The Types of Retinal Ganglion Cells: Current Status and Implications for Neuronal Classification

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Abstract

In the retina, photoreceptors pass visual information to interneurons, which process it and pass it to retinal ganglion cells (RGCs). Axons of RGCs then travel through the optic nerve, telling the rest of the brain all it will ever know about the visual world. Research over the past several decades has made clear that most RGCs are not merely light detectors, but rather feature detectors, which send a diverse set of parallel, highly processed images of the world on to higher centers. Here, we review progress in classification of RGCs by physiological, morphological, and molecular criteria, making a particular effort to distinguish those cell types that are definitive from those for which information is partial. We focus on the mouse, in which molecular and genetic methods are most advanced. We argue that there are around 30 RGC types and that we can now account for well over half of all RGCs. We also use RGCs to examine the general problem of neuronal classification, arguing that insights and methods from the retina can guide the classification enterprise in other brain regions.

Contents

INTRODUCTION

Retinal ganglion cells (RGCs) are the brain's only portal to the visual world. What do these neurons tell the brain, and in what language? Different ganglion cell types are sensitive to distinct visual features and thus send a set of parallel, highly processed images of the world to the rest of the brain. In mice, genetic techniques have accelerated progress in classifying and characterizing RGCs; more than half can now be unambiguously specified. Here we provide a progress report. We also use RGCs as a lens through which to examine the general problem of neuronal classification.

Identification of cell types is critical to understanding the brain: They specify the parts list from which circuits are assembled. Indeed, neuronal classification was a mainstay of neuroscience a century ago (Ramón y Cajal 1892, Polyak 1957), but subsequently, it came to be viewed as mere

"stamp collecting." Recently, however, the tide has turned. An atlas of cell types is a major plank in the BRAIN Initiative platform (**http://www.braininitiative.nih.gov/2025/index.htm**), and activity has intensified on many fronts. Why this change? We note several reasons.

First, clear categorization allows researchers at different times or in different labs to know they are dealing with the same cells. The ability to address identified cells repeatedly has been key to unraveling circuits in invertebrate nervous systems; the same power can now be applied to mammals. Second, once genes expressed selectively in specific cell types are identified, they can be used to gain genetic access to them, so the cells can then be marked and manipulated (Siegert et al. 2009, Madisen et al. 2012, Huang & Zeng 2013). Third, prospective identification of cell types is invaluable for developmental studies: It is hard to unravel the steps by which a neuron achieves its final form and function if it can only be identified after its development is complete. Finally, clear descriptions of neuronal categories enable evolutionary comparisons among model organisms—and with humans.

NEURONAL CLASSES AND TYPES

Linguistic confusion can arise in naming neuronal classes, subclasses, types, and subtypes. Following earlier usage (Wässle & Boycott 1991, Masland 2004), we refer to groups of neurons that can be qualitatively distinguished from other groups as types—a definition we refine below. Classes are groups of types that share common features.

In this nomenclature, there are five classes of neurons in the vertebrate retina: photoreceptors, which detect light; horizontal, bipolar, and amacrine cells, which are interneurons that process the output of photoreceptors; and RGCs, whose axons convey visual information to the rest of the brain through the optic nerve. The neurons are arranged in three cellular (nuclear) layers, separated by two synaptic (plexiform) layers. Photoreceptors occupy the outer nuclear layer, interneurons occupy the inner nuclear layer, and RGCs plus some amacrines populate the ganglion cell layer (**Figure 1**).

Each of these neuronal cell classes is divisible into types. In the mouse (the number of types varies slightly across vertebrate species), there are three types of photoreceptors: rods and two types of cones. There is one type of horizontal cell (two in most species) and around 12 types of

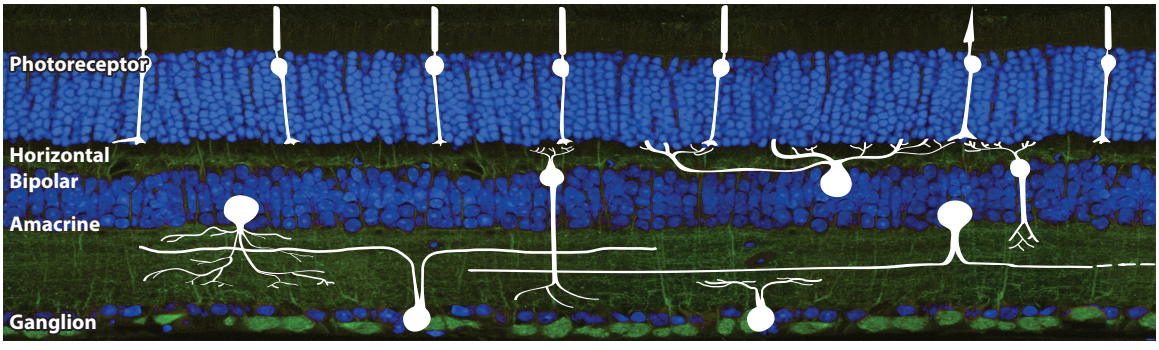

Figure 1

The main cell classes in the mouse retina. In the mouse, there is a single type of horizontal cell. These cells contact cone and rod photoreceptors. Bipolar cell dendrites receive synapses from photoreceptors, and their axons make synapses on amacrine and retinal ganglion cells (RGCs). Amacrine cells synapse on bipolar cells, other amacrine cells, and RGCs; RGCs send axons through the optic nerve. The sketch shows two bipolar cells and two RGCs with different levels of stratification in the inner plexiform layer, as well as a narrow- and a wide-field amacrine cell.

bipolar cells (Peichl & González-Soriano 1994, Euler et al. 2014). Amacrines are less well studied; there may be approximately 40 types. As we describe below, the number of ganglion cell types is somewhere in the neighborhood of 30.

CLASSIFYING RETINAL GANGLION CELLS

How does one classify RGCs—or neurons in general? Ultimately, a neuronal type is a group of neurons that carry out a task different from the tasks of the other neurons. However, lacking functional information for most neurons, we must use other indicators. At present, four criteria define an RGC type:

- Uniform morphology: Morphology was Cajal's original criterion of a type, and it remains an important one.
- Similar gene expression: The expression of unique sets of genes or transgenes is an increasingly used criterion. As discussed below, it remains uncertain how many genes are required to define a type and whether qualitative (yes/no) distinctions, which are clearly preferable to quantitative ones, will suffice.
- Regular spacing: In the retina, neurons of a single type show regular spacing, in the sense that they avoid other members of the same cell type while ignoring cells of other types (Wässle & Riemann 1978, Rockhill et al. 2000, Reese 2008, Kay et al. 2012) (**Figure 2**). This criterion, which seems to represent the retina's opinion of what constitutes a type, is so far (sadly) only applicable to the retina.
- Uniform physiological properties: This criterion is currently the most difficult to fulfill, largely because careful study of receptive fields is a low-throughput enterprise. Multielectrode arrays permit recording from many RGCs at once, but they have been of limited utility because there is no way to assess the molecular or morphological features of the cells from which signals arise. Recently, optical imaging methods have provided a way to circumvent this limitation (Briggman & Euler 2011, Briggman et al. 2011, Baden et al. 2013), so we can anticipate progress on this front.

All RGCs share several features. With rare exceptions, their somata are located in the ganglion cell layer, their spine-free dendrites arborize in the inner plexiform layer, and their axons travel through the optic nerve to the brain (**Figure 3**). They receive synapses on their dendrites, conduct action potentials, and release glutamate from their terminals. Molecularly, pan-RGC markers include the cell surface protein Thy1 (Barnstable & Dräger 1984), transcription factors of the Brn3 (Pou4F) family (Xiang et al. 1995, Badea et al. 2009), and the RNA-binding protein RBPMS (Rodriguez et al. 2014).

Yet RGCs are also heterogeneous in many ways. Early work distinguished between ON- and OFF-center RGCs, which respond best to increases and decreases in light intensity, respectively, delivered to their receptive field centers (Kuffler 1953). Over the next decade, over a dozen further RGC types were described physiologically (Levick 1967). The findings that dendrites of ON and OFF RGCs arborize in the inner and outer halves of the inner plexiform layer linked structure to function (Famiglietti & Kolb 1976, Famiglietti et al. 1977). A landmark finding was that different physiological types corresponded precisely to specific morphological types (Cleland et al. 1975). Investigators made sporadic attempts to overlay molecular labels on these physiological and morphological distinctions (Karten & Brecha 1983), but only in the past decade has molecular classification become routine, mostly in the mouse. Now, by combining morphological, physiological, molecular, and mosaic criteria, we can divide mouse RGCs into at least 25 clear types (Roska & Meister 2014) (**Figure 4** and **Table 1**).

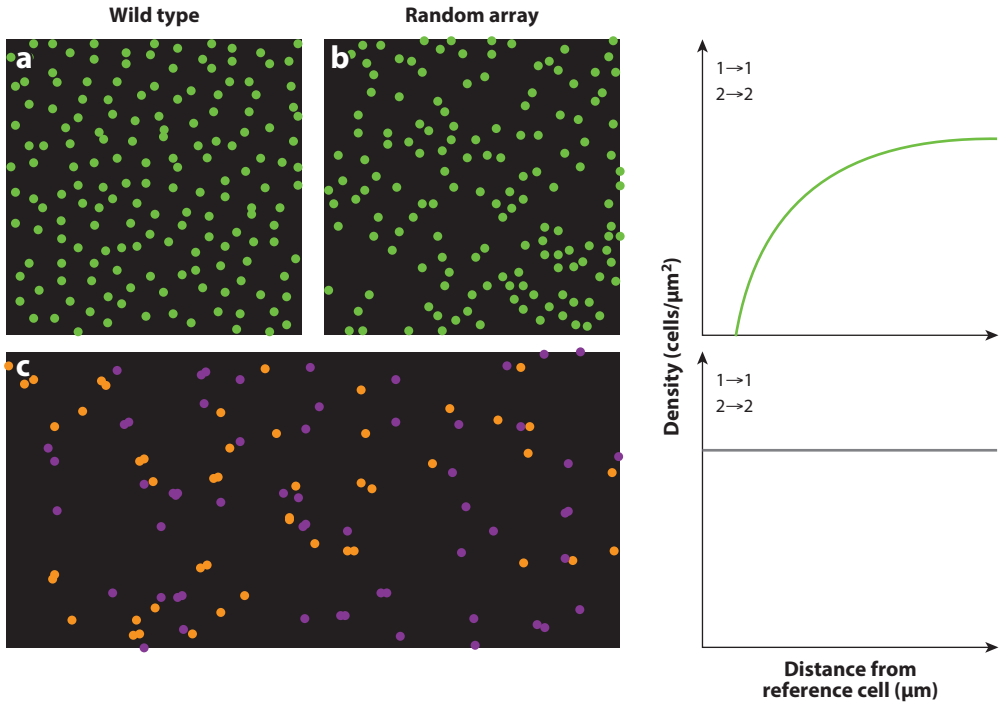

Figure 2

Retinal cells of the same type are evenly spaced in a so-called mosaic arrangement: Cells of a single type are less likely to be near neighbors of other cells of the same type than would be expected by chance. (*a*) A mosaic of retinal neurons. (*b*) The same number of cells randomly positioned. On the right are the density recovery profiles for the two situations. Each cell is taken as the reference cell, and the number of other cells at various distances from the reference cell is measured. Regularly spaced cells exhibit a gap at near distances. (*c*) Retinal neurons are evenly spaced with respect to other cells of the same type but are randomly spaced with respect to cells of different types (Rockhill et al. 2000). Shown are mosaics of two RGC types superimposed (data from Kay et al. 2011). The orange cells represent one cell type, and the purple cells represent a second.

DEFINITIVE TYPES OF GANGLION CELLS IN THE MOUSE

Four Types of ON-OFF Directionally Selective Ganglion Cells

As their name implies, ON-OFF directionally selective ganglion cells (ON-OFF DSGCs or ooDSGCs) respond to both increases and decreases in light intensity (ON and OFF responses, respectively), and they respond best to motion of a stimulus in a particular direction. Initially described in rabbits (Barlow et al. 1964, Barlow & Levick 1965), they are also prominent in mice (Weng et al. 2005) (**Figure 5**).

Two key features justify the conclusion that ON-OFF DSGCs respond selectively to directional movement itself, rather than to some particular pattern of stationary stimuli. First, they report movement in their preferred direction independent of the sign of contrast—they have the same preference for direction whether the stimulus is a dark spot or a light spot, a dark edge or a light edge. Second, the directional preference is observed for movements of stimuli much smaller than the total receptive field, so the cells report direction no matter where within the receptive field the stimulus lies (Barlow & Levick 1965). Although details remain to be worked out, the fundamental engine of the directional discrimination is selective input from directionally selective processes of starburst amacrine cells (for reviews, see Wei & Feller 2011, Vaney et al. 2012).

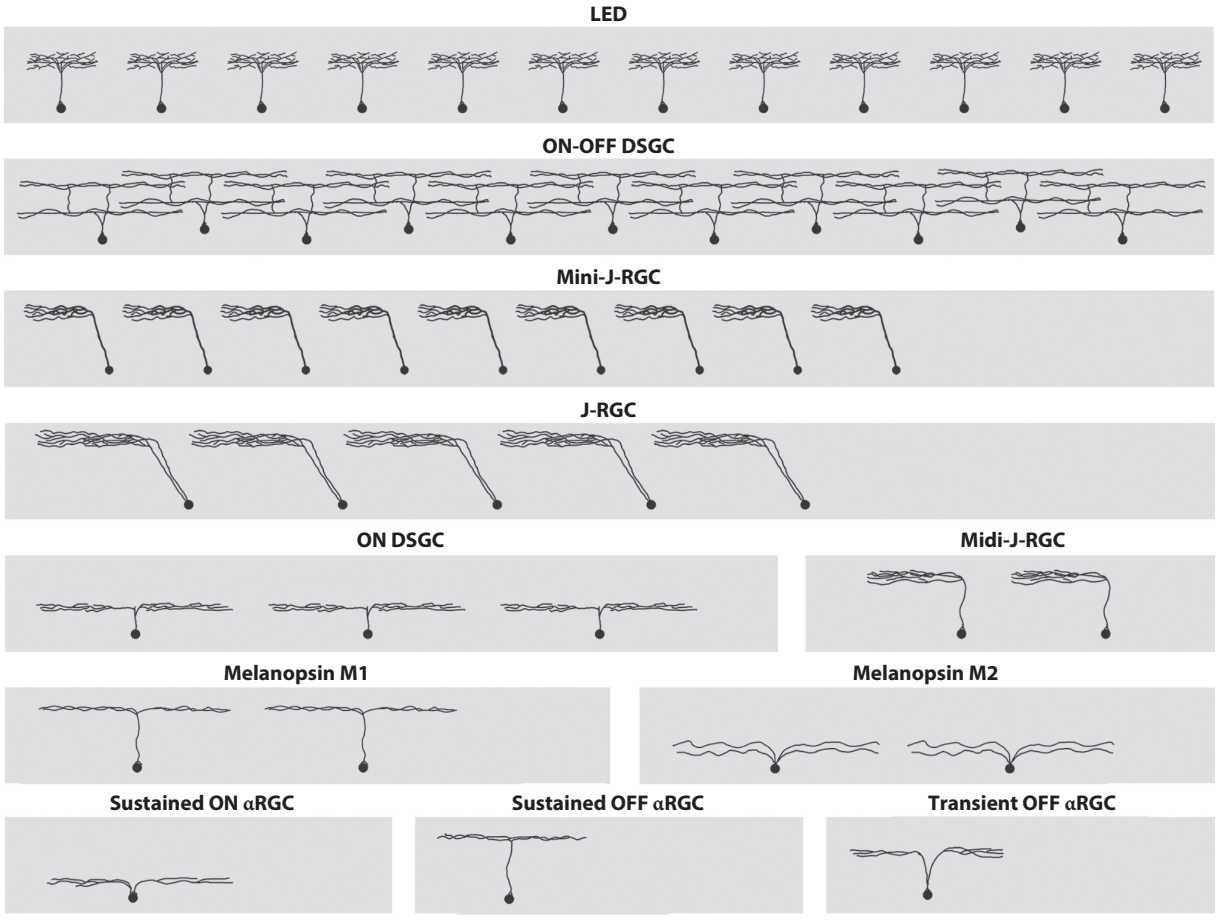

Figure 3

A selection of mouse RGC types shown in cross section. The schematic images of the cells preserve their relative dimensions and stratification. The number of cells of each type is proportional to their approximate frequency in the mouse retina. Abbreviations: DSGC, directionally selective ganglion cell; J-RGC, junctional adhesion molecule B–positive RGC; LED, local edge detector; RGC, retinal ganglion cell.

All ON-OFF DSGCs have a similar bistratified dendritic morphology, and their dendrites are tightly aligned with those of starburst amacrine cells (**Figures 3** and **4**). Moreover, expression of the gene encoding the neuropeptide CART (cocaine- and amphetamine-regulated transcript) distinguishes all ON-OFF DSGCs from other RGCs (Kay et al. 2011). Thus, ON-OFF DSGCs are without argument a coherent group. On the other hand, physiological, mosaic, and molecular criteria all indicate that there are actually four distinct ON-OFF DSGC types. First, in both rabbits and mice, directional preferences sort into four discrete classes, each sensitive to motion in one direction: upward, downward, backward, and forward (Oyster & Barlow 1967, Elstrott et al. 2008). Second, each directional type forms a separate mosaic (Devries & Baylor 1997). Third, transgenic mouse lines have been generated in which ON-OFF DSGC types preferring nasal, ventral, or dorsal plus ventral motion are selectively labeled (Huberman et al. 2009, Kay et al. 2011, Rivlin-Etzion et al. 2011, Trenholm et al. 2011). Kay et al. (2011) also identified endogenous

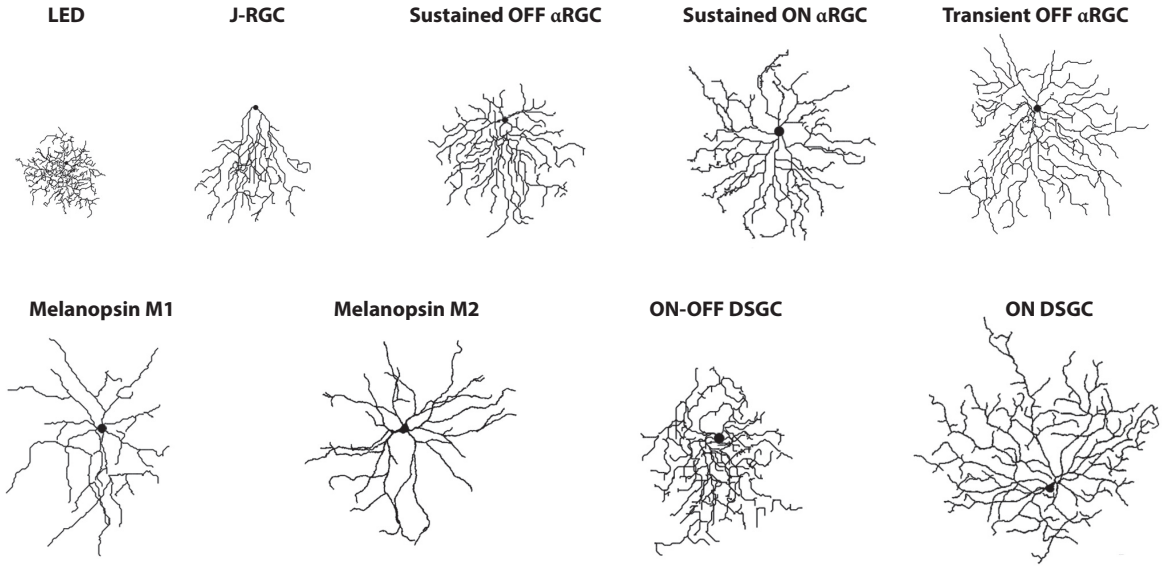

Figure 4

A selection of mouse RGCs shown in en-face or whole-mount views. Note the variation in size, shape, and arbor density among types. Adapted from Sümbül et al. (2014). Abbreviations: DSGC, directionally selective ganglion cell; J-RGC, junctional adhesion molecule B–positive RGC; LED, local edge detector; RGC, retinal ganglion cell.

cell surface molecules, including cadherin 6, collagen XXVα1, and matrix metalloprotease 17, that are selectively expressed by distinct subsets of ON-OFF DSGCs.

These four types also differ in their projection patterns. Although the superior colliculus is the main retinorecipient area in mice, RGCs project to a total of at least twenty areas, with different types projecting to different targets and to different laminae within targets (Hong et al. 2011, Dhande & Huberman 2014, Lawrence & Studholme 2014). Terminal arbors of ON-OFF DSGCs preferring ventral and nasal motion are partially segregated within the dorsal lateral geniculate nucleus. In addition, ON-OFF DSGCs preferring ventral and dorsal but not nasal motion project to the medial terminal nucleus and nucleus of the optic tract (Kay et al. 2011, Rivlin-Etzion et al. 2011).

Three Types of ON Directionally Selective Ganglion Cells

Barlow and Levick (Barlow et al. 1964, Barlow & Levick 1965) described a second group of RGCs in the rabbit that are direction selective but respond only to ON stimuli; a similar group is present in mice (Sun et al. 2006). Unlike ON-OFF DSGCs, these cells respond to flashed stimuli only at ON, rather than ON and OFF. Accordingly, they respond to moving light spots but not dark ones and to moving light but not dark edges. In addition, they are tuned to much slower stimulus motion than the ON-OFF cells. They also have a distinct dendritic morphology: Their monostratified dendrites costratify with the ON starburst cells in the inner plexiform layer, whereas the dendrites of the bistratified ON-OFF DSGCs stratify with both ON and OFF starbursts. They project to the accessory optic system, where they drive the optokinetic reflex (Simpson 1984), which minimizes slip of the image across the retina as the animal moves through the world.

Table 1 Types of ganglion cells in the mouse[a]

Name	Lines and markers	Equivalent to
ON-OFF DSGC dorsal	CART, Cdh6-Creer	PV0
ON-OFF DSGC ventral	CART, HB9-GFP, BD-Creer,Cdh6-Creer	PV0
ON-OFF DSGC nasal	CART, DRD4-GFP, THRH-GFP, MMP17	PV0
ON-OFF DSGC temporal	CART	PV0
ON DSGC ventral	SPIG1-GFP, Hoxd10-GFP	
ON DSGC dorsal	Hoxd10-GFP	
ON DSGC nasal	HoxD10-GFP	
J-RGC	JAM-B-Creer	PV7
Mini-J-RGC		
ON αRGC	Spp1, Kcng4-cre, low Opn4	PV1, M4
Transient OFF αRGC	Spp1, Kcng4-cre, TYW7, CB2-GFP	PV5
Sustained OFF αRGC	Spp1, Kcng4-cre, TYW7	PV6
M1	Opn4	
M2	Opn4	
M3	Low Opn4	
M5	Low Opn4	
W3B	Sdk2-CreER, TYW3	
PV2	PvalbCre	
PV4	PvalbCre	
Sümbül U	Classified morphologically	
Sümbül V	Classified morphologically	
Sümbül W	Classified morphologically	
Sümbül X	Classified morphologically	
Sümbül Y	Classified morphologically	
Sümbül Z	Classified morphologically	

Abbreviations: Cdh6, cadherin 6; CART, cocaine- and amphetamine-regulated transcript; DSGC, directionally selective ganglion cell; GFP, green fluorescent protein; JAM-B, junctional adhesion molecule B; J-RGC, JAM-B-positive RGC; MMP17, matrix metalloprotease 17; Opn4, melanopsin; RGC, retinal ganglion cell.

[a]Blank cells indicate those that are not yet confirmed.

Like ON-OFF DSGCs, the ON DSGCs can be divided into types by preferred direction, but each ON DSGC is tuned to one of three preferred directions rather than four (Sun et al. 2006, Yonehara et al. 2009, Dhande et al. 2013). One set can be marked genetically in a subregion of the retina by the cells' expression of *Fstl4* (also called Spig1) (Yonehara et al. 2008, 2009). *Fstl4*-positive cells form a regular mosaic and project exclusively to the medial terminal nucleus of the accessory optic system, which mediates the optokinetic response. A second group, which forms a separate mosaic, also projects to the medial terminal nucleus but is *Fstl4*-negative (Yonehara et al. 2008). *Fstl4*-positive and -negative ON DSGCs respond preferentially to upward and downward motion, respectively (Yonehara et al. 2009). In a second transgenic line (Hoxd10-GFP), all ON DSGC types are labeled, along with some ON-OFF DSGCs (Dhande et al. 2013).

Three Types of Alpha Retinal Ganglion Cells

Wässle and colleagues first described and named alpha RGCs (αRGCs) in studies of the cat retina (Cleland et al. 1975, Wässle et al. 1981, Peichl 1991). These cells represented the first

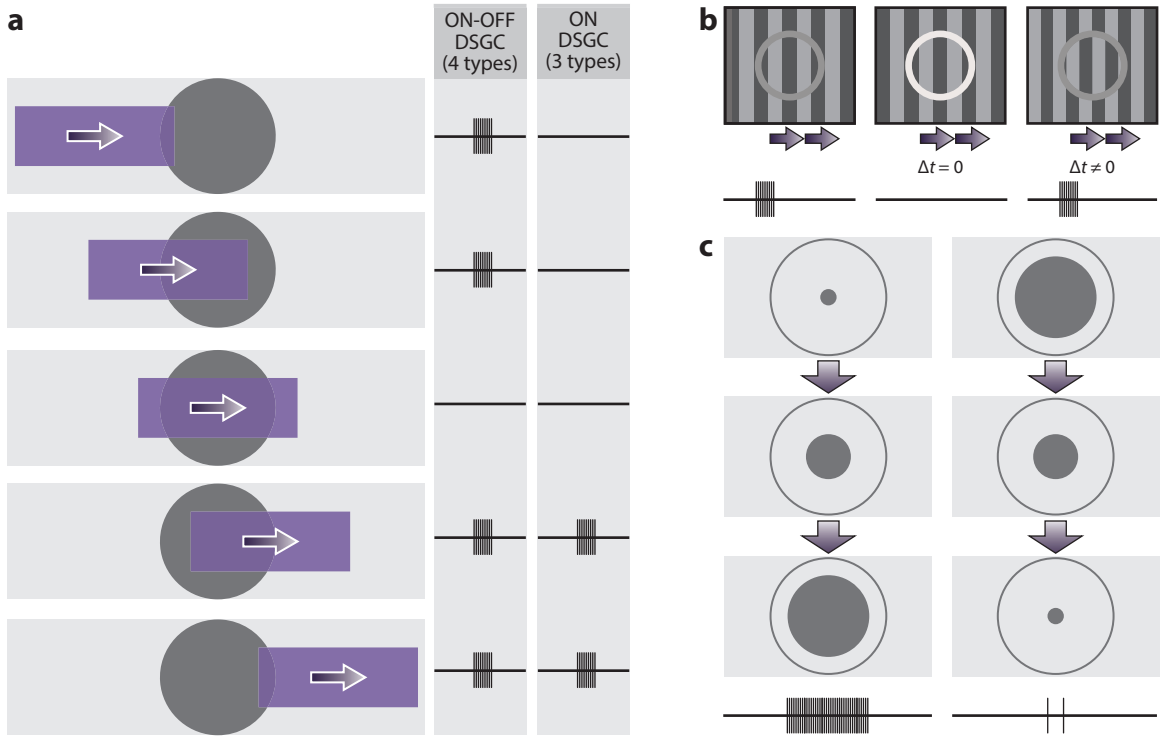

Figure 5

Many RGCs report selectively on moving stimuli. (*a*) ON-OFF and ON DSGCs respond to—and maintain the same direction preference for—both light and dark stimuli. (*b*) A small stimulus moving within the receptive field center excites the W3B-RGC (*left*). If the whole visual field moves coherently, the cell does not respond (*middle*). If there is a temporal separation between movement in the center and movement in the surround, the cell is once again excited (*right*) (modified from A. Krishnaswamy, M. Yamagata, X. Duan, Y.K. Hong & J.R. Sanes, manuscript submitted). (*c*) A dark stimulus with a continuously expanding contour excites the PV5 or transient OFF αRGC, as would an approaching dark object (*left*). If the object is contracting, i.e., receding, the cell does not respond (*right*). Modified from Münch et al. (2009). Abbreviations: DSGC, directionally selective ganglion cell; RGC, retinal ganglion cell.

demonstration of a rigorous match between structural and functional RGC types. In the cat, αRGCs have large somas and wide, dichotomously branching dendrites. Similar cells are known physiologically as the Y cell in the cat and the brisk transient cell in the rabbit (Cleland & Levick 1974a, Caldwell & Daw 1978, Enroth-Cugell & Robson 1984). In terms of physiology, they also closely resemble the parasol cells of the monkey (Polyak 1957, Rodieck 1998). In most mammals, they stain more intensely than other ganglion cells for neurofilaments (often labeled by the monoclonal antibody SMI-32), and this staining is sometimes viewed as diagnostic.

In the mouse, the term αRGCs has (confusingly) come to be applied to many large RGCs that are SMI-32-positive, without regard for their physiological properties or the details of their morphology, which differ in many respects from those of classical αRGCs. Currently, investigators have described three αRGC types in mice (Pang et al. 2003). In addition to being large and neurofilament rich, they all express high levels of *spp1*, which encodes the secreted phosphoprotein osteopontin, and *kcng4*, which encodes a voltage-gated potassium channel subunit (Duan et al. 2015). The three types differ, however, in physiological properties (ON or OFF, sustained or transient) and laminar position: There are sustained ON, sustained OFF, and transient OFF αRGCs, with dendrites stratifying near 70%, 50%, and 30% of the inner plexiform layer depth, respectively.

The transient OFF αRGCs are equivalent to RGCs called PV5 or approach-sensitive RGCs by Münch et al. (2009), who studied them in a parvalbumin-cre mouse line. When tested with expanding or contracting stimuli—the two-dimensional retinal correlate of an object approaching or receding in the world—the cells are seen to respond strongly to simulated approach and weakly to receding stimuli (**Figure 5**). An ecological interpretation is that this specialization could have evolved to detect an approaching predator or its shadow. In real life, however, an expanding retinal stimulus occurs far more frequently during self-generated motion than it does during predator attacks. Self-motion produces an image upon the retina in which virtually all contours are expanding; the transient OFF αRGCs could be involved in computations related to tracking these flow fields as much as they help escape predation. Another twist on the physiology of αRGCs is that at least some αRGCs show blue-green spectral opponency, a potential basis of color vision (Chang et al. 2013).

Molecular differences among the three αRGC types have now been described. The TYWY7 transgenic mouse line labels both transient OFF and sustained OFF αRGCs (Kim et al. 2010). Another line, CB2-GFP, labels only transient OFF αRGCs (Huberman et al. 2008). ON αRGCs express low levels of melanopsin (Estevez et al. 2012). Thus, each of the three αRGC types has a unique molecular signature.

Five Types of Intrinsically Photosensitive Melanopsin-Containing Retinal Ganglion Cells

The classic intrinsically photosensitive melanopsin-containing retinal ganglion cells (ipRGCs) have a large dendritic arbor and an intrinsic sensitivity to light. They express melanopsin, which activates a signaling cascade that opens a cation channel in the membrane. When stimulated with bright light, the response outlasts the stimulus for many seconds. They project primarily to the suprachiasmatic nucleus and play a major role in synchronizing the circadian oscillator (Hattar et al. 2002, 2003; Panda et al. 2002; Qiu et al. 2005; Do & Yau 2010).

Over the past few years, the situation has become more complex. Four additional types of RGCs have been found to express at least trace amounts of melanopsin and show intrinsic photosensitivity. They are called M1–M5, M1 being the classical ipRGC and M4 being the ON αRGC described above (Berson et al. 2010, Estevez et al. 2012, Hu et al. 2013). The other three are all sparse, widely spreading cells, but each has a distinctive pattern of dendritic arborization. These findings have raised the possibility that the cells perform functions beyond circadian ones (Schmidt et al. 2011b, 2014; Allen et al. 2014). Consistent with this idea, melanopsin cells project to multiple brain regions other than the suprachiasmatic nucleus (Hattar et al. 2002, Schmidt et al. 2011a, Münch & Kawasaki 2013).

Local Edge Detectors

Local edge detectors (LEDs) were first described in the rabbit and cat and were named in classic studies by Levick, still unsurpassed as a description of the functional characteristics of many RGC types (Levick 1967, Cleland & Levick 1974b). Other workers have studied their physiology and morphology in detail (Amthor et al. 1989, van Wyk et al. 2006, Baccus et al. 2008, Russell & Werblin 2010). These cells have compact and extensively branched arbors stratifying near the center of the inner plexiform layer. In the mouse, a similar morphology and physiology are displayed by the W3B-RGCs, which form a regular mosaic of yellow fluorescent protein–positive cells in the TWY3 transgenic line (Kim et al. 2010, Zhang et al. 2012). W3B cells have small (approximately 100 μm) dendritic fields, with dendrites that ramify near the center of the inner plexiform layer. In the rabbit, the LEDs represent about 15% of the total ganglion cells (Vaney

et al. 2012); in the mouse, the estimate is 13% near the center of the visual field, tapering toward the periphery (Zhang et al. 2012).

The feature for which LED cells were originally named was that the most effective stimuli must fall entirely within the center of the receptive field. Objects that exceed the size of the receptive field center do not excite the cell. A striking demonstration of this finding is the total silence of W3B-RGCs when a natural scene drifts across the retina (Zhang et al. 2012), a stimulus that dominates a mouse's visual experience (Wallace et al. 2013). This contrasts with most other ganglion cells, which give a clear, if submaximal, response to this stimulus.

In both the rabbit LED and the mouse W3B-RGC, firing is suppressed when there is temporal coherence between the initiation of stimulus movement in the receptive field center and surround—i.e., when the surround stimulus moves at the same time as the center. This may be a consequence of the fact that both the center and the surround response of this cell are phasic—they respond to changes in stimulation, not static stimuli (Levick 1967, Cleland & Levick 1974b). Interestingly, simultaneous timing occurs during fixational eye movements. Because this property allows the cell to distinguish between an object truly moving relative to background and a moving stimulus generated on the retina by eye or head movements, Olveczky et al. (2003) termed the property object motion sensing. In the rabbit and salamander, in which it was first studied, several different cell types exhibit this behavior. It is one of what appears to be a variety of contextual effects on ganglion cell selectivity (Chiao & Masland 2003, Roska & Werblin 2003, Lin & Masland 2006, Baccus et al. 2008).

Levick (1967) proposed that the rabbit LED was especially suited to detect one of a small mammal's predators—a raptor circling in the sky. And indeed, such a stimulus has just the characteristics that drive the W3B cell effectively (Zhang et al. 2012). A tiny dot moving slowly upon a featureless background—just the stimulus generated by a high-flying hawk—will be an extremely effective stimulus. In this way of thinking, the cell has been tuned by evolution to detect narrow and specific features of the animal's environment. An alternative to this feature-detection view is a pixel-based view, implying that every ganglion cell contributes something to the analysis of every image. Opponents of feature detection ask why the retina should devote such a substantial fraction of its resources to a single narrow stimulus—do we know that hawks are a more important predator than foxes or cats? Proponents note the considerable survival advantage of evading this particular predator. This general conceptual issue applies to all RGC types (Sharpee 2013). As it becomes possible by genetic manipulations to modify the output of specific RGC types in behaving animals, we should be able to revisit it in a new way.

Three Types of J-RGCs

Based on studies showing that expression of immunoglobulin superfamily recognition molecules marks RGC subsets in the chick (Yamagata & Sanes 2008, 2012), Kim et al. (2008) screened over 100 such genes in the mouse. One, junctional adhesion molecule B (JAM-B, also called JAM2), labeled a regular mosaic, as demonstrated by in situ hybridization. They generated transgenic mice to mark JAM-B-expressing cells and showed that JAM-B expression labels a population of OFF RGCs, which they called J-RGCs. These cells have strikingly asymmetric dendritic arbors aligned in a dorsal-to-ventral direction across the retina. Their proximal dendrite exits the soma and slants across the inner plexiform layer, expanding in the outer sublamina. They compose a type in that they share morphological and molecular features and form a regular mosaic.

Most J-RGCs have the same set of responses to light: They respond selectively to stimuli moving in a soma-to-dendrite direction. Because the lens reverses the image of the world on the retina, these cells detect upward motion in the visual field. (A small, direction-nonselective subset

is discussed below.) However, the directional behavior is clearly different from that shown by the ON-OFF and ON DSGCs. J-RGCs show a strongly asymmetric receptive field when tested with checkerboard stimuli: They have an OFF center and a highly asymmetric ON surround. These are stationary features, linked to the asymmetry of the dendritic arbor, quite different from other DSGCs, whose directional preference is replicated for many subunits within the receptive field and cannot be predicted from responses to stationary flashing stimuli. Because the direction-selective response is created by a simple asymmetry between ON and OFF regions, the behavior of the cell is different for bright and dark spots. Thus, a directional signal carried by these cells would be much less reliable than for the ON-OFF and ON types.

More recently, two additional RGC types have been identified that do not express JAM-B but share morphological features with J-RGCs in that they have ventrally biased dendrites that laminate in sublayer S1 of the inner plexiform layer. They can be marked as distinct populations by combinatorial expression of a set of transcription factors. Both demonstrate regular mosaic spacing and have a spatial density sufficient to cover the retinal surface. Based on their similarities to J-RGCs and their smaller size, they are provisionally named mini-J-RGCs and midi-J-RGCs, with the expectation that more fitting names will be chosen once more is known more about them (D. Rousso & J.R. Sanes, manuscript in preparation). The features that link them to the original J-RGCs are dendritic asymmetry and stratification in layer 1, so the similarity in their names should not be taken to imply a functional similarity—indeed, mini- and midi-J-RGCs have yet to be characterized physiologically. Nonetheless, their morphological and molecular properties, as well as their mosaic arrangement, make clear that they are definitive RGC types.

GANGLION CELL TYPES IDENTIFIED BUT INCOMPLETELY CHARACTERIZED

The definitive RGC types described thus far account for around half of the 40–50,000 RGCs per mouse retina (see below). What types remain to be described? Good candidates are six RGC types that are ubiquitous in the retinas of other mammals and have been observed physiologically in the mouse retina or lateral geniculate nucleus (which is thought to mirror RGC response properties) but remain to be characterized molecularly or morphologically.

Chromatically Sensitive Ganglion Cells

Blue-ON and Blue-OFF RGCs are apparently universal among mammals, including the rabbit, cat, and monkey (Jacobs & Tootell 1980, Okano et al. 1992, Jacobs 1993, Johnson et al. 1993, Hemmi et al. 2002, Yin et al. 2009, Chen & Li 2012, Sher & Devries 2012). They are preferentially excited or inhibited by short-wavelength (blue) stimuli and inhibited by long wavelengths (green). They receive input via a well-documented type of bipolar cell that in turn receives input exclusively from short wavelength–sensitive cones (Li & DeVries 2004, 2006; Schein et al. 2004; Mills et al. 2014). A similar blue-selective bipolar cell has been characterized in the mouse (Breuninger et al. 2011, Euler et al. 2014). Moreover, chromatically tuned ganglion cells have been reported in the mouse retina (Ekesten & Gouras 2005, Wang et al. 2011, Chang et al. 2013). However, the morphological and/or genetic identity of blue-sensitive ganglion cells in the mouse has not been unequivocally determined.

Orientation-Sensitive Cells

As their name implies, orientation-sensitive cells respond best to elongated stimuli. Investigators originally found them in rabbit and cat retinas (Cleland & Levick 1974b, Vaney et al. 1981,

Bloomfield 1994, He et al. 1998), and they have been seen in the monkey lateral geniculate nucleus (Cheong et al. 2013). The receptive field plotted with small stimuli consists of an excitatory region aligned parallel to an inhibitory region. These cells resemble a cortical simple cell of the cat or monkey (Hubel & Wiesel 1962). Their receptive fields are small, covering only a one-degree visual angle in the central retina of the rabbit. They have recently been observed in the retina of the mouse, as well as in the lateral geniculate nucleus (Marshel et al. 2012, Piscopo et al. 2013, Zhao et al. 2013, Chen et al. 2014), but in the absence of molecular or morphological specification, it remains unclear whether they compose a discrete type.

Suppressed-by-Contrast Cells

Suppressed-by-contrast cells are seen in the retinas of the rabbit and cat and in the lateral geniculate nucleus of the monkey (Stone & Hoffmann 1972, Troy et al. 1989, Tailby et al. 2007) and were initially termed uniformity detectors. They have a high firing rate, which is suppressed by any form of contrast falling within the receptive field center. Piscopo et al. (2013) recently observed them in the lateral geniculate nucleus of the mouse.

Two Types of Beta Cells

Little is certain about beta cells (βRGCs) in the mouse; this is remarkable because, in cats and primates, βRGCs are thought to be the main vehicle of acute vision. They were first named in the cat, and functionally similar cells have been described in the monkey (midget cells) and rabbit (brisk-sustained cells). It is unclear whether cells in these species are truly homologous (Caldwell & Daw 1978, Rodieck 1998, Berson 2008), but investigators agree completely on the existence of a pair of functionally analogous cells—a large cell and a small cell with contrasting properties—in these three retinas. The generic βRGC/X-type/brisk-sustained/midget cell is characterized by a circular or slightly elliptical receptive field with a simple center (ON or OFF), linear summation of stimuli presented within the center, more or less sustained responses to light, and an antagonistic surround. The receptive fields are much smaller than those of αRGCs, and the cells are more numerous, so they provide the high sampling density necessary for acute vision. In the mouse retina, small cells with antagonistic receptive fields and sustained responses have been seen, and these could correspond to the βRGCs (Stone & Pinto 1993, Sagdullaev & McCall 2005).

Additional Morphologically Identified Candidates

Sümbül et al. (2014) classified mouse RGCs by rigorous morphological criteria, focusing on the laminar placement of the dendrites and the shape and size of their dendritic arbor. This exercise yielded 22 RGC types, 16 of which corresponded to types that have been defined genetically and are described above. The other 6 types could not be identified with any known type. These cells were highly stereotyped—their peak stratification positions had standard deviations of <1 μm (**Figure 6**), suggesting that they correspond to novel types.

Farrow et al. (2013) characterized RGCs labeled in a parvalbumin-cre mouse line. Again, several of the types appear to correspond to previously defined types, including ON-OFF DSGCs, the three αRGCs, and J-RGCs. Two others, called PV2 and PV4, appear to be novel, distinct from the well-known types described above. They are an ON and OFF pair with small receptive fields, strong surrounds, and transient responses. A similar effort by Yi et al. (2012) generated overlapping but nonidentical classes.

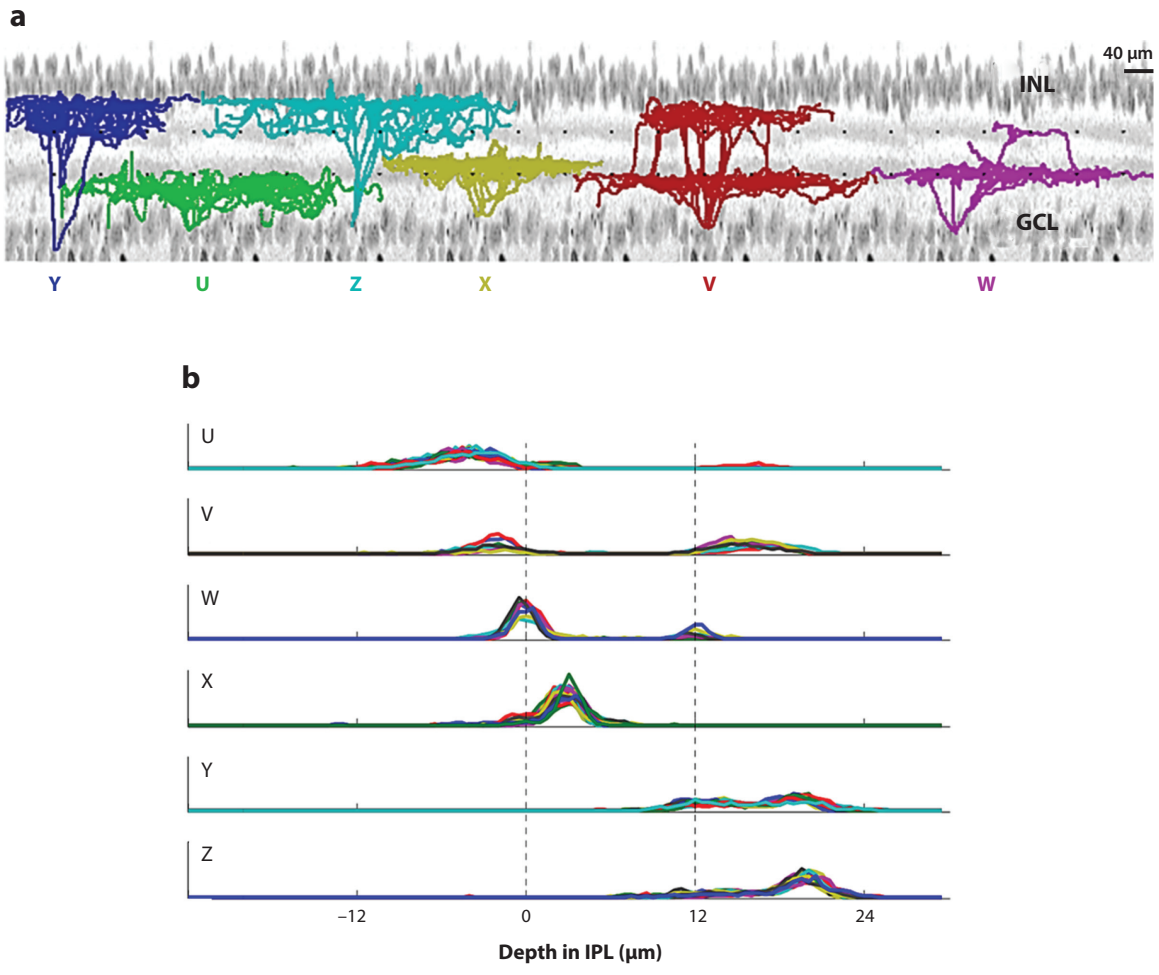

Figure 6

Candidate cell types identified by a combined computational and genetic strategy. Types U through Z were identified by an unsupervised clustering algorithm. (*a*) Projected images of representative examples. (*b*) Stratification of the dendrites. The ordinate shows the normalized voxel density, in arbitrary units. Dashed lines represent the dendritic level of the starburst amacrine cells, used as fiducial marks. Note the precision with which the dendrites are arrayed. In most cases, the stratification profile is enough to uniquely specify the cell type. For a few, other information is necessary, as in the case of cells Y and Z, which stratify similarly but differ in dendritic field width. Modified from Sümbül et al. (2014). Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer.

GETTING TO 100%: THE SEARCH FOR CLOSURE

The ultimate goal of RGC classification is to understand the array of signals that the retina sends to higher visual centers—the basic building blocks of vision. For this goal, we need a complete inventory of RGC types—not just the number of types, but what fraction of the retina's hardware is devoted to each. For example, our understanding of the primate visual system would be far different if parasol cells vastly outnumbered midget cells instead of the reverse. For amacrine cells, a turning point came when a quantitative analysis revealed that the previously characterized cells in this class—at the time thought to have been a fairly complete set—represented only 22%

of the total number of amacrine cells (Strettoi & Masland 1996). How close are we for RGCs? Based on the types known so far, we guess that there are around 30 types that compose at least 1% of the population each (there could be any number of extremely rare types) and that together they account for ≥95% of all RGCs (**Table 1**; see also Roska & Meister 2014).

Of all ganglion cells, how many can be accounted for by the securely known types? Although a systematic study across types has not been done, it is possible to make estimates of cell densities from the original papers on their identities (see references above). A limitation is that the published density is often for the retinal point of highest density, and this varies somewhat from type to type (Zhang et al. 2012, Hughes et al. 2013, Bleckert et al. 2014). Also, different laboratories study mice of different ages, and the density of cells falls substantially as the eye grows. This is a major issue, as the retina more than doubles in size from postnatal day 30 to adulthood, which roughly halves the average density of ganglion cells (Kuhrt et al. 2012). These estimates thus cry out for improvement, but for present purposes a first approximation remains useful if one always recognizes that individual values will undoubtedly be refined. Among the securely known RGC types, the most numerous ganglion cell types encountered thus far are the W3B cells (~250 cells/mm^2) and the mini-J-RGCs (~350 cells/mm^2). They are followed by the JAM-B cells (~200 cells/mm^2), the ON-OFF DSGCs (~125 cells/mm^2 for each of the four types), the midi-J-RGCs (~80 cells/mm^2), and the melanopsin M1 (63 cells/mm^2) and M2 RGCs (56 cells/mm^2). Finally, the three αRGCs and the three ON DSGC types are present at ~40 cells/mm^2 each. The total of ~1,700 cells/mm^2 amounts to ~60% of the ~3,000 cells/mm^2 in the midperipheral retina of the mouse (Jeon et al. 1998).

CHALLENGES TO CELL TYPE IDENTIFICATION AND CLASSIFICATION

At some level, every RGC, like every human being, is a unique individual. For humans, this variability does not stand in the way of classifying us as a single species, but it does complicate attempts to make some finer distinctions—for example, determining a person's race. How can we decide which features of an RGC are invariant enough to be type-specific criteria and which it would be prudent to ignore?

Good and Bad Markers

One requirement for proper categorization is to distinguish canonical from state markers. By this we mean that some labels selectively mark RGCs of a specific type under most commonly encountered conditions, whereas others may be missing or present depending on conditions—for example, neuronal activity, circadian rhythm, hormones, stress, and a variety of other factors—and such regulation can bedevil the unwary. As one example, in dark-adapted retinas, αRGCs have far higher levels of phosphorylated ribosomal protein S6 (pS6) than other RGCs. However, S6 phosphorylation is stimulated by neuronal activity (Knight et al. 2012), so in retinas taken from mice using their visual systems, pS6 levels are high in a variety of RGCs (Duan et al. 2015). Thus, anti-pS6 is a cell-type-specific marker only when light-evoked activity is minimized and is therefore not terribly useful for this purpose.

A related question is the extent to which one can rely on qualitative differences in expression level (expression of gene A is on in RGC type X and undetectable in other types), which are clearly preferable to quantitative distinctions (gene A is expressed at threefold higher levels in RGC type X than in other types). That is not to say that quantitative variations are unimportant. Levels of Eph kinases, for example, vary manyfold along the anterior-posterior and dorsal-ventral axes (McLaughlin & O'Leary 2005). In this case, we are comfortable saying that expression level defines a positional gradient with RGC types, rather than defining types. It remains a troubling

possibility, however, that different levels of marker expression characterize cell types in some cases. Other inadequate markers would be ones that label only some cells of a type or label only transiently during development.

The Perils of Genetic Markers

Although transgenic lines are invaluable for marking and manipulating retinal neurons, they are not necessarily reliable indicators of gene expression. Very few lines have only a single cell type that expresses a marker gene reliably (see below). First, few transgenes, even large ones (as used in the GENSAT collection), carry all of the regulatory information that influences the endogenous gene. Second, transgenes insert randomly into the chromosome, and their expression is frequently influenced by sequences and chromatic conformation near the insertion site. Consequently, transgenes often mark cells that do not express the endogenous gene and fail to mark cells that do express the gene (Haverkamp et al. 2009, Kay et al. 2011). Fortunately, knockin mice, in which an endogenous locus is labeled, are less susceptible to this sort of error.

Another technical pitfall arises because of changing expression during development. An increasingly popular genetic labeling method is the use of lines that express *cre* recombinase under the control of a cell-type-specific gene in combination with a reporter line that expresses green fluorescent protein (GFP) when acted upon by Cre. In these combinations, GFP indelibly marks cells that expressed *cre* at any time in their, or their progenitor's, development—in other words, the integral of all cells that have ever expressed the gene. A way to circumvent this limitation is to use ligand-activated Cre, so that GFP can be activated selectively in cells expressing *cre* at a particular developmental stage.

Choosing Among Objective Criteria

Whatever the limitations of molecular criteria, they are at present the most amenable to objective definition and quantification. For morphology, clustering algorithms have been used to carve categories from what might seem like continua (Badea & Nathans 2004, Kong et al. 2005), but with limited success. The recent study by Sümbül et al. (2014), discussed above, used a more sophisticated computational method of classifying RGCs by dendritic morphology and validated the method by use of molecularly defined types. Digital microscopy and computational methods provide a way to put morphological criteria on a more solid footing. Indeed, connectomic methods (Helmstaedter et al. 2013, Marc et al. 2013) provide access to one of the most basic definitions of a neuronal cell type: its synaptic connections.

For visual responses recorded electrophysiologically, the problem of classification touches one of the deep issues of contemporary neurobiology: the nature of neuronal coding (Shlens et al. 2009, Sharpee 2013). Which of the many stimulus features to which a cell responds is the real signal to the brain, or are they all? In practical terms, the main problems have been (*a*) the lack of a set of standard stimulation parameters to be shared among labs and (*b*) incomplete agreement on what features of the cell's resulting train of action potentials are the meaningful ones. These difficulties render classification from pure physiology currently untenable; it may await a new conceptual framework.

Relationships Among Criteria

Implicit in all this is the belief is that classification by molecular, morphological, and physiological measures will all yield the same set of types. This is not always the case. For example, morphological or biophysical categorization would likely treat all ON-OFF DSGCs as a single type, yet these cells

divide into four types, each with a distinct preferred direction of motion and molecular profile. By contrast, molecular criteria (e.g., expression of parvalbumin) can also often lead to heterogeneous groupings.

The JAM-B-positive J-RGCs represent a different case: heterogeneity within a single type. JAM-B-RGCs throughout most of the retina are morphologically asymmetric and physiologically direction-selective, but those in the dorsal and ventral retinal margins are morphologically symmetric and physiologically direction-nonselective (Kim et al. 2008). Kim and colleagues classified them as a single type based on gene and transgene expression. Thus, the criteria for a cell type are in conflict for these cells. We hope for a principled way to define cell types in the face of discrepant data, but at present we have no choice but to adjudicate discrepancies among criteria on an ad hoc basis.

Single Genes May Not Be Unique Identifiers

Initial forays into neuronal classification led to the hope that RGC types could be identified by expression of a single gene. It is time to disabuse ourselves of this naive view. For example, useful RGC markers are often expressed by other neuronal classes in retina—JAM-B (J-RGCs) in the outer retina (Daniele et al. 2007), KCNG4 (αRGCs) by type 5 bipolar cells (Duan et al. 2014), and CART by several sets of amacrine cells. Thus, using these genes to mark RGC types even within the retina requires combining them with a second marker, such as Thy1, which is expressed by RGCs but not bipolar, amacrine, or photoreceptor cells.

Even considering only RGCs, most genes studied to date mark more than a single type. For example, the TYW3 line mentioned above marks both W3B and W3D cells, the Kcng4 line marks all three types of αRGCs, and Sidekick2 is expressed in W3B and ON αRGCs (Zhang et al. 2012; Duan et al. 2015; A. Krishnaswamy, M. Yamagata, X. Duan, Y.K. Hong & J.R. Sanes, manuscript submitted). Thus, none of these markers suffices on its own to label a single RGC type, but they demarcate single types when used in pairs: Sidekick2+TYW3 uniquely marks W3B-RGCs, and Sidekick2+Kcng4 uniquely marks ON αRGCs.

The question then becomes, how many genes will need to be used to define an RGC type? Two is not a big problem, given current intersectional technology; three is manageable; more than three is a nightmare. The good news, based on experience to date, is that two or three seem likely to suffice.

DEVELOPMENTAL AND EVOLUTIONARY RELATIONSHIPS

Once RGC types have been categorized, one can ask of them, as of species in phylogenetics, what are their relationships? There is reason to hope that this enterprise can provide deep insights into the development and evolutionary conservation of RGC types.

Taxonomy

How are RGC types related to each other? Now that it is feasible to purify individual RGC types (e.g., by fluorescence-activated cell sorting) and subject them to molecular profiling (Kay et al. 2012, Siegert et al. 2012), this issue can be addressed. At one extreme, each type could be related equally closely to all others. Already, however, this seems unlikely. For example, the similarities in structure and function, and the molecular markers the cells share, argue that the four types of ON-OFF DSGCs are each other's closest relatives. Likewise, the three ON DSGC types and the three αRGC types are likely to form subgroups.

From such cases, hierarchies are beginning to emerge (**Figure 7**). All RGCs share morphological, physiological, and molecular features and are likely to be more closely related to each other than they are to other neuronal classes. Next, some groups of RGC types appear to be each other's closest relative: αRGCs (Smi32-, spp1-, and Kcng4-positive), ON-OFF DSGCs (CART-positive), and so on. These groups are then divisible into individual types: the four ON-OFF DSGCs, the three ON DSGCs, the three types of αRGCs, and so on. Eventually, these distinctions may lead to more formalized hierarchies, much as Linnaean classification moves from class to genus to species.

Many questions remain: What are the closest relatives of RGCs generally—other retinal neurons or projection neurons elsewhere in the central nervous system? What are the highest-level subdivisions of RGCs? Are ON DSGCs more closely related to ON-OFF DSGCs or to ON αRGCs (**Figure 7**)? As in organismic systematics, molecular profiling is likely to transform this area.

Development and Disease

Molecular characterization and classification of RGCs can inform our understanding of at least two aspects of RGC development. One is how RGCs diversify to form types, and the other is how they form the specific and stereotyped connections that underlie their physiological roles. In both cases, solutions require identification and analysis of genes expressed in RGC types (see, for example, Sanes & Yamagata 2009, Sanes & Zipursky 2010, Cepko 2014). Similarly, RGC types appear to differ profoundly in their ability to survive insults such as axotomy or diseases such as glaucoma (Della Santina et al. 2013, Duan et al. 2015), suggesting that molecular comparisons among types could lead to identification of protective factors. For these enterprises to succeed, the types themselves must be classified clearly.

Evolution

How can cellular taxonomies inform our understanding of the basic principles of RGC function? We have seen that most RGC types described in the mouse appear to be present in numerous other species, as judged by physiological and morphological criteria (see above and Mangrum et al. 2002, Sun et al. 2002, Naito & Chen 2004, Isayama et al. 2009). Only now, though, with molecular markers available, is it becoming possible to establish correspondences among species and ask whether such conserved types represent conservation or convergent evolution.

It is attractive to imagine that RGC types evolved to devote the greatest neural resources to informationally important aspects of the visual scene. Because the ecological niches of animals contain many shared visual characteristics, some RGC types would be broadly conserved. They might include types that detect motion, luminance, spectral contrast, and edges, as well as ipRGCs that regulate circadian rhythms. In contrast, others might vary with the niche that each species occupies—for example, nocturnal versus diurnal.

LESSONS FOR THE BRAIN

Finally, we consider ways in which what we have learned in the retina can point us toward improved cell type classification in other areas, such as the cerebral cortex or spinal cord—clearly much tougher nuts to crack.

- First, despite lingering skepticism, neurons can be divided into distinct types. Evidence in the retina is conclusive for RGCs, as discussed above, and for photoreceptor, horizontal,

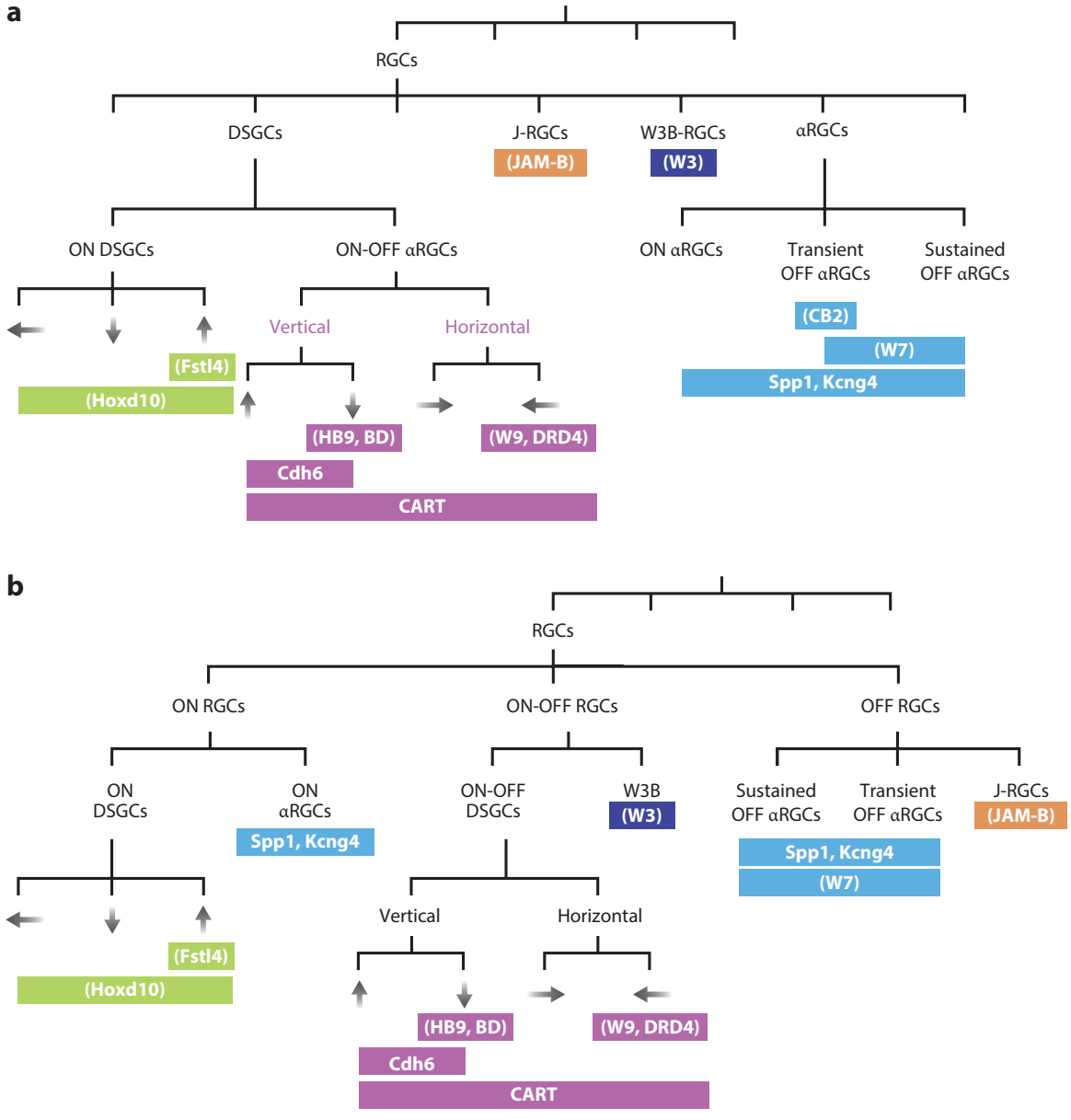

Figure 7

Hierarchies of RGC types. Relationships among RGC types remain unknown; two possibilities are shown here. (*a*) In one, direction-selective RGCs and αRGCs compose major groupings. (*b*) In another, RGCs are grouped according to whether they are ON or OFF cells. In both cases, molecular markers are shown. Those in parentheses are mouse lines in which the transgene may not report accurately on expression of the endogenous gene. Ongoing transcriptomic analysis is providing a new way to distinguish these and other models, generating a rational taxonomy of the diverse RGCs (Macosko et al. 2015). Abbreviations: CART, cocaine- and amphetamine-regulated transcript; DSGC, directionally selective ganglion cell; JAM-B, junctional adhesion molecule B; J-RGC, JAM-B-positive RGC; RGC, retinal ganglion cell.

and bipolar cells (Peichl & González-Soriano 1994, Wässle et al. 2009, Euler et al. 2014). There is little reason to imagine that other regions of the central nervous system will differ in this fundamental respect.

- Molecular markers are at present the leading criteria for neuronal classification. They are objective and quantifiable. However, the brain uses a limited set of genes. Combinations of genes, or combinations of a marker with structural information, are likely to be required to uniquely identify most cell types.
- Among molecular approaches, genetic labeling strategies are critical because they (*a*) show repeated examples of a cell type; (*b*) permit targeted recording, without which an adequate sample of any one cell type can rarely be gathered; and (*c*) allow workers in one laboratory to test and extend models proposed by other groups. They also allow selective manipulation of neuronal types—for example, optogenetic stimulation, silencing, killing, and live imaging (Madisen et al. 2012, Huang & Zeng 2013).
- Structure itself remains a useful tool for classification, especially given new high-throughput light and electron microscope methods, augmented by novel computational approaches. Whatever the methodology, one needs both sparse labeling (so that the structure of an individual cell is clear) and population labeling (so that the cell numbers can be determined).
- In the retina, the existence of a regular mosaic of cells is an invaluable litmus test for a cell type. To our knowledge, investigators have made few attempts to find type-specific mosaics elsewhere in the central nervous system.
- Physiology has been a difficult methodology for classifying neurons. Optical recording methods are increasing the potential throughput, however (Briggman & Euler 2011, Briggman et al. 2011, Baden et al. 2013), and allow recordings from large numbers of cells to be related to molecular and morphological measures—a step that will be even more important in the brain.
- Counting counts. In the absence of reliable numbers, the analysis remains anecdotal and we have gained little over Cajal; to paraphrase Ecclesiastes, of making many types there is no end. In fact, for cell types there is an end, which will come when all of the available cells have been identified, but we can only know that by quantitative analysis of the cell populations.

CONCLUDING REMARKS

The classification of RGCs has come far: More than half of the total population of ganglion cells can now be specified, and the general character of the remaining types is in view. Genetic techniques have been pivotal to recent progress and promise to be equally so in the brain. One of the most powerful methods has been to analyze the spacing of candidate cell types—something as yet unexplored in the brain but which could be invaluable there as well. It is satisfying that for retinal ganglion cells, the definitive end of Cajal's program is in sight.

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