

From Form to Function: the Ways to Know a Neuron

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Abstract: The shape of a neuron, its morphological signature, dictates the neuron's function by establishing its synaptic partnerships. Here, we review various anatomical methods used to reveal neuron shape and the contributions these have made to our current understanding of neural function in the *Drosophila* brain, especially the optic lobe. These methods, including Golgi impregnation, genetic reporters, and electron microscopy (EM), necessarily incorporate biases of various sorts that are easy to overlook, but that filter the morphological signatures we see. Nonetheless, the application of these methods to the optic lobe has led to reassuringly congruent findings on the number and shapes of neurons and their connection patterns, indicating that morphological classes are actually genetic classes. Genetic methods using, especially, GAL4 drivers and associated reporters have largely superseded classical Golgi methods for cellular analyses and, moreover, allow the manipulation of neuronal activity, thus enabling us to establish a bridge between morphological studies and functional ones. While serial-EM reconstruction remains the only reliable, albeit labor-intensive, method to determine actual synaptic connections, genetic approaches in combination with EM or high-resolution light microscopic techniques are promising methods for the rapid determination of synaptic circuit function.

Keywords: Golgi impregnation, GFP, photoreceptor, optic lobe, serial-section EM

INTRODUCTION

Until now, a comprehensive knowledge of the cell types of any single nervous system has been patchy. Exceptions exist, of course, for nervous systems, such as that of *Caenorhabditis elegans*, that have few enough elements and are of sufficiently small dimensions to be serially sectioned and reconstructed *in toto* (White et al., 1986). Other examples are either less complete or are restricted to favorable neuropiles, such as the first neuropile of arthropod visual systems (Macagno et al., 1973; Fahrenbach, 1985; Meinertzhagen & O'Neil, 1991).

The fly's brain has already yielded a great deal of what we know about neurons and their circuits and seems destined to contribute much more toward our understanding of their functions at the systems level (Luo et al., 2008). This short essay addresses the question of how we can understand the cellular composition of a brain, in particular the tiny brain of the fruit fly *Drosophila melanogaster*, from the number and features of its types of neurons. It is dedicated to the many illustrative studies

of Martin Heisenberg and his group, who have contributed so richly to that knowledge.

The Shapes of Neurons: As Cajal Would Have Seen

Others (e.g., Szentagothai, 1975), have commented on the remarkably fortuitous nature of the discovery by Camillo Golgi, that successive immersion of brain tissue in chromate and silver salts could, under appropriate conditions, impregnate neurons in their entirety (Golgi, 1873). The process Golgi discovered is, as we now know, stochastic, but the reasons that any particular neuron is impregnated and not its neighbors, remain as dark as the impregnate itself. The method is simple, but must be repeated on huge numbers of preparations. It requires the eye of a microscopist to recognize the different cell types, the discernment of a systematicist to identify and catalog their differences, and the skill of an artist to depict them accurately. There are always uncertainties as to whether all cell types have been identified, whether some may

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remain refractory to impregnation, or—if they arborise widely—be contained only partially in the sections used to visualize them, and whether all features of the neuron can be seen in a single view. Nevertheless, the Golgi method, very early on, opened the first window on the cellular composition of the brain.

Since this initial discovery, made quite by chance and long before most other approaches to studying brains were possible, the shapes of many types of neurons have been reported, both vertebrate (e.g., Cajal, 1893, 1899) and invertebrate (e.g., Cajal & Sánchez, 1915; Cajal, 1917). These studies, and more recent ones that followed (e.g., Strausfeld, 1976), systematically cataloged many different types of neurons, each from its distinctive shape. In common with many other invertebrate nervous systems, the brain of a fly incorporates cells with remarkably determinate shapes, and these provide very strict criteria to recognize different cell types. Relative to vertebrate neurons, the miniature forms and fixed shapes of insect neurons were a source of wonder to Cajal (1937), who based his observations on the optic lobes of several species (Cajal & Sánchez, 1915), none of them *Drosophila*. Those on *Drosophila* derive from later studies and fully confirm these features. They constitute an especially valuable resource, the gold standard against which the later genetic methods that now supersede Golgi impregnation can be compared. Many significant studies were initiated in the Heisenberg group, in particular those on the optic lobe (Fischbach & Dittrich, 1989), central body (Hanesch et al., 1989), and mushroom body (Technau, 1984) of *Drosophila*. These derive especially from the neuropile regions that are modular in composition, with many repeating processing elements, the so-called glomerular or, as we will refer to them here, modular neuropiles—as opposed to diffuse neuropiles (Hanström, 1928)—and had an ultimate objective to reveal the structural phenotypes of various brain mutants (e.g., Fischbach, 1983; Fischbach & Lyly-Hünerberg, 1983).

Nomenclature: Names Matter

In no region in the *Drosophila* brain has the cellular composition been better characterized than in the optic lobes. The *Drosophila* optic lobe contains four successive neuropils (lamina, medulla, lobula, and lobula plate), each organized as a matrix of strata and columns, cartridges in the lamina, and columns in the medulla (Figure 1). Many of the major cell types in other fly species were already identified long ago by Cajal and Sánchez, (1915), and comprehensive reports by Strausfeld extended considerably the number and range of these, especially in various species of larger fly (e.g., Strausfeld, 1970, 1971). Studies using Golgi impregnation have also been supplemented by related methods that retro- or anterogradely fill cells

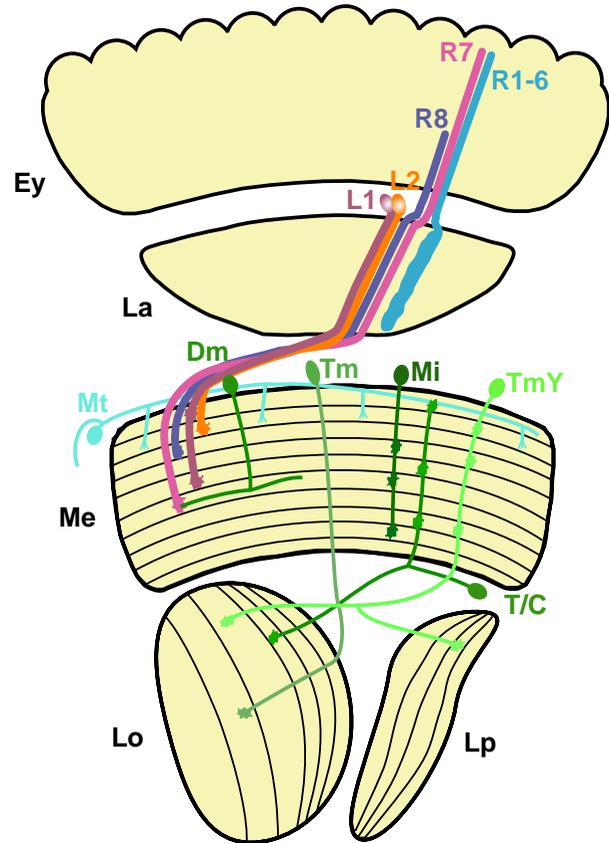


Figure 1. A schematic illustration of the *Drosophila* visual system, including the eye (Ey) and four optic neuropils: lamina (La), medulla (Me), lobula (Lo), and lobula plate (Lp). The photoreceptors are identified in light blue (R1-R6), pink (R7), and dark blue (R8). Lamina neurons (represented by L1, purple; L2, orange) project axons to different medulla strata. Four types of medulla neurons (various shades of green) are shown: Dm (distal medulla or amacrine cell); Mi (medulla intrinsic neuron); Tm (transmedulla neuron); TmY (transmedulla Y neurons); along with Mt, a medulla tangential neuron (turquoise). T/C: T and C neurons (also in green) are centrifugal neurons.

from peripheral nerves, using markers such as horseradish peroxidase (HRP) (Nässel, 1983), cobalt (e.g., Stocker & Schorderet, 1981), fluorescent dextrans (e.g. Consoulas et al., 2005), Lucifer yellow (Strausfeld et al., 1983), or DiI (Landgraf et al., 1997).

Studies of neuron shape attained the status of high art in the classical studies of Cajal, with neurons assigned descriptive names (e.g., bushy, amacrine, etc.), according to the features of their shape, usually reported, however, only from a single view. In this way, the shape of a neuron came to be seen as its morphological signature. But Masland (2004) has pointed out the deficiencies in our nomenclatures for different neuron types, and the failure to adopt the strict code of a true taxonomy, with a hierarchy of taxonomic ranks. Thus, the nomenclature for neurons of the optic lobe adopted by Cajal was essentially

descriptive, with all neurons taking an equivalent rank. Later studies (e.g., Strausfeld, 1970, 1976) distinguish neuron types according to the orientation of their long axis with respect to the surface of the neuropile, and the differing spreads of dendritic arbors, or in *Drosophila*, the primary growth direction of their neurite (Fischbach & Dittrich, 1989). Thus, three main classes of optic lobe neuron, columnar, amacrine, and tangential, were finally distinguished on a developmental basis.

Numbers of Neuron Types: The Magnitude of the Problem

While the differentiated shapes of optic lobe neurons enable us to discriminate each according to very clear features, they also reveal the many classes of neurons, and thus the prospective complexity of connections in visual circuits. As examples from *Drosophila*, at least 109 different types of optic lobe neuron have been reported from Golgi impregnation, more than half in the second neuropile, or medulla, alone. About half of the latter, in turn, are the populous transmedulla (Tm) cell types that penetrate the medulla and innervate the lobula, with at least 26 forms (Fischbach & Dittrich, 1989). Additional new medulla cell types have also been identified by means of genetic methods (Morante & Desplan, 2008; Gao et al., 2008). Several of these, such as Tm5 (Gao et al., 2008), have hitherto unreported subtypes, while larger fly species have yet more described types (Strausfeld, 1970, 1976). As outputs, in *Drosophila*, 44 pathways have been identified between the lobula and brain, from a screen of 4,000 GAL4 enhancer-trap strains (Otsuna & Ito, 2006). These totals give approximate numerical proportions to cell classes, but clearly are only estimates, probably underestimates. It is a matter of perspective whether we should be daunted by these numbers or grateful that they are not yet larger.

A library of cell types from any brain faces the uncertainty of three potential imperfections: whether all cell types are represented; whether the impregnation of each is both complete and accurate; and whether cell types are continuous or discrete. These categories of deficiency are well recognized from Golgi impregnation (e.g., Fischbach & Dittrich, 1989; Masland, 2004) and partially overlap. In favor of the completeness of impregnation, Golgi-EM confirms that the Golgi impregnate fills the entire profiles of a neuron (e.g., Campos-Ortega & Strausfeld, 1973), but samples only a tiny part of that neuron. In favor of the comprehensiveness of Golgi sampling, arguments have been advanced that all neurons are susceptible to impregnation by the Golgi method (Strausfeld, 1980), and thus that all types of neurons should appear if sufficient preparations are made. In the end, this is an empirical issue, however, one that

will be finally resolved only when alternative methods have repeatedly revealed the same neuron types and numbers. Finally, while morphometric analyses support the notion that cell types are discrete, but with discernable morphological variations within each type (Marin et al., 2002), conclusive evidence has come from the genetic methods that have now supplanted classical methods to identify cell types in *Drosophila*.

Neuroanatomy's New Age: Genetic Reporters

Recent evidence especially using the GAL4/UAS system (Brand & Perrimon, 1993) to target expression of reporter genes, such as green fluorescent protein (GFP), or the single-cell mosaic methods (such as the MARCM and flip-out techniques) to generate clones of labeled neurons (Lee & Luo, 1999; Wong et al., 2002), now confirms the many cell shapes previously seen by Golgi impregnation. Recent screens of extensive libraries of random GAL4 enhancer-trap strains provide the first comprehensive images of, for example, optic lobe output neurons (Otsuna & Ito, 2006), auditory projection neurons (Kamikouchi et al., 2006), and mushroom body neurons (Tanaka et al., 2008). The inventories of cells from these screens appear to go considerably beyond previous Golgi studies. However, given the sampling nature and inherent bias of GAL4 enhancer-trap screens, it is also difficult to claim completeness, regardless of the size of the genetic screens. At least one cell type, the large bilateral 5HT-expressing neuron of the optic lobes (Nässel, 1987), has, for example, not been detected in a major screen of optic lobe output neurons (Otsuna & Ito, 2006). Finally, for all they resolve neuron subtypes, genetic reporter lines also raise some of their own ambiguities. Thus, although the labeling pattern of each strain is largely the same, in a few cases, it may vary with the particular UAS reporter line (Ito et al., 2003). Not all lines label one cell type with total specificity, separate from other cells that express a related enhancer (Otsuna & Ito, 2006).

Nonetheless, it is perhaps worth reflecting on the reassurance provided by the similar cell shapes revealed by two such different technical approaches. Thus, in the optic lobe, the form of the lamina cell type, L2, is reported from both Golgi and reporter studies, as is the form of Tm2 (Figure 2). In both cases, the pattern of GFP expression is judged to match closely the form of the corresponding Golgi impregnate. The nature of this match is important. Not only is there a close correspondence in the cell profiles, but also the resolution of confocal images can be enhanced by deconvolution (Figures 2B and 2F), and the stack of such images can be viewed from different rotations, to optimize the match. In addition, the location of the profiles within the neuropil can be determined by using additional cell markers as landmarks. In the case of

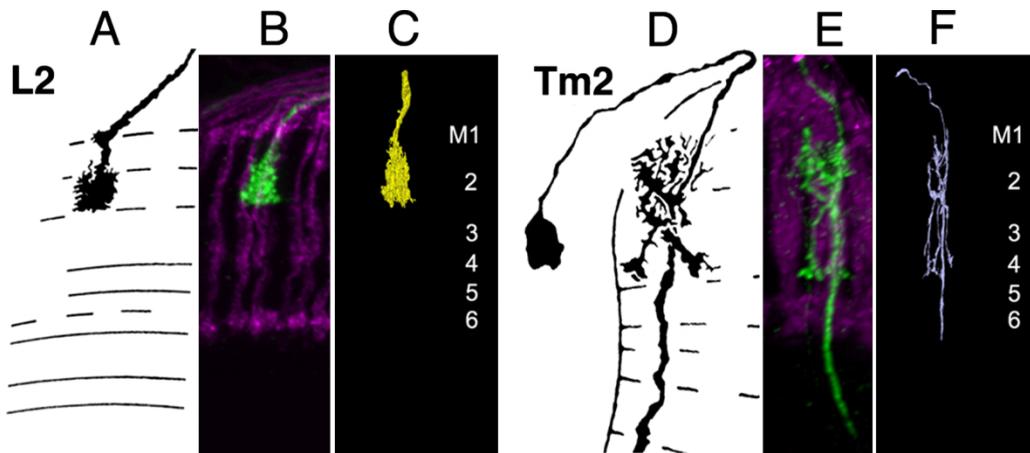


Figure 2. Comparison between the neuron shapes reported from Golgi impregnation, and those revealed by means of a genetic reporter, and serial-EM reconstruction. (A–C) Lamina cell L2. (D–F) Transmedulla cell Tm2. (A, D) Golgi impregnation, from Fischbach and Dittrich (1989), with kind permission of Springer Science + Business Media. (B, E) GFP expression in L2-Gal4/UAS-GFP (B) and Tm2-Gal4;UAS-GFP (E) flies. (C, F) Corresponding serial-section EM reconstruction profiles (from the dataset reported in Takemura et al., 2008).

the lamina, in which all columnar cell types are known from both Golgi (Fischbach & Dittrich, 1989) and EM (Meinertzhagen & O’Neil, 1991) analyses, we can identify L2 confidently from the shape and location of its medulla terminal (Figure 2C). The identification is less secure for the 30 or so Tm cell types, each of which has a complex and type-specific axonal and dendritic form. In that case, identification is based on both positive and negative criteria, the first being the similarity between the Golgi and GFP forms of the cells, with respect to the stratum-specific arborization of axons and dendrites, and the second being the lack of alternative candidate Golgi forms, which would provide a closer match to the GFP form. Overall, the strikingly similar forms of neurons seen by classical and reporter methods indicate that the forms of neurons, at least in part, reflect their patterns of gene expression; these, in turn, provide a more strongly categorical basis to distinguish one type of neuron from another than the more graded features of their respective shapes. This development represents a paradigm shift. Given that many aspects of the developmental history of a neuron could contribute to its final morphological features, however, it is unlikely that every morphologically distinct cell type corresponds exclusively to a single genetic class. Nonetheless, classifying neurons on the basis of their patterns of gene expression is both highly effective and utilitarian and has become the prevailing method to report neurons in *Drosophila*.

Screening randomly inserted GAL4 enhancer-trap lines provides an effective, and perhaps the least biased, way to identify most neuron types (Otsuna & Ito, 2006). However, a targeted, rational search based on promoters of identified genes, such as transcription factors and neurotransmitter synthesis enzymes, has other advantages

(Pfeiffer et al., 2008). Using four enhancer traps of transcription factors, most medulla neurons could be divided into four classes on the basis of their nonoverlapping patterns of expression (Morante & Desplan, 2008). The expression patterns of transcriptional factors are likely to reflect their developmental history and the mechanisms of neuronal subtype diversification. The expression patterns of neurotransmitter synthesis enzymes and receptors, on the other hand, support a more physiological classification of neurons. For example, the *ort*-GAL4 construct (Gao et al., 2008), which contains the promoter of the histamine-gated channel, *ort* (ora transientless; *hclA* = HisCl2; Gengs et al., 2002), effectively identifies candidate target cells for photoreceptor neurons, which are histaminergic (Hardie, 1987; Sarthy, 1991). Further, to refine the classification of cell types further, and select only subpopulations, promoters can be dissected and critical enhancers identified by comparative genomic analysis (Gao et al., 2008). Most importantly, the recently developed “split-GAL4” system has made it possible to combine two different promoters to further differentiate neuron subtypes (Luan et al., 2006). For example, Tm5, reported as a single cell type that extends an arborization in medulla strata M3 and M6 (Fischbach & Dittrich, 1989; Figure 3A), in fact has three subtypes seen from *ort*-GAL4-driven GFP expression (Figures 3B–3D). Each of the three subtypes, Tm5a, b, and c, has a unique dendritic arbor in strata M3 and M6 and that of Tm5c has an additional arbor in stratum M1, a morphological phenotype correlated with Tm5c’s expression of a vesicular glutamate transporter (Figure 3D). There are also corresponding differences in the size and shape of the lobula terminals (data not shown). In addition, the combinatorial use of an *ort* promoter fragment and the

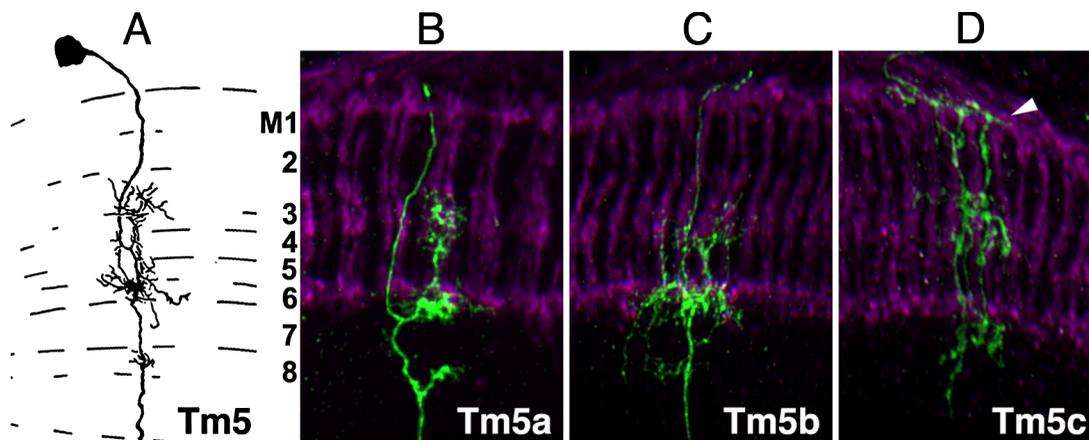


Figure 3. Transmedulla neuron Tm5, reported from Golgi impregnation (Fischbach & Dittrich, 1989) in A (with kind permission of Springer Science + Business Media), has, in fact, three subtypes, as revealed by GFP expression in *ort-Gal4*; UAS-GFP flies. (B) Tm5a. (C) Tm5b. (D) Tm5c. All three types of Tm5 neurons extend processes in the M3 and M6 strata, but Tm5c has an additional arbor in stratum M1 (arrowhead).

vesicular glutamate transporter enhancer trap allowed the identification of an amacrine cell, Dm8, which serves as a major synaptic target of R7 photoreceptors. These genetic tools simultaneously provide two advantages. First, they enable the identification of specific neuron subtypes, and second, they assign the specific neurotransmitter system of each identified neuron, thereby providing further insights into its circuit functions.

The most widely used reporter, mCD8::GFP, a fusion protein of the mouse CD8 transmembrane glycoprotein and GFP, labels membrane and, in most cases, provides the clearest pictures of fine neurites (Lee & Luo, 1999). Beyond revealing mere morphologies, other reporters allow the visualization of specific subcellular compartments: Tau-lacZ (or GFP) and Dscam-17.1-GFP mark axonal and dendritic compartments, respectively (O'Keefe et al., 1998; Wang et al., 2004), while HA- and GFP-tagged synaptobrevin (or synaptotagmin) and bruchpilot-GFP label, respectively, the synaptic vesicles and T-bar ribbons, at tentative presynaptic sites (Wagh et al., 2006). On the other hand, no reliable generic report is yet available for postsynaptic sites, although, in some cases, tagged neurotransmitter receptors have proved useful (Schmid et al., 2008). It is worth noting that the effectiveness of these specialized reporters has not yet been fully validated, while some may not be innocuous, their expression possibly leading to morphological changes. Finally, genetically encoded activity reporters can monitor calcium concentration, synaptic vesicle fusion, and membrane potential, thus reporting synaptic transmission by indirect means (reviewed in Guerrero & Isacoff, 2001). The application of such reagents in the visual system has not been reported, however, likely because of their modest temporal resolution.

The Third Method: Serial-Section EM Reconstructions

Questions of anatomical taxonomy should not obscure the fact that it is the functional types of neuron that require identification. Even though neuron shape defines many electrophysiological parameters (e.g., Borst & Haag, 1996), for example, through its cable properties (Jack et al., 1975; Rall et al., 1992), a neuron's branching pattern primarily provides the opportunities for it to form synaptic partnerships with other neurons. Thus, the description of neuron shape is not neuroanatomy's final goal, rather the synaptic contacts between neurons, and the circuits that these establish. Contact between neurons may be chiefly between axon terminal and dendrite, with a sequence of transmission from the first to the second, as assumed by Cajal (Cajal & Sánchez, 1915) and widely thereafter (e.g., Strausfeld & Lee, 1991; Strausfeld et al., 2006). But, exceptions to these rules clearly exist, making the detection of circuits possible only by EM examination, as examples from the medulla reveal (Takemura et al., 2008; Gao et al., 2008). Only when we know the actual circuits of synaptic contact may we interpret the recordings made from a neuron that are necessary to suggest its function. Thus, we start at the anatomy to elucidate the connections and then define the function by interpreting a neuron's electrophysiology.

Based on serial-section EM, three-dimensional (3D) reconstructions provide, finally, a third method to visualize neurons. Here, selectivity in the views of a neuron is introduced by choosing the consecutive profiles to reconstruct. Perhaps, remarkably, even when a neuron is sliced into ultrathin sections and images of its consecutive profiles are aligned and warped to bring them into vertical register, and then reconstructed, the 3D form that is

reconstituted can replicate the many fine details of neuron shape that are seen in the entire stained neuron (Takemura et al., 2008). Thus, the terminal of lamina cell L2 and medulla cell Tm2 appears very similar in many details when a serial-EM reconstruction is compared with a Golgi profile (Figures 2C and 2F). This close correspondence is reassuring, confirming that significant alteration to the shape of a neuron does not result from the process of Golgi impregnation itself, and that the profiles of an EM reconstruction are correctly and completely connected.

Such 3D serial-section EM reconstructions come at a huge human cost, however. They are not suited to address questions of variations in the form of neurons from the same class, or of differences between neurons of different classes, because so few cells can usually be reconstructed (three of each type in a recent analysis of medulla input neurons; Takemura et al., 2008). Such questions are better answered by genetic reporters that label the same cells reliably and repeatedly in different preparations. Yet, the increased resolution and manipulability of serial-EM reconstructions offer alternative advantages, chief among which is that they enable the profiles of all other neurons to be traced and thus reveal the location of, and partnerships at, synaptic sites. The 3D shape of an unlabeled neuron serves only to identify the cell. Once identified, the same EM dataset can then be used to trace out the synaptic partnerships and circuits to which that neuron contributes.

An alternative, and final, strategy currently offers the best of all worlds: the serial-section analysis of cells labeled by a genetic reporter that drives expression of a gene product, such as the marker, HRP-CD2. Successful application in two recent studies (Clements et al., 2008; Gao et al., 2008) indicates that despite a number of outstanding practical difficulties, this method currently provides our best opportunity to identify the synaptic connections of identified neurons. Using this approach, the genetic identity of the cell is known, while the profiles of the cell are revealed from the expression of the HRP-CD2 fusion protein, a membrane-targeted EM marker (Graham & Karnovsky, 1966; Larsen et al., 2003). These reagents, or related ones that await development, thus provide the advantages of serial-EM reconstruction methods on identified neurons, but without having to reconstruct the parent neuron in its entirety (data not shown).

Circuits: The Ultimate Functional Questions

The use of *Drosophila* pioneered many studies on the neural basis of visual behavior (Hecht & Wald, 1933, 1934; Kalmus, 1943; Götz, 1964; and studies reviewed in Heisenberg & Wolf, 1984). Genetic approaches became

widely instrumental in the analysis of the neural basis of many behaviors in the fly, starting especially with the early experiments of Hotta and Benzer (1970, 1972), using gynandromorphs to map the approximate circuits of mating behavior. The same approach was later used by Heisenberg to analyze the *optomotor-blind* mutant that affects the giant neurons of the lobula plate (Heisenberg et al., 1978). These neurons have been shown, in larger fly species, to be intimately involved in the neural processing of visual motion, especially that required to guide flight (see, e.g., Hausen & Egelhaaf, 1989; Borst & Haag, 2002).

In a series of elegant studies, which combined classical genetic and behavioral approaches, Heisenberg and his colleagues lesioned different regions of the *Drosophila* brain and assigned specific functions to distinct neural subsystems (Brunner et al., 1992; Strauss & Heisenberg, 1993; Heisenberg et al., 1985). These studies not only identified the role of the lobula plate in motion detection, but also the central complex in locomotion, and the mushroom body in olfactory learning. Earlier studies had likewise revealed the functional segregation between the two photoreceptor subtypes within ommatidia of the compound eye, into the optomotor functions of R1-R6 and the spectral functions of the two central cells, R7 and R8 (Heisenberg & Buchner, 1977).

Functional analysis at the level of circuits or individual neurons, especially for the visual system, is made difficult by the small size and functional diversity of its cells. While electrophysiological recordings are feasible in the visual system (e.g., Zheng et al., 2006; Joesch et al., 2008), they are by no means easy and attract few practitioners. As an alternative approach, the deoxyglucose method has been used to identify the stimulus-specific patterns of activation of medulla and lobula plate strata (Bausenwein et al., 1990; Bausenwein & Fischbach, 1992). By comparison with the overlaps between the shapes of Golgi impregnates (Bausenwein et al., 1992), these patterns then suggested the involvement of a limited number of parallel pathways from the photoreceptor inputs to the optic lobe. Although this method seldom provides directness and cellular resolution, it paved the way for the further analyses of medulla circuits that have appeared recently (Rister et al., 2007; Katsov & Clandinin, 2008; Gao et al., 2008). These depend on the conditional, temperature-dependent interruption of synaptic transmission, using a UAS-*shi* construct (Kitamoto, 2001). By targeting this construct to a specific cell by means of an appropriate GAL4 line, it is possible to assay the consequence to visual behavior of impairing function in the identified optic lobe neuron. By selectively reinstating function in those same neurons when they lack it, it is then possible to establish not only the necessity for, but also the sufficiency of, the function of

identified neurons (Liu et al., 2006) for identified visual behaviors in *Drosophila*. Such a strategy was recently adopted in the Heisenberg lab to elucidate the behavioral functions of lamina neurons, and thus, for example, at low light intensities, the L2 pathway is not only necessary, but also fully sufficient, for sensing front-to-back motion (Rister et al., 2007). A subsequent study using related strategies now reveals that the medulla intrinsic amacrine cell, Dm8, is both necessary and sufficient for ultraviolet phototaxis (Gao et al., 2008), and the list seems set to continue for many of the identified cell types and much of the fly's visual behavior.

The success of this genetic dissection approach to circuit function will depend on the convergence of three methods: the quantitative analysis of behavior, the design and use of appropriate genetic driver lines, and the availability of synaptic circuit information to interpret the effects of functional dissections using genetic reagents. Quantitative tests of behavior rely on a number of paradigms in fly vision that have been intensively studied for many years (Heisenberg & Wolf, 1984; Tang et al., 2004; Liu et al., 2006) and others of more recent design (Katsov & Clandinin, 2008). The selection of appropriate genetic drivers is absolutely crucial, because their specificity defines the resolution of analysis. As the available GAL4 lines rarely give rise to highly specific expression pattern, promoter dissection and the recently developed split-GAL4 system are attractive approaches to refine the expression pattern and pinpoint the critical elements of the circuit (Gao et al., 2008). Finally, interpretation of the role of the particular neuron requires detailed information on the synaptic contacts made by the neuron and the circuits to which it contributes. In the case of the lamina, this information was already available to the study by Rister et al. (2007) from previous studies based on serial EM (Meinertzhagen & O'Neil, 1991; Meinertzhagen & Sorra, 2001). For the medulla, those analyses are still incomplete (Takemura et al., 2008; Gao et al., 2008) but an obvious future target.

SUMMARY AND FUTURE DIRECTIONS

The daunting level of complexity common in the nervous systems of most advanced animals, and the large numbers of cell types even in the deceptively simple visual system of *Drosophila*, pose a considerable challenge to the analysis of synaptic circuits and their functions, against which we can now set the superior methods of labeling and identification advanced here. These allow the visualization of neuronal form by three quite independent methods. Each confers advantages but comes at its own cost. First, classical Golgi methods are simple, applicable

to any nervous system, and provide high-contrast images of stained neurons. But, in general, these can be seen only as a single projection image that must be drawn and classified with great care to compare it with other cell types. Second, genetic reporter methods are superior in essentially all ways to classical methods of staining neurons, but are applicable only in *Drosophila* and other genetically manipulable species. In *Drosophila*, they have essentially supplanted other light microscopic methods. Third, serial-section EM reconstructions can be used to reveal the 3D form of a neuron, as a requirement to studying its synaptic connections. A combination method, the EM analysis of cells labeled by an EM marker driven by a genetic reporter, provides the best combination of all methods in *Drosophila*. Perhaps, not remarkably, but nevertheless reassuringly, all three methods yield surprisingly similar images of neuron shape, confirming that our morphological distinctions among different cell types, indeed, recognize the genetic decisions made by *Drosophila*, and providing different ways to tackle the functional problem of the synaptic circuits formed by identified neurons.

In the state of our current techniques, serial-section EM reconstruction still remains the only method to identify reliably synaptic contacts in a complex, densely packed neuropile. The prospect of advances in automatic ultrathin sectioning systems (e.g., Hayworth et al., 2006) and in computer programs for segmenting and tracing neurite profiles, are likely soon to reduce considerably the human labor and subjectivity of these tasks, but are unlikely to automate all steps fully, nor to annotate the resulting reconstructions. No substitute is yet in sight for the human brain. Attractive alternatives that allow the rapid assessment of synaptic connections include genetic techniques such as the GRASP (GFP reconstitution across synaptic partners) method (Feinberg et al., 2008), or recently developed super-resolution microscopic techniques, which aim to break the diffraction limit and deliver the resolution required to identify synapses at the light microscopic level (Klar et al., 2001; Betzig et al., 2006; Huang et al., 2008). For reporting neuronal activity, genetically encoded optical voltage reporters have been greatly optimized to deliver submillisecond temporal resolution (Sjulson & Miesenböck, 2008). The application of these new tools to the *Drosophila* visual system is eagerly awaited. After some decades of relative neglect, the *Drosophila* visual system seems finally ready to yield the secrets of its cells and the wiring of their circuits.

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