The Physiological and Pathological Roles of Microglia-Neuron Interactions in the Central Nervous System

Chloe N. Winston[1,2,3], Katherine E. Prater[3], Gwenn A. Garden[4]
[1]Department of Neuroscience, University of Washington, Seattle, Washington 98195; [2]Department of Computer Science, University of Washington, Seattle, Washington 98195; [3]Department of Neurology, University of Washington, Seattle, Washington 98195; [4]Department of Neurology, University of North Carolina, Chapel Hill, North Carolina 27599

Microglia are the resident macrophages of the central nervous system (CNS). Numerous lines of research support a high frequency of microglia-neuron interactions in the brain and spinal cord. Often, interactions between microglia and neurons correspond with changes in individual synaptic strength, as assessed by neural activity, as well as synaptic or neuronal lifespan. Microglia-neuron interactions also contribute to large scale remodeling of neural networks during development and learning. This review first summarizes the relevant literature on the presence of microglia-neuron interactions in the resting state and how these interactions can mediate synaptic formation, neurogenesis, synaptic pruning, and synaptic strengthening or weakening. This review will then discuss how dysfunction in signaling mechanisms between microglia and neurons may lead to disease states, with examples from neurodegeneration, epilepsy, and psychiatric disorders.

Abbreviations: AD – Alzheimer's Disease; BDNF – Brain-Derived Neurotrophic Factor; C4 – Complement Component 4; C4A – Complement Component 4A; CNS – Central Nervous System; C1q – Complement Component 1q; CR3 – Complement Receptor 3; CSF1 – Colony Stimulating Factor 1; CSF1R – Colony Stimulating Factor 1 Receptor; C3 – Complement Component 3; CX3CL1 – Fractalkine; CX3CR1 – Fractalkine Receptor; dLGN – Dorsal Lateral Geniculate Nucleus; GABA – Gamma-Aminobutyric Acid; GWAS – Genome Wide Association Study; IFN-γ – Interferon-Gamma; IGF-1 – Insulin-Like Growth Factor-1; IL-4 – Interleukin-4; IL-1β – Interleukin-1-Beta; MHC – Major Histocompatibility Complex; PD – Parkinson's Disease; PET – Positron Emission Tomography; SE – Status Epilepticus; 6-OHDA – 6-hydroxydopamine; TLE – Temporal Lobe Epilepsy; TNF-α – Tumor Necrosis Factor-Alpha; TTX – Tetrodotoxin; US – Unpredictable Stress

Keywords: Multipartite Synapse; Microglia Surveillance; Filopodia Formation; Phagocytosis; Synaptic Pruning; Neurogenesis; Neuroinflammation; Synaptic Plasticity; Neurodegeneration; Epilepsy; Psychiatric Disorders

Introduction

The concept of a multipartite synapse refers to the idea that synapses consist of neurons, glia, and other cells. This was first discussed in the literature in the early 1900s when Ramon y Cajal suggested glia cells as the "third element" of the synapse (García-Marín et al. 2007). Since then, this concept has greatly expanded as researchers have discovered new cells that interact with synapses and new modes of interaction between these components of the synapse.

In the traditional bipartite synapse, both cell bodies and dendritic spines, which are small protrusions from dendrites, may receive input

from synaptic boutons, which are specialized areas in presynaptic cells that contain vesicles of neurotransmitters (Berry and Nedivi 2017). Synapses are the connections made between presynaptic boutons and postsynaptic elements such as cell bodies or dendritic spines (Morris et al. 2013). Synapses connect large scale networks or circuits of neurons. There are several processes by which synapses are modified, including plasticity and remodeling.

Synaptic plasticity occurs when specific sets of synapses increase or decrease in strength (the amount of signal transmission between two neurons; Citri and Malenka 2008). This arises from changes in neurotransmitter levels or functional changes in proteins involved in synaptic transmission (Citri and Malenka 2008). In many cases, though not all, synaptic strength increases following repetitive activation of the pre-synaptic neuron (Citri and Malenka 2008). The process of synaptic plasticity contributes to overall changes in neural circuitry and is important in learning (Citri and Malenka 2008).

Synaptic remodeling refers to the formation of new synapses or the elimination of existing ones. Synaptic formation is typically preceded by the formation and stabilization of filopodia, thin protrusions from axons or dendritic spines (Ziv and Smith 1996). Filopodia are highly motile and tend to move towards a pre-synaptic element on another neuron (Ziv and Smith 1996). A synapse typically forms once filopodia contact a pre-synaptic element and stabilize (Ziv and Smith 1996). Synapses may also be eliminated from circuitry. Synaptic pruning refers to the engulfment of synapses or synaptic elements by other cells (Piochon et al. 2016). This abolishes the connection between two synaptic elements. During development, synapses are pruned to establish appropriate connections in the nervous system (Piochon et al. 2016). Memories are encoded in neural circuitry as patterns of activated synapses (Thompson and Krupa 1994). The changes in synaptic connections described by synaptic plasticity and remodeling support learning and memory. In the healthy brain, microglia play a key role in modulating synaptic remodeling (Morris et al. 2013).

Microglia are the primary immune cells of the central nervous system (Kim and de Vellis 2005). Microglia are highly dynamic during inflammation but are engaged in interactions with other cells even in the healthy brain (Morris et al. 2013). Even though their somas are usually stationary in homeostatic conditions, microglia continuously extend and retract processes into and from the surrounding microenvironment (Morris et al. 2013). Microglia tile in the CNS, meaning that they are uniformly distributed through tissue such that each microglia surveys non-overlapping regions (Parkhurst and Gan 2010; Morris et al. 2013). Microglia make transient contacts with surrounding structures including neurons, blood vessels, and other glial cells (Morris et al. 2013). The purpose of some of these contacts is unclear, but microglia provide dynamic surveillance of the CNS, including individual synapses (Morris et al. 2013).

The mechanisms and regulation of signaling between microglia and neuronal synapses are gradually being uncovered and are the subject of this review of literature. The literature on this topic supports several hypotheses. (1) Neural activity drives the frequency and nature of microglia-neuron crosstalk. (2) Microglia-neuron crosstalk occurs in one of two ways: physical contact or signaling through cytokines. (3) Microglia-neuron interactions can cause changes in synaptic strength or lifespan and thereby modulate neuronal growth, synapse formation and degradation, and large-scale synaptic remodeling.

Microglia-Neuron Interactions in the Healthy Brain

Synaptic Activity Modulates Microglia Surveillance of the CNS Environment

In the healthy brain, microglia actively survey their surrounding microenvironment (Morris et al. 2013). According to two-photon time-lapse imaging, in homeostatic conditions, microglia have long, highly ramified, and motile processes that make contact with blood vessels, neurons, and other cells (Li et al. 2012; Morris et al. 2013). Near synapses, microglia processes form rounded bulbous endings, with a diameter

larger than that of dendritic spines (Morris et al. 2013). These endings occasionally contact neuronal elements, often with different processes of a single microglia making contact with different neurons (Tremblay et al. 2010).

The occurrence of microglia-synapse contact raises the question of what regulates microglia surveillance and what drives microglia to extend processes towards or contact specific synapses. Numerous studies suggest that neural activity directs microglia-synapse interactions (Nimmerjahn et al. 2005; Fontainhas et al. 2011; Li et al. 2012). Both exposure to neurotransmitters and changes in sensory experience are used to experimentally alter neural activity levels in order to study its effect on microglia-neuron interactions. The primary excitatory neurotransmitter of the brain, glutamate, is often utilized in these studies to promote neuronal activity. Generally, increases in neural activity appear to enhance microglia process motility in some settings, though this trend is not completely supported by experiments with sensory experience.

In the optic tectum of larval zebrafish, microglia processes moved towards neurons activated by light stimuli (Li et al. 2012). Glutamate uncaging, the experimental release of the excitatory neurotransmitter glutamate into extracellular space, directed tips of microglia processes towards neurons activated by the glutamate in the optic tectum in vivo (Li et al. 2012). These results suggest that neural activity steers microglia processes.

The type of synaptic activity may affect microglia surveillance dynamics, including the extent of microglia ramification, process motility, and frequency of contact with neurons in different ways. In retinal explants, ionotropic glutamate transmission increased microglia ramification and the motility of microglia processes, whereas inhibitory GABAergic transmission decreased these measures of microglia surveillance (Fontainhas et al. 2011). Similar results were found in the tectum of zebrafish larvae where glutamate uncaging increased microglia contact with neurons (Li et al. 2012), and in the mouse neocortex where application of a GABA receptor blocker increased the volume that microglia were surveying (Nimmerjahn et al. 2005). In hippocampal slices, however, microglia did not appear to respond to the application of glutamate or GABA agonists (Wu and Zhuo 2008). Additionally, in spinal cord slices, microglia motility was not affected by application of glutamate or GABA (Chen et al. 2010). Though experimental differences may account for these findings, these results may suggest regional differences in the mechanisms regulating the motility of microglia processes where mechanisms present in some regions may override the microglia response to neural activity. For example, microglia appear to be more responsive to neurotransmission and changes in neural activity in regions of the CNS that process sensory experience, such as the retina and tectum, than in other regions. It would be informative to further study the effects of glutamate and GABA on microglia surveillance dynamics in different parts of the CNS using a consistent approach to verify and characterize this potential regional difference. This could also shed light on the controversial effects of changes in sensory experience on microglia-neuron dynamics, which will be discussed next.

Several studies have investigated the effects of sensory deprivation on microglia surveillance (Tremblay et al. 2010; Grier et al. 2016; Sipe et al. 2016). As demonstrated by confocal imaging and *in vivo* two-photon imaging, olfactory deprivation induced by nostril occlusion led to activation of microglia in the olfactory bulb (decreased microglia process length and increased microglia branching), as well as a greater motility of microglia processes, allowing more dynamic surveillance of the surrounding microenvironment (Grier et al. 2016). In addition, olfactory deprivation was associated with increased microglia-neuron contacts and microglia engulfment of neurons (Grier et al. 2016). On the other hand, two-photon imaging and electron microscopy in mice that underwent visual deprivation showed an expansion of microglia process area and a decrease in the motility of microglia processes in the visual cortex (Tremblay et al. 2010; Sipe et al. 2016). Microglia also made more contacts with synaptic clefts during visual deprivation, although the contact frequency with other synaptic elements seemed unchanged (Tremblay et al. 2010; Sipe et al. 2016). These contacts

were shown to be more extensive than under light exposure, with a greater perimeter of each contact (Tremblay et al. 2010). This result implies a more involved interaction like phagocytosis under visual deprivation. Indeed, microglia phagocytosis of other cells, including synaptic elements, increased with visual deprivation, according to electron microscopy (Tremblay et al. 2010). Upon re-exposure to light, the overall area that microglia covered decreased and returned to its original size, but the frequency of contact with synaptic clefts did not decrease (Tremblay et al. 2010). Further investigation of the effects of longer re-exposure periods may be needed to conclude whether the frequency of contact with neurons decreases at later timepoints in re-exposure. This will shed light on whether different mechanisms are responsible for changes in microglia surveillance and changes in microglia-neuron interactions. These studies on sensory deprivation demonstrate that changes in sensory experience, which generally influence synaptic activity, affects microglia surveillance and microglia-neuron interactions, though there is not a clear trend. Although olfactory deprivation and visual deprivation both increased the frequency of microglia-neuron contacts, they had opposite effects on microglia morphology and process motility.

Comparing the results from sensory deprivation to those from neurotransmitter application, there is a general consensus that synaptic activity regulates microglia ramification. It is possible that the increase in contacts resulting from glutamate application may be solely for the purpose of increasing microglia surveillance whereas that resulting from sensory deprivation may allow for synaptic pruning of less active neurons. This would explain why the sensory deprivation, which typically reduces neural activity, would lead to an increase in microglia-neuron contact frequency. However, the overall effect of synaptic activity on the number of microglia contacts is inconclusive and further research is needed to explain this. To summarize, microglia process outgrowth is directed towards regions of higher neural activity, and changes in neural activity affects microglia surveillance dynamics and the frequency of microglia-neuron interactions. These mechanisms elucidate one aspect of microglia-neuron crosstalk—how neural activity regulates microglia interactions with neurons.

Microglia Depletion and Behavior

Since microglia-neuron interactions are regulated by neural activity, are microglia a required participant in normal synaptic function? To address this question, experiments utilizing microglia depletion have been performed. Depletion of microglia is observed to limit synaptic remodeling and thereby impair certain behavioral tasks, although some controversy surrounds this. In one study, auditory cue fear conditioning, which is the pairing of an auditory cue with a fear-inducing stimulus (Curzon et al. 2009), was less effective in mice depleted of microglia (Parkhurst et al. 2013). This was measured by the freezing response, when mice become immobile in response to a fear-eliciting stimulus (Curzon et al. 2009). Microglia depletion also correlated with a decreased exploration time for novel objects which implies impaired memory (Parkhurst et al. 2013). Learning was also impaired, as measured with the rotarod motor learning task where microglia depletion impaired performance improvement (Parkhurst et al. 2013). However, as microglia repopulated the brain, mice improved in these behavioral tasks (Parkhurst et al. 2013). Oddly, a different study found that microglia depletion did not have any cognitive or behavioral effects (Elmore et al. 2015). It is possible that this discrepancy could have resulted from the different microglia depletion paradigms utilized by the two studies. The mice in the first study expressed the diphtheria toxin receptor in microglia and were administered diphtheria toxin, and behavioral studies were done after complete depletion of microglia (Parkhurst et al. 2013). On the other hand, the mice in the second study were administered Plexxikon (PLX3397), which inhibits CSF1-CSF1R signaling, a signaling pathway necessary for microglia viability, and behavioral studies were carried out during microglia depletion (Elmore et al. 2015). Diphtheria toxin administration and Plexxikon administration may vary in effects on other cells in the CNS, or the effect of microglia depletion may vary over time. Hence, the discrepancy in

results may be clarified by using a consistent microglia depletion paradigm and testing the behavioral and cognitive effects of microglia depletion at various timepoints throughout the depletion paradigm.

Although the contributions of microglia to behavior are controversial, microglia dysfunction is observed in multiple behavioral disorders, as will be discussed later in the review. Moreover, microscopy reveals that microglia modulate synaptic plasticity, which plays a key role in learning and memory, and in synaptic remodeling (Miyamoto et al. 2016). Microglia depletion was found to decrease synaptic remodeling in the late postnatal period and young adulthood of mice (Parkhurst et al. 2013). Specifically, microglia depletion was associated with decreased rates of postsynaptic dendritic spine formation and dendritic spine elimination both in baseline conditions and in motor learning experiments (Parkhurst et al. 2013). The next sections will discuss potential mechanisms by which microglia contribute to synaptic remodeling and synaptic plasticity.

Microglia-Mediated Synaptic Formation

Synapses usually form between dendritic spines and axon terminals. Filopodia are postsynaptic protrusions that precede dendritic spines (Ziv and Smith 1996; Weinhard et al. 2018). Typically, filopodia are transient, and only a few mature into spines to form permanent synapses (Weinhard et al. 2018). Filopodia can form a new contact with a presynaptic bouton, or it can direct the original bouton to contact a different spine (Weinhard et al. 2018).

In both the hippocampus (Weinhard et al. 2018) and the somatosensory cortex (Miyamoto et al. 2016), microglia contact with dendrites appears to increase the probability of filopodia formation (Figure 1). In the hippocampus, formation of filopodia on dendritic spines was associated with elongation of the spine towards the microglia process (Weinhard et al. 2018). Occasionally, newly formed filopodia mediated switching of synapses (Figure 1D; Weinhard et al. 2018). In these cases, the spine head was directed to the tip of the filopodia where it established a synapse with a different presynaptic bouton (Weinhard et al. 2018). This phenomenon correlated with longer filopodia lifetimes (Weinhard et al. 2018), supporting the idea that the filopodia play a role in establishing the new synapse. Intriguingly, in the somatosensory cortex, microglia seemed to target mature spine heads and excitatory synapses to induce filopodia formation (Miyamoto et al. 2016). This is consistent with the previous discussion of microglia processes being directed towards areas of higher synaptic activity. The purpose of

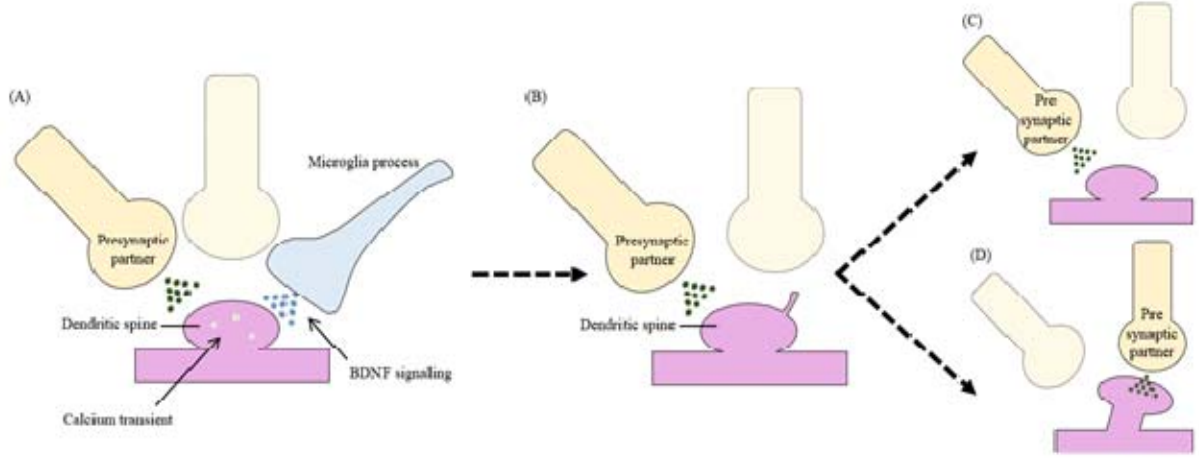

Figure 1: Schematic of microglia contact-mediated filopodia formation on dendritic spine. Darker presynaptic bouton indicates the presynaptic partner of the spine at any given point. (A) Both microglia release of BDNF and dendritic calcium transients are suggested in microglia contact-mediated filopodia formation. (B) Filopodia formation may follow microglia contact. (C) Filopodia are transient structures and may disappear. (D) Occasionally, filopodia mediate synaptic switching.

this selectiveness may be a productive area of future research. These findings support the idea that microglia contact with neurons supports synapse formation.

There are several proposed molecular pathways that are involved in microglia-mediated synaptic formation. One of them involves calcium signaling (Figure 1). In the somatosensory cortex, microglia contact with dendritic spines was sometimes followed by local calcium transients that typically lasted the duration of the contact (Miyamoto et al. 2016). These calcium transients often preceded the formation of filopodia, likely due to an accumulation of F-actin, a key component of filopodia (Miyamoto et al. 2016). In addition, contact-induced calcium transients increased the rates of local filopodia formation and the lifetimes of filopodia formed on the contacted dendrite (Miyamoto et al. 2016). In a different study in the hippocampus though, weak local calcium transients promoted filopodia growth, whereas higher levels inhibited filopodia extension, potentially inhibiting growth of "competing" filopodia (Lohmann et al. 2005). While it is unclear whether these calcium transients were microglia-mediated, this raises the question of whether microglia may induce varying magnitudes of calcium transients through contact with neurons. Nevertheless, these findings support the involvement of calcium transients in microglia contact-induced filopodia formation.

Brain-derived neurotrophic factor (BDNF) may also be involved in microglia contact-mediated synaptic formation (Figure 1). Specific depletion of microglia BDNF was found to impair motor learning-induced dendritic spine formation in mice (Parkhurst et al. 2013). This may be explained by the apparent contribution of BDNF signaling to the aforementioned calcium transients. In vitro studies of rat hippocampus revealed that BDNF signaling contributed to local calcium transients at synapses, and this required the activation of TrkB, the primary receptor of BDNF (Lang et al. 2007). Microglia activation by lipopolysaccharide (LPS), a bacterial molecule which induces neuroinflammation, was found to induce BDNF release (Gomes et al. 2013), so the involvement of microglia release of BDNF in synaptic formation is consistent with the finding that microglia activation is required for contact-induced filopodia formation. Microglia contact-induced filopodia formation was observed only in young rodents at ages during which microglia are known to be activated (Miyamoto et al. 2016). Even more strikingly, minocycline administration, which inhibits microglia activation, reduces the frequency of microglia-mediated filopodia formation (Miyamoto et al. 2016). Not only does this support the role of BDNF signaling in synapse formation, but it also suggests the presence of other inflammatory cytokines released by activated microglia that may contribute to synapse formation and are yet to be uncovered. From these results together, it appears that microglia release of BDNF and microglia contact with neurons may induce local calcium transients, which may stabilize filopodia by inducing F-actin accumulation.

It is unclear whether microglia initiate filopodia formation or if they simply support growing filopodia. The first hypothesis seems to be more supported as filopodia formation appeared to follow microglia contact. At the same time, calcium transients were found to prevent filopodia outgrowth (Lohmann et al. 2005), and this suggests that the calcium transients mediated by microglia contact with dendrites may serve to stabilize existing filopodia. Further research is needed to understand the timeline of microglia contact and filopodia formation. Additionally, the subset of neurons that microglia contact to induce filopodia formation should be determined as selectivity in microglia contact-induced synapse formation may suggest a more complex way by which neural circuitry is shaped.

Microglia-Mediated Neurogenesis

Several studies suggest crosstalk between neural progenitor cells and microglia may modulate the proliferation of neural progenitor cells and their differentiation into neurons (Walton et al. 2006). Microglia release type-II interferon-gamma (IFN-γ) which supports neurogenesis, and also release tumor necrosis factor-alpha (TNF-α) which has an antiproliferative role in neurogenesis (Iosif et al. 2006). Microglia also release insulin-like

growth factor-1 (IGF-1; Arcuri et al. 2017). In layer V of the cortex, microglia were found to be essential for survival of neurons during development (Ueno et al. 2013). These findings suggest that microglia may modulate neurogenesis in a contact-independent manner that involves the release of various cytokines instead. In vivo and in vitro experiments revealed that suppression of microglia activation reduced proliferation and differentiation of stem cells in the subventricular zone, and both interleukin-1-beta (IL-1β) and IFN-γ were found to be involved in this, further supporting this idea (Shigemoto-Mogami et al. 2014).

In cocultures of neural progenitor cells and microglia, different cytokines impacted how microglia influenced neurogenesis and early differentiation. For example, one study found that microglia activated by IFN-γ better supported neurogenesis than microglia activated by interleukin-4 (IL-4), based on the number of neurons derived from the neural progenitor cells (Butovsky et al. 2006). Additionally, neurons formed in culture with IFN-γ activated microglia had longer processes and neurons formed with IL-4 activated microglia had more branched dendritic trees (Butovsky et al. 2006). These findings highlight a fascinating interplay between neurons and microglia which appear to be dependent on cytokine release rather than contact. Further research should aim to elucidate what cytokines microglia release in developing structures and the effects on neurogenesis throughout development. In addition, the effect of the environment and the cytokines present in the tissue on what cytokines are released by microglia should also be studied. This would provide a clearer picture of how microglia shape neurogenesis.

Microglia-Mediated Synaptic Pruning

Synaptic pruning is a process of synapse elimination that occurs throughout CNS development. Microglia are directly involved in synaptic pruning of specific subsets of neurons. Microglia-mediated synaptic pruning occurs in three steps: recognition of target, engulfment of target, and digestion (Vilalta and Brown 2018). The mouse retinogeniculate system is a common model to study synaptic pruning. During development, inputs from the left and right eyes overlap in the dorsal lateral geniculate nucleus (dLGN; Bickford et al. 2010; Kerschensteiner and Guido 2017). Over time, these inputs "compete" for space and typically, the weaker inputs are pruned from this region (Bickford et al. 2010; Kerschensteiner and Guido 2017). Eventually, these inputs are segregated into eye-specific regions with little overlap (Bickford et al. 2010; Kerschensteiner and Guido 2017).

Multiple studies have demonstrated microglia engulfment of specific inputs from areas of the nucleus to facilitate the formation of these non-overlapping areas (Schafer et al. 2012; Stephan et al. 2012). Based on these studies, two separate factors distinguish the neurons and synapses that microglia target for pruning: neural activity and synaptic labels. Microglia preferentially phagocytose neurons based on neuronal activity. For example, in one paradigm (Schafer et al. 2012), one eye was treated with forskolin which upregulates synaptic activity or tetrodotoxin (TTX) which decreases synaptic activity. Microglia in the dLGN preferentially phagocytosed "weaker" inputs, synaptic inputs from the eye with lower synaptic activity, suggesting activity-dependent synaptic pruning (Schafer et al. 2012).

An alternative method by which microglia recognize potential targets in synaptic labeling. Neurons may express certain molecular tags that microglia recognize for phagocytosis. The complement system appears to be involved in this labeling. Specifically, complement components such as C3 may label synapses for phagocytosis (Stephan et al. 2012). Complement proteins are upregulated during peak synaptic pruning time in the mouse retinogeniculate system, supporting this hypothesis (Schafer et al. 2012). Furthermore, complement knockout decreases segregation of inputs likely due to decreased engulfment of synapses by microglia (Schafer et al. 2012). It is hypothesized that specific synapses express complement, marking them for phagocytosis. Microglia, the primary cells in the brain expressing complement receptor CR3 (Lian et al. 2016), recognize the labeled synaptic elements and phagocytose them. Despite this, a recent study using the developing mouse postnatal hippocampus as a model showed that CR3 knockout has no effect on the engulfment of synapses in the mouse postnatal hippocampus (Weinhard et al. 2018).

The age of the mice used in this study and other experimental constructs may explain this contradiction, but it does suggest the presence of other mechanisms that may compensate for the loss or deactivation of the complement system.

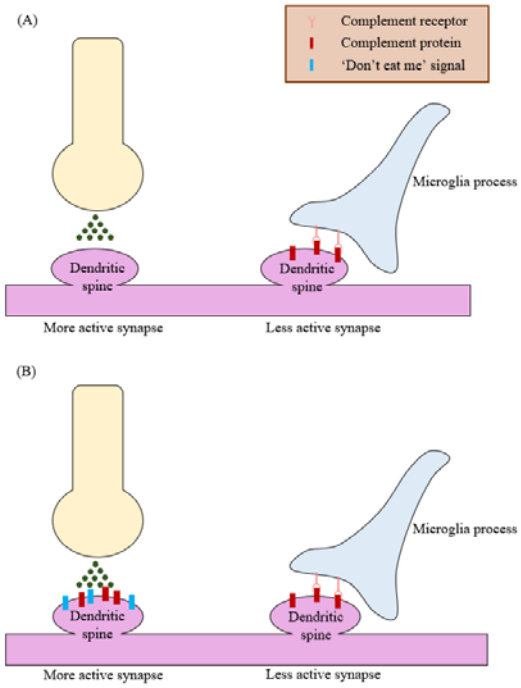

Figure 2: Schematic of hypotheses regarding preferential microglia phagocytosis of less active synapses. (A) One hypothesis is that complement preferentially tags less active synapses, leading to microglia phagocytosis of less active synapses. (B) An alternative hypothesis is that complement tags all synapses, but 'don't eat me' signals protect more active synapses from phagocytosis.

The prior studies raise the question of how synaptic labeling and neuronal activity together modulate synaptic pruning (Figure 2). One possibility is that components of the complement system preferentially tag less active synapses, and microglia phagocytose these. Alternatively, a wider set of synapses may be tagged, but complement inhibitors or 'don't eat me' signals protect more active synapses. There is some evidence for this hypothesis. CD47 is a transmembrane protein that prevents phagocytosis by immune cells (Lehrman et al. 2018). CD47 was found to be enriched in the dLGN during peak pruning and becomes more uniformly spread as eye-specific segregation completes (Lehrman et al. 2018). CD47 knockout increased engulfment of retinal ganglion cell inputs in the dLGN and thereby caused excessive presynaptic pruning (Lehrman et al. 2018). Administration of TTX to one eye to increase competition between inputs led to CD47 localization to inputs from the more active eye, suggesting that this 'don't eat me' signal is degraded or removed from less active neurons (Lehrman et al. 2018). These data together with the data on complement upregulation during peak pruning indicate opposing roles for the complement system and 'don't eat me' molecules that balance each other in regulating synaptic pruning.

Once microglia recognize target synapses, they eliminate them using a variety of methods. Microglia may phagocytose synapses (Schafer et al. 2012), but one study using advanced imaging techniques demonstrated microglia trogocytosis (rapid capture of membrane elements) instead (Weinhard et al. 2018). In this study, less than 50% of each spine that was engulfed was contacted, and only parts of contacted spines were engulfed (Figure 3; Weinhard et al. 2018). Additionally, no phagocytic cups were observed on the microglia, and engulfment was rapid (Weinhard et al. 2018). At first, this finding of trogocytosis rather than phagocytosis of entire synaptic elements was taken to contradict the occurrence of phagocytosis (Weinhard et al. 2018). However, the data are inconclusive and could suggest separate mechanisms for synaptic pruning. The same imaging techniques used in these experiments should be used in the dLGN and other sites of significant synaptic pruning in order to determine whether both phagocytosis and trogocytosis occur in the brain. If this is true, it would be beneficial to probe what signaling pathways underlie the differential engulfment mechanisms in different parts of the brain.

Microglia-Mediated Synaptic Strengthening and Weakening

Microglia can also mediate selective synaptic strengthening or weakening via the release of various cytokines. This was suggested in the mouse somatosensory cortex where the depletion of microglia changed the spatial

distribution of strengths of inputs from different layers (Miyamoto et al. 2016). Further research using a variety of models has elucidated some of the mechanisms by which microglia regulate synaptic strength. For example, TNF-α, which may be released by microglia (Wang et al. 2015), promoted the accumulation of glutamatergic receptors and the removal of GABAergic receptors on hippocampal neurons (Stellwagen et al. 2005). In organotypic entorhino-hippocampal slice cultures, denervation, which induced synaptic strengthening, was accompanied by an increase in mRNA expression of TNF receptors in various layers (Becker et al. 2015). TNF receptor deficiency decreased the synaptic strengthening following denervation (Becker et al. 2015). However, TNF receptor deficiency did not abolish this strengthening, suggesting the role of other signals in long term synaptic scaling (Becker et al. 2015). Fractalkine signaling and BDNF signaling are suggested to influence synaptic scaling as well. Fractalkine (CX3CL1) is found mostly on neurons, and its receptor (CX3CR1) is found on microglia in the rat brain (Harrison et al. 1998). CX3CR1 loss of function in mice impaired long-term potentiation (Béchade et al. 2013). BDNF may also play a role in modulating synaptic strength as it was shown to reverse the direction of GABA currents (Kettenmann et al. 2013). Through the release of such factors, microglia can regulate synaptic strength.

Regulation of Outcome of Microglia-Neuron Interactions

The variety of findings in the studies discussed thus far raises the question of what causes microglia to promote formation versus engulfing synapses. Future research in this area should focus on distinguishing the signaling pathways that lead to different microglia-neuron interactions to clarify how microglia are signaled to do one action over the other. Perhaps, it depends on the region of the brain, or on age, or on the cytokine environment. For example, in the optic tectum of the larval zebrafish, deprivation of light exposure altered the subset of synapses contacted and the changes that microglia led to on contacted synapses (Tremblay et al. 2010). While microglia contacted smaller spines that subsequently grew in resting state, microglia more strongly tended to contact larger spines that subsequently shrank under light deprivation, suggesting synaptic depression or phagocytosis (Tremblay et al. 2010). This supports a hypothesis that amidst highly active neurons as is present in the optic tectum when exposed to light, microglia are more likely to phagocytose synapses than to support them. However, one study found that microglia preferentially induced filopodia formation on excitatory synapses (Miyamoto et al. 2016), so the set of mechanisms regulating the type of microglia-neuron interaction remains unclear.

One model that may be especially helpful to elucidate how the outcomes of microglia-neuron interactions are determined may be the developing hippocampus where contact-induced synaptic trogocytosis, contact-induced filopodia formation, and microglia-mediated synaptic plasticity have been observed (Stellwagen et al. 2005; Becker et al. 2015; Weinhard et al. 2018). A detailed study of the environment and the signaling pathways involved during these processes could shed light on how formation and elimination of synapses and neurons are regulated in the healthy brain.

Together, these studies demonstrate the variety of microglia-neuron interactions in the healthy brain. Neural dynamics instruct microglia dynamics and interactions with neurons. Multiple studies applying neurotransmitters onto tissue suggest that increased neural activity upregulates microglia surveillance and contacts with neuron (Nimmerjahn et al. 2005; Fontainhas et al. 2011; Li et al. 2012). However, this is controversial due to inconsistent results from neurotransmitter application studies and sensory deprivation studies (Wu and Zhuo 2008; Chen et al. 2010; Tremblay et al. 2010; Grier et al. 2016; Sipe et al. 2016). Microglia also instruct neural dynamics, and this may lead to behavioral effects, though this is also controversial in the literature (Parkhurst et al. 2013; Elmore et al. 2015). Nevertheless, microglia shape synaptic remodeling in a variety of ways. In developing structures, microglia contact with dendritic

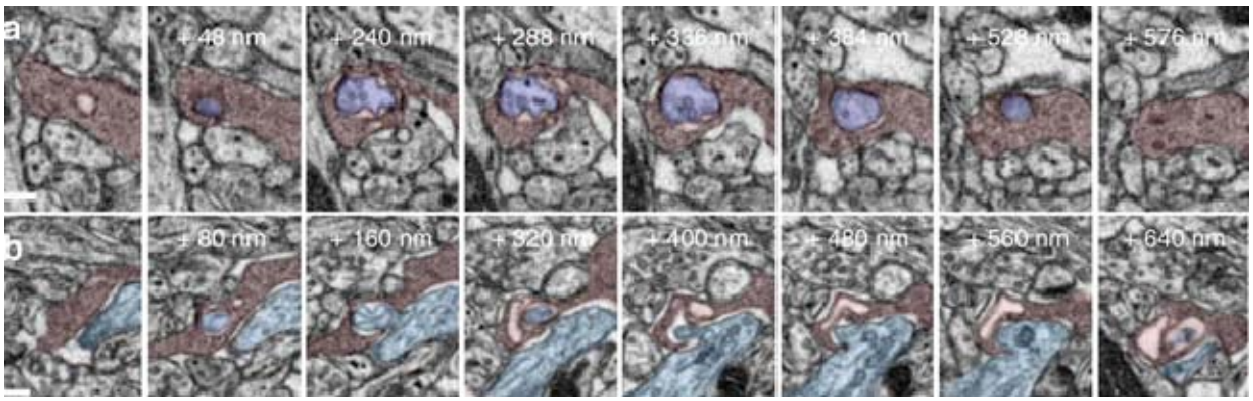

Figure 3: Representative sequences of focused ion beam scanning electron microscopy images. Used from Weinhard et al. 2018 under Creative Commons license (http://creativecommons.org/licenses/by/4.0/). (A) Complete engulfment of a presynaptic bouton (purple) by a microglia (red). (B) Partial engulfment of axonal material (blue) by microglia (red).

spines was found to promote formation of filopodia (Miyamoto et al. 2016; Weinhard et al., 2018). This may be mediated by BDNF signaling or calcium signaling (Lohmann et al. 2005; Lang et al. 2007; Parkhurst et al. 2013; Miyamoto et al. 2016). In addition, microglia appear to regulate neurogenesis through release of various cytokines, and different cytokines have different effects on neurogenesis (Butovsky et al. 2006; Iosif et al. 2006; Walton et al. 2006; Shigemoto-Mogami et al. 2014; Arcuri et al. 2017). Microglia also prune synapses in developing structures, with both trogocytosis and phagocytosis observed to be mediated by microglia contact (Schafer et al. 2012; Weinhard et al. 2018). This is likely directed by neural activity (Schafer et al. 2012) and synaptic labeling (Schafer et al. 2012; Stephan et al. 2012; Lehrman et al. 2018; Weinhard et al. 2018). The mechanism of synaptic labeling is still unclear, though both complement proteins and 'don't eat me' signals have been implicated in this process. Both microglia-mediated filopodia formation and microglia-mediated synaptic pruning may be directed towards selected sets of neurons based on neural activity (Tremblay et al. 2010; Miyamoto et al. 2016). Overall, it remains unclear what regulates the various types of microglia-neuron interactions, and a study of the environments and signaling pathways involved in each may elucidate the directing factors involved. To summarize, neural dynamics appear to influence microglia surveillance and contact. Microglia surveillance, contact, and cytokine release lead to various synaptic and neural processes, including synaptic formation, neurogenesis, and synaptic pruning. The next section will focus on how microglia-neuron interactions are impacted in disease states.

Microglia-Neuron Interactions in Disease States

Altered synaptic transmission or maturation is involved in numerous neurological and psychiatric disorders. Because of the significant role microglia play in synaptic remodeling and transmission, "microgliopathy" (Arcuri et al. 2017) appropriately describes many disorders where microglia dysfunction is a primary cause of the disease. Typically, in such diseases, a signaling pathway is disrupted in some way, leading to aberrant microglia activation which damages the neuronal network.

Neurodegenerative Diseases

Some neurodegenerative diseases involve excessive phagocytosis of neurons by microglia. This may occur because of disrupted synaptic labeling or excessive microglia activation. Further research in these areas may open doors to new therapeutic targets for such diseases.

Alzheimer's disease (AD) is characterized by neurofibrillary tangles composed of hyperphosphorylated tau and

amyloid plaques composed of aggregated amyloid-beta (Rajendran and Paolicelli 2018). The precise causes of this dementia are not entirely clear, but the presence of activated microglia in AD brains (Udeochu et al. 2016) suggest microglia involvement. Because the inhibition of the complement system was found to decrease plaque-related synapse loss, one hypothesis is that the complement system is reactivated in Alzheimer's disease (Rajendran and Paolicelli 2018). Beta amyloid, the key component of amyloid plaques, may increase the expression of complement components in neurons (Rajendran and Paolicelli 2018). Microglia may then phagocytose complement-tagged neurons. Deficits in fractalkine signaling also led to decreased beta amyloid deposition, as well as fewer microglia surrounding amyloid plaques (Lee et al. 2010). Because microglia are the primary cell in the CNS that express the fractalkine receptor (Harrison et al. 1998), it is likely that fractalkine signaling between microglia and neurons impairs the ability of microglia to clear beta amyloid in the presence of other chemokines that may be present in AD. These findings suggest that amyloid plaques cause microglia to aberrantly phagocytose synapses and that complement activation and fractalkine signaling may be involved.

Parkinson's disease (PD) is a movement disorder accompanied with the degeneration of dopaminergic neurons in the substantia nigra (DeMaagd and Philip 2015). A recent study demonstrated that microglia activation may aggravate PD (Kuter et al. 2018). The study used a toxin (6-OHDA) that caused degeneration of dopaminergic neurons (Kuter et al. 2018). This toxin caused motor dysfunction in rats that eventually reversed (Kuter et al. 2018). However, pharmacological activation of microglia inhibited this reversal (Kuter et al. 2018). Additionally, microglia activation accelerated the loss of dopaminergic neurons in the substantia nigra (Kuter et al. 2018). The loss or impairment of BDNF signaling may underlie these observations as BDNF levels were found to decrease in PD (Bathina and Das 2015). Fractalkine signaling and complement activation may also be involved in PD as they are in AD, but further research is needed to elucidate the specific mechanisms that underlie the involvement of microglia in PD.

Epilepsy

Epilepsy involves occurrences of excessive neuronal excitation (Fisher et al. 2005). Multiple studies suggest that dysfunctional microglia-neuron crosstalk is involved in epilepsy. Microglia exhibit an activated phenotype in human epileptic brains (Wyatt et al. 2017) and in rodent epilepsy models (Zhao et al. 2018). Additionally, the administration of minocycline, which inhibits microglia activation, decreased neurodegeneration associated with kainic acid induced status epilepticus (SE) in rodents (Shin et al. 2015).

The complement system is one aspect that seems to be affected in epilepsy. Rodent SE and human temporal lobe epilepsy (TLE) were associated with increased complement levels in the brain (Aronica et al. 2007; Wyatt et al. 2017). Moreover, hippocampal levels of iC3b, fragments of complement proteins C1q and C3, positively correlated with seizure frequency (Schartz et al. 2018). Increased levels of complement may suggest increased labeling of synapses for phagocytosis, as previously discussed, which may lead to excessive microglia activation and phagocytosis of synapses in epilepsy. However, the cause of increased complement labeling in epilepsy remains unclear and additional research is needed.

Fractalkine signaling is also implicated in epilepsy. As discussed earlier, fractalkine signaling involves ligand CX3CL1 in neurons and receptor CX3CR1 on microglia (Harrison et al. 1998). Increased levels of CX3CR1 were found both in pilocarpine-induced SE in rodents and in TLE in humans (Yeo et al. 2011; Roseti et al. 2013). In one study, fractalkine infusion further increased neurodegeneration that resulted from SE, while administration of antibodies against fractalkine mitigated neurodegeneration, suggesting that fractalkine signaling contributes to neural damage in epilepsy (Yeo et al. 2011). Potentially, this observed neurodegeneration resulted from microglia phagocytosis of neurons, as infusion of fractalkine into non-epileptic rodents was

found to induce microglia activation (Yeo et al. 2011). However, further research is needed to confirm the mechanism of neurodegeneration. Intriguingly, in another study CX3CL1 application prevented the decrease in GABA-evoked currents that followed seizures, suggesting a neuroprotective role of fractalkine signaling in epilepsy (Roseti et al. 2013). This is consistent with the aforementioned contribution of fractalkine signaling to long-term potentiation in the healthy brain (Béchade et al. 2013). These findings suggest that multiple modes of action of fractalkine may be involved in epilepsy, involving both neuroprotective effects on neurotransmission imbalances and deleterious effects aggravating neurodegeneration. Future research is needed to explore how fractalkine signaling leads to these apparently contrasting effects.

Psychiatric Disorders

Many psychiatric disorders occur when neuroplasticity in the corticolimbic area of the brain is altered. Microglia are implicated in several psychiatric conditions. Psychological stress alters microglia morphology in the unpredictable stress (US) paradigm in rodents, which models depression (Kreisel et al. 2014). Inhibition of microglia activation and IL-1 signaling in these models suppressed some depressive phenotypes (Kreisel et al. 2014). These findings suggest that depression is associated with neuroinflammation that involves microglia activation. In humans, major depressive disorder was associated with increased microglia activation, measured by total distribution volume of translocator protein, supporting the role of microglia activation in depression (Setiawan et al. 2018). Future research may attempt to elucidate the specific signaling pathways involved in microglia involvement in depression.

Schizophrenia is another psychiatric disorder that involves various types of cognitive impairments (Andreasen and Flaum 1991). PET scans revealed microglia activation in humans with recent-onset schizophrenia, suggesting the involvement of microglia in schizophrenia (van Berckel et al. 2008). GWAS studies show that MHC and C4A are the two genes most associated with schizophrenia, and schizophrenia patients have higher levels of C4A (Schizophrenia Working Group of the Psychiatric Genomics Consortium et al. 2016). C4A is a complement protein that activates C3, which may lead to tagging of neurons and eventual phagocytosis by microglia (Schizophrenia Working Group of the Psychiatric Genomics Consortium et al. 2016). This hypothesis was supported by a study on neuron-microglia cocultures derived from humans with and without schizophrenia (Sellgren et al. 2019). Increased phagocytosis of synapses by microglia was found in cocultures derived from schizophrenia patients compared to control (Sellgren et al. 2019). Minocycline, which decreases microglia activation, mitigated the over-pruning observed in schizophrenic-derived cocultures (Sellgren et al. 2019). Levels of C4 correlated with the frequency of synaptic phagocytosis (Sellgren et al. 2019). These findings support the idea that microglia are overactivated in schizophrenia, leading to excessive pruning of synapses which likely results from increased expression of complement.

Together, these results demonstrate the role of microglia in a plethora of neurological conditions. In each disorder discussed above, microglia were shown to be overactivated in disease models or diseased individuals. Excessive microglia phagocytosis is suggested to be partially responsible for many forms of neurodegeneration, and complement signaling and fractalkine signaling are implicated in most of these disorders. Parkinson's Disease may additionally involve changes in microglia BDNF signaling (Bathina and Das 2015), and microglia may contribute to depression through various neurotransmitters though the mechanisms remain unclear (Kreisel et al. 2014). These signaling pathways are involved in microglia-mediated neuronal processes in healthy state but may be abnormally activated in disease. Future research should explore the mechanisms behind the abnormal activation of these pathways.

Generally, microglia appear to promote disease pathology, suggesting microglia function as a potential therapeutic target. However, microglia did appear to play a neuroprotective role in schizophrenia. The balance between the protective and deleterious effects of microglia in

disease and how this may be regulated would be an important point of future research. This would further elucidate how microglia are involved in disease and potentially uncover future therapeutical targets.

Conclusion

Numerous research studies support the existence of microglia-neuron interaction in both healthy and diseased states. At a whole organism level, such interactions are involved in learning and memory (Parkhurst et al. 2013), and, at a cellular level, in CNS surveillance and synaptic plasticity (Morris et al. 2013). As described above, current literature demonstrates that microglia continuously survey the tissue microenvironment in the CNS, and neural activity can steer this surveillance (Li et al. 2012). Microglia contact neurons, and neural activity modulates this (Li et al. 2012). Through contacts with neurons, microglia can mediate synaptic formation, neurogenesis, synaptic pruning, and synaptic strengthening and weakening (Butovsky et al. 2006; Miyamoto et al. 2016; Schafer et al. 2012). Neural activity, cytokines, and the complement system regulate the mode and frequency of these interactions. Disruptions in these pathways are involved in a variety of pathological conditions including Alzheimer's disease (Rajendran and Paolicelli 2018) and schizophrenia (Sellgren et al. 2019). In these conditions, microglia activation, complement regulation, fractalkine signaling, or other microglia signaling pathways have been demonstrated to be abnormal. These pathologies highlight the importance of microglia-neuron crosstalk for the maintenance of physiological conditions in the CNS. Despite the progress made in understanding microglia-neuron interactions, numerous key questions remain unanswered. For example, what is the significance of physical contact between microglia and neurons? What causes microglia to release a neurogenesis-promoting cytokine versus phagocytosing a synapse? Further studies on microglia interactions with neurons will elucidate the molecular mechanisms of such crosstalk and suggest new therapeutic targets for diseases involving dysfunctional microglia.

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Corresponding Author

Chloe Winston
University of Washington
www.depts.washington.edu/neurolog
Department of Neurology
Box 356465
1959 NE Pacific St.
Seattle WA 98195-6465
wincnw@gmail.com

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