Retinal Pathoconnectomics: A Window into Neurodegeneration

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Abstract

Over the past decade, the field of retinal connectomics has made huge strides in describing the precise topologies underlying retinal visual processing. The same techniques that allowed these advancements are also applicable to understanding the progression of rewiring in retinal remodeling: retinal pathoconnectomics. Pathoconnectomics is unique in its unbiased approach to understanding the impacts of deafferentation on the remaining network components and identifying aberrant connectivities leading to visual processing defects. Pathoconnectomics also paves the way for identifying underlying rules of rewiring that may be recapitulated throughout the nervous system in other neurodegenerative diseases.

Keywords

Retinal remodeling · Connectomics · Pathoconnectome · Ultrastructure · Early retinal degeneration · Neurodegeneration · Neural networks

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1 Introduction

The idea of distributed networks underlying nervous system function dates back to the Roman physician Galen. As our knowledge of neuroanatomy has progressed, understanding neuronal and glial diversity, along with the molecular contributions underlying neural networks has proven invaluable. Yet, a precise understanding of how neurons are connected to one another, and their relationships with glia, is an ideal application for connectomics approaches. Detailing neuronal class membership and their precise synaptic connections with one another is the basis for the field of ultrastructural connectomics [1].

A connectome is an audacious undertaking, requiring the expertise of a team skilled in anatomy, histology, electron microscopy, chemistry, and computational resources to capture, assemble, annotate, and analyze the resulting large data sets required to encode canonical volumes of neural tissue [2]. Connectomics approaches reveal the network architecture ground truth that have remained hidden to prior techniques. Connectomics initiatives have revealed numerous specific cell classes possessing greater diversity and network complexity than expected, with retina leading the way in describing precise network topologies [3–12].

The retina presents a unique opportunity to explore neuronal networks of the central nervous system (CNS). Retina is compact, highly

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organized, and consists of complex, yet complete, networks that perform all of the algorithmic computations associated with visual primitives, organized in a matrix of repeating motifs allowing for robust exploration and quantification. Within the past 10 years, retinal connectomics has identified novel circuits including ON-OFF bipolar cell crossover motifs [4], crossover motifs involving rod and cone vision [5], nested amacrine cell networks [6], novel network topologies between amacrine cells and ganglion cells [7], and numerous gap junctional coupling motifs below light microscopy resolutions [8] that are highly conserved across the retina. All these network architectures underly components of retinal visual processing, illustrating connectomics approaches for understanding how networks takes seemingly simple input (photons) and transforms it via neuronal and glial networks into the components necessary for interpreting the visual world around us.

2 Remodeling

Because neural systems are interrelated networks, damage to one cell type or region does not occur without wider network consequences. This phenomenon was coined "diaschisis" by von Monakow in 1914 [13] by describing affected remote regions connected in some way to the region of injury. Today, noninvasive imaging methods including diffusion tensor imaging (DTI) and functional magnetic resonance imaging (fMRI) demonstrate pathological disease spread following injury (e.g., stroke, seizures, and concussion) and in neurodegenerative disease (e.g., Alzheimer's disease, Parkinson's disease, and Huntington's disease) [14]. Redundancy in parallel neural networks compensates for losses to some degree, but the fundamental question is: How much damage can neural networks take before failing? Understanding the underlying components contributing to disease spread and how the affected networks breakdown is fundamental to developing therapies, and we propose that retina is an ideal model to explore network and molecular components of neural injury.

Negative plasticity has long been known in the hippocampus and other regions of CNS [15, 16], and retina is no different in its response to deafferentation, exhibiting remodeling processes in trauma or diseases that compromise photoreceptors. Remodeling is not restricted to photoreceptors in outer retina that comprise the sensory retina. Following injury (e.g., retinal detachment, light-induced retinal degeneration) [17–19] or disease (e.g., retinitis pigmentosa, age-related macular degeneration) [20–26], the retina undergoes a progressive series of revisions including neuritic sprouting, alterations in glutamate channel expression, aberrant synaptogenesis, and glial responses [27–29]. Of particular interest to connectomics is the neurite sprouting and aberrant synaptogenesis underlying rewiring.

Early investigations of retinal remodeling and rewiring were accomplished through immunohistochemical analyses [29]. These studies demonstrated a robust, reproducible progression associated with the severity of photoreceptor degeneration, regardless of initial insult. Retinal remodeling is phased revision; in phase 1, rod photoreceptors become stressed and extend neurite sprouts beyond their normal synaptic partners (rod-contacting horizontal cells (HCs) and rod bipolar cells (RodBCs) [24, 26, 30]. Simultaneously, RodBCs and HCs begin dendritic retraction from the rod photoreceptors with a subset aberrantly contacting cone photoreceptors [31]. In phase 2, photoreceptors (including cones) continue to degenerate and undergo cell death. As photoreceptors degenerate, bipolar cells (BCs) and HCs continue to lose synaptic input, with BCs becoming completely deafferented following the complete loss of photoreceptors [21, 22]. HCs extend processes deep into inner retina, though what contacts they make is unknown [21–23]. Also, during phase 2, amacrine cells (ACs) and ganglion cells (GCs) begin to sprout anomalous neurites that extend outside of their normal patterns, and make new synapses, though up to this point precise network motifs were unknown [20, 25, 28]. Phase 3 remodeling is characterized by a complete absence of photo-

receptors. At this point, all cell classes have initiated neurite sprouting, coalescing into synaptic groupings termed "microneuromas" [25]. Within these microneuromas, ultrastructural anatomy is consistent with the presence of functional synapses as is evident by the presence of ribbons, vesicle clouds, and associated post-synaptic densities.

Combined, these studies demonstrated the clear presence of network altering morphologies and individual synapses, but are insufficient to fully describe the precise ways in which the networks as a whole are altered.

3 Pathoconnectomics and RPC1

Connectomics has demonstrated that complete network diagrams are required to understand neural circuitry. A building literature illustrates that we cannot guess at topologies, we have to map them [1]. Because networks are altered in disease, connectomes of pathological tissue, or "pathoconnectomes," are therefore necessary to determine how network topologies are changed, and rules that underly rewiring. Just as retinas were ideal for constructing the first connectome, they also make an ideal platform for creating and exploring the first pathoconnectomes, retinal pathoconnectome 1 (RPC1) [32].

RPC1 was constructed from a 10-month-old transgenic rabbit model of autosomal dominant human retinitis pigmentosa, originally created in the Kondo laboratory with a rhodopsin proline 347 to leucine mutation [33]. We previously detailed progression of degeneration in this rabbit [34] and found that it progresses through cone-sparing retinal degeneration analogous to that seen in humans [27, 35, 36]. RPC1 was selected for a reduced photoreceptor layer thickness and shortened rod outer segments, but is prior to complete rod outer segment loss. This early time point of retinal degeneration allows the description of partial deafferentation of rod connected HCs, RodBCs, and what effects this has on the downstream neurons in the network.

4 Early Findings

Analysis of RPC1 began by examining RodBCs as they are the source of the first synapse in the visual system and function as the interneuron connecting sensory to neural retina, via rod synapses. As predicted from earlier studies, RodBCs extend processes toward cone axon terminals. However, in RPC1, we not only confirmed the presence of synaptic contacts from RodBCs onto cone pedicles but also demonstrated some cone inputs were not from the conventional cone pedicle, but rather from secondary terminals found on sprouts off of cones not normally observed in healthy retina. We also found these novel connections with cones occur prior to complete deafferentation of RodBCs from rod photoreceptors, meaning that at least for part of retinal degeneration, there is mixed input from both rods and cones. Next, we evaluated RodBC connections in inner retina and were surprised to find that although chemical synapse number and partnerships appear unchanged, gap junctions emerged between RodBCs and the Aii amacrine cell [32]. Gap junctions are electrical synapses coupling many neuronal classes in retina. Within retina, gap junctions convey polarization state between Aii amacrine cells and ON-type cone bipolar cells, allowing RodBCs making chemical synapses onto Aii amacrine cells to inject their signals to GCs. The emergence of gap junctional coupling directly between RodBCs and Aii amacrine cells is a network corruption causing a feedback loop never found in healthy retina, likely impacting the duration and timing of glutamate release by RodBCs. More altered circuit topologies are currently under investigation as understanding the complete network and how it fails in retinal degenerative disease is our goal. We have continued exploration of the Aii amacrine cell and discovering alterations in synapse partners and numbers as degeneration progresses. In addition, RPC1 contains numerous copies of ACs and GCs that are demonstrating neurite sprouting, indicating this process initiates earlier in retinal degeneration than was previously predicted.

5 Future of Pathoconnectomics

The next steps are clear as we continue this work in later stages of retinal degeneration in the next two pathoconnectomes produced from retinas in later stages of degeneration: RPC2 and RPC3. These next pathoconnectomes will reveal how the network is altered at more advanced stages of disease progression. In a more global sense, we believe pathoconnectomics as a field will inform the wider neuroscience community not only on the progression of specific neurological diseases but will also demonstrate specific stereotyped rules in the way networks change in disease and will play a crucial role due to the difficulties in doing whole-brain ultrastructural connectomics.

We have detailed in previous sections, the advantages of the size, organization, and repeated motifs of the retina lending it to ultrastructural connectomics. In addition, our recent work suggests the retina recapitulates neurodegeneration by entering a stage of progressive neurodegeneration following prolonged remodeling, which appears to only end once all of the neurons of the retina have degenerated [29]. We further find similarities between retinal degeneration and some of the classic neurodegenerative diseases (Parkinson's disease and Alzheimer's disease) through alterations in α-synuclein and phosphorylated α-synuclein expression [29]. These features make the retina ideal to observe how proteinopathies spread from neuron to neuron as a potential driving component of neurodegeneration. Lastly, the detailed network topographies already mapped from retina make it unique in our ability to identify and track network changes initiated during rewiring, potentially revealing new, fundamental mechanisms of neurodegeneration.

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