Published in final edited form as:

Trends Neurosci. 2017 February ; 40(2): 63–71. doi:10.1016/j.tins.2016.11.007.

Weighing the Evidence in Peters' Rule: Does Neuronal Morphology Predict Connectivity?

Christopher L. Rees¹, Keivan Moradi¹, and Giorgio A. Ascoli^{1,*}

¹Kransnow Institute for Advanced Study, George Mason University, Fairfax, VA 22030, USA

Abstract

Although the importance of network connectivity is increasingly recognized, identifying synapses remains challenging relative to the routine characterization of neuronal morphology. Thus, researchers frequently employ axon–dendrite colocations as proxies of potential connections. This putative equivalence, commonly referred to as Peters' rule, has been recently studied at multiple levels and scales, fueling passionate debates regarding its validity. Our critical literature review identifies three conceptually distinct but often confused applications: inferring neuron type circuitry, predicting synaptic contacts among individual cells, and estimating synapse numbers within neuron pairs. Paradoxically, at the originally proposed cell-type level, Peters' rule remains largely untested. Leveraging Hippocampome.org, we validate and refine the relationship between axonal–dendritic colocations and synaptic circuits, clarifying the interpretation of existing and forthcoming data.

Big-Data Connectomes and the Usage of Potential-Synapse Proxies

Worldwide big-data endeavors such as the BRAIN Initiative [1] and the Human Brain Project [2] aspire to map the human brain connectome with detailed representations of individual neurons and their dynamic interactions. However, existing experimental approaches, including dense electron microscopy reconstructions, electrophysiological paired recording, and synaptic marker localization by light microscopy, are proving difficult to scale. Collecting, integrating, and interpreting data for up to 10^{11} neurons [3–5] and 10^{15} synapses [6] is thus a daunting challenge. Even disregarding interindividual diversity or temporal changes and focusing on small volumes of tissue in simpler species, the undertaking remains formidable. Recognizing that these highly expected data may remain a decade or more away, many researchers focus on neuronal morphology to answer the question of where synapses might be. While certain connections may be genetically or developmentally specified, an as-yet-undetermined proportion of synapses appear to form non-selectively between any crossing axonal and dendritic arbors. Accordingly, although individual synapses are contingent on molecular recognition and activity-dependent turnover, the spatial **apposition** (see Glossary) between axons and dendrites could to a certain degree provide a statistical summary of the connectome.

*Correspondence: ascoli@gmu.edu (G.A. Ascoli).

The identification of neurite overlaps with potential connections was originally proposed over four decades ago by Peters and Feldman for the specific projections from the lateral geniculate nucleus to the granular layer of the primary visual cortex in rats [7]. Twenty-two years later Braitenberg and Schüz broadly extended the idea to all cortical synapses, coining the phrase ‘Peters’ rule’ [6]. The principle, little heralded at its inception, has rapidly gained traction in recent times with the rising emphasis on connectomics. Unfortunately, this surge of popularity was accompanied by widespread confusion regarding the original intent and even the proper spelling of Peters’ rule. Google Scholar searches for “Peter’s rule”, “Peters rule”, and “Peters’s rule” all return neuroscience-relevant results intermingled with hits pertaining to: (i) the reign of Tsar Peter the Great; (ii) Peter’s half-slope method to estimate the depth of buried magnetic sources; and (iii) the Peter principle, according to which employees continue to be promoted until they reach their level of incompetence. Even among the citations genuinely pertaining to synaptic connectivity, common and self-perpetuating misconceptions about the intended scope of Peters’ rule conflate already fervent debates on the correctness and utility of this pragmatic approximation to neural circuitry.

Peters’ Rule: Original Intent and Promiscuous Generalizations

On critically reviewing the relevant literature, we here distinguish, for the first time, three conceptually separate usages of Peters’ rule (Box 1) roughly corresponding to increasing levels of resolution: the originally formulated inference of circuitry among **neuron types**, the identification of connectivity among individual neurons, and the estimation of the number of synapses within a neuron pair. Regrettably, as exploitation and scrutiny of Peters’ rule proliferated over time, the same term has been indiscriminately adopted for each of these three variants without regard to their conceptual differences (Figure 1). The ensuing muddy pool of literature comprises results that are not directly comparable.

Level 1: Inferring Circuitry among Neuron Types

In its foundational formulations [6,7], Peters’ rule postulated that, if neurons of type A project their axons to brain regions i, j , and k , the existence and amount of their synaptic connections to neurons of type X and Y primarily depend on the dendritic extent of X and Y in those same regions. Conversely, if the dendrites of neuron type Z invade region l , the existence and amount of the synapses that they receive from neuron types B and C are principally determined by the axonal presence of B and C in l . In other words, circuit connectivity among neuron types is statistically determined based on the regional colocation of the potential presynaptic axons and postsynaptic dendrites.

More precisely, the closing paragraph of Peters and Feldman’s seminal article [7] contained just a qualitative assertion regarding the presence of synapses among any neuron types with collocated axons and dendrites: ‘every component in layer IV capable of forming an asymmetric synapse is a potential recipient for a thalamocortical axon...in the sense that no specific types of neurons receive the efferents. Instead all elements in layer IV that are capable of forming asymmetric synapses may be involved.’ It was Braitenberg and Schüz who reformulated this notion quantitatively on designating Peters’ rule [6]: ‘we would

expect the proportion of synapses of different types on any dendritic tree to be the same as the proportion of the corresponding elements in its environment’.

At this level of neuron types, Peters’ rule has been applied in diverse species and neural systems, including the hatchling frog tadpole spinal cord [8], the cat primary visual cortex [9,10], the rodent hippocampus [11], and the mouse retina [12–15] and neocortex [16] as well as large-scale studies of the rat somatosensory cortex [17] and fly protocerebral bridge [18] (Table 1). The actual synaptic connectivity was in several cases found to corroborate the statistical impartiality of neuron types, thus quantitatively confirming Peters’ rule [9,15,19]. Other reports challenged the hypothesis by describing substantial connection biases among certain types [10,12–15,20].

Most of these studies, however, question only the stoichiometry of the connections rather than casting explicit doubts on the corresponding neurons types ‘being involved’ in the circuit blueprint. For instance, in the famous case of White’s exception [6,20], ‘They do agree with previous descriptions to the extent that axons entering the cortex make some synaptic contacts with every sort of potential postsynaptic element available within the territory of their terminal arborization. But, if the frequency with which the different kinds of elements are contacted are established by careful quantitative electron microscopy, they are sometimes found to be different from the frequency of these elements in the tissue.’ In other words, it is just the relative ‘proportions’ of synapses that is subject to debate and never the underlying network schematic.

Level 2: Assessing Potential Connectivity among Individual Neurons

Peters’ rule has also been invoked to assess potential connections among individual neurons. Consider two groups of neurons, $a_1 \dots a_n$ of type A and $b_1 \dots b_m$ of type B, and assume that neuron type A serves as presynaptic to type B (for example, A could be thalamic projection neurons while B could be layer IV spiny stellate cells in V1). For a synapse to be present, it is obviously necessary for the presynaptic axon to be juxtaposed to the postsynaptic dendrite, but this condition is not sufficient: a given neuron of type A synapses with only a subset of those neurons of type B whose dendrites are in close proximity to its axon (a proportion termed the **connectivity fraction** [21]).

This extension of Peters’ rule posits that the probability p_{ij} of neuron a_i being connected with neuron b_j is proportional to the spatial apposition of their respective axonal and dendritic arbors. Such an assumption is nontrivial since several factors might affect synapse formation, including the number of neurons of each type, their 3D **neurogeometries** [22] (including location, orientation, and **branch morphometrics** [9,23]), the density of junctures at given **touch distances** [17,22,24], the kind of synaptic elements (spines, shafts, gap junctions, and terminal boutons), the dynamic nature of synaptogenesis [25], and the communication method (chemical, electrical, or even volume transmission, whose longer-distance interactions are not subject to Peters’ rule).

In practice, testing this hypothesis requires the inspection of a large number of neurons of types A and B seeking axonal–dendritic juxtapositions. The prediction is that the extent of spatial proximity between A axons and B dendrites should be statistically greater among

connected neurons than among non-connected ones. Examining potential connectivity at this resolution is complicated by the low **connection density** in the brain. The potential pool of a typical cortical neuron's synaptic partners ($\sim 10^6$) far outnumbers its actual synapses ($\sim 10^4$) and the fraction of connected neurons diminishes further when multisynaptic connectivity is taken into account. Even in the eminently autoassociative hippocampal network of CA3 pyramidal cells, finding two connected neurons requires the examination of more than 100 neuronal pairs on average [26]. Although axonal–dendritic appositions are more common than synapses, they still occur in <10% of CA3 pyramidal cell pairs [27].

Despite the experimental challenge, this level of analysis constitutes a widespread application referred to with the name of Peters' rule, with resultant evidence both confirming [28,29] and, more frequently, refuting [30–34] the prediction (Table 1).

Level 3: Estimating Synapse Numbers within Neuronal Pairs

Peters' rule has been recently applied with subcellular scope to correlate the number of synapses within pairs of connected neurons with the count of their axonal–dendritic juxtapositions. This is an important open question because the mechanisms of synapse selection are poorly understood and the occurrence of mono- versus multisynaptic connectivity between neurons is likely to be activity dependent. Dense 3D reconstructions of entire neuropil volumes and sophisticated computational analyses again yielded mixed results supporting [16,17,35–38] and opposing [24,34,39] the predicted relation (Table 1).

Since two disconnected neurons can be seen as a particular case of a pair with zero synapses, it is reasonable to consider levels 2 and 3 as instantiations of the same phenomenon, whereby synapses independently form among all axonal–dendritic appositions with a fixed probability. This model, however, represents a conceptual departure from the original Level 1 intent of Peters' rule, even in Braitenberg's 1998 quantitative formulation. A double-dissociation thought experiment can demonstrate this divergence. Consider the axons of neuron type A invading region r to synapse with dendrites of types B and C. Suppose dendrites B to be twice as dense in r as dendrites C. Further, assume equal numbers of A–B and A–C appositions in r . Level 1 would predict a double number of A synapses with B relative to those with C, whereas according to the Level 2 hypothesis those numbers should be the same. Both of these scenarios would be consistent with Peters' 1976 qualitative affirmation since both B and C receive A efferents in light of their dendritic presence in region r .

Testing the Accuracy of Peters' Rule (Level 1) in the Hippocampal Formation

In its more general sense, the (original) circuitry-level version of Peters' rule simply affirms that the colocation of axonal and dendritic arbors reasonably forecasts at least some level of connectivity between the corresponding neuron types. Paradoxically, this relatively simple hypothesis remains to be tested systematically.

To evaluate available data, we take advantage of Hippocampome.org, a freshly compiled open-access catalog of all known hippocampal neuron types [11] annotated from ~14 000

pieces of experimental evidence through a multiyear literature-mining effort. This resource tracks the axonal and dendritic presence of 122 neuron types across 26 subregions and layers of the rodent hippocampal formation (HF) (<http://hippocampome.org/morphology>), including the dentate gyrus (DG), CA3/CA2/CA1, the subiculum (SUB), and the entorhinal cortex (EC). Applying Peters' rule at Level 1 for the 122×122 pairs of neuron types yields a potential connectome of >3300 potential synapses by identifying all axonal–dendritic colocations in any anatomical parcel [40]. In other words, Peters' rule is useful in excluding approximately 80% of all-to-all type–level connectivity. Nearly 10% of the remaining potential connections have been directly confirmed or refuted experimentally (e.g., via electron microscopy or dual electrophysiological recordings). Notably, these data are not restricted to synapses to or from principal cells, as 25% of all confirmed potential connections are between interneurons (<http://hippocampome.org/connectivity>; view evidence for known connections by clicking on the green '+' icons and for refuted connections by clicking on a red 'x'). This dataset provides a unique opportunity to assess the efficacy of Peters' rule for prediction of cell-type circuitry in the hippocampal formation.

The results of this meta-analysis, overall and by subregion, are displayed in Table 2. In the rodent hippocampus, the original version of Peters' rule is correct in 75.3% of investigated cases (219 confirmed connections compared with 72 refuted), with the lowest accuracy in CA3 (56.5%) and the highest for connections that project across subregion boundaries. Interestingly, the vast majority of exceptions are attributable to just two families of neurons with well-known targeting specificity: **axo-axonic cells** [41–44], which avoid interneurons and connect exclusively to the axon initial segment of principal cells, and **interneuron-specific interneurons** [43–46], which synapse preferentially with other GABAergic neurons. When accounting for these connectivity preferences in a more nuanced version of Peters' rule, the predictive accuracy jumps to a remarkable 99% (219 corroborations compared with two incorrect cases).

As a cautionary note, it is worth observing that the connectivity literature itself is likely to be inclined towards reports of confirmed synapses over refuted synapses. This is due to both a positive-results publication bias and the difficulty of disproving connectivity with existing electrophysiological methodology. Nevertheless, the data at hand suggest that most hippocampal cell types may connect with a broad selection of potential partners as determined by their respective axonal and dendritic morphologies.

Reconciling Opposing Views in the Community

Progress in neuroscience has been fettered not only by neural complexity and technological hurdles but also by ambiguous terminologies. Investigations of neural connectivity at the macroscale (whole-brain tracts among regions or voxels) differ fundamentally from those at the microscale (ultrastructural mapping of synaptic contacts) [47,48]. Light microscopy identification of putative contacts provides a practical mesoscale bridge between these connectomic levels. Peters' rule formalizes this link by explicitly tying network connectivity to axonal and dendritic morphology. This primer illuminated the distinct conceptual interpretations of that hypothesis for: (i) defining neuron type circuitry; (ii) determining the connection probability among individual neurons; and (iii) estimating the number of

synapses within connected neuronal pairs. A large portion of the polarizing discourse on Peters' rule [13,16,24,49–51] can be ascribed to confusion and disagreement over these different levels. Recognizing and clarifying the multiple notions behind this single name might thus 'explain away' many results that initially seem contradictory.

Regardless of the application, however, Table 1 shows that Peters' rule works in some instances and not in others. It may thus be possible to progressively refine this 40-year-old connectivity rule by introducing appropriate corrections for different orders of specificity [25,52]. To a first approximation, certain connections are based on the axonal–dendritic coincidence [53]. Describing the network in this fashion is useful to expose the backbone architecture underlying the main signal propagation paths and local processing unit blocks [40]. Next, accounting for cell-type-specific targeting (e.g., axo-axonic and interneuron specific cells in the hippocampus) in Level 1 violation of Peters' rule may reveal precise functional ramifications, including direct inhibition or disinhibition of key players within the larger network [54]. Non-synaptic junctional diffusion (e.g., via serotonin, acetylcholine, or dopamine) [55] is also exempt from Peters' rule. Last, some (Level 2 and 3) connectivity is likely to be functionally specific, where synapsing (or lack thereof) is not determined by neuronal morphology or genetics but rather by the temporal flux of physiological activity (see Outstanding Questions).

In the intensely investigated rodent hippocampal formation, Peters' rule is strikingly predictive once corrected for modest and well-known exceptions. As information grows regarding other neural systems, suitably tiered rules might emerge to describe compactly yet accurately overall circuitry and synaptic specificity. For example, a recent study of the adult mouse visual cortex summarized the connectivity of interneurons into three broad categories [56]: 'master regulators' connected nonspecifically to all types in proximity to their axons; cells preferentially targeting pyramidal neurons and other interneurons of their own type; and interneuron specific interneurons synapsing primarily with other GABAergic cells except those of their own type. It will be interesting to explore the robustness and generalizability of these rules to other species, regions, and developmental stages (see Outstanding Questions).

A variety of applications over the past four decades has blurred the original intent of Peters' rule. The time is ripe to re-sharpen and refine the basic principles of neuron-type circuitry. Reconciling contrasting views in the community may pave the path towards the derivation of a 'draft' connectome from neuronal morphology to help guide and interpret forthcoming synaptic data.

Acknowledgments

The authors thank Diek Wheeler, Charise White, Alexander Komendantov, David Hamilton, Siva Venkadesh, and all of the contributors to this project (<http://hippocampome.org/thx>) for their suggestions and help. This work was supported in part by grants R01NS39600 (NIH), IIS-1302256 (NSF), and MURI N00014-10-1-0198 (ONR).

Glossary

Apposition

spatial proximity between neurites, sometimes (but not always) expressly specified via a given touch distance (see below).

Axo-axonic cells

also known as chandelier cells; a GABAergic, fast-spiking, parvalbumin-containing type of neuron that synapses exclusively with the axon initial segment of glutamatergic principal neurons, providing inhibition.

Branch morphometrics

any of several measures quantifying the shape and size of neurite arbors, including length, surface area, fractal dimension, branch order, number of bifurcations, and 3D orientation.

Connection density

the number of connections made divided by the square of the number of elements in the network.

Connectivity fraction

the ratio of actual to potential connections.

Interneuron-specific interneurons

any GABAergic cell type with biased affinity for synapsing with other interneuron types, often in direct conflict with the net distributions of those types in a given volume.

Neurogeometry

the geometric shapes of axons and dendrites, which can be quantified using various branch morphometrics.

Neuron type

a particular set of neurons grouped because they share one or more morphological, molecular, electrophysiological, or other properties; within-type diversity varies according to the definition and the type.

Potential connection

a location where axon-dendrite proximity permits a synapse to form.

Touch distance

a specified maximum distance between axons and dendrites under which all adjacencies or crossings, typically identified via electron microscopy, are referred to as ‘touches’.

References

1. Insel TR, et al. The NIH BRAIN Initiative. *Science*. 2013; 340:687–688. [PubMed: 23661744]
2. Markram H, et al. Reconstruction and simulation of neocortical microcircuitry. *Cell*. 2015; 163:456–492. [PubMed: 26451489]
3. Williams RW, Herrup K. The control of neuron number. *Annu. Rev. Neurosci.* 1988; 11:423–453. [PubMed: 3284447]
4. Azevedo FA, et al. Equal numbers of neuronal and non-neuronal cells make the human brain an isometrically scaled-up primate brain. *J. Comp. Neurol.* 2009; 513:532–541. [PubMed: 19226510]

5. Herculano-Houzel S. The human brain in numbers: a linearly scaled-up primate brain. *Front. Hum. Neurosci.* 2009; 3:31. [PubMed: 19915731]
6. Braitenberg, V., Schüz, A., editors. *Cortex: Statistics and Geometry of Neuronal Connectivity*. Springer-Verlag; 1998.
7. Peters A, Feldman ML. The projection of the lateral geniculate nucleus to area 17 of the rat cerebral cortex. I. General description. *J. Neurocytol.* 1976; 5:63–84. [PubMed: 1249593]
8. Li WC, et al. Axon and dendrite geography predict the specificity of synaptic connections in a functioning spinal cord network. *Neural. Dev.* 2007; 2:17. [PubMed: 17845723]
9. Binzegger T, et al. A quantitative map of the circuit of cat primary visual cortex. *J. Neurosci.* 2004; 24:8441–8453. [PubMed: 15456817]
10. Potjans TC, Diesmann M. The cell-type specific cortical microcircuit: relating structure and activity in a full-scale spiking network model. *Cereb. Cortex.* 2014; 24:785–806. [PubMed: 23203991]
11. Wheeler DW, et al. Hippocampome.org: a knowledge base of neuron types in the rodent hippocampus. *Elife.* 2015; 4:e09960. [PubMed: 26402459]
12. Morgan JL, et al. Development of cell type-specific connectivity patterns of converging excitatory axons in the retina. *Neuron.* 2011; 71:1014–1021. [PubMed: 21943599]
13. Helmstaedter M, et al. Connectomic reconstruction of the inner plexiform layer in the mouse retina. *Nature.* 2013; 500:168–174. [PubMed: 23925239]
14. Kim JS, et al. Space-time wiring specificity supports direction selectivity in the retina. *Nature.* 2014; 509:331–336. [PubMed: 24805243]
15. Krishnaswamy A, et al. Sidekick 2 directs formation of a retinal circuit that detects differential motion. *Nature.* 2015; 524:466–470. [PubMed: 26287463]
16. Packer AM, et al. Axo-dendritic overlap and laminar projection can explain interneuron connectivity to pyramidal cells. *Cereb. Cortex.* 2013; 23:2790–2802. [PubMed: 22941716]
17. Reimann MW, et al. An algorithm to predict the connectome of neural microcircuits. *Front. Comput. Neurosci.* 2015; 9:120. [PubMed: 26500529]
18. Lin CY, et al. A comprehensive wiring diagram of the protocerebral bridge for visual information processing in the *Drosophila* brain. *Cell Rep.* 2013; 3:1739–1753. [PubMed: 23707064]
19. Peters A. Thalamic input to the cerebral cortex. *Trends Neurosci.* 1979; 2:1183–1185.
20. White EL, Keller A. Intrinsic circuitry involving the local axon collaterals of corticothalamic projection cells in mouse SmI cortex. *J. Comp. Neurol.* 1987; 262:13–26. [PubMed: 3624546]
21. Escobar G, et al. Structural plasticity of circuits in cortical neuropil. *J. Neurosci.* 2008; 28:8477–8488. [PubMed: 18716206]
22. Stepanyants A, Chklovskii DB. Neurogeometry and potential synaptic connectivity. *Trends Neurosci.* 2005; 28:387–394. [PubMed: 15935485]
23. Jefferis GS, et al. Comprehensive maps of *Drosophila* higher olfactory centers: spatially segregated fruit and pheromone representation. *Cell.* 2007; 128:1187–1203. [PubMed: 17382886]
24. Kasthuri N, et al. Saturated reconstruction of a volume of neocortex. *Cell.* 2015; 162:648–661. [PubMed: 26232230]
25. Reid RC. From functional architecture to functional connectomics. *Neuron.* 2012; 75:209–217. [PubMed: 22841307]
26. Guzman SJ, et al. Synaptic mechanisms of pattern completion in the hippocampal CA3 network. *Science.* 2016; 353:1117–1123. [PubMed: 27609885]
27. Ropireddy D, Ascoli GA. Potential synaptic connectivity of different neurons onto pyramidal cells in a 3D reconstruction of the rat hippocampus. *Front. Neuroinform.* 2011; 5:5. [PubMed: 21779242]
28. Fino E, Yuste R. Dense inhibitory connectivity in neocortex. *Neuron.* 2011; 69:1188–1203. [PubMed: 21435562]
29. Rieubland S, et al. Structured connectivity in cerebellar inhibitory networks. *Neuron.* 2014; 81:913–929. [PubMed: 24559679]
30. Hamos JE, et al. Synaptic circuits involving an individual retinogeniculate axon in the cat. *J. Comp. Neurol.* 1987; 259:165–192. [PubMed: 3584556]

31. Shepherd GM. Geometric and functional organization of cortical circuits. *Nat. Neurosci.* 2005; 8:782–790. [PubMed: 15880111]
32. Mishchenko Y, et al. Ultrastructural analysis of hippocampal neuropil from the connectomics perspective. *Neuron.* 2010; 67:1009–1020. [PubMed: 20869597]
33. Briggman KL, et al. Wiring specificity in the direction-selectivity circuit of the retina. *Nature.* 2011; 471:183–188. [PubMed: 21390125]
34. Lee WCA, et al. Anatomy and function of an excitatory network in the visual cortex. *Nature.* 2016; 532:370–374. [PubMed: 27018655]
35. Stepanyants A, et al. Local potential connectivity in cat primary visual cortex. *Cereb. Cortex.* 2008; 18:13–28. [PubMed: 17420172]
36. Czajkowski R, et al. Superficially projecting principal neurons in layer V of medial entorhinal cortex in the rat receive excitatory retrosplenial input. *J. Neurosci.* 2013; 33:15779–15792. [PubMed: 24089485]
37. van Pelt J, van Ooyen A. Estimating neuronal connectivity from axonal and dendritic density fields. *Front. Comput. Neurosci.* 2013; 7:160. [PubMed: 24324430]
38. Merchán-Pérez A, et al. Three-dimensional spatial distribution of synapses in the neocortex: a dual-beam electron microscopy study. *Cereb. Cortex.* 2014; 24:1579–1588. [PubMed: 23365213]
39. Takemura SY, et al. Synaptic circuits and their variations within different columns in the visual system of *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 2015; 112:13711–13716. [PubMed: 26483464]
40. Rees CL, et al. Graph theoretic and motif analyses of the hippocampal neuron type potential connectome. *Eneuro.* 2016; 3:e0205.
41. Li XG, et al. Axonal and dendritic arborization of an intracellularly labeled chandelier cell in the CA1 region of rat hippocampus. *Exp. Brain Res.* 1992; 90:519–525. [PubMed: 1385200]
42. Bernard C, Wheal HV. Model of local connectivity patterns in CA3 and CA1 areas of the hippocampus. *Hippocampus.* 1994; 4:497–529. [PubMed: 7889123]
43. Freund TF, Buzsáki GY. Interneurons of the hippocampus. *Hippocampus.* 1996; 6:347–470. [PubMed: 8915675]
44. Klausberger T, Somogyi P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science.* 2008; 321:53–57. [PubMed: 18599766]
45. Acsády L, et al. Different populations of vasoactive intestinal polypeptide-immunoreactive interneurons are specialized to control pyramidal cells or interneurons in the hippocampus. *Neuroscience.* 1996; 73:317–334. [PubMed: 8783252]
46. Gulyás AI, et al. Interneurons containing calretinin are specialized to control other interneurons in the rat hippocampus. *J. Neurosci.* 1996; 16:3397–3411. [PubMed: 8627375]
47. DeFelipe J. From the connectome to the synaptome: an epic love story. *Science.* 2010; 330:1198–1201. [PubMed: 21109663]
48. Martone ME, Ascoli GA. Connecting connectomes. *Neuroinformatics.* 2013; 11:389–392. [PubMed: 24096724]
49. White EL. Specificity of cortical synaptic connectivity: emphasis on perspectives gained from quantitative electron microscopy. *J. Neurocytol.* 2002; 31:195–202. [PubMed: 12815239]
50. Briggman KL, Denk W. Towards neural circuit reconstruction with volume electron microscopy techniques. *Curr. Opin. Neurobiol.* 2006; 16:562–570. [PubMed: 16962767]
51. Markram H. The Blue Brain Project. *Nat. Rev. Neurosci.* 2006; 7:153–160. [PubMed: 16429124]
52. Ohki K, Reid RC. Specificity and randomness in the visual cortex. *Curr. Opin. Neurobiol.* 2007; 17:401–407. [PubMed: 17720489]
53. Ascoli GA, Wheeler DW. In search of a periodic table of the neurons: axonal–dendritic circuitry as the organizing principle. *Bioessays.* 2016; 38:969–976. [PubMed: 27516119]
54. Yoshimura Y, Callaway EM. Fine-scale specificity of cortical networks depends on inhibitory cell type and connectivity. *Nat. Neurosci.* 2005; 8:1552–1559. [PubMed: 16222228]
55. Selmeczy Z, et al. Role of nonsynaptic communication in regulating the immune response. *Neurochem. Int.* 2008; 52:52–59. [PubMed: 17640770]

56. Jiang X, et al. Principles of connectivity among morphologically defined cell types in adult neocortex. *Science*. 2015; 350:1055.
57. Ascoli GA, et al. NeuroMorpho.org: a central resource for neuronal morphologies. *J. Neurosci.* 2007; 27:9247–9251. [PubMed: 17728438]
58. Ramaswamy S, et al. Intrinsic morphological diversity of thick-tufted layer 5 pyramidal neurons ensures robust and invariant properties of in silico synaptic connections. *J. Physiol.* 2012; 590:737–752. [PubMed: 22083599]
59. Thomson AM, et al. Synaptic connections and small circuits involving excitatory and inhibitory neurons in layers 2–5 of adult rat and cat neocortex: triple intracellular recordings and biocytin labelling *in vitro*. *Cereb. Cortex.* 2002; 12:936–953. [PubMed: 12183393]

Trends

Ongoing attempts to map the structural and functional connectivity of the nervous system focus on two separate endeavors: Systems-resolution tract-tracing approaches (projectomes) reveal the regional architecture of brain-wide network projections but are blind to individual-neuron details. Synapse-resolution projects (synaptomes) generate rich data but at a much slower pace and only in limited volumes of tissues.

Peters' rule predicts connectivity among neuron types based on the anatomical colocation of their axonal and dendritic arbors, providing a statistical summary of neural circuitry at mesoscopic resolution.

A conceptual extension of Peters' rule has also been applied to assess connectivity at the individual cell and subcellular levels, leading to confusion and disagreement about its correctness.

Recent knowledge bases provide morphological potential-connectivity data, making the original version of Peters' rule testable.

Box 1

Three Conceptually Distinct Interpretations of Peters' Rule

Level 1 (cell type)		As originally proposed, Peters' rule predicts synapsing among neuron types with anatomically colocated axonal and dendritic arbors. Quantitatively interpreted, the number of synapses increases with the coincident availability of axons and dendrites, but the connectivity of individual cells within each type is not considered.
Level 2 (individual neuron pairs)		Peters' rule has also been invoked to mean that spatial overlap of axons and dendrites predicts synapsing among individual neurons. Actual connectivity heavily depends on at least two factors: the spatial geometry (e.g., branching patterns, relative orientation, overlap) and the specificity of the neurites in question.
Level 3 (synapse number)		When applied with subcellular resolution, Peters' rule is taken to predict the number of synapses between connected neurons based on the number of their axonal–dendritic overlaps at a set touch distance.

Outstanding Questions

What percentage of connections is genetically or developmentally specified as opposed to formed through experience-dependent plasticity among the happenstantial juxtapositions of neurites?

How do connectivity fractions vary across species, brain regions, and developmental stages?

How well does neuron morphology predict Peters' original rule (i.e., at the intended level of cell types) in brain regions other than the hippocampus?

How many layers of increasing refinement are needed to 'correct' Peters' rule? If identifying exceptions based on connectivity-specific groupings can increase the accuracy of Peters' rule, are there functional-level rules to subdivide the groups further?

Is there a systematic relationship between actual synapse formation and touch distance? Does this optimal distance vary according to presynaptic specializations (*en passant* and terminal varicosities, filopodial extensions) and post-synaptic elements (e.g., spine heads, spine necks, dendritic shafts, axonal initial segments, somata)?

Are there specific motifs affecting the conditional probability of connectivity among specific neuron classes such as excitatory, inhibitory, and neuromodulatory?

Do connection probabilities vary with intersomatic distance?

Does Peters' rule apply to connections with non-neuronal partners such as glia, muscles, and the microvasculature?

Do diseases and other conditions affect Peters' rule (connectopathies)?

Usage of Peters' rule by level

Trends in Neurosciences

Figure 1.
Citations of Peters' Rule Have Proliferated Over Time Concomitant with Increasing
Confusion about the Intended Interpretation Level.

Table 1

The Applications of Peters' Rule: Abridged Synthesis from the Past 5 Years

Refs	Level	Application
[33] ^a	2	Reconstructed 24 starburst amacrine cells and six direction-selective ganglion cells from the mouse retina using electron microscopy and two-photon calcium imaging. Found the types to be connected, but connections between individual neurons were highly specific depending on the preferred direction of the ganglion cell.
[28]	2	Utilizing two-photon uncaging stimulation to map connections systematically with single-cell resolution; found nearly full connectivity between somatostatin-positive GABAergic interneurons and pyramidal cells in mouse frontal cortex. No speculation about whether dense connectivity is due to Peters' rule overlap or to more selective mechanisms.
[12]	1	Examined synaptic development of three glutamatergic bipolar cell types converging onto a common postsynaptic retinal ganglion cell. Determined that neurogeometry predicts synaptogenesis during early developmental periods. However, synapses became specific with maturation, breaking down Peter's rule.
[27] ^a	2 and 3	Digitally embedded 3D reconstructions from NeuroMorpho.Org [57] and reported on the potential connectivity from various presynaptic principal cells and interneurons across rat hippocampal subregions onto pyramidal neuron dendrites.
[58]	3	Identified axodendritic appositions among thick-tufted layer 5 pyramidal neurons packed and modeled in a volume of rat somatosensory cortex and analyzed the resulting microcircuit.
[36]	3	Found the density of retrosplenial cortex axonal fibers to be linearly proportional to the number of their synaptic contacts on medial entorhinal cortex layer V cell dendrites.
[13]	1	Densely reconstructed 950 neurons and their mutual contacts in mouse retina from electron microscopy data by crowd-sourced manual annotation and machine learning. The absence of contacts between certain cell types with extensively co-mingled neurites suggests violations of Peters' rule.
[18] ^a	1	Examined 3D reconstructions to propose a comprehensive wiring diagram based on the intersections among 194 neuron types in the <i>Drosophila</i> protocerebral bridge.
[16]	1 and 3	Investigated whether Peters' rule could explain the nonspecific connectivity between somatostatin-positive neurons and parvalbumin-positive neurons to pyramidal cells in the neocortex. Concluded that the spatial profile of the connectivity maps and even the postsynaptic position of interneuron contacts could result from axodendritic overlap of their arbors.
[37]	3	Derived an expression for the expected number of contacts between two neurons based on the overlap of their axonal and dendritic density fields that accurately estimated the number of synapses between neuron pairs.
[14]	1	Using crowd-sourced reconstructions, identified contact areas between starburst amacrine cell types and bipolar cell types. Showed that fairly subtle violations of Peters' rule may be important for visual function.
[38]	3	In neuropil samples of layer III of the young rat somatosensory cortex, fully reconstructed ~1700 synaptic junctions while determining synapse position and analyzing spatial distributions. Results indicated that the distribution of synaptic junctions was nearly random.
[10]	1	Derived a modified version of earlier connectivity maps [9,59] to correct for the excitatory/inhibitory target specificity of certain cell types in a cortical network model.
[29]	2	Investigated the connectivity of the interneuron network in the molecular layer of the cerebellum by graph-theoretic techniques to determine that connectivity among cell pairs is random but connectivity among triplets is structured.
[24]	3	Used Peters' rule to explore whether redundant synapses form among random appositions between excitatory axons and dendritic spines. Refuted the idea that physical proximity is sufficient to predict the formation of a synapse.
[15]	1	In the mouse retina, detected strong and weak connectivity between types (by optogenetic stimulation) that was partially attributable to the fraction of individual cells connected and was not explainable by the overlap of arbors.
[17]	3	Applied Peters' rule extensively to predict connectivity from morphology and digitally reconstructed the complete synaptic connectivity between neuron types in a contained volume of cortical tissue.
[39]	3	In the <i>Drosophila</i> visual system, determined that synapse numbers did not correlate with the area of contact between pairs of neurons.

Refs	Level	Application
[11]	1	Constructed a knowledge base for neuron types and their properties in the rodent hippocampal formation. In the absence of information on known connectivity between types, inferred potential connectivity by identifying axon–dendrite colocalizations in 26 anatomically defined parcels.
[34]	2 and 3	In the rodent primary visual cortex, found that functionally specific connections do not result from the spatial arrangement of the neuropil and that multiple synapses between pairs of neurons occurred at far above chance levels.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2

Meta-analysis of [Hippocampome.org](https://www.hippocampome.org) Data Reveals that the Vast Majority of Exceptions to Peters' Rule Involve Restricted Numbers of Neuron Types with Well-Known Targeting Specificity

Connections	HF	DG	CA3	CA2	CA1	SUB	EC	Inter subregional
Potential, by Peters' rule	3302	263	451	25	1075	8	722	758
Experimentally confirmed	219	45	26	9	77	2	6	54
Experimentally refuted (total)	72	11	20	0	35	0	1	5
Axo-axonic refuted	60	11	20	0	26	0	0	3
Interneuron specific refuted	10	0	0	0	9	0	0	1
Other refuted	2	0	0	0	0	0	1	1
Current known accuracy of original Peters' rule	75.3%	80.4%	56.5%	100.0%	68.8%	100.0%	85.7%	91.5%
Current known accuracy of nuanced Peters' rule	99.1%	100.0%	100.0%	100.0%	100.0%	100.0%	85.7%	98.2%
Sample size	8.8%	21.3%	10.2%	36.0%	10.4%	25.0%	1.0%	7.8%