Molecular mechanisms underlying synaptic material clearance by microglia after CNS injury

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Table of contents

Chapter 1

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Chapter 2

Introduction

This thesis is the culmination of my studies into the functions of microglia during CNS Wallerian degeneration that made up the bulk of my graduate work. I have focused on the role of neuronal activity as a driving force in microglial activation as well as what phagocytic receptors are necessary for microglial debris clearance.

In this chapter I will introduce the basic concepts of phagocytosis, highlighting the critical role performed by microglia in development and conditions of CNS pathology. Discussion will include the role of cell death and clearance of cells and their processes in development, the continued engulfment of neuronal progenitors in adult neurogenesis, and macrophage/ microglial function following acute injury at the site of injury and at sites of Wallerian degeneration downstream of CNS injury. Further, I will discuss the role of complement in these settings and during chronic CNS pathology. Finally, I will summarize these concepts and principles with the rationale for my thesis work.

Microglia in the embryonic CNS

Recently, microglia have been confirmed as arising from immune progenitors in the fetal yolk-sac (Ginhoux et al., 2010, 2013). Entering the CNS as early as embryonic day 9.5 (E9.5) microglia are present in the CNS much earlier than astrocytes and even before the start of true cortical neurogenesis which commences at approximately E12 (Miller and Gauthier, 2007). Microglia are unique in that these cells exhibit a unique transcriptional program in comparison to

other tissue resident macrophage and that they are also yolk-sac derived for the lifespan of the mouse with little to no contribution from fetal monocyte progenitors or adult monocytes (Ajami et al., 2007; Hoeffel et al., 2015).

Documentation of what signals recruit microglial entry into the developing brain has been controversial. Colony stimulating factor 1 (M-CSF-R) or its ligand, IL-34, has been well documented in their role of microglia differentiation/maintenance, as either blockade of M-CSF-R or deletion of IL-34 yields an absence of microglia within the brain (Squarzoni et al., 2014; Ulland et al., 2015; Wang et al., 2012)

Surprisingly, fractalkine or its receptor CX3CR1 was shown to be dispensable for microglial entry into the developing brain (Squarzoni et al., 2014). This was also shown in a study which added that chemokine receptor signaling was minimally important for microglial brain entry with CCR1, CCR2, CXCR3 or *Tyrobp* (DAP12) deficient mice displaying normal numbers of microglia within the developing brain (Kierdorf et al., 2013). Positive data regarding microglial entry, however, has been shown in the developing zebrafish, where neural progenitor cell (NPC) death and subsequent release of purinergic signals, such as ATP, was necessary for proper microglial infiltration and consequent NPC engulfment (Casano et al., 2016). Thus, ATP release may be a critical mediator of microglial brain entry and movement properties overall, as ATP sensing is the key mediator of microglial process extension to focal sites of injury in adulthood (Davalos et al., 2005). Regardless of mechanisms of microglial entry to the mouse CNS, their role in the clearance of NPCs during mammalian CNS development has been well documented. Before I proceed in discussing the roles of microglial phagocytosis in development, it will be helpful to have a brief overview of the concepts of apoptotic cell clearance and its regulation by specialized molecular cues (“find me” and “eat me” signals).

Apoptotic cell clearance

A key element of homeostasis in nearly all living animals is the clearance of apoptotic cell corpses and debris. This process is essential even in baseline tissue maintenance as billions of cells undergo apoptosis every day (Arandjelovic and Ravichandran, 2015). Failure to complete this debris clearance is disruptive to both the tissue and organism, with impaired organ processes and autoimmunity pursuant to failed clearance of dying cells and debris (Nagata, 2007).

Clearance of debris and dying cells is largely regulated by two types of phagocytes, "professional" and "non-professional". Professional phagocytes are defined as hematopoietic cells of the innate immune system while non-professional phagocytes are organ cells that are non-immune derived and consist classically of epithelial cells, endothelial cells, and fibroblasts. Professional phagocytes have a generally higher phagocytic capacity on a per cell basis, with specialized genetic and organelle specification to allow for a wider diversity of phagocytic recognition and increased digestive capacity. This is mostly due to a more diverse array of phagocytic receptor expression and increased lysosomal capacity which allows for the clearance and continued phagocytosis of many targets (Parnaik et al., 2000; Rabinovitch, 1995). Within the CNS parenchyma, microglia serve as the professional phagocytes and tissue resident macrophages (Sierra et al., 2014; Brown and Neher, 2014; Kettenmann et al., 2013), while non-professional phagocyte cells consist of astrocytes (Chung et al., 2013), oligodendrocytes (Nguyen and Pender, 1998), brain endothelial cells (Howland et al., 2015; Lam et al., 2010), pericytes (Winkler et al., 2014), neural progenitor cells (Lu et al., 2011), and, in limited instances, even neurons (Bowen et al., 2007). Further examination of these cell types in engulfment of material in health and CNS disease will be provided in later sections of this introduction.

"Find me" and "Eat me", essential instructions for phagocytes

Despite the variety and ubiquity of phagocyte types, the number of molecules informing phagocytes where to eat ("find me" signals) and what to eat ("eat me" signals) that are known to date is limited and conserved in many different tissues types (Medina and Ravichandran, 2016; Hochreiter-Hufford and Ravichandran, 2013; Green et al., 2016). Major constituents of find me signals are functional intracellular components released from dying cells during various death processes. This is the first element in a reoccurring theme of phagocytic target recognition where intracellular cell contents are either released into the extracellular space (find me signals) or where intracellular elements of the plasma membrane are exposed to the outside surface of a cell (eat me signals) (Medina and Ravichandran, 2016).

Cell death can be robustly induced through cell extrinsic means via ligand-receptor interactions (Fas, CD95, TRAIL) or though secreted factors (TNF) (Walczak, 2013). Cells may also die by cell-intrinsic means related to growth factor withdrawal, toxins, cell stress, or mechanical disruption. Once cell death is initiated, diffusible "find me" signals are released including S1P, fractalkine, and nucleotides such as ATP and UTP (Medina and Ravichandran, 2016). Fractalkine has been shown to be a key component of microglial biology as neuronal expression of fractalkine and its reception by microglia at baseline is thought to maintain microglial homeostasis (Paolicelli et al., 2014). Its role as a find me signal has also been highlighted with *Cx3cr1*$^{-/-}$ mice exhibiting equal efficiency in phagocytosis of targets but associating less with apoptotic debris (Sokolowski et al., 2014).

Once potential phagocytes have localized themselves to their phagocytic target, changes in the target cell membrane occur, such as display of calreticulin (Gardai et al., 2005; Fricker et al., 2012) and/or oxidized low-density lipoprotein (Fernandez-Castaneda et al., 2013) and flipping of

the inner plasma membrane to display phosphatidylserine residues (Lemmon, 2008), which allow for phagocytes to engage the target cell or debris. With engagement of the target, cytoskeletal rearrangement then proceeds to allow for engulfment in a process largely mediated by the Rho-family GTP-binding protein RAC1 (Castellano et al., 2000).

Cytoskeletal rearrangement then leads to the formation of the plasma membrane-enclosed target material known as the phagosome. Through a process known as phagosome maturation, the phagosome itself becomes increasingly more acidified through fusion with low pH lysosomes within the phagocyte cytosol whose proteases and low pH contribute to the destruction of the ingested material (Kinchen and Ravichandran, 2008).

An interesting feature of professional phagocyte ingestion of apoptotic cells is that the process is highly anti-inflammatory, with phagocyte uptake of material yielding inhibition of TNF release, production of IL-10, and production of transformation of growth factor β (TGF-β) (Turner et al., 2003; Ryu et al., 2012).

Microglial phagocytosis of NPCs and neuronal processes in development

Programmed cell death is a key driver of organ formation across the body, including the developing brain. The theory behind this process in the brain is that individual neurons, who extend axons to distal brain regions or to their target organs in the periphery, receive key survival signals that allow for reward of successfully targeted axons and death for neurons incapable of successful axon targeting. Evidence for this theory includes the well documented NGF-TrkA signaling pathway documenting neuronal survival in development from neural crest cells and the aberrant

growth seen in developing brains of Caspase 3,9 and APAF knockout mice (Dekkers et al., 2013; Nijhawan et al., 2000).

Neuronal and neural progenitor death in the developing brain is robust, with extensive death of progenitors seen in both the developing cortex and cerebellum (Blaschke et al., 1996; Perez-Pouchoulen et al., 2015). Extensive cell death within the developing brain proceeds almost concurrently with the seeding of microglia at E9.5 in the developing mouse brain (Ginhoux et al., 2010).

Microglial mechanisms of engulfment of NPCs in the developing zebrafish have been well characterized with microglia localized to dying NPCs triggering their rapid engulfment (Mazaheri et al., 2014). Despite this striking phenotype in zebrafish, This was not seen in the developing mouse forebrain, with microglial localization appearing to be independent of dying cells (Squarzoni et al., 2014). Microglia were, however, associated with axonal extensions, especially in the E14.5 thalamus with microglia depletion or activation yielding a respective extension or contraction of thalamic projections (Squarzoni et al., 2014). Notable was the fact that this localization was also CX3CR1 dependent. Microglial depletion also yielded an increase in the number of LHX6+ interneurons, implying that microglial phagocytosis or activity is necessary for maintaining interneuron numbers during cortical development.

While microglia were clearly shown to limit the number of neurons and their extensions during this early period of cortical development, it was also shown that in the postnatal cortex, microglia were necessary for survival of cortical neurons through their secretion of IGF-1 both in vivo and in vitro (Ueno et al., 2013). A role for microglial-derived IGF-1 in vitro has also been shown previously, with the secretion of microglial cytokines controlling neural progenitor number as well as fate direction (Butovsky et al., 2006a).

IL-1β has also been postulated to be a microglial-derived cytokine capable of inducing direct NPC death through its p53-mediated stimulation of PUMA contributing to NPC death in vitro (Guadagno et al., 2015).

Within the mouse cerebellum it has also been shown that developmental Purkinje cell death is seen within the first postnatal week. When Purkinje cell death was measured by cleaved caspase 3, it was shown that these dying cells are nearly all surrounded by microglial processes (Marín-Teva et al., 2004). Microglial depletion induced by incubation of cerebellar slices with clodronate-loaded liposomes also led to significantly higher numbers of Purkinje cells, indicating that microglia are also necessary for maintaining proper neuronal populations within the cerebellum (Marín-Teva et al., 2004). Incubation of cerebellar slices with control liposomes also showed that nearly all microglia were liposome-positive indicating their highly phagocytic capacity while astrocytes in the slice culture were not seen ingesting liposomes (Marín-Teva et al., 2004). In the developing rat cerebellum, it was also shown that microglia are highly phagocytic of neural precursors (Perez-Pouchoulen et al., 2015), with their engulfment enriched to the granule cell layer on postnatal week three.

While much of the focus of this introduction so far has focused on the engulfment of whole cells in development, the engulfment of cell processes by glia has been a well-documented and growing phenomenon in both homeostatic and disease conditions (Jung and Chung, 2018; Frost and Schafer, 2016; Cronk and Kipnis, 2013).

This concept was highlighted first with contemporary tools in the neuromuscular junction (NMJ) during axon withdrawal following lack of growth factor signaling (Bishop et al., 2004). By identifying immature NMJs occupied by two neuronal projections (with mature NMJs receiving projections from one neuron) through dual fluorescent labeling, researchers could observe process

retraction of the axon not receiving survival signals. Of note was the fact that the axon itself was not succumbing to Wallerian degeneration as it had intact 40 nm vesicles and mitochronidae. Also seen by TEM, was that Schwann cell membranes were in close contact with the withdrawing axon and that remnants of "axoplasm" where also seen within neighboring Schwann cells, implying that Schwann cells were important for clearing these axon remnants during PNS development (Bishop et al., 2004).

Within the CNS, the nature of microglial interaction with synapses was first conceptualized through novel technology that allowed for viewing microglia dynamics in real time. This was achieved through two-photon live imaging of CX3CR1-GFP mice (Jung et al., 2000) through a thin-skulled technique allowing for repeated imaging sessions in healthy animals or by imaging ex vivo slices of CNS tissue. A striking phenotype of rapidly moving microglial processes (1.5 μm per minute) were observed contacting many elements of the CNS, including blood vessels and the cell bodies of neurons and astrocytes (Nimmerjahn et al., 2005). This study also showed that microglia were capable of rapid process extension in the wake of injury and neurological disease, with further studies concluding that this was largely due to ATP-sensing from microglial P2RY12 receptors (Davalos et al., 2005; Eyo et al., 2014; Gu et al., 2016; Maeda et al., 2010).

Within the CNS, the best-described system involving engulfment of neuronal material by glia is during a critical period in the developing retinogeniculate visual system. Initially, cortical projecting thalamic relay neurons receive input from the eye by up to ten individual neurons which are dramatically reduced to one or two by activity dependent stimulation that starts prior to eye opening and continues to postnatal day 30 (P30) (Hooks and Chen, 2006). Additionally, it was shown at that time that astrocytes are strong inducers of synapse formation (Christopherson et al., 2005; Ullian et al., 2001). Consequently, a co-culture of retinal ganglion cells (RGCs) and

astrocytes yielded data showing that the complement system, a critical element of innate immunity, was markedly upregulated in RGCs upon astrocyte co-culture (Stevens et al., 2007). Investigation in to complement's role showed that C1q, an opsonin that tags dying and foreign material for destruction by cells (Ricklin and Lambris, 2013; Orsini et al., 2014), was critical for neurodevelopment. These experiments showed that C1q-deficient mice had impairment of synapse elimination within the LGN and that in glaucomatous retinae, C1q presence signaled an early sign of disease (Stevens et al., 2007).

Further studies identified that C1q production was mediated by retinal astrocytic secretion of TGF-β that led to RGC production and transport of C1q to the LGN (Bialas and Stevens, 2013). C1q tagging lead to clearance of presynaptic material by microglia in a process that was also C1q, C3, and CR3 (CD11b) dependent (Schafer et al., 2012). It was also shown that neuronal activity can drive microglial engulfment of material. Dampening of neuronal activity by tetrodotoxin (TTX) treatment increased engulfment of weak synapses over controls while increased neuronal activity through forskolin treatment lead to a decrease in engulfed material compared to untreated controls (Schafer et al., 2012). However, whether neuronal activity controls complement deposition by RGCs has not been directly measured.

Further examining the clearance of synaptic material within the developmental LGN, the role of astrocytes were characterized with the ALDH1L1-GFP mouse which allowed for the labeling of all astrocytes with intracellular GFP (Tsai et al., 2012). This study showed that at the same time points when microglia were phagocytic of retinogeniculate terminals, astrocytes were phagocytic as well, consuming five to ten times as much synaptic material. This study also showed that engulfment was mediated by the phagocytic receptor MER on both microglia and astrocytes, while astrocytes had additional contribution from the phagocytic receptor MEGF10 (Chung et al.,

2013). While MEGF10 was also shown to be utilized by astrocytes in a C1q-dependent manner to clear NPCs (Iram et al., 2016), the role of C1q in astrocyte-mediated synaptic material clearance was not assessed (Chung et al., 2013).

Prior to the works focusing on the LGN of the visual system, previous works had looked at development in the mouse hippocampus and visual cortex. Within the hippocampus, a CX3CR1-mediated engulfment of post-synaptic material by microglia (Paolicelli et al., 2011) was shown in the early postnatal period, with CX3CR1 deficiency resulting in a transient increase in microglial cell number and immature synapses with a resultant increase in synaptic activity and a reduction in seizure susceptibility (Paolicelli et al., 2011).

Microglial contact with synaptic elements was also described using the role of critical periods within the visual cortex (Tremblay et al., 2010). Through usage of TEM and 2-photon fluorescent imaging in live animals with Thy1.1-YFP constructs to label neuronal processes and CX3CR1-GFP to label microglia, it was observed that microglia were in close proximity to dendritic spines of visual cortex neurons and that the contact of microglial processes with spines significantly increased the chances of synapse elimination (Tremblay et al., 2010). Finally, this group showed that animals that were dark adapted during a critical period within the visual cortex showed microglia with much slower process motility and reduced numbers of dendritic spines (Tremblay et al., 2010). This was also shown in instances of ischemia or with TTX treatment to the eye (Wake et al., 2009). Later studies investigating microglia during critical periods of the visual cortex would show that this process was largely mediated by the microglial ATP-sensing receptor P2RY12 (Sipe et al., 2016).

While the focus of this introduction thus far has been focused on the role of microglia and their phagocytic function in neural development, the following sections will detail their function

in postnatal neurogenesis, at the site of injury and in distal locations during acute CNS spinal cord and nerve injury, and during chronic CNS disease states.

Microglial phagocytosis during postnatal neurogenesis

In the postnatal zones of adult neurogenesis, the phagocytic roles of microglia have also been well documented. Postnatal neurogenesis is largely relegated to two major zones of continued proliferation and differentiation of neural precursors, the subventricular zone of the lateral ventricle (SVZ) and the subgranular zone of the dentate gyrus of the hippocampus (SGZ) (Ming and Song, 2011; Malatesta et al., 2008; Romero-Grimaldi et al., 2011). SGZ neurogenesis is important as it generates new glutamatergic dentate gyrus granule cell neurons that contribute to the discrimination of similar type objects in a process known as pattern separation (Sahay et al., 2011). SVZ neurogenesis also gives rise to neuroblasts that, unlike their SGZ brethren which migrate on the order of microns to the nearby granule zone, proceed through chain migration through the rostral migratory stream (RMS) to reach their ultimate destination of the olfactory bulb (Doetsch et al., 1997). Once in the olfactory bulb, they mature into GABAergic interneurons critical for control of olfactory discrimination (Moreno et al., 2009).

In the SGZ, it was shown that the degree of microglial phagocytosis is coupled to the degree of NPC proliferation with the number of engulfed cells dropping in the first several months of age (Sierra et al., 2010). Further, an inflammatory insult induced by LPS led to decreased rates of in vivo phagocytosis by SGZ microglia indicating that neuroinflammation may impact microglial phagocytic processes.

Within the SVZ, observations of NPC engulfment by microglia are much more rare, with engulfment of NPCs more readily seen within the olfactory bulb (Ribeiro Xavier et al., 2015).

Despite this fact, depletion of SVZ and RMS microglia through CD11b-mediated delivery of saporin toxin led to a dramatic increase in the number of SVZ NPCs (Ribeiro Xavier et al., 2015). Subsequently, this also led to a decrease in migrated NPCs to the olfactory bulb, indicating that SVZ microglia contribute to the successful migration of SVZ NPCs (Ribeiro Xavier et al., 2015).

Attenuation of microglial activation through minocycline treatment also led to a drop in NPC number within the SVZ that correlated with a decrease in cytokines known to be released by microglia; including IL-1β, TNF, and IL-6 (Shigemoto-Mogami et al., 2014). This indicates that the influence of microglia on NPC function within neurogenic niches may be more than just through phagocytic means and could consist of aiding differentiation and survival through cytokine production, which has been shown robustly in vitro (Butovsky et al., 2006b).

Neuronal cell death from spinal cord and CNS nerve injury

Before the responses of glia to acute neurodegeneration is assessed, a brief overview of neural mechanisms of death in acute injury should be examined. Acute CNS injury is a relatively common malady in the United States with 1-2% of the population showing long-term disability (mostly from falls, motor vehicle accidents, and violence) (Hempill, 2016). When injury directly affects the spinal cord, serious complications arise due to paralysis below the injury site itself, with life-long immobility a frequent result. Common injuries to the nerves of the face, which include the trigeminal nerve (Cranial nerve V) and facial nerve (cranial nerve VII), are far less common and can be idiopathic, seen in Bell's Palsy, or due to accidents, violence, or maxillofacial surgery procedures including local anesthesia delivery (Miloro and Kolokythas, 2012; Renton et al., 2010; Atolini Junior et al., 2009). CNS injury is unlike that seen in other tissues, with little to no neuroregeneration observed, which leads to permanent neurological loss or dysfunction. Reasons

for this fatal outcome are largely due to factors that prevent neural regeneration itself or those that contribute to secondary neuronal death, which can affect neuronal health in the days following the initial insult.

Death of neurons occurs in two phases, with neuronal death in the first phase resulting from the initial injury through physical disruption of neuronal membrane on the cell body, distal dendrites, or the axon/presynaptic terminals. Secondary death of neurons is a consequence of the initial incident and can be elicited by a myriad of factors, including excitotoxicity through excessive glutamate release, free radicals inducing oxidative stress, tissue swelling, hypoxia, loss of trophic support, and inflammatory responses from surrounding cells (Yoles and Schwartz, 1998; Stoica and Faden, 2010).

Neuronal death in CNS injury can lead to both necrotic or apoptotic death. Apoptotic death is largely characterized histopathologically by preserved membrane integrity and cell shrinkage with nuclear and organelle condensation. Later stages also show a distinct "blebbing" morphology as the cell itself is pinched off to form smaller components (Yakovlev and Faden, 2004). Characteristics of necrotic death include loss of membrane integrity and cell swelling, organelle damage, and oftentimes cell lysis, which leads to more profound inflammation when compared to apoptotic cell death. Important to note, is that in an vivo context there is most often a combination of cell death processes, including apoptosis and necrosis, with contemporary studies identifying "new" forms of cell death at steady rates (Kroemer et al., 2009; Tait et al., 2014).

Neurons damaged during acute pathology either succumb to direct insult from the injury itself or through damage from their distal axons, which results in concurrent death of the distal axon "stump" and the cell body itself in a process known as Wallerian degeneration (Rotshenker, 2011; Gaudet et al., 2011). First characterized in the glossopharyngeal and hypoglossal nerves of

the frog by Augustus Volney Waller in the mid 19th century, this process leads to a clearing of debris that allows for possible regeneration to occur (Waller, 1850). Dynamics of Wallerian degeneration in the peripheral vs. central nervous system vary dramatically, with CNS Wallerian degeneration proceeding much more slowly compared to the peripheral system, with failed regeneration as an often consequence (Vargas and Barres, 2007; BrosiusLutz and Barres, 2014). This is due to a myriad of factors including, but not limited to, the activity of Schwann cells in the PNS vs. oligodendrocytes in the CNS, and the presence of infiltrating macrophages in PNS tissue with debris clearance facilitated by opsonizing antibodies derived from the blood (Vargas and Barres, 2007). Schwann cells are seen to stop their production of myelin within 12 hours post injury in the PNS and proceed to clear debris via TAM-receptor mediated phagocytosis and the attraction of peripheral myeloid cells by CCL2 release, all of which are crucial elements necessary for clearance and subsequent regeneration (Jessen and Mirsky, 2016; Brosius Lutz et al., 2017). Oligodendrocytes are not as beneficial to nerve outcome during neurodegeneration with their fate resulting in either apoptosis or quiescence due to loss of contact from the degenerating axon. This leads to survival of 60-70 percent of oligodendrocytes within the optic nerve after nerve crush with myelin debris seen up to 22 months post injury in rats (Ludwin, 1990; Vaughn and Pease, 1970).

The initiation of neuronal death during Wallerian degeneration is fast, with intracellular calcium flux being a major driver in the minutes and hours after injury. Disintegration of the axon is rapid with hundreds of microns disintegrated by 30 minutes on both proximal and distal axon segments (Kerschensteiner et al., 2005). The first morphological appearance of disintegration is the swelling and beading of the axon in an unknown molecular process that is independent of calcium and calpain activation (Vargas and Barres, 2007). Calcium influx allows for the

consequential activation of calpain proteases which are critical for the granular disintegration of the axonal cytoskeleton (GDC) (Simons et al., 2014). Microtubule breakdown proceeds first, with the ubiquitin-proteasome required (Zhai et al., 2003). Neurofilaments are further degraded by calpains which themselves are sufficient to induce axon breakdown in healthy neurons when supplemented by calcium ionophores (George et al., 1995).

Despite the passive nature of calcium influx and activation of the ubiquitin-protease response, Wallerian degeneration is a very active process, controlled by a recently discovered cadre of downstream mediators (Gerdts et al., 2016a). Long thought to be a passive process, this "active theory of axon degeneration" originated with the observation of significantly delayed degeneration in a naturally occurring mouse strain (Lunn et al., 1989; Tsao et al., 1994) termed Wallerian degeneration slow or *Wld*^{S}. Mice with a single copy of the *Wld*^{S} allele exhibited structurally and metabolically intact axons that lasted up to two weeks with no physical connection to the cell body, while WT axons degenerated in less than two days (Tsao et al., 1994). *Wld*^{S} mice also exhibited axon protection in many models of chronic neurodegeneration including glaucoma and motor neuron disease, suggesting that axonal degeneration resultant from pathology had a shared common pathway to both acute and chronic neurodegenerative conditions (Beirowski et al., 2008; Ferri et al., 2003).

The *Wld*^{S} mice led to mechanistic insights into axonal degeneration as the *Wld*^{S} allele produced a novel fusion protein allowing for increased levels of NAD+. This allowed for persistent ATP levels and cell processes to continue (Gerdts et al., 2016a). Further works in drosophila and mouse nerve models of neuroregeneration offered additional insight, elucidating required roles for DLK (Watkins et al., 2013; Miller et al., 2009) and SARM1 (Yang et al., 2015) activity to facilitate the coordinated destruction of the axon.

Building on Fire: sounding the "alarmin" in CNS injury

How is damage conveyed by the injured neuron and how does the immune system receive this signal? A conceptual evolution occurred in the early 90s when the canonical view of the immune system being poised to react to self vs. non-self was modified to include the immunological concept of "danger". Posited by the pioneering immunologist Polly Matzinger. This theory held that immune activation would only proceed in the presence of significant damage to the tissue or organism, thus activating the immune system through release of molecules coined "alarmins" (Matzinger and Kamala, 2011; Matzinger, 1994; Pradeu and Cooper, 2012). Thus, signals emerging from pathogens (pathogen associated molecular patterns or PAMPs) and signals emerging from host tissue damage (damage associated molecular patterns or DAMPs) would alarm and equip immune cells for commensurate responses.

DAMP release and exposure are important features of acute neurodegeneration, resulting in the large degree of myeloid recruitment and activation at the site of injury. Key alarmins which have been shown to play important roles in CNS injury include HMGB1 (Kim et al., 2006), ATP (Davalos et al., 2005), and IL-33 (Gadani et al., 2015a; b). Similar to the "find me" signals discussed previously, alarmins serve to alert and inform the cellular milieu surrounding the injury site. DAMPs allow for proper immune responses including: inflammasome-dependent cyotkine release, peripheral monocyte recruitment, local cell migration, and process extension.

Sensing of both PAMPS and DAMPs occurs through common pattern recognition receptors (PRRs) shared on many different types of glia and immune cells (Gadani et al., 2015a). Notable examples include: NOD-like receptors (NLRs), RIG receptors, C-type lectin receptors, and toll-like receptors (TLRs) (Ransohoff and Brown, 2012; Walsh et al., 2014a; Gadani et al.,

2015a). Variation within these receptor families are myriad, with humans exhibiting 32 types of NLRs and TLRs while mice are known to have 46 different NLR and TLR molecules (Bryant and Monie, 2012). One of the most well characterized TLRs is TLR4 which has been shown to play a key role in sensing the canonical PAMP molecule lipopolysachharide (LPS) in addition to a range of DAMPs which include: heat shock proteins, fibrinogen, heparin sulfate, and HGMB1, among others (Erridge, 2010). A central hub of alarmin signaling is the molecule Myd88, which acts downstream of all TLRs in the mouse except TLR3 (Lu et al., 2008). Myd88 further signals activation through a master transcription factor of immune activation, NF-κB. With NF-κB activity yielding the transcription of hundreds of downstream targets necessary for cell responses to infection and injury, TLR, and subsequent NF-κB mediated activation, are essential for the immune response to DAMPs and PAMPs (Gilmore, 2014).

Release of alarmins and NF-κB activity leads to an amplification of the immune response by both infiltrating and resident immune cells through production of soluble factors, including TNF, IL-6, IL-1β, and IL-1α (Ransohoff and Brown, 2012). TNF has been shown to be especially detrimental in the early phase of the immune response after SCI, with TNF blockade significantly improving disease outcome (Esposito and Cuzzocrea, 2011).

IL-1β production post injury is tightly regulated by IL-1β being made initially in a pro-form and then cleaved by caspase 1 and NLRs in a complex known as the inflammasome (Lukens and Kanneganti, 2014; Lukens et al., 2012; Lamkanfi and Kanneganti, 2010). Inflammasomes form large multiprotein structures composed of an adaptor molecule (typically ASC), an NLR (NLRP1, NLRP3, AIM2, and NLRC4 are classic examples), as well as caspase 1 (Walsh et al., 2014a). The range of DAMPs recognized by NLRs is extensive with AIM2 sensing double-stranded DNA and NLRP3 sensing a myriad of cell stress molecules including ATP, Amyloid β,

silica, cholesterol, intracellular ROS, and potassium (Jo et al., 2016). Thus, inflammasome signaling is uniquely poised to serve as a signaling hub that simultaneously senses immune "danger" and releases a potent cytokine in response.

IL-1α has received recent attention as a critical CNS alarmin. Unique from its related cytokine IL-1β, IL-1α is also made as a pro form that is cleaved through a process dependent on calcium and calpains (Brough and Denes, 2015). This can be mediated through inflammasome activation as described previously, through the activation of the necroptotic pathway which includes the proteins RIPK1 and RIPK3, and through a mechanism independent of these two pathways. Works investigating IL-1α have been limited, with single knockout experiments lacking in both number and depth. However, experiments have shown that platelet-derived IL-1α can induce activation of endothelial cells by adhesion molecule upregulation (Thornton et al., 2010) and that IL-1α deficient animals have decreased lesion size, myeloid recruitment, and improved Basso Mouse Score compared to wild type mice in a mouse model of spinal cord injury (Bastien et al., 2015).

The immune molecule IL-33 has also been ascribed a key role in the CNS (Gadani et al., 2015a). Shown previously to be a key alarmin in the lung during both infection and asthmatic conditions, IL-33 has also been shown to promote hypernociception in the skin (Liew et al., 2010). Our lab has also described a specific role for IL-33 during CNS injury with myeloid cell recruitment and activation of meningeal innate lymphoid cells (ILCs) being IL-33 dependent (Gadani et al., 2016, 2015b). Alarmin signaling is crucial to many pathologies within biology and specific to the CNS. In the next section I will examine one of these, CNS nerve injury.

Mouse models of spinal cord and CNS nerve injury

Many models of nerve injury in the mouse exist and are extensively reviewed in the literature (Angius et al., 2012; Costigan et al., 2009; Shechter and Schwartz, 2013). In this introduction I would like to focus on three models that are heavily utilized in the investigation of immune interactions with nerve injury in the CNS: spinal cord injury, facial nerve injury, and optic nerve crush injury.

There is extensive work from our lab and others utilizing spinal cord injury as a platform to investigate the immune effects of acute and long term neurodegeneration as well as concepts such as neuroprotection and regeneration (Basso et al., 2006; Shechter and Schwartz, 2013; Zhang et al., 2014). In brief, these models utilize a laminectomy surgery to expose the spinal cord after which an impact probe is lowered onto the surface of the spinal dura with a preassigned quantity of force. Though there are mouse strain differences, standardized scoring methods by blinded researchers are used to determine as the Basso Mouse Score (BMS) (Basso et al., 2006). Female mice are often used for spinal cord injury models as mice with thoracic injury lose bladder control necessitating manual expulsion. This is extremely traumatic to male mice due to their smaller urethral size. Onset of debilitation is rapid with spinal shock in the hours post-injury subsiding to a gradual plateau of behavioral stability at around three weeks post-injury. BMS score is also dependent on injury severity with full recovery not seen (Basso et al., 2006).

Mouse facial nerve injury is one of the most utilized models of peripheral nerve injury with studies conducted in many vertebrate species (Serpe et al., 1999; Moran and Graeber, 2004; Jinno and Yamada, 2011; Sanders and Jones, 2006). The facial nerve exits the skull through the stylomastoid foramen, allowing for it to be easily accessible for injury. This nerve solely innervates the muscles of the face, and thus unilateral manipulation allows for nerve injury with no significant

ill health effects to the animal. Crush injury for the facial nerve allows for regeneration of the peripheral nerve and functional recovery around 2-3 weeks while transection results in non-regeneration and allows for study of neuroprotection (Jones et al., 2005; Sanders and Jones, 2006; Moran and Graeber, 2004). In this model, it was found that peripheral T cells are necessary for neuronal survival four weeks after injury, with T cell reconstitution sufficient to restore neuroprotection (Jones et al., 2005; Serpe et al., 1999). Microglia are also active players in the model, secreting a variety of neurogenic factors including NGF, NT-4/5, TGF-β, GDNF, FGF, and IL-3 which all affect neuronal survival (Nakajima et al., 2007). Microglia are also highly phagocytic of processes innervating the facial nerve nucleus in a process known as "synaptic stripping" (Blinzinger and Kreutzberg, 1968), which will be discussed later.

Optic nerve crush injury is a common model utilized by our lab and others to detail processes of both neuroprotection and neuroregeneration (Schwartz, 2004; Tang et al., 2011; Templeton and Geisert, 2012). The retina is a complex CNS structure with sensory cells allowing for vision and only one set of projection neurons. Termed retinal ganglion cells (RGCs), there are up to 30 different RGC types that send their processes to brain targets, which include the superior colliculus, lateral geniculate nucleus of the thalamus (LGN), the suprachiasmatic nucleus of the hypothalamus, and the pretectal nuclei (Martersteck et al., 2017). The fact these neurons are isolated from surrounding grey matter in the optic nerve just distal to the optic nerve head allows for a unique circumstance where Wallerian degeneration can be investigated in a large territory by injury to a small area, with little trauma to the surrounding tissue and mouse itself, aside from blindness. Crush is applied by reclosing forceps, so that a defined pressure can be employed on multiple subjects to improve reproducibility. Crush is preferable to transection in this model as the optic nerve itself contains ophthalmic arteries and veins which would be transected along with the

nerve, prompting unnecessary trauma to the eye. To study neuroprotection, survival of RGCs is quantified post-crush by either retrograde tracing from the brain or by quantifying specific markers of RGCs, which include RBMPS, which labels nearly all mammalian RGCs (Rodriguez et al., 2014) or Brn3a which labels retinogeniculate projecting neurons (Nadal-Nicolás et al., 2009; Quina et al., 2005). Anterograde labeling can also be employed with either fluorescent probes conjugated to dextran, cholera toxins, or through transcription from viruses. RGC loss in the mouse is rapid, as crush injury is total across a majority of axons in the mouse with a 50% reduction in RGC counts seen one-week post-injury and 90% loss by three weeks post-injury.

Glial and peripheral immune cell responses at the site of CNS injury

An important factor when discussing the immune response in nerve injury is the behavior of glia and immune cells at the site of injury. This response is rapid and can translate to both pathological outcome and the possibility for regeneration.

As noted previously, the relative differences between oligodendrocyte and Schwann cell responses to injury are what help define the major differences in PNS and CNS Wallerian degeneration (Vargas and Barres, 2007; BrosiusLutz and Barres, 2014).

Astrocyte responses at the site of injury within the CNS are fast, as these cells undergo a hypertrophy and an upregulation of structural proteins such as glial fibrillary acidic protein (GFAP) and laminin. Extracellular matrix proteins, including chondroitin sulfate proteoglycans (CSPGs), are also secreted, which form a major component of the “glial scar” seen frequently after nerve or spinal cord injury (Sofroniew, 2005). Despite the large amount of literature and scientific effort ascribing a negative role to the astrocytic glial scar as blocking possible axon regeneration,

recent works have shown that this structure is actually a scaffold essential to the axon pathfinding that is typical with regeneration (Anderson et al., 2016).

Astrocytes have been recently ascribed a role in cytotoxicity following optic nerve crush, through their secretion of a triad of key immune activators which include IL-1α, TNF, and C1q (Liddelow et al., 2017). These factors, especially TNF and IL-1α, were shown to be critical for RGC survival after optic nerve crush, with microglial depletion not affecting RGC survival (Liddelow et al., 2017; Hilla et al., 2017) (a phenotype we have also observed at multiple time points). While these experiments indicate that microglia-derived TNF and IL-1α may not be involved in neuronal survival, there could be a possibility of astrocyte-microglial amplification of neurodegeneration, or a critical role for an additional cell type in IL-1α, TNF, and C1q production post-injury.

Peripheral cells are also recruited to the site of injury, with their role as beneficial or detrimental being a point of controversy still ongoing in the field of neuroimmunology (Shechter et al., 2009; Yawata et al., 2008; Russo and McGavern, 2015; Rezai-Zadeh et al., 2009). The arrival of peripheral immune cells comes in waves, with neutrophils first on the scene, followed by the chemokine-mediated arrival of monocytes (which are often impossible to distinguish from resident microglia), and finally T and B lymphocytes (Gadani et al., 2015a).

Neutrophils are the first cell type to arrive in nearly any tissue with breach of the vascular system or infection, with CNS injury being no different. Neutrophils subsequently proceed to conduct a profound anti-bacterial response (regardless of injury sterility), releasing lytic enzymes, cytokines, chemokines, sometimes secreting their DNA in a web-like fashion known as neutrophil "nets", and phagocytosing infectious organisms (Summers et al., 2010). Neutrophil recruitment occurs most likely through chemokines, however a powerful role for purinergic receptors has

recently been assigned to a sterile model of TBI by the McGavern lab (Roth et al., 2014). The role of neutrophils is controversial with studies obscured by differential effects of depleting antibody (anti-GR-1, which is composed of Ly6G and Ly6C expressed on monocytes) that can lead to depletion of monocytes as well (Stirling et al., 2009). A purely beneficial role has also been ascribed to neutrophils with their depletion depriving the retina of the key growth factor oncomodulin after optic nerve crush injury (Kurimoto et al., 2013; de Lima et al., 2012).

Monocyte-derived macrophages arrive in a chemokine-dependent fashion after spinal cord injury or lesion to the CNS, where they promote tissue remodeling through angiogenesis and scarring and are also critical mediators of debris clearance. Monocyte type is classified in the blood by expression of Ly6C, with cells exhibiting lower expression of Ly6C (Ly6Clo) acting as patrolling "tissue-resident" blood macrophages, expressing CX3CR1 and utilizing LFA1 for extravasation from the blood (Auffray et al., 2007). Ly6Chi monocytes are in higher frequency and express CCR2, with their tissue entry mediated by the chemokine CCL2 (Gadani et al., 2015b; Muessel et al., 2000; Jakubzick et al., 2013). When both cells enter a tissue, however, surface expression of monocyte markers are lost, making it a considerable task to define macrophage vs microglial identity (Greter et al., 2015). Our work, described in Chapter 3, utilized fate-mapping of endogenous microglia to track whether inflammatory monocytes seeded the injured LGN post optic-nerve crush injury. While little influx was seen in this region, we did see robust infiltration of periphery-derived Iba1+ cells in the optic nerve crush site.

Recent fate-mapping work has also shown robust infiltration of monocytes within the retina after injury (Ma et al., 2017). This study showed that usage of CCR2-RFP was an unreliable marker to denote monocyte tracking, as peak CCR2-RFP+ cell numbers were seen at three days post injury with that number sharply declining by day 7. This was in stark contrast to fate-mapping

experiments which showed that nearly half of all Iba1+ cells in the retina were peripheral-derived at 30 days post-injury while none of these cells expressed CCR2 (Ma et al., 2017). Experiments investigating this phenomenon in the spinal cord have not been performed, though the differential role of resident microglia and periphery-derived macrophages has been attempted.

Utilizing a transgenic mouse driving GFP expression under the *LysM* promoter, experiments were performed to examine the relative contribution of microglia and peripheral macrophages to macrophage populations in the spinal cord after injury (Greenhalgh and David, 2014; Greenhalgh et al., 2016). This is controversial as this mouse brightly labels neutrophils with GFP, which, as discussed previously, are indeed present in the spinal cord injury site (Faust et al., 2000). Despite this, these labeling studies showed that arginase-1, an enyzme necessary for macrophage responses post injury, was only seen in LysM-GFP+ macrophages (Greenhalgh et al., 2016). Phagocytic properties of macrophages and microglia were also ascribed in the LysM-GFP+ model, with LysM-GFP+ macrophages phagocytosing greater quantities of myelin debris on a per cell basis and for longer periods after injury, though myelin phagocytosis was shown to be much more detrimental to LysM-GFP+ peripheral macrophages, inducing higher rates of cell death after ingestion (Greenhalgh and David, 2014). Phagocytosis of myelin debris in the injury site and along the injured spinal cord or CNS nerves is controversial, as incomplete oligodendrocyte death and quiescence promotes unresolved myelin clearance which is in stark contrast to PNS neurodegeneration (BrosiusLutz and Barres, 2014; Vargas and Barres, 2007). Survival and proliferation of RGCs and their progenitors have also been shown to be mediated by peripheral monocytes, with monocyte entry to the retina exhibiting a high degree of neuroprotection after injury and capable of secreting neuroprotective factors (London et al., 2011; Benhar et al., 2016; Barouch and Schwartz, 2002).

T and B lymphocytes have also been shown to play a dramatic role in neuroprotection after CNS injury. The major arm of the adaptive immune system, T and B lymphocytes are unique in that they can rearrange their genome during development, creating specific receptors that recognize unique pathogens. The B cell receptor is a cell surface bound molecule, that when activated can also be excreted as an antibody during inflammation or constitutively by plasma cells after their conversion from B cells. T Cell receptors are surface bound and help the cell interact with the innate arm of the immune system through TCR-MHC interactions. Given the proper molecular context and valence of immune signal, this interaction promotes T cell activation and differentiation to proliferate and effectively combat the particular threat. T cells (and B cells) are absent in the brain parenchyma at baseline and their presence during pathology is normally associated with poor outcome and disease state. Given this fact, it was surprising that early experiments utilizing T cell deficient mice showed significantly worse outcome during models of CNS injury (Schwartz and Kipnis, 2011; Jones et al., 2005; Moalem et al., 1999a; b). Despite TCR molecules playing a key role in recognition of self and immune defense for pathogens, a TCR non-specific role for neuroprotection has been described by our lab, with a key function assigned to sensing of endogenous DAMPs by CD4 T cells through the sensor Myd88 and subsequent secretion of IL-4, which was necessary for neuronal survival and neurite outgrowth (Walsh et al., 2015). Regulatory T cells, necessary for restricting CD4 T cell responses in infection, were also shown to be critical for response to CNS injury through tuning of CD4 T cell production of IL-4 and modulation of the immune response in multiple locations including at the site of injury, within the retina promoting RGC survival, and in the draining lymph nodes of the CNS (Walsh et al., 2014b).

Phagocytosis of neuronal processes during Wallerian degeneration

While nerve injury produces dramatic effects at the site of injury, less investigation has been made into the anterograde lesion and how glial cells clear debris despite the knowledge that presynaptic elements are rapidly lost, often at speeds far greater than axonal or cell body loss (Mack et al., 2001; Gillingwater et al., 2003, 2006).

Experiments utilizing transmission electron microscopy (TEM) in a variety of organisms in the mid 20th century were able to visualize synaptic degeneration from injury to the optic nerve and glial engulfment of neuronal material (Ghetti et al., 1972; Vaccarezza et al., 1970; Wong-Riley, 1972), though modern works describing these processes have been limited. This is largely due to a limitation in reagents necessary to observe phagocytosis. To facilitate this process reagents must be sufficient to label projections from neurons while they must also be able to withstand the low pH of the phagocytic lysosome. In our experiments (detailed in Chapter 2) we utilized Cholera toxin B subunit (CTB) conjugated to various fluorochromes which are detailed in Chapters 3 and 4. Since CTB binds robustly to neuronal membranes through interactions with M1 gangliosides and subsequent trafficking by endocytosis through neuronal processes, CTB is capable of staining the cell body and axonal processes in their entirety (Angelucci et al., 1995; Alisky et al., 2002).

Immunohistochemistry of ingested targets is largely insufficient to monitor phagocytosis as lysosomal activity renders all antibody staining of phagocytic targets controversial due to the low pH and proteases of the phagosome and even lower pH of the lysosome. This would denature epitopes necessary for antibody binding, rendering difficult the interpretation of true positive staining. Despite this, studies have attempted to document endogenous phagocytosis of neuronal material during Wallerian degeneration through a variety of means.

Engulfment of degenerative neuronal material using GFP reporter systems has been well documented in *Drosophila* injury models to the mushroom body and maxillary palp with glial clearance by phagocytosis the critical step in debris clearance (Broadie, 2004; Logan et al., 2012a; MacDonald et al., 2006). A key role for the fly engulfment receptor Draper was shown in a series of experiments utilizing maxillary palp ablation experiments (Logan et al., 2012b; MacDonald et al., 2006). Draper was also shown to play a functional role in neuronal corpse clearance via the *C. Elegans* homolog of Draper, Jedi-1 or as it is known in *M. Musculus,* MEGF10 (Wu et al., 2009). Astrocytic MEGF10 also has been shown to play a critical role in the clearance of neuronal processes in development (Chung et al., 2013), though astrocyte phagocytic responses were not seen in our model of clearance post optic nerve crush injury (Chapter 3).

In the mouse spinal cord, microglial and macrophage phagocytosis was shown by imaging autoflourescent granules which were interpreted to be ingested myelin debris (Greenhalgh and David, 2014). Caution should be applied to this interpretation though, as activated macrophages and microglia do have an intrinsic degree of autofluorescence (Misharin et al., 2013; Brunk and Terman, 2002; Schnell et al., 1999; Xu et al., 2008). The marker, Oil Red O (ORO), was also employed post spinal cord injury to mark phagocytic macrophages (Guo et al., 2016; Gaudet et al., 2015). Limitations of this marker are that they indicate, in a binary fashion, whether a cell is phagocytic and are impossible to assess the relative clearance of myelin debris.

The concept of "synaptic stripping" has been well documented in the facial and chorda tympani nerve injury models (Neumann et al., 2009; Bartel, 2012) where microglial are highly proliferative and activated following facial nerve axotomy (Jinno and Yamada, 2011; Rappert, 2004; Tay et al., 2017). The classic observation of "stripping" in these works was through TEM,

which though it has unquestionable resolution, is a laborious process (Streit and Kreutzberg, 1988; Graeber et al., 1988).

A recent work in a model of sciatic nerve injury showed that in the dorsal column where axonal processes from the DRG converge in the spinal cord, that microglia were activated and proliferative (Guan et al., 2015). This proliferation was mediated by CSF production from the DRG which shunted CSF towards the spinal cord, promoting microglial proliferation. Deletion of CSF from the DRG utilizing *Advillin*^{Cre} mice prevented microglial proliferation and a hyperalgesia phenotype associated with this nerve injury. Since we observed a similar microglial proliferation during Wallerian degeneration after optic nerve injury, a similar mechanism is tempting to ascribe, however, in the aforementioned model, the cell body of the DRG is left intact, whereas with optic nerve injury, this connection from the retina to the LGN is disrupted with optic nerve crush.

Microglial phagocytic responses to chronic CNS disease: a key role for complement-mediated synapse elimination

In chronic neurodegeneration, dystrophic or degenerating neuronal processes are often times a critical driver of glial activation. Glial cells then may serve as an amplifier of neuronal dysfunction, which often times leads to an exacerbation of the underlying neuronal pathology or wholesale neuronal death. During this process, glia often have active roles in the clearance of whole neuronal cells, processes, or waste products.

In the mouse model of multiple sclerosis (MS), known as experimental autoimmune encephalomyelitis (EAE), differential roles for microglia and infiltrating CCR2-expressing monocytes were ascribed using the CCR2-RFP/CX3CR1-GFP (Red/Green) mouse (Yamasaki et al., 2014). This mouse, along with advance block-face scanning electron microscopy, allowed for

a precise examination of moncoyte-derived macrophage vs. microglial roles in the stripping of myelin from damaged axons, a process frequently seen in both MS and EAE. These researchers observed that macrophages were the active removers of myelin surrounding nodes of Ranvier, with microglia operating in a scavenging role, picking up the debris remains. This supports the notion that monocyte-derived macrophages are key mediators of demyelination and disease pathology driven by GMCSF producing CD4 T cells (Croxford et al., 2015; Mrdjen et al., 2018). This condition may not be indicative of other neurodegenerative diseases as EAE is typified by robust immune infiltration of T cells, which may alter microglial function, monocyte-derived macrophages which are robust phagocytes themselves, and deposition of IgG which profoundly enhances phagocytic ability. In fact, EAE was shown recently to be unique compared to other neurological diseases as the surface profile of nearly all microglia was seen to shift as a unit, while in other conditions like aging and mouse models of Alzheimer's disease, a small population of microglial cells was shown to change in terms of their surface marker profile (Mrdjen et al., 2018).

A receptor-mediated role for myelin clearance was also shown recently with TREM2-deficient mice exhibiting impaired clearance of myelin debris in the cuprizone model of demyelination (Poliani et al., 2015). *Trem2*$^{-/-}$ mice exhibited impaired clearance of debris that was also associated with impaired microglial proliferation during demyelination post cuprizone treatment, as well as during healthy aging. However, a strict microglia role for TREM2-mediated clearance of myelin was not addressed in this paper as peripheral myeloid cell engraftment is classically seen with cuprizone treatment (Lampron et al., 2015).

Trem2 has also been shown to play a critical role in the recognition of amyloid plaques in Alzheimer's disease as TREM2 is capable of recognizing many lipid moieties and motifs on amyloid plaques, including APOE, LDL, and Clusterin (Yeh et al., 2016). When crossed to the

5XFAD mice, a severe mouse model of Alzheimer's disease (Oakley et al., 2006), increased plaque burden and a decrease in microgliosis were observed, indicating that microglial TREM2 was necessary for proper proliferation and activation in Alzheimer's disease models. This was further reinforced by transcriptional profiling through RNA sequencing which showed that TREM2 was necessary for formation of Disease Associated Microglia, or DAMs (Keren-Shaul et al., 2017). TREM2 dysfunction was also shown to be important to microglial metabolism with microglia deficient in TREM2 increasing autophagic processes which ultimately led them to be more susceptible to cell death (Ulland et al., 2017). In fact, rescue of metabolic deficiencies with cyclocreatine supplementation to mice led to enhanced microglial energy balance and a rescue of neuronal dysfunction (Ulland et al., 2017).

These works indicate that TREM2 may be essential for promoting a neuroprotective "activated" phenotype of microglia, allowing for proper containment and response to amyloid plaques and pathology. Additional works pointing to this hypothesis emerged when two types of Alzheimer's disease models were crossed to IL-10 deficient mice. IL-10 has been documented as a master anti-inflammatory cytokine, capable of suppressing immunity and ensuring tissue protection (Ouyang et al., 2011; Couper et al., 2008). In two works published in the journal Neuron in 2015, it was found that IL-10 deficient animals crossed to two different mouse models of Alzheimer's disease were shown to have enhanced phagocytic capacity of amyloid plaques, preservation of synaptic material, and restoration of cognitive function (Guillot-Sestier et al., 2015; Chakrabarty et al., 2015). These data point to a model of "protective activation" in microglia that may aid in easement of Alzheimer's pathology, specifically synapse loss.

While the phagocytosis of amyloid plaques in mouse models of Alzheimer's disease is a growing topic of basic and translational study (Lai and McLauring, 2012; Fiala et al., 2007;

Koenigsknecht, 2004) an emerging body of literature has pointed to the role of complement proteins (discussed previously concerning developmental synapse engulfment) in the removal of synapses during Alzheimer's pathology at time points that occur both before amyloid deposition and cognitive decline, with the hypothesis that complement mediated removal of synapses by microglia may be a key pathology of Alzheimer's disease models in mice and in human pathology.

Complement has been shown to be associated with Alzheimer's disease in a recent GWAS study (Tosto and Reitz, 2013). Its role in synapse loss during animal models in the early stages of disease was also shown with a marked deposition of C1q occurring in the J20 mouse model of Alzheimer's disease at only three months of age, well before plaque formation (Hong et al., 2016). This coincided with synapse loss and microglial engulfment of synaptic terminals that led to neuronal dysfunction that was blocked by antibody mediated blockade of C1q (Hong et al., 2016).

A mouse model of frontotemporal dementia (FTD) by germline deletion of the progranulin gene ($Grn^{-/-}$) also showed a key role for complement in microglia-mediated synapse loss (Lui et al., 2015). Reduction of complement by crossing these mice to $C1qa^{-/-}$ mice led to a complete reversal of the synapse loss phenotype as well as reversal of aberrant neuronal dysfunction in the ventral thalamus and a reduction in obsessive compulsive like behavior (Lui et al., 2015).

Surprisingly, complement was shown to play a minimal role in the MPTP-mediated mouse model of Parkinson disease, despite an increased presence of complement in Parkinsonian tissue and CSF (Depboylu et al., 2011; Orsini et al., 2014). MPTP did induce a marked upregulation of C1q in the substantia nigra coinciding with loss of tyrosine hydroxylase positive neurons. However, microglial activation or substantia nigra neuron loss was unchanged following MPTP treatment in $C1qa^{-/-}$ mice compared to their wild type littermates (Depboylu et al., 2011). This study was limited, however, in that the researchers only looked within the substantia nigra and did

not investigate alterations to synapses downstream of the substantia nigra or the presence of Lewy bodies in *C1qa*$^{-/-}$ mice with MPTP treatment.

Complement pathway activation has also been seen in spinal cord tissue and CSF from amyotrophic lateral sclerosis (ALS) patients (Orsini et al., 2014). Microglia have been highlighted in ALS as key drivers of pathology, with complement and other pro-inflammatory pathways upregulated in the spinal cord of mouse models of ALS (Butovsky et al., 2015; Chiu et al., 2009). Direct targeting of microglial NF-κB also showed that ALS neuronal pathology could be reversed, placing a key role for microglial inflammation as a contributor to neurological dysfunction in ALS, as astrocyte deletion of NF-κB had no effect on disease outcome (Frakes et al., 2014). A specific role for C1q was also shown in the mutant SOD1 mouse model of ALS. SOD1 mutant mice were crossed to *C1qa*$^{-/-}$ mice which resulted in an increase in presynaptic inputs to motor neurons. Despite this, there was no change to overall motor neuron survival or disease outcome (Lobsiger et al., 2013). The same phenotype in ALS mouse models was also seen for mice deficient in C3 (Lobsiger et al., 2013) or C4 (Chiu et al., 2009) complement proteins. These data collectively point to a role for complement specifically in synapse elimination, since no change in overall motor neuron cell numbers were seen in this model of neurodegeneration.

Complement proteins have also been implicated in the progressive neurodegenerative disorder known as Huntington's disease, where neuronal and microglial dysfunction is seen resulting from an expansion of the PolyQ repeat segment of the Huntingtin protein (Singhrao et al., 1999; Crotti and Glass, 2015). Future studies will most likely show a microglial-dependent complement deposition underlying some form of neuronal dysfunction in a pattern seen with most neurodegenerative disorders.

While there is controversy as to whether Schizophrenia is a bona fide neurodegenerative disorder (Kochunov and Hong, 2014; Kulhara and Gupta, 2010; Pérez-Neri et al., 2006), a role for complement and immune activation were hinted at with GWAS studies in Schizophrenia patients identifying the MHC as a region with the strongest genetic link to disease pathology (Ripke et al., 2014). A further examination into patient genetics and postmortem tissue showed that the complement protein C4 was associated with synaptic processes in the hippocampus (Sekar et al., 2016). Its further role in engulfment of synaptic material in LGN development was shown by C4-deficient mice exhibiting overlap of presynaptic terminals within the dLGN (Sekar et al., 2016).

Synaptic and neuronal dysfunction are also disease hallmarks of chronic brain infections where viruses or pathogenic organisms may persist and directly affect neuronal function, activate the immune system and induce neuronal dysfunction, or decrease CD4 T cell counts which yield cognitive impairment. These are all factors shown to ultimately lead to cognitive decline (Ene et al., 2016; Hong and Banks, 2015; Kipnis et al., 2008; Samaan et al., 2016; Carson et al., 2006). Two models that will be discussed in terms of synaptic dysfunction, glial activation, and the complement system are infection by the protozoan *Toxoplasma gondii* (Toxo), and the flavivirus West Nile (WNV).

Capable of infecting a variety of warm-blooded hosts, Toxo infection consists of a two-phase process in mice, with the acute phase consisting of fast-replicating tachyzoite infection which is cleared from the periphery in a matter of weeks, followed by a chronic infection of fairly immunoquiescent bradyzoites within the CNS that last for the lifespan of the mouse (Hunter and Sibley, 2012). Despite its relatively innocuous effect on immune activation in the chronic phase in terms of visible pathology, infiltration of T cells and peripheral monocytes, microglial and astrocytic activation, as well as BBB breach are all evident in the CNS parenchyma (Hunter and

Sibley, 2012). Focal reaction of bradyzoite cysts also leads to localized neuronal death and immune cell activation, which can be further disruptive in an immunocompromised host (Halonen, 2004). Corresponding with this continued activation of immune cells within the brain are neurological symptoms in humans, which may include increased risk of schizophrenia (Torrey and Yolken, 2003), impaired auditory learning, and risk of suicide, as well as possible increases in risk-taking behaviors (Sugden et al., 2016). In mouse models, contextual freezing and extinction of memory are impaired, coinciding with an increase in metabolites for the neurotransmitter dopamine and a decrease in metabolites for both serotonin and norepinephrine (Ihara et al., 2016). These phenotypes were also associated with synapse mislocalization and seizure susceptibility (Brooks et al., 2015), along with decreased axonal density, decreased cortical pyramidal neuron complexity, and reduced synaptic density in both the hippocampus and cortex (Parlog et al., 2014). Despite these neuronal abnormalities, the literature points to an attenuated microglial phenotype during Toxo infection with TGF-β inhibiting iNOS and NO production that could be further detrimental to neuronal function (Rozenfeld et al., 2005). Elevated C1q was also seen in the chronic phase of Toxo infection, with C1q deposition localized to both bradyzoite cysts and neuronal processes (Xiao et al., 2016). Microglial responses with respect to synaptic destruction have not been forthcoming within the Toxo literature, with synaptic alteration or removal by microglia most likely preceding the frank immunopathology seen during the chronic disease stage.

West Nile Virus (WNV) is the most widely distributed arbovirus (a virus delivered by an arthropod such as mosquitos or ticks) with a range that includes every continent except Antarctica (Kramer et al., 2008). Patients with acute encephalitis relating to WNV present with fever, headache, and altered mental status. Unfortunately, patients surviving WNV often experience persistent memory impairment, speech disorders, or cognitive impairment (Sadek et al., 2010).

During these processes it has been shown that the complement pathway, which is important for host defense in the periphery and synapse alteration in development, is also at play during cognitive impairment following WNV (Vasek et al., 2016).

In a model of memory impairment weeks after acute WNV infection, it was shown that microglia are phagocytic of neuronal processes and that a complement-related gene signature was elevated in the hippocampus, possibly interfering with spatial memory (Vasek et al., 2016). A key finding was that removal of microglia via infection of IL-34 deficient mice or by knocking out C3 or the C3a receptor resulted in a rescue of hippocampal synapse loss. Unfortunately, the impact of microglia or C3 on cognitive dysfunction was not tested. A role for the phagocytic receptors MER and AXL (discussed in chapter four) were also investigated in a mouse model of WNV with endothelial cell expression of both these molecules necessary for proper BBB permeability and viral control (Miner et al., 2015).

Thesis rationale

The basis of the experiments and logic behind this thesis work are two-fold and can be summarized by two experimental questions:

1. ***Are clearance mechanisms of synaptic material conserved during development and after CNS trauma?***
2. ***How is microglial engulfment of neuronal debris regulated in the brain parenchyma after CNS injury?***

Detailed in the following chapters will be our work published in the *Journal of Experimental Medicine* detailing the kinetics and mechanisms of microglial clearance of debris following Wallerian degeneration of retinal projections post optic nerve crush injury (Chapter 3). This work documented that contrary to what is seen during development of the visual system, microglia are the key mediators of debris clearance, behaving independently of neuronal activity and performing a classical tissue resident macrophage function of debris clearance. In development, synapse clearance is a well-orchestrated balance of neuronal activity driving "pruning" of improper connections predominantly by astrocytes with microglia also playing a phagocytic role. In contrast, we observed that in acute injury during adulthood, microglia were the key phagocytic cell responsible for debris clearance. This elucidates a critical aspect of microglial biology with neuronal activity driving microglial responses in development, while their activity in pathology resembles a function much more akin to other macrophage populations.

Since such important works in development have shown critical roles for glial phagocytosis in forming the visual system with both microglia and astrocytes contributing to the direct formation of neuronal connections, our studies here provide a clear signal that outside of critical periods, microglia can be the sole phagocytic responders, and showcase that their professional phagocyte function is sufficient to ensure complete clearance of debris, in a manner distinct from developmental glial function and reflective rather of the essential immune process of phagocytosis.

We also observed a profound change in the dLGN microglial transcriptional profile starting 24 hours after injury and changing dramatically again 72 hours post injury, reflective of the very plastic nature of these cells in response to CNS trauma. Surprisingly, we found that this clearance

was mediated by elements of the complement system, a feature of microglial clearance found in both development and CNS pathology.

Further attempts at mechanistic dissections of microglial phagocytosis in our model are examined in Chapter 4. We found that a variety of canonical phagocytic receptors (previewed and discussed in Chapter 4) are dispensable for microglial clearance of debris, pointing to a common feature of tissue resident macrophages where phagocytic receptor repertoire and requirement are specific to tissue and cell type (Gonzalez et al., 2017; Martinez et al., 2009; Gordon and Plüddemann, 2017).

Recent works have sought to categorize microglia as a cell type unique among macrophages, capable of shaping neuronal form and function. While some functions may in fact be unique to microglia, this thesis represents a collective work ascribing a role for microglia similar to that seen for any tissue resident macrophage with neurodegeneration eliciting a macrophage-specific response towards debris clearance.

Chapter 3

Neuronal integrity, not activity, drives synaptic material clearance by microglia after CNS injury

We first assessed the function and kinetics of dLGN microglia in response to neurodegeneration. To this end, we injected the right eyes of wild-type (WT) mice with cholera toxin subunit B (CTB) conjugated to Alexa Fluor 647, 24 h before subjecting the mice to crush injury (Schwartz, 2004). (Fig. 3.1 A). Following the injury, mice were killed at various time points (days 1, 2, 3, 4, 5 ,7, and 28 after crush). Measurement of CTB-tagged retinogeniculate inputs into the dLGN, contralateral to the injected eye, revealed their progressive loss with substantial loss observed on day 3 and an even greater loss (~70%) on day 7 after the crush injury (Fig. 3.2, B and C). By day 28, no CTB-647 signal could be detected (Fig. 3.1, B and C). We observed an increase in the numbers of Iba1+ cells in the dLGN, starting on day 2 after the crush injury and peaking on day 7 (Fig. 3.1, B and D), similar to what has been previously seen in nerve injury models (Bartel, 2012; Tay et al., 2017). Ki67+ (proliferating) microglia were detectable by post-injury day 2, with a significant increase in their numbers on days 3 and 4, followed by a marked reduction on day 5 (Fig. 3.1, B and E).

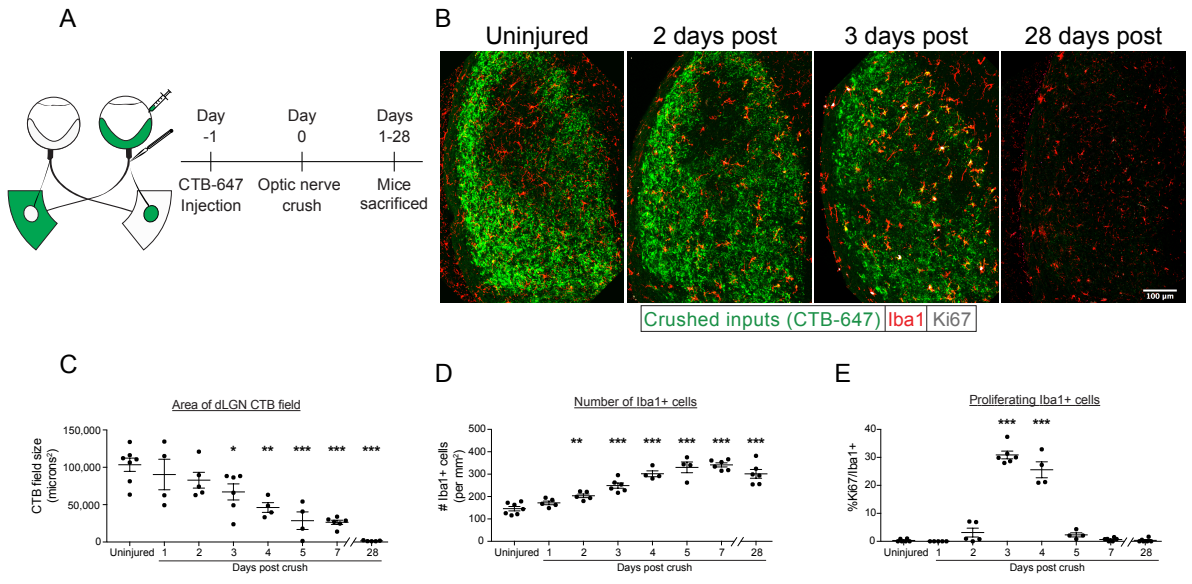

Figure 3.1 **Optic nerve crush induces retinogeniculate terminal degeneration and proliferation of Iba1+ cells.** (A) Scheme of CTB-labeling and optic nerve crush paradigm. (B) Representative images of the degenerating dLGN presynaptic field contralateral to the crushed eye, and Iba1+ and Ki67+ cells (scale bar = 100 microns). (C) Quantification of the degenerating presynaptic debris field shown in (B) (n = 4-7 mice per group; one-way ANOVA with Dunnett's post-hoc test compared to uninjured condition, representative of two independently performed experiments). Quantification of (D) Iba1+ and (E) Iba1/Ki67+ cells in the dLGN following optic nerve crush (n = 4-7 mice per group; one-way ANOVA with Dunnett's post hoc test compared to uninjured condition, representative of two independently performed experiments).

To demonstrate that the gradual disappearance of CTB labeling actually corresponded to the loss of presynaptic terminals, we quantified retinogeniculate synapses in areas of CTB loss within the dLGN contralateral to the crushed eye on day 3 after optic nerve crush injury. Utilizing a method previously employed to count synapses of the mouse visual system (Ippolito and Eroglu, 2010), we observed a significant decrease in retinogeniculate (VGlut2/PSD95 double-positive) synapses (Fig.3.2, A and B), suggesting that the disappearance of CTB observed after the injury was indeed related to the loss of retinogeniculate terminals.

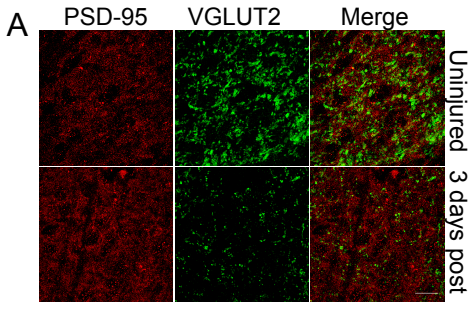

Figure 3.2 **Optic nerve crush injury leads to retinogeniculate synapse loss.** (A) Representative images of VGlut2/PSD95 double positive retinogeniculate synapse loss three days post optic nerve crush (scale bar = 25 microns). (B) Quantification of synapse loss following optic nerve crush (n = 3 mice per group; Student's t-test, representative of two independently performed experiments).

Noting an activated and hypertrophic morphology of microglia post-crush we hypothesized whether the novel microglia-specific marker TMEM119 (Bennett et al., 2016) would still be present on microglia three days post-injury (Fig. 3.3, A and B). Strikingly, we saw a marked downregulation of TMEM119. This data shows that microglial activation leads to loss of TMEM119 positivity, at least during CNS Wallerian degeneration, and that caution should be used when interpreting TMEM119 labeling, other than in naïve mice.

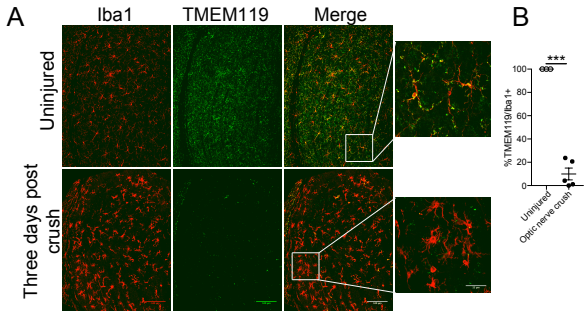

Figure 3.3 **TMEM119 is positivity is lost on Iba1+ cells three days post optic nerve crush injury** (A) Representative confocal images of Iba1 and TMEM119 staining from uninjured mice and three days post injury. (B) Quantification of TMEM119+ microglia before and three days post optic nerve injury depicted in (A) (mean ± s.e.m; n = 3-5 mice per group; *** = $p < 0.001$, Student's T-test, representative of two independent experiments).

The optic nerve crush injury model is unique in that it allows for the study of cellular responses to neurodegeneration that occur far from the actual site of injury. This is particularly important when attempting to assess the role of microglia, as the injury site is heavily populated by peripheral cells (Walsh et al., 2014b) that may compete and even interfere with microglial

function and complicate microglial identification. Before proceeding with nerve injury and assessment of microglial responses we first needed to confirm that, in the context of our system that microglia could indeed be studied in isolation from contamination by peripheral macrophages. We did this by fate-labeling of microglia, utilizing *Cx3cr1*$^{ERT2Cre/+}$:Ai6 mice. By modifying a previously published protocol (Goldmann et al., 2013), mice were fed tamoxifen-containing chow for one week and then we proceeded to wait 1 month for peripheral myeloid cell turnover (Fig. 3.4, A). After this period, we analyzed the brain and peripheral organs for macrophage expression of ZsGreen. We observed that nearly 100% of microglia retained ZsGreen labeling in the uninjured cerebral cortex and cerebellum (Fig. 3.4, B and D). This contrasted with peripheral macrophage populations which exhibited decreasing percentage of ZsGreen+ macrophage populations in the spleen, liver, and blood, respectively (Fig. 3.4, B-D). We then performed unilateral optic nerve crush and sacrificed the injured mice 3 days post-injury. Examining the injury site in the optic nerve , we observed numerous Iba1^{+}/ZsGreen^{-} cells, indicative of peripheral myeloid engraftment there (Fig. 3.4, E), as has been previously reported (Walsh et al., 2014b).

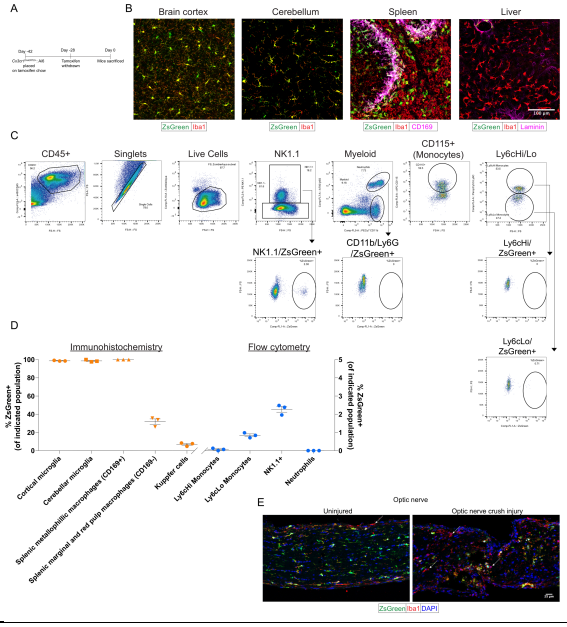

Figure 3.4 **Macrophage ZsGreen expression at baseline in immune organs and optic nerve crush site three days post injury in Cx3cr1$^{ERT2Cre/+}$:AI6 mice** (A) Scheme of fate-mapping experiment. (B) Representative images of macrophage subsets in brain regions, spleen, and liver. Within the dLGN before and after crush injury the vast majority of myeloid cells (Iba1+) were ZsGreen positive (Fig. 3.5, A and B) with no change in number of Iba1+ or Iba1+/ZsGreen+ cells (Fig. 3.5, B and C) Furthermore, there was no difference in the percentages of Iba1+ or Iba1+/ZsGreen+ cells expressing Ki67 in dLGN after injury (Fig. 3.5, C and D), implying that the Iba1+ cells were derived from endogenous microglial populations.

The unique model we describe here incorporates crush injury of the optic nerve and the consequent neurodegeneration in the dLGN, distal to the injury site itself. Thus, this allows a 'closed system' to study phagocytic uptake of neuronal material with no prior priming of microglia BBB disruption, or peripheral myeloid "contamination".

Since microglia have been shown to be prolific phagocytes of synaptic material (Schafer et al., 2012), we examined whether the observed phagocytosis and clearance of presynaptic terminals were attributable to dLGN microglia. Utilizing a previously described method (Schafer et al., 2014), we observed that neuronal debris (presented as the percentage of microglial volume) was indeed present within the microglial soma (Fig. 3.6, A and B). In CX3CR1$^{GFP/+}$ mice, co-staining for engulfed neuronal debris alongside the phagolysosomal marker CD68 revealed a high degree of internalized CTB co-localized with the microglial phagolysosome (Fig. 3.7, A), indicative of microglial phagocytosis.

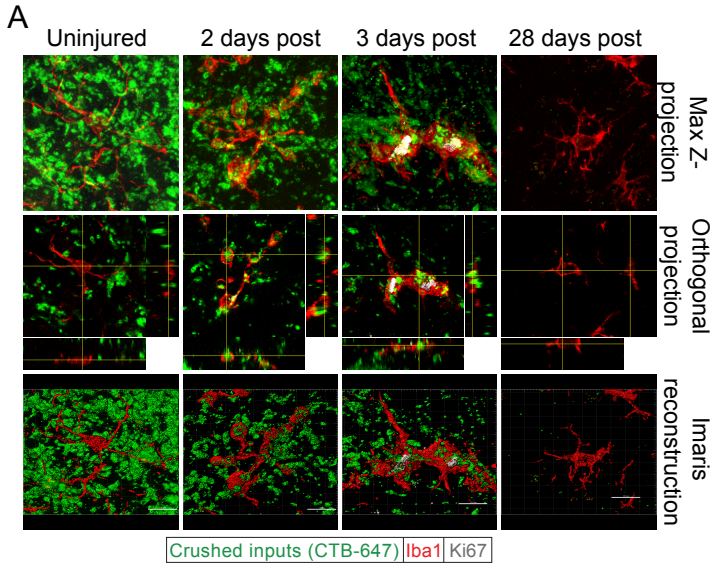

Figure 3.6 **Engulfment of neuronal debris peaks at three days post optic nerve injury in microglia** (A) Z-projections, orthogonal slices, and Imaris 3D reconstructions of microglial phagocytosis of crushed retinogeniculate inputs (scale bar = 25 microns). (B) Quantification of microglial engulfment of neuronal debris depicted in (L) (n = 4-6 mice per group; one-way ANOVA with Dunnett's post-hoc test compared to uninjured dLGN microglia, representative of two independently performed experiments).

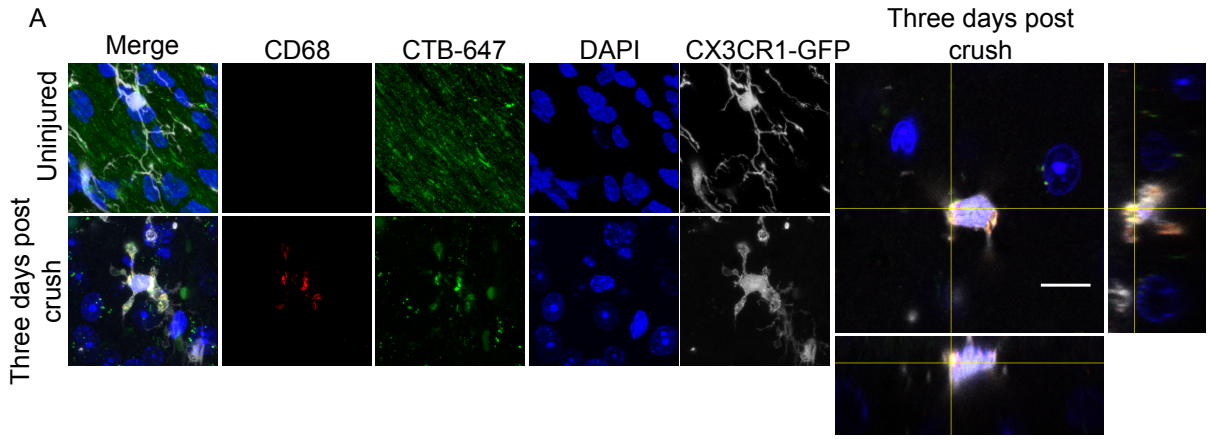

Figure 3.7 **Engulfed neuronal debris is localized to the microglial phagolysosome** (A) Representative confocal images and orthogonal sections of microglia three days post optic nerve crush in *Cx3cr1*$^{GFP/+}$ mice showing localization of engulfed neuronal debris to the CD68+ phagolysosome.

Microglia are known to play a role in the pruning of synapses during the developmental period of eye-specific segregation at post-natal day 5 (P5), with the soma of microglia exhibiting a higher proportion of ingested material compared to astrocytes. However, due to their increased number compared to microglia at that developmental time period, the dominant LGN phagocytes in terms of total material consumed are astrocytes (Chung et al., 2013). To assess the possibility that astrocytes could be phagocytic of neuronal debris following optic nerve crush injury we quantified the number of cells positive for the astrocyte marker, glial fibrillary acidic protein (GFAP), post injury (Fig. 3.8, A). No change was seen in GFAP+ cell number at three days post crush, despite a robust increase in microglial number (Fig. 3.8, A and B). To analyze the engulfment of debris by astrocytes we performed our assessment of phagocytosis by examining the amount of CTB+ debris in both Iba1+ and GFAP+ processes. With crush no change in engulfed material was seen in GFAP+ processes despite an increase seen within the microglial Iba1+ soma and processes (Fig 3.8, C and D).

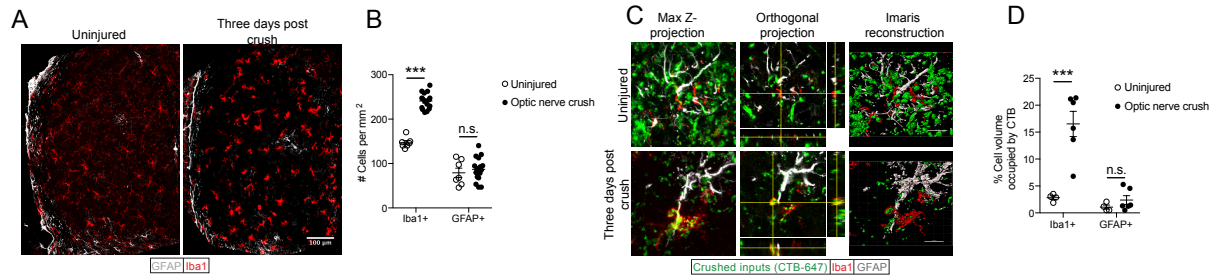

Figure 3.8 **Change in cell number and engulfment of neuronal debris is not seen in GFAP+ cells three days post optic nerve crush injury** (A) Representative images of Iba1+ and GFAP+ cell number in the LGN in uninjured LGN and three days post optic nerve crush injury. (B) Quantification of Iba1+ and GFAP+ cells seen in (A) (mean ± s.e.m; n = 7 mice for uninjured and n=16 for crushed groups; *** = $p < 0.001$, two-way ANOVA with Tukey's post-hoc test, representative of two independent experiments). (C) Max Z-projections, orthogonals, and Imaris reconstructions of engulfment by Iba1+ and GFAP+ cells in uninjured LGN and three days post optic nerve crush injury. (D) Quantification of Iba1+ and GFAP+ cell engulfment seen in (C) (mean ± s.e.m; n = 4 mice for uninjured and n=6 for crushed groups; *** = $p < 0.001$, two-way ANOVA with Tukey's post-hoc test, representative of two independent experiments).

Relying on GFAP as marker for engulfment assays is limiting due the minimal filling of astrocyte soma and incomplete expression by astrocytes that reside outside of the white matter (Lundgaard et al., 2014). Due to these limitations we further assessed the phagocytic capabilities of LGN astrocytes and microglia during acute Wallerian degeneration using flow cytometry. Bilateral CTB-A647 injection and crush injury were performed, followed by LGN microdissection three days later (Fig. 3.9, A). Cell populations were verified by gating on nucleated Hoechst+ events, single cell events (singlets), and cells not positive for the dead cell dye Zombie NIR (live cells). To mark astrocytes, we employed the extracellular marker GLAST. GLAST- cells where then selected on CD11b/CD45 positivity to define microglia (Fig. 3.9, B). While a significant increase in the amount of CTB was only seen in the microglial population, we did see a small, yet not significant, increase in CTB+ astrocyte populations with CTB injected eyes in both uninjured and post-crush conditions (Fig 3.9, C and D). To distinguish whether this positivity was due to internalization of CTB or extracellular attachment, we utilized a cholera toxin B subunit

conjugated to the pH-sensitive dye CypHer 5E. This compound is maximally fluorescent at low pH with minimal fluorescence at neutral pH (Cooper et al., 2002). Thus, only cells with engulfed CTB should exhibit CypHer positivity. After bilateral eye injection with CTB-CypHer followed by optic crush, only CypHer+ microglia, and not astrocytes, were observed (Fig. 3.9, E and F).

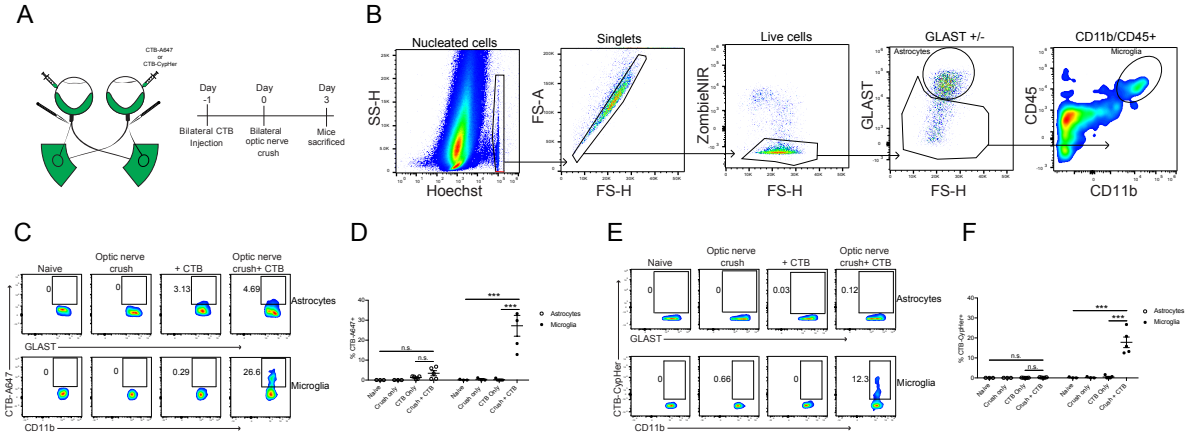

Figure 3.9 **Engulfment of neuronal material is seen in LGN microglia and not astrocytes three days post optic nerve crush injury** (A) Scheme of flow cytometry experiments to identify LGN phagocytes following optic nerve crush injury. (B) Gating strategy to obtain astrocytes (GLAST+) and microglia (CD11b+CD45+) from the LGN. (C) Representative FACS plots of astrocytic and microglial engulfment of CTB-A647 labeled neuronal debris. (D) Quantification of engulfment depicted in (C) (mean ± s.e.m; n = 3-5 mice per group; *** = $p < 0.001$, two-way ANOVA for effect of cell type and treatment with Holm-Sidak post hoc test, representative of three independent experiments). (E) Representative FACS plots of astrocytic and microglial engulfment of CTB-CypHer labeled neuronal debris. (F) Quantification of engulfment depicted in (E) (mean ± s.e.m; n = 3-5 mice per group; *** = $p < 0.001$, two-way ANOVA for effect of cell type and treatment with Holm-Sidak post hoc test, representative of three independent experiments).

To assess the necessity of microglia in the clearance of neuronal debris after injury in adult mice, we eliminated microglia in our system by utilizing the colony stimulating factor 1 receptor (CSF1R) inhibitor PLX5622, a compound previously shown to efficiently deplete microglia in the CNS (Elmore et al., 2014). After 3 weeks of treatment with PLX5622 chow, mice received bilateral injections of CTB intravitreally and were subjected 24 h later to unilateral optic nerve crush injury. As previously shown (Hilla et al., 2017), PLX5622 treatment also had no effect on RGC survival

three (Fig. 3.10, A and B) and seven (Fig. 3.10, C and D) days post optic nerve crush injury. As expected, PLX5622 also resulted in a marked reduction in microglial counts (Fig. 3.11, A and B). Subsequently, CTB+ dLGN fields contralateral to crushed optic nerves were significantly larger three days post optic nerve crush injury in PLX5622-fed mice than in mice fed control chow, suggesting that following optic nerve crush injury, microglia are necessary for the optimal clearance of neuronal debris (Fig. 3.11, A and C). In fact, microglial depletion and debris presence persisted in PLX5622-fed mice up to seven days post optic nerve crush injury (Fig. 3.12, A-C). Notable was that at both three and seven days post optic nerve injury control inputs had no differences between control and PLX5622-fed mice.

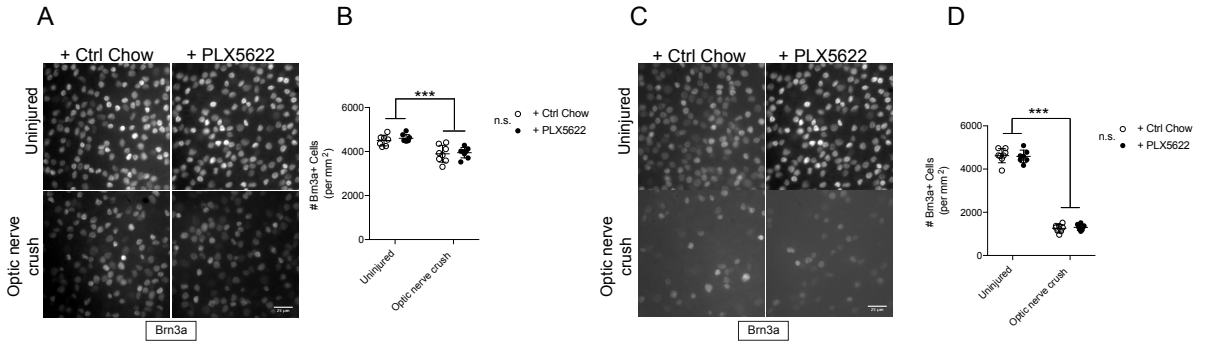

Figure 3.10 **PLX5622 treatment has no effect on RGC survival at baseline or with optic nerve crush injury** (A) Representative images of uninjured and three days post- optic nerve crushed injury-experienced RGCs in the retina of PLX5622 and control chow-fed mice. (C) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 7 retinas per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test, representative of three independent experiments). (C) Representative images of uninjured and seven days post-optic nerve crushed injury-experienced RGCs in the retina of PLX5622 and control chow-fed mice. (D) Quantification of RGC counts seen in (C) (mean ± s.e.m; n = 4-5 retinas per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test, representative of three independent experiments).

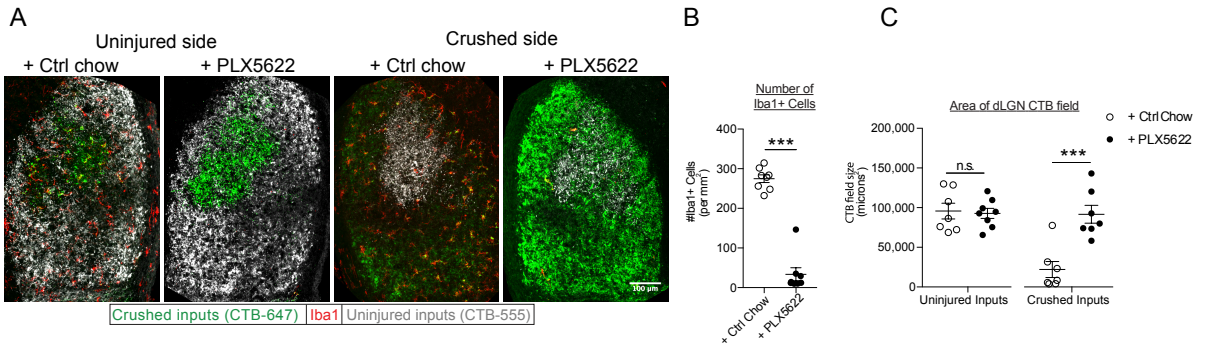

Figure 3.11 **PLX5622 reduces microglia number and prevents clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the uninjured and optic nerve crushed dLGN fields three days post injury in PLX5622 and control chow-fed mice (scale bar = 100 microns). (B) Quantification of Iba1+ cells (depicted in A) in the crushed contralateral field three days post optic nerve crush in PLX5622 and control chow-fed mice (n = 7-8 mice per group; Student's t-test, representative of three independently performed experiments). (C) Quantification of the CTB+ field (depicted in A) contralateral to uninjured and crushed eyes three days post optic nerve crush in PLX5622 and control chow-fed mice (n = 7-8 mice per group. Student's t-test, representative of three independently performed experiments). Error bars represent mean ± S.E.M. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

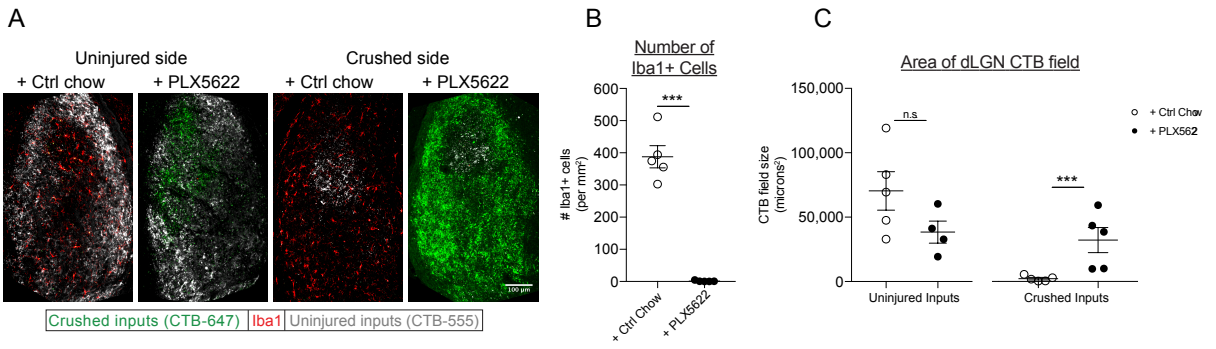

Figure 3.12 **PLX5622 reduces microglia number and prevents clearance of neuronal debris seven days post optic nerve crush injury** (A) Representative images of the uninjured and optic nerve crushed dLGN fields seven days post injury in PLX5622 and control chow-fed mice (scale bar = 100 microns). (B) Quantification of Iba1+ cells (depicted in A) in the crushed contralateral field seven days post optic nerve crush in PLX5622 and control chow-fed mice (n = 5 mice per group; Student's t-test, representative of two independently performed experiments). (C) Quantification of the CTB+ field (depicted in B) contralateral to uninjured and crushed eyes seven days post optic nerve crush in PLX5622 and control chow-fed mice (n = 4-5 mice per group. Student's t-test, representative of two independently performed experiments. Error bars represent mean ± S.E.M. *** = $p < 0.001$).

The rapid response of dLGN microglia observed after optic nerve crush prompted us to investigate whether neuronal activity from the retina, shown to be crucial for engulfment of neuronal material in development (Schafer et al., 2012), might regulate microglial activation in response to optic nerve injury, especially since all neuronal activity from the retina is known to be

rapidly disrupted following optic nerve crush in the mouse. To address this question we utilized *Wld*^{S} mice (Coleman et al., 1998), which demonstrate delayed axonal and synaptic degeneration after acute injury to the peripheral and central nervous systems (Vargas and Barres, 2007; Gillingwater et al., 2006; Wright et al., 2010). Thus, by studying the microglia in the acutely injured *Wld*^{S} dLGN, we were able to examine a system in which neuronal activity had ceased (as in the injured WT), but presynaptic degeneration was markedly delayed (Fig. 3.13, A and B). Before examining the dLGN we counted the RGCs in *Wld*^{S} mice 3 days after optic nerve crush and observed no changes in their numbers (Fig. 3.14, A and B). We then examined the dLGN and found significantly less microglia in the dLGN contralateral to the crushed eye of *Wld*^{S} mice than in their WT counterparts, indicating that little or no dLGN microglial proliferation had occurred in the *Wld*^{S} mice by day 3 after the optic nerve crush (Fig. 3.15, A and B). The CTB+ field of the dLGN contralateral to the crushed optic nerve was also significantly larger in the *Wld*^{S} mice, which indicated a reduced microglial clearance of the CTB+ presynaptic field in *Wld*^{S} mice (Fig. 3.15, A and C). CTB+ field size in the dLGN of *Wld*^{S} mice contralateral to the uninjured optic nerve were also unchanged (Fig. 3.15, A and C), indicating that—as shown previously (Hoopfer et al., 2006)—there were no developmental changes in retinogeniculate projections in *Wld*^{S} mice. Since we noticed a robust proliferation of WT microglia on days 2-4 post optic nerve crush we also stained for the proliferative marker Ki67 to ascertain whether microglial proliferation was attenuated in *Wld*^{S} mice and thus with delayed neuronal degeneration. We observed that along with the reduction in microglia number was also a decrease in Ki67+ microglia, implying that microglial proliferation is greatly diminished when neurodegeneration is delayed (Fig. 3.16, A and B).

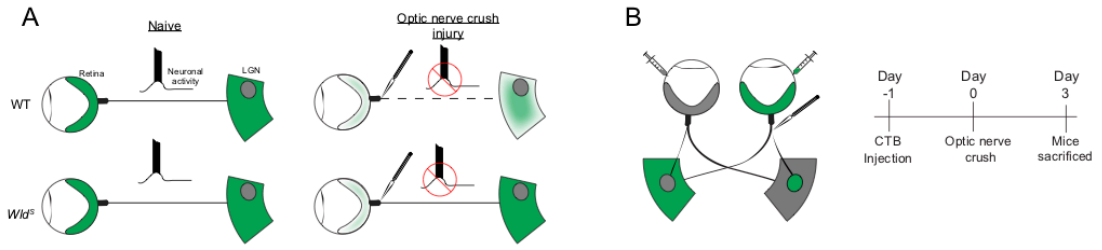

Figure 3.13 **Scheme of optic nerve crush injury in *Wld*^{S} mice** (A) Scheme of neuronal activity and Wallerian degeneration following optic nerve crush in WT and *Wld*^{S} mice. (B) Experimental setup of optic nerve crush injury in WT and *Wld*^{S} mice.

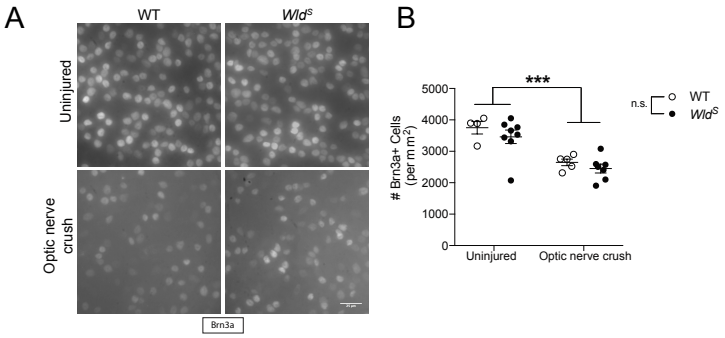

Figure 3.14 **RGC counts are unchanged at baseline or three days post optic nerve crush injury in *Wld*^{S} mice** (A) Representative images of uninjured and three days post optic nerve crushed injury-experienced RGCs in the retina of *Wld*^{S} and WT mice. (B) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 4-8 retinas per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test, representative of three independent experiments.

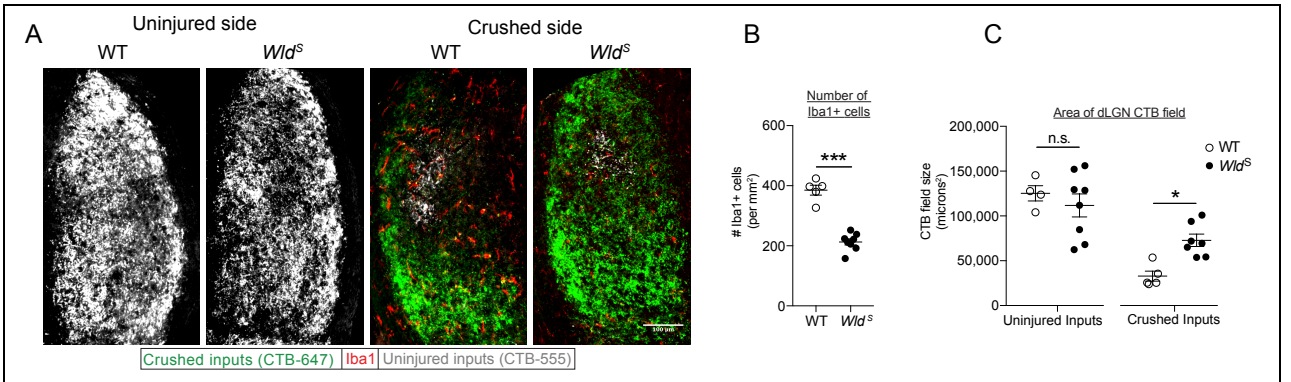

Figure 3.15 **Microglia number and clearance of neuronal debris is reduced in *Wld*^{S} mice three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in WT and *Wld*^{S} mice. (B) Quantification of Iba1+ cells (depicted in C) three days post optic nerve crush in WT and *Wld*^{S} mice (n = 5-8 mice per group; Student's t-test, representative of three independent experiments). (C) Quantification of uninjured and crushed CTB fields (depicted in A) three days post optic nerve crush in WT and *Wld*^{S} mice (n = 4-8 mice per group; Student's t-test, representative of three independent experiments).

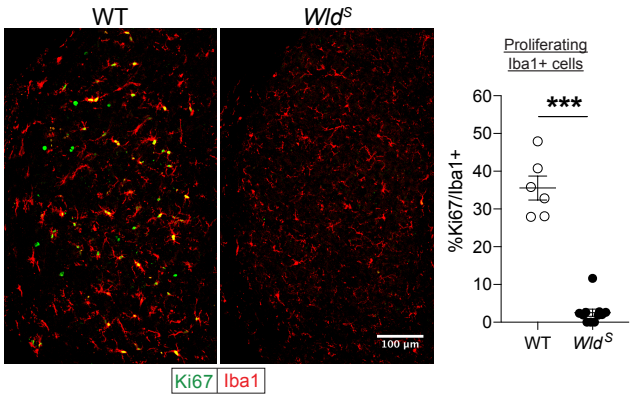

Figure 3.16 **Microglia proliferation is reduced in *Wld*^{S} mice three days post optic nerve crush injury** (A) Representative images of Ki67+ dLGN microglia three days post optic nerve crush in WT and *Wld*^{S} mice. (B) Quantification of Ki67/Iba1+ cells (depicted in A) three days post optic nerve crush in WT and *Wld*^{S} mice (n = 5-8 mice per group; Student's t-test, representative of three independent experiments).

Since no proliferation or apparent activation was visible in *Wld*^{S} microglia of the dLGN after injury we performed our phagocytosis assay to see if these cells may still be phagocytic, despite an activated morphology. Our analysis of phagocytosis of crushed neuronal inputs in the *Wld*^{S} mice also revealed a much less activated morphology resembling uninjured WT microglia when imaging at high resolution. Phagocytic analysis also showed that *Wld*^{S} mice had significantly less engulfed neuronal debris than in WT microglia at three days post injury (Fig. 3.17, A and B).

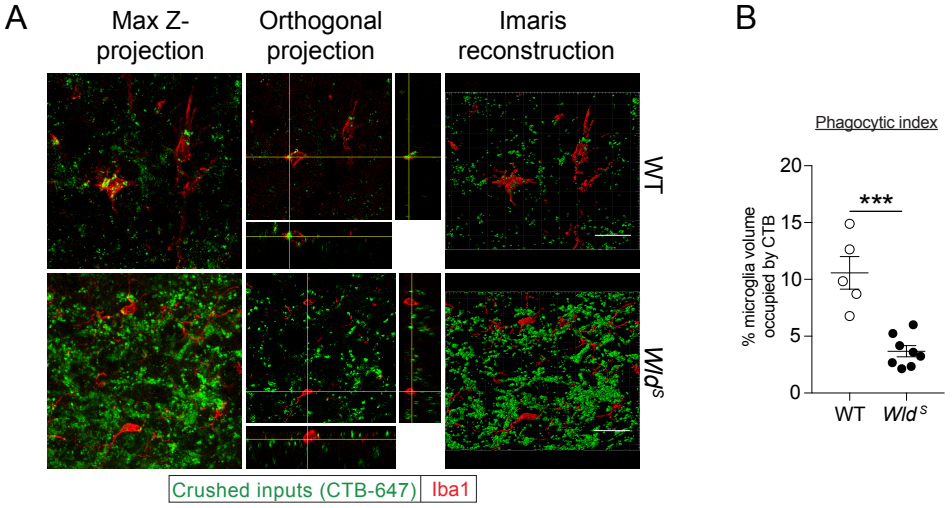

Figure 3.17 **Engulfment of neuronal material is reduced in *Wld*^{S} mice three days post optic nerve crush injury** (A) Z-projections, orthogonal slices, and Imaris 3D reconstructions of microglial phagocytosis of crushed retinogeniculate inputs in WT and *Wld*^{S} mice three days post optic nerve crush. (B) Quantification of microglial engulfment of neuronal debris in WT and *Wld*^{S} mice depicted in (A) (n = 5-8 mice per group; Student's t-test, representative of three independent experiments).

To further determine whether presynaptic terminals lacking neuronal activity from the retina could be engulfed by injury-activated microglia, we took advantage of the dLGN architecture (consisting of a small ipsilateral patch within the large contralateral projection area). Using pharmacological attenuation of neuronal activity in the ipsilateral eye concurrently with optic nerve crush in the contralateral eye, we tested whether injury-activated microglia would engulf weakened inputs (from the ipsilateral patch, Fig. 3.18, A). To this end, we labeled retinogeniculate inputs with CTB conjugated to Alexa Fluor dyes, and 24 h later applied tetrodotoxin (TTX) or its carrier (citrate buffer) to one eye, effectively blocking action-potential mediated activity from the eye, while crush-injuring the contralateral optic nerve. To confirm that TTX had an effect on dampening neuronal activity from the eye, we examined the pupillary constriction response (Su et al., 2011; Shanks et al., 2016) in our mice 24 h and 48 h after the above procedures. This test is a measure of activity from the retina to the Edinger-Westphal nuclei driven via activity from melanopsin expressing RGCs that sense light activity independent from the rods and cone photoreceptor cells (Provencio et al., 2000; Hattar et al., 2002). Thus, TTX treatment will attenuate action potentials from dLGN-projecting neurons as well as those projecting to the Edinger-Westphal Nuclei, resulting in a diminished pupillary reflex. Significant effects of both the optic nerve crush and the TTX application on pupillary constriction reflexes were observed both at 24 and at 48 h after the injury, with no detectable effect on naïve eyes or on citrate buffer-treated controls (Fig. 3.18, A-F). With confirmation of the efficacy of TTX, mice were killed three days post-crush, during peak phagocytosis of crushed neuronal inputs. Examination at that time (3 days after crush injury) showed that there were no effects of TTX on RGC survival (Fig. 3.19, A and B). dLGN microglia counts were also unchanged contralateral to

eyes treated with TTX, with only dLGN microglia contralateral to the crushed eye showing an increase in number (Fig. 3.20, A and B). We also found that the CTB+ presynaptic field sizes contralateral to TTX-treated eyes were unchanged compared to either naive or citrate buffer-treated eyes, with only dLGN CTB fields contralateral to crushed eyes decreasing in size (Fig. 3.20, A and C).

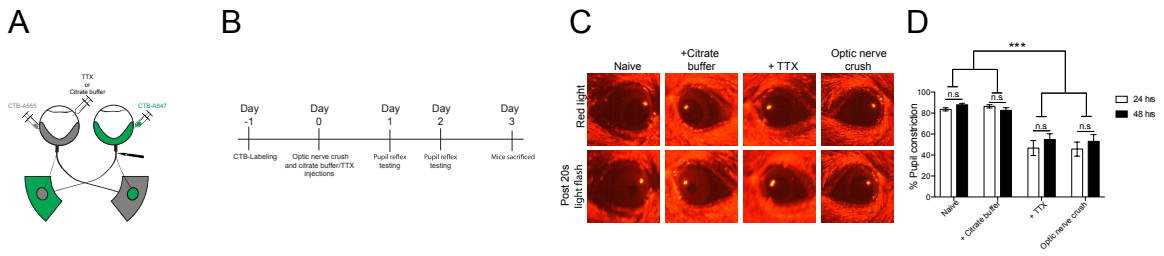

Figure 3.18 **Experimental scheme and pupil responses of TTX treatment concurrent with optic nerve crush injury** (A) Scheme and (B) time course of TTX treatment concurrent with optic nerve crush and pupil response testing. (C) Representative images of pupil responses in eyes of naïve, citrate buffer-treated, TTX-treated, and optic nerve crush injured eyes. (D) Quantification of pupil responses depicted in (C) (mean ± s.e.m; n = 5-10 eyes per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush and TTX treatment with Holm-Sidak post hoc test, representative of two independent experiments).

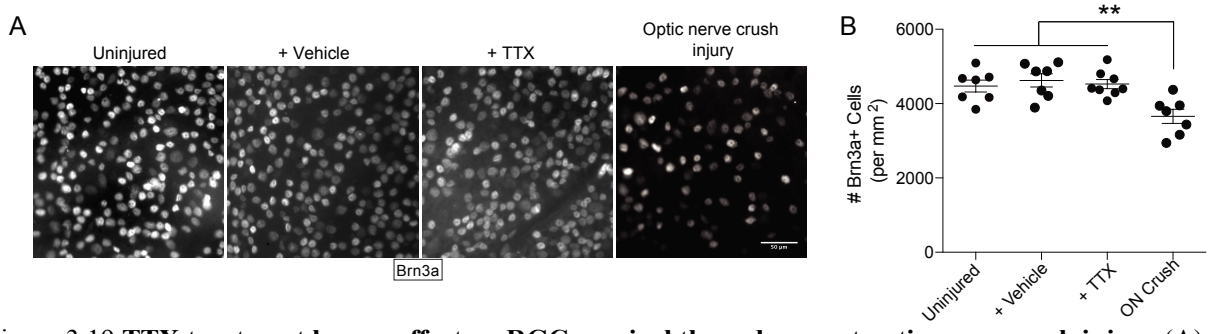

Figure 3.19 **TTX treatment has no effect on RGC survival three days post optic nerve crush injury** (A) Representative images of uninjured, citrate buffer, TTX, and optic nerve crush injury-experienced RGCs from mouse retina. (B) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 7-8 retinas per group; ** = $p < 0.01$, one-way ANOVA with Dunnett's post hoc test, representative of two independent experiments).

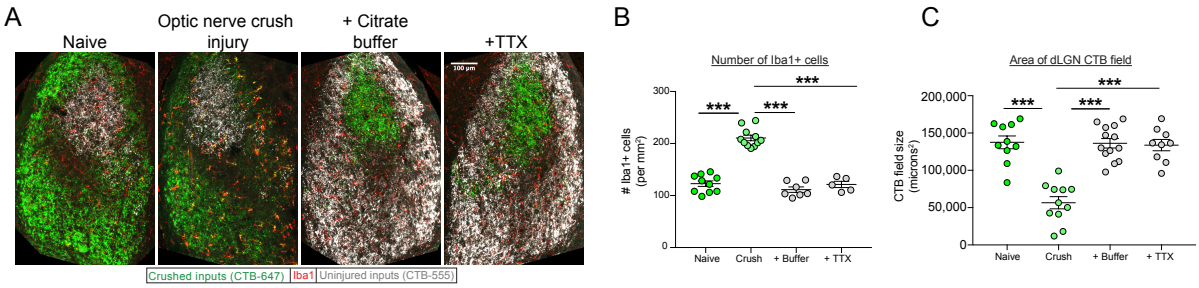

Figure 3.20 **TTX treatment has no effect on microglial number or CTB clearance three days post optic nerve crush injury** (A) Representative images of dLGN fields contralateral to naïve, citrate buffer, TTX, and optic nerve crushed eyes. (B) Quantification of Iba1+ cells (depicted in A) in dLGN fields contralateral to naïve, citrate buffer, TTX, and optic nerve crushed eyes (n = 5-11 mice per group; one-way ANOVA with Holm-Sidak post hoc test, representative of two independent experiments). (C) Quantification of CTB fields (depicted in A) in the dLGN contralateral to naïve, citrate buffer, TTX, and optic nerve crushed eyes (n = 5-13 mice per group; one-way ANOVA with Holm-Sidak post hoc test, representative of two independent experiments).

Next, we examined whether injury-activated microglia would phagocytose TTX-treated uninjured presynaptic terminals (Fig. 3.21, A). We found that microglia engulfed neuronal material from the crushed eye but did not engulf TTX-treated inputs (Fig. 3.21, B and C), which were deficient in action-potential mediated activity from the eye.

While TTX application did serve to block activity emanating from the eye, spontaneous release of neurotransmitter from presynaptic terminals would not be affected by TTX treatment (Chesselet, 1984). These results demonstrate that in the adult mouse, microglia become activated in response to synaptic degeneration, while cessation of neuronal activity has no effect on microglial phagocytic activity.

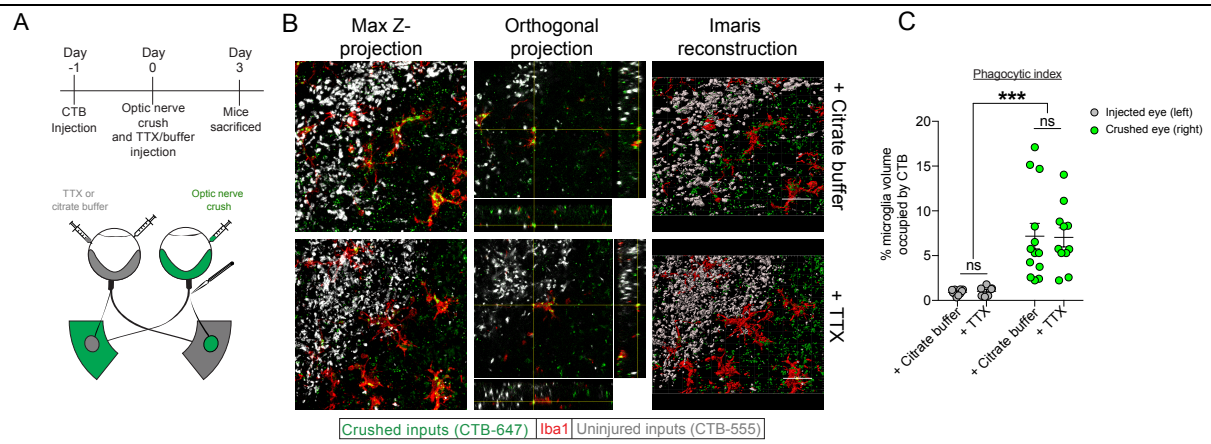

Figure 3.21 **TTX treatment with optic nerve crush injury has no effect on microglial engulfment of neuronal debris engulfment three days post optic nerve crush injury** (A) Scheme of TTX application concurrent with optic nerve crush injury in adulthood. (B) Z-projections, orthogonal slices, and Imaris 3D reconstructions of microglial phagocytosis of crushed retinogeniculate inputs in the dLGN following TTX or citrate buffer treatment concurrent with optic crush (scale bar = 25 microns). (C) Quantification of microglial debris engulfment (depicted in B) three days post optic nerve crush in mice with TTX or citrate buffer treatment concurrent with optic nerve crush (n = 7-13 mice per group, pooled from two independent experiments; two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test). Error bars represent mean ± S.E.M. * = $p < 0.05$, *** = $p < 0.001$.

Since microglia are the main phagocytes in response to neurodegeneration in the dLGN, these findings provide an amenable system for studying molecular mechanisms governing microglial engulfment of endogenous material. Thus, in order to identify the genetic programs necessary for removal of neuronal debris by dLGN microglia, we isolated CD11b+ cells (using AutoMACS) from an uninjured state and at 1 and 3 days after bilateral optic nerve crush (Fig. 3.22, A). RNA-seq was performed on our samples after flow cytometry confirmed that each sample was at least 90% CD11b+CD45+ (Fig. 3.22, B-D).

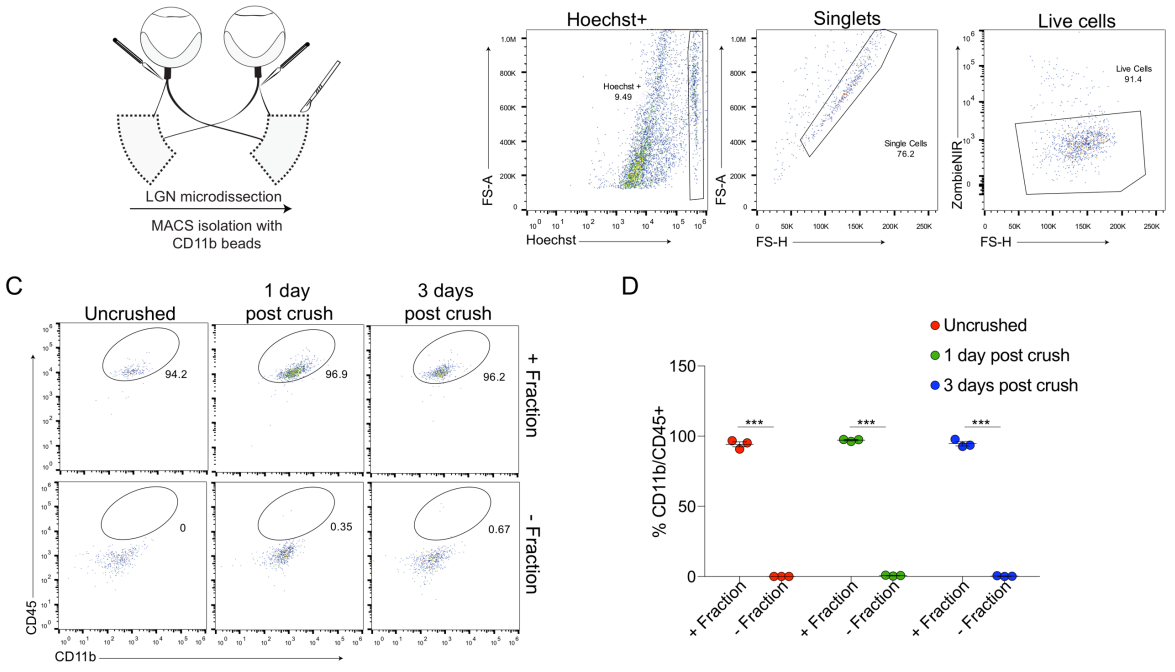

Figure 3.22 **Schematic and flow cytometric validation of purity from LGN-isolated microglia after optic nerve crush injury** (A) Experimental scheme for the isolation of dLGN microglia post optic nerve crush. (B) Representative FACS plots documenting gating strategy of microglia to assess purity of MACS prior to RNAseq. (C) Representative FACS plots of positive and negative fractions following CD11b-based MACS sorting. (D) Quantification of microglia purity in positive and negative fractions following CD11b-based MACS sorting (mean ± s.e.m; n = 3 samples per group; *** = $p < 0.001$, two-way ANOVA for effect of sample type with Holm-Sidak post hoc test, representative of two independent experiments).

Principal component analysis of all statistically significant transcripts revealed three discretely clustered populations, indicating a unique transcriptional signature for post-injury microglia at both time points tested (Fig. 3.23 A). Notably, day 1 and day 3 microglial subsets did not cluster together more closely than in the uninjured condition, indicating a dynamic transcriptional profile of microglia in the acute phase of Wallerian degeneration. This stands in contrast to transcriptional and surface marker profiles of microglia in disease states, where largely the profile is indicative of a progressive unidirectional shift to an activated phenotype over time (Matcovitch-Natan et al., 2016; Mrdjen et al., 2018; Keren-Shaul et al., 2017; Butovsky et al., 2015; Chiu et al., 2013). To assess the differences between genes expressed on days 1 and 3 after

crush injury, we compared the uniquely enriched genes of each condition to uninjured controls (Fig. 3.23, B). We found 132 common genes enriched in microglia on both days 1 and 3 after crush injury (adjusted *P*-value < 0.05), with 3-day post-injury microglia expressing the higher amount of uniquely enriched genes after the injury (1216 unique genes on day 3 compared to 697 unique genes on day 1 after crush). To illustrate the functional differences between dLGN microglia at 1 and 3 days after crush injury, we identified gene sets enriched on days 1 and 3 by performing a gene-set enrichment analysis. This revealed that a number of gene sets were downregulated in microglia on day 1 after crush injury, including those associated with the extracellular matrix and biological adhesion (Fig. 3.23, C). Upregulated gene sets on day 1 after injury included those associated with sensing molecular 'danger', such as complement (Fig. 3.24, A), and NOD-like receptor and Toll-like receptor signaling pathways (Fig. 3.24, B), indicating that microglia at 1 day after crush injury indeed sense and respond to the ongoing accumulation of neuronal debris. Gene sets downregulated on day 3 after the injury included regulation of hydrolase, GTPase, and catalytic activity, as well as regulation of immune-system processes. Upregulated gene sets included genes necessary for ribosome and proteasome production, cell-cycle processes, oxidative phosphorylation, phagocytosis (Fig. 3.24, C), and Alzheimer's disease (Fig. 3.24, D). This diverse array of gene sets would be expected for microglial cells that are surrounded by neuronal debris, actively engaged in phagocytosis, and undergoing cell division. Interestingly, the transcriptional signature three days post-injury is distinct from that one-day post-injury, with just 132 genes enriched on both post-injury days compared to non-injured condition.

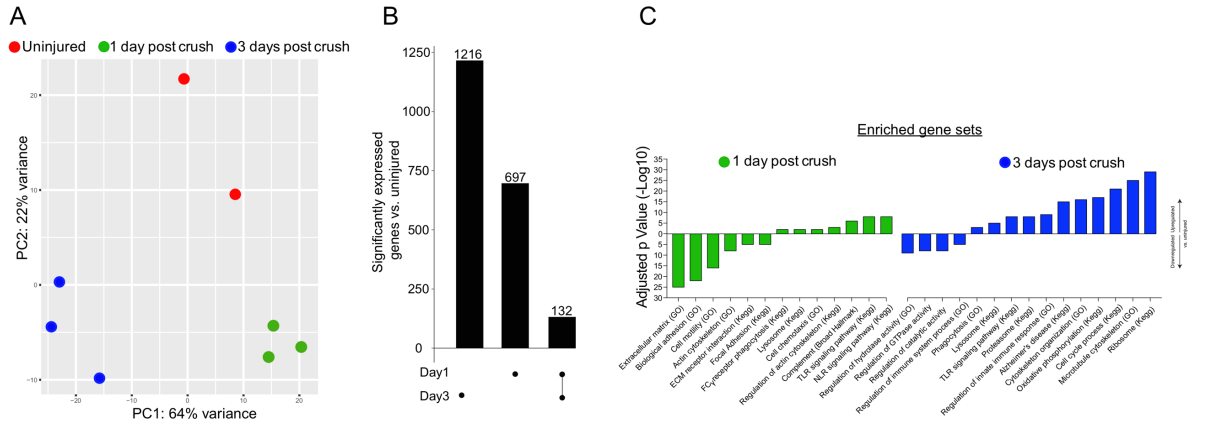

Figure 3.23 **LGN microglia exhibit a dynamic RNA transcriptome following optic nerve crush injury** (A) PCA analysis of uninjured dLGN microglia and microglia obtained one and three days post optic nerve crush injury. (B) UpSet plot detailing uniquely enriched genes on days one, three, and common to both days post-crush. (C) Gene sets obtained from KEGG, GO, and Broad Hallmark pathways that are enriched in dLGN microglia one and three days post optic nerve crush.

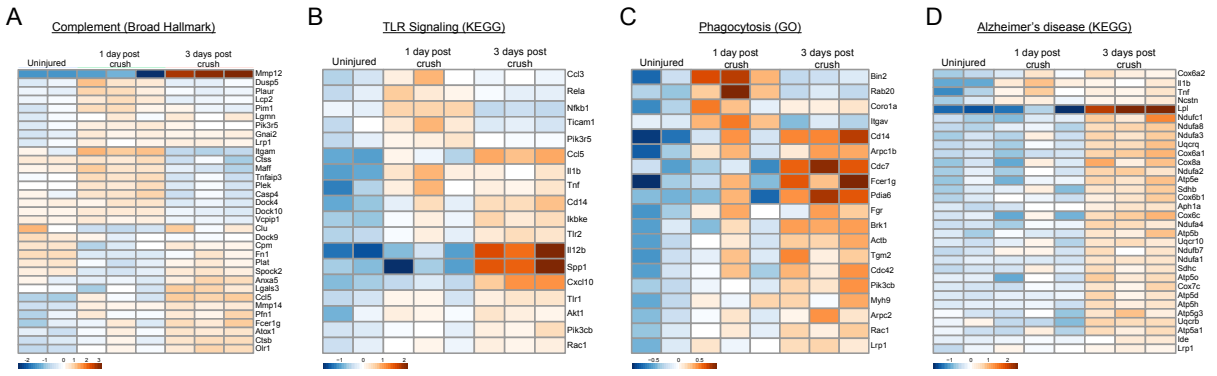

Figure 3.24 **Gene sets related to immune activation and phagocytosis are upregulated in LGN microglia following optic nerve crush injury** Heat maps of (A) complement, (B) TLR signaling, (C) phagocytosis, and (D) Alzheimer's disease gene sets that are upregulated in dLGN microglia following optic nerve crush (n = 2 samples for uninjured microglia and n = 3 samples for one and 3 days post injury group, Fisher's test, performed once).

Because the complement gene set was upregulated on day 1 after crush injury, we examined specific complement genes associated with the microglial engulfment of neuronal material during development (Fig. 3.25, A). These results indicated that *Itgam* (CD11b or C3R)

was highly upregulated in microglia on day 1 after optic nerve crush. We also observed a slight (not statistically significant) increase in transcription of *C1qb* and *C1qc*.

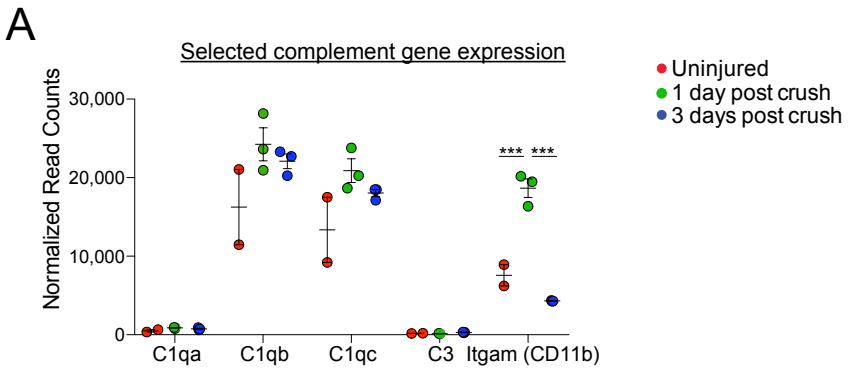

Figure 3.25 **Complement genes associated with developmental pruning are altered in LGN microglia following optic nerve crush injury** (A) Normalized read counts of selected complement genes following optic nerve crush (n = 2 samples for uninjured microglia and n = 3 samples for one and 3 days post injury group, Fisher's test, performed once).

To determine whether the complement system plays a role in the microglial clearance of neuronal debris after optic nerve crush, we repeated optic nerve crush injury experiments using *C1qa*$^{-/-}$ and *Itgam*$^{-/-}$ mice. First, we examined whether RGC survival could be influenced by C1q and found that neuronal survival of RGCs was not different between WT and C1q-deficient mice at baseline or three days post injury (Fig. 3.26, A and B). We therefore examined the dLGN of C1q-deficient mice on day 3 after optic nerve crush and observed no change in the number of microglia (Fig. 3.27, A and B) while significantly more residual neuronal debris was seen compared to WT mice (Fig. 3.27, A and C).

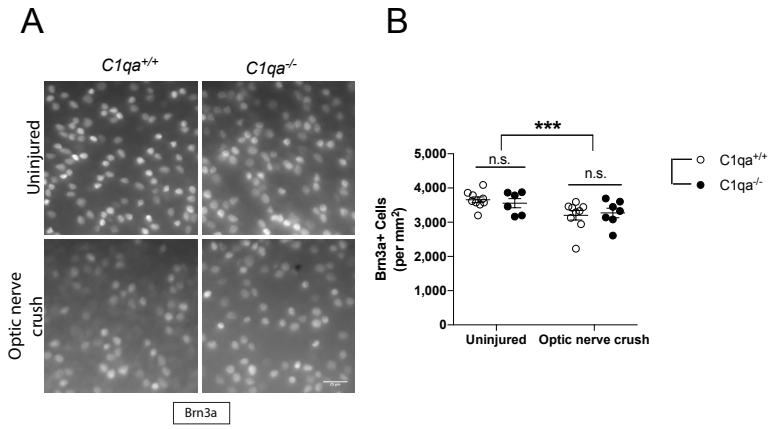

Figure 3.26 **Deletion of C1qa has no effect on RGC survival at baseline or three days post optic nerve crush injury** (A) Representative images of uninjured and optic nerve crushed injury-experienced RGCs in the retina of *C1qa*$^{+/+}$ and *C1qa*$^{-/-}$ mice. (B) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 6-9 retinas per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test, representative of two independent experiments).

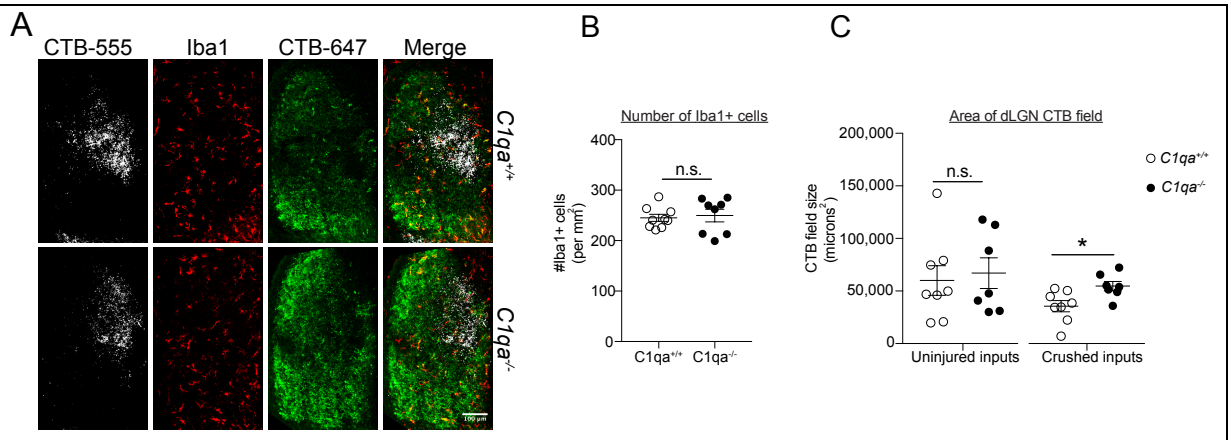

Figure 3.27 **C1qa is necessary for optimal clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in *C1qa*$^{+/+}$ and *C1qa*$^{-/-}$ mice. (B) Quantification of Iba1+ cell (depicted in A) counts three days post optic nerve crush depicted in (J) of *C1qa*$^{+/+}$ and *C1qa*$^{-/-}$ mice (n = 8-9 mice per group; Student's t-test, representative of two independent experiments). (C) Quantification of uninjured and crushed contralateral CTB field three days post optic nerve crush depicted in (A) of *C1qa*$^{+/+}$ and *C1qa*$^{-/-}$ mice (n = 7-8 mice per group; Student's t-test, representative of two independent experiments).

The complement receptor 3, also known as the integrin CD11b, was also shown to be critical for engulfment of synaptic material in development. Similar to that observed in C1q-deficient mice, we found that *Itgam*$^{-/-}$ mice had no change in neuronal survival at baseline or three days post-injury (Fig. 3.28, A and B). *Itgam*$^{-/-}$ mice also showed no change in the number of dLGN

microglia after injury (Fig. 3.29, A and B), and exhibited more neuronal debris in the dLGN contralateral to the crushed eye as compared to their WT counterparts (Fig. 3.29, A and C). Altogether, these experiments indicated an active role for complement in the microglial clearance of neuronal debris pursuant to Wallerian degeneration, implicating complement as a key driver of neuronal material clearance by microglia in Wallerian degeneration, shown previously in development (Stephan et al., 2012), Alzheimer's disease (Hong et al., 2016), schizophrenia (Sekar et al., 2016), and viral infection (Vasek et al., 2016).

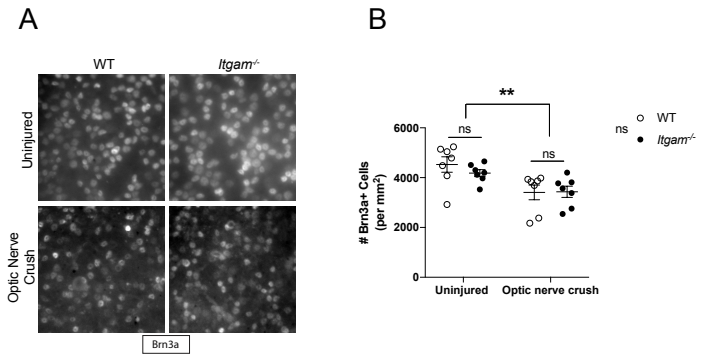

Figure 3.28 **Deletion of CD11b has no effect on RGC survival at baseline or three days post optic nerve crush injury** (A) Representative images of uninjured and optic nerve crushed injury-experienced RGCs in the retina of WT and *Itgam*$^{-/-}$ mice. (B) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 7 retinas per group; ** = $p < 0.01$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test, representative of two independent experiments).

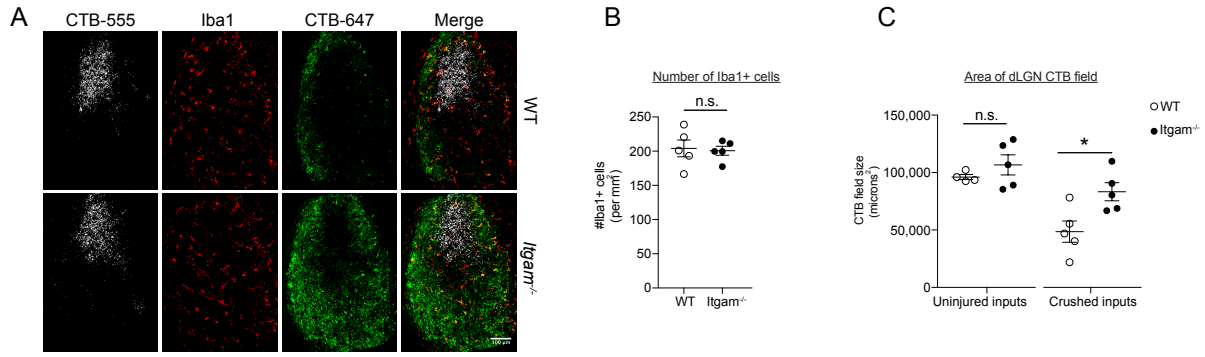

Figure 3.29 **CD11b is necessary for optimal clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in WT and *Itgam*$^{-/-}$ mice. (B) Quantification of Iba1+ cell counts three days post optic nerve crush depicted in (A) of WT and *Itgam*$^{-/-}$ mice (mean ± s.e.m; n = 5 mice per group; Student's t-test, representative of two independent experiments). (C) Quantification of uninjured and crushed contralateral CTB field three days post optic nerve crush depicted in (A) of WT and *Itgam*$^{-/-}$ mice (n = 4-5 mice per group; Student's t-test, representative of two independent experiments). Error bars represent mean ± S.E.M. * = $p < 0.05$

Chapter 4

Canonical phagocytic receptors are not necessary for clearance of neuronal debris

In our experiments defining the role of dLGN microglia in clearing neuronal debris we assayed a variety of phagocytic receptors known to the field of both phagocytosis and microglial biology. Discussed below will be the rationale and findings from those experiments.

With our initial findings detailing the key importance of both microglial presence within the dLGN and that they were highly phagocytic, we put forth a concerted effort to investigate the possible role of known phagocytic receptors (Brown and Neher, 2014) in clearance of neuronal debris during Wallerian degeneration. We also saw in our RNAseq dataset that many genes associated with microglial phagocytosis and proliferation were also differentially regulated in dLGN microglia post-crush (Fig. 4.1, A).

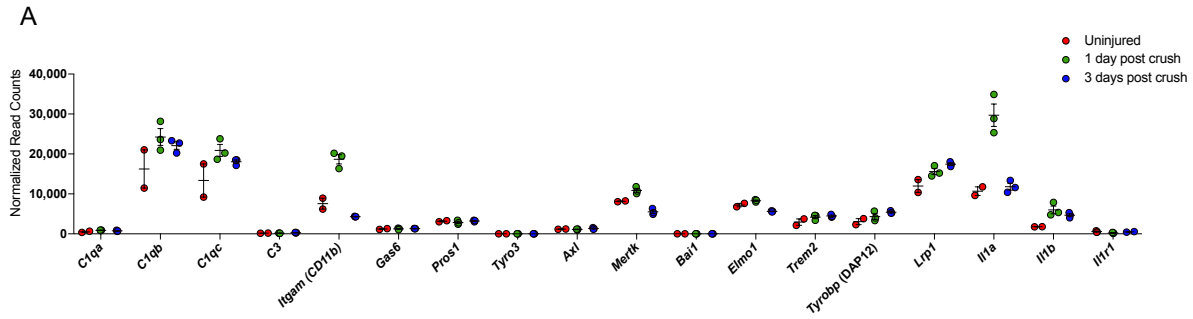

Figure 4.1 **dLGN Microglial expression of canonical phagocytosis genes following optic nerve crush injury** (A) Normalized read counts obtained from RNAseq data of microglia isolated by CD11b MACs in uninjured mice and those receiving bilateral optic nerve crush injury.

The first candidate receptor we investigated was due to its high expression on microglia and its established role as a marker of tissue resident macrophages and phagocytic activity was MER (Gautier et al., 2012; Fourgeaud et al., 2016; Rothlin et al., 2015). Encoded by the gene *Mertk* and a member of the TAM (TYRO3/AXL/MER) family of phagocytic receptors, MER has been shown to be critical for aspects of microglial biology relating to the clearance of neural progenitor cells (Fourgeaud et al., 2016) and engulfment of neuronal material by microglia in development (Chung et al., 2013) and has also been shown to be critical in microglial clearance of debris after viral infection (Tufail et al., 2017).

TAM receptor interaction with dying material or debris is critically mediated by bridging molecules that directly link the receptor to the phagocytic target by direct interaction with phosphatidylserine (Rothlin et al., 2015). This interaction is further complicated with regards to MER as the two canonical TAM bridging molecules Protein S and GAS6 have both been shown to be sufficient for MER-mediated phagocytosis (Lew et al., 2014). All three of these proteins (protein S, GAS6, and MERTK) are critical to retinal function as retinal pigmented epithelial cells utilize this axis of the TAM pathway for clearance of diurnal clearance of spent rod and cone processes that are critical for maintenance of vision (Burstyn-Cohen et al., 2012). While mutations that affect RPE cell function do in fact have stark vision loss, RGC phenotypes and projections (the critical component of our study) are largely unaffected even under the most severe phenotypes of vision loss (Lin and Peng, 2013; Mazzoni et al., 2008).

Thus, we proceeded to dissect whether GAS6 or Protein S may be involved in the clearance of dLGN debris by microglia during Wallerian degeneration. To achieve this, we performed our optic nerve crush protocol on *Gas6*$^{-/-}$ mice to target the bridging molecule GAS6 and on *LysM*$^{Cre/+}$*:Pros1*$^{fl/fl}$ mice to specifically delete Protein S in the myeloid compartment. Conditional

deletion of protein S is essential as genomic deletion results in embryonic lethality brought on by severe coagulopathy (Burstyn-Cohen et al., 2009).

We first looked to see if RGC survival was differentially affected by optic nerve crush injury in both *Gas6*$^{-/-}$ and *LysM*$^{Cre/+}$*:Pros1*$^{fl/fl}$ mice. We found that three days post injury or at baseline there was no change in survival of *Gas6*$^{-/-}$ LGN-projecting Brn3a+ RGCs (Fig. 4.2, A and B). This was also true for RGC survival post-injury in *LysM*$^{Cre/+}$*:Pros1*$^{fl/fl}$ mice (Fig. 4.2, C and D). Since there was no effect of these critical retinal proteins on neuronal survival in optic nerve crush injury we then proceeded to interrogate whether MER associated phagocytosis was necessary for clearance of neuronal debris within the LGN.

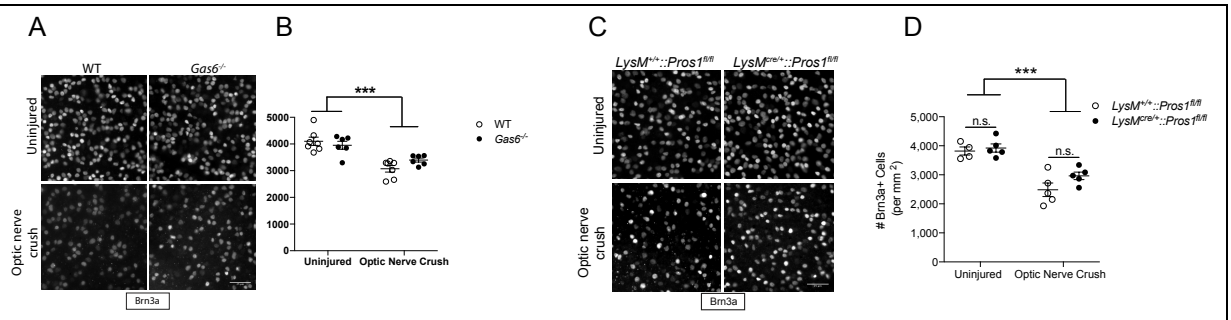

Figure 4.2 **Genomic deletion of Gas6 or myeloid deletion of protein S has no effect on RGC survival at baseline or three days post optic nerve crush injury** (A) Representative images of uninjured and optic nerve crushed injury-experienced RGCs in the retina of WT and *Gas6*$^{-/-}$ mice. (B) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 6-7 retinas per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test. (C) Representative images of uninjured and optic nerve crushed injury-experienced RGCs in the retina of *LysM*$^{Cre/+}$*:Pros1*$^{fl/fl}$ and *LysM*$^{+/+}$*:Pros1*$^{fl/fl}$ mice. (B) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 4-5 retinas per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test.

Strikingly we saw that *Gas6*$^{-/-}$ mice had reduced numbers of microglia within the LGN, with no change seen in clearance of neuronal debris or the size of control CTB+ fields (Fig 4.3, A-D). While no change in Iba1+ cell number was seen in the dLGN after injury in *LysM*$^{Cre/+}$*:Pros1*$^{fl/fl}$ mice (Fig 4.4, A and B), a similar pattern of no change in uninjured (Fig 4.4, A and C) or crushed (Fig 4.4, A and D) inputs was also seen similar to that of *Gas6*$^{-/-}$ mice.

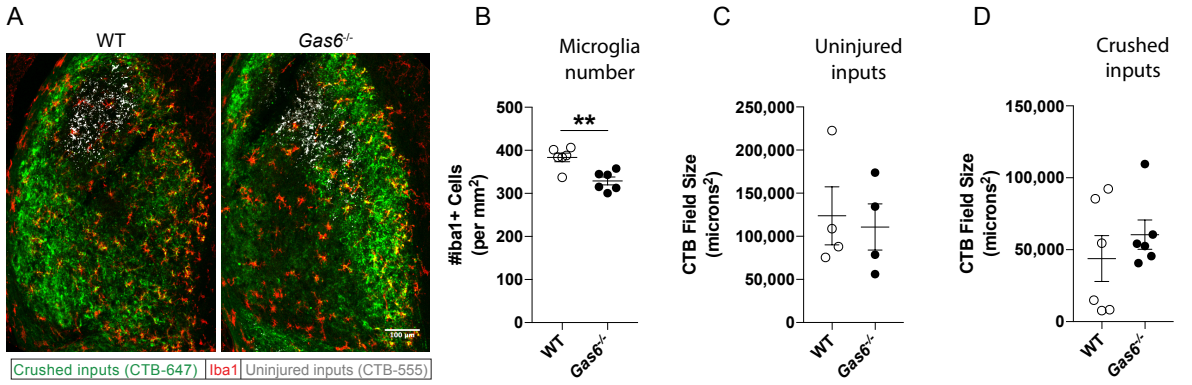

Figure 4.3 **Gas6 is not necessary for optimal clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in WT and *Gas6*$^{-/-}$ mice. (B) Quantification of Iba1+ cell (depicted in A) counts three days post optic nerve crush depicted in (A) of WT and *Gas6*$^{-/-}$ mice (n = 5-6 mice per group; Student's t-test, representative of two independent experiments).

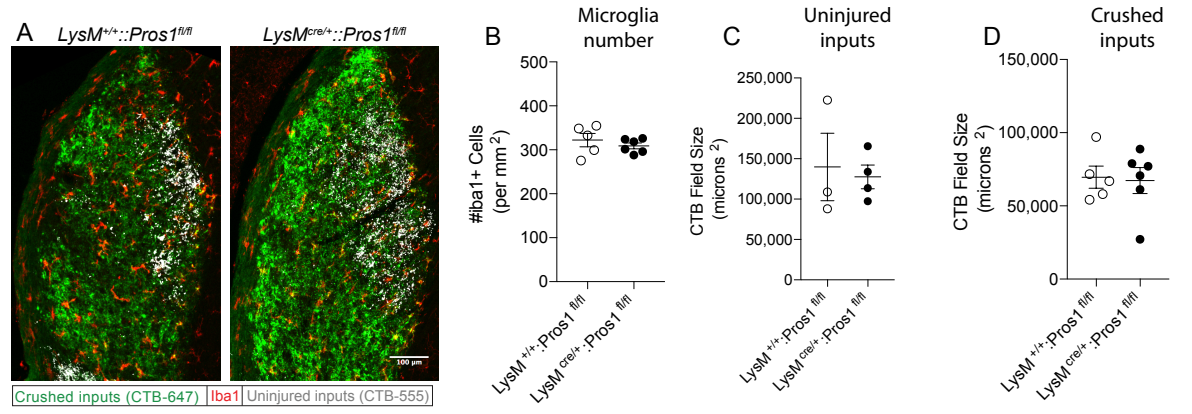

Figure 4.4 **Myeloid expression of Protein S is not necessary for optimal clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in *LysM*$^{Cre/+}$*:Pros1*$^{fl/fl}$ and *LysM*$^{+/+}$*:Pros1*$^{fl/fl}$ mice. Quantification of (B) Iba1+ cell number, (C) uninjured CTB field size, and (D) crushed CTB field size (depicted in A) three days post optic nerve crush depicted in (A) of *LysM*$^{Cre/+}$*:Pros1*$^{fl/fl}$ and *LysM*$^{+/+}$*:Pros1*$^{fl/fl}$ mice (n = 5-6 mice per group; Student's t-test, representative of two independent experiments).

Since both GAS6 and protein S bridging molecules may be involved in MER-mediated clearance of debris we also assessed whether clearance of debris was mediated by MER itself. First, we assessed whether optic nerve crush injury to *Mertk*$^{-/-}$ mice would affect neuronal survival and found no difference in RGC survival at baseline or three days post optic nerve injury (Fig. 4.5,

A and B). Examining the LGN we saw a trend towards a decrease in microglial cell number after crush in *Mertk*$^{-/-}$ mice (Fig. 4.6, A and B) that was similar to *Gas6*$^{-/-}$ mice. In regards to clearance of debris we saw no difference in either uninjured control CTB inputs or those degenerating three days post optic nerve crush injury (Fig. 4.6, A and C).

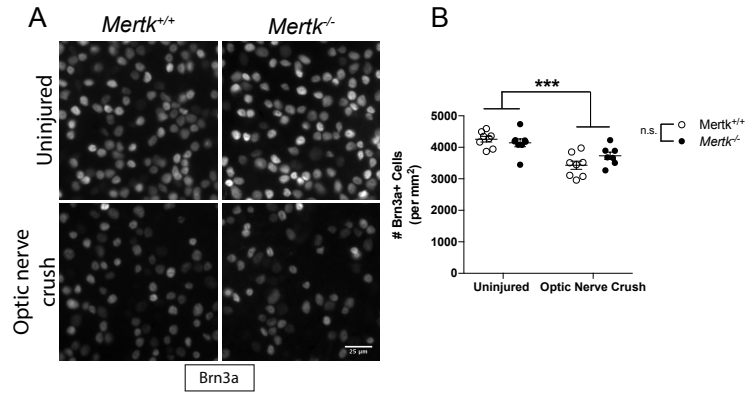

Figure 4.5 **Deletion of Mertk has no effect on RGC survival at baseline or three days post optic nerve crush injury** (A) Representative images of uninjured and optic nerve crushed injury-experienced RGCs in the retina of *Mertk*$^{+/+}$ and *Mertk*$^{-/-}$ mice. (B) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 6-7 retinas per group; ** = $p < 0.01$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test.

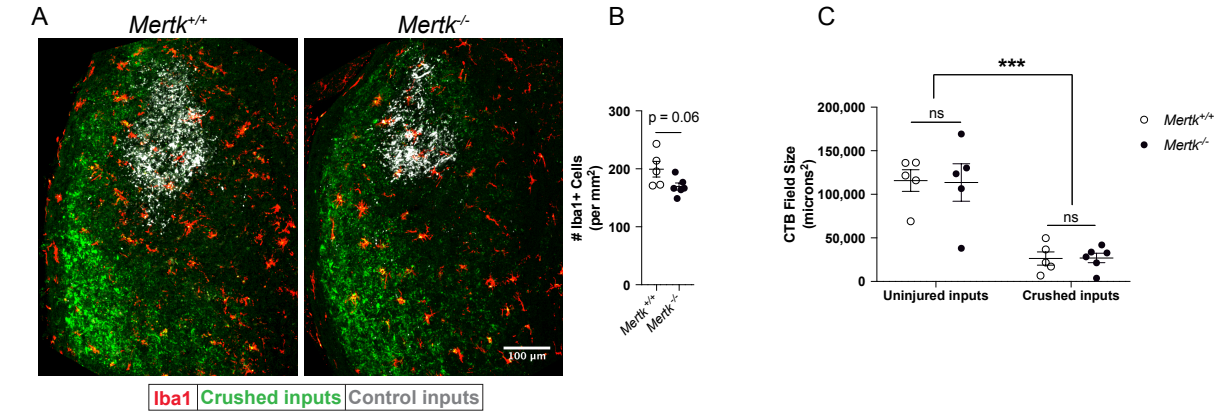

Figure 4.6 **Mertk is not necessary for optimal clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in *Mertk*$^{+/+}$ and *Mertk*$^{-/-}$ mice. (B) Quantification of Iba1+ cell (depicted in A) counts three days post optic nerve crush depicted in (A) of *Mertk*$^{+/+}$ and *Mertk*$^{-/-}$ mice (n = 5-6 mice per group; Student's t-test, representative of two independent experiments). (C) Quantification of uninjured and crushed contralateral CTB field three days post optic nerve crush depicted in (A) of WT and *Itgam*$^{-/-}$ mice (n = 4-5 mice per group; Student's t-test, representative of two independent experiments). Error bars represent mean ± S.E.M. *** = $p < 0.001$.

Because of the reduction in microglial number in both *Gas6*$^{-/-}$ and *Mertk*$^{-/-}$ mice we decided to quantify microglial numbers in a larger cohort and found a significant reduction in LGN microglial numbers post-crush (Fig. 4.7, A and B). This was not due to a lack of proliferation as the number (Fig 4.7, A and C) and percentage (Fig 4.7, A and D) of Ki67+ dLGN microglia was not changed in *Mertk*$^{-/-}$ mice. However, there was a significant difference in microglial counts in the uninjured cortex of *Mertk*$^{-/-}$ mice, indicating that there may be a developmental necessity for MER in maintaining the number of microglia within the CNS (Fig. 4.7, A and E).

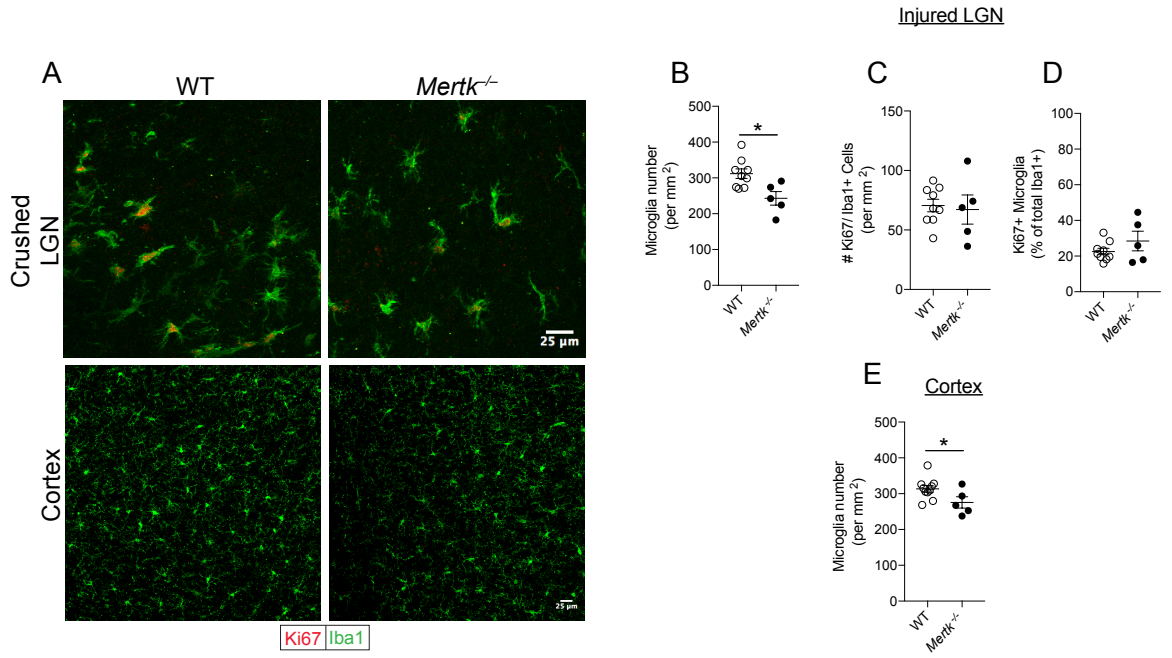

Figure 4.7 **Reduced microglial number is seen after injury in the dLGN and cortical regions of *Mertk*$^{-/-}$ mice**. (A) Representative images of the dLGN and cortex three days post optic nerve crush in *Mertk*$^{+/+}$ and *Mertk*$^{-/-}$ mice. (B) Quantification of Iba1+ cell (depicted in A) counts three days post optic nerve crush of *Mertk*$^{+/+}$ and *Mertk*$^{-/-}$ mice. Quantification of the (C) number and (D) % of microglia expressing Ki67 within the dLGN three days post crush. (E) quantification of microglia number within the cortex of *Mertk*$^{-/-}$ mice three days post optic nerve crush (n = 5-9 mice per group; Student's t-test, representative of two independent experiments. Error bars represent mean ± S.E.M. * = $p < 0.05$.)

Unlike the TAM receptor tyrosine kinase phagocytic receptors, the receptor brain angiogenesis inhibitor 1 (BAI1) has been shown to directly interact with phosphatidylserine while simultaneously exhibiting signaling properties of it's own, primarily through the adaptor ELMO1

(Park et al., 2007). BAI1 engagement allows for cytoskeletal alteration and formation of the phagocytic cup by ELMO acting directly acting as a guanine exchange factor for RAC1 (Arandjelovic and Ravichandran, 2015).

The phagocytic role of BAI1 has been ascribed to a variety of cell types including peritoneal macrophages (Fond et al., 2015), bone marrow derived-macrophages, thymic phagocytes, colonic epithelial cells (Lee et al., 2016), Sertoli cells of the testes (Elliott et al., 2010a), and Zebrafish microglia (Mazaheri et al., 2014).

Due to BAI play a critical role in many different phagocytes including microglia we utilized our optic nerve crush paradigm in mice which exhibit constitutively elevated levels of the BAI1 protein which is localized to the Rosa26 locus with a STOP-lox construct. Thus crossing of these mice to those which harbor Cre recombinase under the *E2A* promoter will produce mice which constitutively express BAI1 in nearly all cell types (BAI1-Tg) (Fond et al., 2015; Lakso et al., 1996).

We first measured measured RGC cell loss in BAI1-Tg mice at very early time points post crush. Since we hypothesized that BAI1-Tg mice would have accelerated phagocytosis of neuronal debris we were not sure of when this acceleration could have an impact on phagocytosis if LGN debris or RGC survival during our crush paradigm. Thus, we proceeded to crush and analyze BAI-Tg and WT control mice at 6, 24, 48, and 72 hours post-injury. We first noticed no difference in RGC counts at time points of 6, 24, and 48 hours (Fig. 4.8, A-C), with a crush effect seen 72 hours post injury with no difference between BAI1-Tg and WT mice (Fig. 4.8, D).

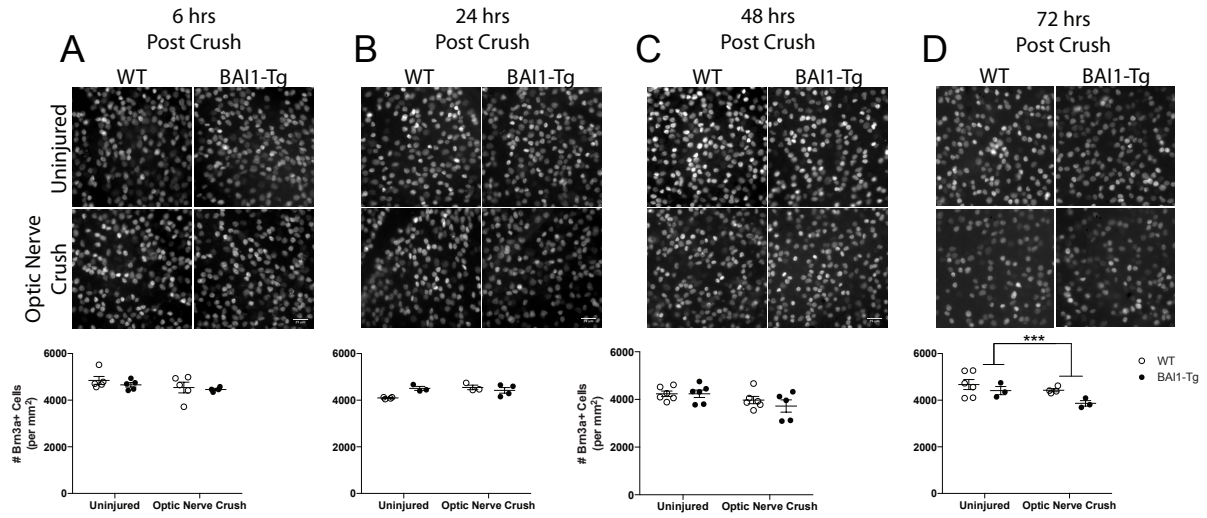

Figure 4.8 **BAI1 overexpression does not affect RGC survival after optic nerve crush injury** Representative images and quantification of uninjured and optic nerve crushed injury-experienced RGCs in the retina of WT and BAI1-Tg mice at (A) 6, (B) 24, (C) 48, and (D) 72 hours post crush (mean ± s.e.m; n = 3-6 retinas per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush.

Knowing that overexpression of BAI1 had no impact on RGC survival we then proceeded to measure the clearance of CTB-labeled RGC inputs to the dLGN by microglia. We initially observed no change in microglia number by Iba1 staining at all time points measured (Fig. 4.9, A and B). Further we also saw no change in the clearance of neuronal debris from BAI1-Tg mice from 6-72 hours post crush (Fig 4.9, A and C). Imaging at higher magnification for documentation of debris engulfment by microglia also showed no differences between BAI-Tg and WT mice from 6-72 hours post crush (Fig. 4.10, A and B).

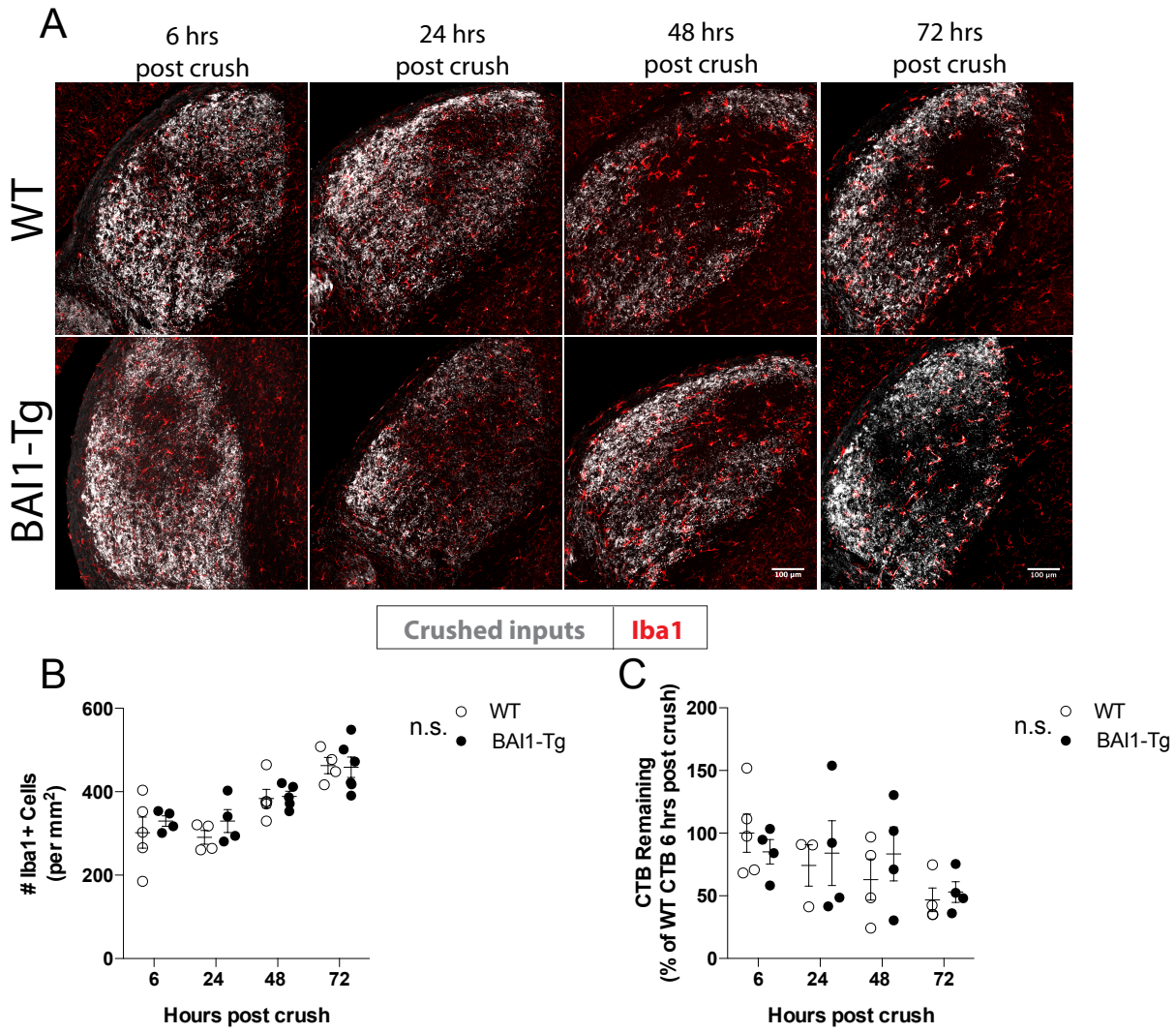

Figure 4.9 **BAI1 overexpression does not affect microglia number or clearance of neuronal debris survival after optic nerve crush injury** (A) Representative images of the dLGN 6-72 hours post optic nerve crush in BAI1-Tg mice. Quantification of (B) Iba1+ cell number and (C) crushed CTB field size (depicted in A) post optic nerve crush depicted in (A) of WT and BAI1-Tg mice (n = 3-5 mice per group; Two-way ANOVA with Holm-Sidak post hoc test, performed once).

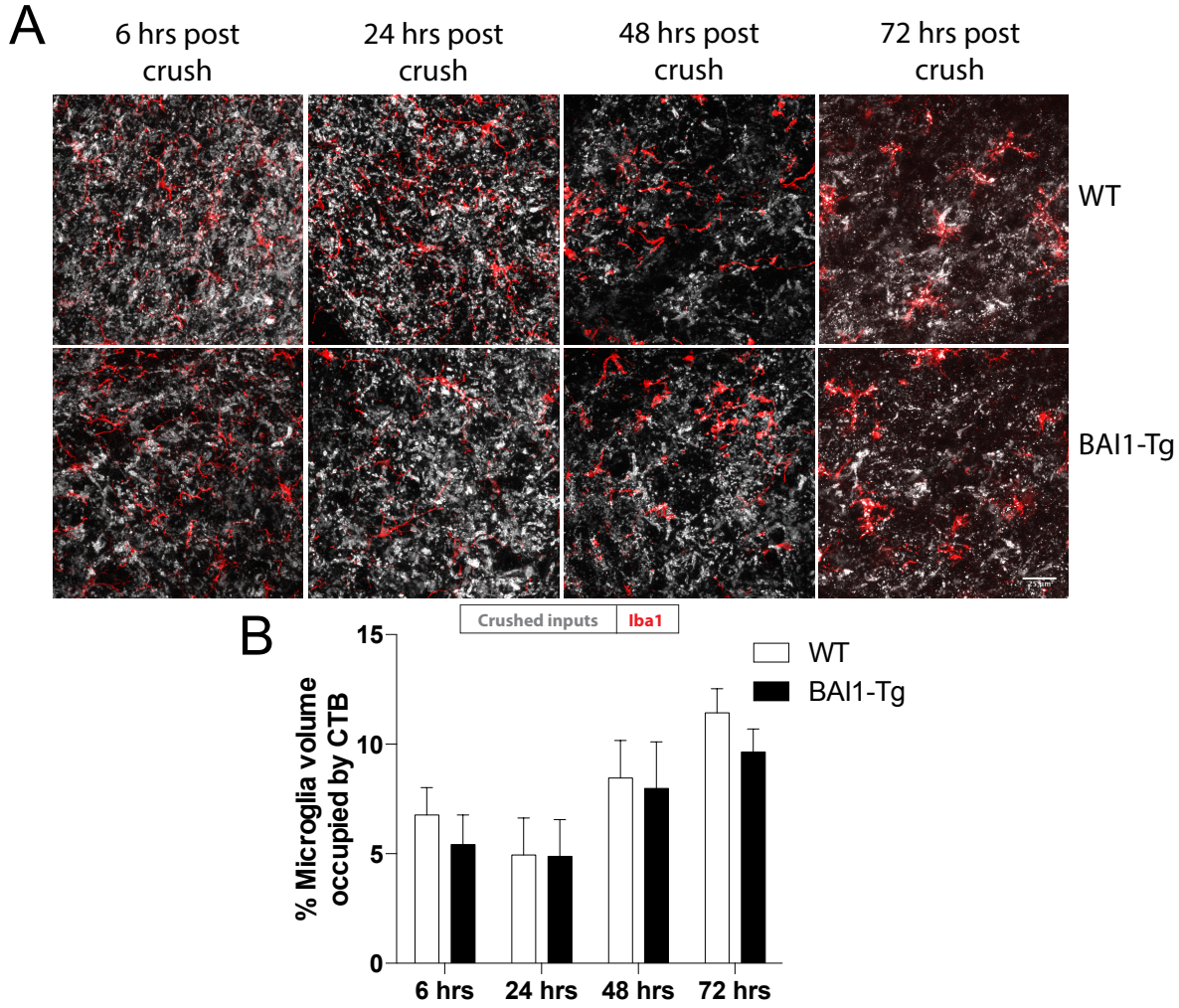

Figure 4.10 **Bai1 overexpression does not affect microglial engulfment of neuronal debris after optic nerve crush injury** (A) Representative images of microglial engulfment of debris 6-72 hours post optic nerve crush in BAI1-Tg mice. (B) Quantification of engulfment (depicted in A) of WT and BAI1-Tg mice (n = 3-5 mice per group; Two-Way ANOVA, performed once).

With overexpression of BAI1 not serving to accelerate or alter phagocytosis of neuronal debris in our system we then proceeded to examine whether BAI1 or its downstream mediator ELMO1 were necessary for microglial engulfment of debris. To accomplish this, we assayed RGC survival and clearance of neuronal debris in *Bai1*$^{-/-}$ and *Elmo1*$^{-/-}$ mice. Three days post optic nerve crush we observed no difference in RGC survival in homozygotes or heterozygotes of *Elmo1* or

Bai1 mice, indicating that the BAI1 pathway may play little role in RGC survival after acute optic nerve crush injury (Fig. 4.11, A-D). Analysis of microglial counts in *Elmo1*$^{-/-}$ mice three days post crush injury also showed no difference with no change in clearance of neuronal debris or the uninjured control CTB+ field (Fig. 4.12, A-D).

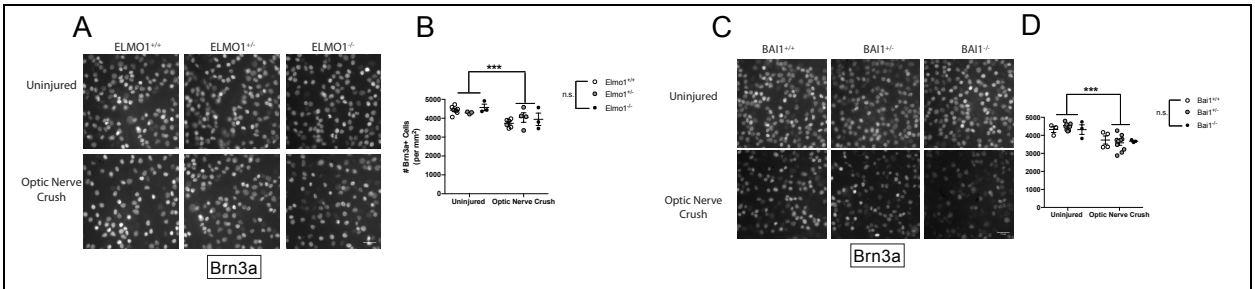

Figure 4.11 **Deletion of ELMO1 or BAI1 has no effect on RGC survival at baseline or three days post optic nerve crush injury** (A) Representative images of uninjured and optic nerve crushed injury-experienced RGCs in the retina of WT and *Elmo1*$^{-/-}$ mice. (B) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 3-6 retinas per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test. (C) Representative images of uninjured and optic nerve crushed injury-experienced RGCs in the retina of WT and *Bai1*$^{-/-}$ mice. (D) Quantification of RGC counts seen in (C) (mean ± s.e.m; n = 3-10 retinas per group; *** = $p < 0.001$, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test.

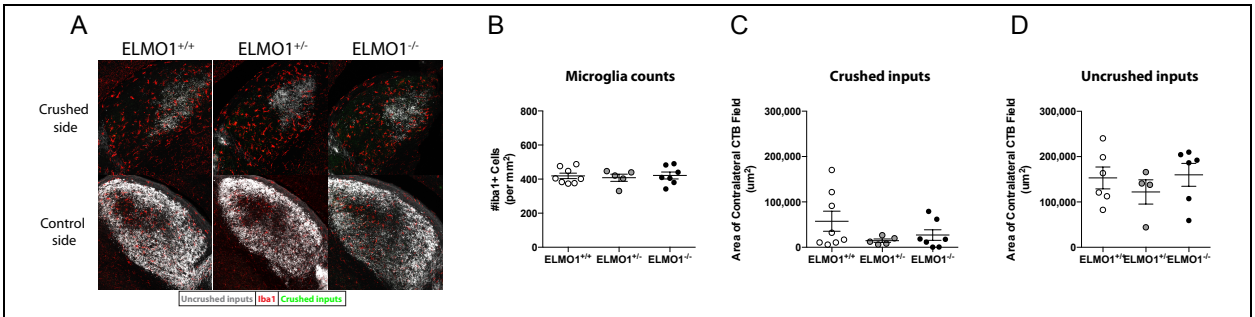

Figure 4.12 **ELMO1 is not necessary for optimal clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in *Elmo1*$^{+/+}$, *Elmo1*$^{+/-}$, and *Elmo1*$^{-/-}$ mice. Quantification of (B) Iba1+ cell number, (C) uninjured CTB field size, and (D) crushed CTB field size (depicted in A) three days post optic nerve crush depicted in (A) of *Elmo1*$^{+/+}$, *Elmo1*$^{+/-}$, and *Elmo1*$^{-/-}$ mice (n = 5-8 mice per group; One-way ANOVA with Holm-Sidak post hoc test, representative of two independent experiments).

Bai1$^{-/-}$ mice had a similar phenotype to that seen in *Elmo1*$^{-/-}$ mice with no change in microglial counts, clearance of the crushed CTB+ debris field or size of the uninjured CTB+ field (Fig. 4.13, A-D).

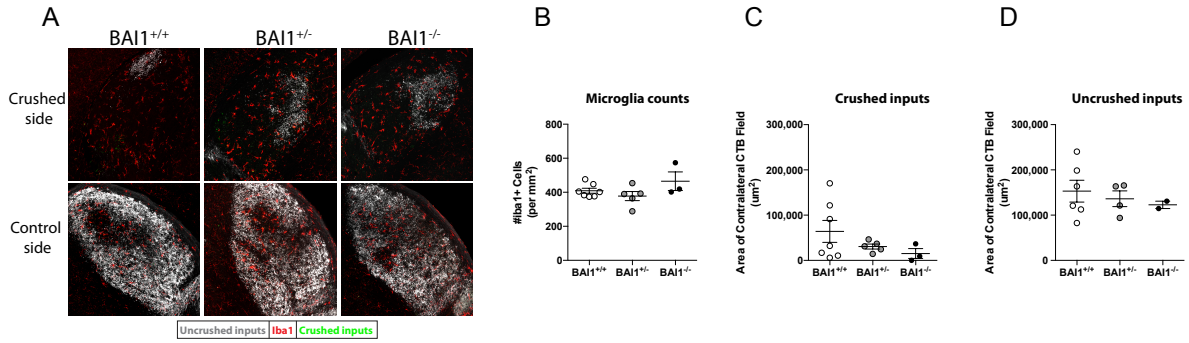

Figure 4.13 **BAI1 is not necessary for optimal clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in *Bai1*$^{+/+}$, *Bai1*$^{+/-}$, and *Bai1*$^{-/-}$ mice. Quantification of (B) Iba1+ cell number, (C) crushed CTB field size, and (D) uninjured CTB field size (depicted in A) three days post optic nerve crush depicted in (A) of *Bai1*$^{+/+}$, *Bai1*$^{+/-}$, and *Bai1*$^{-/-}$ mice (n = 3-7 mice per group; One-way ANOVA with Holm-Sidak post hoc test, representative of two independent experiments).

Research focusing on the phagocytic receptor TREM2 has had a major shift in focus recently since it was identified in a genome-wide association study (GWAS) of risk associated single nucleotide polymorphisms (SNPs) associated with development of Alzheimer's disease (Jonsson et al., 2013; Guerreiro et al., 2013). Since then its role in Alzheimer's disease and clearance of myelin debris during cuprizone-induced demyelination has been illustrative. Examples of TREM2 function include the containment of myeloid plaques and ensuring proper remyelination through clearance of debris (Poliani et al., 2015; Wang et al., 2015a). Further, it's role in the activation of microglia during Alzheimer's disease was shown with TREM2 necessary for the the induction of inflammatory microglia transcription profile seen in mouse models of Alzheimer's and ALS (Keren-Shaul et al., 2017). With these insights we then proceeded to place *Trem2*$^{-/-}$ mice in to our protocol of optic nerve crush injury.

Three days post optic nerve crush injury we analyzed the dLGN of *Trem2*$^{-/-}$ mice and observed a trend towards a decrease in microglial number (Fig. 4.14, A and B). This was similar to what was seen during cuprizone-induced demyelination where microglial numbers were greatly reduced during the demyelination phase of cuprizone treatment (Poliani et al., 2015). Despite this reduction in Iba1+ cell number, the clearance of CTB-labeled terminals was unaffected in *Trem2*$^{-/-}$ mice (Fig. 4.14, A and C).

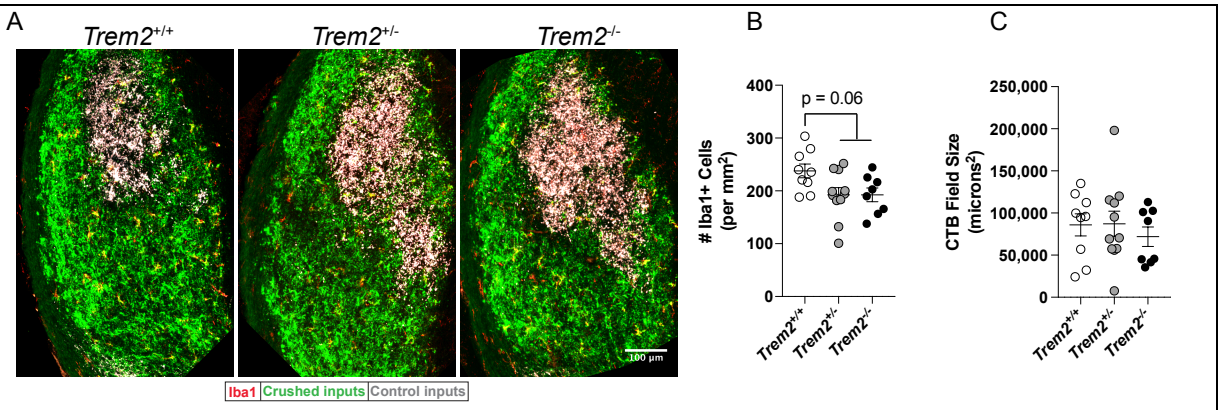

Figure 4.14 **TREM2 is not necessary for optimal clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in *Trem2*$^{+/+}$, *Trem2*$^{+/-}$, and *Trem2*$^{-/-}$ mice. Quantification of (B) Iba1+ cell number, and (C) crushed CTB field size (depicted in A) three days post optic nerve crush depicted in (A) of *Trem2*$^{+/+}$, *Trem2*$^{+/-}$, and *Trem2*$^{-/-}$ mice (n = 8-11 mice per group; One-way ANOVA with Holm-Sidak post hoc test, representative of two independent experiments).

The low-density lipoprotein receptor-related protein 1 (LRP1) has been shown to be a potent scavenger receptor involved in both the clearance of myelin debris as well as apoptotic and necrotic cells (Fernandez-Castaneda et al., 2013; Gardai et al., 2005; Gaultier et al., 2009). Further, it's expression on microglia has been shown recently to restrain rampant autoimmune processes that occur during experimental autoimmune encephalopathy (EAE) (Chuang et al., 2016). In light of this data, we sought to examine whether microglial LRP1 expression may aid in clearance of neuronal debris during Wallerian degeneration.

Before analyzing the effect of LRP1 deletion from microglia in the context of Wallerian degeneration we first analyzed whether there was sufficient deletion from microglia in mice with microglia specific deletion of LRP1. To test this we isolated microglia from *Cx3cr1*$^{ERT2Cre/+}$:*Lrp1*$^{fl/fl}$ and *Cx3cr1*$^{+/+}$:*Lrp1*$^{fl/fl}$ mice one month post a single pulse of tamoxifen to induce excision of LRP1 from microglia and allow peripheral myeloid cell turnover (Goldmann et al., 2013). We then analyzed the MFI of LRP1 by flow cytometry. After gating on microglia and non-microglial cells by CD11b/CD45 positivity (Fig. 4.15, A), we analyzed the relative MFI of LRP1 on non-microglial cells and found no difference in LRP1 between non-microglial cells of *Cx3cr1*$^{ERT2Cre/+}$:*Lrp1*$^{fl/fl}$ and *Cx3cr1*$^{+/+}$:*Lrp1*$^{fl/fl}$ mice. In contrast, CD11b/CD45+ cells of *Cx3cr1*$^{ERT2Cre/+}$:*Lrp1*$^{fl/fl}$ mice exhibited a robust deletion of LRP1 relative to their Cre negative controls.

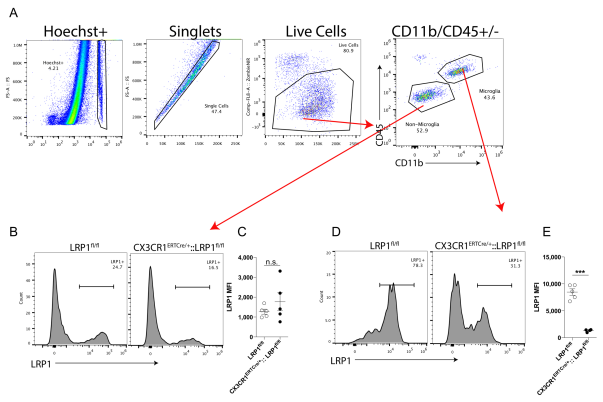

Fig 4.15 **Excision of LRP1 from microglia and not other cells with application of tamoxifen to *Cx3cr1*$^{ERT2Cre/+}$:*Lrp1*$^{fl/fl}$ mice.** (A) Gating strategy to obtain non-microglia and microglia (CD11b+CD45+) from the optic nerve. (B) LRP1 expressing fractions of LRP1 on non-microglial cells one month after tamoxifen treatment. (C) Quantification of LRP1 MFI on non-microglial cells. (D) LRP1 expressing fractions of microglial cells one month after tamoxifen treatment. (E) Quantification of LRP1 MFI on microglial cells one month after tamoxifen treatment. (mean ± s.e.m; n = 4-5 mice per group; *** = $p < 0.001$, Student's t-test, representative of two independent experiments).

We then analyzed the survival of RGCs three days post optic nerve crush injury and found no effect of microglial deletion of LRP1 on RGC number at baseline or post-injury (Fig. 4.16, A and B). Within the LGN, no effect of LRP1 deletion was also seen on both the number of microglia or clearance of neuronal debris (Fig. 4.17, A-C).

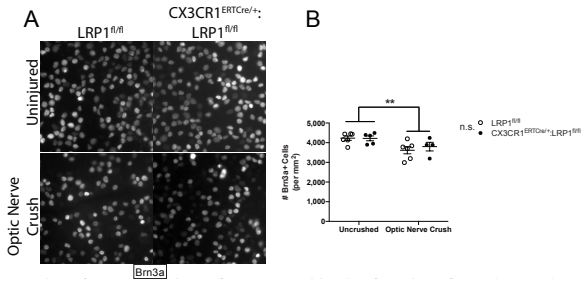

Figure 4.16 **Deletion of LRP1 on microglia has no effect on RGC survival at baseline or three days post optic nerve crush injury** (A) Representative images of uninjured and optic nerve crushed injury-experienced RGCs in the retina of *Cx3cr1*$^{ERT2Cre/+}$:*Lrp1*$^{fl/fl}$ and *Cx3cr1*$^{+/+}$:*Lrp1*$^{fl/fl}$ mice. (B) Quantification of RGC counts seen in (A) (mean ± s.e.m; n = 4-5 retinas per group; ** = p < 0.01, two-way ANOVA for effect of optic nerve crush with Holm-Sidak post hoc test.

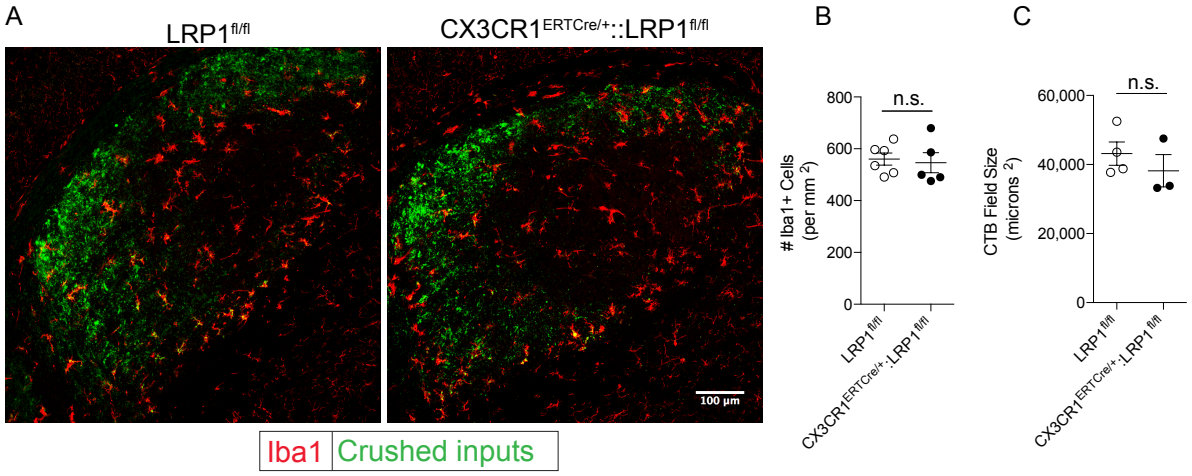

Figure 4.17 **Microglial LRP1 is not necessary for clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in *Cx3cr1*$^{ERT2Cre/+}$:*Lrp1*$^{fl/fl}$ and *Cx3cr1*$^{+/+}$:*Lrp1*$^{fl/fl}$ mice. Quantification of (B) Iba1+ cell number, and (C) crushed CTB field size depicted in (A) three days post optic nerve crush (n = 5-6 mice per group; Student's t-test, representative of two independent experiments experiment).

Recently a paper demonstrated genetic ablation of microglia utilizing the diphtheria toxin receptor driven by the promoter for *Cx3cr1* (Bruttger et al., 2015). In this study it was shown that inhibition of the receptor for IL-1β (IL-1R1) with IL-1Ra treatment, was sufficient to block repopulation of microglial through inhibition of their proliferation. Further, it was also shown that constitutive deletion of IL-1R1 on microglia yielded fewer microglia in many brain regions at baseline. Since IL-1β and IL-1α were two of the most highly upregulated transcripts in our system

(Fig. 4.1, A), and that microglia were highly proliferative during LGN Wallerian degeneration (Fig. 3.1, A and E), we sought to determine whether deletion of IL-1R1 would affect dLGN microglial proliferation in response to optic nerve crush injury.

To achieve this, we put *Il1r1*$^{-/-}$ mice through our RGC-labeling and optic nerve crush protocol. *Il1r1*$^{-/-}$ mice and WT controls were then sacrificed three days post injury when WT mice are both maximally phagocytic and proliferative. We observed however, that dLGN microglia counts in *Il1r1*$^{-/-}$ mice were no different than that in WT mice and had a slight trend towards an increase in cell number (Fig. 4.18, A and B). Further, no difference was also seen in regards to clearance of CTB-labeled neuronal debris (Fig. 4.18, A and C).

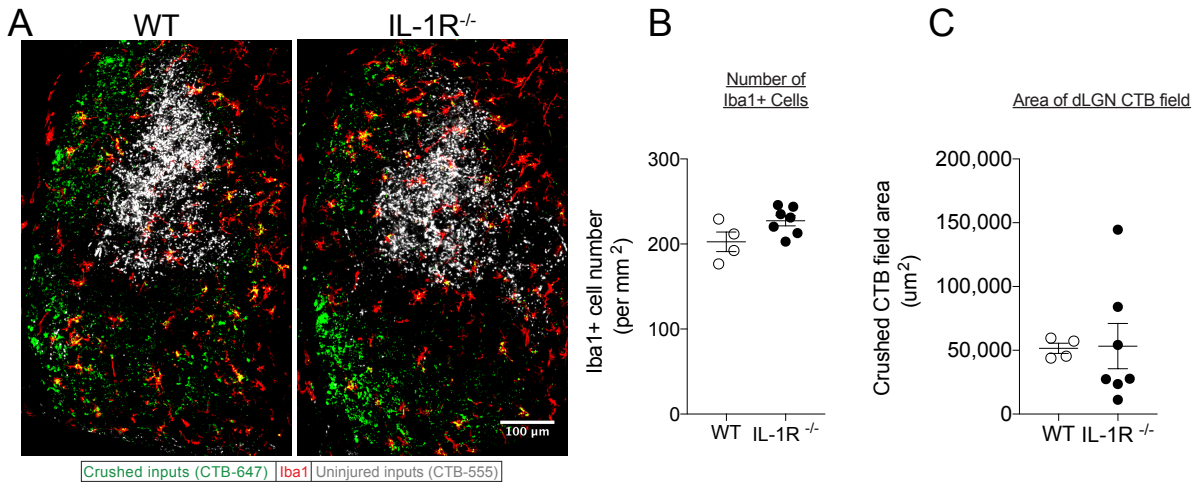

Figure 4.18 **IL-1R is not required for clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in WT and *Il1r*$^{-/-}$ mice. Quantification of (B) Iba1+ cell number, and (C) crushed CTB field size depicted in (A) three days post optic nerve crush (n = 4-7 mice per group; One-way ANOVA with Holm-Sidak post hoc test, representative of one experiment).

Myd88 has been shown to be a central mediator of downstream signaling for TLR 1-9 (except TLR3 which signals through TLR3) (Lu et al., 2008; Gadani et al., 2015a). Our lab has also shown that Myd88 is critical for neuroprotection during optic nerve crush injury, with

Myd88$^{-/-}$ mice exhibiting impaired neuroprotection and transfer of *Myd88*$^{-/-}$ bone marrow in to WT mice phenocopying the enhanced neuronal loss seen with germline deletion of Myd88 (Walsh et al., 2015). Further, we also saw that TLR and NLR signaling pathways were upregulated in microglia following optic nerve crush injury (Fig. 3.23, C). With this data in hand we proceeded to perform optic nerve crush injury to investigate whether Myd88 signaling may mediate microglial activation and subsequent clearance of debris. Surprisingly we found that dLGN microglia numbers were decreased after injury (Fig. 4.19, A and B). This could be due to baseline differences in number of microglia in *Myd88*$^{-/-}$ mice or due to impaired proliferation in response to injury. Despite this decrease in number we observed that there was no difference in the clearance of neuronal debris post-crush, with also no change in baseline CTB fields from uninjured eyes (Fig. 4.19, A and C). Collectively this informs us that Myd88 signaling may be dispensable for the activation of microglia and their subsequent synaptic debris clearance during Wallerian degeneration, despite its role in immune cell protection of RGCs within the retina (Walsh et al., 2015).

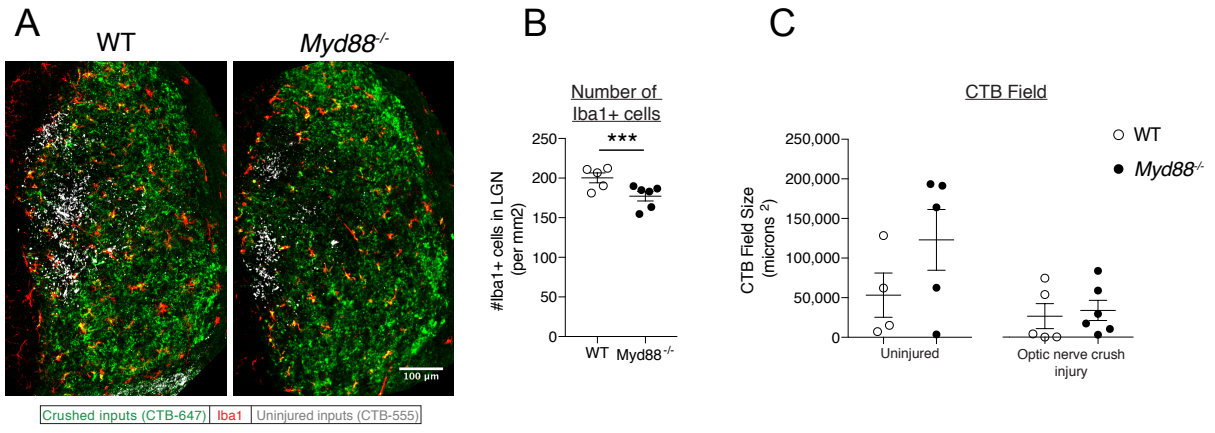

Figure 4.19 **Myd88 signaling is not required for clearance of neuronal debris three days post optic nerve crush injury** (A) Representative images of the dLGN three days post optic nerve crush in WT and *Myd88*$^{-/-}$ mice. Quantification of (B) Iba1+ cell number, and (C) crushed CTB field size depicted in (A) three days post optic nerve crush (n = 5-6 mice per group; Student's t-test, representative of two independent experiments experiment).

Chapter 5

Discussion and future directions

Any breach or external perturbation of an organism, sterile or otherwise, produces an immune response commensurate with that disturbance. Whether it be the wheal-and-flare of an allergic reaction of the skin, a scab forming over a minor scrape of the knee, or the sore throat resulting from the adaptive immune response to rhinovirus. This is no different in the immune system of the CNS parenchyma where the umbrella term glia is applied to the organ's specialized fibroblasts (astrocytes) or tissue resident macrophages (microglia). As discussed in the introduction, there are significant responses from astrocytes and especially microglia to parenchymal shifts in homeostasis whether they be acute or chronic. In development, the phagocytic nature of both astrocytes and microglia have been well characterized showing that microglia and astrocytes can shape the very structure of the brain itself, and thus affect brain function (Stephan et al., 2012; Jung and Chung, 2018). How do microglia commence, engage, and regulate this process? Are these mechanisms conserved in disease states? These two major questions have been key drivers of my thesis work and are presented here. As discussed in the introduction, phagocytes and other immune cells are directed by environmental cues that allow for recognition, engagement, and clearance of debris or intruders. Is this dynamic preserved in the interaction of CNS phagocytes with neuronal material during development? Is this dynamic altered during acute degeneration or chronic disease states?

Prior to the completion of this work the observation and necessity of microglial phagocytosis in pathology was somewhat in question. While microglia clearly engulf synaptic

processes in development (Schafer et al., 2012) their role was minimal when compared to astrocytes in development, with astrocytes consuming 5-10 times the amount of neuronal material compared to microglia (Chung et al., 2013). Recent papers have also made much acclaim of the opportunity for microglial phagocytosis of amyloid plaques in Alzheimer's disease but it appears that microglia are only capable of maintaining plaque compactness (Wang et al., 2015b) and are unable to clear totally the amyloid plaque burden seen in mouse models of Alzheimer's disease (Lai and McLauring, 2012). Further, it appears that microglial phagocytosis may serve a pathologic role in the early phases of Alzheimer's disease (Hong et al., 2016).

One of our major questions prior to performing the experiments that form the foundation of this thesis was: are microglia responsible for the initiation of synaptic "pruning" in development and destructive phagocytosis in pathology? Or, rather, could neurons themselves induce a response in microglia through membrane alterations or secreted factors that ultimately govern microglial behavior? Answering this question would assign a role for microglia either as causative agent in CNS formation and destruction or place microglia as crucial responders to neuronal biology in development, homeostasis, and disease.

In this thesis I have attempted to understand how microglia respond and interact with neuronal debris during acute neurodegeneration, focusing on what signals drive microglial activation and what complement of receptors are necessary for debris clearance. I have described the response of microglia as the key phagocyte during CNS Wallerian degeneration. I have also shown that differing from the site of injury there is no contribution from peripheral myeloid cells. Unique to microglial function in this context is that they are irresponsive to neuronal activity, behaving much more like classical professional phagocytes recognizing immunogenic cues. The transcriptional profile of microglia in response to acute injury was also examined, with a dramatic

change found in transcriptional profiling just 48 hours apart which exhibits the plastic nature of microglial cells specifically and macrophages as a whole. Finally, I have also shown a specific requirement for complement and the phagocytic receptor CD11b in the clearance of presynaptic debris, with phagocytic receptors normally associated with microglia, dispensable during Wallerian degeneration.

In the course of my graduate work I have seen a large amount of significant works detailing microglial function in both health and disease. While some of these works do of course exhibit their shortcomings, in total this has been an exciting and opportune time to study microglial biology. The arrival of the *Cx3cr1*^{ERT2Cre} mouse (Goldmann et al., 2013) allowing for microglial fate-mapping and manipulation is really just the beginning with works in very recent years detailing master transcription factors for microglia (Buttgereit et al., 2016), their gradual maturation process in development through adulthood (Matcovitch-Natan et al., 2016), sex-dependent effects of microbiota exposure (Thion et al., 2017), and the role of micro RNAs (Varol et al., 2017) and epigenetics (Datta et al., 2018) in regulation of microglial homeostasis and inflammation.

I anticipate that future works will build upon this success and offer new insights into microglia-specific responses (using fate-mapping) in various contexts where peripheral myeloid cell engraftment is present including Alzheimer's disease, SCI, and traumatic brain injury. Studies utilizing single cell transcriptomics and proteomics will also most likely emerge which are essential in cases of CNS pathology where microglial responses are uniquely focal (Keren-Shaul et al., 2017). I would surmise that future works will yield insight in to the properties of microglia with respect to aging, as microglial transcriptional identity (Matcovitch-Natan et al., 2016) alters significantly with normal aging in to adulthood which is magnified further in advanced age

(Hickman et al., 2013). Whether aging has an effect on in vivo microglial phagocytic capacity remains to be seen and promises to be an exciting area of further research. Related to the question of microglia in the aged brain are microglial metabolomics. Phagocytosis is an extremely active process metabolically (Biswas and Mantovani, 2012) and it remains to be seen whether microglial metabolism changes during critical periods of development, acute and chronic pathologic conditions, or with physiological aging. Recently the metabolic role of microglia was shown to play a key role in a mouse model of Alzheimer's disease (Ulland et al., 2017) and I would anticipate that this may be the case during phagocytic or inflammatory microglial events.

The work in this thesis adds a role for microglia and its phagocytic capacity to a list of known functions in both health and disease (Kettenmann et al., 2013; Cronk and Kipnis, 2013; Kettenmann et al., 2011). In this chapter I will discuss the results of previous chapters in more detail, with the goal of placing them in a larger biological context and discussing their implications. Questions arising from my thesis work and possible experiments to address them will also follow.

The neuronal membrane as alarmin / "eat me" signal

There is much focus as to what regulates microglial phagocytosis in development and disease, as this action can prove critical in a variety of circumstances (discussed in the introduction). Central to this question in development is how microglia interact with neurons. Neurons are very well characterized as modulating their processes self-autonomously, independent of any microglial influence (Sala et al., 2001; Matus, 1988; Riccomagno and Kolodkin, 2015). Thus, in discussing phagocytosis in development, a role of agency must be ascribed to either the neuron or the microglial phagocyte in terms of specific elimination of processes. Recent works have showcased that, in fact, astrocytes and microglia are indeed excellent phagocytes of neuronal

material but how this process is initiated by the neuron has not been shown. One possibility that has arisen through recent study is through the alarmin IL-33, discussed in the introduction and shown to be critical for the immune response in spinal cord and optic nerve injury (Gadani et al., 2015b; a). Specifically, this group demonstrated that IL-33 can regulate microglial engulfment of neuronal material (Vainchtein et al., 2018). Although this group showed that germline deletion of IL-33 results in lower engulfment of microglial processes, no mechanistic attempt was provided to show how IL-33 regulated this process. In fact IL-33+ astrocytes were shown to have enhanced expression of MEGF10, shown to be critical for astrocytic engulfment of material (Chung et al., 2013). Despite this, analysis of astrocyte engulfment in the context of IL-33 removal was not addressed. Still to this date no work has shown how the presynaptic compartment changes in development allowing for the phagocyte to properly recognize and clear a marked terminal. While complement is clearly needed for this process to occur (Stephan et al., 2012), how IL-33 and C1q interact in this system has not been examined. Is IL-33 an alarmin guiding developmental phagocytosis of neuronal processes? If so, more works demonstrating how presynaptic terminals are tagged for elimination are needed.

In pursuit of alarmins in our system of Wallerian degeneration, some of the highest upregulated transcripts in microglia were *Il1a* and *Il1b* (Fig. 4.1), two key inflammatory cytokines that are readily involved in both infectious and sterile immunity (Garlanda et al., 2013). In fact, the role of IL-1 has been shown in a variety of sterile contexts during CNS inflammation including: Alzheimer's disease, ALS, macular degeneration, MS, Parkinson's disease, and stroke (Lukens et al., 2012). The role of IL-1 signaling has also been described in acute brain injury as well (Murray et al., 2015; Brough et al., 2011; Giles et al., 2015). Thus, it was surprising for us to see that no effect on microglial number or clearance of debris was seen in *Il1r*$^{-/-}$ mice three days post crush

injury (Fig. 4.18). We thought that IL-1 signaling could also be behind complement deposition as IL-1 has been shown to affect complement levels independent of serum complement in both the brain (Youm et al., 2013) and kidney (Gerritsma et al., 1996). Recent works have also shown that complement can also impact the release of IL-1 family cytokines (Laudisi et al., 2013). While we did not examine complement deposition in *Il1r*$^{-/-}$ mice three days post crush injury it appears that IL-1 signaling is not essential for microglial clearance of debris. Often times IL-1 signaling is downstream of the initial alarmin signal and helps to tune and amplify sources of local inflammation (Gadani et al., 2015a). At the acute time points investigated in our system it appears that IL-1 signaling is not needed. However, this cytokine may play a more critical role at later time points or during chronic pathology.

In development C1q was shown to be produced by RGCs and trafficked to the presynapse (Bialas and Stevens, 2013). Dependent on TGF-β release from astrocytes, neuronal C1q deposition has not been shown to be regulated by neuronal activity even though neuronal activity has been shown to direct engulfment. Future experiments should thus discern how and if neuronal activity is capable of regulating C1q self deposition on neurons. In our system, during Wallerian degeneration, we found that neuronal activity was indispensable for microglial engulfment of neuronal material. This is in direct contrast with what is seen in development. This begs the question, what does neuronal activity do to neuronal membranes in development? Experimentally it would be informative to perform proteomic and structural analysis of presynaptic terminals at baseline, neuronal activity induced (Forskolin treatment), and activity suppressed (TTX treatment) conditions. In phagocytosis literature the signal for engulfment is exposure of cytosolic proteins or membrane to the outer leaflet of the plasma membrane. This is the case for molecules such as

calreticulin or phosphatidylserine. Does this circumstance occur during development or Wallerian degeneration and is this the key alarmin and "eat me" signal for microglia?

We attempted to answer this question in our system by injection of Annexin V (which blocks phosphatidylserine residues directly) and utilization of the pSIVA reagent which is fluorescent only when bound to exposed phosphatidylserine residues. Additionally, pSIVA was shown to stain phosphatidylserine residues on degenerating cells in the cortex during microglial activation and clearance of dying cells (Tufail et al., 2017). This was possible as the pSIVA reagent could be applied to the top of the skull in living animals, which were later sacrificed for analysis. Due to the deep location of the dLGN, experiments in our system or in development have proven difficult. Experiments utilizing reporter mice could also be used for presynaptic targeting. Still, exposure of "eat me" signal could differ in neurodegenerative or developmental situations as these processes have been shown to be governed by distinct biological pathways (Gerdts et al., 2016b; Hoopfer et al., 2006).

During viral infection, it was shown that prevention of phosphatidylserine flipping from the inner to outer membrane leaflet was sufficient to block both microglial activation and phagocytosis. This was achieved through viral delivery in the cortex of an shRNA blocking the scramblase necessary for phosphatidylserine exposure (PLSCR1). In theory, if phosphatidylserine exposure governed either C1q deposition and/or glial engulfment of neuronal material in development, viral delivery of an shRNA against PLSCR1 should be sufficient to block engulfment and at least recapitulate the phenotype seen in complement-deficient mice. Elimination of PLSCR1in our system of Wallerian degeneration could also be advantageous and similar to the scenario seen in *Wld*^{S} mice if in fact phosphatidylserine exposure is driving dLGN microglial activation after optic nerve crush injury. This might not be the case as cell death in viral infection

and during Wallerian degeneration may have very different effects on what "eat me" signals are exposed on neuronal membranes and also what alarmins contribute to glial activation.

The difference between presynaptic membrane profile may also underlie differences in phagocytic phenotype between developmental synapse engulfment and engulfment seen with Wallerian degeneration. We observed that TTX treatment concurrent with optic nerve crush did not induce microglial engulfment of TTX treated terminals. This may be due to the membrane properties between presynaptic terminals undergoing degeneration (and presumably maximal phosphatidylserine exposure) and those receiving TTX treatment which may have reduced or non-existent phosphatidylserine exposure.

Classically there are defined cues for phagocytes to either enter a tissue or be drawn to targets requiring phagocytosis. The same has been shown in the CNS where fractalkine (CX3CL1) acted as a "find me" signal necessary for microglial migration and subsequent engulfment (Sokolowski et al., 2014; Noda et al., 2011; Truman et al., 2008; Paolicelli et al., 2014). While this system of "find me" signals is useful for advertising the location of cells necessary for clearance, such a system may not be needed for recognition of presynaptic terminals in either development or degenerative conditions. This may be due to the constant surveillance of microglial processes at baseline (Davalos et al., 2005; Liu et al., 2015; Gu et al., 2016; Eyo et al., 2014), groping their surroundings while theoretically monitoring neuronal activity at synapses but also perhaps sampling membrane integrity of neuronal processes. By constantly surveilling the dense tissue mass that is the mammalian CNS microglia may be uniquely poised to circumvent the need for true "find me" signals.

Perhaps our most puzzling piece of data came during analysis of *Myd88*$^{-/-}$ mice (Fig. 4.19, A-C). Discussed in the introduction, Myd88 is a central signaling hub of TLR signaling, capable

of signaling transduction through all mouse TLRs aside from TLR3, which is mediated through TRIF (Lu et al., 2008; Gadani et al., 2015a). Despite this central role in macrophage activation and the capability of TLRs to sense both foreign PAMPs and sterile injury-related DAMPs we found that Myd88-deficient animals had no change in clearance of neuronal debris from the LGN, indicating that dLGN microglial activation may in fact be Myd88 independent. Such a circumstance has actually been shown before in the case of macrophage activation proceeding independently of Myd88 when responding to extracellular nematode parasites (Mylonas et al., 2013) and with macrophage sensing and activation from LPS occurring independently from Myd88 through IRF3 (Kawai et al., 2001). Still our results were surprising given that we have shown previously that Myd88 signaling through immune cells plays a profound role in RGC neuroprotection during optic nerve crush injury (Walsh et al., 2015). It is thus possible that clearance of debris during Wallerian degeneration is Myd88-independent while neuroprotection is Myd88 dependent. This may be due to the nature of degeneration in our system as microglia are completely surrounded by their phagocytic targets and possibly their signal for activation, with presynaptic membrane alteration itself serving as both a profound "eat me" signal and sufficient alarmin to activate microglia. This puts phosphatidylserine as a central mediator of microglial activation which has been shown before during viral death within the brain (Tufail et al., 2017), though this role during sterile injury has not been shown.

This theory also posits that phosphatidylserine exposure as a unique activating signal on macrophages in specific circumstances even though phosphatidylserine has been canonically documented as a profoundly "anti-inflammatory" molecule (Birge et al., 2016). Judging from these results and the massive volume of literature documenting phosphatidylserine's role as a central mediator of phagocyte function (Kiss et al., 2006; Fond et al., 2015), it appears likely that the role

of the presynaptic membrane may be critically important in microglial mediated elimination of debris, either in development or pathology.

The point must also be made that most studies of phagocytic biology are reliant on the model of the phagocyte as a motile cell encountering an apoptotic cell body. In our context of Wallerian debris clearance microglia are centimeters away from any apoptotic neuronal cell body and are instead surrounded by degenerating cell processes which provide simultaneous signals of activation and phagocytosis. It thus seems that our normal linguistic constructs of "activation", "anti-inflammatory", and "immunosuppressive" in the classic sense of phagocytosis and immunology may be insufficient to account for all biological circumstances and open questions within the field of neuroimmunology and biology at large.

If neuronal membrane integrity is in fact the signal controlling microglial activation in our system of Wallerian degeneration, then what is the receiver on microglia? Can a single phagocytic receptor account for both microglial activation and engulfment of neuronal debris?

A "complementary" role for microglia

The role for complement in neurodevelopmental engulfment and clearance of synaptic material in a variety of inflammatory contexts has been discussed in the introduction. In our studies we found that during Wallerian degeneration, the complement gene set is highly enriched in microglia. We also found one our highest enriched transcripts was for *Itgam* (CD11b) which was shown to be critical for regulating engulfment during development (Schafer et al., 2012). The same was true in our system, with CD11b-deficient mice exhibiting impaired debris clearance. We also noticed that LGN C1q deposition was seen following optic nerve crush with microglial depletion by PLX5622 sufficient to prevent any C1q placement. This appears to separate C1q's mechanism

of action from that seen in our system vs. that seen in development where C1q is self-deposited by RGCs.

In fact, microglial complement deposition is also seen in healthy aging, where C1q levels rise in nearly all brain regions. Further, aged *C1qa*$^{-/-}$ mice exhibit reduced synaptic density and improved cognitive ability on learning tasks (Stephan et al., 2013). Presumably this is also the case in mouse models of Alzheimer's disease. In one study, J20 mice had improved long-termed potentiation (LTP) with deletion of C1q, presumably due to prevention of pathologic deposition by microglia (Hong et al., 2016). Despite the implication that C1q was deposited by microglia in this study, this conclusion was purely speculative as no attempt was made to delete microglia and see if this early deposition of C1q did, in fact, occur in the absence of microglia.

With C1q also important in development, it would also be intriguing to know if C1q drives astrocyte engulfment. Importantly, astrocytic engulfment was claimed by the authors to be independent of C1q in 2013, though this data was not shown (Chung et al., 2013). This is important to verify as astrocytes collectively eat around five to ten times as much total neuronal material in development compared to microglia (Chung et al., 2013). Further, it was shown that astrocytes can also phagocytose apoptotic corpses through a C1q-MEGF10 axis (Iram et al., 2016). Thus, an examination of C1q in relation to astrocyte engulfment during development is essential. In our system, C1q does seem to play a key role in regulating microglial engulfment, though its effect is not total, with *C1qa*$^{-/-}$ mice exhibiting impaired but not completely abolished phagocytic capacity. It may be that within the context of Wallerian degeneration, C1q deposition simply accelerates phagocytosis with other phagocytic receptors capable of compensation.

The two most highly upregulated phagocytic receptors in our dataset proved to *Itga5* and *Itgam* (CD11b). Due to CD11b being a classic marker for both macrophages and microglia as well

as essential for microglia-mediated engulfment of neuronal material in development (Schafer et al., 2012), we analyzed *Itgam*$^{-/-}$ mice in our system and found that CD11b was also essential for optimal debris clearance. This would add to the hypothesis that CD11b is an essential receptor for microglial-mediated phagocytosis. While CD11b is a canonical phagocytic receptor it has a myriad of functions including activation, chemotaxis, cytotoxicity, and tolerance induction and is expressed on a wide variety of cells (Rosetti and Mayadas, 2016).

Systemic lupus erythematous (SLE) is an autoimmune disease where the highest risk factor is a mutation in the *Itgam* gene (Fagerholm et al., 2013). Despite CD11b impacting chemotaxis and activation, it was shown that CD11b-mediated phagocytosis was the key function in cells carrying this risk allele (Fossati-Jimack et al., 2013). While the theory behind SLE pathology is that failed engulfment of cells yields necrotic cell death and autoimmune antibodies against self-DNA, could deficient CD11b function also yield impaired clearance of cell processes apart from whole cell phagocytosis? Moreover, could this contribute to failed debris clearance of cell processes and impact autoimmunity? Study of this possible phenomenon is problematic as the full repertoire for a cell to engage cell processes vs. a cell in its entirety is unknown. Furthermore, the phagocytic receptor requirement for specialized removal of processes may be organ, phagocyte, and context-dependent.

One receptor to rule them all?

This question of phagocyte receptor specificity has long intrigued the field of phagocyte biology. Since many cells exhibit a wide range of phosphatidylserine recognizing receptors, what variety is needed for engulfment? Is the variety target dependent? Are these receptors interchangeable on various cell types? A prime example of this conundrum is in comparison of phagocytic function between microglia and two other major phagocytes, the retinal pigmented epithelial (RPE) cell of the retina and Sertoli cells of the testes.

RPE cells, while not professional phagocytes, play a critical role in retinal homeostasis through phagocytosis with disruption of their phagocytic capacity or loss of RPE cells resulting in blindness to the organism (Arandjelovic and Ravichandran, 2015; Burstyn-Cohen et al., 2012). Sertoli cells are specialized phagocytes of the testis, responsible for clearance of dying spermatocytes that are a natural consequence of healthy spermatogenesis (Arandjelovic and Ravichandran, 2015; Elliott et al., 2010b). While phagocytic processes are often difficult to observe during homeostasis this is not the case for these two cell populations as Sertoli cells clear dead spermatocytes on a constant basis and also are responsible for elimination of the residual-body that is removed during spermiation (Wang et al., 2006). RPE cells are diurnally phagocytic as soon as an organism wakes. Upon waking RPE cells proceed to "trim" the apical tips of photoreceptors that are about to shed in a phosphatidylserine-mediated process, allowing for the production of new photoreceptor segments to take place of the old (Ruggiero et al., 2012). In an elegant series of experiments it was shown that a MER/Protein S/Gas6 mediated axis was necessary for optimal phagocytosis of photoreceptor processes, with deletion of any one of these three elements leading to retinal degeneration (Burstyn-Cohen et al., 2012). In a similar fashion, Sertoli cell function is mediated through MER, with male mice deficient in MER signaling infertile

as a consequence of spermatogenic failure due to apoptotic corpse accumulation in the testes (Chen et al., 2009). With such a common core requirement of MER in both these phagocyte populations one would hypothesize that a rescue experiment overexpressing a phagocytic receptor in *Mertk*$^{-/-}$ mice would be sufficient to rescue phagocytic deficits in both the testes and retina. This was attempted by overexpression of the canonical phagocytic receptor BAI1 (discussed in Chapter 4) in *Mertk*$^{-/-}$ mice (Penberthy et al., 2017). While overexpression of BAI1 was sufficient to rescue phagocytic activity in Sertoli cells, RPE cells were unable to commence phagocytosis with stable overexpression of BAI1 (Penberthy et al., 2017). These experiments highlight that depending on the cell of interest some receptors (MER) may be necessary for two types of phagocytes while another receptor type (BAI1) may be sufficient to restore phagocytosis in one cell type (Sertoli cells) and not the other (RPE cells). Thus, caveats must be made when interpreting phagocytic cell types, as there is obviously not a universal "Phagocyte" and phagocytic receptors are not functional in a one-size-fits-all approach. This was also shown across multiple organs recently in another study detailing that organ professional phagocytes (Lung alveolar macrophages, liver Kupffer cells, intestinal macrophages, splenic macrophages, and bone marrow macrophages) each have a differential requirement for classic phagocytic receptors (MER, TIMD4, MFGE8, and LXRs) (Gonzalez et al., 2017).

This collective data helped elucidate a puzzling phenomenon in our studies when we investigated the role of MER in microglial phagocytosis of debris pursuant to Wallerian degeneration. As mentioned, MER is a canonical phagocyte receptor across multiple cells in both professional and non-professional phagocytes and is also a marker for tissue resident macrophages (Rothlin et al., 2015; Jung and Chung, 2018; Graham et al., 2014; Gautier et al., 2012). Further, its role has been recently widened to be critical for macrophage function with MER being required

for the engulfment of neural progenitors cells within areas of adult neurogenesis (Fourgeaud et al., 2016) and for the activation of microglia and clearance of viral infected neural cells in a phosphatidylserine dependent manner (Tufail et al., 2017). With this collection of data, it would thus make perfect sense that if neurodegeneration were necessary for microglial activation and engulfment in our system, then the MER phagocytic system must play a role in the clearance of neuronal debris. Further, the tiny bits of degenerating membrane in our system seem quite similar to that of degenerating photoreceptor tips whose phagocytosis again is dependent on the MER pathway (Burstyn-Cohen et al., 2012). Despite this strong evidence for MER as a canonical phagocytic receptor, deletion of MER or its bridging molecules Protein S or Gas6 had no effect on clearance of debris during Wallerian degeneration. MER may thus be useful in a cell, organ, or context-dependent situation. To investigate this, it would be insightful to see if MER-dependent phagocytosis was mediated by target type by treating microglia in culture with apoptotic neural progenitor cells or purified synaptosomes. The result of this experiment would thus reveal if MER-mediated phagocytosis was dependent on the phagocytic target itself. MER could also be important as an activating receptor rather than phagocytic mediator per se since MER can also induce activation signals through MEK, ERK, and NF-κB signaling (Cummings et al., 2013). Thus, in terms of microglial biology MER may act principally as an activating receptor. Therefore, in situations where microglia are surrounded by activating debris such as in Wallerian degeneration, MER may be dispensable.

A similar phenomenon may apply to BAI1, TREM2, and LRP1 in our model. While BAI1 was clearly shown to play a role in the developmental phagocytosis of neural progenitor cells in zebra fish (Mazaheri et al., 2014), transcripts in dLGN were considerably low at baseline and did not change following optic nerve crush injury (Fig. 4.1).

TREM2, much like MER, has been shown to be a critical phagocytic receptor and key regulator of microglial metabolism in the context of chronic disease (Poliani et al., 2015; Ulland et al., 2017). TREM2 has also been shown to interact with a variety of lipid ligands, including phosphatidylserine, though to a lesser degree when compared to sulfatide and sphingomyelin which are much more enriched in myelin (Poliani et al., 2015). Therefore, TREM2, though it was upregulated in our system (Fig. 4.1), may operate like MER in that it functions primarily to change the activation profile of microglia or it could be a more useful phagocytic receptor for myelin-rich lipids, while the presynaptic terminals in the dLGN are unmyelinated (Crish et al., 2010) and thus not feasible targets for TREM2-mediated phagocytosis. Experiments utilizing in vitro phagocytic assays with WT and *Trem2*$^{-/-}$ microglia fed myelin-rich and myelin-deficient synaptosomes or membrane would aid in answering this question. DAP12, the downstream mediator of TREM2, was also upregulated in dLGN microglia following optic nerve crush (Fig. 4.1). However, DAP12 is downstream of a variety of surface receptors and its upregulation may not be a reflection of TREM2 mediated action (Turnbull and Colonna, 2007).

LRP1 displayed a continuous upregulation from days 1 to 3 post-injury (Fig 4.1). Despite this, and its role as a microglial regulator of immunity (Chuang et al., 2016), we found no difference in the clearance of neuronal debris with microglial-specific deletion of LRP1. This could be due to LRP1's primary role as a scavenger receptor capable of interacting with hundreds of substrates (Fernandez-Castaneda et al., 2013) and thus its phagocytic ability could be dispensable for microglial phagocytosis.

Collectively, these data demonstrate a key need for microglia and microglia-mediated complement deposition in order to clear synaptic debris. Future studies should attempt to determine if this complement requirement is due to the size of phagocytic target, or whether

microglial complement deposition is a key component in either acute or chronic neurodegenerative states.

Taking out the trash, implications for microglial dereliction of duty

Discussed in the previous section, failure of phagocytosis can have critical outcomes in the context of both the retina and the testes, where blindness and infertility may result. Such catastrophic consequences would obviously have considerable evolutionary pressure and are indeed rare in hereditary circumstances. As such, most highly evolved organisms have a phagocytic capacity on the order of billions of cells with evidence of this process difficult to discern unless under severe pathology or with dramatic deletion of critical receptors. What then would be the consequence of failed microglial engulfment?

In the context of optic nerve crush, we are lucky as the Fischer group reported a study in which this question was asked in the context of neuronal regeneration after optic nerve crush injury (Hilla et al., 2017). While this group also found that depletion of microglia had no effect on neuroprotection up to three weeks post injury, they furthered this knowledge by showing that axonal regeneration (induced by prior lens injury prior to optic nerve crush) was unaffected by microglial depletion. While this may in fact be true, preconditioning lens injury by itself is a very low threshold to assess regeneration. Further, these researchers only observed axonal growth to 3 mm past the optic nerve head. While no effect of maximal axon growth or density was seen in the optic nerve with microglial depletion, the astrocytic scar was significantly smaller owing possibly to a communication between myeloid cells and astrocytes at the sight of injury (Hilla et al., 2017). While this may not be essential at the three week time point post-injury, we know now that the glial scar is a critical bridge and platform for axon regeneration in spinal cord injury (Anderson et

al., 2016). Thus, this absence of scar could be essential for longer time points in which neuronal regeneration may occur. Recently, models have been developed to allow for functional guidance from the retina all the way to the dLGN that restore visual function through optogenetic stimulation (Lim et al., 2016) or deletion of PTEN from RGCs along with injection of Zymosan and CPT-cAMP (de Lima et al., 2012). It would be quite interesting to see if deletion of microglia in these circumstances would allow for full axon regrowth, especially if the response of astrocytes to microglial cues is necessary for supporting the proper formation of the glial scar in response to injury. Further, this would assign a neuroprotective role to microglia-astrocyte crosstalk in an inflammatory circumstance, going against the current dogma that microglia are instigators of disease-amplifying astrocytes (Liddelow et al., 2017).

A recent study on the role of microglia in ALS came to a surprising result in a novel model of ALS where human TDP-43 is overexpressed (Spiller et al., 2018). Once TDP-43 production was halted this study found that microglia were required to clear toxic TDP-43 and this depletion of microglia led to a reduced number of both neuromuscular junctions and motor neuron number in the spinal cord (Spiller et al., 2018). This is at odds with previous ALS literature in ALS models, where microglia were positioned as distinctly disease-inducing (Frakes et al., 2014).

This sort of neuroprotective effect is what we would predict to happen in our current system. With microglial depletion leading to a persistence of uncleared debris by at least one-weak post injury, we would hypothesize that the thalamic relay neurons (TRNs), which are the projection neurons of the LGN, may be aberrantly active leading to further neuronal dysfunction in the visual circuit and possibly impeding the functional plasticity that can occur in the absence of visual stimuli (Merabet and Pascual-Leone, 2010; Rauschecker, 1995). Since microglia have been shown to be critical mediators in both plasticity of the visual system (Frost and Schafer, 2016; Tremblay

et al., 2010) and in our model of debris clearance, future experiments delving into the possibility of microglia preventing excitotoxicity and enhancing compensatory plasticity may aid in our overall understand of glial biology and CNS function.

Concluding remarks

Starting my journey into graduate work I had no idea that the fields of neuroscience and immunology even converged, let alone had become one of the fastest growing and exciting arenas of neuroscience. The neuroimmunology field is growing rapidly with new insights emerging in areas such as neurodevelopment (Stephan et al., 2012), lymphatic biology (Louveau et al., 2015), neural control of immunity (Pavlov and Tracey, 2017), immune influence on neurodegenerative disorders (Colonna and Butovsky, 2017), adaptive immune influence of cognition (Derecki et al., 2010), and the emerging influence of the immune system on developmental neurological disorders (Derecki et al., 2012; Cronk et al., 2015). I have learned that neuroimmune interactions are far more complicated than simple antagonism, and that their interactions are a necessary and fascinating aspect of biology itself. To be a small part of this growing field is both thrilling and humbling. In this thesis I have attempted to place these collected findings in the larger context of microglial biology and the immune response to acute CNS injury, offering an introduction to the basic concepts of phagocyte and microglial biology in the context of CNS perturbance, discussing the role of microglia during CNS Wallerian degeneration and what phagocytic repertoire they possibly require to clear debris, as well as positing theories describing our experimental results and providing possible experiments to test them and continue our understanding of microglia during CNS insult.

What I have not shown and discussed here are the countless hypotheses and unreported data from both myself and scientists in our field that have led to this collected knowledge. If it is true that Newton once said that he was merely standing on the shoulder of giants, then those giants must have indeed sunk their feet in the mountain of failed endeavor and rejected hypotheses that form the foundation of science. I sometimes like to say that one of the best feelings in science is knowing exactly the reason why an experiment failed or a hypothesis was disproven. It is through this understanding that I have grown as both a scientist and person. This along with the collective insight, inspiration, support, criticism, and comradery that I have received from my peers have been the guideposts on the start of my scientific journey.

Materials and Methods

Mice

C57/Bl6 and *Cx3cr1*-eGFP mice were obtained from Jackson Laboratory, stocks 000664 and 005582, respectively. B6.Cg-*Gt(ROSA)26Sor*$^{tm6(CAG-ZsGreen1)Hze}$/J (Ai6) mice were generously provided by the Harris Lab (University of Virginia) and originally obtained from Jackson Laboratories (Stock 007906). *Wld*^{S} mice were generously provided by the Deppmann Lab (University of Virginia) and originally obtained from Jackson Laboratories (008820) and bred with congenic FVB/N mice to obtain heterozygous *Wld*^{S} mice and wild-type controls. *Itgam*$^{-/-}$ mice and their congenic C57/Bl6J controls were purchased from Jackson Laboratories (stock 003991 and 000664, respectively). Heterozygous *C1qa*$^{+/-}$ mice were obtained from Jackson Laboratories (stock# 022307) and bred to obtain *C1qa*$^{-/-}$ and *C1qa*$^{+/+}$ control mice. *Mertk*$^{-/-}$, BAI1-Tg, *Elmo1*$^{-/-}$, and *Bai1*$^{-/-}$ mice were all provided by the Ravichandran Lab at the University of Virginia. *Trem2*$^{-/-}$ mice were provided by the Colonna Lab at Washington University of St. Louis, St. Louis, MS. *Cx3cr1*$^{ERT2Cre/+}$:*Lrp1*$^{fl/fl}$ mice were generously donated by the Gaultier Lab, University of Virginia. *Il1r*$^{-/-}$ mice were provided by the Lukens Lab, University of Virginia. All strains were kept in identical housing conditions. All animals were housed in temperature and humidity-controlled rooms, maintained on a 12-hour light/dark cycle (lights on 7:00 a.m.), mice were utilized from 2-4 months old with mice age-matched in each experiment described. For survival surgeries, mice were anesthetized with either 200 µl of Ketamine/Xylazine (1 ml KetamineHCl (1mg/ml), 1 ml of 2% Xylazine, 8 ml saline), or inhaled isoflurane. All procedures complied with regulations of the Institutional Animal Care and Use Committee (ACUC) at the University of Virginia. *Gas6*$^{-/-}$ and

LysM$^{Cre/+}$*:Pros1*$^{fl/fl}$ mice were kindly provided by Tal Burstyn-Cohen and bred and housed at the Hebrew University of Jerusalem, Ein Kerem campus. All experimentation prior to imaging and analysis were performed there under supervision of their University Ethics Committee for the Care and Use of Laboratory Animals.

Fate-mapping of microglia

B6.Cg-*Gt(ROSA)26Sor*$^{tm6(CAG-ZsGreen1)Hze}$/J mice (Ai6) were crossed to the *Cx3cr1*$^{ERT2cre/ERT2cre}$ mice to obtain *Cx3cr1*$^{ERT2/+}$:Ai6 heterozygotes. Mice were placed on a tamoxifen-supplemented (250 mg/kg) diet for one week (TD.130856 Envigo). After return to normal chow, a period of one month was allowed to ensure turnover of the peripheral immune system, allowing only microglia to retain the ZsGreen label (Goldmann et al., 2013). Mice were then subjected to analysis or optic nerve crush injury and sacrificed three days post injury.

PLX5622 administration

PLX5622 was provided by Plexxikon Inc. and formulated in AIN-76A standard chow by Research Diets Inc. at 1200mg/kg. Mice were kept on PLX5622 or AIN-76A control diets for three weeks prior to experimentation and maintained on their respective diets until sacrifice.

CTB-CypHer preparation

Cholera toxin β subunit (Sigma-Aldrich, C9903) was resuspended at 10mg/ ml in PBS and labeled with CypHer5 (10 μM, GE Healthcare) for 1h at 20 °C. Free dye was removed by gel filtration using Zeba desalting column (Thermo, 89890) according to manufacturer's instructions.

CTB injection and optic nerve injury

Following anesthesia, mice received intravitreal injections of cholera toxin-β subunit (CTB) conjugated to Alexa 555 dye into the left eye and CTB conjugated to Alexa 647 (Invitrogen, Eugene, OR) dye into the right eye (1 μl per eye at a concentration of 1 μg/μL in sterile saline). Optic nerve crush was performed 24 hours post CTB injection. Briefly, mice were anesthetized and the tissue around the nerve was carefully dissected and the optic nerve exposed. N5 self-closing forceps were closed around the optic nerve 5 mm behind the globe and held for three seconds. The mice were then allowed to recover at 37C on a warming pad before returning to their cages and sacrificed at time points indicated in the text (Mice that did not receive sufficient dLGN dye fills were omitted from analysis).

Tetrodotoxin (TTX) administration and measurement of pupil response

Once anesthetized, 1 μL tetrodotoxin (5μM) or its carrier solution (20 mM citrate buffer) was injected intravitreally with a Hamilton syringe contralateral to the eye used for optic nerve crush. Pupil response was measured as described previously (Shanks et al., 2016). Briefly, at time points 24 and 48 hours post optic nerve crush injury and TTX administration, mice were acclimated to a dark room for 30 minutes prior to testing. While under a red light (Black Diamond Cosmos), the pupil size was obtained using a Leica IC80HD camera fixed to a Leica M220 dissecting microscope. The mouse was then subjected to a 15 second exposure of bright white light and the pupil size was imaged instantly following return to red light conditions. The percentage pupil constriction was calculated as the difference in pupil size between red and bright white light conditions.

Tissue preparation and Immunofluorescence

The brain and eyes were harvested from animals after perfusion with heparinized (5U/mL) PBS and 4% PFA. Following 24 hours post-fixation in 4% PFA, the eye was dissected by cutting through the sclera just posterior to the ciliary body. The lens was removed and four cuts were made toward the optic disc to allow the retina to lay flat. The retina was then separated from the sclera and pigmented epithelium and placed in to 96-well plates for staining. Brain tissue was post-fixed for 48 hours in 4% PFA, followed by cryoprotection in 30% sucrose for 48 hours. Brains were then frozen in optimal cutting temperature (OCT) compound (Tissue-Tek) on the freezing element of a Leica CM3050 S cryostat. 40µm sections were sliced into 24-well plates containing PBS with 0.05% sodium azide. Slices were permeabilized with 0.5% Triton for 15 minutes then washed 2x 5 minutes in PBS and blocked for 1hr at room temperature in blocking buffer (5% serum of the secondary's species) followed by overnight incubation in 0.5% BSA with primary antibody at 4 degrees C. The following antibodies were used for immunofluorescence staining: Rabbit anti-Iba1 (Biocare Medical CP-290; 1:300), Goat anti-BRN3a (Santa Cruz sc- 31984; 1:300), Rabbit anti-Ki67 (Abcam ab15580; 1:300), Goat anti-Iba1(Abcam ab5076; 1:300), Rat anti-CD68 (Biolegend FA-11), Rabbit anti-TMEM119 (Abcam ab209064**)** Guinea Pig anti-Vglut2 (EMD Millipore AB2251) Rabbit anti-PSD-95 (Invitrogen 51-6900). Slices were washed 3x 5 minutes, incubated for 2 hours at RT with the appropriate secondary antibodies (all from Life Technologies; 1:1000), washed again 3x5 minutes and mounted with Aquamount (Thermo Scientific) and DAPI.

RGC Image acquisition and quantification

For assessing RGC survival, images of Brn3a+ RGCs from four quadrants of the retina were taken at equal distances from the optic disc using an Olympus IX-71 microscope with 40x objective. The

pictures were counted in a blinded fashion to determine the number of RGCs/field. RGC counts were established from each image utilizing a modified method described previously (Danias et al., 2002). Briefly, each image was inverted with the contrast enhanced (1%) and background subtracted (rolling ball 55). Each image was then set to a threshold to encompass all Brn3a+ cells. Once binary, the Watershed feature was applied and the Analyze Particle function used to count all RGCs. All images were acquired and quantified by a user blinded to the experiment.

dLGN Image quantification and analysis

For each animal, 3-4 sections of medial dLGN were chosen for imaging and quantification of RGC inputs to the dLGN, Iba1+ cell counts and microglial engulfment analysis. Images were acquired on a Leica SP8 microscope at 20x magnification with 1 μm *z*-steps for CTB field size measurements and Iba1+ cell counts and at 60x magnification using 0.2 μm *z*-steps for engulfment analyses. For each animal, 4-7 dLGN fields were imaged. Obtained images were processed and quantified using ImageJ (NIH) for CTB field size and Iba1+ cell number and Imaris software (Bitplane) for engulfment analyses. For CTB field size and Iba1+ cell number, ImageJ was utilized to obtain ROIs of contralateral dLGN fields for crushed and uninjured inputs. Each ROI was then thresholded to include all CTB+ inputs and the area measured. Iba1+ counts were made using the Cell Counter plugin (ImageJ). *In vivo* microglia phagocytosis assays and analyses were performed as previously described in detail (Schafer et al., 2012, 2014). Imaris software (Bitplane) was used to create 3D volume surface renderings for microglia and engulf debris. To visualize and measure debris volume, any fluorescence that was not within the microglia volume was subtracted from the image using the mask function of Imaris. The remaining engulfed fluorescence was surface rendered and total volume of engulfed debris was calculated. To determine percentage engulfment

the following calculation was used: volume of internalized debris /volume of microglial cell x 100. Image acquisition, quantification, and analyses were performed blind.

Retinogeniculate synapse quantification

Quantification of retinogeniculate synapses was performed as described previously (Ippolito and Eroglu, 2010). Briefly, dLGN brain sections from uninjured and three days post crush mice were stained with pre- (VGlut2) and post-synaptic (PSD95) markers. 5 μm thick confocal z-stacks (optical section depth 0.33 μm, 15 sections/z-stack) within the outer shell were imaged at 60x magnification on a Leica SP8 confocal laser-scanning microscope. Maximum projections of three consecutive sections (corresponding to 1 μm total depth) were generated from the original z-stack. The Puncta Analyzer plugin that was developed by Barry Wark for ImageJ (available at http://labs.cellbio.duke.edu/Eroglu/Eroglu_Lab/Publications. html) was used to count the number of co-localized puncta.

Flow cytometry of LGN astrocytes and microglia to assess engulfment of neuronal material

Following anesthesia, mice received intravitreal injections of cholera toxin-β subunit (CTB) conjugated to Alexa 647 dye or CypHer in to both eyes (1 μl per eye at a concentration of 1 μg/μL in sterile saline). Optic nerve crush was performed 24 hours post CTB injection. Three days post bilateral optic nerve crush, mice were perfused with PBS containing heparin (5U/mL) until livers and tongues were exsanguinated to minimize peripheral immune cell contamination. The entire brain was then removed and meninges and choroid plexus carefully separated and discarded from the rest of the brain tissue. The brain was then placed in a coronal brain matrix (Roboz SA-2165) and a razor blade used to isolate a brain slice containing the dLGN. The dLGN was dissected from

surrounding brain regions and placed in 1.5 ml Accutase (Stempro A1110501**)** in a 1.5 mL Eppendorf tube. Samples were then incubated at 37º C for 15 minutes, followed by gentle trituration five times up and down with a 1000 μL Eppendorf pipette. Samples were again incubated at 37º C for 10 minutes, followed by a second gentle trituration five times up and down with a 1000 μL Eppendorf pipette. At this point, cells were well dissociated. The 1.5 mL suspension was then filtered gently through a 70 μm cell strainer and placed in 15 ml tubes containing 14 mL DMEM/F12/ with 10% FBS. Cells were then pelleted at 300 RCF for 10 minutes. Cells were then stained for 15 minutes at four degrees C using antibodies against CD11b (PercpCy5.5), CD45 (FITC) (Both from R&D Systems), GLAST (PE, Miltenyi) as well as the viability dye ZombieNIR (Biolegend) and Hoechst dye (Molecular Probes) to label all nucleated cells. Cells were then washed and spun at 300 RCF for 10 minutes at 4 degrees and resuspended in FACS buffer (2mM EDTA, 0.5% BSA in PBS) and ran on a Gallios flow cytometer (Becton Dickinson). Positive fraction cells gated on Hoechst positivity, singlet events, and live cells before their respective gates. CTB positivity was set on naïve populations.

dLGN dissection, MACS isolation of microglia, and RNA extraction

Three days post bilateral optic nerve crush, mice were perfused with PBS containing heparin (5U/mL) until livers and tongues were exsanguinated to minimize peripheral immune cell contamination. The entire brain was then removed and meninges and choroid plexus carefully separated and discarded from the rest of the brain tissue. The brain was then placed in a coronal brain matrix (Roboz SA-2165) and a razor blade used to isolate a brain slice containing the dLGN. The dLGN was dissected from surrounding brain regions and placed in 1 ml HBSS (containing Mg and Ca), 4 U/ml papain, 50 u/ml DNASE-I (Sigma) in a 1.5 mL Eppendorf tube. Bilateral

dLGNs were pooled from four mice to serve as one biological sample (three samples per condition). Samples were then incubated at 37º C for 15 m, followed by gentle trituration five times up and down with a 1000 μL Eppendorf pipette. Samples were again incubated at 37º C for 15 m, followed by a second gentle trituration five times up and down with a 1000 μL Eppendorf pipette. At this point, cells were well dissociated. The 1 mL suspension was then filtered gently through a 70 μm cell strainer and placed in 15 ml tubes containing 14 mL DMEM/F12/ with 10% FBS. Cells were then pelleted at 300 RCF for 10 minutes. top of the gradient was then removed. Cells were then labeled with microglia CD11b+ magnetic selection beads (Miltenyi). Cells were positively selected by AutoMACS twice using the Possel setting. All 15 mL tubes prior to use were coated with sterile BSA in DEPC-treated PBS to ensure optimal recovery of microglia. Flow cytometry was used to ensure purity of the positive and negative fraction of AutoMACS sorted cells. 5% of each fraction was isolated and stained for 15 minutes at four degrees C using antibodies against CD11b (PE-Cy7) CD45 (BB515) (Both from R&D Systems) as well as the viability dye ZombieNIR (Biolegend) and Hoechst dye (Molecular Probes) to label all nucleated cells. Cells were then washed and spun at 300 RCF for 10 minutes at 4 degrees and resuspended in 1% PFA in PBS and samples ran on a Gallios flow cytometer (Becton Dickinson). Positive fraction cells gated on Hoechst positivity, singlet events, and live cells were > 90% pure CD45/CD11b+ microglia with the negative fraction < 1%. RNA was collected using the RNAqueous micro kit (Ambion) according to the manufacturer's protocol and stored at −80°C until use.

RNA Sequencing

RNA integrity was verified using the Qubit RNA assay kit (Life Technologies) and the Agilent RNA Pico kit (Agilent). Amplification and cDNA construction was performed using Nugen Ovation RNA-seq V2 kit with manufacturer's recommended protocol. Sample clean-up post amplification was performed using Qiagen PCR purification kit (Qiagen). Paired-end (50bp reads) RNA sequencing was performed using an Illumina HiSeq (Illumina) yielding 50-70 million reads per sample. All procedures were performed by HudsonAlpha (Huntsville, Alabama).

RNA-seq analysis.

The raw sequencing reads (FASTQ files) went through two stages of preprocessing to remove low quality reads and bases. First, they were chastity filtered, which removes any clusters that have a higher than expected intensity of the called base compared to other bases. Then they were trimmed with Trimmomatic (Bolger et al., 2014) to remove low quality bases (minimum read length after trimming = 36). After preprocessing, the quality of the reads was evaluated using FastQC (Andrews, 2010),and after passing quality control (QC), were aligned to the UCSC mm9 genome (Harrow et al., 2012) using the splice-aware read aligner STAR (Dobin et al., 2013).The quality of the alignments was next assessed by SAMStat (Lassmann et al., 2011), and any low quality alignments were removed with samtools (Li et al., 2009) (MAPQ < 10). Next, the number of reads aligning to each gene was quantified with HTSeq (Anders et al., 2015), and then the Bioconductor package DESeq2 (Love et al., 2014) was used to normalize the raw counts, perform exploratory analysis (e.g., PCA), and differential expression analysis. The Benjamini–Hochberg false discovery rate procedure was used to correct the p-values for multiple testing. Heat maps of the differentially expressed genes generated with the R package pheatmap (Kolde, 2015). UpSet plots

were created with the R package UpSetR (Conway et al., 2017),and the R implementation of Fisher's exact test, *fisher.test*, was used to identify enriched gene sets in the differentially expressed genes using the gene set collections from MSigDB (Subramanian et al., 2005)and the gene families from the Hugo Gene Nomenclature Committee (HGNC) (Gray et al., 2015).

Statistical Analyses

Statistical tests were performed using Prism (GraphPad) as described in the text and figure legends. In all figures, error bars represent mean ± SEM; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Accession number

All sequencing data has been uploaded to the GEO repository under accession number GSE107635

Bibliography

Ajami, B., J.L. Bennett, C. Krieger, W. Tetzlaff, and F.M. V Rossi. 2007. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. 10:1538–1543. doi:10.1038/nn2014.

Alisky, J.M., C.I. van de Wetering, and B.L. Davidson. 2002. Widespread dispersal of cholera toxin subunit b to brain and spinal cord neurons following systemic delivery. *Exp. Neurol.* 178:139–146. doi:10.1006/exnr.2002.8031.

Anders, S., P.T. Pyl, and W. Huber. 2015. HTSeq-A Python framework to work with high-throughput sequencing data. *Bioinformatics*. 31:166–169. doi:10.1093/bioinformatics/btu638.

Anderson, M.A., J.E. Burda, Y. Ren, Y. Ao, T.M. O'Shea, R. Kawaguchi, G. Coppola, B.S. Khakh, T.J. Deming, and M. V. Sofroniew. 2016. Astrocyte scar formation AIDS central nervous system axon regeneration. *Nature*. 532:195–200. doi:10.1038/nature17623.

Andrews, S. 2010. FastQC A Quality Control tool for High Throughput Sequence Data http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ by S. Andrews.

Angelucci, A., F. Clasc, and M. Sur. 1995. Anterograde axonal tracing with the subunit B of cholera toxin : a highly sensitive immunohistochemical protocol for revealing fine axonal morphology in adult and neonatal brains. 65.

Angius, D., H. Wang, R.J. Spinner, Y. Gutierrez-Cotto, M.J. Yaszemski, and A.J. Windebank. 2012. A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. *Biomaterials*. 33:8034–8039. doi:10.1016/j.biomaterials.2012.07.056.

Arandjelovic, S., and K.S. Ravichandran. 2015. Phagocytosis of apoptotic cells in homeostasis. *Nat. Immunol.* 16:907–17. doi:10.1038/ni.3253.

Atolini Junior, N., J. Jarjura, J. Junior, V. De, F. Gignon, A.T. Kitice, L. Suriano De, A. Prado, V. Gracia, and W. Santos. 2009. Facial Nerve Palsy: Incidence of Different Ethiologies in a Tertiary Ambulatory. *Arch. Otorhinolaryngol. Intl. Arch. Otorhinolaryngol*. 1313:175–167.

Auffray, C., D. Fogg, M. Garfa, and F. Geissmann. 2007. Monitoring of Blood Vessels and Tissues by a Population of Monocytes with Patrolling Behavior. *Science (80-.).* 317:666–671.

Barouch, R., and M. Schwartz. 2002. Autoreactive T cells induce neurotrophin production by immune and neural cells in injured rat optic nerve: implications for protective autoimmunity. *FASEB J.* 16:1304–6. doi:10.1096/fj.01-0467fje.

Bartel, D.L. 2012. Glial responses after chorda tympani nerve injury. *J. Comp. Neurol.* 520:2712–2729. doi:10.1002/cne.23069.

Basso, D.M., L.C. Fisher, A.J. Anderson, L.B. Jakeman, D.M. Mctigue, and P.G. Popovich. 2006. Basso Mouse Scale for Locomotion Detects Differences in Recovery after Spinal Cord Injury in Five Common Mouse Strains. *J. Neurotrauma*. 23:635–659. doi:10.1089/neu.2006.23.635.

Bastien, D., V. Bellver Landete, M. Lessard, N. Vallieres, M. Champagne, A. Takashima, M.-E. Tremblay, Y. Doyon, and S. Lacroix. 2015. IL-1 Gene Deletion Protects Oligodendrocytes after Spinal Cord Injury through Upregulation of the Survival Factor Tox3. *J. Neurosci.* 35:10715–10730. doi:10.1523/JNEUROSCI.0498-15.2015.

Beirowski, B., E. Babetto, M.P. Coleman, and K.R. Martin. 2008. The WldS gene delays axonal but not somatic degeneration in a rat glaucoma model. *Eur. J. Neurosci.* 28:1166–1179.

doi:10.1111/j.1460-9568.2008.06426.x.

Benhar, I., K. Reemst, V. Kalchenko, and M. Schwartz. 2016. The retinal pigment epithelium as a gateway for monocyte trafficking into the eye. *EMBO J.* 35:1219–1235. doi:10.15252/embj.201694202.

Bennett, M.L., F.C. Bennett, S.A. Liddelow, B. Ajami, J.L. Zamanian, N.B. Fernhoff, S.B. Mulinyawe, C.J. Bohlen, A. Adil, A. Tucker, I.L. Weissman, E.F. Chang, G. Li, G.A. Grant, M.G. Hayden Gephart, and B.A. Barres. 2016. New tools for studying microglia in the mouse and human CNS. *Proc. Natl. Acad. Sci.* 113:E1738–E1746. doi:10.1073/pnas.1525528113.

Bialas, A.R., and B. Stevens. 2013. TGF-β signaling regulates neuronal C1q expression and developmental synaptic refinement. *Nat. Neurosci.* 16:1773–82. doi:10.1038/nn.3560.

Birge, R.B., S. Boeltz, S. Kumar, J. Carlson, J. Wanderley, D. Calianese, M. Barcinski, R.A. Brekken, X. Huang, J.T. Hutchins, B. Freimark, C. Empig, J. Mercer, A.J. Schroit, G. Schett, and M. Herrmann. 2016. Phosphatidylserine is a global immunosuppressive signal in efferocytosis, infectious disease, and cancer. *Cell Death Differ.* 23:962–978. doi:10.1038/cdd.2016.11.

Bishop, D.L., T. Misgeld, M.K. Walsh, W.B. Gan, and J.W. Lichtman. 2004. Axon branch removal at developing synapses by axosome shedding. *Neuron*. 44:651–661. doi:10.1016/j.neuron.2004.10.026.

Biswas, S.K., and A. Mantovani. 2012. Orchestration of metabolism by macrophages. *Cell Metab.* 15:432–437. doi:10.1016/j.cmet.2011.11.013.

Blaschke, a J., K. Staley, and J. Chun. 1996. Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. *Development*. 122:1165–1174.

Blinzinger, K., and G. Kreutzberg. 1968. Displacement of synaptic terminals from regenerating motoneurons by microglial cells. *Zeitschrift für Zellforsch. und Mikroskopische Anat.* 85:145–157.

Bolger, A.M., M. Lohse, and B. Usadel. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*. 30:2114–2120. doi:10.1093/bioinformatics/btu170.

Bowen, S., D.D. Ateh, K. Deinhardt, M.M. Bird, K.M. Price, C.S. Baker, J.C. Robson, M. Swash, W. Shamsuddin, S. Kawar, T. El-Tawil, J. Roos, A. Hoyle, C.D. Nickols, C.H. Knowles, A.H. Pullen, P.J. Luthert, R.O. Weller, M. Hafezparast, R.J.M. Franklin, T. Revesz, R.H.M. King, O. Berninghausen, E.M.C. Fisher, G. Schiavo, and J.E. Martin. 2007. The phagocytic capacity of neurones. *Eur. J. Neurosci.* 25:2947–2955. doi:10.1111/j.1460-9568.2007.05554.x.

Broadie, K. 2004. Axon pruning: An active role for glial cells. *Curr. Biol.* 14:302–304. doi:10.1016/j.cub.2004.03.049.

Brooks, J.M., G.L. Carrillo, J. Su, D.S. Lindsay, M.A. Fox, and I.J. Blader. 2015. Toxoplasma gondiiInfections Alter GABAergic Synapses and Signaling in the Central Nervous System. *MBio*. 6:e01428-15. doi:10.1128/mbio.01428-15.

Brosius Lutz, A., W.-S. Chung, S.A. Sloan, G.A. Carson, L. Zhou, E. Lovelett, S. Posada, J.B. Zuchero, and B.A. Barres. 2017. Schwann cells use TAM receptor-mediated phagocytosis in addition to autophagy to clear myelin in a mouse model of nerve injury. *Proc. Natl. Acad. Sci.* 201710566. doi:10.1073/pnas.1710566114.

BrosiusLutz, A., and B.A. Barres. 2014. Contrasting the Glial Response to Axon Injury in the Central and Peripheral Nervous Systems. *Dev. Cell*. 28:7–17. doi:10.1016/j.devcel.2013.12.002.

Brough, D., and A. Denes. 2015. Interleukin-1α and brain inflammation. *IUBMB Life*. 67:323–

330. doi:10.1002/iub.1377.

Brough, D., P.J. Tyrrell, and S.M. Allan. 2011. Regulation of interleukin-1 in acute brain injury. *Trends Pharmacol. Sci.* 32:617–622. doi:10.1016/j.tips.2011.06.002.

Brown, G.C., and J.J. Neher. 2014. Microglial phagocytosis of live neurons. *Nat. Publ. Gr.* 15:209–216. doi:10.1038/nrn3710.

Brunk, U.T., and A. Terman. 2002. Lipofuscin: Mechanisms of age-related accumulation and influence on cell function. *Free Radic. Biol. Med.* 33:611–619. doi:10.1016/S0891-5849(02)00959-0.

Bruttger, J., K. Karram, S. Wörtge, T. Regen, F. Marini, N. Hoppmann, M. Klein, T. Blank, S. Yona, Y. Wolf, M. Mack, E. Pinteaux, W. Müller, F. Zipp, H. Binder, T. Bopp, M. Prinz, S. Jung, and A. Waisman. 2015. Genetic Cell Ablation Reveals Clusters of Local Self-Renewing Microglia in the Mammalian Central Nervous System. *Immunity*. 43:92–107. doi:10.1016/j.immuni.2015.06.012.

Bryant, C.E., and T.P. Monie. 2012. Mice, men and the relatives: cross-species studies underpin innate immunity. *Open Biol.* 2:120015–120015. doi:10.1098/rsob.120015.

Burstyn-Cohen, T., M.J. Heeb, and G. Lemke. 2009. Lack of Protein S in mice causes embryonic lethal coagulopathy and vascular dysgenesis. *J. Clin. Invest.* 119:2942–2953. doi:10.1172/JCI39325.

Burstyn-Cohen, T., E.D. Lew, P.G. Través, P.G. Burrola, J.C. Hash, and G. Lemke. 2012. Genetic Dissection of TAM Receptor-Ligand Interaction in Retinal Pigment Epithelial Cell Phagocytosis. *Neuron*. 76:1123–1132. doi:10.1016/j.neuron.2012.10.015.

Butovsky, O., M.P. Jedrychowski, R. Cialic, S. Krasemann, G. Murugaiyan, Z. Fanek, D.J. Greco, P.M. Wu, C.E. Doykan, O. Kiner, R.J. Lawson, M.P. Frosch, N. Pochet, R. El Fatimy, A.M. Krichevsky, S.P. Gygi, H. Lassmann, J. Berry, M.E. Cudkowicz, and H.L. Weiner. 2015. Targeting miR-155 restores abnormal microglia and attenuates disease in SOD1 mice. *Ann. Neurol.* 77:75–99. doi:10.1002/ana.24304.

Butovsky, O., G. Landa, G. Kunis, Y. Ziv, H. Avidan, N. Greenberg, A. Schwartz, I. Smirnov, A. Pollack, S. Jung, and M. Schwartz. 2006a. Induction and blockage of oligodendrogenesis by differently activated microglia in an animal model of multiple sclerosis. 116. doi:10.1172/JCI26836.Research.

Butovsky, O., Y. Ziv, A. Schwartz, G. Landa, A.E. Talpalar, S. Pluchino, G. Martino, and M. Schwartz. 2006b. Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol. Cell. Neurosci.* 31:149–60. doi:10.1016/j.mcn.2005.10.006.

Buttgereit, A., I. Lelios, X. Yu, M. Vrohlings, N.R. Krakoski, E.L. Gautier, R. Nishinakamura, B. Becher, and M. Greter. 2016. Sall1 is a transcriptional regulator defining microglia identity and function. *Nat. Immunol.* 17:1397–1406. doi:10.1038/ni.3585.

Carson, P.J., P. Konewko, K.S. Wold, P. Mariani, S. Goli, P. Bergloff, and R.D. Crosby. 2006. Long-Term Clinical and Neuropsychological Outcomes of West Nile Virus Infection. *Clin. Infect. Dis.* 43:723–730. doi:10.1086/506939.

Casano, A.M., M. Albert, and F. Peri. 2016. Developmental Apoptosis Mediates Entry and Positioning of Microglia in the Zebrafish Brain. *Cell Rep.* 1–10. doi:10.1016/j.celrep.2016.06.033.

Castellano, F., P. Montcourrier, and P. Chavrier. 2000. Membrane recruitment of Rac1 triggers phagocytosis. *J. Cell Sci.* 113 (Pt 1:2955–2961.

Chakrabarty, P., A. Li, P. Das, T.E. Golde, P. Chakrabarty, A. Li, C. Ceballos-diaz, J. a Eddy, C.C.

Funk, B. Moore, N. Dinunno, A.M. Rosario, P.E. Cruz, C. Verbeeck, A. Sacino, and S. Nix. 2015. IL-10 Alters Immunoproteostasis in APP Mice , Increasing Plaque Burden and Worsening Cognitive Article IL-10 Alters Immunoproteostasis in APP Mice , Increasing Plaque Burden and Worsening Cognitive Behavior. *Neuron*. 85:519–533. doi:10.1016/j.neuron.2014.11.020.

Chen, Y., H. Wang, N. Qi, H. Wu, W. Xiong, J. Ma, Q. Lu, and D. Han. 2009. Functions of TAM RTKs in regulating spermatogenesis and male fertility in mice. *Reproduction*. 138:655–666. doi:10.1530/REP-09-0101.

Chesselet, M.F. 1984. Presynaptic regulation of neurotransmitter release in the brain: Facts and hypothesis. *Neuroscience*. 12:347–375. doi:10.1016/0306-4522(84)90058-7.

Chiu, I.M., E.T.A. Morimoto, H. Goodarzi, J.T. Liao, S. O'Keeffe, H.P. Phatnani, M. Muratet, M.C. Carroll, S. Levy, S. Tavazoie, R.M. Myers, and T. Maniatis. 2013. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep.* 4:385–401. doi:10.1016/j.celrep.2013.06.018.

Chiu, I.M., H. Phatnani, M. Kuligowski, J.C. Tapia, M.A. Carrasco, M. Zhang, T. Maniatis, and M.C. Carroll. 2009. Activation of innate and humoral immunity in the peripheral nervous system of ALS transgenic mice. *Proc. Natl. Acad. Sci.* 106:20960–20965. doi:10.1073/pnas.0911405106.

Christopherson, K.S., E.M. Ullian, C.C.A. Stokes, C.E. Mullowney, J.W. Hell, A. Agah, J. Lawler, D.F. Mosher, P. Bornstein, and B.A. Barres. 2005. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell*. 120:421–433. doi:10.1016/j.cell.2004.12.020.

Chuang, T.Y., Y. Guo, S.M. Seki, A.M. Rosen, D.M. Johanson, J.W. Mandell, C.F. Lucchinetti, and A. Gaultier. 2016. LRP1 expression in microglia is protective during CNS autoimmunity. *Acta Neuropathol. Commun.* 4:68. doi:10.1186/s40478-016-0343-2.

Chung, W.-S., L.E. Clarke, G.X. Wang, B.K. Stafford, A. Sher, C. Chakraborty, J. Joung, L.C. Foo, A. Thompson, C. Chen, S.J. Smith, and B. a Barres. 2013. Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. *Nature*. 504:394–400. doi:10.1038/nature12776.

Coleman, M.P., L. Conforti, E.A. Buckmaster, A. Tarlton, R.M. Ewing, M.C. Brown, M.F. Lyon, and V.H. Perry. 1998. An 85-kb tandem triplication in the slow Wallerian degeneration (Wlds) mouse. *Proc. Natl. Acad. Sci. U. S. A.* 95:9985–90. doi:10.1073/pnas.95.17.9985.

Colonna, M., and O. Butovsky. 2017. Microglia Function in the Central Nervous System During Health and Neurodegeneration. *Annu. Rev. Immunol.* 35:441–468. doi:10.1146/annurev-immunol-051116-052358.

Conway, J.R., A. Lex, and N. Gehlenborg. 2017. UpSetR: An R package for the visualization of intersecting sets and their properties. *Bioinformatics*. 33:2938–2940. doi:10.1093/bioinformatics/btx364.

Cooper, M.E., S. Gregory, E. Adie, and S. Kalinka. 2002. pH-Sensitive Cyanine Dyes for Biological Applications. *J. Fluoresc.* 12:425–429. doi:10.1023/A:1021366010681.

Costigan, M., J. Scholz, and C.J. Woolf. 2009. Neuropathic Pain: A Maladaptive Response of the Nervous System to Damage. *Annu. Rev. Neurosci.* 32:1–32. doi:10.1146/annurev.neuro.051508.135531.

Couper, K.N., D.G. Blount, and E.M. Riley. 2008. IL-10: The Master Regulator of Immunity to Infection. *J. Immunol.* 180:5771–5777. doi:10.4049/jimmunol.180.9.5771.

Crish, S.D., R.M. Sappington, D.M. Inman, P.J. Horner, and D.J. Calkins. 2010. Distal axonopathy with structural persistence in glaucomatous neurodegeneration. *Proc. Natl. Acad. Sci. U. S. A.* 107:5196–5201. doi:10.1073/pnas.0913141107.

Cronk, J.C., N.C. Derecki, E. Ji, Y. Xu, A.E. Lampano, I. Smirnov, W. Baker, G.T. Norris, I. Marin, N. Coddington, Y. Wolf, S.D. Turner, A. Aderem, A.L. Klibanov, T.H. Harris, S. Jung, V. Litvak, and J. Kipnis. 2015. Methyl-CpG Binding Protein 2 Regulates Microglia and Macrophage Gene Expression in Response to Inflammatory Stimuli. *Immunity*. 42:679–691. doi:10.1016/j.immuni.2015.03.013.

Cronk, J.C., and J. Kipnis. 2013. Microglia - the brain's busy bees. *F1000Prime Rep.* 5:53. doi:10.12703/P5-53.

Crotti, A., and C.K. Glass. 2015. The choreography of neuroinflammation in Huntington's disease. *Trends Immunol.* 36:364–373. doi:10.1016/j.it.2015.04.007.

Croxford, A.L., M. Lanzinger, F.J. Hartmann, B. Schreiner, F. Mair, P. Pelczar, B.E. Clausen, S. Jung, M. Greter, and B. Becher. 2015. The Cytokine GM-CSF Drives the Inflammatory Signature of CCR2+ Monocytes and Licenses Autoimmunity. *Immunity*. 502–514. doi:10.1016/j.immuni.2015.08.010.

Cummings, C.T., D. DeRyckere, H.S. Earp, and D.K. Graham. 2013. Molecular pathways: MERTK signaling in cancer. *Clin. Cancer Res.* 19:5275–5280. doi:10.1158/1078-0432.CCR-12-1451.

Danias, J., F. Shen, D. Goldblum, B. Chen, J. Ramos-Esteban, S.M. Podos, and T. Mittag. 2002. Cytoarchitecture of the retinal ganglion cells in the rat. *Invest. Ophthalmol. Vis. Sci.* 43:587–594.

Datta, M., O. Staszewski, E. Raschi, M. Frosch, N. Hagemeyer, T.L. Tay, T. Blank, M. Kreutzfeldt, D. Merkler, S. Ziegler-Waldkirch, P. Matthias, M. Meyer-Luehmann, and M. Prinz. 2018. Histone Deacetylases 1 and 2 Regulate Microglia Function during Development, Homeostasis, and Neurodegeneration in a Context-Dependent Manner. *Immunity*. 48:514–529.e6. doi:10.1016/j.immuni.2018.02.016.

Davalos, D., J. Grutzendler, G. Yang, J. V Kim, Y. Zuo, S. Jung, D.R. Littman, M.L. Dustin, and W.-B. Gan. 2005. ATP mediates rapid microglial response to local brain injury in vivo. *Nat. Neurosci.* 8:752–8. doi:10.1038/nn1472.

Dekkers, M.P.J., V. Nikoletopoulou, and Y.A. Barde. 2013. Death of developing neurons: New insights and implications for connectivity. *J. Cell Biol.* 203:385–393. doi:10.1083/jcb.201306136.

Depboylu, C., K. Schorlemmer, M. Klietz, W.H. Oertel, E. Weihe, G.U. Höglinger, and M.K.-H. Schäfer. 2011. Upregulation of microglial C1q expression has no effects on nigrostriatal dopaminergic injury in the MPTP mouse model of Parkinson disease. *J. Neuroimmunol.* 236:39–46. doi:10.1016/j.jneuroim.2011.05.006.

Derecki, N.C., A.N. Cardani, C.H. Yang, K.M. Quinnies, A. Crihfield, K.R. Lynch, and J. Kipnis. 2010. Regulation of learning and memory by meningeal immunity: a key role for IL-4. *J. Exp. Med.* 207:1067–80. doi:10.1084/jem.20091419.

Derecki, N.C., J.C. Cronk, Z. Lu, E. Xu, S.B.G. Abbott, P.G. Guyenet, and J. Kipnis. 2012. Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature*. 484:105–9. doi:10.1038/nature10907.

Dobin, A., C.A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, and T.R. Gingeras. 2013. STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*. 29:15–21. doi:10.1093/bioinformatics/bts635.

Doetsch, F., A. García-Verdugo, and J. Alvarez-Buylla. 1997. Cellular Composition and Three-Dimensional Organization of the Subventricular Germinal Zone in the Adult Mammalian Brain. *J. Neurosci.* 17(13):5046–61.

Elliott, M.R., S. Zheng, D. Park, R.I. Woodson, M. a Reardon, I.J. Juncadella, J.M. Kinchen, J. Zhang, J.J. Lysiak, and K.S. Ravichandran. 2010a. Unexpected requirement for ELMO1 in clearance of apoptotic germ cells in vivo. *Nature*. 467:333–337. doi:10.1038/nature09356.

Elliott, M.R., S. Zheng, D. Park, R.I. Woodson, M. a Reardon, I.J. Juncadella, J.M. Kinchen, J. Zhang, J.J. Lysiak, and K.S. Ravichandran. 2010b. Unexpected requirement for ELMO1 in clearance of apoptotic germ cells in vivo. *Nature*. 467:333–337. doi:10.1038/nature09356.

Elmore, M.R.P., A.R. Najafi, M.A. Koike, N.N. Dagher, E.E. Spangenberg, R.A. Rice, M. Kitazawa, B. Matusow, H. Nguyen, B.L. West, and K.N. Green. 2014. Colony-Stimulating Factor 1 Receptor Signaling Is Necessary for Microglia Viability, Unmasking a Microglia Progenitor Cell in the Adult Brain. *Neuron*. 82:380–397. doi:10.1016/j.neuron.2014.02.040.

Ene, L., T.D. Marcotte, A. Umlauf, C. Grancea, A. Temereanca, A. Bharti, C.L. Achim, S. Letendre, and S.M. Ruta. 2016. Latent toxoplasmosis is associated with neurocognitive impairment in young adults with and without chronic HIV infection. *J. Neuroimmunol.* 299:1–7. doi:10.1016/j.jneuroim.2016.08.003.

Erridge, C. 2010. Endogenous ligands of TLR2 and TLR4: agonists or assistants? *J. Leukoc. Biol.* 87:989–999. doi:10.1189/jlb.1209775.

Esposito, E., and S. Cuzzocrea. 2011. Anti-TNF therapy in the injured spinal cord. *Trends Pharmacol. Sci.* 32:107–115. doi:10.1016/j.tips.2010.11.009.

Eyo, U.B., J. Peng, P. Swiatkowski, A. Mukherjee, A. Bispo, and L.-J. Wu. 2014. Neuronal Hyperactivity Recruits Microglial Processes via Neuronal NMDA Receptors and Microglial P2Y12 Receptors after Status Epilepticus. *J. Neurosci.* 34:10528–10540. doi:10.1523/JNEUROSCI.0416-14.2014.

Fagerholm, S.C., M. Macpherson, M.J. James, C. Sevier-Guy, and C.S. Lau. 2013. The CD11b-integrin (ITGAM) and systemic lupus erythematosus. *Lupus*. 22:657–663. doi:10.1177/0961203313491851.

Faust, N., F. Varas, L.M. Kelly, S. Heck, and T. Graf. 2000. Insertion of enhanced green fluorescent protein into the lysozyme gene creates mice with green fluorescent granulocytes and macrophages. *Blood*. 96:719–726.

Fernandez-Castaneda, A., S. Arandjelovic, T.L. Stiles, R.K. Schlobach, K.A. Mowen, S.L. Gonias, and A. Gaultier. 2013. Identification of the low density lipoprotein (LDL) receptor-related protein-1 interactome in central nervous system myelin suggests a role in the clearance of necrotic cell debris. *J. Biol. Chem.* 288:4538–4548. doi:10.1074/jbc.M112.384693.

Ferri, A., J.R. Sanes, M.P. Coleman, J.M. Cunningham, and A.C. Kato. 2003. Inhibiting Axon Degeneration and Synapse Loss Attenuates Apoptosis and Disease Progression in a Mouse Model of Motoneuron Disease. *Curr. Biol.* 15:669–673. doi:10.1016/S.

Fiala, M., D.H. Cribbs, M. Rosenthal, and G. Bernard. 2007. Phagocytosis of Amyloid-. *J. Neurosci.* 11:457–463.

Fond, A.M., C.S. Lee, I.G. Schulman, R.S. Kiss, and K.S. Ravichandran. 2015. Apoptotic cells trigger a membrane-initiated pathway to increase ABCA1. 1:1–11. doi:10.1172/JCI80300.for.

Fossati-Jimack, L., G.S. Ling, A. Cortini, M. Szajna, T.H. Malik, J.U. McDonald, M.C. Pickering, H.T. Cook, P.R. Taylor, and M. Botto. 2013. Phagocytosis Is the Main CR3-Mediated Function Affected by the Lupus-Associated Variant of CD11b in Human Myeloid Cells.

PLoS One. 8. doi:10.1371/journal.pone.0057082.

Fourgeaud, L., P.G. Través, Y. Tufail, H. Leal-Bailey, E.D. Lew, P.G. Burrola, P. Callaway, A. Zagórska, C. V. Rothlin, A. Nimmerjahn, and G. Lemke. 2016. TAM receptors regulate multiple features of microglial physiology. *Nature*. 1–15. doi:10.1038/nature17630.

Frakes, A.E., L. Ferraiuolo, A.M. Haidet-Phillips, L. Schmelzer, L. Braun, C.J. Miranda, K.J. Ladner, A.K. Bevan, K.D. Foust, J.P. Godbout, P.G. Popovich, D.C. Guttridge, and B.K. Kaspar. 2014. Microglia induce motor neuron death via the classical NF-κB pathway in amyotrophic lateral sclerosis. *Neuron*. 81:1009–1023. doi:10.1016/j.neuron.2014.01.013.

Fricker, M., M.J. Oliva-Martín, and G.C. Brown. 2012. Primary phagocytosis of viable neurons by microglia activated with LPS or Aβ is dependent on calreticulin/LRP phagocytic signalling. *J. Neuroinflammation*. 9:196. doi:10.1186/1742-2094-9-196.

Frost, J.L., and D.P. Schafer. 2016. Microglia: Architects of the Developing Nervous System. *Trends Cell Biol.* 26:587–597. doi:10.1016/j.tcb.2016.02.006.

Gadani, S.P., I. Smirnov, A.T. Smith, C.C. Overall, and J. Kipnis. 2016. Characterization of meningeal type 2 innate lymphocytes and their response to CNS injury. *J. Exp. Med.* 1–14.

Gadani, S.P., J.T. Walsh, J.R. Lukens, and J. Kipnis. 2015a. Dealing with Danger in the CNS: The Response of the Immune System to Injury. *Neuron*. 87:47–62. doi:10.1016/j.neuron.2015.05.019.

Gadani, S.P., J.T. Walsh, I. Smirnov, J. Zheng, and J. Kipnis. 2015b. The Glia-Derived Alarmin IL-33 Orchestrates the Immune Response and Promotes Recovery following CNS Injury. *Neuron*. 85:703–709. doi:10.1016/j.neuron.2015.01.013.

Gardai, S.J., K.A. McPhillips, S.C. Frasch, W.J. Janssen, A. Starefeldt, J.E. Murphy-Ullrich, D.L. Bratton, P.A. Oldenborg, M. Michalak, and P.M. Henson. 2005. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. *Cell*. 123:321–334. doi:10.1016/j.cell.2005.08.032.

Garlanda, C., C.A. Dinarello, and A. Mantovani. 2013. The Interleukin-1 Family: Back to the Future. *Immunity*. 39:1003–1018. doi:10.1016/j.immuni.2013.11.010.

Gaudet, A.D., P.G. Popovich, and M.S. Ramer. 2011. Wallerian degeneration: Gaining perspective on inflammatory events after peripheral nerve injury. *J. Neuroinflammation*. 8:110. doi:10.1186/1742-2094-8-110.

Gaudet, A.D., D.R. Sweet, N.K. Polinski, Z. Guan, and P.G. Popovich. 2015. Galectin-1 in injured rat spinal cord: Implications for macrophage phagocytosis and neural repair. *Mol. Cell. Neurosci.* 64:84–94. doi:10.1016/j.mcn.2014.12.006.

Gaultier, A., X. Wu, N. Le Moan, S. Takimoto, G. Mukandala, K. Akassoglou, W.M. Campana, and S.L. Gonias. 2009. Low-density lipoprotein receptor-related protein 1 is an essential receptor for myelin phagocytosis. *J. Cell Sci.* 122:1155–1162. doi:10.1242/jcs.040717.

Gautier, E.L., T. Shay, J. Miller, M. Greter, C. Jakubzick, S. Ivanov, J. Helft, A. Chow, K.G. Elpek, S. Gordonov, A.R. Mazloom, A. Ma'ayan, W.-J. Chua, T.H. Hansen, S.J. Turley, M. Merad, G.J. Randolph, A.J. Best, J. Knell, A. Goldrath, B. Brown, V. Jojic, D. Koller, N. Cohen, P. Brennan, M. Brenner, A. Regev, A. Fletcher, K. Elpek, A. Bellemare-Pelletier, D. Malhotra, S. Turley, R. Jianu, D. Laidlaw, J. Collins, K. Narayan, K. Sylvia, J. Kang, R. Gazit, B.S. Garrison, D.J. Rossi, F. Kim, T.N. Rao, A. Wagers, S. a Shinton, R.R. Hardy, P. Monach, N. a Bezman, J.C. Sun, C.C. Kim, L.L. Lanier, T. Heng, T. Kreslavsky, M. Painter, J. Ericson, S. Davis, D. Mathis, and C. Benoist. 2012. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat. Immunol.* 13:1118–1128. doi:10.1038/ni.2419.

George, E.B., J.D. Glass, and J.W. Griffin. 1995. Axotomy-induced axonal degeneration is mediated by calcium influx through ion-specific channels. *J. Neurosci.* 15:6445–6452. doi:10.1523/JNEUROSCI.5397-08.2009.

Gerdts, J., D.W. Summers, J. Milbrandt, and A. DiAntonio. 2016a. Axon Self-Destruction: New Links among SARM1, MAPKs, and NAD+ Metabolism. *Neuron*. 89:449–460. doi:10.1016/j.neuron.2015.12.023.

Gerdts, J., D.W. Summers, J. Milbrandt, and A. DiAntonio. 2016b. Axon Self-Destruction: New Links among SARM1, MAPKs, and NAD+ Metabolism. *Neuron*. 89:449–460. doi:10.1016/j.neuron.2015.12.023.

Gerritsma, J.S.J., A.F. Gerritsen, C. Van Kooten, L.A. Van Es, and D.M. R. 1996. INTERLEUKIN-la ENHANCES THE BIOSYNTHESIS OF C3 AND FACTOR B BY HUMAN TUBULAR EPITHELIAL CELLS IN WZ- ‘ RO. *Mol. Immunol.* 33:847–854.

Ghetti, B., D.S. Horoupian, and H.M. Wisniewski. 1972. Transsynaptic response of the lateral geniculate nucleus and the pattern of degeneration of the nerve terminals in the Rhesus monkey after eye enucleation. *Brain Res.* 45:31–48. doi:10.1016/0006-8993(72)90214-4.

Giles, J. a., A.D. Greenhalgh, C.L. Davies, A. Denes, T. Shaw, G. Coutts, N.J. Rothwell, B.W. McColl, and S.M. Allan. 2015. Requirement for interleukin-1 to drive brain inflammation reveals tissue-specific mechanisms of innate immunity. *Eur. J. Immunol.* 45:525–530. doi:10.1002/eji.201444748.

Gillingwater, T.H., C.A. Ingham, M.P. Coleman, and R.R. Ribchester. 2003. Ultrastructural correlates of synapse withdrawal at axotomized neuromuscular junctions in mutant and transgenic mice expressing the Wld gene. *J. Anat.* 203:265–276. doi:10.1046/j.1469-7580.2003.00214.x.

Gillingwater, T.H., C.A. Ingham, K.E. Parry, A.K. Wright, J.E. Haley, T.M. Wishart, G.W. Arbuthnott, and R.R. Ribchester. 2006. Delayed synaptic degeneration in the CNS of Wlds mice after cortical lesion. *Brain*. 129:1546–1556. doi:10.1093/brain/awl101.

Gilmore, T. 2014. Nf-kB Gene targets. *https://www.bu.edu/nf-kb/gene-resources/target-genes/*.

Ginhoux, F., M. Greter, M. Leboeuf, S. Nandi, P. See, S. Gokhan, M.F. Mehler, S.J. Conway, L.G. Ng, E.R. Stanley, I.M. Samokhvalov, and M. Merad. 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*. 330:841–5. doi:10.1126/science.1194637.

Ginhoux, F., S. Lim, G. Hoeffel, D. Low, and T. Huber. 2013. Origin and differentiation of microglia. *Front. Cell. Neurosci.* 7:45. doi:10.3389/fncel.2013.00045.

Goldmann, T., P. Wieghofer, P.F. Müller, Y. Wolf, D. Varol, S. Yona, S.M. Brendecke, K. Kierdorf, O. Staszewski, M. Datta, T. Luedde, M. Heikenwalder, S. Jung, and M. Prinz. 2013. A new type of microglia gene targeting shows TAK1 to be pivotal in CNS autoimmune inflammation. *Nat. Neurosci.* 16:1618–26. doi:10.1038/nn.3531.

Gonzalez, N.A., J.A. Quintana, S.G. Silva, M. Mazariegos, A. González, D. Aleja, J.A.N. Ávila, W. Walter, J.M. Adrover, G. Crainiciuc, V.K. Kuchroo, C. V Rothlin, H. Peinado, A. Castrillo, M. Ricote, and A. Hidalgo. 2017. Phagocytosis imprints heterogeneity in tissue-resident macrophages. 1–16. doi:10.1084/jem.20161375.

Gordon, S., and A. Plüddemann. 2017. Tissue macrophages: Heterogeneity and functions. *BMC Biol.* 15:1–18. doi:10.1186/s12915-017-0392-4.

Graeber, M.B., W. Tetzlaff, W.J. Streit, and G.W. Kreutzberg. 1988. Microglial cells but not astrocytes undergo mitosis following rat facial nerve axotomy. *Neurosci. Lett.* 85:317–321. doi:10.1016/0304-3940(88)90585-X.

Graham, D.K., D. DeRyckere, K.D. Davies, and H.S. Earp. 2014. The TAM family: phosphatidylserine-sensing receptor tyrosine kinases gone awry in cancer. *Nat. Rev. Cancer*. 14:769–785. doi:10.1038/nrc3847.

Gray, K.A., B. Yates, R.L. Seal, M.W. Wright, and E.A. Bruford. 2015. Genenames.org: The HGNC resources in 2015. *Nucleic Acids Res.* 43:D1079–D1085. doi:10.1093/nar/gku1071.

Green, D.R., T.H. Oguin, and J. Martinez. 2016. The clearance of dying cells: Table for two. *Cell Death Differ.* 23:915–926. doi:10.1038/cdd.2015.172.

Greenhalgh, A.D., and S. David. 2014. Differences in the phagocytic response of microglia and peripheral macrophages after spinal cord injury and its effects on cell death. *J. Neurosci.* 34:6316–22. doi:10.1523/JNEUROSCI.4912-13.2014.

Greenhalgh, A.D., R. Passos dos Santos, J.G. Zarruk, C.K. Salmon, A. Kroner, and S. David. 2016. Arginase-1 is expressed exclusively by infiltrating myeloid cells in CNS injury and disease. *Brain. Behav. Immun.* 56:61–67. doi:10.1016/j.bbi.2016.04.013.

Greter, M., I. Lelios, and A.L. Croxford. 2015. Microglia versus myeloid cell nomenclature during brain inflammation. *Front. Immunol.* 6:1–7. doi:10.3389/fimmu.2015.00249.

Gu, N., U.B. Eyo, M. Murugan, J. Peng, S. Matta, H. Dong, and L.J. Wu. 2016. Microglial P2Y12 receptors regulate microglial activation and surveillance during neuropathic pain. *Brain. Behav. Immun.* 55:82–92. doi:10.1016/j.bbi.2015.11.007.

Guadagno, J., P. Swan, R. Shaikh, and S.P. Cregan. 2015. Microglia-derived IL-1β triggers p53-mediated cell cycle arrest and apoptosis in neural precursor cells. *Cell Death Dis.* 6:e1779-10. doi:10.1038/cddis.2015.151.

Guan, Z., J.A. Kuhn, X. Wang, B. Colquitt, C. Solorzano, S. Vaman, A.K. Guan, Z. Evans-Reinsch, J. Braz, M. Devor, S.L. Abboud-Werner, L.L. Lanier, S. Lomvardas, and A.I. Basbaum. 2015. Injured sensory neuron–derived CSF1 induces microglial proliferation and DAP12-dependent pain. *Nat. Neurosci.* doi:10.1038/nn.4189.

Guerreiro, R., A. Wojtas, J. Bras, M. Carrasquillo, E. Rogaeva, E. Majounie, C. Cruchaga, J.S.K. Kauwe, S. Younkin, L. Hazrati, J. Lambert, P. Amouyel, A. Goate, A. Singleton, J. Hardy, and The Alzheimer Genetic Analysis Group. 2013. TREM-2 variants in AD. 368:117–127. doi:10.1056/NEJMoa1211851.TREM2.

Guillot-Sestier, M.-V., K.R. Doty, D. Gate, J. Rodriguez, B.P. Leung, K. Rezai-Zadeh, and T. Town. 2015. Il10 Deficiency Rebalances Innate Immunity to Mitigate Alzheimer-Like Pathology. *Neuron*. 85:534–548. doi:10.1016/j.neuron.2014.12.068.

Guo, L., A.J. Rolfe, X. Wang, W. Tai, Z. Cheng, K. Cao, X. Chen, Y. Xu, D. Sun, J. Li, X. He, W. Young, J. Fan, and Y. Ren. 2016. Rescuing macrophage normal function in spinal cord injury with embryonic stem cell conditioned media. *Mol. Brain*. 9:1–14. doi:10.1186/s13041-016-0233-3.

Halonen, S.K. 2004. Immune response to Toxoplasma Gondii in the Central Nervous system. *In* Opportunistic Infections: Toxoplasma, Sarcocystis, and Microsporidia. World Class Parasites.

Harrow, J., A. Frankish, J.M. Gonzalez, E. Tapanari, M. Diekhans, F. Kokocinski, B.L. Aken, D. Barrell, A. Zadissa, S. Searle, I. Barnes, A. Bignell, V. Boychenko, T. Hunt, M. Kay, G. Mukherjee, J. Rajan, G. Despacio-Reyes, G. Saunders, C. Steward, R. Harte, M. Lin, C. Howald, A. Tanzer, T. Derrien, J. Chrast, N. Walters, S. Balasubramanian, B. Pei, M. Tress, J.M. Rodriguez, I. Ezkurdia, J. Van Baren, M. Brent, D. Haussler, M. Kellis, A. Valencia, A. Reymond, M. Gerstein, R. Guigó, and T.J. Hubbard. 2012. GENCODE: The reference human genome annotation for the ENCODE project. *Genome Res.* 22:1760–1774.

doi:10.1101/gr.135350.111.

Hattar, S., H.-W. Liao2, M. Takao4, D.M. Berson, and K.-W. Yau. 2002. Melanopsin-Containing Retinal Ganglion Cells: Architecture, Projections, and Intrinsic Photosensitivity. *Science (80-.).* 295:1065–1070. doi:10.1126/science.1069609.Melanopsin-Containing.

Hempill, J.C. 2016. Traumatic brain injury: Epidemiology, classification, and pathophysiology. *Uptodate*. https://ww.

Hickman, S.E., N.D. Kingery, T.K. Ohsumi, M.L. Borowsky, L.C. Wang, T.K. Means, and J. El Khoury. 2013. The microglial sensome revealed by direct RNA sequencing. *Nat. Neurosci.* 16:1896–1905. doi:10.1038/nn.3554.

Hilla, X.A.M., H. Diekmann, and X.D. Fischer. 2017. Microglia Are Irrelevant for Neuronal Degeneration and Axon Regeneration after Acute Injury. *J. Neurosci.* 37:6113–6124. doi:10.1523/JNEUROSCI.0584-17.2017.

Hochreiter-Hufford, A., and K.S. Ravichandran. 2013. Clearing the dead: Apoptotic cell sensing, recognition, engulfment, and digestion. *Cold Spring Harb. Perspect. Biol.* 5:1–20. doi:10.1101/cshperspect.a008748.

Hoeffel, G., J. Chen, Y. Lavin, D. Low, F.F. Almeida, P. See, A.E. Beaudin, J. Lum, I. Low, E.C. Forsberg, M. Poidinger, F. Zolezzi, A. Larbi, L.G. Ng, J.K.Y. Chan, M. Greter, B. Becher, I.M. Samokhvalov, M. Merad, and F. Ginhoux. 2015. C-Myb+ Erythro-Myeloid Progenitor-Derived Fetal Monocytes Give Rise to Adult Tissue-Resident Macrophages. *Immunity*. 42:665–678. doi:10.1016/j.immuni.2015.03.011.

Hong, S., and W.A. Banks. 2015. Role of the immune system in HIV-associated neuroinflammation and neurocognitive implications. *Brain. Behav. Immun.* 45:1–12. doi:10.1016/j.bbi.2014.10.008.

Hong, S., V.F. Beja-glasser, B.M. Nfonoyim, A. Frouin, S. Li, S. Ramakrishnan, K.M. Merry, Q. Shi, A. Rosenthal, B.A. Barres, C.A. Lemere, D.J. Selkoe, and B. Stevens. 2016. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science*. 352:712–716.

Hooks, B.M., and C. Chen. 2006. Distinct Roles for Spontaneous and Visual Activity in Remodeling of the Retinogeniculate Synapse. *Neuron*. 52:281–291. doi:10.1016/j.neuron.2006.07.007.

Hoopfer, E.D., T. McLaughlin, R.J. Watts, O. Schuldiner, D.D.M. O'Leary, and L. Luo. 2006. Wlds Protection Distinguishes Axon Degeneration following Injury from Naturally Occurring Developmental Pruning. *Neuron*. 50:883–895. doi:10.1016/j.neuron.2006.05.013.

Howland, S.W., C.M. Poh, and L. Rénia. 2015. Activated Brain Endothelial Cells Cross-Present Malaria Antigen. *PLoS Pathog.* 11:1–24. doi:10.1371/journal.ppat.1004963.

Hunter, C.A., and L.D. Sibley. 2012. Modulation of innate immunity by Toxoplasma gondii virulence effectors. *Nat. Rev. Microbiol.* 10:766–778. doi:10.1038/nrmicro2858.

Ihara, F., M. Nishimura, Y. Muroi, M.E. Mahmoud, N. Yokoyama, K. Nagamune, and Y. Nishikawa. 2016. Toxoplasma gondii Infection in Mice Impairs Long-Term Fear Memory Consolidation through Dysfunction of the Cortex and Amygdala. *Infect. Immun.* 84:2861–2870. doi:10.1128/IAI.00217-16 [doi].

Ippolito, D.M., and C. Eroglu. 2010. Quantifying synapses: an immunocytochemistry-based assay to quantify synapse number. *J. Vis. Exp.* 2–9. doi:10.3791/2270.

Iram, T., Z. Ramirez-Ortiz, M.H. Byrne, U.A. Coleman, N.D. Kingery, T.K. Means, D. Frenkel, and J. El Khoury. 2016. Megf10 Is a Receptor for C1Q That Mediates Clearance of Apoptotic Cells by Astrocytes. *J. Neurosci.* 36:5185–5192. doi:10.1523/JNEUROSCI.3850-15.2016.

Jakubzick, C., E.L. Gautier, S.L. Gibbings, D.K. Sojka, A. Schlitzer, T.E. Johnson, S. Ivanov, Q.

Duan, S. Bala, T. Condon, N. van Rooijen, J.R. Grainger, Y. Belkaid, A. Ma'ayan, D.W.H. Riches, W.M. Yokoyama, F. Ginhoux, P.M. Henson, and G.J. Randolph. 2013. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity*. 39:599–610. doi:10.1016/j.immuni.2013.08.007.

Jessen, K.R., and R. Mirsky. 2016. The repair Schwann cell and its function in regenerating nerves. *J. Physiol.* 594:3521–3531. doi:10.1113/JP270874.

Jinno, S., and J. Yamada. 2011. Using comparative anatomy in the axotomy model to identify distinct roles for microglia and astrocytes in synaptic stripping. *Neuron Glia Biol.* 7:55–66. doi:10.1017/S1740925X11000135.

Jo, E.-K., J.K. Kim, D.-M. Shin, and C. Sasakawa. 2016. Molecular mechanisms regulating NLRP3 inflammasome activation. *Cell. Mol. Immunol.* 13:148–159. doi:10.1038/cmi.2015.95.

Jones, K.J., C.J. Serpe, S.C. Byram, C.A. DeBoy, and V.M. Sanders. 2005. Role of the immune system in the maintenance of mouse facial motoneuron viability after nerve injury. *Brain. Behav. Immun.* 19:12–19. doi:10.1016/j.bbi.2004.05.004.

Jonsson, T., H. Stefansson, S. Steinberg, I. Jonsdottir, P. V. Jonsson, J. Snaedal, S. Bjornsson, J. Huttenlocher, M.D. Allan I. Levey, M.D. James J. Lah, and K. Stefansson. 2013. Variant of TREM2 associated with the risk of AD. *N. Engl. J. Med.* 368:107–116. doi:10.1056/NEJMoa1211103.Variant.

Jung, S., J. Aliberti, P. Graemmel, M.J. Sunshine, G.W. Kreutzberg, A. Sher, and D.R. Littman. 2000. Analysis of Fractalkine Receptor CX3CR1 Function by Targeted Deletion and Green Fluorescent Protein Reporter Gene Insertion. *Mol. Cell. Biol.* 20:4106–4114. doi:10.1128/MCB.20.11.4106-4114.2000.

Jung, Y.-J., and W.-S. Chung. 2018. Phagocytic Roles of Glial Cells in Healthy and Diseased Brains. *Biomol. Ther. (Seoul).* 8:1–8. doi:10.4062/biomolther.2017.133.

Kawai, T., O. Takeuchi, T. Fujita, J. -i. Inoue, P.F. Muhlradt, S. Sato, K. Hoshino, and S. Akira. 2001. Lipopolysaccharide Stimulates the MyD88-Independent Pathway and Results in Activation of IFN-Regulatory Factor 3 and the Expression of a Subset of Lipopolysaccharide-Inducible Genes. *J. Immunol.* 167:5887–5894. doi:10.4049/jimmunol.167.10.5887.

Keren-Shaul, H., A. Spinrad, A. Weiner, O. Matcovitch-Natan, R. Dvir-Szternfeld, T.K. Ulland, E. David, K. Baruch, D. Lara-Astaiso, B. Toth, S. Itzkovitz, M. Colonna, M. Schwartz, and I. Amit. 2017. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell*. 169:1276–1290.e17. doi:10.1016/j.cell.2017.05.018.

Kerschensteiner, M., M.E. Schwab, J.W. Lichtman, and T. Misgeld. 2005. In vivo imaging of axonal degeneration and regeneration in the injured spinal cord. *Nat. Med.* 11:572–577. doi:10.1038/nm1229.

Kettenmann, H., U. Hanisch, M. Noda, and A. Verkhratsky. 2011. Physiology of Microglia. 461–553. doi:10.1152/physrev.00011.2010.

Kettenmann, H., F. Kirchhoff, and A. Verkhratsky. 2013. Microglia: New Roles for the Synaptic Stripper. *Neuron*. 77:10–18. doi:10.1016/j.neuron.2012.12.023.

Kierdorf, K., D. Erny, T. Goldmann, V. Sander, C. Schulz, E.G. Perdiguero, P. Wieghofer, A. Heinrich, P. Riemke, C. Hölscher, D.N. Müller, B. Luckow, T. Brocker, K. Debowski, G. Fritz, G. Opdenakker, A. Diefenbach, K. Biber, M. Heikenwalder, F. Geissmann, F. Rosenbauer, and M. Prinz. 2013. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat. Neurosci.* 16:273–80. doi:10.1038/nn.3318.

Kim, J.-B., J. Sig Choi, Y.-M. Yu, K. Nam, C.-S. Piao, S.-W. Kim, M.-H. Lee, P.-L. Han, J. -s.

Park, and J.-K. Lee. 2006. HMGB1, a Novel Cytokine-Like Mediator Linking Acute Neuronal Death and Delayed Neuroinflammation in the Postischemic Brain. *J. Neurosci.* 26:6413–6421. doi:10.1523/JNEUROSCI.3815-05.2006.

Kinchen, J.M., and K.S. Ravichandran. 2008. Phagosome maturation: Going through the acid test. *Nat. Rev. Mol. Cell Biol.* 9:781–795. doi:10.1038/nrm2515.

Kipnis, J., N.C. Derecki, C. Yang, and H. Scrable. 2008. Immunity and cognition: what do age-related dementia, HIV-dementia and "chemo-brain" have in common? *Trends Immunol.* 29:455–63. doi:10.1016/j.it.2008.07.007.

Kiss, R.S., M.R. Elliott, Z. Ma, Y.L. Marcel, and K.S. Ravichandran. 2006. Apoptotic Cells Induce a Phosphatidylserine-Dependent Homeostatic Response from Phagocytes. *Curr. Biol.* 16:2252–2258. doi:10.1016/j.cub.2006.09.043.

Kochunov, P., and L.E. Hong. 2014. Neurodevelopmental and neurodegenerative models of schizophrenia: White matter at the center stage. *Schizophr. Bull.* 40:721–728. doi:10.1093/schbul/sbu070.

Koenigsknecht, J. 2004. Microglial Phagocytosis of Fibrillar -Amyloid through a 1 Integrin-Dependent Mechanism. *J. Neurosci.* 24:9838–9846. doi:10.1523/JNEUROSCI.2557-04.2004.

Kolde, R. 2015. pheatmap: Pretty Heatmaps. Implementation of heatmaps that offers more control over dimensions and appearance. https://CRAN.R-project.org/package=pheatmap. *https://CRAN.R-project.org/package=pheatmap*.

Kramer, L.D., L.M. Styer, and G.D. Ebel. 2008. A Global Perspective on the Epidemiology of West Nile Virus. *Annu. Rev. Entomol.* 53:61–81. doi:10.1146/annurev.ento.53.103106.093258.

Kroemer, G., L. Galluzzi, P. Vandenabeele, J. Abrams, E.S. Alnemri, E.H. Baehrecke, M. V. Blagosklonny, W.S. El-Deiry, P. Golstein, D.R. Green, M. Hengartner, R.A. Knight, S. Kumar, S.A. Lipton, W. Malorni, G. Nuñez, M.E. Peter, J. Tschopp, J. Yuan, M. Piacentini, B. Zhivotovsky, and G. Melino. 2009. Classification of cell death: Recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ.* 16:3–11. doi:10.1038/cdd.2008.150.

Kulhara, P., and S. Gupta. 2010. What is schizophrenia: A neurodevelopmental or neurodegenerative disorder or a combination of both? A critical analysis. *Indian J. Psychiatry*. 52:21. doi:10.4103/0019-5545.58891.

Kurimoto, T., Y. Yin, G. Habboub, H.-Y. Gilbert, Y. Li, S. Nakao, A. Hafezi-Moghadam, and L.I. Benowitz. 2013. Neutrophils Express Oncomodulin and Promote Optic Nerve Regeneration. *J. Neurosci.* 33:14816–14824. doi:10.1523/JNEUROSCI.5511-12.2013.

Lai, A.Y., and J. McLauring. 2012. Clearance of amyloid-b peptides by microblia and macrophages:the issue of what, when and where. *Futur. Nerology*. 7:165–176. doi:10.2217/fnl.12.6.Clearance.

Lakso, M., J.G. Pichel, J.R. Gorman, B. Sauer, Y. Okamoto, E. Lee, F.W. Alt, and H. Westphal. 1996. Efficient in vivo manipulation of mouse genomic sequences at the zygote stage. *Proc. Natl. Acad. Sci. U. S. A.* 93:5860–5. doi:10.1073/pnas.93.12.5860.

Lam, C.K., T. Yoo, B. Hiner, Z. Liu, and J. Grutzendler. 2010. Embolus extravasation is an alternative mechanism for cerebral microvascular recanalization. *Nature*. 465:478–482. doi:10.1038/nature09001.

Lamkanfi, M., and T.D. Kanneganti. 2010. Nlrp3: An immune sensor of cellular stress and infection. *Int. J. Biochem. Cell Biol.* 42:792–795. doi:10.1016/j.biocel.2010.01.008.

Lampron, A., A. Larochelle, N. Laflamme, P. Préfontaine, M.-M. Plante, M.G. Sánchez, V.W. Yong, P.K. Stys, M.-È. Tremblay, and S. Rivest. 2015. Inefficient clearance of myelin debris by microglia impairs remyelinating processes. *J. Exp. Med.* 212:481–495. doi:10.1084/jem.20141656.

Lassmann, T., Y. Hayashizaki, and C.O. Daub. 2011. SAMStat: Monitoring biases in next generation sequencing data. *Bioinformatics*. 27:130–131. doi:10.1093/bioinformatics/btq614.

Laudisi, F., R. Spreafico, M. Evrard, T.R. Hughes, B. Mandriani, M. Kandasamy, B.P. Morgan, B. Sivasankar, and A. Mortellaro. 2013. Cutting Edge: The NLRP3 Inflammasome Links Complement-Mediated Inflammation and IL-1 Release. *J. Immunol.* 191:1006–1010. doi:10.4049/jimmunol.1300489.

Lee, C.S., K.K. Penberthy, K.M. Wheeler, I.J. Juncadella, P. Vandenabeele, J.J. Lysiak, and K.S. Ravichandran. 2016. Boosting Apoptotic Cell Clearance by Colonic Epithelial Cells Attenuates Inflammation In Vivo. *Immunity*. 44:807–820. doi:10.1016/j.immuni.2016.02.005.

Lemmon, M.A. 2008. Membrane recognition by phospholipid-binding domains. *Nat. Rev. Mol. Cell Biol.* 9:99–111. doi:10.1038/nrm2328.

Lew, E.D., J. Oh, P.G. Burrola, I. Lax, A. Zagórska, P.G. Través, J. Schlessinger, and G. Lemke. 2014. Differential TAM receptor-ligand-phospholipid interactions delimit differential TAM bioactivities. *Elife*. 3:1–23. doi:10.7554/eLife.03385.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 25:2078–2079. doi:10.1093/bioinformatics/btp352.

Liddelow, S.A., K.A. Guttenplan, L.E. Clarke, F.C. Bennett, C.J. Bohlen, L. Schirmer, M.L. Bennett, A.E. Münch, W.-S. Chung, T.C. Peterson, D.K. Wilton, A. Frouin, B.A. Napier, N. Panicker, M. Kumar, M.S. Buckwalter, D.H. Rowitch, V.L. Dawson, T.M. Dawson, B. Stevens, and B.A. Barres. 2017. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 541:481–487. doi:10.1038/nature21029.

Liew, F.Y., N.I. Pitman, and I.B. McInnes. 2010. Disease-associated functions of IL-33: The new kid in the IL-1 family. *Nat. Rev. Immunol.* 10:103–110. doi:10.1038/nri2692.

Lim, J.-H.A., B.K. Stafford, P.L. Nguyen, B. V Lien, C. Wang, K. Zukor, Z. He, and A.D. Huberman. 2016. Neural activity promotes long-distance, target-specific regeneration of adult retinal axons. *Nat. Neurosci.* 19. doi:10.1038/nn.4340.

de Lima, S., Y. Koriyama, T. Kurimoto, J.T. Oliveira, Y. Yin, Y. Li, H.-Y. Gilbert, M. Fagiolini, A.M.B. Martinez, and L. Benowitz. 2012. Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors. *Proc. Natl. Acad. Sci. U. S. A.* 109:9149–54. doi:10.1073/pnas.1119449109.

Lin, B., and E.B. Peng. 2013. Retinal Ganglion Cells are Resistant to Photoreceptor Loss in Retinal Degeneration. *PLoS One*. 8. doi:10.1371/journal.pone.0068084.

Liu, J., D. Tian, M. Murugan, U.B. Eyo, C.F. Dreyfus, W. Wang, and L.J. Wu. 2015. Microglial Hv1 proton channel promotes cuprizone-induced demyelination through oxidative damage. *J. Neurochem.* 135:347–356. doi:10.1111/jnc.13242.

Lobsiger, C.S., S. Boillee, C. Pozniak, A.M. Khan, M. McAlonis-Downes, J.W. Lewcock, and D.W. Cleveland. 2013. C1q induction and global complement pathway activation do not contribute to ALS toxicity in mutant SOD1 mice. *Proc. Natl. Acad. Sci.* 110:E4385–E4392. doi:10.1073/pnas.1318309110.

Logan, M.A., R. Hackett, J. Doherty, A. Sheehan, S.D. Speese, and M.R. Freeman. 2012a. Negative regulation of glial engulfment activity by Draper terminates glial responses to axon injury. *Nat. Neurosci.* 1–10. doi:10.1038/nn.3066.

Logan, M.A., R. Hackett, J. Doherty, A. Sheehan, S.D. Speese, and M.R. Freeman. 2012b. Negative regulation of glial engulfment activity by Draper terminates glial responses to axon injury. *Nat. Neurosci.* 15:722–730. doi:10.1038/nn.3066.

London, A., E. Itskovich, I. Benhar, V. Kalchenko, M. Mack, S. Jung, and M. Schwartz. 2011. Neuroprotection and progenitor cell renewal in the injured adult murine retina requires healing monocyte-derived macrophages. *J. Exp. Med.* 208:23–39. doi:10.1084/jem.20101202.

Louveau, A., I. Smirnov, T.J. Keyes, J.D. Eccles, S.J. Rouhani, J.D. Peske, N.C. Derecki, D. Castle, J.W. Mandell, K.S. Lee, T.H. Harris, and J. Kipnis. 2015. Structural and functional features of central nervous system lymphatic vessels. *Nature*. 523:337–341. doi:10.1038/nature14432.

Love, M.I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15:550. doi:10.1186/s13059-014-0550-8.

Lu, Y.C., W.C. Yeh, and P.S. Ohashi. 2008. LPS/TLR4 signal transduction pathway. *Cytokine*. 42:145–151. doi:10.1016/j.cyto.2008.01.006.

Lu, Z., M.R. Elliott, Y. Chen, J.T. Walsh, A.L. Klibanov, K.S. Ravichandran, and J. Kipnis. 2011. Phagocytic activity of neuronal progenitors regulates adult neurogenesis. *Nat. Cell Biol.* 13:1076–83. doi:10.1038/ncb2299.

Ludwin, S. 1990. Phagocytosis in the rat optic nerve following Wallerian degeneration. *Acta Neuropathol.* 80:266–73.

Lui, H., J. Zhang, S.R. Makinson, M.K. Cahill, K.W. Kelley, H.Y. Huang, Y. Shang, M.C. Oldham, L.H. Martens, F. Gao, G. Coppola, S.A. Sloan, C.L. Hsieh, C.C. Kim, E.H. Bigio, S. Weintraub, M.M. Mesulam, R. Rademakers, I.R. Mackenzie, W.W. Seeley, A. Karydas, B.L. Miller, B. Borroni, R. Ghidoni, R. V. Farese, J.T. Paz, B.A. Barres, and E.J. Huang. 2015. Progranulin Deficiency Promotes Circuit-Specific Synaptic Pruning by Microglia via Complement Activation. *Cell*. 165:921–935. doi:10.1016/j.cell.2016.04.001.

Lukens, J.R., J.M. Gross, and T.D. Kanneganti. 2012. IL-1 family cytokines trigger sterile inflammatory disease. *Front. Immunol.* 3:1–12. doi:10.3389/fimmu.2012.00315.

Lukens, J.R., and T.-D. Kanneganti. 2014. Beyond canonical inflammasomes: emerging pathways in IL-1-mediated autoinflammatory disease. *Semin. Immunopathol.* 36:595–609. doi:10.1007/s00281-014-0434-8.

Lundgaard, I., M.J. Osório, B.T. Kress, S. Sanggaard, and M. Nedergaard. 2014. White matter astrocytes in health and disease. *Neuroscience*. 276:161–173. doi:10.1016/j.neuroscience.2013.10.050.

Lunn, E.R., V.H. Perry, M.C. Brown, H. Rosen, and S. Gordon. 1989. Absence of Wallerian Degeneration does not Hinder Regeneration in Peripheral Nerve. *Eur. J. Neurosci.*

Ma, W., Y. Zhang, C. Gao, R.N. Fariss, J. Tam, and W.T. Wong. 2017. Monocyte infiltration and proliferation reestablish myeloid cell homeostasis in the mouse retina following retinal pigment epithelial cell injury. *Sci. Rep.* 7:1–18. doi:10.1038/s41598-017-08702-7.

MacDonald, J.M., M.G. Beach, E. Porpiglia, A.E. Sheehan, R.J. Watts, and M.R. Freeman. 2006. The Drosophila Cell Corpse Engulfment Receptor Draper Mediates Glial Clearance of Severed Axons. *Neuron*. 50:869–881. doi:10.1016/j.neuron.2006.04.028.

Mack, T.G., M. Reiner, B. Beirowski, W. Mi, M. Emanuelli, D. Wagner, D. Thomson, T.

Gillingwater, F. Court, L. Conforti, F.S. Fernando, A. Tarlton, C. Andressen, K. Addicks, G. Magni, R.R. Ribchester, V.H. Perry, and M.P. Coleman. 2001. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. *Nat. Neurosci.* 4:1199–1206. doi:10.1038/nn770.

Maeda, M., M. Tsuda, H. Tozaki-Saitoh, K. Inoue, and H. Kiyama. 2010. Nerve injury-activated microglia engulf myelinated axons in a P2Y12 signaling-dependent manner in the dorsal horn. *Glia*. 58:1838–1846. doi:10.1002/glia.21053.

Malatesta, P., I. Appolloni, and F. Calzolari. 2008. Radial glia and neural stem cells. *Cell Tissue Res.* 331:165–78. doi:10.1007/s00441-007-0481-8.

Marín-Teva, J.L., I. Dusart, C. Colin, A. Gervais, N. Van Rooijen, and M. Mallat. 2004. Microglia Promote the Death of Developing Purkinje Cells. *Neuron*. 41:535–547. doi:10.1016/S0896-6273(04)00069-8.

Martersteck, E.M., K.E. Hirokawa, M. Evarts, A. Bernard, X. Duan, Y. Li, L. Ng, S.W. Oh, B. Ouellette, J.J. Royall, M. Stoecklin, Q. Wang, H. Zeng, J.R. Sanes, and J.A. Harris. 2017. Diverse Central Projection Patterns of Retinal Ganglion Cells. *Cell Rep.* 18:2058–2072. doi:10.1016/j.celrep.2017.01.075.

Martinez, F.O., L. Helming, and S. Gordon. 2009. Alternative Activation of Macrophages: An Immunologic Functional Perspective. *Annu. Rev. Immunol.* 27:451–483. doi:10.1146/annurev.immunol.021908.132532.

Matcovitch-Natan, O., D.R. Winter, A. Giladi, S.V. Aguilar, A. Spinrad, S. Sarrazin, H. Ben-Yehuda, E. David, F.Z. Gonzalez, P. Perrin, H. Keren-shaul, M. Gury, D. Lara-Astaiso, C.A. Thaiss, M. Cohen, K. Halpern Bahar, K. Baruch, A. Deczkowska, E. Lorenzo-Vivas, S. Itzkovitz, E. Elinav, M.H. Sieweke, M. Schwartz, and I. Amit. 2016. Microglia development follows a stepwise program to regulate brain homeostasis. *Sci. Press*. 8670.

Matus, A. 1988. Microtubule-Associated Proteins: Their potential role in determining neuronal morphology. *Annu. Rev. Neurosci.* 11:29–44.

Matzinger, P. 1994. Tolerance, Danger, and the Extended Family. *Annu. Rev. Immunol.* 12:991–1045.

Matzinger, P., and T. Kamala. 2011. Tissue-based class control: the other side of tolerance. *Nat. Rev. Immunol.* 11:221–230. doi:10.1038/nri2940.

Mazaheri, F., O. Breus, S. Durdu, P. Haas, J. Wittbrodt, D. Gilmour, and F. Peri. 2014. Distinct roles for BAI1 and TIM-4 in the engulfment of dying neurons by microglia. *Nat. Commun.* 5:4046. doi:10.1038/ncomms5046.

Mazzoni, F., E. Novelli, and E. Strettoi. 2008. Retinal ganglion cells survive and maintain normal dendritic morphology in a mouse model of inherited photoreceptor degeneration. *J Neurosci*. 28:14282–14292. doi:10.1523/JNEUROSCI.4968-08.2008.

Medina, C.B., and K.S. Ravichandran. 2016. Do not let death do us part: “find-me” signals in communication between dying cells and the phagocytes. *Cell Death Differ.* 23:979–989. doi:10.1038/cdd.2016.13.

Merabet, L.B., and A. Pascual-Leone. 2010. Neural reorganization following sensory loss: The opportunity of change. *Nat. Rev. Neurosci.* 11:44–52. doi:10.1038/nrn2758.

Miller, B.R., C. Press, R.W. Daniels, Y. Sasaki, J. Milbrandt, and A. Diantonio. 2009. A dual leucine kinase-dependent axon self-destruction program promotes Wallerian degeneration. *Nat. Neurosci.* 12:387–389. doi:10.1038/nn.2290.

Miller, F.D., and A.S. Gauthier. 2007. Timing Is Everything: Making Neurons versus Glia in the Developing Cortex. *Neuron*. 54:357–369. doi:10.1016/j.neuron.2007.04.019.

Miloro, M., and A. Kolokythas. 2012. Management of Complications in Oral and Maxillofacial Surgeryle. Chapter 25 pp.

Miner, J.J., B.P. Daniels, B. Shrestha, J.L. Proenca-Modena, E.D. Lew, H.M. Lazear, M.J. Gorman, G. Lemke, R.S. Klein, and M.S. Diamond. 2015. The TAM receptor Mertk protects against neuroinvasive viral infection by maintaining blood-brain barrier integrity. *Nat. Med.* 21:1464–72. doi:10.1038/nm.3974.

Ming, G.-L., and H. Song. 2011. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron*. 70:687–702. doi:10.1016/j.neuron.2011.05.001.

Misharin, A. V., L. Morales-Nebreda, G.M. Mutlu, G.R.S. Budinger, and H. Perlman. 2013. Flow Cytometric Analysis of Macrophages and Dendritic Cell Subsets in the Mouse Lung. *Am. J. Respir. Cell Mol. Biol.* 49:503–510. doi:10.1165/rcmb.2013-0086MA.

Moalem, G., R. Leibowitz-Amit, E. Yoles, F. Mor, I.R. Cohen, and M. Schwartz. 1999a. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. *Nat. Med.* 5:49–55. doi:10.1038/4734.

Moalem, G., A. Monsonego, Y. Shani, I.R. Cohen, and M. Schwartz. 1999b. Differential T cell response in central and peripheral nerve injury: connection with immune privilege. *FASEB J.* 13:1207–17.

Moran, L.B., and M.B. Graeber. 2004. The facial nerve axotomy model. *Brain Res. Rev.* 44:154–178. doi:10.1016/j.brainresrev.2003.11.004.

Moreno, M.M., C. Linster, O. Escanilla, J. Sacquet, A. Didier, and N. Mandairon. 2009. Olfactory perceptual learning requires adult neurogenesis. *Proc. Natl. Acad. Sci.* 106:17980–17985. doi:10.1073/pnas.0907063106.

Mrdjen, D., A. Pavlovic, F.J. Hartmann, B. Schreiner, S.G. Utz, B.P. Leung, I. Lelios, F.L. Heppner, J. Kipnis, D. Merkler, M. Greter, and B. Becher. 2018. High-Dimensional Single-Cell Mapping of Central Nervous System Immune Cells Reveals Distinct Myeloid Subsets in Health, Aging, and Disease. *Immunity*. 0:1–16. doi:10.1016/j.immuni.2018.01.011.

Muessel, M.J., N.E.J. Berman, and R.M. Klein. 2000. Early and specific expression of Monocyte Chemoattractant Protein-1 in the thalamus induced by cortical injury. *Brain Res.* 870:211–221. doi:10.1016/S0006-8993(00)02450-1.

Murray, K.N., A.R. Parry-Jones, and S.M. Allan. 2015. Interleukin-1 and acute brain injury. *Front. Cell. Neurosci.* 9:1–17. doi:10.3389/fncel.2015.00018.

Mylonas, K.J., M.A. Hoeve, A.S. MacDonald, and J.E. Allen. 2013. Alternative activation of macrophages by filarial nematodes is MyD88-independent. *Immunobiology*. 218:570–578. doi:10.1016/j.imbio.2012.07.006.

Nadal-Nicolás, F.M., M. Jiménez-López, P. Sobrado-Calvo, L. Nieto-López, I. Cánovas-Martinez, M. Salinas-Navarro, M. Vidal-Sanz, and M. Agudo. 2009. Brn3a as a marker of retinal ganglion cells: Qualitative and quantitative time course studies in naïve and optic nerve-injured retinas. *Investig. Ophthalmol. Vis. Sci.* 50:3860–3868. doi:10.1167/iovs.08-3267.

Nagata, S. 2007. Autoimmune diseases caused by defects in clearing dead cells and nuclei expelled from erythroid precursors. *Immunol. Rev.* 220:237–50. doi:10.1111/j.1600-065X.2007.00571.x.

Nakajima, K., Y. Tohyama, S. Maeda, S. Kohsaka, and T. Kurihara. 2007. Neuronal regulation by which microglia enhance the production of neurotrophic factors for GABAergic, catecholaminergic, and cholinergic neurons. *Neurochem. Int.* 50:807–820. doi:10.1016/j.neuint.2007.02.006.

Neumann, H., M.R. Kotter, and R.J.M. Franklin. 2009. Debris clearance by microglia: An essential

link between degeneration and regeneration. *Brain*. 132:288–295. doi:10.1093/brain/awn109.

Nguyen, K.., and M.P. Pender. 1998. Phagocytosis of apoptotic lymphocytes by oligodendrocytes in experimental autoimmune encephalomyelitis. *Acta Neuropathol.* 95:40–46.

Nijhawan, D., N. Honarpour, and X. Wang. 2000. a Poptosis in N Eural D Evelopment and D Isease. *Proteins*. 37:73–87.

Nimmerjahn, A., F. Kirchhoff, and F. Helmchen. 2005. Resting Microglial Cells Are Highly Dynamic Surveillants of Brain Parenchyma in Vivo. *Science*. 308:1314–1319. doi:10.1126/science.1110647.

Noda, M., Y. Doi, J. Liang, J. Kawanokuchi, Y. Sonobe, H. Takeuchi, T. Mizuno, and A. Suzumura. 2011. Fractalkine attenuates excito-neurotoxicity via microglial clearance of damaged neurons and antioxidant enzyme heme oxygenase-1 expression. *J. Biol. Chem.* 286:2308–2319. doi:10.1074/jbc.M110.169839.

Oakley, H., S.L. Cole, S. Logan, E. Maus, P. Shao, J. Craft, A. Guillozet-Bongaarts, M. Ohno, J. Disterhoft, L. Van Eldik, R. Berry, and R. Vassar. 2006. Intraneuronal beta-Amyloid Aggregates, Neurodegeneration, and Neuron Loss in Transgenic Mice with Five Familial Alzheimer's Disease Mutations: Potential Factors in Amyloid Plaque Formation. *J. Neurosci.* 26:10129–10140. doi:10.1523/JNEUROSCI.1202-06.2006.

Orsini, F., D. De Blasio, R. Zangari, E.R. Zanier, and M.-G. De Simoni. 2014. Versatility of the complement system in neuroinflammation, neurodegeneration and brain homeostasis. *Front. Cell. Neurosci.* 8:1–19. doi:10.3389/fncel.2014.00380.

Ouyang, W., S. Rutz, N.K. Crellin, P.A. Valdez, and S.G. Hymowitz. 2011. Regulation and Functions of the IL-10 Family of Cytokines in Inflammation and Disease. *Annu. Rev. Immunol.* 29:71–109. doi:10.1146/annurev-immunol-031210-101312.

Paolicelli, R.C., K. Bisht, and M.-Ã. Tremblay. 2014. Fractalkine regulation of microglial physiology and consequences on the brain and behavior. *Front. Cell. Neurosci.* 8:1–10. doi:10.3389/fncel.2014.00129.

Paolicelli, R.C., G. Bolasco, F. Pagani, L. Maggi, M. Scianni, P. Panzanelli, M. Giustetto, T.A. Ferreira, E. Guiducci, L. Dumas, D. Ragozzino, and C.T. Gross. 2011. Synaptic pruning by microglia is necessary for normal brain development. *Science*. 333:1456–8. doi:10.1126/science.1202529.

Park, D., A.-C. Tosello-Trampont, M.R. Elliott, M. Lu, L.B. Haney, Z. Ma, A.L. Klibanov, J.W. Mandell, and K.S. Ravichandran. 2007. BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature*. 450:430–434. doi:10.1038/nature06329.

Parlog, A., L.-A. Harsan, M. Zagrebelsky, M. Weller, D. von Elverfeldt, C. Mawrin, M. Korte, and I.R. Dunay. 2014. Chronic murine toxoplasmosis is defined by subtle changes in neuronal connectivity. *Dis. Model. Mech.* 7:459–469. doi:10.1242/dmm.014183.

Parnaik, R., M.C. Raff, and J. Scholes. 2000. Differences between the clearance of apoptotic cells by professional and non-professional phagocytes. *Curr. Biol.* 10:857–860. doi:10.1016/S0960-9822(00)00598-4.

Pavlov, V.A., and K.J. Tracey. 2017. Neural regulation of immunity : molecular mechanisms and clinical translation. 20. doi:10.1038/nn.4477.

Penberthy, K.K., C. Rival, L.S. Shankman, M.H. Raymond, J. Zhang, J.S.A. Perry, C.S. Lee, C.Z. Han, S. Onengut-Gumuscu, K. Palczewski, J.J. Lysiak, and K.S. Ravichandran. 2017. Context-dependent compensation among phosphatidylserine-recognition receptors. *Sci. Rep.* 7:1–14. doi:10.1038/s41598-017-15191-1.

Pérez-Neri, I., J. Ramírez-Bermúdez, S. Montes, and C. Ríos. 2006. Possible mechanisms of neurodegeneration in schizophrenia. *Neurochem. Res.* 31:1279–1294. doi:10.1007/s11064-006-9162-3.

Perez-Pouchoulen, M., J.W. VanRyzin, and M.M. McCarthy. 2015. Morphological and Phagocytic Profile of Microglia in the Developing Rat Cerebellum. *eNeuro*. 2:1–15. doi:10.1523/ENEURO.0036-15.2015.

Poliani, P.L., Y. Wang, E. Fontana, M.L. Robinette, Y. Yamanishi, S. Gilfillan, and M. Colonna. 2015. TREM2 sustains microglial expansion during aging and response to demyelination. *J. Clin. Invest.* 125:2161–2170. doi:10.1172/JCI77983DS1.

Pradeu, T., and E.L. Cooper. 2012. The danger theory: 20 years later. *Front. Immunol.* 3:1–9. doi:10.3389/fimmu.2012.00287.

Provencio, I., I.R. Rodriguez, G. Jiang, W.P. Hayes, E.F. Moreira, and M.D. Rollag. 2000. A Novel Human Opsin in the Inner Retina. *J. Neurosci.* 20:600–605.

Quina, L.A., W. Pak, J. Lanier, P. Banwait, K. Gratwick, Y. Liu, T. Velasquez, D.D.M.O. Leary, M. Goulding, and E.E. Turner. 2005. Brn3a-Expressing Retinal Ganglion Cells Project Specifically to Thalamocortical and Collicular Visual Pathways. 25:11595–11604. doi:10.1523/JNEUROSCI.2837-05.2005.

Rabinovitch, M. 1995. Professional and non-professional phagocytes: an introduction. *Trends Cell Biol.* 5:85–87. doi:10.1016/S0962-8924(00)88955-2.

Ransohoff, R.M., and M.A. Brown. 2012. Innate immunity in the central nervous system. *J. Clin. Invest.* 122. doi:10.1172/JCI58644.1164.

Rappert, A. 2004. CXCR3-Dependent Microglial Recruitment Is Essential for Dendrite Loss after Brain Lesion. *J. Neurosci.* 24:8500–8509. doi:10.1523/JNEUROSCI.2451-04.2004.

Rauschecker, J.P. 1995. Compensatory plasticity and sensory substitution in the cerebral cortex. *Trends Neurosci.* 18:36–43. doi:10.1016/0166-2236(95)93948-W.

Renton, T., D. Adey-Viscuso, J.G. Meechan, and Z. Yilmaz. 2010. Trigeminal nerve injuries in relation to the local anaesthesia in mandibular injections. *Br. Dent. J.* 209:1–5. doi:10.1038/sj.bdj.2010.978.

Rezai-Zadeh, K., D. Gate, and T. Town. 2009. CNS infiltration of peripheral immune cells: D-Day for neurodegenerative disease? *J. Neuroimmune Pharmacol.* 4:462–475. doi:10.1007/s11481-009-9166-2.

Ribeiro Xavier, A.L., B.T. Kress, S.A. Goldman, J.R. Lacerda de Menezes, and M. Nedergaard. 2015. A Distinct Population of Microglia Supports Adult Neurogenesis in the Subventricular Zone. *J. Neurosci.* 35:11848–11861. doi:10.1523/JNEUROSCI.1217-15.2015.

Riccomagno, M.M., and A.L. Kolodkin. 2015. Sculpting Neural Circuits by Axon and Dendrite Pruning. *Annu. Rev. Cell Dev. Biol.* 31:779–805. doi:10.1146/annurev-cellbio-100913-013038.

Ricklin, D., and J.D. Lambris. 2013. Complement in Immune and Inflammatory Disorders: Therapeutic Interventions. *J. Immunol.* 190:3839–3847. doi:10.4049/jimmunol.1203200.

Ripke, S., B.M. Neale, A. Corvin, J.T.R. Walters, K.H. Farh, P.A. Holmans, P. Lee, B. Bulik-Sullivan, D.A. Collier, H. Huang, T.H. Pers, I. Agartz, E. Agerbo, M. Albus, M. Alexander, F. Amin, S.A. Bacanu, M. Begemann, R.A. Belliveau, J. Bene, S.E. Bergen, E. Bevilacqua, T.B. Bigdeli, D.W. Black, R. Bruggeman, N.G. Buccola, R.L. Buckner, W. Byerley, W. Cahn, G. Cai, D. Campion, R.M. Cantor, V.J. Carr, N. Carrera, S. V. Catts, K.D. Chambert, R.C.K. Chan, R.Y.L. Chen, E.Y.H. Chen, W. Cheng, E.F.C. Cheung, S.A. Chong, C.R. Cloninger, D. Cohen, N. Cohen, P. Cormican, N. Craddock, J.J. Crowley, D. Curtis, M.

Davidson, K.L. Davis, F. Degenhardt, J. Del Favero, D. Demontis, D. Dikeos, T. Dinan, S. Djurovic, G. Donohoe, E. Drapeau, J. Duan, F. Dudbridge, N. Durmishi, P. Eichhammer, J. Eriksson, V. Escott-Price, L. Essioux, A.H. Fanous, M.S. Farrell, J. Frank, L. Franke, R. Freedman, N.B. Freimer, M. Friedl, J.I. Friedman, M. Fromer, G. Genovese, L. Georgieva, I. Giegling, P. Giusti-Rodríguez, S. Godard, J.I. Goldstein, V. Golimbet, S. Gopal, J. Gratten, L. De Haan, C. Hammer, M.L. Hamshere, M. Hansen, T. Hansen, V. Haroutunian, A.M. Hartmann, F.A. Henskens, S. Herms, J.N. Hirschhorn, P. Hoffmann, A. Hofman, M. V. Hollegaard, D.M. Hougaard, et al. 2014. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*. 511:421–427. doi:10.1038/nature13595.

Rodriguez, A.R., L.P. de Sevilla Müller, and N.C. Brecha. 2014. The RNA binding protein RBPMS is a selective marker of ganglion cells in the mammalian retina. *J. Comp. Neurol.* 522:1411–1443. doi:10.1002/cne.23521.

Romero-Grimaldi, C., M. Murillo-Carretero, M.A. López-Toledano, M. Carrasco, C. Castro, and C. Estrada. 2011. ADAM-17/tumor necrosis factor-α-converting enzyme inhibits neurogenesis and promotes gliogenesis from neural stem cells. *Stem Cells*. 29:1628–1639. doi:10.1002/stem.710.

Rosetti, F., and T.N. Mayadas. 2016. The many faces of Mac-1 in autoimmune disease. 175–193.

Roth, T.L., D. Nayak, T. Atanasijevic, A.P. Koretsky, L.L. Latour, and D.B. McGavern. 2014. Transcranial amelioration of inflammation and cell death after brain injury. *Nature*. 505:223–8. doi:10.1038/nature12808.

Rothlin, C. V, E.A. Carrera-Silva, L. Bosurgi, and S. Ghosh. 2015. TAM receptor signaling in immune homeostasis. *Annu. Rev. Immunol.* 33:355–391. doi:10.1146/annurev-immunol-032414-112103.

Rotshenker, S. 2011. Wallerian degeneration: The innate-immune response to traumatic nerve injury. *J. Neuroinflammation*. 8:109. doi:10.1186/1742-2094-8-109.

Rozenfeld, C., R. Martinez, S. Seabra, C. Sant'anna, J.G.R. Gonçalves, M. Bozza, V. Moura-Neto, and W. De Souza. 2005. Toxoplasma gondii prevents neuron degeneration by interferon-gamma-activated microglia in a mechanism involving inhibition of inducible nitric oxide synthase and transforming growth factor-beta1 production by infected microglia. *Am. J. Pathol.* 167:1021–31. doi:167/4/1021 [pii].

Ruggiero, L., M.P. Connor, J. Chen, R. Langen, and S.C. Finnemann. 2012. Diurnal, localized exposure of phosphatidylserine by rod outer segment tips in wild-type but not Itgb5-/- or Mfge8-/- mouse retina. *Proc. Natl. Acad. Sci. U. S. A.* 109:8145–8. doi:10.1073/pnas.1121101109.

Russo, M. V., and D.B. McGavern. 2015. Immune Surveillance of the CNS following Infection and Injury. *Trends Immunol.* 36:637–650. doi:10.1016/j.it.2015.08.002.

Ryu, K.-Y., G.-S. Cho, H.Z. Piao, and W.-K. Kim. 2012. Role of TGF-β in Survival of Phagocytizing Microglia: Autocrine Suppression of TNF-α Production and Oxidative Stress. *Exp. Neurobiol.* 21:151–7. doi:10.5607/en.2012.21.4.151.

Sadek, J., S. Pergram, J. Harrington, L. Echevarria, L. Davis, D. Goade, J. Harnar, R. Nofchissey, C. Sewell, P. Ettestad, and K. Haaland. 2010. Persistent neuropsychological impairment associated with West Nile virus infection. *J Clin Exp Neuropsychol*. 32:81–87.

Sahay, A., D. a Wilson, and R. Hen. 2011. Pattern separation: a common function for new neurons in hippocampus and olfactory bulb. *Neuron*. 70:582–8. doi:10.1016/j.neuron.2011.05.012.

Sala, C., V. Piëch, N.R. Wilson, M. Passafaro, G. Liu, and M. Sheng. 2001. Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron*. 31:115–130.

doi:10.1016/S0896-6273(01)00339-7.

Samaan, Z., S.M. Vaz, M. Bawor, T.H. Potter, S. Eskandarian, and M. Loeb. 2016. Neuropsychological impact of west nile virus infection: An extensive neuropsychiatric assessment of 49 cases in Canada. *PLoS One*. 11:1–15. doi:10.1371/journal.pone.0158364.

Sanders, V.M., and K.J. Jones. 2006. Role of immunity in recovery from a peripheral nerve injury. *J. Neuroimmune Pharmacol.* 1:11–19. doi:10.1007/s11481-005-9004-0.

Schafer, D.P., E.K. Lehrman, C.T. Heller, and B. Stevens. 2014. An engulfment assay: a protocol to assess interactions between CNS phagocytes and neurons. *J. Vis. Exp.* e51482. doi:10.3791/51482.

Schafer, D.P., E.K. Lehrman, A.G. Kautzman, R. Koyama, A.R. Mardinly, R. Yamasaki, R.M. Ransohoff, M.E. Greenberg, B.A. Barres, and B. Stevens. 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*. 74:691–705. doi:10.1016/j.neuron.2012.03.026.

Schnell, S. a, W. a Staines, and M.W. Wessendorf. 1999. Reduction of lipofuscin-like autofluorescence in fluorescently labeled tissue. *J. Histochem. Cytochem.* 47:719–730. doi:10.1177/002215549904700601.

Schwartz, M. 2004. Optic nerve crush: Protection and regeneration. *Brain Res. Bull.* 62:467–471. doi:10.1016/S0361-9230(03)00076-5.

Schwartz, M., and J. Kipnis. 2011. A conceptual revolution in the relationships between the brain and immunity. *Brain. Behav. Immun.* 25:817–9. doi:10.1016/j.bbi.2010.12.015.

Sekar, A., A.R. Bialas, H. de Rivera, A. Davis, T.R. Hammond, N. Kamitaki, K. Tooley, J. Presumey, M. Baum, V. Van Doren, G. Genovese, S.A. Rose, R.E. Handsaker, M.J. Daly, M.C. Carroll, B. Stevens, and S.A. McCarroll. 2016. Schizophrenia risk from complex variation of complement component 4. *Nature*. 530:177–183. doi:10.1038/nature16549.

Serpe, C.J., A.P. Kohm, C.B. Huppenbauer, V.M. Sanders, and K.J. Jones. 1999. Exacerbation of facial motoneuron loss after facial nerve transection in severe combined immunodeficient (scid) mice. *J. Neurosci.* 19:RC7.

Shanks, J.A., S. Ito, L. Schaevitz, J. Yamada, B. Chen, A. Litke, and D.A. Feldheim. 2016. Corticothalamic Axons Are Essential for Retinal Ganglion Cell Axon Targeting to the Mouse Dorsal Lateral Geniculate Nucleus. *J. Neurosci.* 36:5252–63. doi:10.1523/JNEUROSCI.4599-15.2016.

Shechter, R., A. London, C. Varol, C. Raposo, M. Cusimano, G. Yovel, A. Rolls, M. Mack, S. Pluchino, G. Martino, S. Jung, and M. Schwartz. 2009. Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. *PLoS Med.* 6. doi:10.1371/journal.pmed.1000113.

Shechter, R., and M. Schwartz. 2013. CNS sterile injury: Just another wound healing? *Trends Mol. Med.* 19:135–143. doi:10.1016/j.molmed.2012.11.007.

Shigemoto-Mogami, Y., K. Hoshikawa, J.E. Goldman, Y. Sekino, and K. Sato. 2014. Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. *J. Neurosci.* 34:2231–43. doi:10.1523/JNEUROSCI.1619-13.2014.

Sierra, A., S. Beccari, I. Diaz-Aparicio, J.M. Encinas, S. Comeau, and M.-È. Tremblay. 2014. Surveillance, phagocytosis, and inflammation: how never-resting microglia influence adult hippocampal neurogenesis. *Neural Plast.* 2014:610343. doi:10.1155/2014/610343.

Sierra, A., J.M. Encinas, J.J.P. Deudero, J.H. Chancey, G. Enikolopov, L.S. Overstreet-Wadiche, S.E. Tsirka, and M. Maletic-Savatic. 2010. Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell*. 7:483–495.

doi:10.1016/j.stem.2010.08.014.
Simons, M., T. Misgeld, and M. Kerschensteiner. 2014. A unified cell biological perspective on axon-myelin injury. *J. Cell Biol.* 206:335–345. doi:10.1083/jcb.201404154.
Singhrao, S.K., J.W. Neal, B.P. Morgan, and P. Gasque. 1999. Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease. *Exp. Neurol.* 159:362–376. doi:10.1006/exnr.1999.7170.
Sipe, G.O., R.L. Lowery, M.-È. Tremblay, E.A. Kelly, C.E. Lamantia, and A.K. Majewska. 2016. Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. *Nat. Commun.* 7:10905. doi:10.1038/ncomms10905.
Sofroniew, M. V. 2005. Reactive astrocytes in neural repair and protection. *Neuroscientist*. 11:400–407. doi:10.1177/1073858405278321.
Sokolowski, J.D., C.N. Chabanon-Hicks, C.Z. Han, D.S. Heffron, and J.W. Mandell. 2014. Fractalkine is a â€œfind-meâ€ signal released by neurons undergoing ethanol-induced apoptosis. *Front. Cell. Neurosci.* 8:1–10. doi:10.3389/fncel.2014.00360.
Spiller, K.J., C.R. Restrepo, T. Khan, M.A. Dominique, T.C. Fang, R.G. Canter, C.J. Roberts, K.R. Miller, R.M. Ransohoff, J.Q. Trojanowski, and V. M-Y Lee. 2018. Microglia-mediated recovery from ALS-relevant motor neuron degeneration in a mouse model of TDP-43 proteinopathy. *Nat. Neurosci.* doi:10.1038/s41593-018-0083-7.
Squarzoni, P., G. Oller, G. Hoeffel, L. Pont-lezica, P. Rostaing, D. Low, A. Bessis, F. Ginhoux, and S. Garel. 2014. Report Microglia Modulate Wiring of the Embryonic Forebrain. *CellReports*. 8:1271–1279. doi:10.1016/j.celrep.2014.07.042.
Stephan, A.H., B. a. Barres, and B. Stevens. 2012. The Complement System: An Unexpected Role in Synaptic Pruning During Development and Disease. *Annu. Rev. Neurosci.* 35:369–389. doi:10.1146/annurev-neuro-061010-113810.
Stephan, A.H., D. V Madison, J.M. Mateos, D. a Fraser, E. a Lovelett, L. Coutellier, L. Kim, H.-H. Tsai, E.J. Huang, D.H. Rowitch, D.S. Berns, A.J. Tenner, M. Shamloo, and B. a Barres. 2013. A dramatic increase of C1q protein in the CNS during normal aging. *J. Neurosci.* 33:13460–74. doi:10.1523/JNEUROSCI.1333-13.2013.
Stevens, B., N.J. Allen, L.E. Vazquez, G.R. Howell, K.S. Christopherson, N. Nouri, K.D. Micheva, A.K. Mehalow, A.D. Huberman, B. Stafford, A. Sher, A.M. Litke, J.D. Lambris, S.J. Smith, S.W.M. John, and B. a. Barres. 2007. The Classical Complement Cascade Mediates CNS Synapse Elimination. *Cell*. 131:1164–1178. doi:10.1016/j.cell.2007.10.036.
Stirling, D.P., S. Liu, P. Kubes, and V.W. Yong. 2009. Depletion of Ly6G/Gr-1 Leukocytes after Spinal Cord Injury in Mice Alters Wound Healing and Worsens Neurological Outcome. *J. Neurosci.* 29:753–764. doi:10.1523/JNEUROSCI.4918-08.2009.
Stoica, B.A., and A.I. Faden. 2010. Cell Death Mechanisms and Modulation in Traumatic Brain Injury. *Neurotherapeutics*. 7:3–12. doi:10.1016/j.nurt.2009.10.023.
Streit, W.J., and G.W. Kreutzberg. 1988. Response of endogenous glial cells to motor neuron degeneration induced by toxic ricin. *J. Comp. Neurol.* 268:248–263. doi:10.1002/cne.902680209.
Su, J., C. V. Haner, T.E. Imbery, J.M. Brooks, D.R. Morhardt, K. Gorse, W. Guido, and M.A. Fox. 2011. Reelin Is Required for Class-Specific Retinogeniculate Targeting. *J. Neurosci.* 31:575–586. doi:10.1523/JNEUROSCI.4227-10.2011.
Subramanian, A., P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, and J.P. Mesirov. 2005. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.

Proc. Natl. Acad. Sci. 102:15545–15550. doi:10.1073/pnas.0506580102.

Sugden, K., T.E. Moffitt, L. Pinto, R. Poulton, B.S. Williams, and A. Caspi. 2016. Is Toxoplasma Gondii Infection related to brain and behavior impairments in humans? Evidence from a population-representative birth cohort. *PLoS One*. 11:1–14. doi:10.1371/journal.pone.0148435.

Summers, C., S.M. Rankin, A.M. Condliffe, N. Singh, A.M. Peters, and E.R. Chilvers. 2010. Neutrophil kinetics in health and disease. *Trends Immunol.* 31:318–324. doi:10.1016/j.it.2010.05.006.

Tait, S.W.G., G. Ichim, and D.R. Green. 2014. Die another way – non-apoptotic mechanisms of cell death. *J. Cell Sci.* 127:2135–2144. doi:10.1242/jcs.093575.

Tang, Z., S. Zhang, C. Lee, A. Kumar, P. Arjunan, Y. Li, F. Zhang, and X. Li. 2011. An Optic Nerve Crush Injury Murine Model to Study Retinal Ganglion Cell Survival. *J. Vis. Exp.* 6–8. doi:10.3791/2685.

Tay, T.L., D. Mai, J. Dautzenberg, F. Fernández-Klett, G. Lin, Sagar, M. Datta, A. Drougard, T. Stempfl, A. Ardura-Fabregat, O. Staszewski, A. Margineanu, A. Sporbert, L.M. Steinmetz, J.A. Pospisilik, S. Jung, J. Priller, D. Grün, O. Ronneberger, and M. Prinz. 2017. A new fate mapping system reveals context-dependent random or clonal expansion of microglia. *Nat. Neurosci.* doi:10.1038/nn.4547.

Templeton, J.P., and E.E. Geisert. 2012. A practical approach to optic nerve crush in the mouse. *Mol. Vis.* 18:2147–52. doi:10.1016/j.brainres.2003.10.029.

Thion, M.S., D. Low, A. Silvin, J. Chen, P. Grisel, J. Schulte-Schrepping, R. Blecher, T. Ulas, P. Squarzoni, G. Hoeffel, F. Coulpier, E. Siopi, F.S. David, C. Scholz, F. Shihui, J. Lum, A.A. Amoyo, A. Larbi, M. Poidinger, A. Buttgereit, P.-M. Lledo, M. Greter, J.K.Y. Chan, I. Amit, M. Beyer, J.L. Schultze, A. Schlitzer, S. Pettersson, F. Ginhoux, and S. Garel. 2017. Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific Manner. *Cell*. 1–17. doi:10.1016/j.cell.2017.11.042.

Thornton, P., B.W. McColl, A. Greenhalgh, A. Denes, S.M. Allan, and N.J. Rothwell. 2010. Platelet interleukin-1?? drives cerebrovascular inflammation. *Blood*. 115:3632–3639. doi:10.1182/blood-2009-11-252643.

Torrey, E.F., and R.H. Yolken. 2003. Toxoplasma gondii and Schizophrenia. *Emerg. Infect. Dis.* 9:1375–1380. doi:10.3201/eid0911.030143.

Tosto, G., and C. Reitz. 2013. Genome-wide association studies in Alzheimer's disease: A review topical collection on dementia. *Curr. Neurol. Neurosci. Rep.* 13. doi:10.1007/s11910-013-0381-0.

Tremblay, M.-È., R.L. Lowery, and A.K. Majewska. 2010. Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi:10.1371/journal.pbio.1000527.

Truman, L., C. Ford, M. Pasikowska, and Jd. 2008. CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. *Blood*. 112:5026–5036. doi:10.1182/blood-2008-06-162404.The.

Tsai, H.-H., H. Li, L.C. Fuentealba, A. V Molofsky, R. Taveira-Marques, H. Zhuang, A. Tenney, A.T. Murnen, S.P.J. Fancy, F. Merkle, N. Kessaris, A. Alvarez-Buylla, W.D. Richardson, and D.H. Rowitch. 2012. Regional astrocyte allocation regulates CNS synaptogenesis and repair. *Science*. 337:358–62. doi:10.1126/science.1222381.

Tsao, J.W., M.C. Brown, M.J. Carden, W.G. Mclean, and V.H. Perry. 1994. Loss of the Compound Action Potential : an Electrophysiological , Biochemical and Morphological Study of Early

Events in Axonal Degeneration in the C57BL / OIa Mouse. 6:516–524.

Tufail, Y., D. Cook, L. Fourgeaud, C.C.O. Shea, G. Lemke, and A. Nimmerjahn. 2017. Phosphatidylserine Exposure Controls Viral Innate Immune Responses by Microglia. *Neuron*. 93:1–13. doi:10.1016/j.neuron.2016.12.021.

Turnbull, I.R., and M. Colonna. 2007. Activating and inhibitory functions of DAP12. *Nat. Rev. Immunol.* 7:155–161. doi:10.1038/nri2014.

Turner, C., a Devitt, K. Parker, M. MacFarlane, M. Giuliano, G.M. Cohen, and C.D. Gregory. 2003. Macrophage-mediated clearance of cells undergoing caspase-3-independent death. *Cell Death Differ.* 10:302–312. doi:10.1038/sj.cdd.4401170.

Ueno, M., Y. Fujita, T. Tanaka, Y. Nakamura, J. Kikuta, M. Ishii, and T. Yamashita. 2013. Layer V cortical neurons require microglial support for survival during postnatal development. *Nat. Neurosci.* 16:543–51. doi:10.1038/nn.3358.

Ulland, T.K., W.M. Song, S.C.C. Huang, J.D. Ulrich, A. Sergushichev, W.L. Beatty, A.A. Loboda, Y. Zhou, N.J. Cairns, A. Kambal, E. Loginicheva, S. Gilfillan, M. Cella, H.W. Virgin, E.R. Unanue, Y. Wang, M.N. Artyomov, D.M. Holtzman, and M. Colonna. 2017. TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease. *Cell*. 170:649–663.e13. doi:10.1016/j.cell.2017.07.023.

Ulland, T.K., Y. Wang, and M. Colonna. 2015. Regulation of microglial survival and proliferation in health and diseases. *Semin. Immunol.* 27:410–415. doi:10.1016/j.smim.2016.03.011.

Ullian, E.M., S.K. Sapperstein, K.S. Christopherson, and B.A. Barres. 2001. Control of synapse number by glia. *Science (80-.).* 291:657–661. doi:10.1126/science.291.5504.657.

Vaccarezza, O.., T.A. Reader, E. Pasqualini, and J. Pecci-Saavedra. 1970. Temporal Course of Synaptic Degeneration in the Lateral Geniculate Nucleus. *Exp. Neurol.* 28:277–285.

Vainchtein, I.D., G. Chin, F.S. Cho, K.W. Kelley, J.G. Miller, E.C. Chien, S.A. Liddelow, P.T. Nguyen, H. Nakao-Inoue, L.C. Dorman, O. Akil, S. Joshita, B.A. Barres, J.T. Paz, A.B. Molofsky, and A. V. Molofsky. 2018. Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development. *Science (80-.).* 3589:1–8. doi:10.1126/science.aal3589.

Vargas, M.E., and B.A. Barres. 2007. Why Is Wallerian Degeneration in the CNS So Slow? *Annu. Rev. Neurosci.* 30:153–179. doi:10.1146/annurev.neuro.30.051606.094354.

Varol, D., A. Mildner, T. Blank, A. Shemer, N. Barashi, S. Yona, E. David, S. Boura-Halfon, Y. Segal-Hayoun, L. Chappell-Maor, H. Keren-Shaul, D. Leshkowitz, E. Hornstein, M. Fuhrmann, I. Amit, N. Maggio, M. Prinz, and S. Jung. 2017. Dicer Deficiency Differentially Impacts Microglia of the Developing and Adult Brain. *Immunity*. 46:1030–1044.e8. doi:10.1016/j.immuni.2017.05.003.

Vasek, M.J., C. Garber, D. Dorsey, D.M. Durrant, B. Bollman, A. Soung, J. Yu, C. Perez-Torres, A. Frouin, D.K. Wilton, K. Funk, B.K. DeMasters, X. Jiang, J.R. Bowen, S. Mennerick, J.K. Robinson, J.R. Garbow, K.L. Tyler, M.S. Suthar, R.E. Schmidt, B. Stevens, and R.S. Klein. 2016. A complement–microglial axis drives synapse loss during virus-induced memory impairment. *Nature*. 534:538–543. doi:10.1038/nature18283.

Vaughn, J.E., and D.C. Pease. 1970. Electron microscopic studies of Wallerian degeneration in rat optic nerves. II. Astrocytes, oligodendrocytes and adventitial cells. *J. Comp. Neurol.* 140:207–225. doi:10.1002/cne.901400205.

Wake, H., A.J. Moorhouse, S. Jinno, S. Kohsaka, and J. Nabekura. 2009. Resting Microglia Directly Monitor the Functional State of Synapses In Vivo and Determine the Fate of Ischemic Terminals. *J. Neurosci.* 29:3974–3980. doi:10.1523/JNEUROSCI.4363-08.2009.

Walczak, H. 2013. Death receptor-ligand systems in cancer, cell death, and inflammation. *Cold Spring Harb. Perspect. Biol.* 5:1–18. doi:10.1101/cshperspect.a008698.

Waller, A. 1850. Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. *Philos. Trans. R. Soc. London*. 140:423–429.

Walsh, J.G., D.A. Muruve, and C. Power. 2014a. Inflammasomes in the CNS. *Nat. Rev. Neurosci.* 15:84–97. doi:10.1038/nrn3638.

Walsh, J.T., S. Hendrix, F. Boato, I. Smirnov, J. Zheng, J.R. Lukens, S. Gadani, D. Hechler, G. Gölz, K. Rosenberger, T. Kammertöns, J. Vogt, C. Vogelaar, V. Siffrin, A. Radjavi, A. Fernandez-castaneda, A. Gaultier, R. Gold, T. Kanneganti, and R. Nitsch. 2015. MHCII-independent CD4 + T cells protect injured CNS neurons via IL-4. *J. Clin. Invest.* 125:699–714. doi:10.1172/JCI76210DS1.

Walsh, J.T., J. Zheng, I. Smirnov, U. Lorenz, K. Tung, and J. Kipnis. 2014b. Regulatory T Cells in Central Nervous System Injury: A Double-Edged Sword. *J. Immunol.* 193:5013–5022. doi:10.4049/jimmunol.1302401.

Wang, H., H. Wang, W. Xiong, Y. Chen, Q. Ma, J. Ma, Y. Ge, and D. Han. 2006. Evaluation on the phagocytosis of apoptotic spermatogenic cells by Sertoli cells in vitro through detecting lipid droplet formation by Oil Red O staining. *Reproduction*. 132:485–792. doi:10.1530/rep.1.01213.

Wang, Y., M. Cella, K. Mallinson, J.D. Ulrich, K.L. Young, M.L. Robinette, S. Gilfillan, G.M. Krishnan, S. Sudhakar, B.H. Zinselmeyer, D.M. Holtzman, J.R. Cirrito, and M. Colonna. 2015a. TREM2 Lipid Sensing Sustains the Microglial Response in an Alzheimer's Disease Model. *Cell*. 160:1061–1071. doi:10.1016/j.cell.2015.01.049.

Wang, Y., M. Cella, K. Mallinson, J.D. Ulrich, K.L. Young, M.L. Robinette, S. Gilfillan, G.M. Krishnan, S. Sudhakar, B.H. Zinselmeyer, D.M. Holtzman, J.R. Cirrito, and M. Colonna. 2015b. TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. *Cell*. 160:1061–1071. doi:10.1016/j.cell.2015.01.049.

Wang, Y., K.J. Szretter, W. Vermi, S. Gilfillan, C. Rossini, M. Cella, A.D. Barrow, M.S. Diamond, and M. Colonna. 2012. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nat. Immunol.* 13:753–760. doi:10.1038/ni.2360.

Watkins, T. a, B. Wang, S. Huntwork-Rodriguez, J. Yang, Z. Jiang, J. Eastham-Anderson, Z. Modrusan, J.S. Kaminker, M. Tessier-Lavigne, and J.W. Lewcock. 2013. DLK initiates a transcriptional program that couples apoptotic and regenerative responses to axonal injury. *Proc. Natl. Acad. Sci. U. S. A.* 110:4039–44. doi:10.1073/pnas.1211074110.

Winkler, E.A., A.P. Sagare, and B. V. Zlokovic. 2014. The pericyte: A forgotten cell type with important implications for alzheimer's disease? *Brain Pathol.* 24:371–386. doi:10.1111/bpa.12152.

Wong-Riley, M.T.T. 1972. Terminal degeneration and glial reactions in the lateral geniculate nucleus of the squirrel monkey after eye removal. *J. Comp. Neurol.* 144:61–91. doi:10.1002/cne.901440104.

Wright, A.K., T.M. Wishart, C.A. Ingham, and T.H. Gillingwater. 2010. Synaptic protection in the brain of WldS mice occurs independently of age but is sensitive to gene-dose. *PLoS One*. 5. doi:10.1371/journal.pone.0015108.

Wu, H.-H., E. Bellmunt, J.L. Scheib, V. Venegas, C. Burkert, L.F. Reichardt, Z. Zhou, I. Fariñas, and B.D. Carter. 2009. Glial precursors clear sensory neuron corpses during development via

Jedi-1, an engulfment receptor. *Nat. Neurosci.* 12:1534–41. doi:10.1038/nn.2446.

Xiao, J., Y. Li, K.L. Gressitt, H. He, G. Kannan, T.L. Schultz, N. Svezhova, V.B. Carruthers, M. V. Pletnikov, R.H. Yolken, and E.G. Severance. 2016. Cerebral complement C1q activation in chronic Toxoplasma infection. *Brain. Behav. Immun.* 58:52–56. doi:10.1016/j.bbi.2016.04.009.

Xu, H., M. Chen, A. Manivannan, N. Lois, and J. V. Forrester. 2008. Age-dependent accumulation of lipofuscin in perivascular and subretinal microglia in experimental mice. *Aging Cell*. 7:58–68. doi:10.1111/j.1474-9726.2007.00351.x.

Yakovlev, A.G., and A.I. Faden. 2004. Mechanisms of Neural Cell Death: Implications for Development of Neuroprotective Treatment Strategies. *NeuroRx*. 1:5–16. doi:10.1602/neurorx.1.1.5.

Yamasaki, R., H. Lu, O. Butovsky, N. Ohno, A.M. Rietsch, R. Cialic, P.M. Wu, C.E. Doykan, J. Lin, A.C. Cotleur, G. Kidd, M.M. Zorlu, N. Sun, W. Hu, L. Liu, J.-C. Lee, S.E. Taylor, L. Uehlein, D. Dixon, J. Gu, C.M. Floruta, M. Zhu, I.F. Charo, H.L. Weiner, and R.M. Ransohoff. 2014. Differential roles of microglia and monocytes in the inflamed central nervous system. *J. Exp. Med.* 211:1533–1549. doi:10.1084/jem.20132477.

Yang, J., Z. Wu, N. Renier, D.J. Simon, K. Uryu, D.S. Park, P.A. Greer, C. Tournier, R.J. Davis, and M. Tessier-Lavigne. 2015. Pathological axonal death through a Mapk cascade that triggers a local energy deficit. *Cell*. 160:161–176. doi:10.1016/j.cell.2014.11.053.

Yawata, I., H. Takeuchi, Y. Doi, J. Liang, T. Mizuno, and A. Suzumura. 2008. Macrophage-induced neurotoxicity is mediated by glutamate and attenuated by glutaminase inhibitors and gap junction inhibitors. *Life Sci.* 82:1111–1116. doi:10.1016/j.lfs.2008.03.010.

Yeh, F.L., Y. Wang, I. Tom, L.C. Gonzalez, and M. Sheng. 2016. TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia. *Neuron*. 91:328–340. doi:10.1016/j.neuron.2016.06.015.

Yoles, E., and M. Schwartz. 1998. Degeneration of spared axons following partial white matter lesion: implications for optic nerve neuropathies. *Exp. Neurol.* 153:1–7. doi:10.1006/exnr.1998.6811.

Youm, Y.H., R.W. Grant, L.R. McCabe, D.C. Albarado, K.Y. Nguyen, A. Ravussin, P. Pistell, S. Newman, R. Carter, A. Laque, H. Münzberg, C.J. Rosen, D.K. Ingram, J.M. Salbaum, and V.D. Dixit. 2013. Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell Metab.* 18:519–532. doi:10.1016/j.cmet.2013.09.010.

Zhai, Q., J. Wang, A. Kim, Q. Liu, R. Watts, E. Hoopfer, T. Mitchison, L. Luo, and Z. He. 2003. Involvement of the ubiquitin-proteasome system in the early stages of Wallerian degeneration. *Neuron*. 39:217–225. doi:10.1016/S0896-6273(03)00429-X.

Zhang, N., M. Fang, H. Chen, F. Gou, and M. Ding. 2014. Evaluation of Spinal Cord Injury Animal Models. *Neural Regen. Res.* 2008–2012.