Review

Neuroplasticity in stroke recovery. The role of microglia in engaging and modifying synapses and networks

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Abstract

Neuroplasticity after ischemic injury involves both spontaneous rewiring of neural networks and circuits as well as functional responses in neurogenic niches. These events involve complex interactions with activated microglia, which evolve in a dynamic manner over time. Although the exact mechanisms underlying these interactions remain poorly understood, increasing experimental evidence suggests a determining role of pro- and anti-inflammatory microglial activation profiles in shaping both synaptogenesis and neurogenesis. While the inflammatory response of microglia was thought to be detrimental, a more complex profile of the role of microglia in tissue remodeling is emerging. Experimental evidence suggests that microglia in response to injury can rapidly modify neuronal activity and modulate synaptic function, as well as be beneficial for the proliferation and integration of neural progenitor cells (NPCs) from endogenous neurogenic niches into functional networks thereby supporting stroke recovery. The manner in which microglia contribute towards sculpting neural synapses and networks, both in terms of activity-dependent and homeostatic plasticity, suggests that microglia-mediated pro- and/or anti-inflammatory activity may significantly contribute towards spontaneous neuronal plasticity after ischemic lesions. In this review, we first

introduce some of the key cellular and molecular mechanisms underlying neuroplasticity in stroke and then proceed to discusses the crosstalk between microglia and endogenous neuroplasticity in response to brain ischemia with special focus on the engagement of synapses and neural networks and their implications for grey matter integrity and function in stroke repair.

Introduction

Ischemic stroke accounts for 87 % of all stroke incidents (Mozaffarian *et al.*, 2016) and is caused by embolic or thrombotic obstruction of blood supply to the brain, which triggers a complex molecular cascade including slowed cellular energy metabolism, cell membrane depolarization, excitotoxicity, reactive oxygen species (ROS) production, a complex inflammatory response by activated microglia, and disruption of the blood–brain barrier, leading to necrotic and apoptotic cell death (Hossmann, 2006; Ahmad *et al.*, 2014). The severity of focal ischemic injury and lesion sequelae are contingent on the restoration of brain energy metabolism, and largely determined by degree of ischemia, lesion location and size and duration of vessel occlusion (Vise *et al.*, 1977; Grefkes & Ward, 2014).

Stroke patients usually demonstrate a degree of spontaneous improvement in the sub-acute phase, which can be further enhanced with appropriate prolonged rehabilitation. The observed functional outcomes appear to be consistent with partial rewiring of surviving neural networks and recruitment of intact synapses, which tends to occur contralateral, but also ipsilateral to the lesion (Cramer & Chopp, 2000; Askim *et al.*, 2009; Askim *et al.*, 2010; Harrison *et al.*, 2013; Alia *et al.*, 2017). Depending on the severity of the stroke and the type of clinical treatment and/or rehabilitation paradigm, these mechanisms may account for spontaneous functional recovery which may be observed in certain stroke patients 30-90 days post-ischemia, especially with regard to alleviation of language and cognitive impairments, but also motor impairments such as voluntary maximum arm extension (Grefkes & Ward, 2014). Furthermore, evidence from animal studies provides valuable insights into the molecular mechanisms of spontaneous recovery after stroke, including release of anti-inflammatory cytokines, angiogenesis, structural remodelling at the axonal, dendritic, and

synaptic level, changes in the extracellular matrix (ECM) as well as activation and migration of endogenous neural stem cells (Hermann & Chopp, 2012; Kazanis, 2013; Kazanis *et al.*, 2015).

Intrinsic responses, such as axonal outgrowth and synaptic plasticity, can be influenced by the regulation of extracelullar proteolytic cascades by the serine protease tissue-type plasminogen activator (tPA). Particularly useful insights as to how extracellular proteolytic activity may regulate plasticity have been derived from studies of monocular deprivation in mice during the critical developmental period (Mataga *et al.*, 2002). These studies have shown that sensory deprivation not only results in elevated tPA activity levels in both brain hemispheres in response to altered inhibitory-excitatory balance; moreover the tPA proteolytic cascade promotes downstream structural and functional remapping with restoration of ocular dominance plasticity (Mataga *et al.*, 2002). This experience-dependent plasticity during the critical period is mediated through ECM and cell adhesion protein degradation by tPA release, which in turn increases permissiveness to afferent sprouting axons providing synaptic input from the open eye to the visual cortex (Mataga *et al.*, 2002; Hensch, 2005).

In a similar manner, induced focal ischemia into the rat motor cortex has been found to increase the activity of the ECM proteases tPA and matrix metalloproteinase (MMP)-9 and to promote ECM remodelling by reducing the number of perineuronal nets (PNN) in the ipsilesional somatosensory cortex (Quattromani *et al.*, 2018). Furthermore, multisensory stimulation through environmental enrichment at the early stages after stroke was found to increase proteolytic tPA and MMP-9 activity and promote ECM remodeling and PNN degradation, resulting in improved functional outcomes suggestive of experience-dependent plasticity (Quattromani *et al.*, 2018). Analysis of post-mortem brain tissue obtained from stroke patients has revealed equivalent tPA, MMP-9, MMP-2 and PNN expression profiles in brain regions proximal but also distal to the infarct (Quattromani *et al.*, 2018).

In the last decade, increased responsiveness in terms of onset-to-treatment times, improved availability of thrombectomy and thrombolysis in combination with enhanced rehabilitation strategies, has improved the overall survival rate of stroke patients by 20% (Askim *et al.*, 2012; Askim *et al.*, 2013; Askim *et al.*, 2014; Hokstad *et al.*, 2015; Cavanaugh *et al.*, 2017; Cavanaugh & Huxlin, 2017). Despite this progress, stroke remains the leading cause of adult disability, with 50% of survivors suffering from severe sensorimotor and cognitive impairments (Modo *et al.*, 2013). As a result, ongoing research effort focuses on the development of alternative or complementary approaches.

Numerous experimental studies, including recent studies from our group, have demonstrated the potential of exogenously or endogenously derived stem cells alone, or in combination with *in situ* tissue engineering strategies and/or pharmacotherapeutics in promoting transplant-mediated repair with functional restoration through such diverse mechanisms as neuroprotection, cell replacement, remyelination, tissue/vascular remodelling and *de novo* neurogenesis (Lindvall & Kokaia, 2011; Bible *et al.*, 2012; Kokaia & Lindvall, 2012; Medberry *et al.*, 2013; Massensini *et al.*, 2015; Sandvig *et al.*, 2015; Jendelová *et al.*, 2016; Augestad *et al.*, 2017). Although highly promising and extremely valuable in elucidating relevant recovery mechanisms at the experimental level, clinical translation of such approaches tends to be confounded by significant challenges, such as allograft rejection as well as the intricacies inherent in trying to create safe, functional *in situ* biointerfaces that could effectively re-establish functional connectivity in multiple foci (Sandvig *et al.*, 2017). This is exemplified by the fact that numerous past and ongoing clinical trials of various stem cell based therapies have largely failed to promote significant and/or long-lasting functional benefits in stroke patients (Sinden *et al.*, 2017). Furthermore, translational failure can be attributed to a number of factors, including poor experimental design, evaluation of functional outcomes measures, and overestimation of efficacy as a result of unpublished negative data (Lapchak et al, 2013).

On the other hand, increasing evidence supports various forms of plasticity triggered after a stroke event and their potential contribution to recovery. As mentioned earlier, these plastic responses include rewiring of surviving neural networks and axonal ramification (Medberry *et al.*, 2013), the recruitment of intact synapses post-lesioning (Cossetti *et al.*, 2012), ECM remodeling (Quattromani *et al.*, 2018), as well as the activation of endogenous neuro/gliogenic niches, including germinal layer-derived sites but also ectopic ones, and the migration of neuronal and glial progenitors towards the injury site (Frisén, 2016). Such processes are reminiscent of the high level of plasticity observed during development, which reiterates a widely-shared view in regenerative neuroscience, i.e. the principle that regeneration in the adult mammalian central nervous system (CNS) may (partially) rely on the recapitulation of the high degree of neuroplasticity underscoring development (Curinga & Smith, 2008; Askim *et al.*, 2009; Nori *et al.*, 2017).

Interestingly, there is increasing consensus regarding a central role of microglia in regulating neuroplasticity. In this review, we discuss endogenous neuroplasticity in response to brain ischemia, with special focus on the role of microglia in engaging synapses and neural networks and their implications for stroke repair.

Microglial-neuron interactions in the uninjured brain

In the adult brain, there is an abundance of resident microglia across all brain regions. There are, however, substantial differences in microglia population densities between different brain areas: more microglia reside in the grey matter compared to white matter, while particularly large numbers can be found in the substantia nigra, basal ganglia, hippocampus and olfactory telencephalon, as opposed to the cerebellum and brainstem (Lawson *et al.*, 1990). In the uninjured brain, resident microglia are characterized by a highly branched ramified phenotype and contribute to homeostasis by scanning their microenvironment and being in apposition with synapses without disturbing neural networks (Davalos *et al.*, 2005; Hanisch & Kettenmann, 2007; Ekdahl *et al.*, 2009; Kettenmann *et al.*, 2011;

Kettenmann & Ransom, 2012). Specifically, histological evidence from experimental animal studies has shown that microglia engulf presynaptic and postsynaptic elements and that their processes display a high level of motility, alternately contacting and retracting from pre- and postsynaptic terminals (Wake *et al.*, 2009; Tremblay *et al.*, 2012; Eyo & Wu, 2013; Kettenmann *et al.*, 2013). The exact mechanisms underlying these interactions under brain homeostasis remain largely unknown (Kettenmann *et al.*, 2013). However, recent studies of monocular deprivation in mice have provided new insights by identifying a critical role for mature microglia in experience-dependent visual cortex plasticity *via* P2Y12 signalling, a purinergic receptor exclusively expressed by resident brain microglia (Sipe *et al.*, 2016). Specifically, while P2Y12 was shown to mediate rapid recruitment of hyper-ramified microglia after sensory deprivation, followed by interactions with synaptic elements and phagocytic activity, disruption of P2Y12 signalling severely impacted experience-dependent plasticity after monocular deprivation (Sipe *et al.*, 2016).

Furthermore, highly branched microglia with radial processes can be found in the neuropil, while along axon tracts, microglia processes may be extended and aligned parallel or perpendicular to axon fibers (Lawson *et al.*, 1990; Lawson *et al.*, 1992). The striking differences in the distribution and phenotypic characteristics of resident microglia in the brain indicate high sensitivity to microenvironmental inputs and, likely, distinct functions in the maintenance of brain homeostasis. A recent study provided evidence of two functionally and mechanistically different forms of microglia motility in brain surveillance and response to homeostatic disturbance (Madry *et al.*, 2018); (i) maintainenance of microglial ramification and surveillance are crucially dependent on the tonic activity of the two-pore domain K^+ channel THIK-1, irrespective of P2Y12 activity; and (ii) directed microglia motility depends on purinergic receptor P2Y12 activity, but is independent of THIK-1 activity (Madry *et al.*, 2018). With regard to phenotypic diversity and how it may be coupled to distinct microglial functions, extensive genomic, microRNA and quantitative proteomic analyses have identified a unique molecular signature of murine and human microglia, distinguishing them

from neurons, astrocytes and oligodendrocytes, as well as monocytes recruited to the CNS (Butovsky *et al.*, 2014). The expression of the associated 106 unique genes was found to be regulated by transforming growth factor beta (TGF-β) activity (Butovsky *et al.*, 2014). Furthermore, a separate study applied direct RNA sequencing and identified 100 genes, uniquely expressed by microglia in healthy adult and aged mice, which can determine microglial sensitivity to changes in the brain microenvironment (Hickman *et al.*, 2013). This suggested that microglia possess a sensome, which enables them to rapidly respond to environmental changes (Hickman *et al.*, 2013).

Experimental evidence strongly supports the view that microglial arborizations correlate with both increased growth as well as elimination of dendritic spines, but also high responsiveness to neuronal activity (Tremblay *et al.*, 2010; Tremblay *et al.*, 2012). For example, ATP released by neurons during neurotransmission has been shown to enhance surveillance by microglial processes (Wake *et al.*, 2009; Fontainhas *et al.*, 2011; Li *et al.*, 2012; Dissing-Olesen *et al.*, 2014; Eyo *et al.*, 2014) and to stimulate microglial process outgrowth (Sipe *et al.*, 2016). The latter however, may be predominantly/selectively controlled by glutamatergic AMPA, NMDA, and kainate receptor activation on microglia processes (Dissing-Olesen *et al.*, 2014; Eyo *et al.*, 2014). Furthermore, it has been shown that tumor necrosis factor α (TNF-α) mediates synaptic scaling, i.e. a feedback mechanism whereby consistent electrical output is facilitated by scaling of synaptic strength through the insertion or deletion of postsynaptic AMPA receptor (Stellwagen & Malenka, 2006; Wu *et al.*, 2015). This results in microglial process outgrowth or retraction from synapses, a response that may differ markedly between different brain regions, as for example process outgrowth in the hippocampus and cortex *versus* retraction in the striatum (Li *et al.*, 2012; Wu *et al.*, 2015). It follows that ATP-mediated communication between resident microglia and neurons may be region specific. This communication is characterized by the reciprocal nature of microglia-neuron interactions (Eyo & Wu, 2013), effectively establishing a feedback loop that regulates neuronal activity. This supports

the view that surveillance of the brain by resting state microglia is a highly active process. However, its exact implications on synaptic function and role in plasticity remain to be elucidated.

Microglial responses to injury

Following pathological alterations in the brain, such as ischemic insult, resident microglia respond to changes in neuronal integrity, synaptic inputs and activity by rapidly switching morphology and migrating towards the injury (Pocock & Kettenmann, 2007; Kettenmann & Ransom, 2012; Morrison & Filosa, 2013). Specific interactions between microglia and neurons are shaped by an intricate dynamic pattern of environmental cues, resulting in transient changes in the exact nature of microglial activity and morphology, which can influence the evolution of stroke lesion pathology in the acute, sub-acute, and chronic stages in a positive or detrimental manner (Hanisch & Kettenmann, 2007; Ekdahl *et al.*, 2009; Morrison & Filosa, 2013; Zhao *et al.*, 2017).

Activated microglia tend to be considered antigenically indistinguishable from recruited macrophages, based on immunostaining with antibodies against epitopes shared by both populations (Perego *et al.*, 2011; Patel *et al.*, 2013). However, as mentioned previously, the purinergic receptor P2Y12 is exclusively expressed in microglia (Sipe *et al.*, 2016) and can be used a microglial-specific marker to distinguish them from macrophages (Hickman *et al.*, 2013; Butovsky *et al.*, 2014; Zarruk *et al.*, 2018). Furthermore, following ischemic injury, the protein and mRNA expression profiles of inflammatory cytokines and chemokines by microglia and macrophages are markedly different (Zarruk *et al.*, 2018).

Microglia polarization in response to injury includes what has been traditionally described as the pro-inflammatory M1 (classic) and anti-inflammatory M2 (alternative) phenotype (Hu *et al.*, 2012). Notwithstanding the extensive application of the specific nomenclature in the literature, and subsequently in this review, it should be acknowledged that the classification of activated microglia

into M1 and M2 phenotypes must be reconsidered. This designation may adequately describe the shared *in vitro* phenotypes of activated microglia and macrophages (Porta *et al.*, 2009), however, it falls short of delineating the distinct role of microglia in the brain or adequately capturing the highly complex profile of microglial activation *in vivo*. Thus, rather than representing absolute states of bivalent microglia activation following disturbance to brain homeostasis,, M1 and M2 must be considered as opposite ends on a broad spectrum of morphological and functional microglia phenotypes and distribution along complex molecular gradients and temporospatial scales (Perego *et al.*, 2011; Lampron *et al.*, 2013; Fumagalli *et al.*, 2015).

Induction of M1 polarization involves a host of different signaling pathways including interferon γ (IFNγ)-mediated activation of signal transducer and activator of transcription 1 (STAT1) factor, which in turn triggers ROS and nitric oxide (NO) production and the secretion of pro-inflammatory cytokines, including TNF-α, interleukin-1β (IL-1β), and IL-12 (Nathan *et al.*, 1983; Zhao *et al.*, 2017). Furthermore, Toll-like receptor 4 (TLR4) activity can also induce M1 polarization through the formation of an activation complex, which includes members of the interferon regulatory factor family (IRF), which regulate inducible nitric oxide synthase (iNOS) secretion and major histocompatibility complex (MHC) II activity (Escalante *et al.*, 1998; Lawrence & Natoli, 2011; Zhao *et al.*, 2017).

On the other hand, M2 polarization is mediated by exposure to IL-4 and IL-10 or IL-13 (Wang *et al.*, 2014) and involves the upregulation of factors such as non-TLR pattern recognition receptor Dectin-1, dendritic cell-specific ICAM-grabbing non-integrin **(**DC-SIGN), mannose receptor, and scavenger receptors A and B (Martinez *et al.*, 2009). M2 microglia have a distinct function from M1 microglia and can ameliorate tissue damage through secretion of neuroprotective factors, including glial cell-line derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) (Neumann *et al.*, 2006; Nakajima *et al.*, 2008), as well as anti-inflammatory cytokines, such as IL-10 and TGF-

β (Gordon, 2003; Iadecola & Anrather, 2011; Grailer *et al.*, 2014). For example, it has been shown that genetic depletion of BDNF from microglia downregulates experience-dependent synaptic structural plasticity, an outcome that recapitulates the effects observed after complete microglia depletion (Parkhurst *et al.*, 2013).

Activated microglia infiltrating the lesion site, together with other components such as reactive astrocytes, and ECM molecules, especially chondroitin sulfate proteoglycans (CSPGs) contribute to the formation of the glial scar (Sandvig *et al.*, 2004; Fitch & Silver, 2008; Moeendarbary *et al.*, 2017). For example, M2 type microglia promote ECM deposition and also prevent its degradation by inducing the secretion of FIZZ 1 protein and the heparin-binding lectin Ym1, respectively (Pepe *et al.*, 2014; Arcuri *et al.*, 2017). Although the glial scar constitutes a chemical and physical barrier inhibiting axonal outgrowth, it has a critical role in limiting damage during the acute phase after injury by sealing the lesion site, restoring homeostasis, preserving spared tissue, and regulating immune responses (Rolls *et al.*, 2009; Martino *et al.*, 2011). On the other hand, as discussed earlier, ECM remodeling can promote gain of function through experience-dependent plasticity (Mataga *et al.*, 2002; Quattromani *et al.*, 2018). Furthermore, selective degradation of CSPG components of the ECM, as for example, through enzymatic digestion of PNN with chondroitinase ABC administration, can significantly promote restoration of locomotor function (Gherardini *et al.*, 2015). Interestingly ECM remodelling seems to recapitulate the increased neuroplasticity observed during the critical developmental period; closure of the critical period coincides with ECM maturity and PNN formation, while PNN removal reactivates experience-dependent plasticity (Pizzorusso *et al.*, 2002; Hensch, 2005). Activated microglia constitute an integral part of such dynamic responses to brain injury and, following ischemic stroke, they may thus determine whether endogenous plastic responses become adaptive or maladaptive in nature.

Microglia and synaptic remodeling – lessons from development

A highly relevant perspective is the role of microglia during CNS development, especially with a view to synaptic remodeling. The first microglia that appear during embryogenesis are derived from myeloid progenitors and are the only glial cells of the CNS at this stage, preceding both astrogliogenesis and oligodendrogliogenesis (Ginhoux *et al.*, 2010; Kettenmann *et al.*, 2013). Thus, in the absence of astrocytes, microglia play an active role in early embryonic synaptogenesis, while during later stages of pre- and postnatal development, microglia become a key regulator of synaptic pruning (Paolicelli *et al.*, 2011; Kettenmann *et al.*, 2013; Wu *et al.*, 2015) and, in this way, promote neural circuit refinement. CX_3 chemokine ligand 1 (CX_3CL1), also known as fractalkine, can be either secreted or membrane bound and its receptor, CX_3CR1, is exclusively expressed by CNS microglia (Harrison *et al.*, 1998; Jung *et al.*, 2000). Although the exact mechanism by which microglia promote synaptic maturation is not known, it is likely that CX_3CL1 secreted by neurons provides a cue that attracts microglia and binds CX_3CL1 through CX_3CR1 (Harrison *et al.*, 1998; Lauro *et al.*, 2015). It has been reported that deficits in microglial function that affect CX_3CL1/CX_3CR1 binding can lead to developmental disorders or induce neurotoxicity (Cardona *et al.*, 2006; D'Haese *et al.*, 2010; van Meer *et al.*, 2012; Yu & Koretsky, 2014). On the other hand, a recent study in CX_3CR1 knockout mice showed that early or late forms of activity-dependent plasticity in the visual cortex were independent of fraktalkine signaling (Lowery *et al.*, 2017). This suggests that rather than being a universal regulator of synaptic plasticity, fraktalkine may be selectively regulating such process in a region and/or age related manner (Lowery *et al.*, 2017). Moreover, while microglia elimination by selective CX3CR1 gene targeting resulted in severe functional deficits (Parkhurst *et al.*, 2013), elimination of microglia though inhibition of colony-stimulating factor 1 receptor (CSFR1) signaling did not induce any functional or cognitive deficits (Elmore *et al.*, 2014).

Apart from the above, the classical complement cascade is another set of molecules implicated in synaptic remodeling during development (Wu *et al.*, 2015). In the postnatal brain, resident microglia express complement receptor 3 (CR3), which binds to C3 protein localized, along with C1q, in a

subset of immature synapses. The upstream regulator of C1q, is TGF-β (Stevens *et al.*, 2007; Bialas & Stevens, 2013). It has been proposed that C1q serves as a tag for synapses that need to be eliminated (Stevens *et al.*, 2007; Kettenmann *et al.*, 2013). Thus, expression of complement proteins at developing synapses is crucial for their elimination through engulfment by phagocytic microglia. This process largely depends on neuronal activity, as microglia preferentially phagocytose less active presynaptic inputs (Wu *et al.*, 2015). The contribution of microglia in sculpting neural circuits in the postnatal brain is both activity dependent and complement dependent, however the latter only occurs in the absence of neuroinflammation (Schafer *et al.*, 2012; Wu *et al.*, 2015).

Neural network plasticity in response to cerebral ischemia

Neural networks and circuits are highly interconnected, thus damage induced by a brain injury such as focal cerebral ischemia will inadvertently affect loci distal to the original injury site (Pizzorusso *et al.*, 2006). This effect, originally described as diaschisis (Von Monakow, 1969), explains how neuronal loss in one brain region affects neuronal excitability and impairs function in remote brain regions (Xerri *et al.*, 2014). This process is characterized by evolution of the related pathology and dissipation of the injury in the brain in a spatiotemporal manner. Conversely, diaschisis may explain the manner in which intra- and interhemispheric neural network remodeling occurs in response to injury, constituting evidence of spontaneous or experience-dependent plasticity (Xerri *et al.*, 2014). As a result, gain of function could be attributable to the partial restoration of neural networks previously involved in impaired function or alternatively, to a compensatory or substitution mechanism, which involves recruitment of other networks (Ogden & Franz, 1917; Stein, 2012; Xerri *et al.*, 2014).

Apart from clinical findings, much of the evidence of network remodeling in response to brain ischemia is derived from animal models by application of a range of molecular, neuroanatomical, neurophysiological, and neuroimaging techniques, including functional magnetic resonance imaging

(fMRI), which have provided valuable insights into morphology-activity relationships and dynamic changes at the network and synaptic level (Rossini & Dal Forno, 2004; Okabe *et al.*, 2016).

One such example is the manner in which ischemic injury affecting the primary motor cortex and/or somatosensory cortex results in early reorganization of topographic representations of spared neural circuits within the perilesional area, characterized by a redistribution of neuronal receptive fields (Reinecke *et al.*, 1999; Brown *et al.*, 2010; Fujioka *et al.*, 2010; Xerri *et al.*, 2014). Furthermore, this unmasking of latent synaptic inputs is related to reorganization of thalamocortical networks and involves inputs that inhibit or promote cortical neuron excitability (Ferezou *et al.*, 2007; Xerri *et al.*, 2014). Relevant experimental findings showed that despite the fact that the emerging neural networks were characterized by loss of selectivity, i.e. they processed information from multiple limbs instead of a single contralateral limb, experience-dependent plasticity through learning of specific motor tasks significantly contributed to functional recovery at the chronic stage post-ischemia (Xerri *et al.*, 2014).

Changes in excitatory and inhibitory neurotransmission within the ischemic core and perilesional area are consistent with the evolution of ischemic pathology over time and can thus influence perilesional remapping acutely but also at the chronic stage after ischemic stroke. For example, experimental evidence suggests that hyperexcitability in the sensorimotor cortex proximal to the lesion is observed acutely after brain ischemia, peaking at 7 days post-lesioning, and thereafter gradually declines over several months (Schmidt *et al.*, 2012). This effect has been attributed to a downregulation of GABAergic inhibition mediated by a transient reduction in the binding density of $GABA_A$ receptor and/or $GABA_A$ receptor subunits at the infarct core and surrounding tissue (Schiene *et al.*, 1996; Qü *et al.*, 1998; Que *et al.*, 1999b; Xerri *et al.*, 2014). Microglia can confer neuroprotection in the injured adult brain by stripping inhibitory GABAergic synapses from neuronal somata, which results in synchronized firing of excitatory NMDARs in the γ- frequency band, with subsequent elevation of intracellular Ca^{2+} levels and increased transcription of neuroprotective genes *via* CREB phosphorylation (Chen *et al.*, 2014).

Similarly, a reduction in the binding density of NMDA, AMPA and kainate receptors was observed after ischemic injury affecting the somatosensory cortex, which peaked at 30 days post-lesioning, at which stage an upregulation of NMDA receptor binding was observed in the perilesional area (Que *et al.*, 1999a; Xerri *et al.*, 2014). Furthermore, the relevant findings suggest that alterations of inhibitory and excitatory inputs at the core and perilesional area, especially during the first week after injury, occur in an opposite manner. Moreover, in tandem with perilesional remapping, morphological changes can also be observed with increased dendritic spine turnover as well as plastic changes in dendrite arborization. Interestingly, this form of plasticity was found to be more pronounced in cortical regions distal to the infarct area, compared to the perilesional area (Brown *et al.*, 2010; Mostany *et al.*, 2010; Xerri *et al.*, 2014).

The fact that microglia contribute towards sculpting neural synapses and networks, both in an activity- and complement dependent manner, suggests that microglia-mediated pro- or anti-inflammatory activity may significantly contribute towards spontaneous neuronal plasticity after ischemic lesion. One such example is evidence from experimental studies supporting that microglial CR3 activation by inflammatory stimulus can trigger long-term synaptic depression (LTD) in surrounding neurons *via* NADPH oxidase, one of the main mediators of neurotoxicity in stroke (Zhang *et al.*, 2014). Specifically, superoxide production from NADPH oxidase induced LTD *via* activation of PP2A and GluA2-mediated AMPAR internalization (Zhang *et al.*, 2014). This indicates that an enhanced neuroinflammatory response in microglia can rapidly modify neuronal activity and modulate synaptic function. Furthermore, another study revealed that activity-dependent connections between microglia and synapses are markedly prolonged after cerebral ischemia, with contact between microglial processes and neuronal synapses maintained for ~1 h, compared to ~5 min in the uninjured brain, and subsequent disappearance of the presynaptic bouton and synaptic elimination (Wake *et al.*, 2009).

Further elucidation of such mechanisms may provide important insights as to the manner in which compromised synaptic efficiency may result in functional deficits after stroke. Additionally, such insights may clarify the potential role of microglia in detecting the functional state of affected

synapses and attempting to preserve function or eliminate them (Wake *et al.*, 2009). Conversely, selective targeting of such responses, as for example by AMPA receptor removal (Collingridge & Peineau, 2014), may determine whether homeostatic plasticity can be maintained by sustained synaptic input or whether synaptic strength can be redistributed in a manner that favors the wiring of coincidently active pathways, i.e. *via* the formation of Hebbian synapses (Hebb, 1949; Turrigiano & Nelson, 2004; Butts *et al.*, 2007; Dissing-Olesen *et al.*, 2014).

Endogenous neurogenesis

Another form of plasticity in which microglia may play an instrumental role in the context of stroke repair is endogenous neurogenesis. It has been established that neurogenesis, a term which encompasses the generation of all cells of neural lineage, i.e. neurons, astrocytes, and oligodendrocytes, continues throughout adult life. In the brain, the main neurogenic niches generating neural progenitor cells (NPCs) are the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) in the dentate gyrus of the hippocampus. Furthermore, relatively recent evidence increasingly supports the view that adult neurogenesis also occurs in several other CNS regions including the spinal cord, neocortex, cerebellum, striatum, amygdala, substantia nigra and hypothalamus (Horner *et al.*, 2002; Kokoeva *et al.*, 2005; Luzzati *et al.*, 2006; Gould, 2007; Kriegstein & Alvarez-Buylla, 2009; Nishiyama *et al.*, 2009; Martino *et al.*, 2011).

Cerebral ischemia, activates both neurogenic niches and non-neurogenic brain regions. Interestingly, such reactive responses seem to have a functional rather than constitutive character. Extensive experimental evidence from animal models of brain ischemia has demonstrated that NPCs generated in the ipsilesional SVZ, instead of migrating to the olfactory bulb through the rostral migratory stream (RMS), i.e. their regular migratory pathway, start migrating towards the infarct site. Interestingly, a distinct population of microglia, characterized by low expression of purinoceptors and lack of ATP-elicitable chemotaxis, appear to regulate NPC migration (Xavier et al., 2015). Similarly, post-mortem tissue from stroke patients obtained 5-15 days post ischemia revealed NPC proliferation and migration towards the infarct from the ipsilesional SVZ. Remarkably, in cases where such progenitors

reach the perilesional area, they can terminally differentiate into medium spiny neurons, as evidenced in a number of animal studies (Arvidsson *et al.*, 2002; Thored *et al.*, 2007; Martino *et al.*, 2011). This suggests that the neurogenic niche response may play a role in functionally integrated cell replacement (Martino *et al.*, 2011). The extent of SVZ-derived NPC proliferation and migration can be positively associated with the severity of the lesion. Furthermore, this response seems to be sustained at the chronic stage post-lesioning (Kazanis *et al.*, 2013). Strikingly, NPC proliferation at the chronic stage is restricted to those regions directly affected by stroke (Gould, 2007).

Earlier studies demonstrated that endogenous neurogenic responses can be detrimentally affected by local inflammation mediated by activated microglia (Ekdahl *et al.*, 2003; Pluchino *et al.*, 2008; Ekdahl *et al.*, 2009). Specifically, *in vivo* and *in vitro* experiments have shown that factors such as TNF-α, interferon γ (IFN-γ), IL-1, and IL-6, suppressed neurogenesis and also inhibited NSC survival, an effect that could be partially controlled through pharmacological modulation of the inflammatory response, as for example by IL-6 neutralization or administration of minocycline (Ben-Hur *et al.*, 2003; Ekdahl *et al.*, 2003; Cacci *et al.*, 2005; Iosif *et al.*, 2008; Koo & Duman, 2008). However, a more complex profile of the role of microglia in neurogenesis has been emerging, suggesting that instead of being detrimental to endogenous neurogenesis, activated microglia may indeed be beneficial for NPC proliferation and integration into functional networks supporting stroke recovery.

For example, it has been shown that stimulation of microglia with low levels of IFN-γ can promote early neurogenesis after stroke (Butovsky *et al.*, 2006). Furthermore, it has been reported that at the chronic stage (16 weeks) after ischemia, microglia within the ipsilesional SVZ exhibited a ramified or intermediate morphology (suggestive of an M2 phenotype), compared to the amoeboid morphology (M1 phenotype) of microglia found in the perilesional striatum. Additionally, there was an upregulation in the numbers of SVZ microglia expressing IGF-1 (Ekdahl *et al.*, 2009). This indicates that the prolonged presence of such microglia within the neurogenic niche can be supportive

of sustained NPC proliferation, a process that may effectively promote tissue remodelling after stroke through prolonged availability of migrating NPCs towards the infarct site. Furthermore, experimental evidence suggests that there is differential inflammatory response to injury in the SVZ neurogenic niche, compared to other neurogenic niches (Goings et al., 2006), and also contingent on the degree of demyelination caused to associated white matter tracts, such as the corpus callosum (Hills et al, 2016).

Similarly, microglia can play a crucial role in NPC migration towards the infarct through the secretion of chemokines such as stromal cell-derived factor 1α (CDF-1α) whose receptor, CXC4, is highly expressed in NPCs (Ni *et al.*, 2004). In this manner, CDF-1α upregulation in response to ischemia may thus fuel NPC proliferation and migration (Imitola *et al.*, 2004; Ekdahl *et al.*, 2009). The above findings suggest that selective modulation of microglia or microglia subtype activation may positively influence short- and long-term neurogenic responses. Furthermore, considering that activated microglia secrete cytokines and growth factors such as TNF-α and BDNF, they may also influence morphology-activity relationships, including excitatory and inhibitory synaptic transmission as well as dendritic spine arborization and thus promote functional integration of newly generated NPCs within the lesion hemisphere (Amo et al., 2014; Pickering *et al.*, 2005; Ekdahl *et al.*, 2009; Caleo, 2015; Wu *et al.*, 2015).

Again, there are clear similarities between the manner in which microglia actively promote NPC proliferation, survival, migration and functional integration after stroke, and microglial support of equivalent developmental processes. One such example is the instrumental role of microglia in axonal pathfinding, survival and network formation by layer V neurons during early development (Ueno *et al.*, 2013).

Finally, considering the fact that neurogenic niche function and responses are modulated in the ageing

brain and the fact that stroke risks increases with ageing, it is worth considering potential differential roles of microglia in regulating these mechanisms in the aged brain (Sato, 2015; Hamilton et al,., 2013; Ruddy and Morshead, 2018).

Conclusions

Neuroplasticity after ischemic injury involves both spontaneous rewiring of neural networks and circuits as well as functional responses in neurogenic niches. Such events involve complex interactions with activated microglia which evolve in a dynamic manner over time. Although the exact mechanisms underlying these interactions remain poorly understood, increasing experimental evidence suggests a determining role of pro- and anti-inflammatory microglial activation profiles in shaping networks and synapses. Ability to elucidate as well as to engage such mechanisms in a selective manner, also in the context of microglia-neuron-astrocyte interactions, can be envisaged to play a significant role in harnessing endogenous plasticity to promote stroke repair with functional outcome.

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Competing interests

The authors have no competing interests to disclose.

Author contributions

IS contributed to the manuscript concept and design, literature search, review and selection, manuscript preparation and editing and figure design. ILA contributed to the literature search, review

and selection, manuscript proofing, figure design and preparation. AKH contributed to the literature review and selection, manuscript preparation and editing. AS contributed to the manuscript design, literature search, review and selection, manuscript preparation and editing.

Abbreviations

BDNF	Brain-derived neurotrophic factor
CSFR1	Colony-stimulating factor 1 receptor
CSPGs	Chondroitin sulphate proteoglycans
CDF	Stromal cell-derived factor
CNS	Central nervous system
CX_3CL1	CX_3 chemokine ligand 1
DC-SKIN	Dectin-1, dendritic cell specific ICAM-grabbing non-integrin
ECM	Extracellular matrix
GDNF	Glial cell line-derived neurotrophic factor
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IFN	Interferon
IRF	Interferon regulatory factor
LTD	Long-term depression
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
NO	Nitric oxide
PNN	Perineuronal nets
RMS	Rostral migratory stream
ROS	Reactive oxygen species

SGZ	Subgranular zone
SVZ	Subventricular zone
TGF	Transforming growth factor
TNF	Tumour necrosis factor
tPA	Tissue-type plasminogen activator
TRL	Toll-like receptor

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