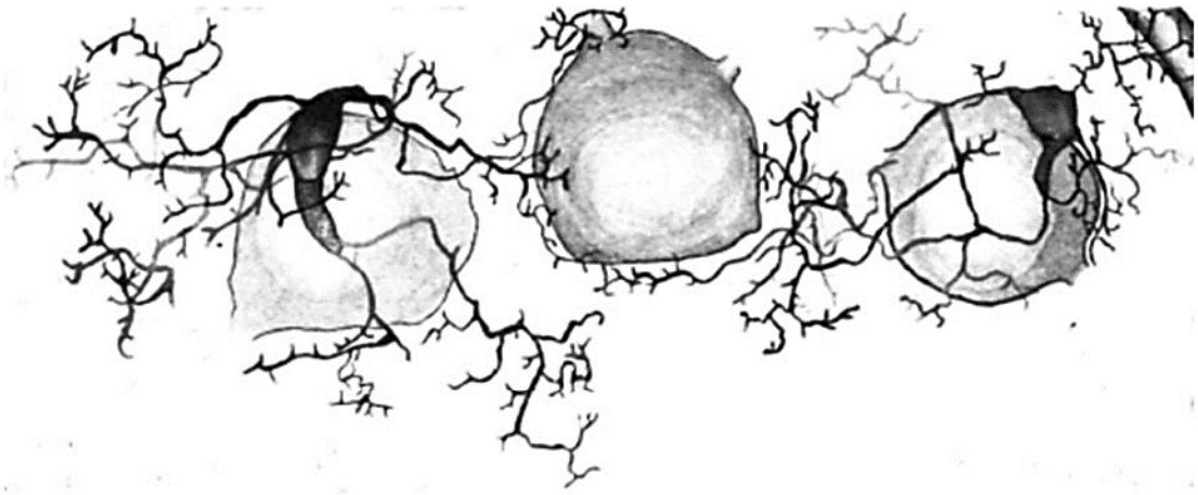
THE FUNCTION OF MICROGLIA IN THE DEVELOPING BRAIN

BACHELOR THESIS



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ABSTRACT

Microglia are increasingly acknowledged as key players in the development of the brain. Although the exact mechanisms are not well understood, recent years more evidence has come up about the ontogeny and the exact functions of microglia in the developing brain. They appear to emerge from 'primitive' macrophages in the yolk sac during embryonic development. Mature microglia control the patterning and wiring of the developing brain. They regulate the neurogenesis and axonal outgrowth by secretion of soluble molecules. Microglia also play a crucial role in the refinement of the brain. They actively regulate neuronal numbers by supporting programmed cell death and phagocytosis of the apoptotic neurons. Survival of the useful neurons is supported by secreted neurotrophic factors. In the process of activity-dependent synapse maturation, microglia are also involved. Improper synapses express signals which trigger microglia to phagocytose them. In this paper, the mechanisms of these processes in the brain development will be described in more detail.

The figure on the front page is adapted from: Penfield, W. Oligodendroglia and its relation to classical neuroglia. *Brain* 1924; 47:430-452.

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INTRODUCTION

Microglia account for 5-20% of all glial cells (consisting of astrocytes, microglia and oligodendrocytes) and are mainly known for their immune function in the brain[1]. They are the only resident macrophages in the central nervous system and therefore serve as a first line of defense against pathogens. In a homeostatic situation, microglia are in a 'resting state', taking a typical ramified shape[2]. When they are being activated by signals from their environment, they retract their branches and take an amoeboid shape, in which they perform effector functions. Activated microglia are often subclassified as classically activated (M1) and alternatively activated (M2)[3]. The phenotype they adapt, depends on the signal they receive. The M1 state is adapted in response to pro-inflammatory signals, such as IL-1β, TNF-α or high levels of ATP[4]. The M2 state appears in response to anti-inflammatory signals, such as IL-4, IL-13 and IL-10[3]. Classically activated microglia have a pro-inflammatory phenotype to eliminate unwanted materials, such as pathogens and misfolded proteins[4]. They actively create an inflammatory environment by secreting cytokines, such as IL-1β, IL-6 and TNF-α[2]. Besides, they release toxic molecules, such as reactive oxygen species (ROS) and nitric oxide (NO) to cause damage to present pathogens. Inflammatory phagocytosis can be induced by activating the receptor CD11b/CD18 on microglia[5]. Alternatively activated microglia have an anti-inflammatory phenotype to restore the homeostasis in the brain[3]. This phenotype is mainly related to tissue remodeling and repair. The alternatively activated phenotype is characterized by the secretion of neurotrophic factors, such as nerve growth factor (NGF), neurotrophin 3 (NT-3) and brain derived neurotrophic factor (BDNF). Non-inflammatory phagocytosis can be induced by triggering the TREM2 receptor on microglia[6].

The research on microglia was for a long time focused on the activated phenotype of microglia in the diseased and damaged brain. Recent years, more studies put their focus on the role of the 'resting' microglia in the healthy brain. This upcoming field showed that microglia are highly active in healthy conditions, continuously extending and retracting their branches[7,8]. Improved models and techniques led to groundbreaking observations that 'resting' microglia are actively scanning its environment and physically interact with other neural cells, including neurons and astrocytes. In fact, the population of microglia is able to sample the entire brain within a few hours[8]. In recent years it was found that microglia are involved in neuronal functioning due to bidirectional communication between microglia and neurons[9]. In this way microglia mediate neuronal plasticity in the adult central nervous system (CNS) by regulating activity-dependent synaptic plasticity[10,11] and neurogenesis[12,13], and are thus considered to be involved in learning and memory[14].

In addition to the microglial behavior in the healthy adult brain, the function of microglia in the immature developing brain has also become subject of research. Questioned is how and where microglia originate in the development and what role they play in the patterning, wiring and refinement of the brain. The patterning of the brain includes the genesis, differentiation and migration of neurons and the wiring of the brain means the outgrowth of axons and dendrites and the formation of synapses. The refinement is the selection of synapses which will be preserved through adulthood.

This paper will discuss the current understanding of the origin and function of microglia in the developing healthy brain. The patterning, wiring and refinement of the brain and the involvement of microglia in these processes will be described. Although much is known about the neuronal aspects of the development of the brain, microglia are increasingly acknowledged to be a crucial factor in this process. Here, insight will be provided about the two-way communication between neurons and microglia in the different steps of brain development.

PATTERNING THE BRAIN

ONTOGENY OF MICROGLIA

The origin of microglia in the development remains subject of discussion. It is long known that microglia can be found during early development, suggesting that microglia emerge from embryonic progenitors[15]. Regarding the shared phagocytic properties, it is likely that microglia and macrophages arise from the same progenitors. They would then emerge from the peripheral circulating monocytes and their precursors in the bone marrow. The precursors in the bone marrow emerge from hematopoietic stem cells (HPCs). Imaging studies on the ontogeny of mice and zebrafish show that there are two different hematopoietic sources from which HPCs and certain tissue macrophages emerge; the aorto-gonado-mesonephros (AGM) region[16] and the yolk sac respectively[17].

On one hand, HSCs emerge from the AGM region at the mouse embryonic day 8.5 (E8.5) and they migrate to the liver at E10.5, which then will serve as the main hematopoietic organ during the rest of the embryonal development[18]. During later development, the HSCs will migrate to other parts of the body, where they will form the definitive hematopoietic organs, such as the bone marrow. In human, the genesis of HPCs in the AGM region starts at day 27 of development and the colonization of the liver starts at day 30[19].

On the other hand, a genesis of 'primitive' macrophages was found in blood islands in the yolk sac[17]. Between E6.5 and E8.5 an expansion of the primitive macrophages is observed in the yolk sac, which start to migrate into the embryo at E9.5. Between E9.5 and E10.5, a colonization of macrophages in the embryo is taking place, including the brain (figure 1)[20]. In human, primitive macrophages in the yolk sac emerge at day 19 of development and start migrating into the embryo after the onset of blood circulation at day 21[19].

Summarizing, the AGM region is responsible for the formation of hematopoietic organs, whereas the blood islands in the yolk sac form macrophages that are distributed in different tissues. The question now remains whether microglia emerge from the HSCs, and thus from the peripheral circulating monocytic lineage, or from the primitive macrophages in the yolk sac. Recent years more evidence has come up that support the hypothesis that microglia are derived from the primitive macrophages.

First, Ajami et al. showed that microglia can proliferate independently from the bone-marrow[21]. Because circulating monocytes are not able to proliferate anymore, it is not likely that microglia emerge from circulating monocytes. This implies that microglia originate from other progenitors earlier in the development.

Secondly, various studies followed the development of primitive macrophages over time. It was found that these macrophages infiltrated the brain before HPCs were formed[20]. The

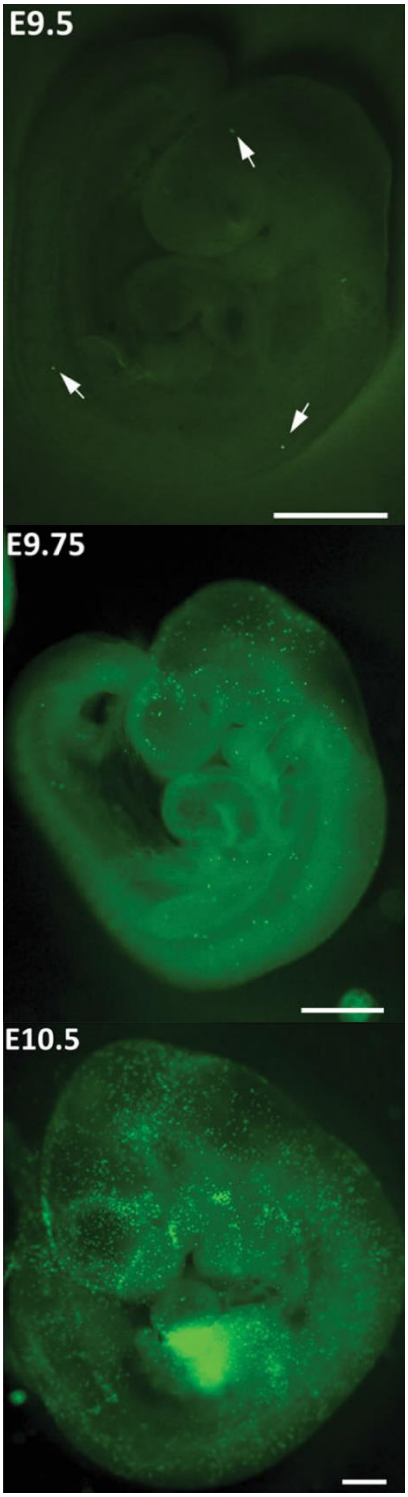

FIGURE 1. Distribution of yolk sac derived macrophages on day 9.5, 9.75 and 10.5 of embryonic development. (Adapted from Schulz et al., 2012)

exact route to the infiltration of the brain is yet not completely understood, but there is evidence that migration takes place along epithelial structures[22]. Once in the brain, the microglial progenitors start to proliferate and form clusters in the subcortical and cerebellar white matter, which are often referred to as 'fountains of microglia'[23,24]. From there, terminally differentiated microglia will emerge.

Schulz et al. (2012) showed that the transcription factor Myb, which is necessary for the generation of HSCs, is not required for the generation of microglia[20]. $Myb^{-/-}$ mice are deficient for bone marrow derived monocytes/macrophages, but they possess a normal amount of microglia. It was shown that microglia emerge around E7.5 from progenitors that are Myb-negative and express a PU.1-dependent Csf1-receptors. These cells are distinct from Myb-dependent HSCs, from which peripheral circulating monocytes and macrophages emerge.

These studies show that it is likely that microglia emerge from the primitive macrophages in the yolk sac, which are distributed all over the embryo. It is less likely that microglia originate from HSCs, which form the definitive hematopoietic organs[25]. However, this does not say that there are no microglia-like cells in the brain that emerge from peripheral circulating monocytes. In certain conditions, such as inflammation, macrophages/monocytes infiltrate the brain[26]. They take the same amoeboid shape as microglia and they behave in the same way, which makes them difficult to distinguish.

Neurogenesis

Neurogenesis is the process of generating new neurons, which takes place in early stages of the development of the brain. The brain develops from the walls of the three primary vesicles, which will form the three main structures in the brain; the forebrain, midbrain and hindbrain (figure 2)[27]. The layers of the wall adjacent to the vesicles contain radial glial cells (neural progenitors), from which neurons, astrocytes and oligodendrocytes emerge[28]. These layers are called the ventricular and subventricular zones. Here, radial glial cells proliferate and differentiate into neurons and glial cells. In the process of proliferation, they perform a 'dance', which leads to cell division (figure 2a)[29]. First, the cell extends a fiber which reaches the pial surface of the brain. Then the nucleus migrates from the ventricular zone towards the pial surface and vice versa. During this migration, the DNA is replicated. After this, the radial glial cell retracts its arm and divides. Vertical cleavage of the radial glial cell leads to two new radial glial cells, which remain in the ventricular zone to proliferate (figure 2b). Horizontal cleavage leads to two different daughter cells as a result of asymmetric cell division (figure 2c). The daughter cell lying closest to the pial surface becomes a neuroblast, which differentiates to a neuron, oligodendrocyte or astrocyte. The daughter cell closest to the ventricular surface becomes a radial glial cell, which remains in the ventricular zone. The radial glial cell extends a thin fiber towards the pial surface, along which the neuroblasts migrate to the marginal zone, where they will further differentiate and form the cortex.

In the earlier stages of the development of the central nervous system, vertical cleavage occurs most to increase the number of neural stem cells. Later in the development, horizontal cleavage takes place. Differentiation of neurons occurs first and in later stages the differentiation of astrocytes and oligodendrocytes takes place. At the stage of the formation of neurons, microglia are already present in the ventricular and subventricular layers. They appear to play a supporting role in the genesis of neurons and the formation connections with other neurons.

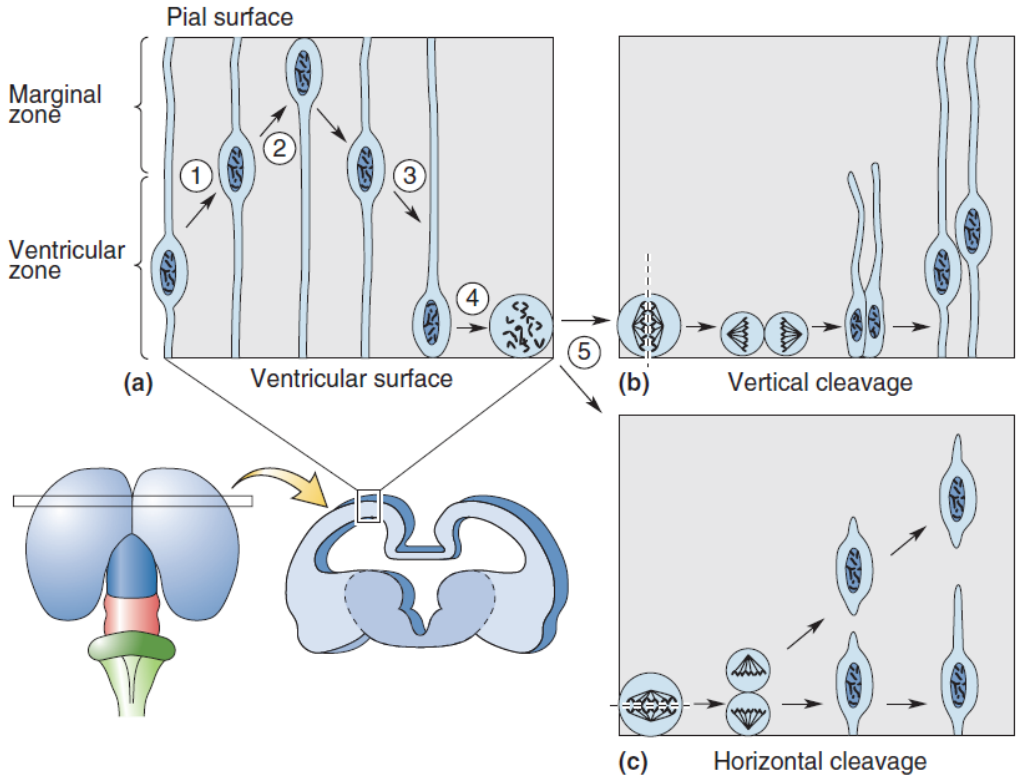

FIGURE 2. The process of neurogenesis in the walls of the primary vesicle. Microglia are present in the ventricular zone. **(a)** The 'dance' of the radial glial cell during which its DNA replicates. **(b)** Vertical cleavage of the neural stem cell. The formed daughter cells become radial glial cells. **(c)** Horizontal cleavage of the neural stem cell. The daughter cell which lays closest to the pial surface becomes a neuroblast and migrates along the fiber of a radial glial cell to the marginal zone. The daughter cell which lays closest to the ventricular surface becomes a radial glial cell. (Adapted from Bear et al., 2007)

Microglia have trophic effects on the neural progenitors. Antony et al. (2011) have done an *in vitro* study using cultured embryonic neural precursors from the cortex of PU.1$^{-/-}$ mice, which lack microglia[30]. A reduced proliferation of the neural precursors and reduced astrogenesis was observed, but there was no effect on neural survival and neurogenesis. Another *in vivo* study showed that a reduced amount of microglia in the brain of Csf1r$^{-/-}$ mice was correlated with a disrupted brain development, which implies a role for microglia in the neurogenesis[31]. This role is further investigated by Shigemoto-Mogami et al. (2014), who found that activated microglia are able to enhance neurogenesis and oligodendrogenesis through releasing cytokines like IL-1β and IFN-γ[32]. However, the blocking of various factors individually (IL-1β, IL-6, TNF-α, IFN-γ) did not show enhancement of neurogenesis and oligodendrogenesis, which suggests that microglia enhance neurogenesis and oligodendrogenesis through combinations of neurotrophic cytokines.

Instead of supporting the proliferation of neural precursors, microglia also have the capacity of reducing the size of the neural precursor pool[33]. Mainly in the later stages of cortical development there is a correlation between the amount of activated microglia and the number of neural precursors. In the presence of more activated microglia, there were less neural precursors found. The microglia eliminate the abundant cells by engulfing them (phagocytosis). This shows that microglia have the function of removing excess neural precursors. It is yet unknown whether microglia facilitate the cell death, phagocytize them, or do both.

Altogether, it could be said that microglia regulate the size of the neural precursor pool by supporting proliferation and differentiation of neural progenitors and eliminate unnecessary cells. The question remains however, if microglia do this actively or passively. It is yet unclear whether microglia autonomously determine if a cell must survive or die, or they just react on signals sent by the cells.

GENESIS OF CONNECTIONS

After the formation, migration and differentiation, neurons start to make connections with each other, which eventually will form the neural circuit. Neurons first extend their axons and dendrites, which in this early stage are called neurites[29]. The tip of the neurite is called the growth cone, which determines the path for neurite elongation. A growth cone does so by continuously scanning its environment by extending and retracting branches called filopodia, in search for guiding cues. The guiding molecules can either be chemoattractants or chemorepellants, which pull the growth cone toward the source or push away from the source of the signal respectively. The path a growth cone is directed in, depends on the spatiotemporal expression of the guidance molecules[34]. In this way the growing axon or dendrite finds its way to its final destination.

Microglia may also have an effect on the growth and guidance of axons. In the postnatal period, activated microglia are found in the white matter where axons are growing[35]. Although it is known that microglia promote axonal growth and guidance *in vitro* by excreting factors, there is no direct *in vivo* evidence, but just indications[36,37]. Microglia express several axonal growth factors, such as NT-3[38] and thrombospondin[39,40] and they also express guidance molecules, such as Slit, Netrin[41] and Semaphorin 7a[42].

Once the growth cone has found its target area, it initially generates synapses abundantly. This is mainly initiated by the spines on the dendrites of target neurons. The spines continually reach out in search for innervation. When they make contact with an axon that might be passing by, adhesion molecules serve as glue that hold them together. Structural changes and recruitment of neurotransmitter receptors will lead to the formation of the final synapse. [29]

PROGRAMMED CELL DEATH OF NEURONS

Simultaneously with the formation of connections between neurons, a selection of these neurons is taking place[29]. In this process of refinement, large-scale elimination of useless neurons and synapses takes place, whereas the useful neurons and synapses strengthen their connections. In this manner only useful networks are maintained. Microglia appear to have an important function in the refinement of the neuronal network[43]. They facilitate cell death of the useless neurons and support the survival of the useful ones.

NEURON-INITIATED CELL DEATH

According to the neurotrophic theory, cell death in the developing brain is the result of 'competition for trophic factors'[29,44]. The presynaptic neurons (input neurons) are competing with each other for trophic factors secreted by the postsynaptic neurons (target neurons). The neurons that receive enough factors will be prevented from cell death, whereas the neurons that receive an insufficient amount of factors will undergo programmed cell death (apoptosis). In this manner a proper match of presynaptic and postsynaptic neurons is achieved. Microglia are often colocalized with dying neurons, suggesting that they have a supporting role in the regulation of neuronal numbers[45,46]. They appear to do so by both secreting neurotrophic factors[47] and facilitating cell death[48]. An example for their induction of neuronal survival is the secretion of IGF1. An *in vivo* study showed that IGF1 secreted by microglia is important for the survival of layer V neurons[47]. Blocking of IGF1 signaling significantly increased the apoptosis of neurons in mice.

Interesting are the neurotrophins NGF, BDNF and NT-3, because they have a regulating function in both survival and death of neurons[44]. Whether the neurotrophins induce neuronal survival or death depends on the receptors on the neuronal membrane. There are two classes of receptors on which neurotrophins can act: the tyrosin kinase receptors (Trk) and neurotrophin receptor p75 (p75NTR)[49]. p75NTR can be stimulated by NGF, BDNF and NT-3 and their precursors proNGF, proBDNF and proNT-3. The proneurotrophins have a higher affinity to p75NTR than the neurotrophins. Stimulation of p75NTR induces apoptosis by triggering the intrinsic pathway for apoptosis. Among the Trk receptors are TrkA, TrkB and TrkC. Their activation will lead to inhibition of apoptosis and thus to survival of the neuron. TrkA, activated by NGF, and TrkC, activated by NT-3, are so called dependence receptors. This means that they induce apoptosis in absence of their ligand. They do so by colocalizing with p75NTR and cut an intracellular death domain off p75NTR. This death domain then executes its apoptotic function by triggering the intrinsic apoptotic pathway. When NGF and NT-3 are bound TrkA and TrkC respectively, they inhibit this effector function and thus inhibit apoptosis[50]. TrkB is no dependence receptor, which means that it induces cell survival when bound to its ligand[44]. When TrkB is not bound, there is no effect. Summarizing, neuronal survival can be obtained by stimulating the Trk family and neuronal death can be obtained by either upregulating p75NTR or the absence of neurotrophins.

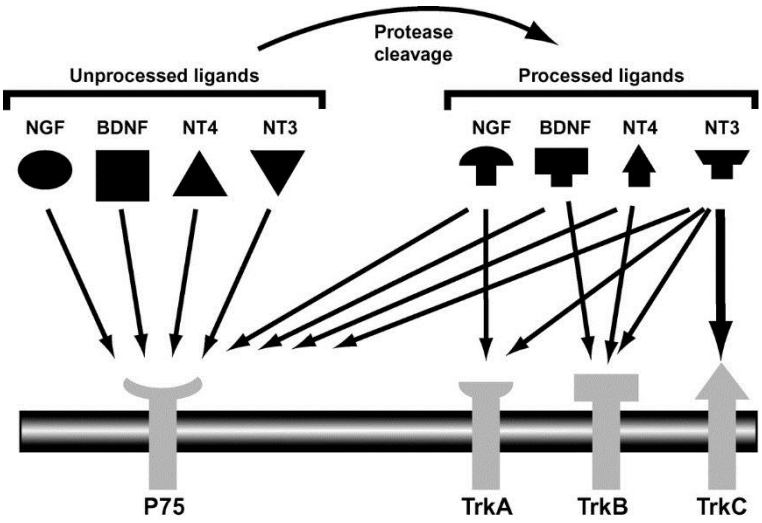

FIGURE 3. Neurotrophins and their target receptors. Both unprocessed proneurotrophins and processed neurotrophins can induce apoptosis of neurons by activating neurotrophin receptor P75. Neurotrophins however, have a lower affinity to p75 than proneurotrophins. Neurotrophins also induce neuronal survival by activating Trk receptors. NGF binds to TrkA, and BDNF and NT4 bind to TrkB. NT3 binds preferably to TrkC, but can activate all Trks. (Adapted from Segal et al., 2003)

This process occurs mainly autonomously by neurons. Once apoptosis is put in motion, they secrete 'find me' signals, such as LPC, CX3CL1 and ATP/UTP to recruit microglia (table 1)[51]. An important chemokine in the recruitment of microglia is fractalkine (CX3CL1), because microglia are the only cells in the central nervous system that express the corresponding receptor CX3CR1[52]. After activation of this receptor, microglia will become activated and migrate towards the source of fractalkine. The apoptotic neuron also expresses 'eat me' signals, such as phosphatidylserine (PS) and TREM2 ligands[53,54]. These signals are the trigger for microglia to phagocytose the apoptotic neuron. Depending on the 'eat me' signal, inflammatory or non-inflammatory phagocytosis occurs[55]. The inflammatory response is initiated by PS, on which the complement factor C3bi is deposited[54]. C3bi is recognized by CD11b on microglia, followed by secretion of reactive oxygen species (ROS)[56]. The damaged neuron will then be phagocytized by microglia. The non-inflammatory phagocytosis is initiated by TREM2 ligands[6]. They stimulate the receptor TREM2 on microglia, which phosphorylates DAP12. DAP12 induces cytoskeleton reorganization and phagocytosis.

	Ligand	**Receptor**	**Microglial response**
'Find me' signals	CX3CL1	CX3CR1	Migration to the source of ligand
	LPC	G2A	Migration to the source of ligand
	ATP/UTP	P2Y12 purinergic receptor	Migration to the source of ligand
'Eat me' signals	PS/C3bi	CD11b	C3bi bound to phosphatidylserine on neurons initiates secretion of reactive oxygen species by microglia
	TREM2 ligands	TREM2/DAP12	Non-inflammatory phagocytosis

TABLE 1. 'Find me' and 'eat me' signals expressed by neurons and the microglial response after the corresponding receptor is activated.

MICROGLIA-INITIATED CELL DEATH

Recent years more evidence showed that microglia not only clear the debris of apoptotic cells, but also that they may be able to actively induce the developmental death of neurons. Microglia seem to mediate the process of apoptosis by activating p75NTR with proNGF, the precursor for NGF[48,57]. In the developing chick retina, it was found that in the absence of microglial proNGF, the developmental neuronal cell death was significantly reduced[48]. Similar results were found in the rat retina[57]. It is known that the precursors of neurotrophins, proneurotrophins, preferentially bind to p75NTR, whereas mature neurotrophins activate Trk receptors[58]. This may explain the apoptotic effects of proNGF and the trophic effects of the neurotrophins.

Microglia are also able to eliminate viable (non-apoptotic) neurons by phagocytosis. As shown in various studies, microglia can actively phagocytose viable neural cells in the adult brain[33,59,60]. The same seems to happen in the developing brain. Peri and Nüsslein-Volhard (2008) have demonstrated that phagocytosis of neurons takes place in the developing brain of vertebrates[45]. No apoptotic cell debris was found outside the microglia, suggesting that the neurons were not damaged, but viable or dying. In another study, it was found that microglia induce cell death of Purkinje cells in the postnatal murine cerebellum by releasing superoxide ions and engulfing them[56]. Neuronal cell death was also observed in the postnatal murine hippocampus after a secretion of superoxide ions by microglia. This was a result of activation of the receptors CD11b (PS-initiated) and DAP12 (TREM2 ligand-initiated) on microglia[61]. Blocking of CD11b led to decreased neuronal cell death, indicating that the release of superoxide ions is indeed responsible for this. Additionally, DAP12 is known to be involved in the non-inflammatory phagocytosis of neurons[6]. Although this shows that microglia may be able to actively induce cell death, there is not enough evidence to conclude this with certainty.

SYNAPTIC REFINEMENT

The abundant synapses, which were formed when an axon reached its target area, are not all correct[29]. A process of rearrangement takes place to eliminate the improper synapses and strengthen the correct ones. This rearrangement is activity-dependent, which means that the synapses with a higher activity are preserved, whereas the synapses with a lower activity are eliminated (synaptic pruning). The axon of the low-activity synapse will then be partially degraded (axonal pruning). Two simple rules describe whether a synapse is useful or has to be eliminated[29]:

1. *Neurons that fire together, wire together.* If the presynaptic axon is active at the same time that the postsynaptic dendrite is strongly activated by other inputs, this synapse will be strengthened.
2. *Neurons that fire out of sync, lose their link.* If the presynaptic axon is active while the postsynaptic dendrite is weakly activated by other inputs, the synapse will be weakened and eventually disappear. Also, an inactive axon does not signal at all, so this connection is eliminated as well.

SYNAPTIC REMODELING

As an example for synaptic remodeling, I will describe this on the basis of glutamate signaling. Glutamate, which is secreted by the presynaptic axon after an action potential, has two postsynaptic target receptors; NMDA (calcium channel) and AMPA (sodium channel). When AMPA is activated by glutamate, sodium influx induces an action potential in the postsynaptic neuron. When NMDA is activated by glutamate, an influx of calcium takes place, which results in the expression of more AMPA on the postsynaptic membrane ('AMPAfication'). The more glutamate released, the more AMPA is expressed on the postsynaptic membrane, and the stronger is the signaling. Therefore, the increase of AMPA is part of the postsynaptic strengthening of the connection.[29]

The NMDA receptor however, is not always active. When the postsynaptic neuron is not (or weakly) activated by other inputs, the channel is blocked by a magnesium ion (Mg^{2+}) (figure 4). Activation by glutamate then does not result in calcium influx and there is no further intracellular signaling. This is the case when neurons fire out of sync. However, when the postsynaptic neuron is activated by other inputs, the NMDA receptor is unblocked and activation by glutamate induces a calcium influx, which leads to strengthening of the synapse. This is the case when the two neurons are synchronized.[29]

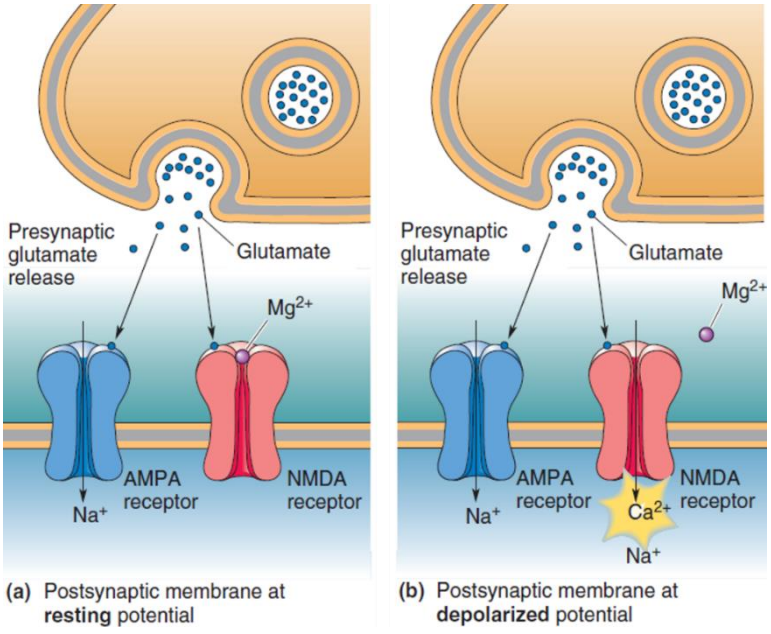

FIGURE 4. Synaptic signaling of glutamate. **(a)** The NMDA receptor on the inactive dendrite is blocked by Mg^{2+}, inhibiting the Ca^{2+} influx. This synapse will not be strengthened. **(b)** The NMDA receptor on the active dendrite is not blocked by Mg^{2+}, allowing the Ca^{2+} influx. This synapse will be strengthened. (Adapted from Bear et al., 2007)

But how does the synchronized neurons strengthen their connection and how are the synapses of unsynchronized neurons eliminated? Prior studies suggested a role of neurotrophins in the strengthening and elimination of neurons[62–64]. Neuronal activity would stimulate the expression of NGF and BDNF. Activation of the unblocked NMDA in synchronized synapses leads to a higher amount of neurotrophins released by the postsynaptic neuron. In unsynchonized synapses, NMDA is inactive, which leads to little or no secretion of neurotorphins.

Recall that neurotrophin signaling inhibits the apoptotic pathway in neurons and thus stimulates survival[44,50]. The absence of neurotrophins however, stimulates the apoptotic pathway. There is also evidence that BDNF is involved with inducing the apoptotic pathway by p75NTR activation[65,66]. In more detail, BDNF-Trk signaling would be (partially) responsive for synaptic strengthening, whereas proBDNF-p75NTR would lead to synaptic pruning[67–69]. The induced apoptotic pathway in the axon eventually leads to degeneration of the axon[70]. Note that this does not have to be the whole axon, but the undesired branches can be individually eliminated[71].

Microglia and synaptic pruning

Microglia appear to have a crucial function in the clearance of unwanted synapses[72–74]. In a resting state, they make once per hour direct contacts of about five minutes with neuronal synapses[72]. The frequency of these contacts is activity-dependent; a reduced neuronal activity is associated with a reduction of the frequency. After ischemia the contacts last longer (about 1h) and are followed by disappearance of the synapse they are in contact with. This suggests that microglia induce the elimination of synapses. Paolicelli et al. (2011) showed that microglia actively engulf synapses in the postnatal development of mice brains[73]. Another study showed that a quarter of the dendritic spines in the juvenile visual cortex disappear after contact with microglia[74]. Deprivation of visual stimuli caused an increase in contact of microglia and dendritic spines, suggesting that a decrease of neuronal activity results in increased elimination of dendritic spines.

The mechanisms of the elimination of synapses by microglia are yet not well understood. In the earlier mentioned study of Paolicelli et al. (2011), a possible role of fractalkine signaling in this process was found[73]. A knock-out of CX3CR1 in mice resulted in a decrease of microglial density and a higher density of dendritic spines in the hippocampus CA1 region. Although there is not much evidence, there are indications that fractalkine is secreted by the synapse that has to be eliminated to recruit microglia[73].

Another molecular pathway that is possibly involved in developmental synaptic pruning is the classical complement cascade[75–77]. Components of this cascade, C1q and C3 that are localized at synapses, initiate synaptic pruning in the postnatal visual system[75]. This complement cascade is part of the innate immune system, where they ‘tag’ cellular material that has to be eliminated by binding to it[78]. The ‘tag’ is then being recognized by phagocytes, which engulf the tagged material. It is possible that C1q and C3 bind synapses to initiate elimination by microglia, the only cells in the brain that express the corresponding receptor, C3R (CD11b)[77]. Further research showed that C3 and C3R knockout mice resulted in reduced engulfment of retinal axons and interrupting in CR3/C3 signaling resulted in an excess synapses into adulthood[75,77].

Summarizing, synapses that are out of sync or not active enough, locally induce apoptosis in the corresponding branches of the axons. These branches will then express signals to recruit microglia that eventually phagocytose the axon.

CONCLUSION AND FUTURE PERSPECTIVES

Findings in recent years have led to a better understanding of microglial functions in different stages of the development of the brain. Knowledge about the ontogeny of microglia helps in understanding their behavior in the healthy (developing) brain. Besides, new findings provide insight on the interactions between neurons and microglia during the patterning, wiring and refinement of the brain. This paper showed an overview of important findings regarding these processes. Contact-dependent communication between microglia and neurons as well as communication through soluble molecules are important in the development of the brain. This communication is crucial during the genesis of new neurons, axonal outgrowth and formation of new synapses. Just as important is the role microglia in the refinement of synapses and neurons.

Remarkable is that we have seen that several processes of the brain development share the same underlying mechanism. In processes where phagocytosis was involved, such as elimination of neurons and synapses, CX3CL1-CX3CR1 and C3b signaling were used. Neurotrophins were also used in multiple processes, such as axonal outgrowth, neuronal survival/apoptosis and strengthening/elimination of synapses and axons. This shows that microglia and neurons use the same mechanisms for different purposes.

Interesting is the role of neurotrophins and the apoptotic pathway in the construction of the neuronal circuit. We have seen that the absence of neurotrophins in the synapse leads to local apoptosis followed by synapse elimination and partial axon degeneration by phagocytosis[70]. We have also seen that the absence of neurotrophins can lead to apoptosis of the complete neuron. In both situations the apoptotic pathway is involved. Dekkers et al. (2013) postulated that the induction of the apoptotic pathway in synapses may eventually lead to apoptosis on cellular level (figure 5)[44]. For example, induction of the apoptotic pathway in synapses due to deprivation of neurotrophins, may induce retrograde pro-apoptotic signaling to the soma of the neuron. Also, insufficient activation of dendrites induces pro-apoptotic signals[44]. Conversely, retrograde signaling of neurotrophin receptors due to the presence of neurotrophic factors may counteract the pro-apoptotic signals. Balancing of pro-survival and death-inducing signals from the different synapses of the neuron, will eventually determine whether a neuron dies or survives. These findings are important for understanding of the underlying signaling of neuron-microglia communication in the refinement of the brain.

Knowledge of the function of microglia in the neural development may also be beneficial for understanding certain neurodevelopmental disorders. Obsessive compulsive disorder and Rett syndrome are known to be associated with microglial dysfunction[79,80]. Compulsive grooming behavior in mice is linked to the *hoxb8* gene, which is required for normal grooming behavior[81]. Homozygous loss of function mutation in *hoxb8* causes compulsive grooming behavior. Besides, Hoxb8 deficiency decreases the number of microglia in the resting state by 15%[80]. This is the result of a reduced number of microglia derived from a $Hoxb8^{+}$ cell lineage. Possibly, knowledge of the exact role of microglia in the development of the brain can lead to better understanding of various neurodevelopmental disorders.

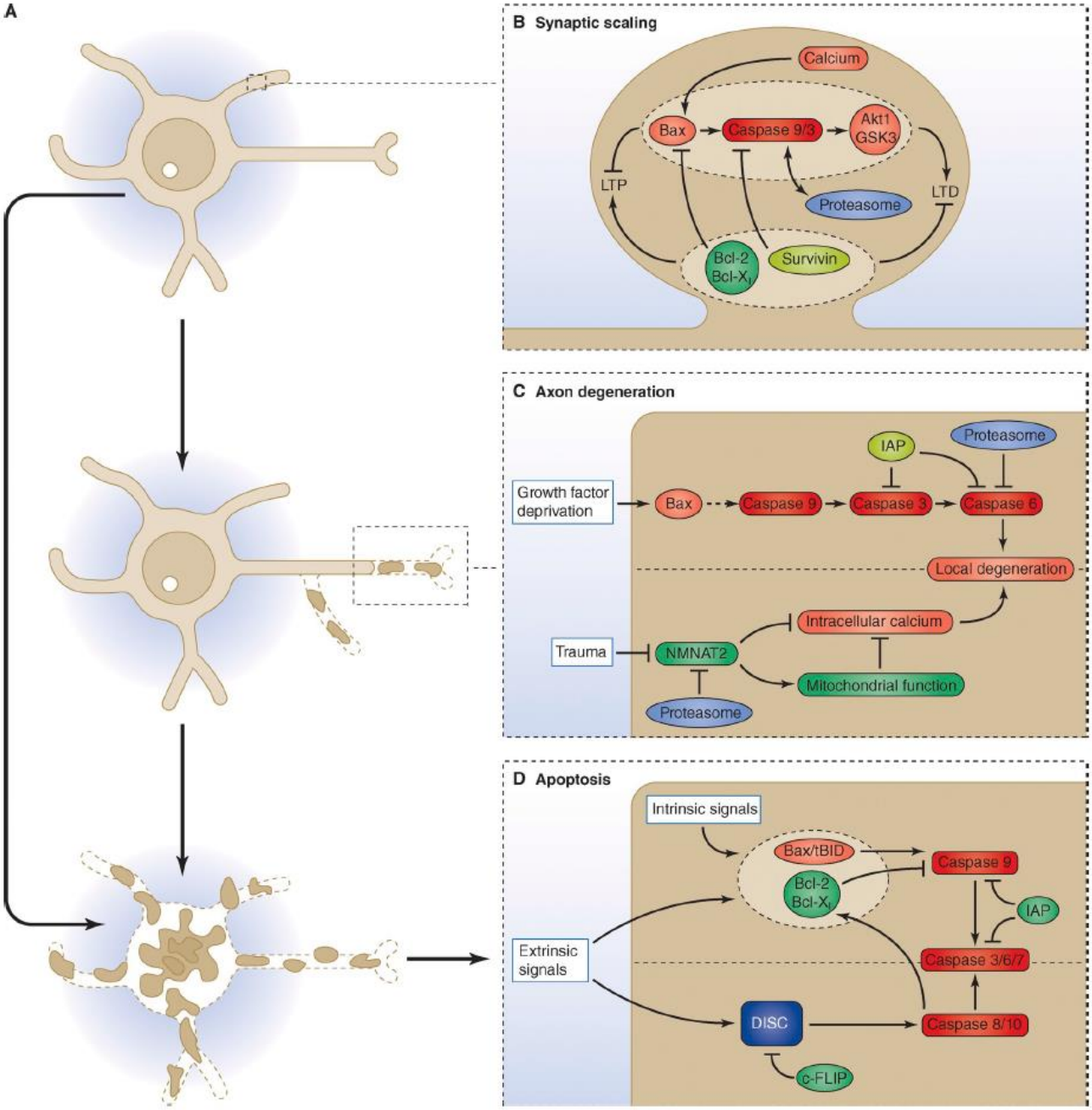

FIGURE 5. The involvement of apoptotic pathways in different processes in the brain development. **(A)** Induction of the apoptotic pathway in synapses on dendrites or axons can eventually lead to apoptosis of the whole neuron. **(B)** The apoptotic pathway is in spines involved in strengthening (long term potentiation [LTP]) and weakening (long term depression [LDP]) of synapses. **(C)** Growth factor deprivation and trauma result in induction of the apoptotic pathway, which causes axon degeneration. **(D)** Intrinsic and extrinsic signals induce the apoptotic pathway, which results in apoptosis of the entire neuron. Intrinsic signals can be signals from the soma as well as from dendrites and axons. (Adapted from Dekkers et al., 2013)

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