NEURAL REGENERATION RESEARCH
www.nrronline.org

# Glial regenerative response in the imaginal discs of *Drosophila melanogaster*

**Sergio B. Velarde, Antonio Baonza***

Glial cells play a key role during nervous system development and actively participate in all the cellular processes involved in maintaining its structural robustness and functional plasticity. In response to neuronal damage, glial cells proliferate, migrate to the injured region and change their morphology, function, and behavior (Gallo and Deneen, 2014; Kato et al., 2018). This glial regenerative response is associated with the repairing function of these cells and is found across species, suggesting that it may reflect a common underlying genetic mechanism (Kato et al., 2018). In mammals, while the central nervous system has very limited capacity to regenerate after traumatic injury or disease, the peripheral nervous system (PNS) exhibits a far greater capacity for regeneration and damaged peripheral nerves can be totally restored (Brosius Lutz and Barres, 2014; Gallo and Deneen, 2014). The PNS largely owes its regenerative potential to the ability of the main glial cells present in the PNS, myelin, and non-myelin (Remak) Schwann cells, to convert to cells devoted to repairing after injury (Nocera and Jacob, 2020). During the regeneration of peripheral nerves in vertebrates, Schwann cells function as a central hub, collecting signals from neurons and other cell types and undergoing a complex process of reprogramming which converts them into a specialized cell for repair. Even though many aspects of regeneration in peripheral nerves have been studied, there is still a lack of understanding regarding the genetic network that controls the flexible differentiation state of PNS neurons and Schwann. The identification of those signals is essential for getting new insight to develop innovative regenerative therapies. In this scenario, the use of relatively simple model organisms, amenable to genetic, cellular, and molecular analysis is fundamental to study the behavior of glial cells in response to damage in their natural context.

The fruit-fly *Drosophila* offers a powerful system to investigate *in vivo* fundamental biology, and has proven to be an extremely powerful model organism to discover evolutionary conserved gene function and networks. Consequently, this organism has been used to characterize elementary features of cell biology, including basic mechanisms that regulate damage response in the central nervous system (Kato et al., 2018). Additionally, the regenerative response after damaging the PNS has also been analyzed in *Drosophila*. Most of these studies have focused on the resulting degeneration and regeneration of axons and dendrites observed after damage (Fang and Bonini, 2012; Brace and DiAntonio, 2017). However, the response of glial cells upon PNS damage in terms of proliferation, migration, and functional changes, as well as the molecular and genetic mechanisms that might regulate this response have remained largely unexplored up to date. Thereupon, we approached this issue by exploring the behavior of glial cells after inducing neural apoptosis in different regions of the PNS. With this aim, we first used the eye imaginal discs as a biological model.

The adult eye of *Drosophila* develops from the imaginal eye discs (**Figure 1**). The cells that form the eye discs proliferate throughout most of larval life as a simple epithelial sheet and only begin to differentiate in the third instar. During this stage, an indentation known as the morphogenetic furrow (MF) sweeps across the eye field from posterior to anterior triggering the onset of neural differentiation. Thus, posterior to the MF cells begin to differentiate as neurons (photoreceptors) and accessory cells that will become the individual units of the compound eye, known as ommatidia. In addition to these cell types, the eye discs also contain glial cells (**Figure 1**). Unlike the photoreceptors and the other cells that will form the retina, the precursors of all subretinal glial cells are not generated from the eye disc cells, since they are specified during embryogenesis in the nerve that connects the primordium of the eye disc to the brain (Bolwig nerve). This nerve will become the optic stalk and will maintain the developing imaginal disc connected to the brain (Silies et al., 2010). The precursor glial cells proliferate in the optic stalk during larval development forming more glial cells. When neurogenesis is initiated behind the MF, these glial cells migrate from the optic stalk and enter the eye disc (Silies et al., 2010; Yildirim et al., 2018). This migratory behavior makes it possible to predict the number of glial cells that the eye discs must contain at any moment during the development. Therefore, changes in the proliferation rate or cell migration in response to damage are easily detectable and quantifiable.

There are three main types of glial cells in the eye disc: subperineurial glia, perineurial glia, and wrapping glia (WG). Subperineurial cells, the so-called carpet cells, are two large cells that cover the entire differentiated part of the eye disc epithelium. Below these cells, we find the perineurial glia, which maintains the ability to divide during eye disc development. These cells will eventually form the wrapping glia cells. These later glial cells enwrap all axons produced by the photoreceptors (Yildirim et al., 2018). Interestingly, wrapping glial cells perform functions, which resemble the non-myelinating Schwann cells forming Remak fibbers in the mammalian PNS (Yildirim et al., 2018). Morphologically, it has been described as two more glial cell types. The function of these cells is unknown (Silies et al., 2010; Yildirim et al., 2018).

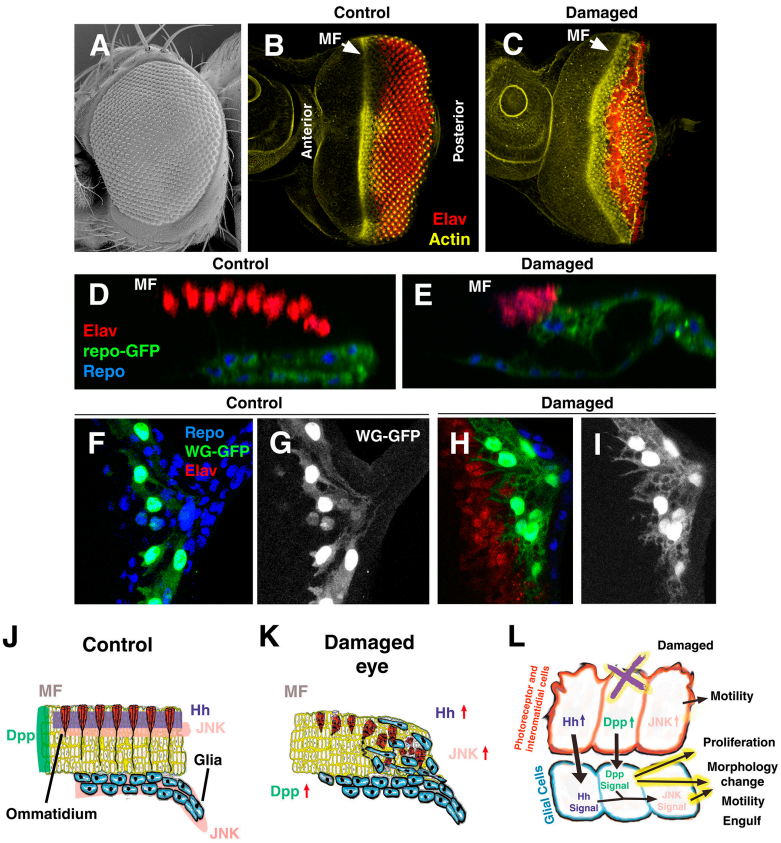


**Figure 1** | **Damage response of glial cells in the eye discs of Drosophila melanogaster.**
(A) Scanning electron microscope image of an adult eye of Drosophila. (B, C) Third instar eye discs stained with anti-Elav (red) and Phalloidin to visualize F-actin (yellow). Control undamaged disc (B) and eye discs after inducing neural apoptosis (C). (D, E) X–Z projections show cross-sections perpendicular to the furrow of a control (D) and a damaged (E) eye disc. The expression of *UAS-GFP* (green) under the control of *repo–Gal4* is restricted to glial cells (anti-repo in blue). Photoreceptors are stained with anti-Elav (red). (D) In control discs, subretinal glial cells are located in the basal layer of the disc. (E) In damaged discs, we observed glial cells in the basal layers, as well as in the middle and apical layers. We even found that some glial cells contact photoreceptors (anti-Elav in red). (F–I) After inducing neural damage wrapping glial cells change their morphology. (F, G) Control wrapping glial cells. (H, I) In damaged eye discs, glial cells produce large cellular projections that can generate complex structures. (J–L) Model representing the signaling network involved in the activation of glial response after cell death induction in eye discs. (J) In control eye discs Dpp is expressed (green) ahead of the MF, whereas Hh is expressed (purple) in the interommatidial and photoreceptors cells (in red), behind the MF. JNK is active (pink) in the glial cells (in blue) and the retina. (K, L) When neural apoptosis is induced, the expression of Hh and Dpp increase in the damaged region, and in turn these signaling pathways are activated in glial cells. The activity of these pathways induces glial cell proliferation and migration and activates the JNK pathway that facilitates glial migration and engulfment. Dpp: Decapentaplegic; Elav: embryonic lethal abnormal vision; GFP: green fluorescent protein; Hh: Hedgehog; JNK: c-Jun N-terminal kinase; MF: morphogenetic furrow; Repo: reversed polarity; WG: wrapping glia. Unpublished data.

The development of the glial cells in the eye discs has been used as a model system to study the mechanisms that regulate the coordinated development of neurons and glial cells (Silies et al., 2010; Bauke et al., 2015). However, the response of glial cells to neuronal damage in the retina and the signaling pathways that might be mediating this function has remained unexplored.

Alternative to the eye disc, the behavior of PNS glial cells in response to neural damage could be studied in the developing *Drosophila* leg imaginal disc. These epithelial structures contain different sense organs arranged in a precise and reproducible pattern. Each sensory organ is formed by neuron and glial cells that, like eye discs, are specified during larval development in the nerve that connects the leg disc anlage and the ventral nerve cord. As in eye discs, glial cells have to migrate through the nerve into the forming leg (Sasse and Klambt, 2016).

We took advantage of the rich palette of genetic tools available in *Drosophila* to induce targeted cell death of photoreceptors and retinal cells of the eye disc, as well as of epithelial cells of the leg discs, including neurons of the sensory organs. To this end, we used the *Gal4/UAS/Gal80ts* system to transiently overexpress the pro-apoptotic gene *reaper* (*rpr*) under the control of *Gal4* lines that were specifically expressed in the eye or leg discs (Velarde et al., 2021). Since glial cells do not originate in the discs, these drivers are not active in glial cells, and therefore any change in glial behavior is due to the signals emitted by dying neural tissue. In this sense, the eye disc is an excellent model to define the signals produced by damaged neuronal tissue that promote the response of the glial cells.

We found that cell death induction, in both the retinal region of eye discs and in leg discs, results in an increase in the number of glial cells. In eye discs, this effect is a consequence of increased glia proliferation and over migration. However, in leg discs we have not detected an excess of mitotic glial cells, suggesting that glial cell proliferation may not be the cause. Nevertheless, in our analysis, we only have examined glial cell proliferation in the leg discs epithelium, but not all along the nerve until the ventral nerve cord. Therefore, we cannot rule out that glial proliferation might be increased in the nerve near the central nervous system/PNS transition zone, where leg glial cells are specified. Glial cells also respond to neural damage by undergoing morphological changes, which will ultimately grant them phagocytic abilities (**Figure 1**; Velarde et al., 2021).

We have identified different signaling pathways involved in triggering the glial response. After cell death induction in the eye discs, the BMP2/4 type morphogen Decapentaplegic (Dpp) and Hedgehog (Hh) are transcriptionally activated in photoreceptor neurons and other cells that form the retina. The high levels of these proteins in the retina region, in turn, non-autonomously activate the corresponding *dpp* and *hh* signaling pathways in glial cells. The function of these signaling pathways is required to promote glial proliferation and the over-migration of glial cells to the eye discs (**Figure 1**). We found a similar effect in the leg disc, where both *dpp* and *hh* are transcriptionally upregulated in the damaged leg epithelium. However, in contrast to eye discs, only *dpp* signaling is activated in glial cells of leg discs. Accordingly, we found that in the leg *dpp* signaling is sufficient to trigger glial response, whereas in the eye discs both Dpp and Hh are involved, and they seem to have a redundant function (Velarde et al., 2021). The different requirements of these signaling pathways could be due to the particular functional relationship between *hh* and *dpp* signaling in the eye disc. Unlike other structures, in the eye, both pathways have a redundant function controlling several development processes. Therefore, it is possible that *dpp* could be the main signal mediating glial response in the developing PNS since it is necessary in both tissues. It would be interesting to analyze whether *hh* is mediating glial response in other regions of the PNS.

Our results also indicate that the depletion of *dpp* and *hh* signaling was not sufficient to totally suppress glial response, suggesting that additional signaling pathways must be involved in this process. It would be interesting to test whether other pathways required for controlling glial behavior, such as epidermal growth factor receptor and fibroblast growth factor, are also involved in regulating glial regenerative response and the possible genetic network that they might form.

In line with previous observations in other regions of the PNS (Macdonald et al., 2013), we found that in response to cell death induction in the retina or in leg disc, c-Jun N-terminal kinase (JNK) signaling is activated in the damaged region, as well as in glial cells. We assayed the activity of this signaling using reporters that contain binding sites for Activator Protein 1 (a heterodimeric Jun/Fos transcription factor targeted by JNK). Our data suggest that in response to damage, JNK signals can activate a transcriptional program that promotes a specific aspect of the glial response. We found that JNK signaling down-regulation in either the damaged region or in glial cells reduces the accumulation of glial cells observed upon neural damage, without affecting glial proliferation. This observation suggests that in response to damage, JNK signaling is necessary to promote glial motility but not proliferation. This effect seems to be only permissive, as the ectopic activation of JNK signaling was not sufficient for increasing glia migration.

Our observations indicate that upon apoptosis induction, a diffusible signal is generated that not only activates JNK signaling in the damaged region, but also in glial cells. Surprisingly, we found that the principal ligand of this pathway in Drosophila, the tumor necrosis factor termed Eiger, is not the signal emitted from the damaged region, as the reduction of its activity does not affect glial response. We have not established the mechanism by which the transduction pathway of this signal is activated in the damaged tissue or in glial cells. Interestingly, the ectopic expression of *dpp* in the retina region was sufficient to activate JNK signaling in glial cells, although the details of the mechanism remain unclear. This regulatory mechanism is necessary to facilitate the migration of glial cells upon *dpp* over-expression, as the over-migration phenotype caused by the ectopic activation of *dpp* signaling in glial cells is totally suppressed when JNK signaling is blocked.

Neural apoptosis also causes changes in the morphology and function of glial cells. We found that most wrapping and some perineurial glial cells expand their membrane surface, generating glial processes (**Figure 1**). Moreover, they develop phagocytic abilities and can engulf and phagocytize apoptotic debris. These behavioral and morphological changes resemble the regenerative response of non-myelinating Schwann cells in vertebrate PNS (Nocera and Jacob, 2020). The ability of Schwann glia to convert into cells specialized to support regeneration is a fundamental step required to drive the major processes necessary for tissue repair. One of the earliest events upon damage induction in peripheral nerves of mammals is the activation of the transcription factor c-Jun (Nocera and Jacob, 2020). This signal is involved in regulating the major aspects of injury response, including Schwann cell dedifferentiation. Alike in vertebrates PNS, we observed that neural damage induces the activation of JNK signaling in wrapping glia. The down-regulation of JNK signaling in WG partially suppresses some of the morphological changes provoked by damage. Hence, wrapping glial cells depleted of the JNK signal do not develop the long membrane projections observed in transformed WG. However, these transformed WG mutant cells still exhibit morphological changes. In addition, the ectopic activation of JNK signaling in WG was not sufficient to transform these cells. Altogether, these data suggest that the coordinated action of JNK signaling and other signaling pathways orchestrate the transformation of wrapping glial after neural damage.

To conclude, we have shown that after inducing neural apoptosis during the development of the eye and leg discs, glial cells respond by increasing their number and undergoing morphological changes that confer their phagocytic activity. We have identified a signaling network, constituted by *dpp*, *hh*, and *JNK* pathways, involved in the regulation of this response (**Figure 1**). Our analysis has been carried out during the development of the discs, hence the response may be different or more pronounced than in the adult fly, it would be important to explore whether in adult tissues glial cells respond to damage in a similar manner.

We find many similarities between this response and the response of glial cells in the PNS of vertebrates. Therefore, the imaginal discs might serve as a good experimental model and a platform to establish the basis about the signaling pathway network involved in regulating glial cells response upon damage induction in the PNS of vertebrates.

**Sergio B. Velarde, Antonio Baonza***
Centro de Biología Molecular "Severo Ochoa", CSIC/UAM, Madrid, Spain
***Correspondence to:** Antonio Baonza, PhD, abaonza@cbm.csic.es.
https://orcid.org/0000-0003-3252-0038 (Antonio Baonza)
**Date of submission:** October 6, 2021
**Date of decision:** November 12, 2021
**Date of acceptance:** December 10, 2021
**Date of web publication:** April 25, 2022

**https://doi.org/10.4103/1673-5374.339479**
**How to cite this article:** *Velarde SB, Baonza A (2023) Glial regenerative response in the imaginal discs of Drosophila melanogaster. Neural Regen Res 18(1):109-110.*


## References

Bauke AC, Sasse S, Matzat T, Klambt C (2015) A transcriptional network controlling glial development in the Drosophila visual system. Development 142:2184-2193.
Brace EJ, DiAntonio A (2017) Models of axon regeneration in Drosophila. Exp Neurol 287:310-317.
Brosius Lutz A, Barres BA (2014) Contrasting the glial response to axon injury in the central and peripheral nervous systems. Dev Cell 28:7-17.
Fang Y, Bonini NM (2012) Axon degeneration and regeneration: insights from Drosophila models of nerve injury. Annu Rev Cell Dev Biol 28:575-597.
Gallo V, Deneen B (2014) Glial development: the crossroads of regeneration and repair in the CNS. Neuron 83:283-308.
Kato K, Losada-Perez M, Hidalgo A (2018) Gene network underlying the glial regenerative response to central nervous system injury. Dev Dyn 247:85-93.
Macdonald JM, Doherty J, Hackett R, Freeman MR (2013) The c-Jun kinase signaling cascade promotes glial engulfment activity through activation of draper and phagocytic function. Cell Death Differ 20:1140-1148.
Nocera G, Jacob C (2020) Mechanisms of Schwann cell plasticity involved in peripheral nerve repair after injury. Cell Mol Life Sci 77:3977-3989.
Sasse S, Klambt C (2016) Repulsive epithelial cues direct glial migration along the nerve. Dev Cell 39:696-707.
Silies M, Yuva-Aydemir Y, Franzdottir SR, Klambt C (2010) The eye imaginal disc as a model to study the coordination of neuronal and glial development. Fly 4:71-79.
Velarde SB, Quevedo A, Estella C, and Baonza, A (2021) Dpp and Hedgehog promote the glial response to neuronal apoptosis in the developing Drosophila visual system. PLoS Biol 19:e3001367.
Yildirim K, Petri J, Kottmeier R, Klambt C (2018) Drosophila glia: few cell types and many conserved functions. Glia 67:5-26.

*C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y*