CHAPTER

14

Within a Spine's Reach

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OUTLINE

1 INTRODUCTION

One of the most striking features of the brain is its ability to produce adaptive behaviors on the basis of individual experience. From development of human language to problem solving by cephalopods, the diversity of sensory, motor, and cognitive skills displayed across the animal kingdom is staggering. Although task-related specializations are evident

The Rewiring Brain
DOI: http://dx.doi.org/10.1016/B978-0-12-803784-3.00014-7

across species and at all levels of the nervous system, the seeming ease by which animals learn new tasks and acquire lasting memories raises the question of what, if any, common principles are at work.

The foundation for answering this question was laid in the 20th century with three fundamental advances: establishment of the neuron doctrine,[1] demonstration of chemical synaptic transmission,[2–6] and elucidation of the ionic basis of the action potential.[7–11] Increasingly precise tools for delving into the structure and function of synapses, and into the electrical signaling properties of neurons, have provided proof that the brain is a network. Contemporary with this insight was development of artificial neural networks, beginning a history of cross-pollination between the fields of neuroscience and computer science.

Information processing and storage within any network, biological or artificial, can be achieved by modifications in three parameters:

- The weights of each connection.
- The pattern of connections between nodes.
- The rules of integration that occurs at each node.

Interestingly, the dominant theory for learning in neuronal circuits from the 1950s to around 2000 focused mainly on the first parameter: weight changes induced at synapses according to their history of electrical activity. Perhaps, that narrow focus resulted either from the compelling logic (and truth) of Hebb's postulate of temporal precedence,[12] now fully described by rules of spike timing-dependent plasticity,[13–16] or from the discovery of long-term potentiation[17] (LTP) and long-term depression[18] in the 1970s–80s, which provided a path toward unraveling the molecular mechanisms of learning and memory[19–21] and the associated hope of clinical applications.

More recent theoretical and experimental results, however, have demonstrated the crucial role of changes in two other parameters: the pattern of connections (rewiring) and the rules of integration performed by each neuron or dendritic branch. This chapter focuses on developments of approximately the past 15 years beginning with discovery of microstructural dynamics in the living brain. We consider how, in principle, these changes might be harnessed to fine-tune information processing and storage and critically review empirical tests of the input clustering model[22–25] developed by Poirazi and Mel (Fig. 14.1). We identify gaps in current understanding and use potential experiments to fill those gaps. Finally, we speculate on the implications of this emerging understanding for neurorealistic simulation and neuromorphic computing.

2 MICROSTRUCTURAL PLASTICITY: SPINE DYNAMICS AND THE MAKING AND BREAKING OF SYNAPTIC CONNECTIONS

Macroanatomical changes in brain structure have long been known to occur during development and in postdevelopmental circuits following injury, neurodegeneration, or neurogenesis. In contrast, the notion that ongoing changes at the microscale level might provide a substrate for learning and memory was still not widely accepted in 1982 with the publication of Francis Crick's commentary "Do dendritic spines twitch?"[26] The

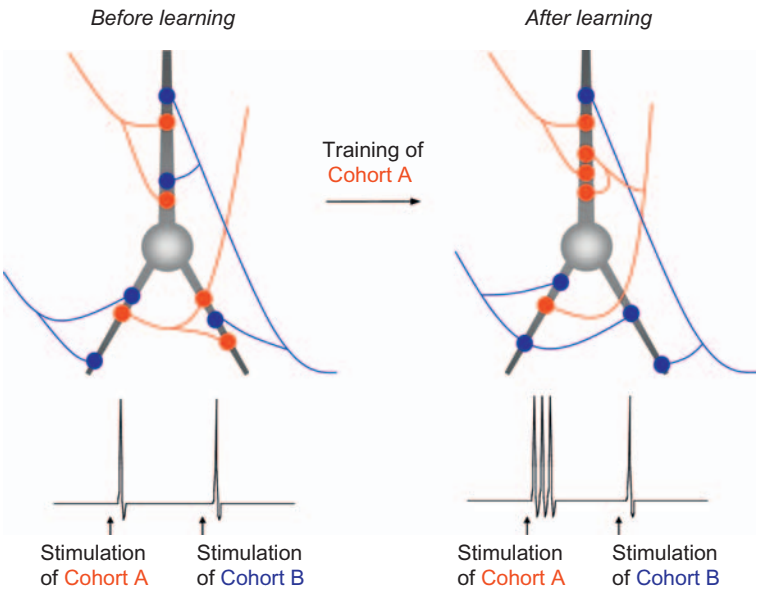

FIGURE 14.1 **The input clustering hypothesis.** Two cohorts of afferent input are depicted in *red* and *blue*. Activity evoked in each cohort is asynchronous with the other. Before learning, synapses are distributed randomly across the dendritic field of the postsynaptic neuron (*gray*). Activation of either cohort evokes a similar response from the soma. During a training period in which cohort A is repeatedly activated, synapses are made with all axons in the local neighborhood. Synapses from cohort A are selectively preserved only if they are located near one another on the same branch of dendrite—an input cluster. Activation of the cluster drives supralinear summation and enhances the response from the soma. That occurs even with the same total number and strengths of synapses present before training. The storage capacity of circuits is predicted to be greater when input clustering is employed than when plasticity is limited to changes in synaptic weight.

postulate was that cytoskeletal-driven changes in the morphology of dendritic spines would alter their electrical properties, thereby effecting synapse-specific weight change that encoded memories. Indeed this "twitching" would be experimentally verified *in vivo* two decades later (discussed in the following paragraph) and the proposed actin-based mechanism confirmed.[27–29] Therefore, compelling was the notion at the time that synaptic wiring was static, even Crick's prescient article did not include speculation that spine twitching might also lead to the formation of new synaptic connections.

The first observations of chronic dendritic spine dynamics in the living brain were reported in 2002 by two research groups using two-photon microscopy to acquire days to weeks of time-lapse images of dendrites of fluorescently labeled neurons in rodent cortex.[30,31] They found that dendritic spines could completely extend or retract in the adult rodent brain. The structure of dendritic arbors, however, was remarkably stable. In most cases the upper range of spine extension was $\sim 2\,\mu m$. The groups disagreed on the prevalence and frequency of spine dynamics, but subsequent work by many others has confirmed the basic observation that spines are extending and retracting in the living brain,[32–35] charted the behavioral and sensory paradigms that affect motility,[34,36–44]

determined the relationship between spine growth and retraction and the formation and elimination of synapses,[36,45–50,51] begun to define the cell types,[36,37,52,53] the cellular, and molecular mechanisms,[54–59] and, in a recent *tour de force*, demonstrated that spine growth is necessary for learning.[60] As this topic receives detailed treatment in a separate chapter, we summarize the relevant findings here:

- Dendritic and axonal branching patterns in postdevelopmental circuits are stable, absent injury, neurogenesis, or neurodegeneration.[30]
- Spine motility occurs throughout life.[32,33,61] Presynaptically, bouton turnover and microstructural extensions of axonal branchlets and terminal boutons have also been observed.[62–65]
- Spine motility is most prevalent in juveniles and declines in adults where most connections are stable over weeks to months, although a few ($\sim$5%) continue to be dynamic.[32,33]
- In both juveniles and adults, newly sprouted spines that eventually stabilize are associated with newly formed synapses.[36,46] Retraction of previously stable spines is associated with synapse elimination.[30,50,51]
- In conclusion, spine motility provides a substrate for sampling the local neighborhood for new synaptic partners. Because the dendritic and axonal branches are stable, the spatial range of remodeling is limited to approximately one spine's reach.

3 WHAT IS WITHIN A SPINE'S REACH?

Neuropil is a densely packed forest of cellular processes and extracellular matrix. What could a single spine's extension within this milieu realistically hope to achieve?

This question was first addressed using geometric modeling and bulk neuropil statistics.[66,67] Informed by the vital imaging studies described above, a cylindrical zone extending one spine's reach from the surface of each dendrite was identified as the spatial realm of remodeling. The number of actual synapses made onto the dendrite was calculated, and the number of potential synapses estimated from total length of axons positioned within the zone. Filling fraction—the ratio of actual to potential synapses—was calculated from anatomical measurements made independently in seven local circuits populating mouse neocortex, macaque neocortex, and rat hippocampus: pyramidal neuron density (range $2–22 \times 10^4$ mm^{-3}), dendritic length/neuron (1.4–12.3 mm), interbouton interval (3.7–6.2 μm), and average spine length (1.8–2.6 μm). The calculated filling fractions ranged from 0.12 to 0.34. Thus, the majority of potential synaptic partners coursing through the remodeling zone were unconnected with the target dendrite.

These low filling fractions imply a high capacity for information storage. Indeed, the storage capacity per synapse was predicted to be 3–4 bits, exceeding the naive value of 1 bit because of the latent potential connections. Thus, one answer to the question of what is within a spine's reach is a large number of unconnected axons providing opportunity for a very large number of distinct wiring diagrams.

A related model explored whether radially random axonal outgrowth could generate the observed filling fractions.[68] In this simulation, as neuronal density increased from a few neurons to thousands (an anatomically realistic number), the filling fraction fell from

1 and stabilized at $\sim$0.26, in the center of the range calculated by the previous study. In addition, the incidence of unconnected neighboring neurons increased, consistent with experimental data. These modeling results demonstrate that a simple geometric rule for outgrowth, in combination with Hebbian-type synaptic competition, can explain key features of cortical connectivity.

Both modeling studies highlight the need for more precise connectivity measurements. With the advent of tools for structural and functional connectome reconstruction, the desired data are beginning to emerge. One project employed multiscale imaging to analyze a 0.13 mm^3 volume of somatosensory cortex from a young adult mouse.[69] An 80×10^3 μm^3 subvolume was imaged at 3 nm per pixel resolution using scanning electron microscopy (EM), and within it, all neuropil elements (axons, dendrites, spines, and synapses) were reconstructed. This smaller "saturated" volume contained two apical dendrites of pyramidal cells and all of their synaptic inputs. This is the type of primary data needed to definitely address questions of connectivity at the microscale. The first application was to test Peters' rule.

Peters' rule, as formalized by Valentino Braitenberg,[70] postulates that the number of synaptic connections between two neurons can be predicted from geometric overlap of their axonal and dendritic arbors.[71] In this model, axodendritic "touches" are assigned as synapses and the entire population winnowed to match actual bulk synapse density. To test Peters' rule using the saturated volume of neocortex, a geometric bootstrap analysis was performed on all 1037 synapses, 916 axons, 1036 dendritic spines, and 7505 spine touches. Redundant synapses were identified when a single axon made more than one synapse with the target dendrite. In the simulation, redundant synapses were calculated from axospinous touches in each of 80,000 randomization trials of axonal positions. The median number of redundant synapses in these trials, 52, was far lower than the actual number, 78, ($P<0.00001$), indicating that axodendritic overlap is not sufficient to explain connectivity in adult somatosensory cortex.[69] The conclusion was that some factor—a molecular tag or activity-based signal—endowed certain axons with privileged access to the dendrite.

These results, and more recent ones employing unbiased large-scale methods to uncover connectivity principles between finely parsed cell type populations in adult neocortex,[72] lend support to the notion that microscale connectivity is nonrandom. Still, the focus of many studies so far has been to map connectivity by quantifying the strength of the bulk connection (including redundant synapses) between two identified neurons. This can be measured functionally using paired recordings or estimated from light or EM images by summing the number of synapses made between neurons A and B after differentially weighting individual synapses on the basis of proxy measures such as volume, number of synaptic vesicles, or protein content. These methods of quantifying bulk connection strength provide valuable information yet do not account for one potentially crucial aspect of postsynaptic integration, which is not just the number and strength of individual synapses but their dendritic address.

4 DISTRIBUTED VERSUS CLUSTERED INPUTS

Neurons can integrate up to 10,000 synaptic inputs arising from hundreds of input sources. Dendritic fields performing this operation exhibit diverse morphologies speckled

with passive and active properties that shape the integration. To identify the summation rules for excitatory synapses, theoretical studies[24,73–77] have used compartmental models of anatomically and biophysically realistic neurons, and experimental studies[78–85] have employed in vitro slice preparation, whole-cell recording, and stimulation of multiple input locations using glutamate iontophoresis or uncaging. A few common principles have emerged.[1] For excitatory postsynaptic potentials (EPSPs) delivered simultaneously to a: (1) small number of different shaft locations on the same branch, summation is sublinear due to local reductions in driving force; (2) small number of spine synapses on the same dendrite, summation is linear, as the high neck resistance restores linearity by electrically isolating each spine head from local reductions in driving force; (3) larger number of spine synapses clustered on a subregion of dendrite, summation is supralinear because the summed EPSP crosses a local threshold for activation of voltage-dependent channels (sodium channels, calcium channels, or NMDA receptors). Whether this supralinearity results in a dendritic spike,[86–88] the activation of clustered inputs results in strong depolarization at the soma.

How these summation rules interact with weight and wiring changes is the key distinction between two competing cellular models for information processing and storage: distributed versus clustered inputs. The distributed model proposes that excitatory spine synapses originating at different afferent sources are distributed randomly along the dendritic tree.[2] Narrow spine necks isolate each spine from dendritic depolarizations, active conductances serve to amplify more distal EPSPs, and, as a result, summation is linear with each synapse contributing to the neuronal computation according to its weight, not location.[90] The output of this global integration would be influenced by adaptable weight changes and plasticity of intrinsic properties but not by readdressing of inputs already present. The computational power of the network would arise both from these cellular properties and emergent features of distributed processing at higher levels.

In contrast, the clustered model proposes that synaptic inputs bringing synergistic afferent information—for example, independent sensory features needed to construct the postsynaptic neurons' receptive field—would be most effectively positioned as dendritic neighbors. This would increase their chance of crossing the local threshold for amplification and thus increase the capacity of the neuron for pattern detection. Indeed, any position-dependent nonlinearity is predicted to add to the repertoire of neuronal computations.[91] In summary, dendritic readdressing even of preexisting inputs, in combination

[1]Cell-type specific variations in dendritic processing can be striking. For example, the dendrites of cerebellar interneurons typically operate in sublinear mode.[89] The discussion below refers primarily, although not exclusively, to pyramidal neurons from the mammalian neocortex or hippocampus and emergent common principles. Cell-type specific exceptions, when relevant, are noted.

[2]The question of dendritic addressing of different afferent sources refers to sources carrying the same type of input, i.e., excitatory glutamatergic synapses encoding the value of a graded variable such as location of a sensory stimulus. The dendritic addressing of these inputs is believed to be sculpted by stochastic mechanisms and activity-dependent feedback. In contrast, dendritic addressing of afferent sources carrying different types of input (neurochemical or source) is in many cases directed by genomic programs. This chapter focuses on the former, not the latter.

with adaptable weight changes and plasticity of intrinsic properties, is predicted to enhance the computational power of the network.

This input clustering hypothesis predicts (1) that clusters of coactive synapses exist and that the number, strength, and spacing of these *dendritic input clusters* is appropriate to drive supralinear summation on dendrites in response to natural stimuli and (2) that experience-dependent remodeling will drive formation of dendritic input clusters from an initially global distribution (Fig. 14.1).

Regarding the first prediction, cluster composition is expected to depend on synaptic weights and kinetics, dendritic dimensions, and membrane properties and thus will vary from cell to cell, dendrite to dendrite, and across a cell's lifetime as synaptic and intrinsic properties are modified. Using biophysically realistic models of pyramidal and hippocampal neurons, Poirazi and Mel's model predicted supralinear interactions resulting from as few as two moderately strong spine synapses located $<40\,\mu m$ apart on a dendrite. Physiological studies informed by these predictions, across a wider range of cell types and conditions, have found supralinear summation resulting from coactivation of $7-10$[82] or ~ 20[83] nearby synapses or as few as two stronger ones.[80] In accordance with model predictions, experimental results confirm that fewer/weaker input patterns result in linear summation.[78,80,81] Thus, functional clusters occupy a niche within the larger parameter space of spatiotemporal input patterns.

There have been more than 20 tests of the two predictions of the input clustering hypothesis since 2008. These studies were performed in a wide array of species, analyzed diverse circuits, and used different tools for functional (calcium imaging, electrophysiology) or anatomical (confocal and two-photon imaging, EM, array tomography) mapping. In some cases they reached opposing conclusions. We highlight many of the significant findings in the subsequent sections.

5 EXPERIMENTAL TESTS OF THE PREDICTIONS OF THE INPUT CLUSTERING HYPOTHESIS

5.1 Do Dendritic Input Clusters Exist?

Within a few years of publication of Poirazi and Mel's modeling predictions, several independent studies established that coactivation of clustered inputs on dendrites *can* drive supralinear summation[80,82,83]; however, determining whether clustered dendritic inputs are *naturally* coactive and whether they *do* drive supralinear summation required experiments that utilized natural activity patterns, such as spontaneous or sensory-evoked activity. Such studies began to appear in 2010–12.[92–96]

Initial support for the existence of naturally coactive input clusters came from Kleindienst et al. (2011) and Takahashi et al. (2012). Using calcium imaging to monitor spontaneous calcium transients in CA3 pyramidal neurons in organotypic hippocampal slice cultures, both studies found that calcium transients arising from neighboring locations on the dendrite were more likely to be coincident than those arising from distant locations; these local clusters of coactive inputs spanned $16\,\mu m$ in early postnatal slices[95] and $8\,\mu m$ in older slices that had been an additional few weeks in culture.[96] Comparable

results were obtained from dendrites of spontaneously active L2/3 pyramidal neurons *in vivo* in the somatosensory cortex of young adult mice, where local clusters of coactive spines were observed within a 6 μm span of dendrite.[96]

In contrast, Konnerth and colleagues reported no evidence for clustering of coactive inputs on dendrites of L2/3 pyramidal neurons *in vivo* in the sensory cortices of young adult mice in response to sensory stimulation.[92–94] Notably, these pioneering studies provided the first observations of sensory-evoked single-spine activity in mammalian cortical neurons *in vivo*. Using two-photon imaging to monitor dendritic calcium transients in L2/3 neurons of the mouse visual cortex,[92] auditory cortex,[94] or somatosensory cortex,[93] the authors observed that calcium transients evoked by closely related sensory stimuli appeared widely distributed on the dendritic tree, and that adjacent inputs often represented signals of broadly different sensory feature values. The authors concluded that the results of their studies are most consistent with the global integration model in which neurons compute their output by integrating spatially distributed synaptic inputs.

While the data from Konnerth and colleagues did not provide support for the preferential activation of clustered inputs, they did not rule out that certain aspects of receptive field arise due to dendritic input clusters. Indeed, a recent study examining a broader array of stimulus characteristics demonstrated just that. Wilson et al. used *in vivo* two-photon calcium imaging of individual spines on dendrites of L2/3 cells to examine orientation preference and orientation selectivity in the visual cortex of young adult mice.[97] They showed that while reliable in predicting orientation preference, summed synaptic input to individual neurons did not predict orientation selectivity. Instead, orientation selectivity strongly correlated with the spatial clustering of cotuned synaptic inputs. Furthermore, those dendritic branches with more cotuned clusters showed a greater rate of local dendritic calcium events, suggesting that functional clustering of synaptic inputs plays an important role in the dendritic nonlinearities that shape orientation selectivity.[97]

Two recent functional studies provide further support for the existence of dendritic input clusters *in vivo*. Winnubst et al. quantified spontaneous calcium transients *in vivo* in L2/3 cells of the visual cortex of young postnatal mice, demonstrating that, even at these early ages, nearby synapses (<12 μm) were more coactive than synapses far apart.[98] Gokce et al. combined optogenetics and two-photon calcium imaging to map the spatial organization of glutamatergic synapses between L5 pyramidal neurons in mouse neocortical slices, finding that synapses of intralaminar inputs form clusters (4–14 synapses) spanning 30 μm on the basal dendrites of L5 pyramidal neurons.[99]

Anatomical studies have provided complementary data. No evidence for clustering was reported in experiments using correlative light and EM to study focally labeled thalamocortical synapses onto L4 cells.[100] Because of the topographic organization of the visual system, focal labeling should target neurons, and thus axons, that are likely to be coactive. Yet evidence for clustering of labeled inputs onto dendrites was not observed for LM-identified contacts nor for EM-identified synapses.[100] In contrast, a recent study examining thalamocortical synapses onto excitatory L4 neurons showed a significant clustering of inputs onto barrel star pyramid neurons and a trend toward input clustering onto a septal star pyramid neuron but no evidence for clustering on barrel spiny stellate neurons.[101] The authors argue that this clustering could help explain the surprisingly potent ability of

the thalamus to drive cortical activity despite that corticocortical synapses outnumber thalamocortical synapses in a range of 10 to 1.

High-throughput anatomical approaches provide further support for dendritic input clusters. Rah and colleagues used array tomography to examine the spatial distribution of thalamocortical synapses onto L5 neurons in the mouse somatosensory cortex,[102] finding within-branch clustering (5–15 μm) more often than predicted from a random distribution. In another study, Druckmann and colleagues used mGRASP to visualize inputs of presynaptic CA3 neurons to postsynaptic CA1 neurons in the hippocampus.[103] The authors analyzed within branch clustering and found significant deviation from random distribution in 17 of 27 neurons: all exhibited an overabundance of the shorter distances, supporting clusters. Furthermore, by labeling only neurons that were born in a similar time window, input clustering (<1.5 μm) was significantly enhanced, suggesting that neurons born at the same time could be functionally related and signaling together.

5.2 Does Experience-Dependent Remodeling Drive Formation of Dendritic Input Clusters?

The second major prediction of the hypothesis is that learning should drive the redistribution of synapses from a dispersed into a more clustered state.[98,104] Indeed, experience-driven changes in clustering have been observed using naturalistic learning paradigms in owl and mouse, and in both juvenile and adult animals.

The first study to show clustering of inputs on dendrites following a learning paradigm was McBride et al. using juvenile barn owls.[105] When owls are reared wearing prism glasses that shift the frontal visual field, new axonal growth in the projection from the central nucleus of the inferior colliculus (ICC) to the external nucleus (ICX) serves to realign the auditory and visual space maps and drive adaptive localization behavior. Notably, the functionally dormant normal circuit is anatomically preserved alongside the active learned circuit,[106] providing an internal control for cluster analysis. Because of the topographic nature of the auditory projections, focal labeling of neurons in the ICC identifies coactive neurons and thus coactive axonal inputs to the ICX. Measurements of the inter-contact distance (ICD) of labeled axonal inputs to the nearest neighbors measured from the dormant normal circuit in prism-adapted owls were on average <10 μm; however, a significant fraction were >20 μm. In comparison, ICDs >20 μm were almost never observed in the active learned circuit; nearly all inputs resided in clusters. When prisms were removed in adulthood, increased clustering was observed in the now reactivated normal circuit.[107] Interestingly, even the synapses in functionally weak circuits in adult owls resided in clusters. This could represent a trace of learning in the brain—an efficient way of storing a disused input pattern that could be needed later in life. Altogether, these results demonstrate that behaviorally relevant experience can drive the formation or disassembly of dendritic input clusters.

A subsequent study in young adult rodents by Makino and Malinow demonstrated experience-dependent input clustering on dendrites of L2/3 cells in acute slices of somatosensory cortex of mice.[108] Using an optical approach to track the delivery of fluorescently tagged AMPA receptors into spines, the authors were able to monitor single-synapse plasticity. Normal sensory experience preferentially produced synaptic potentiation onto

nearby dendritic synapses (28/95 dendrites), consistent with the input clustering hypothesis. In contrast, sensory deprivation via whisker trimming showed much less correlation (5/68 dendrites) and instead led to a global homeostatic enhancement.

Further evidence supporting learning-induced input clustering on dendrites was first reported in rodents by Fu and colleagues.[41] Using two-photon imaging of GFP-labeled L5 pyramidal neurons in the motor cortex following training on a novel forelimb task, Fu et al. showed that one-third of new spines appeared in clusters (adjacent to existing spines, $<5\ \mu m$), and that these were more resistant to elimination than nonclustered new spines. Cross-training of the mice on a new task as adults resulted in the emergence of new clusters that were largely separated from those associated with the first task. Notably, formation of new spine clusters required repetition of the same motor task; thus, the authors show that clustering of new synapses along dendrites is induced by repetitive activation of cortical circuitry during learning.[41] Reiner and colleagues[109] confirmed these studies in wild-type animals and further showed that FMR1 knockout animals have deficits in learning-induced spine clustering.

Finally, two behavioral studies utilized EM analyses to demonstrate learning-induced synaptic clustering.[110,111] In the first study, Lee et al. trained adult rats in a motor skills task. EM analysis of parallel fiber to Purkinje cell synapses showed that motor skill training promoted the formation of parallel fiber multiple synapse boutons (MSBs) contacting two dendritic spines from the same dendrite of Purkinje cells in the rat cerebellum.[110] Notably, excitatory synapses near MSBs were smaller in motor-trained animals, suggesting a compensatory depression of MSB-neighbor synapses.[110] A second study by Pereira and colleagues set out to define the effects of riluzole, a drug that decreases glutamate release and facilitates astrocytic glutamate uptake, on age-related cognitive decline. They measured both synaptic structure (EM) and memory performance and showed that riluzole-treated rats were protected against age-related cognitive decline and they exhibited a correlated increase in the density and clustering of thin spines.[111]

In summary:

- A diverse array of species (owl,[105,107] rat,[95,96,108,110–112] mouse,[41,92–94,98,99,102,103] cat,[100] ferret[97]) and circuits (inferior colliculus,[105,107] hippocampus,[95,96,103,111,112] primary sensory cortices,[92–94,96–100,102,108] motor cortex,[41] cerebellum[110]) has been studied.
- Most researchers analyzed intact circuitry in vivo, ex vivo, or fixed.
- Nearly all studies focused on excitatory synapses convergent on pyramidal neurons with large dendritic fields.
- Coactive synapses were identified by electrical or optical recordings of spontaneous activity, activity evoked by sensory presentation, or by artificial stimulation. Their dendritic locations were apparent from imaging fields with wide coverage of the postsynaptic neuron.
- *Most, not all, studies found a correlation between co-activity and dendritic location.* Specifically, coactive synapses were more likely to occur within $<20\ \mu m$ of one another, with most results indicating $<10\ \mu m$ and several identifying immediate neighbors as part of the cluster. This empirically determined spatial window is somewhat smaller than predicted by computational modeling.

- Activity-dependent cluster formation has been observed in several systems.[98,112–116] Experience-driven changes in clustering have been observed using naturalistic learning paradigms in owl[105,107] and rodents,[41,108–110] and in both juvenile and adult animals.
- Clusters are not composed exclusively of coactive synapses but are often interdigitated with other input cohorts. As a purely random distribution of afferent cohorts is expected to produce some clusters by chance, convincing demonstration of true clustering requires data sets with near saturating coverage and bootstrap analysis.

6 CELLULAR AND MOLECULAR MECHANISMS DRIVING INPUT CLUSTER FORMATION

The formation of dendritic input clusters could result from the appearance of new coactive neighboring synapses and/or from the elimination of neighboring inactive synapses. Several studies, outlined below, have identified potential molecular and cellular mechanisms.

Selective outgrowth and/or stabilization of new coactive neighboring synapses could be accomplished through local cross talk, as reported by Harvey and colleagues in 2007. This study demonstrated that activation of a single spine in a pattern that induces input-specific LTP of synaptic structure and function[113] also leads to the spread of a signal that converts a subthreshold LTP stimulus into stable LTP at neighboring synapses. This cross talk between synapses was due to an intracellular diffusible molecule that acts on a spatial scale of 10 μm and a temporal scale of 10 min. Later studies identified Ras,[117] Rac1, and RhoA as intracellular diffusible signals[59] that support cross talk and thus selective strengthening of nearby synapses. Such a cross talk mechanism could be responsible for the selective outgrowth of new spines nearby active spines observed in 2008 by De Roo and colleagues.[112]

Alternatively, selective outgrowth and/or stabilization could be accomplished through a synaptic tagging and capture mechanism, as reported by Govindarajan and colleagues in 2011.[118] In this model, a stimulus that drives synaptic strengthening at strongly activated synapses also leads to the protein synthesis-dependent production of PrPs (plasticity-related protein products), which are captured at neighboring weakly activated, tagged synapses. If successful in capturing PrPs, the weak, tagged synapse will undergo long-lasting late LTP. This mechanism is similar to cross talk; however, it operates at a more global spatial scale of 70 μm (vs 10 μm) and temporal scale of 90 min (vs 10 min). Furthermore, synaptic tagging and capture requires protein synthesis, whereas cross talk does not.

Finally, selective outgrowth and/or stabilization could occur through location-dependent plasticity mechanisms that rely upon local dendritic excitability and integrative properties.[116] Specifically, Weber and colleagues report a proximodistally increasing gradient of nonlinear NMDA receptor-mediated amplification of spine Ca^{2+} signals; the enhanced synaptic cooperativity at distal dendritic compartments could promote the formation of input clusters on distal dendrites.

In addition to selective outgrowth and/or stabilization, formation of dendritic input clusters could also occur via the selective weakening and/or elimination of nearby inactive inputs. One such mechanism was reported by Oh and colleagues at synapses of hippocampal pyramidal neurons in slice culture.[115] In that study, the authors observed that robust

stimulation, leading to the growth and strengthening of several inputs on a single dendrite, also led to the selective weakening of nearby (<3.5 μm) inactive synapses. Heterosynaptic shrinkage of inactive spines required activation of calcineurin, metabotropic glutamate receptors, and IP_3 receptors,[115] yet the specific mechanism has not yet been determined. A recent report identified activity-induced competition for beta-catenin as critical for mediating interspine competition during heterosynaptic spine pruning.[119]

An alternative mechanism for weakening of nearby inactive inputs was reported by Winnubst et al. The authors used whole-cell patch clamp to monitor naturally occurring changes in spontaneous activity at individual synapses in L2/3 neurons in the mouse visual cortex in vivo and in hippocampal pyramidal neurons in slice culture.[120] They found that synapses that exhibit low synchronicity with neighbors (<12 μm) become depressed in their transmission frequency, but not in their amplitude, suggesting a retrograde signaling mechanism that influences on presynaptic function. Experimentally increasing local synchronicity stabilized synaptic transmission. Signaling through the high-affinity pro-BDNF receptor $p75^{NTR}$ was required for depression of asynchronously stimulated synapses.[120]

Finally, the formation of dendritic input clusters could rely upon the coordinated local plasticity of both excitatory and inhibitory inputs, as observed by Chen and colleagues.[114] Using Teal-Gephyrin as a marker for inhibitory synapses, Chen et al. were able to simultaneously image putative inhibitory synapses and putative excitatory synapses (dendritic spines) in the visual cortex in vivo. They found that inhibitory synapse and dendritic spine remodeling were spatially clustered, suggesting local coordination of inhibitory and excitatory synaptic rearrangements.[114] Such coordinated rearrangements could play an important role in the establishment of coactive clustered inputs on dendrites.

7 FUTURE EXPERIMENTAL STUDIES

Below we outline four potential areas for future work. The first describes a stringent experiment (currently just outside the realm of feasibility) regarding the functional significance of input clustering. The second poses a new question—how microstructural dynamics drive cluster formation. The final two topics are more open-ended.

7.1 What Is the Functional Significance of Clustering?

The empirical studies summarized earlier derive from an impressive arsenal of technologies. They provide compelling support for the existence of clusters and for the capacity of dendrites to act as supralinear integrators in certain circumstances. Yet direct demonstration of whether naturally occurring input patterns drive supralinear summation through clustered inputs is lacking, primarily because the natural input patterns are unknown. Testing the model with natural patterns as opposed to the artificial patterns used in previous studies would be an important advance because postsynaptic integration—known to depend on timing, number, and strengths of inputs—could differ markedly across input regimes. Moreover, naturalistic inputs could cause state changes[121]

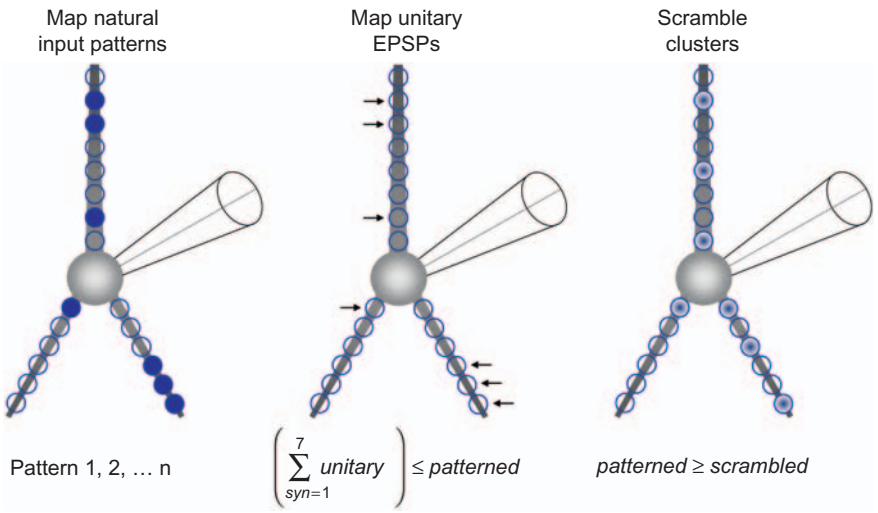

FIGURE 14.2 **Hypothetical experiment.** (A) Map natural input patterns *in vivo*. This could be accomplished via two-photon imaging of calcium transients evoked by natural sensory stimuli. One challenge is developing an imaging system with single synapse resolution and total neuronal coverage (thousands of synapses). Each stimulus would be expected to evoke a different pattern of activation, illustrated for one stimulus by *solid fill* in 7 of 20 synapses. For each pattern, the spatial organization of synchronous versus asynchronous synapses could be analyzed using bootstrap methods to determine deviations from a random pattern. The model predicts different input clusters uniquely associated with different stimuli. (B) Map unitary EPSPs. Whole-cell recordings could be made from the same neuron. Glutamate uncaging could be used to measure EPSPs evoked by individual synapses (*arrows*). Input clustering predicts that patterned activation would produce a stronger response than the sum of unitary activations. (C) Scramble clusters. Multispot photoactivation could be used to simultaneously activate a spatially randomized cohort of synapses (*radial fill*), matching the patterned cohort in its sum of unitary EPSPs. Scrambled activation is predicted to produce a weaker response than patterned activation.

in the neuron via longer lasting biochemical modifications.[117] We propose an experiment with three components (Fig. 14.2).

The first is to determine the spatiotemporal patterns of synaptic input onto the entire dendritic tree of a neuron in vivo. This is not a small task. The challenge lies in visualizing the activations of thousands of afferent synapses because a diverse array of naturalistic sensory stimuli is delivered to an awake animal. Simultaneous optical recording of each synapse is theoretically feasible, and recent unpublished reports suggest that current platforms have achieved the goal of complete dendritic input coverage for some cell types, yet confidence is low that all synaptic events are reliably detected. Meeting this challenge likely depends on the development of higher sensitivity calcium or voltage-based dyes and further tools for high-speed multiphoton imaging, explicit goals of the BRAIN Initiative. Failing complete neuronal coverage, mapping the input pattern to an entire dendrite should suffice, as current evidence supports the dendritic branch as an independent computational subunit.

The second is to measure EPSPs evoked both by each individual synapse and by the naturally occurring input patterns. This could be achieved using multisport photoactivation and electrical or optical recordings, first to quantify (or estimate) the magnitude of

EPSPs resulting from activation of each synapse and second to recapitulate the natural input patterns. Retrospective analysis would determine whether native input clusters are *sufficient* to drive supralinear summation and also should reveal motifs that most effectively drive the neuron. With a diverse array of naturalistic patterns, one might also be able to estimate the neurons capacity for pattern recognition and/or storage.

The third is to manipulate the multispot pattern in artificial ways that probe the *necessity* of clusters. The number, strength, and relative timing of synapses within the effective naturalistic cohort would be preserved but the spatial structure degraded. This would disambiguate the roles of the three parameters that govern function of the synaptic network—location, weight, and integration rules—and provide important new data for computational modeling. Close agreement of the model output and experimental results would provide strong proof of input hypothesis and elevate the hypothesis to paradigm status.

7.2 How Do Clusters Form?

Spine motility is a necessary substrate for cluster formation but in itself does not explain structured dendritic addressing. One possibility is that spines extend randomly into the milieu of their potential inputs and form "tester" synapses with each axon they touch. Those synapses that, by chance, are positioned within an effective cohort receive a reward or consolidation signal, and the synapses would be stabilized. Spines that have formed in the vicinity of asynchronously active synapses would destabilize and be eliminated coincident with spine retraction. This is a straightforward Hebbian model.

The alternative is a "Directed model" in which only coactive dendrites are recruited by an axon.[122] This could be achieved by the release of diffusible factors that guide spine outgrowth in the direction of the axons or by cell surface expression of receptors that signal a match. Activity-based rules would still be needed to confirm specificity and fine-tune the synaptic weight. This Directed model might be expected to rely on fewer tester synapses and thus produce more efficient or faster remodeling. However, it would require a panel of molecular tags (diffusible or cell surface), each encoding a separate activity pattern, to provide target specificity within the milieu and therefore is mechanistically more complex. Occam's razor favors the Hebbian model.

Still, if directed outgrowth were found, the translational significance would be high. Before a search for molecular tags is launched, a simple experiment would be to systematically manipulate the correlative activity between a postsynaptic dendrite and its neighboring axons while monitoring spine dynamics over an extended time period. Is spine outgrowth geometrically random or targeted to processes with registered activity?

7.3 Does Clustering Occur at Higher Levels in the Network?

Lost in the discussion about whether the dendritic branch or neuronal cell body is the fundamental computational subunit is the notion of recurrent structure across scales. This chapter has focused on dendritic input clustering as a mechanism to boost neuronal storage capacity. One open question is whether clustering is an organizing principle at the level of neurons, circuits, and systems.[123] Recordings using high-density multielectrode

arrays (MEAs) have shown that a subset of neurons in mouse somatosensory cortex transfer and receive far more information than others.[124] These "hub neurons" may route as much as 70% or cortical traffic despite representing just 20% of all neurons. A separate theoretical study indicates that neuronal hubs could have an outsized effect on network processing, in particular in orchestrating state changes.[125] Analogous results at the level of macroscale connectomics, based on diffusion tensor imaging tractography with a resolution limited to voxels containing millions of circuit elements, have shown the brain to be a small-world network with hub regions that make and receive significantly more connections than others.[126] It would be interesting to compare the organization of these nonrandom features that appear at markedly different spatial scales

7.4 Did Clustering Evolve in Circuits With Higher Demands for Adaptive Pattern Recognition?

The experiments and models described in this chapter provide good (not conclusive) support of the input clustering model—at least in certain cell types and conditions. However, global integration is hardly ruled out. One could speculate that both have a role in microscale operation.

Few circuits may require the extra capacity afforded by clustering to achieve their purpose. Moreover, many are best left consolidated as they were at the tail end of development. Absent injury, degeneration, or neurogenesis, both the information, input and computed output of these types of circuits, is stationary over the adult lifetime. Building in a seldom-needed capacity for structural plasticity may simply not be worth the metabolic cost. Thus, a comparative analysis of input clustering in circuits that evolved for adaptive pattern recognition would be informative.

This search should not be regarded as a one-way trip through the phylogenetic tree nor up the neuroaxis from spinal cord to cortex. For example, primary visual cortex in mammals—whose capacity for invariant feature detection provides a reliable substrate from which association cortex extracts complex percepts—may be less in need of adaptive pattern recognition than, for example, the inferior colliculus in barn owls, whose role is lifelong adaptation to slight changes in registration of auditory and visual cues necessary for survival. Consistent with this notion, computational estimates of dendritic input discrimination capability (M) across morphologically defined cell types found in fish, insects, amphibians, birds, and mammals revealed large neuron-to-neuron variability[127] that did not correlate with evolutionary complexity. Predicting which circuits employ input clustering demands careful evaluation of each input–output transformation and whether it is stationary over long timescales.

8 FUTURE MODELING STUDIES

As experimental studies move forward to test the sufficiency and necessity of structured dendritic addressing in a variety of circuits—a journey that in our view is more likely to reveal a mixed bag of processing strategies than a canonical one—we consider the

implications of distributed versus clustered processing for two "big science" projects at the intersection of neuroscience and computer science: neurorealistic simulation and neuromorphic computing.

8.1 Neurorealistic Simulation

Quantitative models are ultimately required to demonstrate understanding of complex systems such as Earth's climate or mammalian brains. Simulating *in silico* the biophysical operation of the human brain at the level of individual molecules is infeasible and unnecessary. The question is what level of biophysical detail is required to produce a reasonable emulation of even one semiautonomous neural circuit such as a cortical column. There are at least three schools of thought.

The *structural connectome approach* uses networks built of $\sim 10^3$–10^6 s of anatomically and biophysically realistic neurons represented by compartmental models or computationally simplified two-stage processors. There are two distinct strategies based on *predicted connectivity*[128–136] or *actual connectivity*. One example of the former is the Human Brain Project co-funded by the European Union. To simulate a microcircuit in rat somatosensory cortex, a near complete catalog of resident cell types was assembled from morphological and electrophysiological data gathered from hundreds of independent experiments. The neurons ($\sim$31,000) were superimposed digitally according to laminar landmarks, and connectivity was calculated from axodendritic overlap.[137] The number of synapses ($\sim$37 million) was scaled to match data, and synaptic weights were assigned. Remarkably, this network built of predicted connectivity, as opposed to actual microscale wiring, was able to reproduce an array of functional data including some in vivo features, all without parameter tuning.[138] Such a result is consistent with the distributed model of neural processing.

The general applicability of this strategy, which does not incorporate empirically determined dendritic addresses, is not clear. Many higher order aspects of cortical function, including capacity for learning and memory, have yet to be explored. Indeed, the value of input clustering is maximal when networks are pushed to perform sophisticated pattern recognition and adaptive processing. In this second school of thought, achieving high performance from a neurorealistic simulation will depend on incorporation of *actual connectivity*. Ultimately this may require dense reconstruction of a serial EM volume coregistered with prospective functional mapping.[139] Several such projects are underway but will likely take years to decades to complete.

In the interim, neurorealistic models could be infused with plasticity rules that produce biologically observed connection motifs such as input clusters, sparse connectivity, and other nonrandom features. Toward this goal, an abstract neural network (i.e., not neurorealistic) was constructed to solve a hidden variable estimation task,[140] a type of inference that occurs in the brain. Robust computations were achieved only when both synaptic weights and connectivity were left as adjustable parameters. This result was more striking for sparsely connected networks—of which the brain is one example—than densely connected ones. The network was also trained to mimic a motor learning task under conditions where wiring plasticity was delimited by empirical measurements of the rates and

scope of spine dynamics. Collectively, these theoretical results support the notion that simulation of microstructural dynamics may be essential for maximizing the performance of neurorealistic models.

In a third school of thought, the *functional connectome approach* dispenses with the notion of simulating synaptic signaling and neuronal integration and instead focuses on the real-time activity pattern at millions of neuronal cell bodies.[141] The raw data that would feed such a model is expected to come from advances in high-throughput recording technology, either electrical or optical.[142,143] With days to weeks of recording naturally driven patterns of activity with single neuron and single action potential resolution and circuit-wide coverage, the algorithms performed by canonical circuits might be elucidated. One emerging example of this approach is neural control of prosthetic limbs[144] via real-time analysis of MEA signals implanted in motor cortex of primates.[145] With enhanced coverage and resolution, one could imagine the ability to "readout" of increasingly sophisticated cognitive processes[146] and eventually to piece together holistic emulation of natural behavior.

8.2 Neuromorphic Computing

One other potential application of understanding distributed versus clustered processing is to the field of neuromorphic computing. For more than 80 years, computer cores have employed Von Neumann type architecture in which the sites of processing and memory storage are physically separate. The simple existence of the digital revolution indicates the power of this platform. Yet the design is quite unlike the brain in which the sites of processing and storage are colocated at synapses. Neuromorphic chips, in contrast, loosely mimic real synaptic networks. Prototype designs developed by academic groups at MIT, Stanford, and Zhejiang University (China), and commercial efforts at IBM (True North), Qualcomm (Zeroth NPU), Numenta (NuPIC), and Knowm (memristors) already demonstrate one advantage of such an approach—radically lower power consumption compared with standard processors.[147] This feature alone promises enormous practical advantages for mobile computing and remote sensing applications.

Yet another feature of neuromorphic architecture holds added potential. Recent advances in "deep learning" have produced computer algorithms that, for the first time in history, rival or exceed human performance in complex visual tasks such as object or face recognition.[148] Running these biologically inspired algorithms on Von Neumann architecture, however, is viewed as inefficient. It is believed that embedding deep learning algorithms in neuromorphic chips could maximize the utility of using synaptic networks. The most futuristic musings on this topic predict a full 80 billion neuron simulation by 2025 and human-like artificial intelligence on the near horizon.

Putting aside fantastic claims, unraveling the brain's microscale structure could concretely inform the next generation of neuromorphic designs. This is because current designs are based on a preconnectomics era understanding of synaptic networks and therefore do not implement microscale connection motifs. As neuroscience continues to discover motifs at an accelerating pace—and demonstrate their role in diverse information processing and storage functions—it could provide a deep well of biological inspiration for hardware engineers.

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