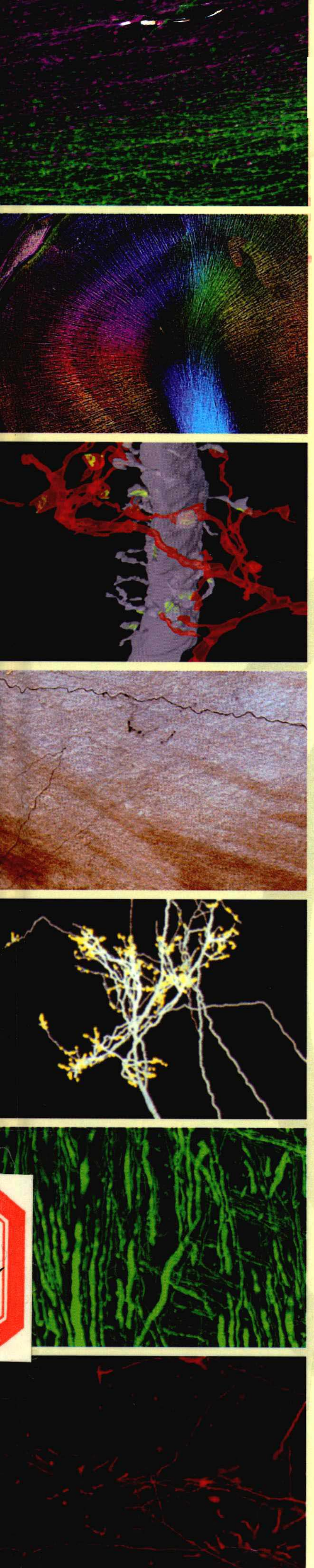

Axons and Brain Architecture

Edited by
Kathleen S. Rockland

AXONS AND BRAIN ARCHITECTURE

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Preface

ax·on
ˈakˌsän/
noun. mid-19th century (denoting the body axis): from Greek *axōn* "axis."
the long threadlike part of a nerve cell along which impulses are conducted from the cell body to other cells (Google).

Axons, and long-distance axons in particular, are attracting increasing attention as the anatomical reality underlying MR imaging techniques. They are also a vital component of microcircuitry. Their contribution to microcircuitry has been relatively neglected, owing to the incompatability of *in vitro* assays with long axons, but it can be expected to figure prominently in the ongoing efforts of whole brain analysis of connectivity (connectome). A major goal of this volume is to bring together in one source an interrelated treatment of single axons both from the perspective of microcircuitry and as substrates of larger scale organization (tractography and brain architecture). We hope by highlighting the span from microcircuitry, axon guidance, and dynamics, to tractography and brain architecture, that this will serve as a convenient reference and facilitate communication between what have tended to be separate communities.

For the sake of focus, the volume concentrates on mammalian systems, largely overlooking the extensive work on "wiring diagrams" of *Caenorhabditis elegans* and *Drosophila*. Myelination, axon transport, and pathological conditions are also only minimally addressed.

Section I is devoted to anatomical investigations of connections at the single axon level, including implications for cortical architecture in particular. A broad coverage of cortical and subcortical connections from different species serves to highlight recurring spatial patterns of divergence, convergence, and collateralization. A background chapter is included to review neuroanatomical tracing techniques in general, with focus on the newer, still developing virus-based tracers.

Section II addresses mechanisms of axon dynamics in normal and pathological conditions, and the temporal aspect (chronnectome) of axonal morphology and circuitry. Section III covers axon guidance in development: how these long distance axons identify and connect with postsynaptic targets, and sort along intermediate points.

Section IV focuses on imaging and tractography. Three chapters discuss histological parameters in relation to high-resolution MRI/Diffusion imaging; and the final chapter describes in detail the relatively new technique of polarized light imaging. This yields high-resolution visualization of nerve fibers in postmortem brains, by the birefringent property of myelin.

The labyrinth on the cover was made by Erwin Reißman, a retired surveyor. It primarily is meant to suggest the axon as an Ariadne's thread and guide through the labyrinth, but is also evocative of a myelinated axon. It was to some extent inspired by a comment of G.H. Bishop: "As a member of the only species capable of comprehending, or even of speculating on its own origins, and looking from this height down along the trunk of our sprawling family tree, no less a filament than the nerve fiber itself will serve as the thread of Ariadne along which may be retraced the course of our brain's evolution."

Reference

Bishop, G.H., 1965. My life among the axons. Annu. Rev. Physiol. 27, 1–20.

Foreword

This book is both timely and exciting in the context of the massive efforts dedicated worldwide (in Europe, United States, Japan, and China) to deciphering connectivity patterns in the brain; i.e., the wiring diagram (connectome) between various brain regions and within local circuits at the synaptic level. With the increasing use of powerful new methods for staining, tracing, stimulating, recording and imaging neuronal processes (e.g., CLARITY, optogenetics, light-sheet imaging), it is becoming increasing clear that brain connectivity is specific, and this is abundantly in evidence at the level of single axons, both at long distances and in local microcircuits.

This raises a few fundamental questions: How do axons navigate so precisely into the appropriate regions and find their target cells under normal conditions? What goes wrong with the route axons take in pathological conditions? What are the functional implications of such intricate circuitry for the processing of spatiotemporal sequences of neural events? For the most part, the consequences of specific innate connectivity for learning, for coding and decoding sensory information, and for implementing specific computations are far from being understood. This book provides an important foundation that will assist and expedite future theoretical advances toward this end.

This volume emphasizes that axons are dynamic devices, especially in the physiological realm. Axonal "hot regions" such as the axon initial segment (AIS), the nodes of Ranvier, and the axonal boutons (release sites), all are capable of undergoing plastic changes. In these sites, the spatial profile, density, and type of ion channels may go through temporal and spatial modifications—affecting the patterning of spikes at the AIS and modifying the impact (the read out) of these spikes at the axon's synaptic release sites.

A key new insight about axons is that a given train of spikes, generated at the AIS, could be interpreted differently by its many postsynaptic (dendritic) "listeners." For a given axon, some synapses might be powerful (generating large postsynaptic potentials (PSPs)) and other synapses might generate small or even no PSPs. Some synapses could have short-term depressing properties and other synapses, arising from the same axon, are facilitatory. These short-term dynamics are themselves remarkably plastic, due to dynamic machinery at both the presynaptic axonal side and at the postsynaptic (dendritic) side. The energy consumption of the axonal spikes is yet another new and fascinating topic of investigation—demonstrating that out of the 20W or so that our brain consumes, the axonal activity is energetically expensive.

The chapters in this book clarify that certain structural motifs, at both global and local scales, are innate and that others are learned. "Running" on this structural foundation are electrical signals (digital in axons and mostly analog in dendrites) representing in our brain the external world and our individual interpretations of it. Axons with their astute (although sometimes fragile, especially in certain diseases) "all or none" spikes serve as a major component in this representation/interpretation scheme. We have somewhat neglected this key component of neurons, as the neuroscience field became fascinated with dendrites—the major input regions of neurons. Now, this comprehensive book beautifully demonstrates that axons and dendrites, and their connecting synapses, work in orchestration to generate the "music" of the brain—a music that continuously changes–largely due to the morphological and biophysical flexibility of axons.

One of many personal inspirations that I took from this book has to do with the development of future computers/robots, behaving in a challenging and unexpected environment. To date, we have built computing machines with essentially static prewired connectivity. For these machines to actually compete with and aspire to replicate the amazing capabilities of the brain in solving real-life (e.g., action-perception) tasks, it seems wise to learn from the brain; that is, combine "innate" wirings (e.g., between particular brain/machine regions, such as the visual and the motor systems) and, at the same time, leave a significant degree of freedom and a crucial reservoir of modifiablity (including in the messages that are transmitted via these wires). We can look forward to brain-inspired machines that rewire themselves both locally and globally, as these seem best fit to handle tasks that require efficient learning and resourceful computations.

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Acknowledgments

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Many thanks also to the students, postdocs, and colleagues over the years who have joined in the work represented by these 18 chapters, and without whose dedication and enthusiasm, little of this would have been possible.

Introduction

Axons figure in axoplasmic transport as substrates of the nerve impulse, as pathfinders in development, as the "wires" in connectivity wiring diagrams. They are at once key players in basic molecular processes, related to axon transport, and presynaptic components of neuronal circuitry and larger scale neural networks. Except for local axons of interneurons and other nonprojecting neurons, axons are long—typically many millimeters in length. This simple fact makes them less accessible to *in vitro* investigations than the more spatially delimited dendrites, and accordingly has led to a comparative neglect despite their central importance.

While research on axons covers as many levels as neuroscience proper, (Waxman et al., 1995; Bagnard, 2007; Feldmeyer and Lubke, 2010; Goldberg and Traktenberg, 2012) this volume is focused on neuroanatomical questions and techniques, largely in relation to a mesoscale of connectivity (i.e., light microscopic). For the sake of general orientation, this short Introduction will briefly address early history of the axon, experimental techniques, and basic questions relating to axons *per se*.

I.1 EARLY HISTORY

Axons have a central place in the history of neuroscience. As macroscopic fiber bundles, axons were already identified by Vesalius in the sixteenth century, predating by several hundred years the identification of neuron cell bodies. The Renaissance investigators in fact sound remarkably modern in their observations of white matter: "To say that the white matter is but a uniform substance like wax in which there is no hidden contrivance, would be too low an opinion of nature's finest masterpiece" (Niels Stenson, quoted in Schmahmann and Pandya, 2006 and see their section "The Era of Gross Dissection").

Closer to the present time, axons were at the heart of at least three major waves of discovery. One is cell biological research on the phenomenon and mechanisms of axonal transport. The early stage of this work coincided with World War II and the effort to improve recovery after peripheral nerve damage. The simple device of nerve constriction demonstrated characteristic swelling, beading, and coiling proximal to the constriction. This was correctly interpreted as an indication of what was initially called "axoplasmic flow" (later modified to "axonal transport," to connote the active nature of the "flow") and inaugurated decades of concentrated research regarding its components, mechanisms, and significance (see Grafstein and Forman, 1980 for an early review).

Second is the pioneering physiological work on the propagation of the nerve impulse and its capstone in the great Hodgkin–Huxley model. Part of this stage is summarized, in the refreshing context of close-up personal experience, in Bishop (1965). Again, the reader will recognize distinctly modern resonances on issues that remain under active discussion, among others: the mystery of unmyelinated axons, the functional significance of the spectrum of small, medium, and large axons; and the increase in the proportion of large fibers in evolution (cf. Perge et al., 2012; Wang et al., 2008). The tone is extraordinarily open-minded and questioning. We hear Bishop, who has spent his research career as a neurophysiologist, pondering, "What is a nerve fiber for, anyway?.... Conduction of an impulse is in fact somewhat incidental to another essential functioning of a neuron, however useful as a sign that the neuron has functioned. Where does one come out if he looks at the neuron as a secretory organ?... To wit: the prime function of a neuron is to produce and apply to other tissues a chemical activator" (Bishop, 1965, p. 12).

Third, in the anatomical domain, axons were of course the focus of heated controversies between "neuronists" and "reticularists" at the end of the nineteenth century. The debate, as is well-known, was resolved in favor of the neuronists, of whom Ramon y Cajal has been generally acknowledged as a key representative. The story has been frequently told, but I would refer the reader to the thoughtful essays of Guillery (2005, 2007). Guillery (Deiters and Guillery, 2013) also has coauthored a biographical account of Otto Deiters who, by fine microdissection of a single large neuron from the ventral horn of the spinal cord, correctly distinguished between the cell's multiple dendrites and its single axon. In effect, this was the first observation of the neuron as a polarized structure, and set the stage for subsequent rigorous work by Cajal and others in support of the neuron doctrine.

The time of Ramon y Cajal and Camillo Golgi, at the beginning of the twentieth century (taking 1906, the year of their shared Nobel Prize award as a reference point), ushered in the highly productive application of the Golgi silver impregnation technique, which lasted well beyond mid-century. Despite the very real limitations of the technique, investigators such as Lorento de No and F. Valverde in Spain, Szentagothai in Hungary, and in the United States, the Scheibels, Ray Guillery, E.G. Jones, and J.S. Lund, among many others, constructed a substantial data set of single axon visualization across the brain of several species. To this day, Golgi preparations are one of the few techniques, with immunohistochemistry, that can be used effectively to visualize cellular and neuropil components in the human brain (e.g., Marin-Padilla, 2015).

I.2 FROM 1970S: THE AGE OF TECHNICAL ADVANCES

I.2.1 Tracer Injections

In the early 1970s, connectivity experiments were transitioning to tracer substances that were transported anterogradely (^{3}H amino acids and autoradiography) or retrogradely (horseradish peroxidase (HRP)) through physiological mechanisms of axonal transport (Morecraft et al., 2015). This was a welcome improvement over the earlier lesion–degeneration methods; but one negative side effect was a decreased attention to the actual projecting axons. Extracellular tracer injections, even if involving only a small volume of cortex (less than 250 μm in diameter), result in what is in effect an average of large numbers of retrogradely filled cells or anterogradely labeled axons and their terminations. The axons of retrogradely labeled neurons are typically not labeled, or labeled only proximally; and many of the anterograde tracers, especially the first generation (e.g., WGA-HRP and autoradiography) result in only dustlike terminal label. Tracers that do produce Golgi-like detail of axon fibers and terminals (PHA-L, biocytin, BDA, and most recently, viral vectors) require laborious collection of histological sections in uninterrupted series and even more laborious reconstruction of labeled profiles across the series—an effort not commonly undertaken. These shortcomings persist to the present, although newer tracers are more successful in demonstrating projections in their entirety (see Section I), and the problem of three-dimensional visualization is being vigorously addressed in Chapter 10 by Beier and Chapter 17 by Mortazavi et al.

I.2.2 Single Axon Visualization

The next step in single axon analysis, after Golgi staining, was the advance to *in vivo* and *in vitro* techniques, where anatomical visualization was achieved by microinjection of HRP in the white matter, by intracellular injection (first, of HRP and subsequently of biocytin or BDA), or by juxtacellular injections (see Section I). Intracellular injections were often subsequent to microelectrode physiological recording, with the obvious advantage of thereby producing a combined data set of physiological and anatomical characteristics.

The approach of intraaxonal recording and HRP fills was instrumental in elucidating basic structural–functional correlations in several systems. Investigations of the retinogeniculocortical projections were among the earliest results. Physiologically identified retinofugal axons were found to have distinct phenotypes and target-specific arborizations that clearly differed in spatial geometry and numbers of boutons (Bowling and Michael, 1980). The projections from lateral geniculate nucleus to visual cortex were mapped by the same method in nonhuman primate (Blasdel and Lund, 1983) and cat (Freund et al., 1985; Humphrey et al., 1985). In both species, distinct structural features were demonstrated that correlated with the known physiological properties of magno- and parvocellular geniculocortical projections; namely, focused parvocellular axon arbors versus the more divergent magnocellular associated, respectively, with color and form sensitivity or transient, contrast-sensitive responses.

Among many other results from this or similar approaches are visualization of: thalamocortical axons to the somatosensory cortex (by microinjections of HRP in the white matter: DeFelipe et al., 1986; Garraghty and Sur, 1990); corticostriatal axons in rat (Cowan and Wilson, 1994); corticothalamic terminations from cat auditory cortex (Ojima et al., 1992). In one groundbreaking study, Katz (1987) injected retrograde tracer *in vivo* to prelabel two populations of projection neurons, and then visualized and intracellularly filled these in a subsequent *in vitro* experiment. The slice preparation did not preserve long distance projection axons, but did allow stunning detail of local intracortical collaterals. In particular, laminar patterns of local collaterals and of dendritic arbors were found to be target-specific, related to extrinsic projections to the claustrum or thalamus.

More recently, combined physiological–anatomical intracellular labeling has become less common. In part, these experiments are not particularly well suited to the investigation of higher order cortical areas, where the neuronal populations are more intermixed, less accessible to intracellular recording and filling, and results are more difficult to interpret than for primary or early sensory cortical areas. For nonhuman primates, another factor in the waning popularity of these experiments is that the awake behaving chronic preparation is now preferred to old-style acute physiological experiments in anesthetized animals. Anatomical studies may be returning, with the renewed interest in the circuitry substrates underlying behaviors and the increasingly routine use of the more controlled optogenetic methods.

The advent of reliably Golgi-like anterograde tracers, such as BDA (~1995) and a repertoire of anterogradely transported viral vectors (see Chapter 10 and Morecraft et al., 2015), gave rise to a small number of anatomical studies where projections were mapped at the level of individual axons. Since axons were typically labeled extracellularly, by relatively large injections, usually only their distal portions, beyond the zone of high density labeling, could be successfully analyzed. Individual axons continue to be investigated, especially in rodents, by the juxtacellular recording-labeling technique (see Section I, and Chapter 2); and injections of low titre Sindbis virus have been particularly successful in demonstrating whole-axon arborizations, again, mainly in rodents (Chapters 1, 3, and 4).

I.3 AXONAL PHENOTYPES IN NEUROANATOMY

Axons are complex in morphology, cell biology, and physiology, in ways that are only partially understood. Axons and their specializations are clearly bound up with the most basic properties of neural organization, from propagation of the nerve impulse to network organization. Elucidating the detailed neuroanatomical phenotypes, as revealed by single axon resolution (see Section I), has functional significance in several domains.

One is the visualization of branching patterns in relation to network organization. In rodents and to some extent primates, extrinsic axons branch (collateralize) to multiple targets. Injections of two or three retrogradely transported, color-differentiated tracers are a popular way of investigating this collateralization. However, injections, even if large, may not involve the relevant termination sites in all the recipient areas. As these are often limited to a territory smaller than 250 µm, the risk of false negatives is high (Rockland, 2013). In addition, while retrograde tracers can identify the cell bodies of collateralizing neurons, they do not show the actual branch points nor the differential density and patterns of termination in the several targets. Visualization of specific branch points is relevant for understanding fasciculation and white matter composition, and thus both for interpreting diffusion images and for better understanding of white matter pathologies.

A second domain is the generation of quantitative data to support neuroanatomically realistic models. Specific data on branching (e.g., branching angle, branch caliber) directly relate to calculations of conduction velocity; and specific data on the distal terminal portions (e.g., size and topology of terminal arbors, and size and number of terminal specializations) provide baseline parameters relevant for microcircuitry and cross-area and cross-species comparisons (Shepherd, 2004 and Chapters 5–7 and 15). For quasiregular structures, such as the cerebellum and hippocampus, a substantial body of morphometric data is available; but even in those cases, major gaps such as subtype specificity and variability remain (Ropireddy et al., 2011). How these parameters are associated with short- or long-term dynamics is a further major area of research (Chapter 12).

A third domain is the categorization into different "types." A few axons are morphologically distinctive, with recognizable phenotypes that are often phylogenetically conserved. Cerebellar parallel fibers are identifiable by their T-shaped branch and narrowly divergent arbors; cerebellar climbing fibers by virtue of their characteristic association with Purkinje cells. Hippocampal "mossy fibers" are distinguishable on the basis of their large boutons, neuronal origin from dentate granular cells, and postsynaptic targets. Corticostriatal afferents are loosely "cruciform" (Cowan and Wilson, 1994, and Chapter 9 in Shepherd, 2004).

Corticothalamic afferents constitute two large groups, in this case based on bouton size (large or small), the shape of terminal arbors (focused or divergent), and the neurons of origin. Classically, large boutons, preferentially targeting proximal sites of postsynaptic neurons, were termed "type 2" (type II or round large (RL)) and small boutons were termed "type 1" (type I or small round (SR)) and targeted mainly distal postsynaptic locations (Ogren and Hendrickson, 1979; Schwartz et al., 1991; Crick and Koch, 1998; Rouiller and Welker, 2000). Work in the 1990s established a further dissociation, according to neurons of origin, such that type 1 (small bouton) originated from neurons in layer 6 and type 2 (large bouton) originated from neurons in layer 5. The two types were considered as having qualitatively different postsynaptic influence (Sherman and Guillery, 2006), as either "driving" (large bouton) or "modulatory" (small bouton). With shifting focus on the physiological roles of the two populations, the favored nomenclature has been revised (Lee and Sherman, 2011) such that "type 1" now often designates "driving" (i.e., classical type 2, RL) and "type 2" designates "modulatory (i.e., classical type 1, SR).

Bouton size, arbor shape, and parent cell identity also dissociate amygdalocortical and amygdalothalamic connections. Namely, the former have small boutons and a divergent arbor, while the latter have large boutons and a small arbor (Miyashita et al., 2007). Corticocortical projections have been broadly classified on the basis of parent cell location, preference of termination layer, and shape of terminal arbor; but there are less obvious differences in bouton size, with the exception of some large neurons, primarily in layer 5. More subtle differences, related to number of beaded versus stalked terminations (respectively, boutons *en passant* and boutons *terminaux*) are still under investigation (Chapters 5 and 6).

Issues of types and diversity of subtypes, based on axonal morphology or correlation with parent cells, are further discussed in several chapters in Section I, as is the significance of different spatial configurations of the distal arbor.

I.4 AXONAL SUBDOMAINS

While the present volume is geared to a relatively meso, light, microscopic scale of organization, important new directions on axon structure and function are emerging from rapidly progressing work on subcellular domains and patterning. The fact of axonal compartments is not new. Myelin and nodes of Ranvier were discovered in the nineteenth century (by, respectively, R. Virchow and L.-A. Ranvier); modern electron microscopic and molecular investigations have provided a wealth of further detail on protein composition (Debanne et al., 2011). Axonal microstructural specializations include differential spacing of nodes of Ranvier; the large heterogeneity of axon initial segments of different neuronal populations, as assayed by subunit composition; density and subcompartmental distribution of voltage-gated sodium and potassium channels (Lorincz and Nusser, 2008); and very recently, the discovery of a periodic actin ring-like organization (D'Este et al., 2015). These aspects can be expected to have implications for microcircuitry, subtypes, and brain architecture; and the cross-disciplinary integration of microstructure data with network organization will be an exciting next phase of brain research.

I.5 OUTLOOK

Looking back, for axon research as for the neuroscience field as a whole, there has been appreciable and impressive progress. The next stages are hard to predict. There will be more data (big data) and better techniques. With the increase in data, the need for synthesis and new theories becomes ever more apparent. Possibly, as expressed in many of the chapters in this book, we are at a crossroads point, where old ideas look less compelling, but newer formulations are not yet in place. Much to do, in moving forward.

Readings

For further in-depth treatment of related topics, the reader is referred to several excellent monographs and selected references, listed below.

Bagnard, D., 2007. *Axon Growth and Guidance*, Advances in Experimental Medicine and Biology. Springer Science, NY.
Bishop, G.H., 1965. My life among the axons. Annu. Rev. Physiol. 27, 1–20.
Blasdel, G.G., Lund, J.S., 1983. Termination of afferent axons in macaque striate cortex. J. Neurosci. 3, 1389–1413.
Bowling, D.B., Michael, C.R., 1980. Projection patterns of single physiologically characterized optic tract fibres in cat. Nature 286, 899–902.
Cowan, R.L., Wilson, C.J., 1994. Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. J. Neurophysiol. 71, 17–32.
Crick, F., Koch, C., 1998. Constrains on cortical and thalamic projections: the no-strong loops hypothesis. Nature 391, 245–250.
Debanne, D., Campanac, E., Bialowas, A., Carlier, E., Alcarez, G., 2011. Axon physiology. Physiol. Rev. 91, 555–602.
D'Este, E., Kamin, D., Gottfert, F., El-Hady, A., Hell, S.W., 2015. STED nanoscopy reveals the ubiquity of subcortical cytoskeleton periodicity in living neurons. Cell Rep. 10, 1246–1251.
DeFelipe, J., Conley, M., Jones, E.G., 1986. Long-range focal collateralization of axons arising from corticocortical cells in monkey sensory-motor cortex. J. Neurosci. 6, 3749–3766.
Deiters, V.S., Guillery, R.W., 2013. Otto Friedrich Karl Deiters. J. Comp. Neurol. 521, 1929–1953.
Feldmeyer, D., Lubke, J.H.R., 2010. New Aspects of Axonal Structure and Function. Springer, NY.
Freund, T.F., Martin, K.A., Whitteridge, D., 1985. Innervation of cat visual areas 17 and 18 by physiologically identified X- and Y-type thalamic afferents. I. Arborization patterns and quantitative distribution of postsynaptic elements. J. Comp. Neurol. 242, 263–274.
Garraghty, P.E., Sur, M., 1990. Morphology of single intracellularly stained axons terminating in area 3b of macaque monkeys. J. Comp. Neurol. 294, 583–593.
Goldberg, J.L., Trakhtenberg, E.F. (Eds.), 2012. Axon growth and regeneration. Int. Rev. Neurobiol. Part 1 105, 2–218.
Grafstein, B., Forman, D.S., 1980. Intracellular transport in neurons. Physiol. Rev. 60, 1168–1283.
Guillery, R.W., 2005. Observations of synaptic structures: origins of the neuron doctrine and its current status. Philos. Trans R. Soc. Lond. B. 360, 1281–1307.

Guillery, R.W., 2007. Relating the neuron doctrine to the cell theory. Should contemporary knowledge change our view of the neuron doctrine? Brain Res. Rev. 55, 411–421.

Humphrey, A.L., Sur, M., Uhlrich, D.J., Sherman, S.M., 1985. Projection patterns of individual X- and Y-cell axons from the lateral geniculate nucleus to cortical area 17 in the cat. J. Comp. Neurol. 233, 159–189.

Katz, L.C., 1987. Local circuitry of identified projection neurons in cat visual cortex brain slices. J. Neurosci. 7, 1223–1249.

Lee, C.C., Sherman, S.M., 2011. On the classification of pathways in the auditory midbrain, thalamus and cortex. Hear. Res. 276, 79–87.

Lorincz, L., Nusser, Z., 2008. Cell-type-dependent molecular composition of the axon initial segment. J. Neurosci. 28, 14329–14340.

Marin-Padilla, M., 2015. Human cerebral cortex Cajal-Retzius neuron: development, structure and function. A Golgi study. Front. Neuroanat 9, 21.

Miyashita, T., Ichinohe, N., Rockland, K.S., 2007. Differential modes of termination of amygdalothalamic and amygdalocortical projections in the monkey. J. Comp. Neurol. 502, 309–324.

Morecraft, R.J., Ugolini, G., Lanciego, J.L., Wouterlood, F.G., Pandya, D.N., 2015. Classic and contemporary neural tract-tracing techniques. In: Johansen-Berg, H., Behrens, T.E.J. (Eds.), Diffusion MRI, second ed. Elsevier, Amsterdam, pp. 359–400.

Ogren, M.P., Hendrickson, A.E., 1979. The morphology and distribution of striate terminals in the inferior and lateral subdivisions of the Macaca monkey pulvinar. J. Comp. Neurol. 188, 179–199.

Ojima, H., Honda, C.N., Jones, E.G., 1992. Characteristics of intracellularly injected infragranular pyramidal neurons in cat primary auditory cortex. Cereb. Cortex 2, 197–216.

Perge, J.A., Niven, J.E., Mugnaini, E., Balasubramanian, V., Sterling, P., 2012. Why do axons differ in caliber? J. Neurosci. 32, 626–638.

Rockland, K.S., 2013. Collateral branching of long-distance cortical projections in monkey. J. Comp. Neurol. 521, 4112–4123.

Ropireddy, D., Scorcioni, R., Lasher, B., Buzsáki, G., Ascoli, G.A., 2011. Axonal morphometry of hippocampal pyramidal neurons semi-automatically reconstructed after *in vivo* labeling in different CA3 locations. Brain Struct. Funct. 216, 1–15.

Rouiller, E.M., Welker, E., 2000. A comparative analysis of the morphology of corticothalamic projections in mammals. Brain Res. Bull. 53, 727–741.

Schmahmann, J.D., Pandya, D.N., 2006. Fiber Pathways of the Brain. Oxford University Press, NY.

Schwartz, M.L., Dekker, J.J., Goldman-Rakic, P.S., 1991. Dual mode of corticothalamic synaptic termination in the mediodorsal nucleus of the rhesus monkey. J. Comp. Neurol. 309, 289–304.

Sherman, S.M., Guillery, R.W., 2006. Exploring the Thalamus and Its Role in Cortical Function, second ed. The MIT Press, Cambridge, MA.

Shepherd, G.M., 2004. The Synaptic Organization of the Brain, fifth ed. Oxford University Press, NY.

Wang, S.S.H., Shultz, J.R., Burish, M.J., Harrison, K.H., Hof, P.R., Towns, L.C., et al., 2008. Functional trade-offs in white matter axonal scaling. J. Neurosci. 28, 4047–4056.

Waxman, S.G., Kocsis, J.D., Stys, P.K., 1995. The Axon. Structure, Function, and Pathophysiology. Oxford University Press, NY.

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AXON GUIDANCE

IV
TRACTOGRAPHY

SECTION I

MICROCIRCUITRY

Overview

Individual axons in their distal, terminal portions have been extensively investigated as presynaptic components of circuits within their various target structures. In their full entirety, including local collaterals and potentially multiple branches, they participate in broader networks; that is, the anatomical substrate of brain organization. Ideally, we want to visualize the totality of an axon (its parent neuron, arbors and terminations, and postsynaptic neurons) in space and time, to learn how the distributed information is used and coordinated in various behaviors. At the network level, however, relatively little information is available about the function and even the overall light microscopic configuration of axons in their entirety.

The first four chapters in this section review axon phenotypes in three different systems; namely, olfactory, basal ganglia, and thalamocortical (TC). In Chapter 1, Kensaku Mori discusses the strikingly different phenotypes of the two major olfactory projection neurons; namely, spatially focal projections of tufted cells and spatially dispersed projections of mitral cells. In this relatively well-studied system, the two phenotypes can be tightly and differentially associated with the inhalation–exhalation respiratory cycle, and are shown to have distinct but complementary spatiotemporal roles.

The next two chapters, by Martin and Andre Parent and by Elisa Mengual and colleagues, elaborate on axonal configurations within the several target structures of the basal ganglia. Notably, fine analysis shows morphological variability within each of the major outputs, in terms of whether neurons terminate in one area alone or branch to several. As remarked by both research groups, the diversity can be taken as a sign of exquisitely precise interactions in the context of complex spatiotemporal sequences across the interconnected network.

In Chapter 4, Francisco Clasca and colleagues present evidence for a substantial revision of the traditional distinction between topographically precise, area-specific TC axons versus divergent TC axons that had been thought to diffusely target the superficial layers of many areas. They demonstrate "multispecific" TC axons that branch in defined patterns to separate cortical areas. From this, a novel architecture emerges, with implications for the dynamics, plasticity, and evolution of TC-based networks.

The chapter by Denis Boire and colleagues focuses on corticocortical axons in the mouse. They demonstrate that projection foci, when dissected to the level of the contributing axons, are averages of highly diversified individual axons, presumably each with different integration properties. From this, the authors stress that a single cortical connection is not a single functional channel. Further implications for computational structure, connectional strengths (the distinction of "driving" vs. "modulatory"), and hierarchical organization are considered.

Issues of brain architecture continue as a theme in the chapter by John Anderson and Kevan Martin, where the authors review an extensive body of work on corticocortical axons and interrogate established ideas on cortical organization (Serial Processing and Lateral thinking). They consider what synaptic data can tell us about design principles of cortical connections and the interactions of local and long-distance connections. As in previous chapters, these authors also return to the issue of diversity: "What has been lost in these generalizations, however, is the variance [within] the projections.... This variance is instantly clear from the composite LM drawing shown in Fig. 2...."

The relation between single axons and populational patterns is further treated in Chapters 7 (Zoltan Kisvarday) and 8 (Kerstin Schmidt). Both authors focus on the still mysterious system of