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Review

Cerebral Innate Immunity in Drosophila Melanogaster

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Abstract: Modeling innate immunity in *Drosophila melanogaster* has a rich history that includes ground-breaking discoveries in pathogen detection and signaling. These studies revealed the evolutionary conservation of innate immune pathways and mechanisms of pathogen detection, resulting in an explosion of findings in the innate immunity field. In *D. melanogaster*, studies have focused primarily on responses driven by the larval fat body and hemocytes, analogs to vertebrate liver and macrophages, respectively. Aside from pathogen detection, many recent mammalian studies associate innate immune pathways with development and disease pathogenesis. Importantly, these studies stress that the innate immune response is integral to maintain central nervous system (CNS) health. Microglia, which are the vertebrate CNS mononuclear phagocytes, drive vertebrate cerebral innate immunity. The invertebrate CNS contains microglial-like cells – ensheathing glia and reticular glia – that could be used to answer basic questions regarding the evolutionarily conserved innate immune processes in CNS development and health. A deeper understanding of the relationship between *D. melanogaster* phagocytic microglial-like cells and vertebrate microglia will be key to answering basic and translational questions related to cerebral innate immunity.

Keywords: brain; *Drosophila melanogaster*; glia; microglia; neuroimmunology; neuroinflammation

Abbreviations: CNS – central nervous system; IMD – immune deficiency; TLR – Toll-like receptor; PRR – pattern recognition receptor; UAS- upstream activation sequence; Gcm – glial cell missing; A-T – ataxia telangiectasia; ATM – ataxia telangiectasia mutated**;** Dnr1 – defense repressor 1**;** BBB – blood brain barrier**;** NF- B – nuclear factor-kappa B; MANF – mesencephalic astrocyte-derived neurotrophic factor**;** Dpr – Draper**;** TNF-α – tumor necrosis factor-α**;** NO – nitric oxide**;** TGF-β – transforming growth factor-β**;** Upd – unpaired; ADGF – adenosine deaminase-related growth factor-A

1. Introduction

The discovery of Toll receptors in *Drosophila melanogaster* provided a molecular context to understand pathogen recognition by the innate immune system [1]. This finding opened the door to the discovery of mammalian Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs), which in turn has illustrated the broad variety of pathogen detection mechanisms, signaling components, immune modulating factors, and innate-adaptive immune cross-talk, which has revolutionized the field of immunology [1–3]. These initial studies in *D. melanogaster*, as well as subsequent invertebrate studies, provided mechanistic insight into peripheral immune responses driven by hemocytes and fat body cells [4,5].

More recent work in the invertebrate central nervous system (CNS) has revealed that dysregulation of cerebral innate immune signaling in glial cells can lead to neuronal dysfunction and degeneration [6–9]. While much more remains to be learned regarding the immune-specific properties and function of invertebrate glia in CNS health, glial biology studies in *D. melanogaster* have identified specific glial subtypes that are hypothesized to perform functions similar to vertebrate glia, ranging from phagocytosis to neurotrophic support, signifying the important role that these enigmatic cells have within the CNS [10,11]. This review will provide anatomical, cellular, and molecular support for the glial analogs found in the *D. melanogaster* CNS and address the promise this field holds for modeling cerebral innate immunity.

2. *D. melanogaster* as a model for vertebrate cerebral innate immunity

Innate immune signaling is highly conserved throughout evolution [12]. In *D. melanogaster,* innate immunity is largely carried out by hemocytes and the fat body, analogous to vertebrate macrophages and liver [4,5]. Pathogen recognition pathways are initiated through genome encoded PRRs, typified by the Toll receptor [3,13]. The high degree of conservation between Toll signaling in fly hemocytes and TLR signaling in vertebrate macrophages enables *Drosophila* to be used as a model system in mechanistic studies of TLR signaling (**Figure 1**).

In mammalian tissues, there are numerous populations of resident, tissue specific, phagocytic cells that are front-line responders to pathogens and injury. In the mammalian CNS, microglia play this role and stand poised to survey the local environment and to mediate innate immune responses. In *D. melanogaster*, glial cells are present within the mushroom body and several studies have characterized these cell populations [14,15]. Transciptome analysis between wild type and glial cell missing (Gcm) mutants identified 45 glial-specific genes with human conservation hovering around 80%[14]. This surprising finding hints at conservation of innate immune roles extending beyond peripheral hemocytes and into the CNS. Since the innate immune system is highly conserved in evolution and is the sole immune system of invertebrates, it allows for the dissection of these molecular pathways that uniquely drive innate immunity. Furthermore, the Gal4-UAS system provides a tractable *D. melanogaster* system, allowing spatial and temporal manipulation of gene expression [16]. Taken together, *D. melanogaster* represents an excellent *in vivo* model to understand basic glial innate immune function that can be applied to vertebrate systems.

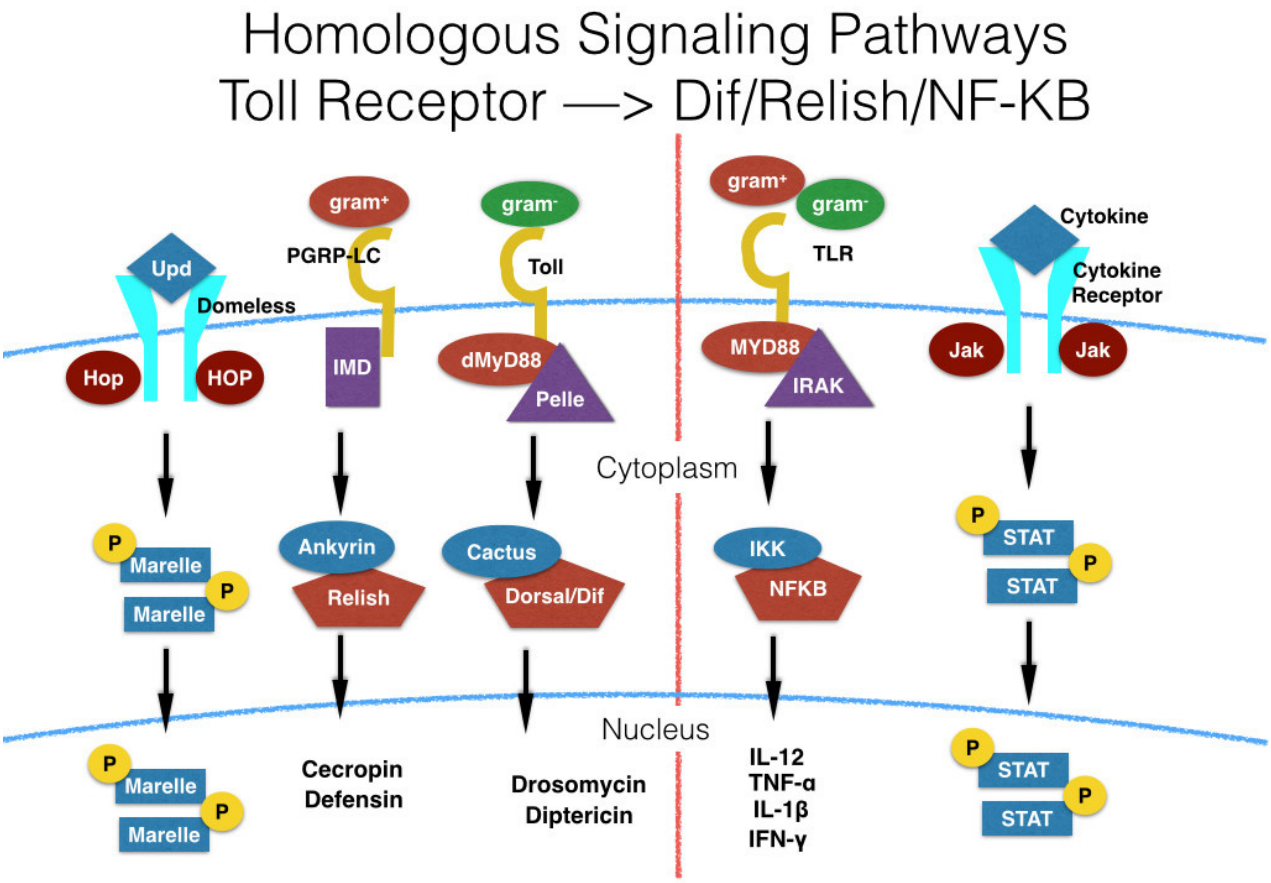

Figure 1. Homologous Signaling pathways Toll, IMD and Upd: The Innate immune signaling pathway between vertebrates and invertebrates is highly conserved. Additionally, the mechanisms by which these proteins promote transcription are very similar. Although the downstream pathways for each pathway are different, they main focus is on the initial immune response that is elicited by the pathogen.

In the mammalian brain, innate immune activation and neuroinflammatory pathways are thought to be major players in neurodegeneration [17]. Several recent studies in *D. melanogaster* have examined the relationship between glia and neurodegeneration. In one paradigm, activation of either of two innate immune pathways leads to neurodegeneration. Specifically, loss of defense receptor 1 (Dnr1), a negative regulator of the IMD innate immune response pathway, resulted in shortened lifespan and age-dependent neuropathology [7,9]. In Dnr1 deficient flies, whole brains exhibited pathologic vacuole formation throughout the neuropil, indicative of neurodgeneration [7]. Furthermore, Dnr1 deficient flies had reduced life span, motor impairment, and increased anti-microbial gene expression. Glial knockdown of Relish – a nuclear factor-kappaB (NF-κB) analog and a key regulatory gene in the IMD pathway – in Dnr1 deficient flies halted neurodegeneration, restored lifespan and improved motor function [9]. Moreover, similar experiments found that bacterial infection in the CNS led to increased anti-microbial gene expression and hastened onset of neurodegeneration. Similarly, knock down of Relish in glia prevented neurodegeneration. These experiments suggest that glial cells can mediate a detrimental form of innate immunity that endorses neuron loss.

3. *D. melanogaster* CNS architecture

While few would argue that the anatomy of the fly brain mimics that of the mammalian brain, there remain key parallels between both structures [18]. The *D. melanogaster* adult CNS is comprised of four, fused major ganglia: the subesophageal ganglion, the protocerebrum, the deutocerebrum, and the tritocerebrum [19]. The largest ganglion is the protocerebrum, which contains the majority of the known brain regions and acts as a hub for environmental inputs from antennae and omatidia, analogous to the vertebrate cerebrum. For the olfactory system, input from the antennae synapse onto the antenna lobe, which then relays parallel projections to the mushroom body (the vertebrate hippocampus analog), and the lateral horn. By comparison, the vertebrate olfactory bulb sends projections to the thalamus and the hippocampus [20,21]. Summarized in **Figure 2**, this olfactory circuitry parallelism demonstrates why the protocerebrum is an ideal brain region to model glial influence on cerebral processing of environmental stimuli, including learning and memory [22].

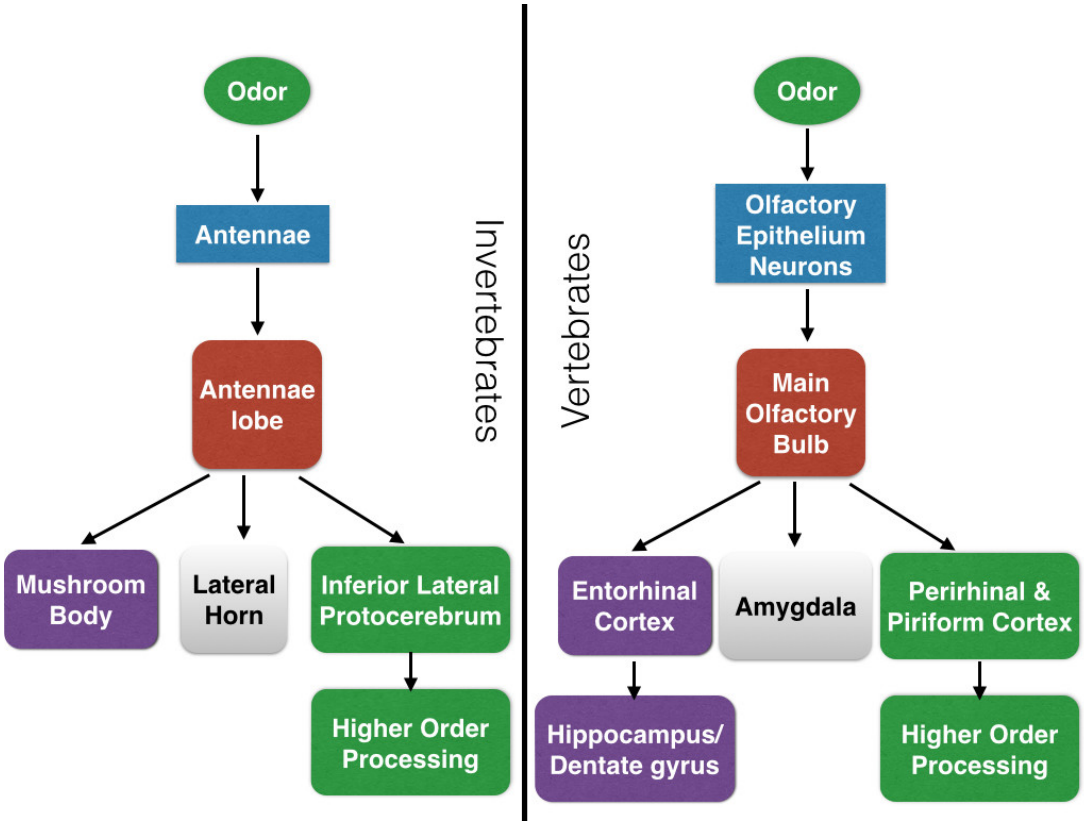

Figure 2. Paralleled olfactory circuitry: The olfactory circuitry in invertebrates and vertebrates are very similar. Although the regional names and structures are different, the functions for each of these regions mirror one another.

In the vertebrate CNS, the most abundant cell type is astroglia; however, in flies, neurons outnumber all other brain cells [23]. Using cell proportions as a basic metric for inter-species comparison, one may conclude different roles for glia. However, across species, glia consistently play critical roles in CNS architecture, neuronal maintenance, axon guidance, debris clearance, and brain barrier formation [10,24–27]. Therefore, invertebrate glial do, at least somewhat, mirror the greater complexity in the vertebrate CNS. Glial cell populations in the vertebrate brain consist of

microglia and macroglia (astroglia and oligodendrocytes) [23]. Although flies do not have oligodendrocytes, they do possess a single population that has microglial and astroglial functions. Despite the lower proportion of cells, glia in the adult *D. melanogaster* are widely distributed, consistent with performing specialized functions.

Glial architecture in the protocerebrum provides evidence that these cells are positioned to support neuronal function in this higher-order brain region that mediates behavioral changes. Within the mushroom body, glial cells are distributed in heterogeneous clusters that ensheathe the mushroom body somas, divide the mushroom body into compartments, and interlace the neuropil in an unorganized mesh-like network [26,28–30]. Although studies have not functionally classified any particular glial subtype in the mushroom body, this relative distribution parallels microglial heterogeneity found in the murine CNS. Early work that enumerated microglial distribution in the mouse CNS found a higher density of microglia in the hippocampus, olfactory telencephalon, basal ganglia, and substantia Niagara [31,32]. Within the microglia-dense mouse hippocampus, there are greater numbers of microglia within the CA1 and CA3 subregions [33,34]. These studies suggest that microglia have a role in learning and memory. However, associations between neuronal activity and microglial density remain inconclusive [31,32]. Since the fly glial populations around the mushroom body are largely uncharacterized, their roles in olfactory learning and memory are not well understood. Nevertheless, functional characterization of analogous structures within the *D. melanogaster* brain can provide valuable information related to vertebrate biology and function [18].

4. Glial cell populations

As previously mentioned, glial cell numbers within the *D. melanogaster* CNS differ substantially from vertebrates. In flies, 10-20% of the cells are glia, while glia make up at least 50% of the vertebrate CNS [23]. In the vertebrate CNS, glial cells are further subdivided into several classes, including microglia, astrocytes, oligodendrocytes, and pericytes, amongst others [10,23]. *D. melanogaster* glia show remarkable morphological and functional similarity to vertebrates; however, functional conservation of specific glial subtypes in flies has been more difficult to determine [35]. Although these differences suggest evolutionarily complex neural function, an important concept to consider is that both anatomical and genetic approaches have suggested at least some degree of interspecies functional overlap. This parallelism provides a unique opportunity to decipher specific glial subtypes in the fly CNS that may inform vertebrate microglia and macroglia function [23]. A comprehensive review on invertebrate glial subtypes can be found elsewhere [26]. For the purpose of this review, we will cover three main cells: pericyte-like cells, astrocyte-like cells, and microglial-like cells.

5. Pericyte-like cells: surface, perineural, and cortex glia

In the mammalian brain, pericytes are endothelial cells that comprise the blood-brain-barrier (BBB), a physical and metabolic barrier that regulates cerebral blood flow [36]. Although flies do not have a closed circulatory system, they do have pericyte analogs that encase the periphery the brain, thereby separating the CNS from the hemolymph, the fluid that acts as the primary distributer of oxygen, water, proteins, fats, and sugars [15,26,37]. The *Drosophila* pericyte analogs – sub-divided into surface, perinurial, and cortex glia – create a hemolymph-brain barrier, which is analogous to the

vertebrate BBB [15,27,38]. Evidence for the role of surface, perineurial, and cortex glia in *D. melanogaster* comes from hemolymph dye injection experiments. Specifically, Moody mutants have dye penetration into the CNS, whereas wild-type flies do not. In Moody mutant flies, Moody knock-in animals expressing the protein on surface, perineural, and cortex glia fully restore dye blockade into the CNS [27]. Although this review will not consider hemolymph-brain-barrier glia in detail, these dye penetration experiments highlight the key homologous properties of surface, perineural, and cortex glia with vertebrate pericytes.

6. Astroglia

While surface, perineural, and cortex glia are analogous to pericytes, *D. melanogaster* astrocytes are homologous to the same cells in vertebrates. In the mammalian CNS, astrocytes are the most abundant glial cell type, and perform a wide array of responsibilities ranging from nutrient transport, metabolism, and maintenance to development, axon guidance, and synaptic function [24,25,39–43]. Astrocytes are easily identifiable in the fly brain and perform similar roles as their vertebrate counterparts. While it has been shown that astrocytes provide neurotrophic support in the fly eye [26], we will focus on astrocytes within the protocerebrum. In the protocerebrum, astrocytes provide neurotrophic support for dopaminergic neurons during development. As evidence of this, Palgi and colleagues ablated *D. melanogaster* mesencephalic astrocyte-derived neurotrophic factor (MANF), in astrocytes during development and observed degenerating dopaminergic axons. When MANF was reintroduced as a knock-in, the phenotype was reversed [44]. These experiments illustrate how astrocytes promote neuronal survival and metabolic support.

7. Microglial homologs: ensheathing glia and reticular glia

Despite being outnumbered by other glia subtypes, microglia receive the majority of the attention in studies of neuroinflammation and neurodegeneration in mammalian systems. Microglia are the CNS resident mononuclear phagocyte in vertebrates, and play a vital role in pathogen clearance, neuronal phagocytosis, and leukocyte recruitment into the brain [45,46]. The last major glial cell type in the *Drosophila* CNS are ensheathing glia. Recent studies demonstrate that these cells are capable of microglial-like functions found in vertebrates. During axonal injury to olfactory neurons, ensheathing glia express the Draper (Dpr) receptor and engage in phagocytic clearance of neuronal debris, while astrocytes and other glia lack Dpr expression and do not have a phagocytic function. To confirm the cell type and temporal specificity of this phenotype, flies with Dpr RNAi knockdown in astrocytes maintained the ability to remove neuronal debris, whereas flies with Dpr RNAi knockdown in ensheathing glia were unable to remove neuronal debris [10,47]. Therefore, these experiments illustrate that ensheathing glia are the main glial cell type that clears neuronal debris. Although more work is needed to further characterize ensheathing glia in *D. melanogaster*, we can look to other vertebrate studies and draw parallels between vertebrates and invertebrates to understand how ensheathing glia may have immune-like properties *in vivo*.

Vertebrate microglia morphology is plastic – alternating between a ramified state with extended mobile processes and an activated, amoeboid shape [48,49]. Interestingly, both Awasaki and Hartenstein have described two potential subtypes of adult fly ensheathing glia based on morphology: cells with flat bodies with either small extensions or highly ramified processes [14,15]. Doherty and

colleagues observed that glia with highly ramified extensions do not express phagocytic genes such as Dpr, and therefore described them to be more astrocyte-like [47]. However, recent evidence illustrates that microglia can play a neurotropic and neuroprotective role, similar to classical astrocytes. For example, live imaging experiments in the optic tectum of larval zebra fish illustrate that resting microglia can perform astrocyte-like roles by extending their processes to reduce neuronal activity [50]. Importantly, astrocyte-like glia are capable of expressing Dpr to engage axonal phagocytosis during metamorphosis [25,42], and therefore should not be confused with canonical astrocytes. To minimize this confusion, Hartenstein asserts that this highly ramified, astrocyte-like glial morphology should be renamed as reticular glia [14]. Taken together, although ensheathing glia and reticular glia have defined roles within the *D. melanogaster* CNS, their roles seem to overlap with vertebrate microglial and thus, both glial subtypes should be considered as microglial homologs.

8. Conservation of ensheathing vs. reticular glia activation states

Vertebrate microglia morphologies are generally categorized into three phenotypes: rounded, extended processes, and "bristled" (or highly ramified). The rounded morphology is not commonly found in the healthy adult CNS, whereas the extended processes and "bristled" phenotypes abound. Microglia with few extended processes are found along neural tracts and highly ramified are commonly found interspersed within the neuropil, surrounding cell bodies and synapses [31]. It is thought that these structural differences can be attributed to microglial polarization states [51]. Using common macrophage polarization terminology, the rounded morphology is generally classified as M1 microglia. On the other hand, ramified cells are largely classified as M2 microglia [48,49,52,53]. A more extensive explanation of the M1/M2 dichotomy can be found elsewhere[53]. However, more recent studies assert that M1/M2 represent only the extremes of a spectrum of polarization states influenced by the local environment, neurons, and other microglia. Additionally, different microglial phenotypes can be simultaneously present in any one brain region [48,54,55], and these dynamic phenotypes offers explanation for the well-recognized heterogeneity amongst microglia [32,48].

Because microglial phenotype polarization is a key functional aspect of these innate immune cells in the vertebrate CNS in both health and disease, it is important to consider parallels in the fly. To this end, several studies have illustrated that both the three cell morphologies and two polarization states exist in flies. First, reticular glia are capable of engulfing mushroom body axonal processes during metamorphosis though a Dpr-mediated process [42]. Although this contradicts a previous study that describes ensheathing glia as the primary cell type to engage in Dpr-mediated phagocytosis [47], it is possible that both ensheathing and reticular glia perform this role under different conditions in the adult fly CNS. Importantly, Dpr expressing ensheathing glia have an aggressive phagocytic state characterized by flattened cell bodies and small extensions, structurally mimicking M1 microglia in mice. On the other hand, the generally anti-inflammatory and phagocytic M2 reticular glia have longer, more ramified extensions, thus morphologically mirroring microglia found in the healthy vertebrate CNS [15,51,53]. **Table 1** summarizes the comparison between ensheathing glia and reticular glia. It is likely that, just like for vertebrate microglia, the polarization phenotype not only impacts phagocytosis, but also expression of inflammatory mediators.

Table 1. Comparison between Reticular/Astrocyte-like Glia and Ensheathing Glia: The function and morphology of these glia are summarized given what is known about these cell types. Additionally, hypothesized comparisons between vertebrate macrophage nomenclature of M1 and M2 are used to describe the similarities found in *Drosophila* glia.

	Astrocyte-like/Reticular Glia	Ensheathing Glia
Phenotype	Larger Cell Body	Flattened and smaller cell body
Process Length	Long	Short
Polarity	M2	M1
Functions	Phagocytosis of unneeded axons	Phagocytose damaged axons
Draper Expression	During Metamorphosis, pruning Mushroom body axons	During axonal damage

9. Neuroinflammatory pathway conservation in *D. melanogaster*: cytokines

Cytokines are secreted factors that instruct surrounding cells respond, and play integral roles in modulating immune responses in both vertebrates and invertebrates. Classical vertebrate innate immune cytokines, such as tumor necrosis factor-alpha (TNF-α) and interferons, have not been found in the fly [56]. However, homologous innate immune pathways do still exist and consist of the immune modulators: nitric oxide (NO), ATP, and transforming growth factor-beta (TGF-β)/Unpaired (Upd) [56–59]. These immune modulators are capable of recruiting and polarizing hemocytes, and also ensheathing and reticular glia.

NO, a small molecule with a diverse set of roles in physiology, neurobiology and immunology, plays beneficial roles as an anti-microbial and immunoregulatory cytokine. Additional functions include vasodilation and cytotoxicity. Studies in flies have shown that nitric oxide synthase (NOS) mutants infected with gram-negative bacteria at both the larval and adult stages are more susceptible to infection compared to wild-type flies. Those authors also demonstrated that feeding NOS inhibitors to wild-type flies resulted in greater susceptibility to infection [56]. While NO is a potent immunomodulator, it also causes collateral tissue damage, often associated with dysregulated M1 responses in macrophages [53]. NO cytotoxicity was demonstrated in flies by exposing NOS mutant and wild-type flies to an airborne fungal compound. Under those conditions, NOS mutants lived longer and were protected from neurodegeneration. Similarly, feeding NOS inhibitors to wild-type flies protected them from neurodegeneration and extended longevity [60]. These results indicate that NO is a conserved immunoregulatory molecule capable of M1 polarization.

Thus far, peripheral hemocytes have been the major immune cell type studied in flies. Similar to vertebrate macrophages, *D. melanogaster* hemocytes respond to immunological challenge. In response to injury, hemocytes acutely express ADGF-A, a homolog of adenosine deaminase 2. ATP is further converted to adenosine, amplifying the inflammatory signal [57]. The origin of

extracellular adenosine is owed either to ATP released by neurons or degenerating axons. Studies in vertebrates show that microglia respond to extracellular ATP via purinergic receptors and initiate an innate immune response [61]. Although more research is needed to show homologous CNS innate immune signaling in flies, ensheathing glia and reticular glia are well-positioned to respond to ATP and participate in the inflammatory cascade. Importantly, these experiments further illustrate how immune modulators activate hemocytes from surveying their environment to responding to pathogens [62].

Cytokines are known to play a critical role in synapse maturation and circuitry formation during development and disease [63]. For example, neurodevelopmental experiments in mice have found that chronic TNF-α exposure to neurons rapidly matures synapses [64]. There is no TNF-α analog or homolog in *D. melanogaster*. However, the TGF-β homolog Upd has been shown to play a dual role in infection and in olfactory learning and memory [65,66]. In the mushroom body, Upd modulates long-term memory by signaling though Dome and short-term memory by modulating the Hop/Stat92E pathway [58]. Studies from invertebrates show that TGF-β signals though the Smad2/3 pathway [66]. Upd is evolutionarily conserved, and is also found in *Caenorhabditis elegans.* In *C. elegans,* Dbl-1, the Upd homolog, is found to be necessary for aversive olfactory learning and memory [5,67,68]. Since the Dbl-1 and Upd pathways are highly conserved across species, this homology in worms and flies corroborates classical Pavlovian conditioning experiments for learning and memory in mammals, supporting that expression of Upd at specific regions along the olfactory circuit strengthen local mushroom body synapses [65,66].

Moving from invertebrate TGF-β homologs to the vertebrate gene, studies from our group have demonstrated that blocking TGF-β signaling in murine CNS-infiltrating peripheral macrophages alleviates learning and memory deficits found in mice with Alzheimer's disease-like pathology [69]. Furthermore, CNS pathogen injection studies show that microglial TGF-β secretion promotes neuronal survival [70]. Additionally, in visual circuitry formation, TGF-β is necessary for synaptic pruning and development [71]. Drawing upon the results in vertebrates, ensheathing and reticular glial-dependent secretion of TGF-β may be necessary for learning and memory. Therefore, the study from Town and coworkers illustrates a potential role for ensheathing or reticular glial Upd signaling within the mushroom body, affecting learning and memory.

Since Pavlovian olfactory-dependent learning experiments are associated with mushroom body function in flies, one study proposed that certain types of learning and memory (e.g., short-term learning, long-term memory, and anesthesia resistant learning) promote characteristic proteomic shifts. From a proteomic screen, one study found proteomic shifts leading to two immune-related genes that were differentially regulated: hemolectin and immune-induced peptide 4 (also known as Dim4) [72]. In one study, Dim4 knock-down forced hemocytes into a more amoeboid shape, suggesting M1 polarization [73]. Similarly, hemolectin is expressed by hemocytes and modifies the dendritic tree during development. Because M2 is the macrophage polarization phenotype that prunes synapses, these results are likely similar to the Dim4 knock-down study [74]. Additionally, hemolectin is a cytokine that has been found in the hemolymph during bacterial infection [4,62,75]. This proteomics study further illustrates how Dim4 and hemolectin could influence ensheathing glia and reticular glia polarity in response to CNS immune challenge. **Figure 3** proposes a putative role for cytokines in the *D. melanogaster* CNS: how Dim4 could suppress M1/ensheathing glial polarization and how hemolectin promotes M2/reticular glial polarization.

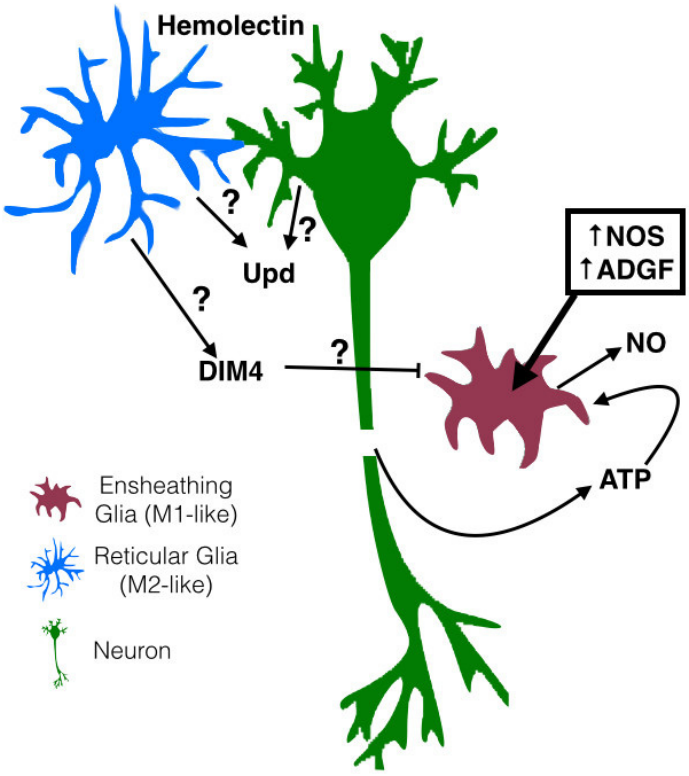

Figure 3. *D. melanogaster* cytokines could polarize glia to perform different functions: Drawing up parallels from hemocyte function, since reticular/astrocytic glia are similar to M2 microglia/macrophages, they could modify the dendritic tree through the expression of Hemolectin or Upd. Alternatively, reticular/astrocytic glia could express DIM4 and could suppress the cytotoxic effect of activating ensheathing glia. On the other hand, ensheathing glia could express NOS and ADGF in response to neuronal damage or pathogens, initiating neuron death.

10. Beyond innate immunity: adaptive immunity

Adaptive immunity uniquely tailors the immune response to a pathogen through genetically-encoded immunological memory. It has been suggested that at least some form of adaptive immunity has been in existence throughout evolution, originating from prokaryotes or early eukaryotes [2,76–79]. Primitive forms of adaptive immunity include clustered, regularly interspaced short palindromic repeats (CRISPR) associated proteins (i.e., CRISPR-Cas; found in prokaryotes) and RNA interference (present in eukaryotes). Both of these enable pathogen recognition through integration of short nucleic acid sequences into the genome, thereby providing "immunological memory" [78,79]. These intracellular defense systems primarily fight against viruses, while evolutionarily recent adaptive immune mechanisms in vertebrates combat a more diverse array of pathogens[80]. Jawed vertebrates (beginning with teleost fish) were the first to develop analogs to mammalian T and B cells, suggesting that these types of adaptive immune responses are evolutionarily restricted, and not present in invertebrates [80–83].

While most agree that *Drosophila melanogaster* does not possess a mammalian-like adaptive immune system, studies have nonetheless found evidence of fly immunological memory. For example, priming flies with a sub-lethal dose of *Streptococcus pneumoniae* improved survival in response to a second, lethal injection of the bacterium [84]. This elegant experiment demonstrates a type of immune memory [85–87]. Subsequent experiments in the mosquito showed that the AgDscam gene contains a hypervariable region. Furthermore, activation of the Toll and IMD pathways activated splicing factors that modified the AgDscam transcript, increasing receptor avidity towards a pathogen–potentially enabling a form of immune memory [88]. *In vitro* experiments using the *Drosophila* hemocyte S2 cell line have shown Dscam-mediated bacterial surface recognition, resulting in pathogen elimination via phagocytosis [89,90]. Therefore, it seems that while *D. melanogaster* has at least some form of adaptive immunity via a hypervariable gene region, the fly eliminates pathogens via phagocytic, innate immune pathways.

11. *D. melanogaster* glia in human disease models

The presence of functional microglia-like cells supporting neuron health and inflammatory processes in the fly CNS suggest that flies could be used to model neurodegenerative disorders. Because glia evolved alongside neurons in both flies and vertebrates, it is hypothesized that these two cell types have conserved mechanisms and analogous glial responses to neurodegeneration [91–93]. By capitalizing on CNS specific immune activation in *D. melanogaster*, one study modeled the neurodegenerative disease, Ataxia-telangiectasia [7]. Ataxia-telangiectasia (A-T) is a multi-system disease, characterized by radiation sensitivity and predisposition to cancer, caused by a mutation in the A-T mutated (*ATM*) kinase, which ensures genomic integrity in response to DNA damage [94,95]. In the human CNS, A-T is characterized by significant neuronal loss [96]. In flies, the use of temperature-sensitive ATM mutant flies has revealed the presence of vacuoles and wide-spread neurodegeneration in the CNS. Additionally, these flies exhibited reduced mobility and an increase in Relish-dependent neurodegenerative immune responses [7]. Furthermore, Relish knock-down experiments in glia reduced CNS neurodegeneration, suggesting a neurotoxic innate immune role. These relish-depeleted flies also showed increased mobility and restored longevity [8]. These studies show how glia have a central role in *Drosophila* CNS innate immune responses, providing evidence that glia could drive this form of immune-dependent neurodegeneration, and illustrating the relationship between immune cell activation and neurodegeneration.

From these A-T experiments, we can infer that glial immune activation can cause learning and motor impairment, an important step towards demonstrating the glia-dependent innate immune activation seen in neurodegenerative pathologies such as Alzheimer's disease [97,98], which is an age-related neurodegenerative disorder that is characterized by memory loss, the cellular deposition of neurotoxic peptides, hyper phosphorylated neurofillaments, gliosis, and neurodegeneration [99,100]. According to the amyloid cascade hypothesis, amyloidogenic processing of amyloid precursor protein releases amyloid-β peptide that is thought to drive Alzheimer's disease pathogenesis [100]. *D. melanogaster* Alzheimer's disease-like models exhibit axonal transport deficits, neurodegeneration, Aβ aggregate formation, and behavioral and motor impairment [97,101–104]. These established Alzheimer's disease-like flies use the fly eye to model neurodegeneration and demonstrate neuronal pathology; however, the fly eye does not correlate with key vertebrate brain regions. Therefore, future studies may go on to utilize the protocerebrum and

mushroom body to examine neuron-glial interactions. Such studies should enable further clarification of the relationship between neurodegeneration and memory loss driven by ensheathing and reticular glia. The studies from A-T fly model stress the role of glial cells in driving neurodegeneration. Investigating the cell type, phenotype, and activation state(s) that drive these pathological outcomes could suggest a novel role for ensheathing/reticular glia in fly neurodegeneration. Furthermore, these experiments would have the potential to establish an innate immune driven response.

12. Conclusion

Microglia are brain-resident myeloid cells that have macrophage-like qualities [105]. Studies have functionally and genetically demonstrated that ensheathing glia and reticular glia can parallel microglia-like behavior. Furthermore, observations have structurally demonstrated that ensheathing glia more closely resemble the phagocytic cells in the *D. melanogaster* CNS, and that reticular glia may also have a hand in phagocytosis. From an anatomical perspective, there seems to be heterogenic distribution of glia surrounding different regions of the mushroom body. These differences may be attributed to the dynamic nature of microglia. Both ensheathing and reticular glia respond to extracellular cytokines and inflammatory signals released into the extracellular space. Current models of neurodegeneration demonstrate the increasingly important role glia may have during the onset of neurodegeneration. Moving forward, we should continue to utilize hemocytes to understand the role of ensheathing and reticular glia within the *D. melanogaster* CNS. Although more research is needed to fully characterize the diverse immune roles that these enigmatic glia play, homologous and analogous comparisons between invertebrate and vertebrate models illustrate significant structural and functional overlap.

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Conflict of interest

All authors declare no conflicts of interest in this review.

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