The brain's polymath: emerging roles of microglia throughout brain development

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Abstract

Microglia, the resident brain immune cells, have garnered a reputation as major effectors of circuit wiring due to their ability to prune synapses. Other roles of microglia in regulating neuronal circuit development have so far received comparatively less attention. Here, we review the latest studies that have contributed to our increased understanding of how microglia regulate brain wiring beyond their role in synapse pruning. We summarize recent findings showing that microglia regulate neuronal numbers and influence neuronal connectivity through a bidirectional communication between microglia and neurons, processes regulated by neuronal activity and the remodeling of the extracellular matrix. Finally, we speculate on the potential contribution of microglia for the development of functional networks and propose an integrative view of microglia as active elements of neural circuits.

Introduction

Over the last decade, the field of neurobiology has undergone a shift towards an all-inclusive view of neuronal circuits, and developmental neurobiology is no exception. This shift includes a greater appreciation of the role of non-neuronal cells. We have witnessed an exciting explosion of studies revealing the many ways by which glial cells regulate neuronal circuit development and function [1]. Among these, microglia —the brain main immune cells— have emerged as crucial regulators of brain wiring. The most studied process by which microglia regulate the development of neuronal circuits is to date synapse elimination [2]. However, the range of processes that microglia participate in is diverse and rapidly expanding (Figure 1). Here, we review recent evidence supporting emerging roles of microglia at embryonic and postnatal stages of brain development. They include the regulation of cell proliferation, neuronal activity, and the remodeling of the extracellular matrix. We also discuss some studies in the adult that can illuminate the role of microglia in developing circuits. We hope to highlight the breadth of microglial functions and identify future areas of research into how microglia regulate brain wiring.

Embryonic development: microglia role in regulating neuronal production

The role of microglia in circuit wiring begins early during neurodevelopment. As one of the earliest glial cells present in the embryonic brain [1] microglia play a role in modulating neuronal numbers through their interactions with neural stem/progenitor cells (NSPCs). Using *in vitro* cultures, microglia have been shown to increase the number of NSPCs through secreted proteins such as osteopontin and TGFβ, while decreasing the number of differentiated cells [3,4]. *In vivo*, however, the role of microglia on NSPCs behavior is more complex. Part of this complexity is due to the inherent nature of microglia. Microglia are immune cells that survey their environment through “sensome” genes – genes that can detect changes in the environment – already expressed during embryonic stages in mice [5] and in humans [6]. In addition, microglia exist in different ‘states’: context-dependent molecularly distinct but dynamic profiles that underlie different functions [7]. Hence, embryonic microglia are equipped to detect local and/or global changes and respond by altering their functional states, [5,8] which allows them to perform different roles [9–11]. This, in turn, can change NSPCs behavior. NSPCs consist of apical progenitors (AP) and basal progenitors (BP). During early-to-mid neurogenesis, APs switch from a symmetric to asymmetric division to produce BPs [12]. In rodents, microglia have been shown to be involved in the production of BPs [9,11]. Depletion of microglia or the reduction of microglia surveillance leads to a decrease in BP numbers with a concurrent increase in APs [9,11]. While the mechanism involved is unknown, it is likely achieved through the secretion of cytokines by microglia as observed in neonates [13]. Towards the end of neurogenesis, instead of assisting in BP production,

microglia phagocytose APs and BPs. In fact, depletion of microglia using liposomal clodronate leads to an increase in both progenitor types [10]. While microglia exhibit transcriptional changes across these different developmental stages [8], it is currently unclear what local cues are instructive in changing microglia function.

Microglia states and, hence, their influence on NSPCs behavior, are also affected by global changes at two levels: organismal (sex, microbiome and immune activity [5,8,14]) and environmental (pollution, exposure to external stress and substances [15,16]). These global changes can impact microglia states both transcriptionally and epigenetically. At the transcriptional level, only subtle sexual dimorphism in microglia is observed during embryogenesis [5]. However, when challenged with gestational cold stress, male but not female hypothalamic microglia alter NSPC behavior and differentiation by increasing the secretion of chemokines such as CCL3 and CCL4 [16]. At the epigenetic level, recent studies demonstrated changes in microglial chromatin accessibility in the absence of microbiota [5,8] and upon infections during embryogenesis [8]. Changes in microglial epigenetic status can significantly impact NSPCs behavior. Su *et al*. have shown that knockdown of the epigenetic remodeler ARID1a alters microglia homeostasis state, leading to a decreased secretion of the proteoglycan PRG3 [17]. This, in turn, perturbs WNT/ß-catenin signaling in NSPCs leading to a reduction in their numbers [17]. These global changes in microglia not only impact NSPC behavior but also have long lasting effects on rodent behavior. In particular, impairment in social interactions, similar to those observed in autism, have been reported [14,16,17]. As such, dissecting how these global changes can impact microglia states during embryonic development may have major implications for our understanding of brain development and related disorders.

Most studies on the role of microglia during neurogenesis have focused on rodents. Recent work in the developing primate brain has highlighted an evolutionary increase in microglia numbers relative to NSPCs [18] and that periventricular microglia in contact with mitotic NSPCs have distinct morphological features [19]. Furthermore, human-specific microglia states defined by their cytokine signatures have been observed in human fetal brains [20]. As cytokines can modulate NSPC behavior [3,4,13], it is possible that these differences in microglia numbers, morphological features and states impact neuronal production and contribute to the evolutionary expansion of the primate brain. In rodents, microglia density does not differ across cortical areas [10]. However, Cunningham *et al*. remarked on the differences in microglial density in the macaque subventricular zone when comparing the occipital lobe with neighboring cortical areas [10]. Why are microglia heterogeneously distributed in the primate brain? It is tempting to speculate that this distribution may contribute

to variation in neuronal production and consequently to a distinct cytoarchitecture across cortical areas. These initial findings on species differences are intriguing. Future work will be needed to shed additional light on their potential contribution to the evolutionary expansion and the development of cortical areas in primate brains.

Postnatal development I: crosstalk between microglia and neuronal activity

The functions of microglia in postnatal development are linked to their ability to sense neuronal activity. Research on the crosstalk between neuronal activity and microglia during development has largely focused on synaptic pruning, which has been extensively reviewed elsewhere [21,22] and will not be discussed in detail here. Although the ability of microglia to sense neuronal activity is key for their recruitment to synapses, the impact of such recruitment may extend well beyond synapse engulfment. Additional roles for the crosstalk between activity and microglia in brain wiring appear likely when considering that (i) physical contacts with synapses do not equate to synapse elimination and (ii) microglia influence neurons through diffusible factors.

Overwhelming evidence supports the prevalent view that microglia eliminate less active synapses [21,22]. However, their contacts may also promote developing synapse stabilization by increasing presynaptic activity. A potential mechanism by which microglia may increase the activity of synaptic boutons is through non-phagocytic physical interactions. Recent work showed that mechanical forces applied to the presynaptic terminal lead to an increase in the release of neurotransmitter [23]. These findings raise the intriguing possibility that the interaction of microglial processes with the presynaptic terminal directly increases neurotransmitter release and therefore presynaptic activity. This would explain why, in some instances, microglia appear to promote the formation rather than the elimination of synapses [24,25].

Recent work in the adult suggests additional activity-dependent mechanisms by which microglia may regulate brain wiring. A prominent purpose of microglia recruitment by neuronal activity is the maintenance of adult neuronal networks within specific bounds of activity [26–28]. Badimon *et al*. showed that the microglial enzyme CD39 converts the ATP released by active neurons into ADP. ADP activates microglial $P2Y_{12}$ receptors that mediate microglia recruitment to active synapses. The enzyme CD73 then converts ADP into adenosine, which reduces presynaptic neuronal activity by acting on neuronal A_1 receptors (A_1Rs) [29]. The potential developmental relevance of these findings is buoyed by recent work where adenosine was shown to regulate synapse development [30]. Gomez-Castro *et al*. demonstrated that adenosine, by acting on neuronal A_{2A} receptors (A_{2A}Rs), detects the activation of newly established GABAergic synapses and promotes their stabilization. This

work raises the intriguing possibility that microglia may weaken (through A_1Rs) or stabilize (through A_{2A}Rs) synapses during development by contributing to the generation of adenosine (Figure 2).

Microglia are also attracted to hypoactive neurons in the adult [31,32]. Chemogenetic, optogenetic, pharmacological neuronal inhibition, sensory deprivation, and general anesthesia all increase microglia surveillance and targeted motility. Unlike hyperactivity-induced microglial responses, this effect is $P2Y_{12}$-independent but requires a reduction in norepinephrine signaling [32,33]. Early postnatal microglia express β2-adrenergic receptors [34], indicating that norepinephrine signaling may also regulate microglia dynamics and their interactions with neurons and synapses during development. Altogether, this indicates that the outcome of microglia recruitment to synapses can be bidirectional and possibly involve different mechanisms. It remains to be determined how microglia “know” what proper activity levels are and how their threshold of responsiveness is set.

Interestingly, the ability of microglia to respond to neuronal activity extends beyond a generic response to hyper- or hypo-excitability. For example, microglia exhibit frequency-specific responses to stimulation. In a mouse model of Alzheimer's disease, 40 Hz – but not random – stimulation induced transcriptional and morphological changes in microglia [35]. The cytokine profile secreted by microglia is different in response to 40 Hz, 20 Hz, random or continuous visual stimulation [36]. Along the same lines, 60 Hz – but not 40 Hz – light flickering triggers microglia-mediated extracellular matrix remodeling [37]. The ability of microglia to functionally tune to specific brain frequencies may have important implications for the modulation of neuronal oscillations and raises the intriguing possibility that these cells may be differentially engaged in specific behavioral states. During transient neonatal periods, endogenous spontaneous rhythms synchronize activity and guide neuronal wiring [38]. We propose that, similar to the adult, postnatal microglia may be functionally tuned to the different patterns of neuronal activity that emerge at multiple stages during development.

As development progresses, specific patterns of activity emerge across cortical layers [39]. How does that shape the interactions between microglia and their neuronal partners? Recent work showed that the relative enrichment of molecularly distinct microglia within different cortical layers is influenced by pyramidal cell identity, possibly through specific ligand–receptor pairs [40]. The layer enrichment of microglia emerges only at the end of the second postnatal week, well after the specification of neuron laminar identity [41]. Further investigation into niche-enriched signals will shed light on how the specification of microglia by neuronal subtypes or by layer-specific patterns of activity may impact their later functions.

Microglia also respond to neuronal activity in ways that are unique to development. In the neonatal but not adult hippocampus, Logiacco *et al*. observed calcium responses in both microglia and astrocytes as a result of Schaffer collateral stimulation. Through a series of pharmacological experiments, the authors determined that $GABA_B$ receptors on hippocampal microglia can sense increases in activity through a conversion of glutamate to GABA signals by astrocytic transporters [42]. By bringing astrocytes into the picture, this work reveals the complex and nuanced ways in which neural activity influences microglia: through interactions that are not only molecularly diverse but also multicellular.

Postnatal development II: remodeling the extracellular matrix and perineuronal nets

Another potential role of microglia during postnatal development is the modulation of connectivity and neuronal properties through the remodeling of the extracellular matrix (ECM). A role for microglia in remodeling the ECM was recently discovered in the context of experience-dependent plasticity. Nguyen *et al*. identified a cytokine-mediated mechanism where neuronal IL-33 directs microglial engulfment of the ECM, which leads to dendritic spine plasticity and memory consolidation [43].

In the context of microglia-mediated ECM remodeling, special attention should be given to microglia as regulators of perineuronal nets (PNNs). PNNs are a specialization of the ECM that is crucial for the onset and closure of developmental critical periods as well as for experience-dependent plasticity underlying learning and memory [44]. In the brain, PNNs primarily wrap the soma of parvalbumin-positive (PV+) interneurons and modulate their cellular and synaptic plasticity [45]. A recent series of studies indicates that microglia are critical effectors of PNN integrity, remodeling and potentially formation across the central nervous system. In the adult brain, microglia depletion increases PNNs [46–48]. In the spinal cord, microglia-mediated degradation of PNN promotes pain [49], an effect also achieved upon optogenetic activation of microglia [50]. Upon ketamine exposure, microglia directly mediate the removal of PNN components in a $P2Y_{12}$ signaling-dependent manner across multiple brain regions [37] (Figure 2). In combination, these studies raise the intriguing possibility that microglia may contribute to the activity-dependent changes in PNN components that occur during normal development and plasticity [45]. In fact, even in absence of any insults, PNNs are not static. By virtue of such dynamics and their ability to control afferent excitatory synaptic inputs and intrinsic properties of PV+ cells, PNN proteins allow these interneurons to adapt to sensory experience [45]. During development, microglia directly influence the wiring of PV+ interneurons by regulating their laminar positioning [51] and

pruning their efferent synapses [52]. It is conceivable that microglia also influence PV+ cell function and connectivity during the critical period by remodeling the PNNs that surround them.

Conclusions and perspectives

Two general principles can be inferred from the growing body of evidence linking microglial actions and neurons. First, they communicate with neurons directly but also indirectly, through other cell types (e.g. astrocytes) or by acting on their environment (e.g. ECM). Second, microglia influence brain wiring by regulating multiple processes in the embryonic and postnatal brain (Figure 1). The role of microglia at these two windows of development is different. Before birth, they act as checkpoint sentinels that contribute to building a blueprint. As such, they ensure that largely genetically predetermined programs are minimally perturbed by local or external factors. In the postnatal brain, in addition to pruning synapses, they undertake more nuanced and sometimes subtle roles. Microglia receive information, discriminate between distinct activity patterns, have integrative properties. By doing so, what could microglia provide to postnatal brain circuits that is not achieved by neuronal and astrocytic regulation? When compared to neurons or other glial cells, two of the many properties of microglia stand out: they survey the environment (both central and peripheral systems) over time and are not “hard-wired” into circuits. These abilities combined place microglia in a unique position to act as spatio-temporal integrators. For example, microglia may act as an “activity dimmer” or an on/off switch to “reset the system” during behavioral states. In this context, it is important to highlight the slow temporal dynamics of microglial regulation. Microglia can regulate neurons through both short- and long-range mechanisms: physical interactions and the release of diffusible factors. Both these routes of action operate on a slower timescale than the millisecond span of neuronal transmission. Therefore, microglia may perform operations across longer time scales that are complementary to more immediate actions of neurons. Whether and to what extent this means that microglia should be considered active elements of neural circuits is open to discussion. Moreover, it will be important to examine the relevance of these unique properties of microglia in the context of developmental dynamics, when interactions between immature neurons are slower, less stereotyped, and rely more on spontaneous activity. During early postnatal development, transient circuits are assembled and disassembled, relative bounds of excitation and inhibition are established, activity shifts from burst to low-amplitude desynchronized patterns, coincidence-detection windows become narrower, and cell-type and regional differences become more pronounced. Do microglia – mobile and adaptive regulators that are available on-demand – contribute to these processes and how? These are pressing questions that beg

for further investigations in order to understand how these cells contribute to the development of functional networks.

Conflict of interest statement

Nothing declared.

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Figure 1. Microglia functions across development. Beyond synaptic pruning, microglia play other prominent roles in regulating brain wiring. They include the regulation of neural stem/progenitor behavior, neuronal numbers and migration, synapse formation, neuronal activity, and extracellular matrix remodeling.

Figure 2. Purinergic and adenosine signaling as mediators of microglia developmental roles. A. Potential dual role of adenosine generated by microglia in synapse development. After sensing ATP through $P2Y_{12}$ receptors ($P2Y_{12}R$), microglia contribute to the generation of adenosine (ADO). Of note, ADO could also originate from presynaptic terminals. During development, ADO of microglial origin may then weaken synapses by acting on presynaptic A_1 receptors (A_1Rs, as described in the adult by Badimon *et al*., red panel). During development, there is a transient increase in A_{2A} receptors ($A_{2A}Rs$) at hippocampal GABAergic postsynapses (green panel). Therefore, in some cases, by activating postsynaptic A_{2A} receptors, ADO of microglial origin may stabilize active synapses (i.e. synapses where GABA is activating $GABA_A$ receptors). As described in Gomez-Castro *et al*., the concomitant activation of A_{2A} receptors and $GABA_A$ receptors converges on cAMP production, which in turn leads to gephyrin phosphorylation and subsequent recruitment of the Slitrk3-PTPσ transsynaptic organizers. **B.** Microglial $P2Y_{12}Rs$ also mediate the removal of PNN components induced by ketamine exposure or 60 Hz light flickering.

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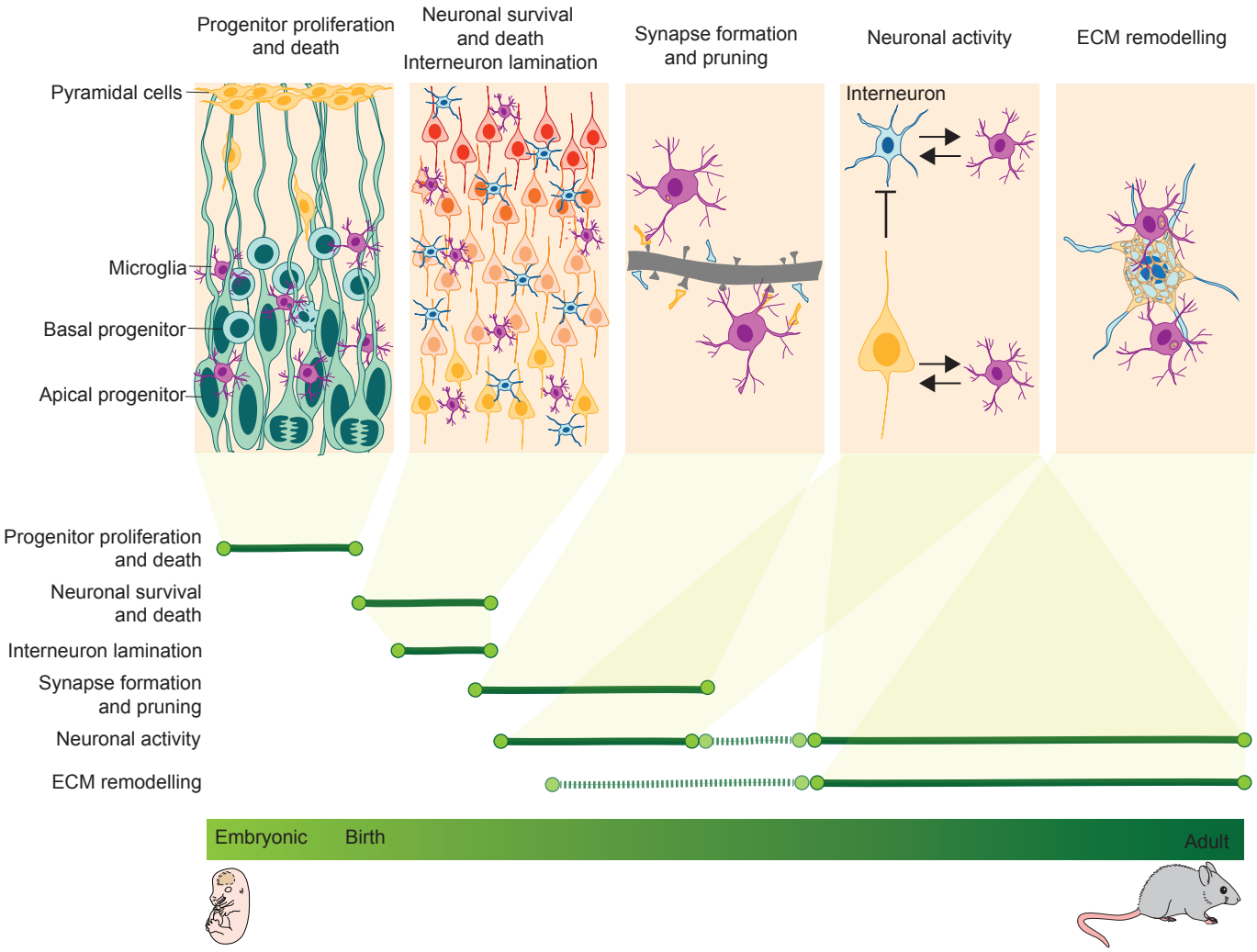

Figure 1 Wong and Favuzzi

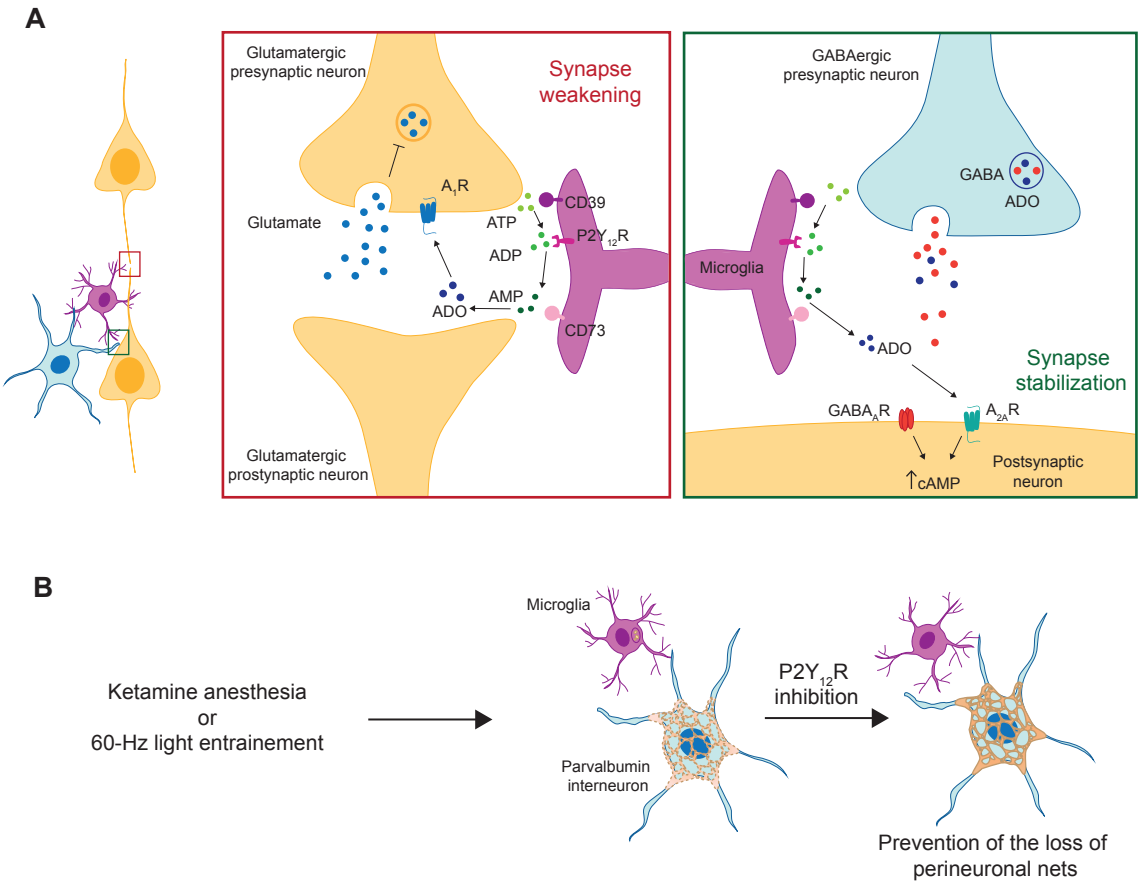

Figure 2 Wong and Favuzzi