Indian Journal of Biochemistry & Biophysics
Vol. 58, August 2021, pp. 334-343

A Review

Applications of electron microscopy in *Drosophila* neurobiology research

Shweta Tandon, Prerna Aggarwal & Surajit Sarkar*
Department of Genetics, University of Delhi, South Campus, New Delhi-110 021, Delhi, India

Received 18 January 2021; *revised 09 March 2021*

Visual evidences undeniably appear more convincing since they can be interpreted easily, though may not be always accurate. The electron microscopy techniques not only allow examining the ultra-structure of various cell/tissue types but also help in proposing the in-depth mechanisms of biological processes. Similarly, comprehensive analysis of the neuronal and synaptic communications, and overall integrity of the brain not only helps us to understand its intricate functioning, but also aids in deciphering the complex human brain disorders. The *Drosophila* brain and the pair of compound eyes have emerged as favoured organs to investigate the fundamentals of nervous system development and disease biology. Various types of electron microscopy techniques have assisted the *Drosophila* neurobiologists to generate significant insights about the development, structure and function of different neuronal cell types and their contribution in the aetiology of neurodegenerative disorders. The present review provides a snapshot of the applications of various electron microscopy methods in *Drosophila* neurobiology research.

Keywords: *Drosophila*, Neurobiology, Neurodegeneration, Ommatidia, Sclerosis, Spinocerebellar ataxia

The electron microscopic techniques allow biologists to unravel the ultra-structure of various living entities and the in-depth mechanisms of biological processes. Electron microscopy (EM) offers a higher resolution and magnification as it uses the shorter electron waves as compared to the longer light waves. For instance, the extent of magnification offered by light microscope is ×1000 - ×1500 times, with a resolution of ~200 nm, whereas, scanning electron microscopy (SEM) allows higher magnifications up to ×100,000 times. EM has emerged as a gold standard to image and construct detailed cellular diagrams and understand the structural complexities such as wiring of neurons, synapses, and the brain itself[1-3]. The recent advances in the EM techniques have allowed the reconstruction of brain circuitry of smaller organisms such as *C. elegans* and *Drosophila* larvae. Such structural information can address essential questions about functions and mechanisms of complex nervous system, which when coupled with biochemical/physiological analyses can yield comprehensive insights into the physiological and pathological workings of the brain[4].

The understanding of the neuronal connections, synaptic communications and overall integrity of the brain not only helps us to understand its intricate functioning, but also aids in deciphering the complex human brain disorders. Human neuronal tauopathies, polyglutamine[poly(Q)] disorders, amyotrophic lateral sclerosis, *etc*. are some neurodegenerative disorders which lack targeted intervention strategies due to insufficient understanding of their aetiology. Some of these disorders, such as Alzheimer's, Parkinson's, and Huntington's disease cause structural changes in the brain which in turn cause gait, memory, and cognition deficits. Due to the limitations associated with human genetics, model systems such as mice, *Drosophila melanogaster* and *C. elegans* have emerged as sophisticated substitutes for deciphering the pathogenesis of these disorders and to develop suitable intervention strategies. The present review attempts to provide a snapshot of the application of electron microscopy in *Drosophila* neurobiology research.

Drosophila as a model system for human neurological disorders

Drosophila is an ideal model system to elucidate the complexities of human neuronal disorders due to a remarkable presence of the functional homologues of more than 50% of all the human disease-causing genes and the striking conservation in the genes and pathways between the fly and human. In addition, it has a well-developed brain and nervous system, and serves as a genetically accessible model organism with complex and well-characterized behaviours such as walking, flight, escape responses, grooming, courtship,

*Correspondence:
E-mail: sarkar@south.du.ac.in

learning, and memory[5-7]. Accordingly, numerous studies on *Drosophila* disease models have enriched our understanding about the complex human brain disorders. Successful generation of the *Drosophila* poly(Q) models of neurodegenerative Spinocerebellar Ataxia 3 (SCA3)[8] and Huntington's disease[9] paved way to model several other human neurological disorders such as Alzheimer's disease, Parkinson's disease, Dementia, Amyotrophic lateral sclerosis, Frontotemporal dementia, *etc*[10,11].

A glimpse into the nervous system of *Drosophila*

Nervous system is one of the most complex systems featuring animals. Studies on the development and functioning of the nervous system, regulation of stimuli reception and response generation, integration of information in the form of memory have always fascinated scientists. *Drosophila* offers an unparalleled system to carry out such experiments *in vivo*. The fly nervous system is composed of both neuronal (~150,000 cells) and glial cells (~15,700 cells) located in a primitive organ resembling brain[12,13]. The neurons and glia are predominantly concentrated in the brain and the compound eyes (in adults), whereas the remaining peripheral system can be considered as a cage of neurons distributed throughout the body of the organism.

The *Drosophila* brain and the pair of compound eyes are the most favoured structures which are exploited to investigate the fundamentals of nervous system development and disease biology. Fly visual system is frequently used to examine the effects of genetic perturbations without directly compromising the viability of the organism. Since the compound eye of *Drosophila* is composed of both neuronal and non-neuronal cells, deliberate expression of the disease-causing human transgene(s) using appropriate driver line(s) generate easily quantifiable degenerative eye phenotype[14].

Electron microscopy allows rapid and accurate screening of the external eye phenotype and identification of the disease causing and disease modifying genes[15,16]. The significance and contribution of SEM and TEM (Transmission Electron Microscopy) in unravelling the in-depths of the human neuronal disorders in the fly system have been discussed in the following sections.

Scanning electron microscopy: Detailed study of the external landscape

SEM, due to its powerful magnification and resolution (~2 nm), has enabled the researchers to gain better insight into multiple areas of biology, especially with an aim to understand the topography, morphology, and composition of a tissue type. Interestingly, SEM has a rich history in *Drosophila* research, such as embryogenesis, development of proboscis, wing, and arrangement of bristles; and ultra-structural analyses of antenna, halteres and gut[17-22].

As already mentioned, *Drosophila* models of neurodegeneration, for instance poly(Q) and tauopathies display characteristic external eye deformities such as ommatidial fusions, roughening of eye surface, depigmentation, and reduced/increased eye curvature that can be observed under the classical light microscope[23-25]. Although, bright field imaging allows the researchers for quick discrimination between a wild type and diseased/mutant eye phenotype on routine basis (Fig. 1A & B), but due to poor magnification and undesired reflection of light from the surface of the eyes, a range of architectural details are often missed. SEM imaging allows the detailed phenotypic analysis of the eye surfaces, including minute ommatidial deformities and imprecise bristle arrangement. For instance, SEM images of tauopathy-expressing fly eyes (Fig. 1D & D′) revealed the differences in the size, surface topography, loss of mechano-sensory bristles and ommatidial fusions[24,26] (compare Fig. 1C & C′ with D & D′). A schematic representation highlights the structural defects in the compound eyes as observed under SEM (Fig. 1E-G). Similarly, SEM analysis of the adult eyes of the *Drosophila* models of human poly(Q) disorders such as Huntington, SCA1 and SCA3 have also revealed loss of ommatidia, misaligned and clustered mechano-sensory bristles and collapsed retina which are indicative of severe tissue degeneration[25,27,28]. Several gene-to-phenotype relationships have been established utilizing SEM. For instance, it has been demonstrated that *Drosophila* eye pigment genes are capable of modulating tau-induced neurodegeneration[29]. Comparative analysis of such identifying features can help evaluating the severity and nature of mutation or disease.

Although, scanning electron microscopy generates a repertoire of information, it fails to provide structural information of internal components which may help in deciphering the underlying defects culminating in the external anomalies. A modified SEM technique that is extensively used to address this challenge is the TEM. A brief overview of the

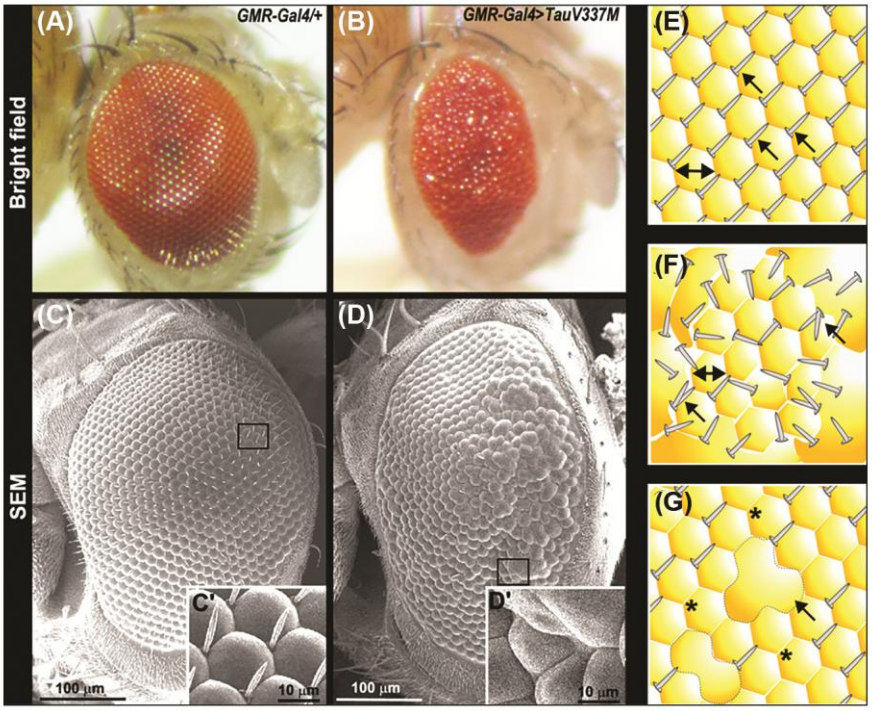

Fig. 1 — Images of *Drosophila* adult compound eyes through different microscopy techniques. (A) A normal looking control (*GMR-Gal4/+*) adult eye; (B) Human mutant-tau expressing adult eye (*GMR-Gal4>TauV337M)* displays reduced curvature and size with roughening of surface as seen under bright-field microscope. C – D′ represent SEM images of the same; (C) A detailed pattern of ommatidia and bristle arrangement in the wild type eye; and (D) Tau-expressing eye shows ommatidial fusions and absence of mechano-sensory bristles. C′ and D′ show the magnified area of the marked regions of C and D, respectively. E-G are schematic representations of defected phenotypes observed in poly(Q) (F) and tauopathy(G) diseases. E is a typical wild type arrangement with uniformly aligned bristles (arrows in E), F represents poly(Q) expressing adult eye surface with reduced ommatidial size (compare the double headed arrows in E and F), clustered bristles (arrows in F) and collapsed retina. G represents human pathogenic tau expressing adult eye surface with ommatidial fusions (arrow in G) and loss of mechano-sensory bristles (indicated by asterisks in G). (Scale: C, D = 100 µM; C′, D′ = 10 µM)

applications of TEM in *Drosophila* neurobiology research has been provided in the following section.

Transmission electron microscopy: Paving way into greater details

Conventionally, fluorescent labelling of protein(s) and/or molecules followed by optical sectioning by a confocal microscope allows the study of internal structures and arrangement of a cell(s). Comprehensive study of the deeper layers of a cell or structure is necessary to distinguish defective components associated with the external phenotypes. However, the limitations in the extent of magnification pertaining to confocal microscopy and inability to identify specific structures due to redundancy in protein expression have opened an avenue for the utilization of TEM. Intriguingly, TEM, due to its capacity to magnify the internal architecture of a specimen up to ×1,000,000 times and generating images with a resolution of about 0.2 nm is one of the most valuable techniques used to out run the technical limitations of confocal microscopy[30]. TEM requires very fine slices of the tissue and generally produces images in black-grey scale where identification of cells and intracellular/extracellular components is made based on prior knowledge of the structure. Recently, specific regions of a TEM micrograph have been painted manually or in a computer-automated fashion in different colour schemes to discriminate different cell types from one another aiding the researchers to develop better understanding of the internal geometry[31-33]. Moreover, TEM, being one of the propitious techniques, has been exploited over other fluorescence labelling techniques to precisely allocate the position of various glial cells at different developmental stages in *Drosophila*[34].

TEM has been used extensively in *Drosophila* research for quite a long time enabling the researchers to extend their knowledge of finer details such as the number, arrangements, and shape of the photoreceptor

cells in the adult eyes, synaptic junctions, mitochondrial morphology and sub-cellular protein aggregates. Further, TEM has enormous implications in the study of manifestation of neurodegenerative conditions modelled in *Drosophila*. Most neurodegenerative conditions are known to be caused due to aberrant expression of some proteins that reorganize themselves in form of proteinaceous insoluble aggregates or inclusion bodies[35]. Examination of tissue samples for the existence of such entities and the study of their structural morphology is essential to annotate the disease condition as well the status of disease progression in an individual. These sub-cellular/inter-cellular components are mostly visible through transmission electron microscopy[36]. Since, rough-eye phenotype and other structural anomalies are often attributed to defective photoreceptor cells and rhabdomeres residing deep down the observable lattice[37,38]. Therefore, TEM offers an extended advantage in understanding the in-depth cellular mechanisms determining these discrete morphological defects. When a processed tangential section of *Drosophila* eye is observed under a conventional TEM, seven photoreceptor cells (R1-R7) are found to exhibit a floral arrangement, whereas the eighth photoreceptor (R8) lies proximal to retina exactly beneath the R7 photoreceptor cell. TEM facilitates detailed examination of any potential degeneration and/or alteration in the arrangement of an embedded and/or internal neuronal structure. Figure 2 depicts a comparative assessment of the arrangement and number of photoreceptor cells in the control (normal) and poly(Q) expressing adult eyes as examined by bright field microscopy (Fig. 2A & C), and TEM (Fig. 2B, B′ & D, D′). TEM has aided in the generation of accurate information, for instance, the role of an endocytic protein, Past1, which was earlier undetermined through SEM, was found to be indispensable in differentiation of R1/R6/R7 photoreceptor cells and cone cells of fly ommatidia as observed in the tangential sections of the adult eye through TEM[39]. Electron microscopy performed on the metabolic model of adrenoleukodystrophy in *Drosophila* showed fenestration in the membrane separating the eye and the brain besides evident disarray in the normal hexagonal pattern of ommatidial structure[40]. Similarly, TEM has also been used to investigate the impact of *yata* mutant on the spatial architecture of the photoreceptor cells. TEM analysis revealed enhanced vacuolization and presence of

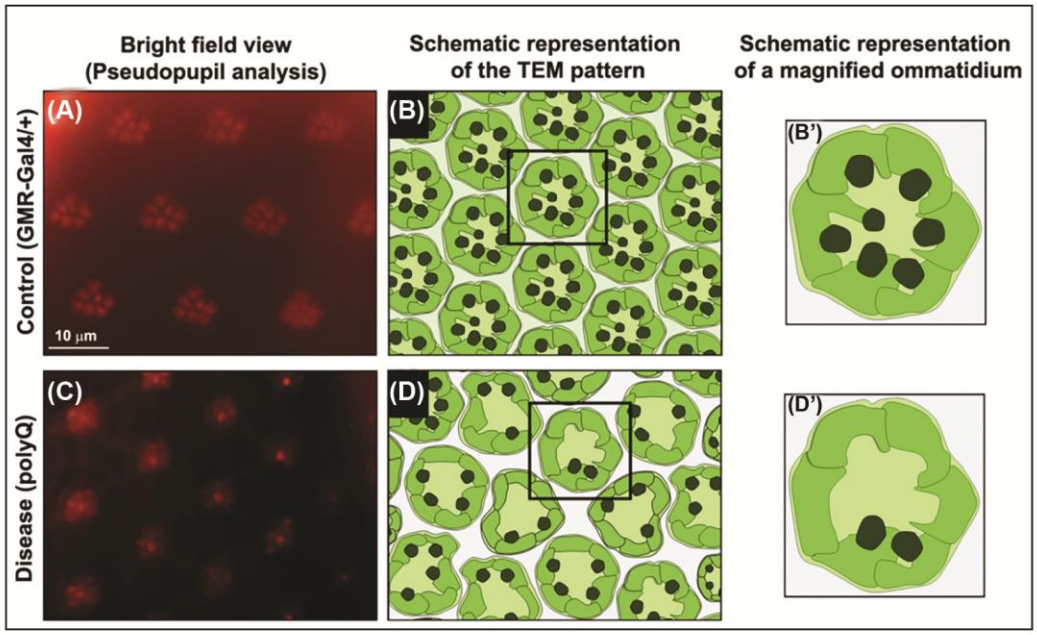

Fig. 2 — Ommatidial architecture of the *GMR-Gal4/+* control and poly(Q) disease bearing adult *Drosophila* eye as observed under bright field microscope and TEM. (A) Deep-pseudopupil analysis of the *GMR-Gal4/+* control adult *Drosophila* eye under bright-field microscope reveals the typical arrangement of a bunch of photoreceptor neurons per ommatidium; (B) Schematic representation of the TEM pattern of image A which reveals detailed structure of the ommatidia and arrangement of photoreceptor cells. (B') Magnified view of an individual ommatidium (boxed area in B) showing the typical arrangement of 7 photoreceptors; (C) Deep-pseudopupil analysis of poly(Q) disease bearing adult *Drosophila* eye showing degenerated photoreceptors and disrupted ommatidial lattice. (D') Schematic representation of the magnified view of an individual ommatidium (boxed area in D) clearly showing the degeneration of the photoreceptors. (Scale: A, C = 10 µM)

abnormal cellular structures in the close vicinity of photoreceptor cells, specifically R7-R8[41]. A range of other studies have also utilized TEM to depict the disordered arrangement of photoreceptor cells[42-45].

TEM has been effectively utilized to generate a better understanding of the pathogenic events that occur in neurodegenerative conditions. TEM analysis of the brain samples of adult flies subjected to Paraquat treatment (to model Parkinsonism) displayed enhanced nuclear membrane, chromatin disintegration and fragmented mitochondria within the cytoplasm[46]. Likewise, TEM-based investigation of the structural organization of cartilage in the laminar region of the brain suggested that pathogenic tau protein can cause presynaptic dysfunction in *Drosophila* disease models. Examination of the laminar region of the brain revealed that accumulation of P301L mutant tau aggregates disrupts the synapse formation and causes abnormally developed synaptic terminals[47]. Interestingly, these aggregates exhibit dynamic structure, transforming from less pathogenic form to more pathogenic form as the disease progresses. Since, such aggregates are visible through TEM, several research groups have utilized this opportunity to investigate the role of the structural dynamics of these aggregates in disease development and progression. In addition to the above, TEM techniques have also facilitated detailed examination of the misfolded and/or abnormally folded ultrastructure of the tau protein isolated from *Drosophila* disease models[48]. Such insights into the disease pathogenesis might pave way for screening of potential drugs or genetic modifiers that could ameliorate the disease condition. A recent study has utilized TEM to examine efficiency of Rose Bengal in regulating the pathogenic tau filaments[49].

With time, several variants of the conventional electron microscopy have been developed to achieve enhanced resolution. An overview of such variants and their applications in *Drosophila* neurobiology research has been provided below.

Variants of the conventional SEM and TEM and their applications in *Drosophila* neurobiology research

The conventional scanning and transmission electron microscopic techniques have immensely aided in understanding the biological structures and increased our knowledge of various neurological disorders. However, the conventional electron microscopy techniques, TEM as well as SEM, have certain limitations that make it inconvenient for converting into automated high-throughput techniques. Some of the limitations associated with the conventional SEM are as follows:

1. Specimen Preparation: Conventional SEM only allows the imaging of dehydrated samples operated under high vacuum to maintain the coherency of the electron beam and to prevent electron scattering by atmospheric gases. Therefore, elaborate processing of the biological samples is required; subjecting them to desiccation, and coating them with heavy metals (gold, palladium), which may alter the native structure and properties of the samples and introduce artefacts[50].Such intricate processing of the biological samples makes it difficult to image them in their native form.
2. High-throughput technology: The conventional electron microscopes image the specimen one pixel at a time, making it time consuming and laborious. Thus, it is difficult to convert these conventional techniques into a high-throughput technology.
3. Specimen thickness: This limitation specifically refers to the conventional TEM, which can produce high resolution images, but is only limited to ultra-thin sections (less than 100 nm). Thus, it fails to offer the structural details of thick or voluminous biological entities.
4. Automation and analysis: Though, the conventional TEM has been paramount in generating high resolution structural images, yet reconstruction of serial sections into 3D constructions and their software-based analysis is not possible with the conventional EM.

To circumvent the above-mentioned limitations of conventional EM, advanced variants of EM have been developed in recent years. Some of the widely used variants are as follows:

Cryo-Electron Microscopy (Cryo-EM)

Cryo-EM is one of the very first techniques that allowed macromolecule structural imaging without fixation, staining, or desiccation of the specimen. It is a combination of three technologies, specimen preparation, electron microscopy and mathematical/ computational approaches[51]. It offers a unique specimen preparation method for preserving the biological samples in their near-native condition by covering it with a thin layer of amorphous ice film and imaging at liquid nitrogen temperature. Cryo-electron microscopy of ultra-thin vitreous sections (CEMOVIS) is a suitable tool to generate high resolution images of

frozen hydrated tissues and cells[52,53] and has been used for the analysis of brain structures such as synapses[54,55]. A schematic representation of the Cryo-electron microscopic image has been provided in (Fig. 3A-C). Combination of cryo-soft X-ray microscopy and cryo-TEM technology has been suggested as a suitable technique to image synapses of the Kenyon cells of the *Drosophila* mushroom body in frozen hydrated brains and ultra-thin vitreous sections[56]. Cryo-electron microscopy has also been used to determine the activity and atomic structure of an amyloid protein- the *Drosophila* cytoplasmic polyadenylation element-binding (CPEB) protein, Orb2, and its role in memory[57].

Atmospheric Scanning Electron Microscopy (ASEM)/Environmental Scanning Electron Microscopy (ESEM)

The conventional SEM is a powerful tool for biological research; however, it requires high vacuum conditions and elaborate specimen processing. Recently designed environmental scanning electron microscope (ESEM) allows imaging of samples in gaseous and vapour conditions at pressures ranging from 10 to 10^3Pa[58]. ESEM allows the imaging of wet (biological) samples without any prior specimen preparation. The primary electron beam, being very energetic, penetrates the water vapour with little scattering and scans across the sample surface, releasing secondary electrons. The water vapour molecules struck by these secondary electrons produce secondary electrons of themselves, which in turn produces more secondary electrons from the adjacent molecules, thus amplifying the cascade[59]. It eliminates the need for the samples to be desiccated and coated with gold-palladium, allowing the preservation of original characteristics of the sample and to be free of artefacts. ESEM makes use of a series of pressure-limiting apertures (PLAs), creating a pressure gradient, with good vacuum at top of the column to protect the electron gun, and poor vacuum conditions in the specimen chamber.

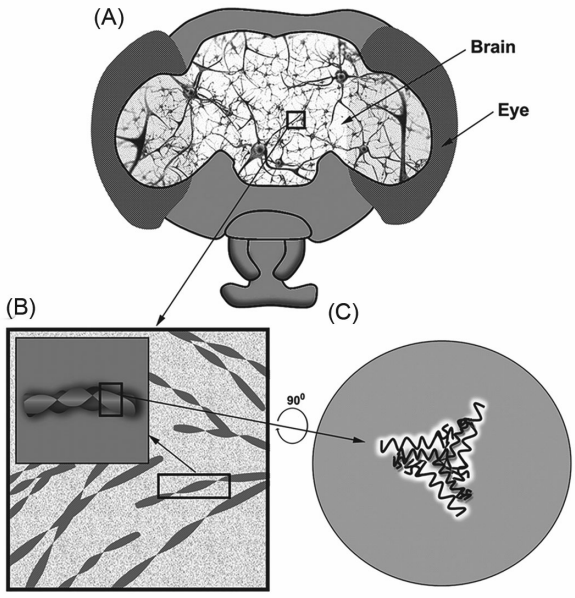

Fig. 3 — Schematic representation of the cryo-electron microscopic pattern of a neuronal protein of adult *Drosophila* brain. (A) Pictorial representation of an adult *Drosophila* head; (B) Representative schematic of cryo-electron micrograph of a neuronal protein of adult *Drosophila* brain with inset depicting the magnified view of a section (boxed area in B); and (C) A schematic representing the cryo-EM reconstruction of the filaments of the neuronal protein at Å resolution in transverse plane

ESEM has been used to study minute details of structure in various biological samples including *Drosophila*[60]. For instance, ESEM examination of *Drosophila* eyes aided in elucidating the role of M6 protein in eye development[61]. ESEM based studies have also allowed the elucidation of the role of crinkled/Myo VII in the formation and organisation of actin filament bundles that ultimately drive the proper shape of cellular projections[62]. ESEM has also determined the role of methionine sulfoxide reductase A (dmrsA) in regulation of FOXO in *Drosophila*[63].

Multi-beam Scanning Electron Microscopy

As mentioned above, conversion of the conventional SEM into high throughput technique is difficult due to slow imaging speed of SEM. To increase the speed of imaging, the beam current will have to be amplified, which means compromising with the resolution of the final image. The multi-beam SEM, instead of a single beam, uses 61 electron beams[64]. The multi-beam produces a pattern of 61 primary foci, arranged in a hexagonal pattern to minimise optical aberrations. The secondary electrons (SE) that emanate from the primary electrons are imaged onto a multi-detector having a specific detection unit for each beam. The sample is scanned over with the primary electron beams and the secondary signal is recorded for each position like the conventional SEM[64]. Therefore, a single round of scanning produces multiple images simultaneously, hence yielding a complete image. A volumetric reconstruction of the mouse brain has been made possible with this technological advancement[65].

Serial section TEM (ssTEM)

TEM has played a vital role in answering important neurobiological questions, such as

synaptic communication[66,67]. Serial section TEM (ssTEM) has paved way for in-depth structural analysis especially in neurobiology, to understand 3D synaptic structures in brain[68-70]. It can accurately identify and measure objects smaller than 250 nm. Many variants of this technique have been developed to achieve large scale serial section electron microscopy such as, serial section electron tomography or SSET; serial section transmission EM or ssTEM; block-face EM or SBEM; focused ion beam scanning EM or FIBSEM[69,71,72]. Though, ssTEM has allowed imaging with higher spatial resolution and a greater signal-to-noise ratio with the same dose of electron beam as conventional EM, however, it still lacks automated collection, handling and imaging of biological samples.

Computer-Assisted Serial Section Electron Microscopy

Modern automated electron microscopic techniques can generate many image tiles; however, their interpretation and construction of volumetric images is not easy[73]. Neuroscience laboratories depend on these automated tools to analyse these vast EM data sets using affordable techniques. Recent developments have allowed the imaging of enormous serial section EM datasets of tissue volumes, for example 86.7 trillion voxel dataset spanning 64 million μM^3 of an adult female *Drosophila* VNC, by a combination technique, the TEMCA-GT[74]. It combines GridTape, a tape substrate that allows automated section collection with an automated TEM camera array (TEMCA)[74]. The GridTape technology has been then used to image the VNC and reconstruct over 1000 sensory and motor neurons that regulate the limb movements, and examining the organisation of peripheral nerves and the leg motor neurons[75].

TrakEM2, an open-source software package, has especially been optimised to reconstruct neural circuits from tera-scale serial sections EM image data sets[73]. This software allows rapid entry, organisation, and navigation through the EM image selections, enabling manipulation, visualisation, reconstruction, annotation, and measurement of the neuronal components embedded in the data. TrakEM2 has been successfully used for the reconstruction of targeted EM micro volumes of *Drosophila* larval central nervous system[3]. Similar strategies have recently allowed the whole-brain EM dataset generation of the adult *Drosophila* brain by utilizing the TEMCA based tools[1]. It also helps in combining confocal stacks of the same tissue with the TEM sections.

Conclusion

Pictorial evidences are undeniably convincing and satisfying since it can be interpreted easily, though may not be always accurate. Microscopes have emerged as indispensable tools for biological research. Particularly, various types of electron microscopy methods have assisted the *Drosophila* neurobiologists to generated significant insights about the development, structure and function of different neuronal cell types and their contribution in the aetiology of neurodegenerative disorders. A transformative advancement in *Drosophila* neurobiology research can be attained by appropriate usage of modern electron microscopy techniques which allow achieving atomic level resolution of biomolecules.

Acknowledgement

Research programs in the laboratory are supported by grants from the Department of Biotechnology (DBT), and Science and Engineering Research Board (SERB), Government of India, New Delhi, India, to Surajit Sarkar. Shweta Tandon and Prerna Aggarwal are supported by research fellowships from University Grant Commission (UGC) and Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi, respectively. We thank Dr. Nisha for the SEM images. We are grateful to Ms. Nabanita Sarkar for technical supports.

Conflict of interest

All authors declare no conflict of interest.

References

1 Zheng Z, Lauritzen JS, Perlman E, Robinson CG, Nichols M, Milkie D, Torrens O, Price J, Fisher CB, Sharifi N, Calle-Schuler SA, Kmecova L, Ali IJ, Karsh B, Trautman ET, Bogovic JA, Hanslovsky P, Jefferis GSXE, Kazhdan M, Khairy K, Saalfeld S, Fetter RD & Bock DD, A complete electron microscopy volume of the brain of adult *Drosophila melanogaster*. *Cell*, 174 (2018) 730.

2 Ryan K, Lu Z & Meinertzhagen IA, The CNS connectome of a tadpole larva of *Ciona intestinalis* (L.) highlights sidedness in the brain of a chordate sibling. *Elife*, 5 (2016) e16962.

3 Cook SJ, Jarrell TA, Brittin CA, Wang Y, Bloniarz AE, Yakovlev MA, Nguyen KCQ, Tang LT, Bayer EA, Duerr JS, Bülow HE, Hobert O, Hall DH & Emmons SW, Whole-animal connectomes of both *Caenorhabditis elegans* sexes. *Nature*, 571 (2019) 63.

4 Ohno N, Katoh M, Saitoh Y, Saitoh S & Ohno S, Three-dimensional volume imaging with electron microscopy toward connectome. *Microscopy*, 64 (2015) 17.

5 Dickinson MH & Muijres FT, The aerodynamics and control of free flight manoeuvres in *Drosophila*. *Phil Trans R Soc B*, 371 (2016) 20150388.

6 Hampel S, Franconville R, Simpson JH & Seeds AM, A neural command circuit for grooming movement control. *Elife*, 4 (2015) e08758.

7 Grotewiel MS, Martin I, Bhandari P & Cook-Wiens E, Functional senescence in *Drosophila melanogaster*. *Aging Res Rev*, 4 (2005) 372.

8 Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL & Bonini NM, Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. *Nat Genet*, 23 (1999) 425.

9 Jackson GR, Salecker I, Dong X, Yao X, Arnheim N, Faber PW, MacDonald ME & Zipursky SL, Polyglutamine-expanded human huntingtin transgenes induce degeneration of *Drosophila* photoreceptor neurons. *Neuron*, 21 (1998) 633.

10 Bolus H, Crocker K, Boekhoff-Falk G & Chtarbanova S, Modeling Neurodegenerative Disorders in *Drosophila melanogaster*. *Int J Mol Sci*, 21 (2020) 3055.

11 Lu B & Vogel H, *Drosophila* models of neurodegenerative diseases. *Annu Rev Pathol*, 4 (2009) 315.

12 Jenett A, Rubin GM, Ngo TT, Shepherd D, Murphy C, Dionne H, Pfeiffer BD, Cavallaro A, Hall D, Jeter J, Iyer N, Fetter D, Hausenfluck JH, Peng H, Trautman ET, Svirskas RR, Myers EW, Iwinski ZR, Aso Y, DePasquale GM, Enos A, Hulamm P, Lam SC, Li HH, Laverty TR, Long F, Qu L, Murphy SD, Rokicki K, Safford T, Shaw K, Simpson JH, Sowell A, Tae S, Yu Y & Zugates CT, A Gal4-Driver line resource for *Drosophila* neurobiology. *Cell Rep*, 2 (2012) 991.

13 Kremer MC, Jung C, Batelli S, Rubin GM & Gaul U, The glia of the adult *Drosophila* nervous system. *Glia*, 65 (2017) 606.

14 Li WZ, Li SL, Zheng HY, Zhang SP & Xue L, A broad expression profile of the GMR-Gal4 driver in Drosophila melanogaster. *Genet Mol Res*, 11 (2012) 1997.

15 Prüßing K, Voigt A & Schulz JB, *Drosophila melanogaster* as a model organism for Alzheimer's disease. *Mol Neurodegener*, 8 (2013) 35.

16 Rimkus SA, Katzenberger RJ, Trinh AT, Dodson GE, Tibbetts RS & Wassarman DA, Mutations in String/ CDC25 inhibit cell cycle re-entry and neurodegeneration in a *Drosophila* model of Ataxia telangiectasia. *Genes Dev*, 22 (2008) 1205.

17 Turner FR & Mahowald AP, Scanning electron microscopy of *Drosophila melanogaster* embryogenesis. III. Formation of the head and caudal segments. *Dev Biol*, 68 (1979) 96.

18 Pyrowolakis G, Veikkolainen V, Yakoby N & Shvartsman SY, Gene regulation during *Drosophila* eggshell patterning. *Proc Natl Acad Sci U S A*, 114 (2017) 5808.

19 Johnson SA & Milner MJ, The final stages of wing development in *Drosophila melanogaster*. *Tissue Cell*, 19 (1987) 505.

20 Benassayag C, Plaza S, Callaerts P, Clements J, Romeo Y, Gehring WJ & Cribbs DL, Evidence for a direct functional antagonism of the selector genes proboscipedia and eyeless in *Drosophila* head development. *Development*, 130 (2003) 575.

21 Szabad J, Bellen HJ & Venken KJ, An assay to detect *in vivo* Y chromosome loss in *Drosophila* wing disc cells. *G3*, 2 (2012) 1095.

22 Kirkpatrick DM, Leach HL, Xu P, Dong K, Isaacs R & Gut LJ, Comparative Antennal and Behavioral Responses of Summer and Winter Morph *Drosophila suzukii* (Diptera: Drosophilidae) to Ecologically Relevant Volatiles. *Environ Entomol*, 47 (2018) 700.

23 Singh MD, Raj K & Sarkar S, *Drosophila* Myc, a novel modifier suppresses the poly(Q) toxicity by modulating the level of CREB binding protein and histone acetylation. *Neurobiol Dis*, 63 (2014) 48.

24 Chanu SI & Sarkar S, Targeted downregulation of dMyc suppresses pathogenesis of human neuronal tauopathies in *Drosophila* by limiting heterochromatin relaxation and tau hyperphosphorylation. *Mol Neurobiol*, 54 (2017) 2706.

25 Singh V, Sharma RK, Athilingam T, Sinha P, Sinha N & Thakur AK, NMR spectroscopy-based metabolomics of *Drosophila* model of Huntington's disease suggests altered cell energetics. *J Proteome Res*, 16 (2017) 3863.

26 Shulman JM& Feany MB, Genetic modifiers of tauopathy in *Drosophila*. *Genetics*, 165 (2003) 1233.

27 Raj K & Sarkar S, Tissue-specific upregulation of *Drosophila* insulin receptor (InR) mitigates poly(Q)-mediated neurotoxicity by restoration of cellular transcription machinery. *Mol Neurobiol*, 56 (2019) 1310.

28 Al-Ramahi I, Pérez AM, Lim J, Zhang M, Sorensen R, de Haro M, Branco J, Pulst SM, Zoghbi HY & Botas J, dAtaxin-2 mediates expanded Ataxin-1-induced neurodegeneration in a *Drosophila* model of SCA1. *PLoS Genet*, 3 (2007) e234.

29 Ambegaokar SS & Jackson GR, Interaction between eye pigment genes and tau-induced neurodegeneration in *Drosophila melanogaster*. *Genetics*, 186 (2010) 435.

30 Winey M, Meehl JB, O'Toole ET & Giddings TH Jr, Conventional transmission electron microscopy. *Mol Biol Cell*, 25 (2014) 319.

31 Cardona A, Saalfeld S, Preibisch S, Schmid B, Cheng A, Pulokas J, Tomancak P & Hartenstein V, An integrated micro- and macroarchitectural analysis of the *Drosophila* brain by computer assisted serial section electron microscopy. *PLoS Biol*, 8 (2010) e1000502.

32 Takemura SY, Xu CS, Lu Z, Rivlin PK, Parag T, Olbris DJ, Plaza S, Zhao T, Katz WT, Umayam L, Weaver C, Hess HF, Horne JA, Nunez-Iglesias J, Aniceto R, Chang LA, Lauchie S, Nasca A, Ogundeyi O, Sigmund C, Takemura S, Tran J, Langille C, Le Lacheur K, McLin S, Shinomiya A, Chklovskii DB, Meinertzhagen IA & Scheffer LK, Synaptic circuits and their variations within different columns in the visual system of *Drosophila*. *Proc Natl Acad Sci U S A*, 112 (2015) 13711.

33 Takemura SY, Nern A, Chklovskii DB, Scheffer LK, Rubin GM & Meinertzhagen IA, The comprehensive connectome of a neural substrate for 'ON' motion detection in *Drosophila*. *Elife*, 6 (2017) e24394.

34 Stork T, Engelen D, Krudewig A, Silies M, Bainton RJ & Klämbt C, Organization and function of the blood-brain barrier in *Drosophila*. *J Neurosci*, 28 (2008) 587.

35 Hipp MS, Park S & Hartl FU, Proteostasis impairment in protein-misfolding and – aggregation disease. *Trends Cell Biol*, 24 (2014) 506.

36 Bauerlein FJB, Fernandez-Busnadiego R & Baumeister W, Investigating the structure of neurotoxic protein aggregates inside cell. *Trends Cell Biol*, 30 (2020) 951.

37 Tomlinson A, Kimmel BE & Rubin GM, Rough, a *Drosophila* homeobox gene required in photoreceptor R2 and R5 for inductive interaction in the developing eye. *Cell*, 55 (1988) 771.

38 Pickup AT, Lamka ML, Sun Q, Yip ML & Lipshitz HD, Control of photoreceptor cell morphology, planar polarity and epithelial integrity during *Drosophila* eye development. *Development*, 129 (2002) 2247.

39 Dorot O, Steller H, Segal D & Horowitz M, *Past1* modulates *Drosophila* eye development. *PLoS One*, 12 (2017) e0174495.

40 Sivachenko A, Gordon HB, Kimball SS, Gavin EJ, Bonkowsky JL & Letsou A, Neurodegeneration in a *Drosophila* model of adrenoleukodystrophy: the role of the Bubblegum and Double bubble acyl-CoA synthetase. *Dis Model Mech*, 9 (2016) 377.

41 Arimoto E, Kawashima Y, Choi T, Unagami M, Akiyama S, Tomizawa M, Yano H, Suzuki E & Sone M, Analysis of a cellular structure observed in the compound eyes of *Drosophila* white; *yata* mutants and *white* mutants. *Biol Open*, 9 (2020) bio047043.

42 Pham H, Yu H & Laski FA, Cofilin/ADF is required for retinal elongation and morphogenesis of the *Drosophila* rhabdomere. *Dev Biol*, 318 (2008) 82.

43 Nie J, Mahato S & Zelhof AC, The actomyosin machinery is required for *Drosophila* retinal lumen formation. *PLoS Genet*, 10 (2014) e1004608.

44 Huang Z, Ren S, Jiang Y & Wang T, PINK1 and Parkin cooperatively protect neurons against constitutively active TRP-channel-induced retinal degeneration in *Drosophila*. *Cell Death Dis*, 7 (2016) e2179.

45 Hebbar S, Lehmann M, Behrens S, Hälsig C, Leng W, Yuan M, Winkler S & Knust E, The splicing regulator Prp31 prevents retinal degeneration in *Drosophila* by regulating Rhodopsin levels. *Biol Open*, 10 (2021) bio052332.

46 Niveditha S, Ramesh SR & Shivanandappa T, Paraquat-Induced movement disorder in relation to oxidative stress-mediated neurodegeneration in the brain of *Drosophila melanogaster*. *Neurochem Res*, 42 (2017) 3310.

47 Zhou L, McInnes J, Wierda K, Holt M, Herrmann AG, Jackson RJ, Wang YC, Swerts J, Beyens J, Miskiewicz K, Vilain S, Dewachter I, Moechars D, De Strooper B, Spires-Jones TL, De Wit J & Verstreken P, Tau association with synaptic vesicles causes presynaptic dysfunction. *Nat commun*, 8 (2017) 15295.

48 Chanu SI & Sarkar S, Targeted downregulation of dMyc restricts neurofibrillary tangles mediated pathogenesis of human neuronal tauopathies in *Drosophila*. *Biochim Biophys Acta Mol Basis Dis*, 1863 (2017) 2111.

49 Dubey T, Gorantla NV, Chandrashekara KT & Chinnathambi S, Photodynamic exposure of Rose-Bengal inhibits Tau aggregation and modulates cytoskeletal network in neuronal cell. *Sci Rep*, 10 (2020) 12380.

50 McKinlay KJ, Scotchford CA, Grant D, Oliver JM, King J & Brown P, Scanning Electron Microscopy of Biomaterials. *Electron Microsc Anan Issue*, 179 (2004) 87.

51 Frank J, Generalized single-particle cryo-EM – a historical perspective. *Microscopy*, 65 (2016) 3.

52 Al-Amoudi A, Norlen LPO & Dubochet J, Cryo-electron microscopy of vitreous sections of native biological cells and tissues. *J Struct Biol*, 148 (2004) 131.

53 Leforestier A, Lemercier N & Livolant F, Contribution of cryoelectron microscopy of vitreous sections to the understanding of biological membrane structure. *Proc Natl Acad Sci U S A*, 109 (2012) 8959.

54 Zuber B, Nikonenko I, Klauser P, Muller D & Dubochet J, The mammalian central nervous synaptic cleft contains a high density of periodically organized complexes. *Proc Natl Acad Sci U S A*, 102 (2005) 19192.

55 Fernández-Busnadiego R, Zuber B, Maurer UE, Cyrklaff M, Baumeister W & Lucic V, Quantitative analysis of the native presynaptic cytomatrix by cryoelectron tomography. *J Cell Biol*, 188 (2010) 145.

56 Leforestier A, Levitz P,Preat T, Guttmann P, Michot LJ & Tchénio P, Imaging *Drosophila* brain by combining cryo-soft X-ray microscopy of thick vitreous sections and cryo-electron microscopy of ultrathin vitreous sections. *J Struct Biol*, 188 (2014) 177.

57 Hervas R, Rau MJ, Park Y, Zhang W, Murzin AG, Fitzpatrick JAJ, Scheres SHW & Si K, Cryo-EM structure of a neuronal functional amyloid implicated in memory persistence in *Drosophila*. *Science*, 367 (2020) 1230.

58 Stokes DJ, Recent advances in electron imaging, image interpretation and applications: environmental scanning electron microscopy. *Phil Trans R Soc A*, 361 (2003) 2771.

59 Muscariello L, Rosso F, Marino G, Giordano A, Barbarisi M, Cafiero G & Barbarisi A, A critical overview of ESEM applications in the biological field. *J Cell Physiol*, 205 (2005) 328.

60 Tardi NJ, Cook ME & Edwards KA, Rapid phenotypic analysis of uncoated *Drosophila* samples with low-vacuum scanning electron microscopy. *Fly*, 6 (2012) 184.

61 Zappia MP, Bernabo G, Billi SC, Frasch AC, Ceriani MF & Brocco MA, A role for the membrane protein M6 in the *Drosophila* visual system. *BMC neurosci*, 13 (2012) 78.

62 Sallee JL, Crawford JM, Singh V & Kiehart DP, Mutations in *Drosophila* crinkled/Myosin VIIA disrupt denticle morphogenesis. *Dev Biol*, 470 (2021) 121.

63 Chung H, Kim AK, Jung SA, Kim SW, Yu K& Lee JH, The *Drosophila* homolog of methionine sulfoxide reductase A extends lifespan and increases nuclear localization of FOXO. *FEBS Lett*, 584 (2010) 3609.

64 Eberle AL, Mikula S, Schalek R, Lichtman J, Tate MLK& Zeidler D, High-resolution, high-throughput imaging with a multibeam scanning electron microscope. *J Microsc*, 259 (2015) 114.

65 Lichtman JW & Denk W, The big and the small: challenges of imaging the brain's circuits. *Science*, 334 (2011) 618.

66 Palay SL & Palade GE, The fine structure of neurons. *J Biophys Biochem Cytol*, 1 (1955) 69.

67 Gray EG, Electron microscopy of synaptic contacts on dendrite spines of the cerebral cortex. *Nature*, 183 (1959) 1592.

68 Ostroff LE, Fiala JC, Allwardt B & Harris KM, Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron*, 35 (2002) 535.

69 Knott G, Marchman H, Wall D & Lich B, Serial section scanning electron microscopy of adult brain tissue using focused ion beam milling. *J Neurosci*, 28 (2008) 2959.

70 Knott GW, Holtmaat A, Wilbrecht L, Welker E & Svoboda K, Spine growth precedes synapse formation in the adult neocortex *in vivo*. *Nat Neurosci*, 9 (2006) 1117.

71 Kubota Y, New developments in electron microscopy for serial image acquisition of neuronal profiles *Microscopy*, 64 (2015) 27.

72 Denk W & Heinz H, Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biol*, 2 (2004) e329.

73 Cardona A, Saalfeld S, Schindelin J, Arganda-Carreras I, Preibisch S, Longair M, Tomancak P, Hartenstein V & Douglas RJ, TrakEM2 Software for Neural Circuit Reconstruction. *PLoS One*, 7 (2012) e38011.

74 Graham BJ, Hildebrand DGC, Kuan AT, Maniates-Selvin JT, Thomas LA, Shanny BL & Lee WCA, High-throughput transmission electron microscopy with automated serial sectioning. *bioRxiv*, 657346 (2019) doi: https://doi.org/10.1101/657346.

75 Phelps JS, Hildebrand DGC, Graham BJ, Kuan AT, Thomas LA, Nguyen TM, Buhmann J, Azevedo AW, Sustar A, Agrawal S, Liu M, Shanny BL, Funke J, Tuthill JC & Lee WA, Reconstruction of motor control circuits in adult *Drosophila* using automated transmission electron microscopy. *Cell*, 184 (2021) 759.