cellular) scales.

One important difference between the macroscopic diagram makers, like Wernicke and Lichtheim, and the microscopic anatomists, especially Ramón y Cajal, was the quality of the data available to them. Benefiting from contemporary technical developments in optics and tissue staining, Ramón y Cajal, Golgi, and others were able to produce very detailed, high-quality images of neurons and microscopic circuits (Figure 1.5). In contrast, the macroscopic diagram makers worked with poorer quality data. For example, Wernicke's work was based on clinicopathological correlation, linking the pattern of symptoms and signs expressed by a few patients in the clinic with the postmortem appearance of their brains (Wernicke, 1970). Even at the time, the unreliability of this method and the diagnostic formulations that followed were sharply criticized by contemporaries, including Sigmund Freud (1891; Figure 1.6b).

Partly as a result of the methodological weakness of postmortem clinicopathological correlations, large-scale network concepts of neurological and psychiatric disorders were somewhat eclipsed for the first half of the twentieth century by more locally anatomical or purely psychological models of cognitive function and brain disorders (Shallice, 1988). The localizationist ambition predominantly focused on how specific psychological processes arose from the function of discrete brain regions; or how specific facets of cognition, emotion, and behavior were anatomically localized and segregated in the brain. Although this tradition has conceptual roots in Gall's discredited phrenology

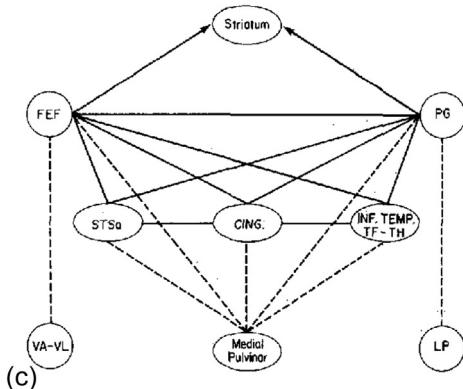
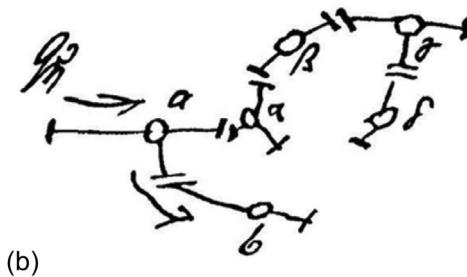
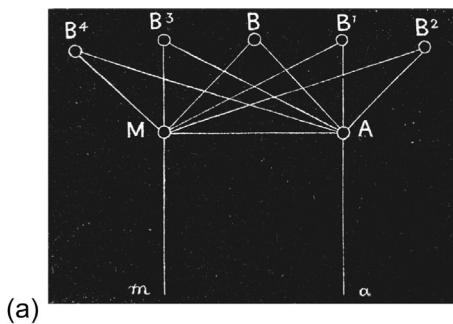


FIGURE 1.6 Early brain graphs based on clinicopathological correlations. (a) Lichtheim's sketch of a large-scale human brain network for language. Broca's and Wernicke's areas, and the arcuate fasciculus which directly connected them, were anatomically localized in the left inferior frontal and superior temporal cortex. However, the number of association areas that should be included in the network was not known, nor was their anatomical location. In this map, Lichtheim speculated that there may be many association areas and that Broca's and Wernicke's areas were the most densely connected nodes in the network. A represents an auditory area (i.e., Wernicke's area), M a motor area (i.e., Broca's area), and B other association areas. (b) A network diagram drawn by Freud, who was a critic of contemporary efforts to map clinical disorders onto large-scale brain networks. Nonetheless, he originally framed his nascent theory of psychoanalysis in terms of a neuronal network. The libido, designated by an enigmatic Q-like glyph, flows across weighted synaptic junctions between charged cells, engendering primary (id) or secondary (ego) process thinking according to the anatomical path of cathectis (or charge of libidinal energy) in the network. Psychoanalysis subsequently moved away from such an explicitly neuronal model, although libido retained a biological meaning in Freud's work until about 1910. (c) Mesulam's later and more anatomically accurate "hub and spoke" model of spatial attention, which comprises multiple cortical and subcortical areas interconnected by white matter tracts; the frontal eye fields (FEF) and posterior parietal cortex (PG) are the most densely connected hubs of the network. Panels (a), (b), and (c) reproduced from Lichtheim (1885), Freud (1891, 1895), and Mesulam (1990), respectively, with permission.

(Fodor, 1983), it received considerable support from famous clinical case studies, such as Broca's Leborgne (Broca, 1861), Harlow's Phineas Gage (Harlow, 1848), and Scoville and Milner's HM (Scoville and Milner, 1957), each of which demonstrated how highly selective cognitive and behavioral deficits could arise from focal brain damage. Further support came from Hubel and Wiesel's (1959) seminal single cell recordings of visual neurons in the cat, and Penfield and Jasper's (1954) intraoperative cortical stimulation studies of human epilepsy patients, both of which demonstrated an extraordinary degree of functional specialization at the level of individual neurons (in the cat) and small patches of cortex (in the human).

More integrative, network-based models of the brain were reinvigorated by the work on disconnection or dysconnectivity syndromes conducted by Norman Geschwind, Marsel Mesulam and colleagues in the 1960s and subsequent years (Geschwind, 1965a,b; Mesulam, 1990). This work showed how many psychological deficits observed clinically could be explained in terms of the network anatomy of the brain. It also demonstrated how normal functions were often not localized to a single, specialized cortical area, but were instead anatomically represented by a large-scale network; for example, spatial attention was linked to a distributed ensemble of frontal and parietal cortical areas and subcortical nuclei that were interconnected by white matter tracts (Figure 1.6c). From this perspective, lesions or other disease processes attacking any component of these networks could be linked to symptomatic disturbances in patients. With better quality data available to link cortical anatomy to psychological functions and clinical symptoms, the importance of large-scale networks for understanding brain function and brain disorders, first advocated in the nineteenth century, was securely reaffirmed around 100 years later.

1.2.3 The Dawn of Connectomics

In the perfect light of hindsight, we can see that the conceptual precedents for connectomics go back a long way in the history of neuroscience. This makes it difficult to say when exactly the first connectome was drawn. The Wernicke-Lichtheim model, for example, is a directed, binary graph; but it was not described or analyzed as such. Mesulam's spatial attention model explicitly included network hubs, but they were not quantitatively defined in terms of degree or any other measure of topological centrality (Chapters 4 and 5). We might therefore characterize these maps as proto-connectomes that preceded the conscious and deliberate application of graph theory to neuroscience data in the 1990s.

The first brain graphs, representing a large number of cortical or subcortical nodes interconnected by axonal edges, were based on tract-tracing data in the cat and the macaque monkey. Tract-tracing measures the axonal propagation of a tracer or signal from the site of its injection to or from all other brain

regions which are directly, anatomically connected to the injection site. It thus offers an excellent technique for exploring the wiring diagram of mammalian cortex, although each experiment will only generate data on the connectivity of a small number of injection sites and many experiments must be combined to estimate the connectivity of large nervous systems. To get around this problem, the first connectomes were generated by collating findings across a large number of published tract-tracing studies. With this approach, David van Essen and Dan Felleman represented the wiring diagram of the macaque visual cortex as a hierarchical system of areal nodes interconnected by directed, lamina-specific edges (Felleman and van Essen, 1991; Figures 1.1a and 1.7a and b). Another topological analysis of the large-scale macaque brain network disclosed a non-trivial organization, with some clusters of highly interconnected areas but overall sparse connectivity (Young, 1992; Figure 1.7c), akin to the modular organization that was later found to be characteristic of many complex networks (Newman, 2003a). These studies also spurred more systematic attempts to collate and organize the findings from large numbers of tract-tracing studies in the macaque, resulting in the first, freely available connectomic data repository—the CoCoMac database (Stephan et al., 2001).

Despite these efforts, the next key step in the formation of connectomics was based not on mammalian tract-tracing data but on the neuronal network of *C. elegans*. The nervous system of this animal was mapped completely at the level of 302 individual neurons, with approximately 5000 chemical synaptic connections between them (as well as 2000 neuromuscular junctions and 600 gap junctions) reconstructed by expert visual inspection of serial electron micrographs (Figure 1.8). The experimental work by John White, Sydney Brenner, and colleagues took more than a decade to complete, and was published as a single 340-page volume of the *Philosophical Transactions of the Royal Society* (White et al., 1986). This canonical dataset on anatomical connectivity at a cellular scale in a relatively small nervous system has since informed many studies examining its spatial and topological organization as a network (Chen et al., 2006; Varshney et al., 2011; Nicosia et al., 2013; Towlson et al., 2013).

In 1998, Watts and Strogatz modeled the *C. elegans* connectome as a binary graph, where the neurons were represented as nodes and the synapses as edges. They showed that this network had a short characteristic path length and high clustering; in other words, its global organization conformed to a small-world topology. In assessing the clustering and path length of the *C. elegans* nervous system, these measures were compared to their values in a **null model**, which is an important step in many network analyses. We return to the concept of using null models to normalize graph theoretical measures on connectivity matrices in Chapter 10.

In addition to their technical novelty, the Watts and Strogatz results on *C. elegans* represented the first point of contact between modern network science and

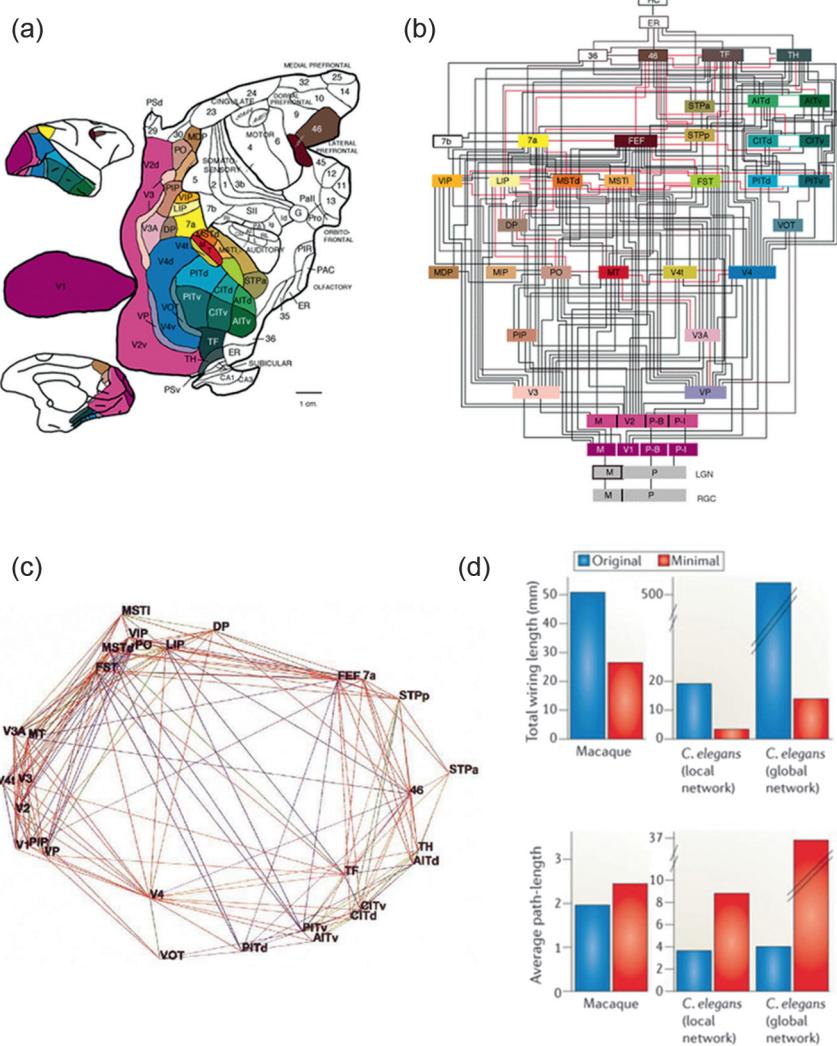


FIGURE 1.7 From brain anatomy to network topology. (a) Felleman and Van Essen (1991) compiled tract-tracing data on 32 regions comprising the macaque visual system (shown anatomically in flat map representation) and (b) used information on the cortical lamina of tract projection and termination to infer a hierarchical wiring diagram. (c) Young (1992) was the first to represent brain network data (in this case, macaque tract-tracing data) in a topological configuration that locates directly connected nodes in close proximity, regardless of the anatomical distance between them. (d) Kaiser and Hilgetag (2006) showed that the macaque and *C. elegans* connectomes could be computationally rewired to minimize their wiring costs (top barchart); but the cost-minimized graphs (red bars in both charts) had longer path length than the naturally selected connectomes (blue bars), pointing to a trade-off between wiring cost and integrative topology in brain networks. (a, b) Reproduced from Felleman and Van Essen (1991), (c) from Young (1992), and (d) from Bullmore and Sporns (2012) with permission.

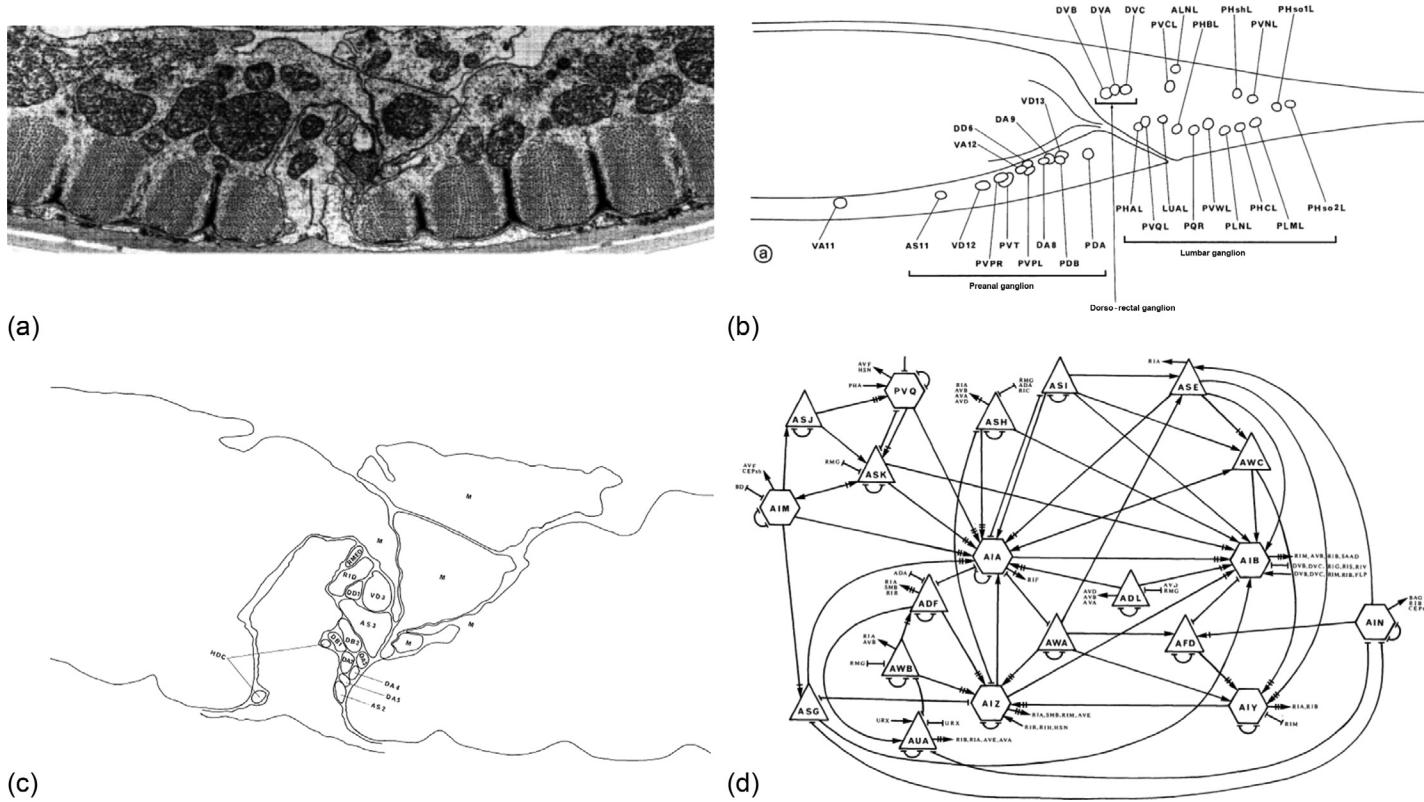


FIGURE 1.8 Mapping the connectome of the nematode worm *C. elegans*. White and colleagues used serial electron micrographs (a) to visually dissect and define synaptic terminals (b) between specific, named cells (c) allowing them to construct circuit maps or wiring diagrams of the anatomical network of synaptic connections between neurons (d). In this representation, sensory neurons are represented as triangles, motor neurons as circles, and interneurons as hexagons. Arrows indicate the directionality of chemical synapses and T-shaped edges represent gap junctions. *Images reproduced from White et al. (1986) with permission.*

neuroscience, the first quantification of the complex topology of a nervous system, and the first hint that the organization of brain networks might share properties in common with other complex networks (Watts and Strogatz, 1998).

In the first years of the twenty-first century, Olaf Sporns, Claus Hilgetag, Rolf Kötter, and others translated the quantitative methods of Watts and Strogatz from the neuronal connectome of *C. elegans* to the large-scale interareal tract-tracing data then available for the cat and the monkey. It was demonstrated that these mammalian networks also had the characteristically small-world property of short path length and high clustering; and it was argued that this architecture might serve a dual function. The short path length of brain networks might favor integrated processing of information over the network as a whole, whereas high clustering might favor segregated processing within functionally specialized cliques of nodes (Sporns et al., 2004). In other words, the small-world organization of the brain offered a topological substrate for both functional segregation and integration, thus resolving the apparent paradox of how these two seemingly opposing tendencies could be supported by a single architecture. Moreover, by computationally rewiring the edges of the *C. elegans* and the macaque brain networks, it was shown that path length was inversely related to wiring cost (Kaiser and Hilgetag, 2006). In other words, the wiring cost of brain networks, approximated by the Euclidean distance between connected nodes, could be reduced in silico by rewiring the connections between nodes specifically to minimize their total physical distance; but in doing so the characteristic path length was increased. This was the first rigorous example of a trade-off between topological and spatial properties of the connectome (Figure 1.7d).

These and other early graph theoretical studies of brain networks demonstrated proof of concept—they showed that the mathematical tools of graph theory were applicable to suitably simplified nervous systems. However, these analyses were limited to historical data on *C. elegans*, the cat or the macaque. Around 2005, the nascent field of connectomics took a decisive step closer to mainstream systems neuroscience with the development of processing pipelines that allowed the application of graph theoretic techniques to human neuroimaging data.

1.2.4 Neuroimaging and Human Connectomics

Since the 1980s, there had been growing interest in the idea of looking at the interactions between pairs of neurophysiological time series recorded simultaneously in two distinct anatomical locations. The simplest version of the analysis is to estimate the correlation coefficient between two time series: brain regions that demonstrate correlated patterns of signal change over time are then said to be functionally connected. More formally, **functional connectivity** is defined as a statistical dependence between the time series of measured neurophysiological signals. This concept was originally developed for the analysis of

spike trains recorded from single units (Gerstein and Perkel, 1969; Aertsen et al. 1989) and was then translated to the analysis of human functional neuroimaging data by Karl Friston, Barry Horwitz, Randy McIntosh, and others (Friston, 1994; McIntosh et al., 1996; Horwitz, 2003). The basic concept—that two locations can be said to be functionally connected if they have coherent or synchronized dynamics—generalizes to many different modalities of neurophysiological imaging, including functional MRI, electroencephalography (EEG), magnetoencephalography (MEG), multielectrode array (MEA) recording of local field potentials (either *in vivo* or *in vitro*), and positron emission tomography (PET; some of these methods are considered in more detail in Chapter 2). For example, analysis of functional connectivity by independent component analysis of resting-state functional MRI data has been used to identify a small set of around ten spatially distributed, large-scale neural systems showing coherent, low-frequency oscillations: so-called resting-state networks (Damoiseaux et al., 2006; Fox and Raichle, 2007; Smith et al., 2009; Fornito and Bullmore, 2010).

The first graph theoretic analyses of human brain functional networks were based on functional connectivity matrices estimated from functional MRI and M/EEG data (Stam, 2004; Eguíluz et al., 2005; Salvador et al., 2005; Achard et al., 2006). In this work, correlation or coherence between time series recorded at different brain locations (nodes) was estimated for every possible pair of nodes and pair-wise correlations were arbitrarily thresholded to define binary edges constituting a graph of the large-scale functional network (Figure 1.9a; Chapters 2 and 3). This work has shown that human brain functional connectivity networks show similar organizational properties to those that had been discovered in the anatomical networks of the macaque, cat, and *C. elegans*, as well as many other naturally complex systems. For example, functional MRI networks are small-world, contain hubs, have a hierarchical modular structure, and appear to be constrained by a pressure to minimize wiring costs (approximated by the Euclidean distance of edges; Vértes et al., 2012; Figure 1.9b). Indeed, trade-offs between spatial constraints (minimization of wiring cost) and topology are a prominent and heritable feature of human brain networks (Bassett et al., 2010; Fornito et al., 2011b; Betzel et al., 2016).

The first graph theoretic analyses of human brain anatomical networks were based on tractographic analysis of diffusion MRI data (Hagmann et al., 2008; Figure 1.9c–e) and structural covariance analysis of conventional MRI data (He et al., 2007; Alexander-Bloch et al., 2013a). Regardless of the method of construction, such human brain anatomical networks have consistently been found to show the same nontrivial organizational properties discovered in other types of brain networks.

While this convergence between brain graphs constructed from tract tracing, structural MRI, diffusion MRI, and functional MRI is encouraging, it is important to note that functional connectivity and structural connectivity are quite

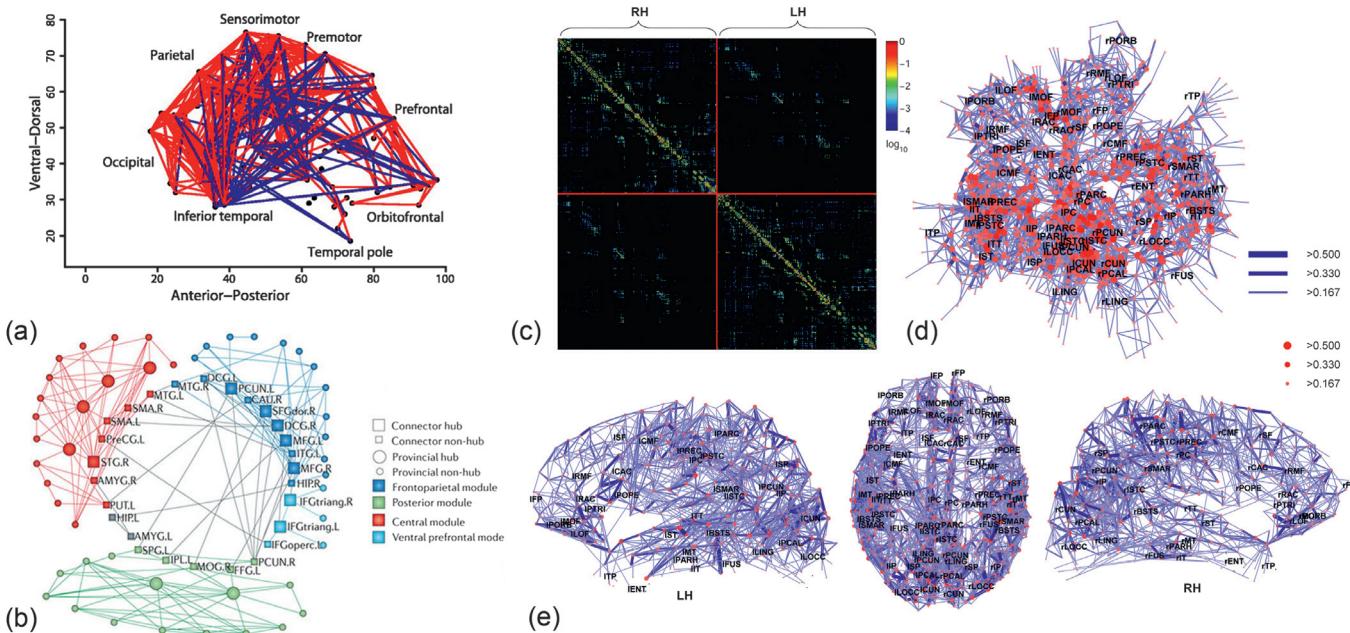


FIGURE 1.9 Brain graphs from human magnetic resonance imaging (MRI). **(a)** Brain graphs can be constructed to represent functional connectivity networks. This is an unweighted, undirected graph representing thresholded correlations (edges) between pairs of resting-state functional MRI time series recorded simultaneously from 90 cortical and subcortical areas (nodes). The nodes were defined by an anatomical parcellation and are located in anatomical space; red edges represent shorter distance connections and blue edges represent longer distance connections. **(b)** The modular organization of a functional MRI graph is shown in a topological configuration. Major modules are differently colored and the connector hubs, which mediate the majority of intermodular links, are highlighted as square nodes. **(c)** Diffusion MRI **tractography** can be used to construct a weighted, undirected connectivity matrix, where each element quantifies the density of axonal projections between a specific pair of regions. This network can be displayed either as a brain graph projected using an algorithmically defined layout to emphasize topological aspects of network organization **(d)**, or with nodes positioned according to their anatomical location **(e)**. In these graphs, edge thickness is proportional to connectivity weight and node size is proportional to the degree of each node. **(a)** Reproduced from Achard et al. (2006), **(b)** from Bullmore and Sporns (2012), and **(c–e)** from Hagmann et al. (2008) with permission.

different concepts in many ways ([Chapter 2](#)). Anatomical connectivity can be defined most fundamentally as an axonal projection from one cell to another, or at coarser spatial scales by a tract of axons projecting from one area to another. Such axonal projections are expected to change only slowly over time. In contrast, functional connectivity is a statistical measure of synchronized activity that does not necessarily imply an underlying anatomical connection, and which can change rapidly over time.

So far, most graph theoretic studies of functional connectivity networks have summarized coherent neurophysiological activity over an extended period with a single scalar metric, such as a correlation coefficient, effectively “averaging out” any dynamic changes in the network configuration over time. Functional connectivity when analyzed in this way is correlated with, although not identical to, anatomical connectivity ([Skudlarski et al., 2008; Honey et al., 2009](#)). In a computational model, the convergence between functional connectivity and the known underlying connectivity between nodes increased as a function of the period of time over which functional connectivity was averaged ([Honey et al., 2007](#)). Thus, as functional connectivity is averaged over longer time periods, it may converge onto structural connectivity, although it is important to remember that structural and functional connectivity are different measures and may thus yield connectomes with different values of some topological parameters ([Zalesky et al., 2012b](#)). Moreover, such a time-averaged analysis of functional connectivity neglects the physiological reality that the brain’s information processing systems must be rapidly reconfigurable in response to changing environmental conditions or experimental task demands ([Palva et al., 2010; Kitzbichler et al., 2011; Bassett et al., 2011; Fornito et al., 2012a](#)). Even in the so-called resting state it is clear that functional connectivity is not stationary ([Chang and Glover, 2010](#)) but spontaneously transitions through a series of different network configurations ([Zalesky et al., 2014](#)). The time-resolved analysis of functional network topology is likely to be an important focus for the future development of connectomics.

Despite the obvious methodological limitations of MRI, such as its limited resolution of network organization in space and time, and the uncertain neurobiological interpretation of MRI estimators of anatomical and functional connectivity, it nonetheless offers the best available tool for characterizing brain connectivity in living humans. MRI-based connectomics will likely continue to offer unique insights into the cognitive and clinical significance of certain organizational properties of brain networks.

The relationship between network topology and cognitive function is an important question that has so far been relatively under-explored, although initial work has shown that interindividual variations in the topological properties of structural and functional networks, including path length, cost-efficiency, and modular organization, correlate with variability in cognitive performance ([Li et al., 2009; van den Heuvel et al., 2009; Bassett et al., 2009, 2011; Zalesky et al., 2011; Fornito et al., 2012a; Dwyer et al., 2014](#)). Indeed,

the modular topology of the human connectome might be expected to correspond somehow to the neophrenological concept of modules as fast, specialized, automatic, faculties for algorithmic information processing (Fodor, 1983). In support of this prediction, meta-analysis of more than 1000 primary functional MRI studies of brain activation showed that the modules of the inter-regional coactivation network were functionally specialized for specific cognitive processes (Crossley et al., 2013). For instance, perception was represented by a module comprising areas of visual cortex localized mainly in the occipital lobe; whereas action was represented by a module comprising areas of motor cortex. Conversely, it may be that other topological features, such as connector hubs and intermodular connections, support more conscious, effortful, domain-general cognitive processes that depend on the costly, integrated activity of a global neuronal workspace (Dehaene et al., 1998). For example, in the same meta-analysis of task-related functional MRI data (Crossley et al., 2013), the most topologically central subset of regions, comprising a rich club in frontal and parietal association cortex, was associated with the greatest diversity of experimental tasks, especially “higher order” executive tasks demanding both cognition and action (Figures 1.10 and 1.11). Using ultra-high field functional

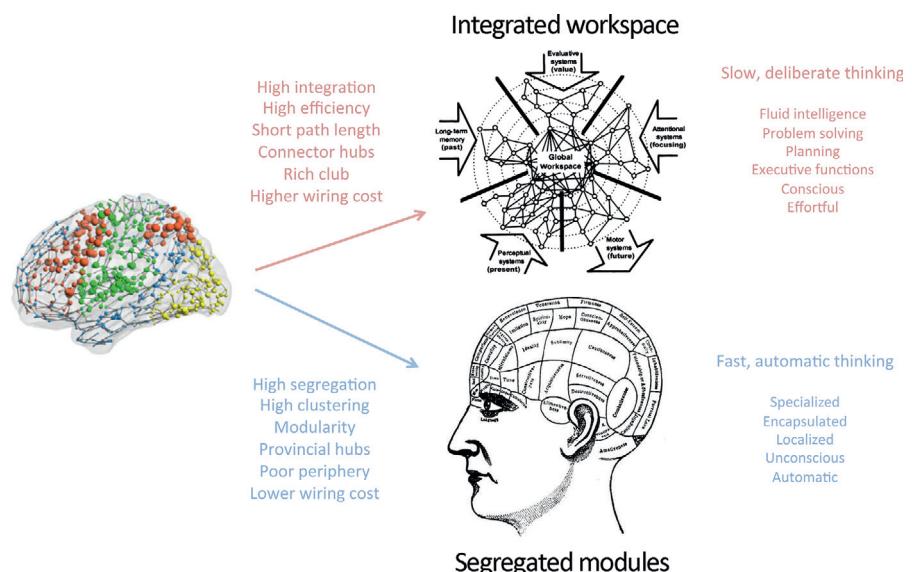


FIGURE 1.10 Schematic linking brain network topology and psychological function. Complex brain network topology can be subdivided into (top stream) high-cost components supporting a so-called global workspace architecture that favors integrated information processing, deliberate thinking, and flexible intelligence; and (bottom stream) low-cost components supporting a segregated architecture that favors fast, specialized, and automated information-processing. *Brain graph reproduced from Crossley et al. (2013) and workspace image from Dehaene et al. (1998), Copyright (2007) National Academy of Sciences, U.S.A., with permission.*

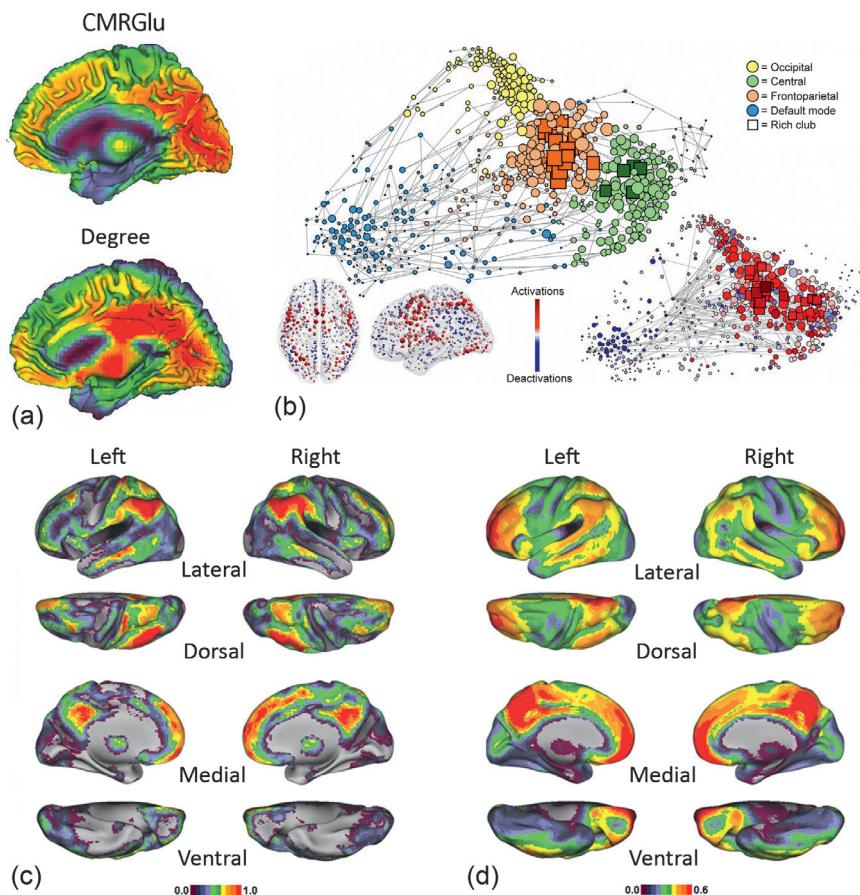


FIGURE 1.11 Human brain network hubs are biologically costly, cognitively valuable, and vulnerable to pathology. **(a)** Hubs are metabolically expensive. Shown here is the anatomical overlap of brain regions with high cerebral metabolic rates of glucose metabolism (CMRglu; top) measured using PET, and brain regions with high degree in functional MRI networks (bottom). **(b)** Hubs are activated by “higher order” cognitive tasks. Meta-analysis of coactivation patterns across more than 1000 task-based functional MRI experiments found that a rich club of hubs (marked as square nodes in a topological representation) was coactivated by a diverse range of experimental conditions, especially executive tasks involving both action and cognition. **(c)** and **(d)** Hubs are commonly implicated in clinical brain disorders. Shown here is the anatomical overlap between regions with high functional connectivity **(c)** and high levels of amyloid deposition measured by PET in patients with Alzheimer’s disease **(d)**.
(a) Reproduced from Tomasi et al. (2013), **(b)** from Crossley et al. (2013), and **(c, d)** from Buckner et al. (2009) with permission.

MRI to optimize the time resolution of functional network changes in response to briefly presented visual stimuli, it was likewise shown that conscious perception was distinctively associated with increased global integration and decreased modularity of network topology (Godwin et al., 2015).

MRI connectomics also has important clinical implications (Fornito et al., 2015; Box 1.1). One key observation that has already been made with remarkable consistency concerns the clinical significance of brain network hubs. In Alzheimer's disease, it was shown that high degree nodes in functional MRI graphs have greater local deposition of amyloid protein (measured using PET) than less topologically central brain regions (Buckner et al., 2009). Across a range of neurodegenerative disorders, node degree and other measures of topological centrality in functional connectivity networks have been positively correlated with local gray matter atrophy measured using MRI (Zhou et al., 2012). More generally, a meta-analysis of case-control MRI studies of 26 different brain disorders demonstrated that the probability of pathological loss of gray matter signal in a given brain region was significantly increased for regions

BOX 1.1 CLINICAL APPLICATIONS OF BRAIN NETWORK ANALYSIS

Like the ripples caused by a stone falling into a pond, pathological perturbations or lesions that are initially localized to specific parts of a nervous system often propagate along axonal fibers to affect the functioning of otherwise intact areas. The ancient Roman physician Galen (c. 129 CE to c. 200 CE) was among the first to note this fact, proposing that animal spirits could flow through nervous pathways to affect distant areas (Galen, 1976). More than 1800 years later, von Monakow (Finger et al., 2004; von Monakow, 1969) coined the term **diaschisis** (Greek for "shocked throughout") to describe the interruption of function in remote, intact brain regions that were connected to an injured site. The importance of neuronal connectivity in disease was also acknowledged by nineteenth and twentieth century proponents of clinicopathological correlation as a means for uncovering the network architecture of the brain (Section 1.2.2), culminating in Geschwind's (1965a,b) characterization of a new class of neurological "disconnection" syndromes.

Connectomics and graph theory offer a powerful framework for mapping, tracking, and predicting patterns of disease spread in brain disorders (Fornito et al., 2015; Stam, 2014; Figure 1.1.1; see also Box 11.2). From this perspective, brain changes in disease can be characterized at the level of network connectivity or topology, and the development of statistical techniques that allow for valid inference on group differences is an active area of research (Chapter 11). The

clinical application of connectomics is now confirming the early insights of Galen, von Monakow, and others by demonstrating that the propagation of pathology in the brain is indeed constrained by its network architecture. For example, neurodegeneration occurs in functionally and structurally connected networks (Raj et al., 2012; Seeley et al., 2009), pathology accumulates in highly connected hub areas of the brain (Buckner et al., 2009; Crossley et al., 2014), and the cognitive sequelae of brain injury or disease are closely related to the connection topology of the affected region (Warren et al., 2014).

Computational models of large-scale brain network dynamics allow neural activity to be simulated on structural network architectures (Deco and Kringelbach, 2014; Deco et al., 2008), and have shown that the functional effects of virtual "lesions" induced *in silico* are determined by the connection topology of the lesioned nodes (Alstott et al., 2009; Cabral et al., 2012; Honey and Sporns, 2008). Moreover, the therapeutic efficacy of invasive and noninvasive brain stimulation therapies across a range of disorders is critically related to the connectivity of the site targeted for stimulation (Fox et al., 2014; Riva-Posse et al., 2014). These findings suggest that an understanding of network topology may allow us to predict expected levels of impairment and prospects for recovery following insult (Fornito et al., 2015), and to select individually tailored interventions with maximum chances of therapeutic success.

BOX 1.1 CLINICAL APPLICATIONS OF BRAIN NETWORK ANALYSIS—CONT'D

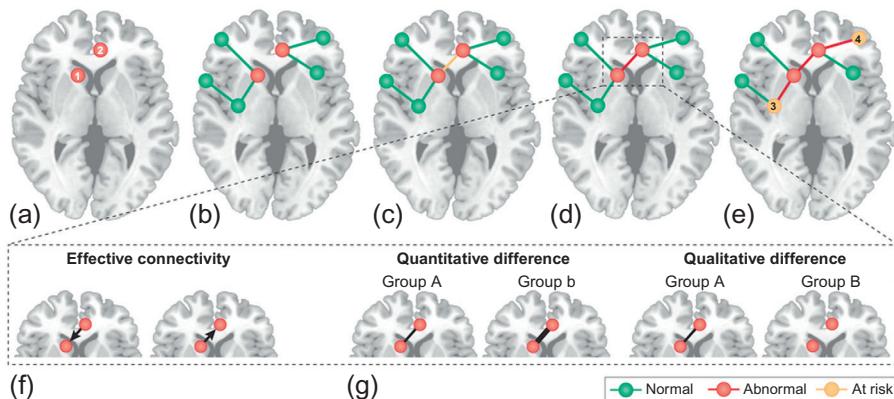


FIGURE 1.1.1 Using brain graphs to map, track, and predict patterns of disease propagation across the connectome.

(a) Historically, most studies of clinical disorders have focused on characterizing pathology in discrete brain regions. For example, in a typical human MRI experiment, a patient and control group are compared on some measure of tissue structure or function at many different locations in the brain. The result is a map identifying areas of localized group differences. In this example, two regions, 1 and 2, show an abnormality in the patient group. This map localizes the abnormalities, but offers no further information about whether these changes are related in some way or are independent. (b) Mapping the connectivity of the brain reveals the broader network context of the local changes. In this case, we see that regions 1 and 2 are connected to other areas, but are not connected to each other, suggesting that they are subject to independent pathophysiological processes. (c) Regions 1 and 2 are connected, but the connection itself is not abnormal. In this case, the pathology may have originated in one area and spread to the other along the intact pathway. Identifying the primary abnormality would require longitudinal analysis, or a model of the causal interactions between regions (i.e., **effective connectivity**, see Chapter 2). The connection between regions 1 and 2 is considered “at-risk” of subsequent deterioration because it links two dysfunctional areas. (d) Regions 1 and 2 and their connection are abnormal. In this case, primary pathology in one area may have resulted in secondary deterioration of its axons and, subsequently, the other area. Alternatively, a primary pathology of the connecting fiber bundle may have caused dysfunction in both regions. (e) Dysfunction in regions 1 or 2 can alter their connectivity with other areas, and place those other areas (regions 3 and 4) at risk of deterioration. (f) Resolving directions of information flow and building a model of effective connectivity allows a more precise characterization of directions of disease spread in brain networks. For example, if region 2 projects to 1, but 1 does not project to 2 (left), it is more likely that pathology propagated from region 1 to 2 than vice versa. (g) Connectivity differences between patients and controls can be either quantitative (left) or qualitative (right). A quantitative difference occurs when there is a difference in connection strength, but patients and controls share the same underlying network architecture. A qualitative difference occurs when a connection is present in one group but not in the other. *Reproduced from Fornito et al. (2015) with permission.*

with high degree in the healthy connectome (Crossley et al., 2014). These and other findings strongly suggest that topologically integrative, but biologically costly network hubs, may be points of special vulnerability for the expression of pathogenetically diverse brain disorders (Figure 1.11).

At a more mechanistic level, computational modeling suggests that the spatial pattern of neurodegeneration in Alzheimer’s and frontotemporal dementia

can be predicted by a relatively simple process of disease diffusion simulated on connectomes reconstructed from diffusion MRI (Raj et al., 2012). Other work indicates that neurodegeneration occurs within structurally and functionally connected networks (Seeley et al., 2009). Collectively, these findings suggest that the topology of large-scale brain networks constrains the way in which neurodegenerative disease spreads throughout the brain. To paraphrase Hebb, it seems that brain areas that are wired together tend to die together. It has also been shown that local reductions of cortical thickness in patients with childhood-onset schizophrenia, a neurodevelopmental disorder, were concentrated within a single module of brain regions that shared a common developmental trajectory. In other words, the normal adolescent processes of brain network development may constrain the expression of brain abnormalities associated with schizophrenia and other psychiatric disorders (Alexander-Bloch et al., 2014). MRI measures of topological centrality and modular organization could thus be important biomarkers for early diagnosis and prediction of clinical outcomes in neurology and psychiatry (Bullmore and Sporns, 2012; Stam, 2014; Fornito et al., 2015).

1.2.5 Back to Basics: From Macro to Meso and Micro Connectomics

By this account of recent history, graph theory impacted decisively on neuroscience at the microscopic scale of *C. elegans* (1998); then jumped to the mesoscopic scale of tract-tracing data on the cat and the macaque (circa 2001); and then translated to the macroscopic scale of human neuroimaging (circa 2005). In the decade since then, graph theoretical studies based on MRI, MEG, and EEG have rapidly proliferated and much of the methodological and conceptual development of connectomics has rested on analysis of human neuroimaging data representing macroscale networks ($\sim 10^2$ m). An exciting recent development, and one that promises to be game-changing for the next decade of connectomics, is the increasing availability of high quality data on brain networks at mesoscales ($\sim 10^{-4}$ m) and microscales ($\sim 10^{-6}$ m).

At the mesoscopic scale of tract-tracing, advances in the automation and standardization of experimental procedures, and improvements in how tracer propagation is imaged and quantified, have driven rapid changes in the scale and richness of data available on the mouse and the macaque monkey (Kennedy et al., 2013; Oh et al., 2014; Zingg et al., 2014). These datasets are sufficiently comprehensive, comprising tens or hundreds of individual tracer injection experiments, to support estimation of the anatomical connectivity between all possible pairs of brain regions defined according to specified anatomical criteria and standard experimental procedures (Figure 1.12; see also Chapter 2).

Pioneering studies using such techniques in the macaque and the mouse have demonstrated that the weight of anatomical connectivity varies over five orders

of magnitude; and that mesoscale connectomes may be more densely connected (with a **connection density** over 60%, in some cases) than had been assumed, due to the presence of many weak and previously unrecognized connections (Markov et al., 2014; Oh et al., 2014). The connectivity matrices generated by these methods can be used to construct **directed graphs**, because tracer propagation is either anterograde or retrograde from the injection site (Figure 1.12). The resulting graphs can also be weighted according to the number or fraction of neurons in the connected region that have been labeled by the tracer, thus providing an estimate of connectivity strength. As will be evident throughout this book, many graph theoretic techniques are readily applicable to such weighted and directed connectomes and often enable a richer characterization of network topology.

At a microscopic scale, there are many major efforts underway to map cellular networks in model organisms ranging from *Drosophila* (Chiang et al., 2011) to zebra fish (Ahrens et al., 2013), and these are supported by significant advances in technologies for reconstructing single cells and their synaptic connections (Lichtman et al., 2008). In the near future, there will certainly be a wave of data available to support graph theoretical analysis of anatomical networks at

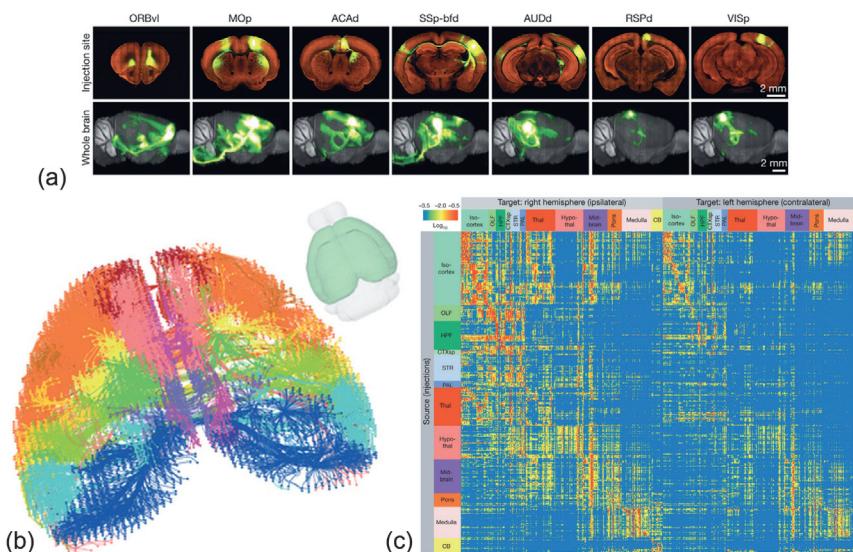


FIGURE 1.12 Large-scale standardized mesoscale connectomics in the mammalian brain. The automation of tissue processing, dissection, and imaging pipelines has enabled the construction of mesoscale connectomes in model species with viral tract tracing. Shown here is one specific pipeline used to map neuronal connectivity in the mouse brain. Separate injections to different brain regions in different animals can be used to map regional connection profiles (a), which can then be tracked and collated to map connectivity between multiple brain regions (b). These data can be used to generate an interregional connectivity matrix (c). Figure reproduced from Oh et al. (2014) with permission.

cellular scale, and to link cellular characteristics to mesoscale and macroscale properties of inter-regional connectivity (Figure 1.13a). It is also likely that there will be greater interest in the graph theoretical analysis of functional networks recorded from neuronal cultures at cellular scale by MEA. In these datasets, each electrode measures a local field potential and the functional connectivity between each pair of electrodes can be estimated to construct a network where nodes represent electrodes (each recording from a few local neurons), and edges represent highly synchronized electrical activity. Preliminary results have again demonstrated the apparent ubiquity of complex topological features: MEA graphs have hubs, cores, modules, and small-world organization, and develop *in vitro* according to a rich-get-richer rule (Downes et al., 2012; Schroeter et al., 2015; Figure 1.13b). This experimental paradigm clearly offers some attractive opportunities for future research to manipulate

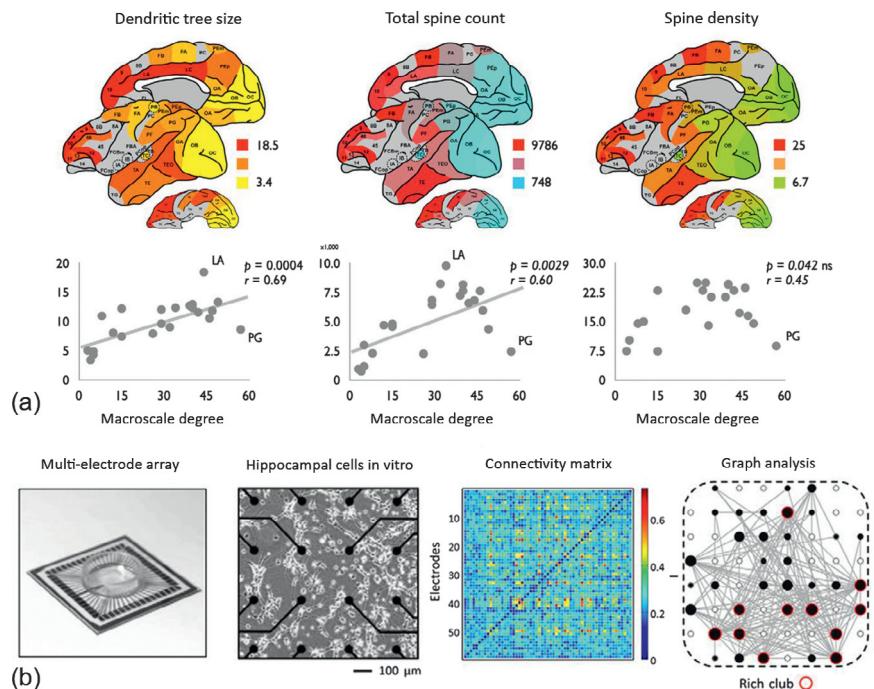


FIGURE 1.13 New approaches to connectomics. (a) Integrating databases that describe regional variations in microscale neuronal properties and macroscale connectivity patterns allows analysis of the relationship between regional cytoarchitecture and interregional connection topology. For example, high degree nodes in the macaque cortical network have higher dendritic tree size and higher spine count. (b) Multi-electrode array recording is another technology with potential to support greater understanding of networks at the cellular scale. Neuronal cultures are grown on an electrode array and the functional connectivity between local field potentials recorded from each pair of electrodes can be summarized as an association matrix and thresholded to generate binary undirected graphs with hubs and rich clubs. (a) Reproduced from Scholtens et al. (2014) and (b) from Schroeter et al. (2015), with permission.

the system pharmacologically, or optogenetically, and so elucidate the molecular and cellular mechanisms driving preferential attachment, the emergence of hubs, and other topological features of structural and functional cellular networks.

1.3 ARE GRAPH THEORY AND CONNECTOMICS USEFUL?

This might seem like an odd question to ask at the end of the first chapter of a book about analyzing brain networks. But any new method demands critical evaluation. In our view, there are two factors arguing in favor of graph theory for neuroscience: its simplicity and its generalizability.

Graphs are fairly simple models. In fact, they are often ruthless abstractions of minutely detailed natural systems, by which the myriad biological intricacies of the brain are reduced to a collection of (often homogenous) nodes and edges. Simplicity is always attractive theoretically, especially in the age of “big data” when there is a real risk of being overwhelmed by terabytes of biological measurements. Moreover, the mathematics of graphs are rigorous while also being accessible to neuroscientists, physiologists, and biologists who may not have a formal training in quantitative sciences. It certainly helps that many of the key concepts of connectomics can be represented graphically and illustrated by analogy to well-known complex systems, such as the global airline network. A brain graph thus has the benefit of providing a conceptually simple model implemented using an accessible mathematical language. However, does a brain graph entirely follow the instruction, sometimes credited to Einstein, that “everything should be made as simple as possible, but not simpler”? Or are brain graphs not so much attractively simple as unacceptably simplistic?

The answer to this question may be in the goals of the scientist. Certainly, a graph cannot be used to model every single detail of the brain. However, it offers a useful, simplified abstraction that allows us to formally address critical questions, such as how does brain network structure constrain function? What are the general organizational principles of brain networks? What developmental processes can give rise to networks that look and function like the brain? If those kinds of questions are of interest, then graph theory is useful. But the challenge to link topological metrics on abstract, simple graphs to biological mechanisms at cellular and molecular levels remains important and has provoked some interesting recent work. For example, the hubs of macroscopic brain structural networks derived from tract-tracing data show distinct microscopic properties related to neuronal morphology and density ([Scholten et al., 2014; Figure 1.13a](#)); associations have been found between network topology and local gene expression profiles ([Wolf et al., 2011; French and Pavlidis, 2011; Rubinov et al., 2015; Fulcher and Fornito, 2016](#)); and the hubs of human

functional MRI networks have been located in brain regions that have high rates of glucose metabolism measured by PET (Tomasi et al., 2013; Figure 1.11). These are all early examples of successful efforts to explore the local biological characteristics of nodal topological properties estimated by the systems-level mathematical analysis of graph theory. We anticipate that as more is known about the biological substrates of brain graphs, topological properties (like hubs and modules) will turn out to be meaningfully linked to underlying biological mechanisms, such as gene expression and developmentally determined patterns of variation in cytoarchitectonics and myeloarchitectonics. In this event, any current concern that brain graphs are unacceptably simplistic will naturally recede.

The generalizability of graph theory is its second major advantage. As we have seen, graphs have already been used to model a diverse array of complex systems. Graph theoretical methods can also be adapted to analyze gene expression arrays and other -omic biological datasets, which typically also have many of the same complex network properties as the connectome (Oldham et al., 2008). In neuroscience, the applicability of graph theory to all kinds of neuroimaging and neurophysiological data opens up the scientific opportunity to look at the same topological and spatial properties of brain networks across a wide range of species, scales, and modalities, thus providing a lingua franca for neuroscientists working in diverse domains. Multiscale measurements of brain networks could serve to translate insights about the topology, dynamics, development, and function of brain networks from one experimentally specialized field of neuroscience to another. Perhaps the ultimate scientific goal in this pursuit is the elucidation of very general selection pressures, or conservation laws, that can explain brain network organization from microscales to macroscales. Graph theory is well suited to address this and other strategic challenges in understanding the network organization of brains.

1.4 SUMMARY

We have seen in this chapter that the importance of understanding brain connectivity was apparent to pioneers, such as Ramón y Cajal and Golgi, from the moment they first peered through the microscope to observe the morphology of neurons and their processes. However, progress in subsequent years was slow, due to a lack of appropriate tools and conceptual frameworks for measuring, mapping, and simulating large-scale neuronal networks. The past few decades have witnessed unprecedented advances not only in our ability to map brain connectivity at micro-, meso- and macroscales, but also in our ability to quantify and generate the connection topology of such intricate systems. These developments have coincided with technical advances in methods for acquiring, curating, and disseminating large-scale data on brain connectivity,

and with the birth of a more general science of complex networks. In particular, graph theory has emerged as a powerful tool for developing a coherent understanding of brain network organization that cuts across spatial and temporal scales, and which allows us to understand how the connectome relates to a much broader class of naturally occurring complex systems.

Our goal in this book is to introduce the fundamentals of graph theory and network science, as applied to the brain. This book is not intended as a general introduction to the mathematics of graph theory and/or the physics of complex networks, for which we refer interested readers to the excellent text book on networks by [Newman \(2010\)](#), as well as detailed review articles by [Newman \(2003a\)](#), [Albert and Barabási \(2002\)](#), and [Boccaletti et al. \(2006\)](#). A more mathematical treatment is provided by [Bollobás \(1998\)](#). More general introductions to connectomics and brain networks have been provided by [Sporns \(2011a, 2012\)](#); see also [Buzsáki, 2006](#). In contrast to these texts, this book will focus in detail on the specific aspects of graph theory that are proving to be the most useful for understanding brain connectivity. We will examine measures that have been adapted from the study of other complex networks, as well as those that have been developed specifically in the neuroscientific context. We will also consider examples of how these measures and methods have been applied to understand brain network organization across a diverse range of species, resolution scales, and experimental techniques. In the next chapter, we begin our discussion of this work with a consideration of perhaps the most fundamental aspect of brain network analysis: how to define nodes and edges.