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University Postgraduate Interdisciplinary Doctoral Study in
Molecular Biosciences

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Interactions between glia and electromagnetic fields in neurological disorders and regeneration of the central nervous system

PhD thesis

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Sveučilište Josipa Jurja Strossmayera u Osijeku
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Međudjelovanje glija stanica i elektromagnetskih polja u neurološkim poremećajima i obnovi središnjeg živčanog sustava

Jasmina Isaković, BSc

Disertacija je izrađena u:
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Prof. dr. sc. Dinko Mitrečić, dr. med.

Kratki sažetak doktorske disertacije:
Poznato je da elektromagnetska polja utječu na stanice zbog njihovog površinskog naboja. Ovaj rad razjašnjava utjecaj urođenih i vanjskih elektromagnetskih polja na neurone i glija stanice. Koristeći matematičko modeliranje, predlaže se uporaba nehomogenih Maxwell-ovih jednadžbi za modeliranje urođenih polja oko neurona. Modulacijom međusobne komunikacije između astrocita i mikroglija, urođena elektromagnetska polja mogu doprinjeti razvoju bolesti, dok vanjska polja pomažu u obnovi tkiva.

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Thesis performed at:
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Short abstract:
Since electromagnetic fields affect electrically charged cells, this thesis elucidates the influence of innate and external electromagnetic fields on neurons and glia. Through mathematical modeling, the use of inhomogeneous Maxwell equations for modeling innate fields around neurons is proposed. Thus, by modulating the microglia-astrocyte crosstalk, innate electromagnetic fields can contribute to disease development while external fields assist in tissue regeneration.

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"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."

— Marie Sklodowska-Curie

Contents

1. INTRODUCTION

1.1. Neural tissue

The term "neural tissue" denotes an organized group of cells and extracellular matrix that play a role in controlling an organism's functioning. Anatomically, the nervous system consists of two parts: the central (CNS) and the peripheral nervous system (PNS). Whilst the peripheral nervous system contains the peripheral nerves, the central nervous system consists of the brain and the spinal cord. At a cellular level, the basic components of nervous tissue are neurons and glial cells. A neuron is the main building block of the nervous system that serves the function of signaling and communicating with other cells. The glial cells, also known as glia or neuroglia, support proper functioning of neurons and are generally classified into astrocytes, microglia, oligodendrocytes and ependymal cells (Fig. 1).

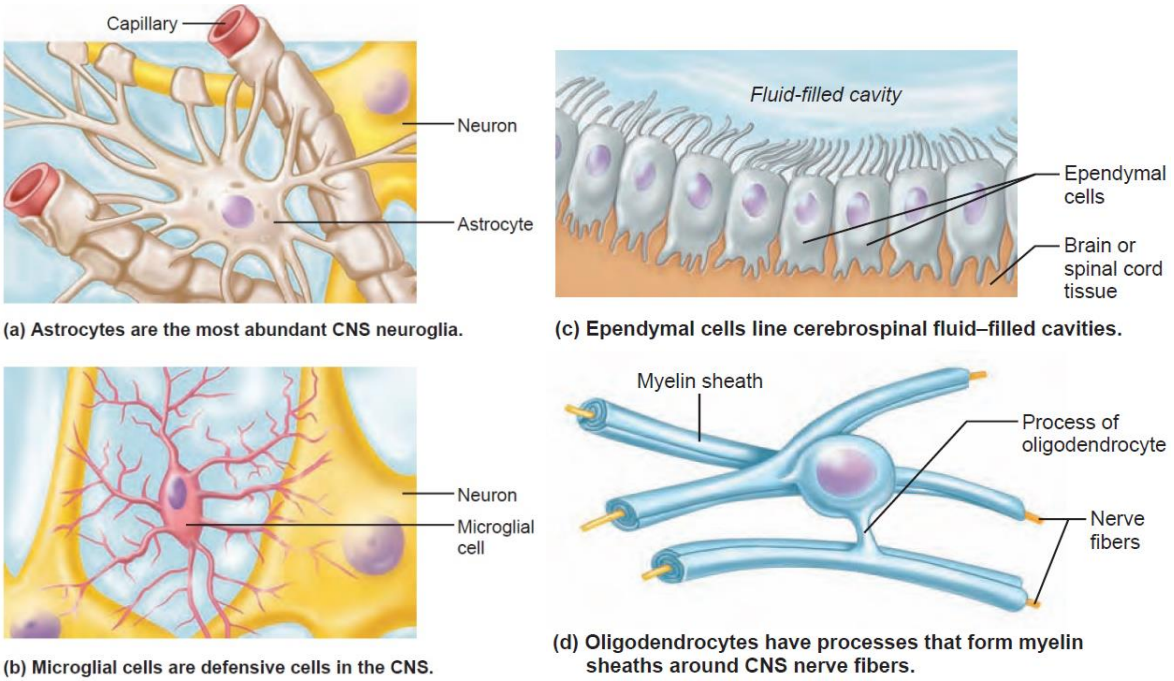


Figure 1. Four types of glial cells within the CNS: astrocytes (a), microglia (b), ependymal cells (c), oligodendrocytes (d) and their respective functions (*adapted from* (1)).

In the central nervous system, microglia cells clean the extracellular space through phagocytosis, maintain homeostasis and present antigens. These cells are involved in initiation of an immune response. On the other hand, astrocytes serve to support the endothelial cells of the blood-brain barrier, maintain ion balance in the extracellular space, and sustain the immune response. Myelin sheaths that wrap neuronal extensions are formed by oligodendrocytes, while

the ependymal cells line cavities containing cerebrospinal fluid.

1.1.1. Neurons

The neuron is the main functional component of the nervous tissue. It is a specialized electrically excitable cell that plays a role in intracellular communication and signal propagation. Neurons generally consist of a soma (cell body) and neurites (cell projections) and, in the case of myelinated neurons, the myelin sheath and nodes of Ranvier (Table 1).

Table 1. The main structural elements of most neurons and their function

Structural element	Function
soma	body of the neuron
axon	cellular projection that conducts electrical impulses from cell body
dendrite	cellular projection, a branched structure that receives (electrical) input
synapse	a structure that serves as an intermediary in passing electrical or chemical signals from one to another neuron or a cell
myelin sheath	a lipid-rich substance that surrounds axons and serves as an insulator in order to protect the axon and increase the speed of signal propagation
node of Ranvier	periodic gap in the myelin sheath rich with ion channels

Depending on specific function and connections, three major types of neurons can be distinguished: multipolar interneurons (Fig. 1.a.), motor neurons (Fig. 1.b) and sensory neurons (Fig. 1.c).

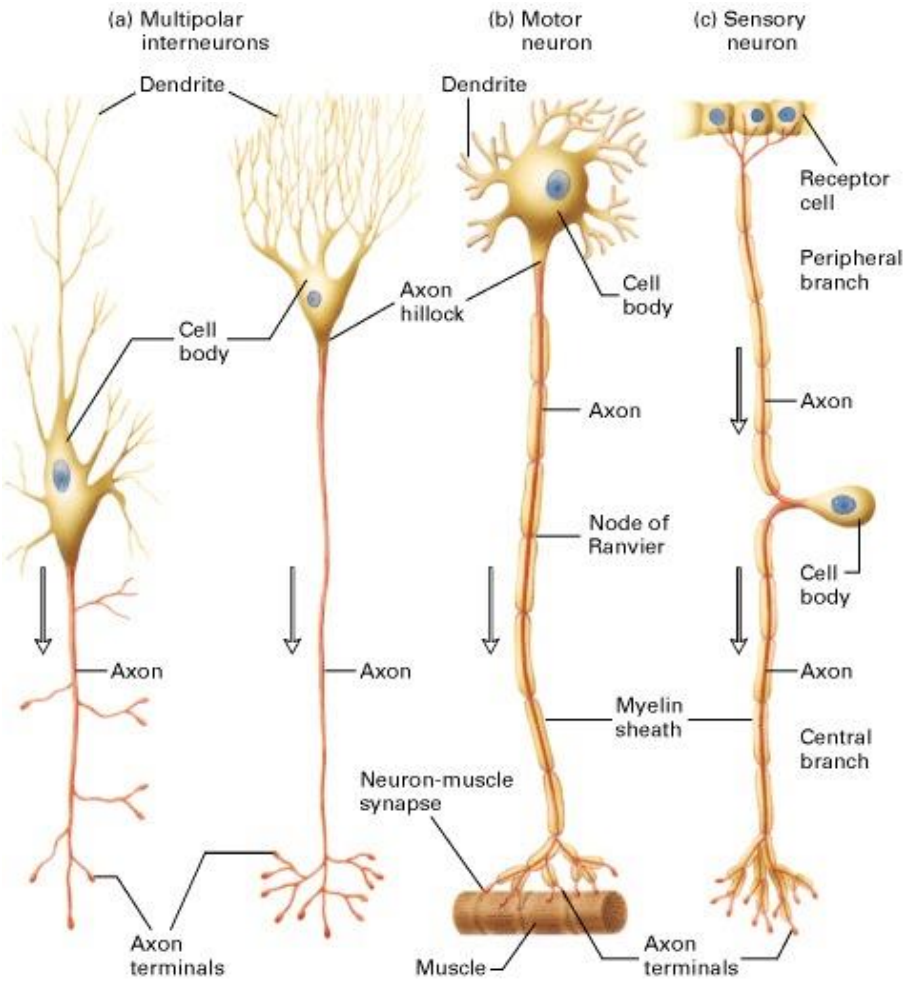


Figure 2. Anatomy of a three major types of neurons: (a) multipolar interneurons, (b) motor neurons, (c) sensory neurons (2).

Neurons can be classified based on the connections they make with surrounding cells, and also based on the number of neurites, properties of dendrites and axonal length (Table 2) (3).

Table 2. Classification of neurons based on the number of neurites, dendrite morphology, types of connections and axon length (3)

Classification		
Number of neurites	unipolar	
	bipolar	
	multipolar	
Dendrites	**stellate cells**	aspinous
		spiny
	pyramidal cells	spiny

Connections	primary sensory neurons
	motor neurons
	interneurons
Axon length	Golgi type I neurons
	Golgi type II neurons

All neurons have two major functions: to receive signals and transmit impulses. Transmission of an impulse, when it comes to cell-to-cell communication, is mainly done in the form of an action potential (AP) – a cellular event consisting of a rapid reversal of the membrane potential and its subsequent return to baseline due to the flow of ions through ion channels embedded in the axonal membrane (4) (Fig. 3).

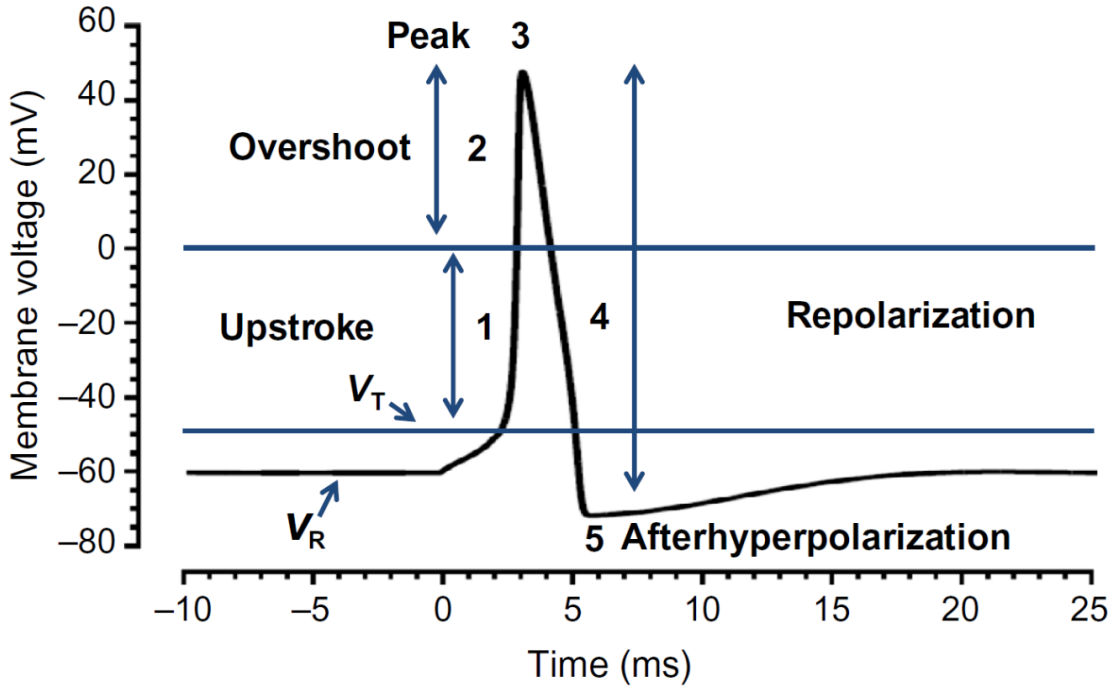


Figure 3. Graphical plot depicting different phases of the action potential (4). *The first stage of AP propagation is a rapid depolarization of the membrane due to Na^+ influx once the membrane potential exceeds the threshold (phase 1). This is followed by a steady rise of the membrane potential which soon becomes positive due to continued membrane depolarization (phase 2) until reaching its peak (phase 3). Once AP has reached its peak, the membrane potential rapidly drops due to K^+ outflux (phase 4). Finally, the membrane potential returns to its resting value, resulting in a more negative membrane potential than at the baseline (phase 5).*

1.1.2. Glial cells

Similar to neurons, most glial cells consist of branching processes (extensions) and a cell body (1). Since microglia and astrocytes are the most active cells within the CNS, their roles are of utmost importance not only in neurodegenerative and neuroimmune response but also in subsequent tissue repair. They are the focus of this work and will generally be referred to as "glial cells" or "glia".

1.1.2.1. Microglia

Microglia are a part of the glial cell group and, together with astrocytes and oligodendrocytes, are the most abundant cell type in the CNS. These cells of myeloid lineage reside in the brain and spinal cord. As resident macrophage cells within the central nervous system (5), microglia play a role in maintaining tissue homeostasis and neuroinflammation (6).

What distinguishes them from other macrophage populations are their cellular projections, 'ramified' branches, which serve as extensions of the cell body to be used for communication with other surrounding cells and neurons (6). Once activated, microglia respond to injury by conferring into an 'amoeboid' activated phenotype and releasing pro-inflammatory mediators (6). Even though their response varies based on the nature of external cues, the most common molecular mediators that microglia release include cytokines, chemokines, reactive oxygen - (ROS) and reactive nitrogen - (RNS) species (RONS), as well as nitric oxide (NO). Deregulation of these signaling molecules can not only cause an increase of the inflammatory response but also contribute to the progression of neurodegenerative, neuroimmune or demyelinating diseases (6–8).

When looking at their structure and function, they express macrophage-associated molecular : cluster of differentiation molecule 11B (CD11b), cluster of differentiation molecule 14 (CD14) and epidermal growth factor (EGF)-like module containing mucin-like hormone receptor-like 1 (EMR1) (6,9). They also boast the expression of colony-stimulating factor 1 receptor (CSF1R) (6,10–12).

More specifically, based on their lineage, microglia are most similar to bone marrow macrophages whose "differentiation and proliferation also requires CSF1 and CSF1R transcription factors" - same molecular markers which form the backbone of microglia

differentiation and proliferation (6). Even though this holds true for bone marrow macrophages, microglia seem to be partly independent of CSF1. This led researchers to suggest that the expression of interleukin-34 (IL-34), a ligand for CSF1R, is what supports microglial activity and function within the CNS (6,13).

Another interesting aspect of microglial activity is their replicative potential and prolonged lifespan. Upon onset of a pathological condition within the CNS, microglia enter a state called microgliosis, in which they respond to an active injury by recruiting other cells of the immune system and increasing their numbers. This implies that they possess an ability to expand their population, a feature that is interesting for delineating both the onset and recovery from pathological conditions of the CNS (6,7,14,15). On top of this, as opposed to tissue macrophages, microglia are self-renewing and have a long half-life (6).

With respect to a specialized function of microglia within the CNS, these cells appear to also possess different activation states, similar to macrophages. These states range from the "classical" (M1-type) to the "alternative" activation (M2-type) state (6,16). *In vitro*, these distinct properties can be associated with macrophages activated through treatment with polarizing ligands (6). The "classical" activation state of macrophages can be achieved by treating them with toll-like receptor (TLR) agonists, such as TLR4 ligand lipopolysaccharide (LPS) or interferon-γ (IFN- γ). On the other hand, the "alternative" activation is induced through treatment with interleukin 4 (IL-4) and interleukin 13 (IL-13) (6).

Classically activated macrophages are present in overactive pro-inflammatory states and diseases which, to a larger extent, include the presence of inflammation (6,17,18). On the other hand, alternatively activated macrophages appear to be involved in tissue repair and are, generally, involved in protection from disease. Thus, these two opposing situations are highly dependent on the type of activation (6).

So far, it seems that microglia might follow similar, or even the same, activation pathways. The fact that precise phenotypes of steady-state (naïve) and deactivated microglia are not fully understood, makes these cells very important for future research. The most meaningful question is whether microglia, once deactivated, return to the same functional naïve state or they maintain memory of prior activation which can, in turn, impact their further activation dynamics and, even more so, function (6).

When in steady state, microglia take on a resting phenotype characterized by ramified cell processes associated with surveillance of the extracellular environment in the CNS (19). Upon occurrence of an insult or injury within the CNS, microglia secrete neurotrophic factors such as nerve growth factor (NGF), transforming growth factor-β (TFG-β), brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF1) in order to modulate the immune response (6,20,21). Besides being involved in permanent surveillance of their surroundings, microglia also participate in synaptic pruning and phagocytosis, supporting normal brain development and neurogenesis, respectively (22).

To maintain microglial resting phenotype, neurons play a role in suppressing activation of microglia through various secreted factors and cell-cell contact (Fig. 4). By releasing CX3C-chemokine ligand 1 (CX3CL1), which interacts with microglial cell-surface receptor CX3C-chemokine receptor 1 (CX3CR1), neurons restrain microglial activity (6).

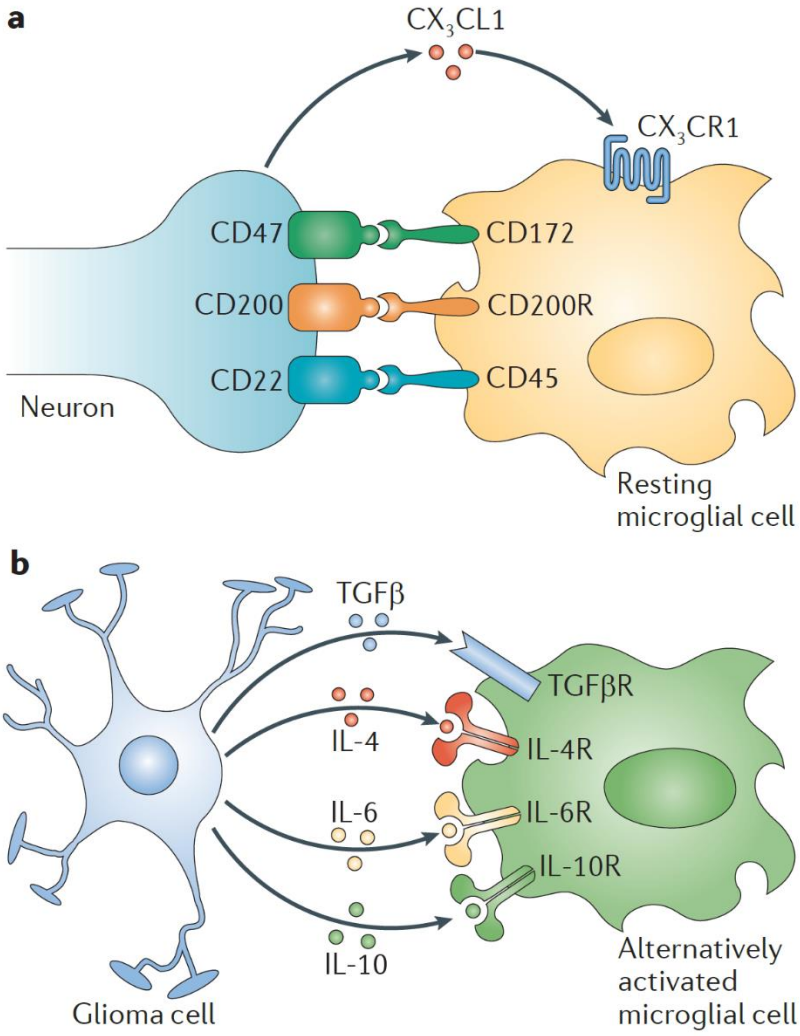


Figure 4. Two main states of microglia: (a) resting and (b) alternatively activated microglia (6).

Another crucial element associated with the resting microglial phenotype is the interaction between cell-surface proteins clusters of differentiation (CsD) 47 (CD47), 200, (CD200) and 22 (CD22) on neurons with receptors on microglia: cluster of differentiation 172 (CD172), 200 (CD200) and 45 (CD45). This causes their further inhibition and retention of their naïve status (23) (Fig. 4.a).

A. Classically activated microglia (M1)

One of the most prominent features of classically activated microglia (M1-type) is their ability to initiate a T cell response. Upon detection of pathogens or tissue injury, activated microglia express class II major histocompatibility complex molecules (MHC class II) and start releasing pro-inflammatory cytokines (tumor necrosis factor-α (TNF), interleukin 1β (IL-1β)) and ROS (6). These, in turn, activate naïve T cells and induce effector T cell differentiation (24). This suggests that microglia are not only a part of the innate immune response but also important players in directing the adaptive immune response within the CNS (Fig. 5).

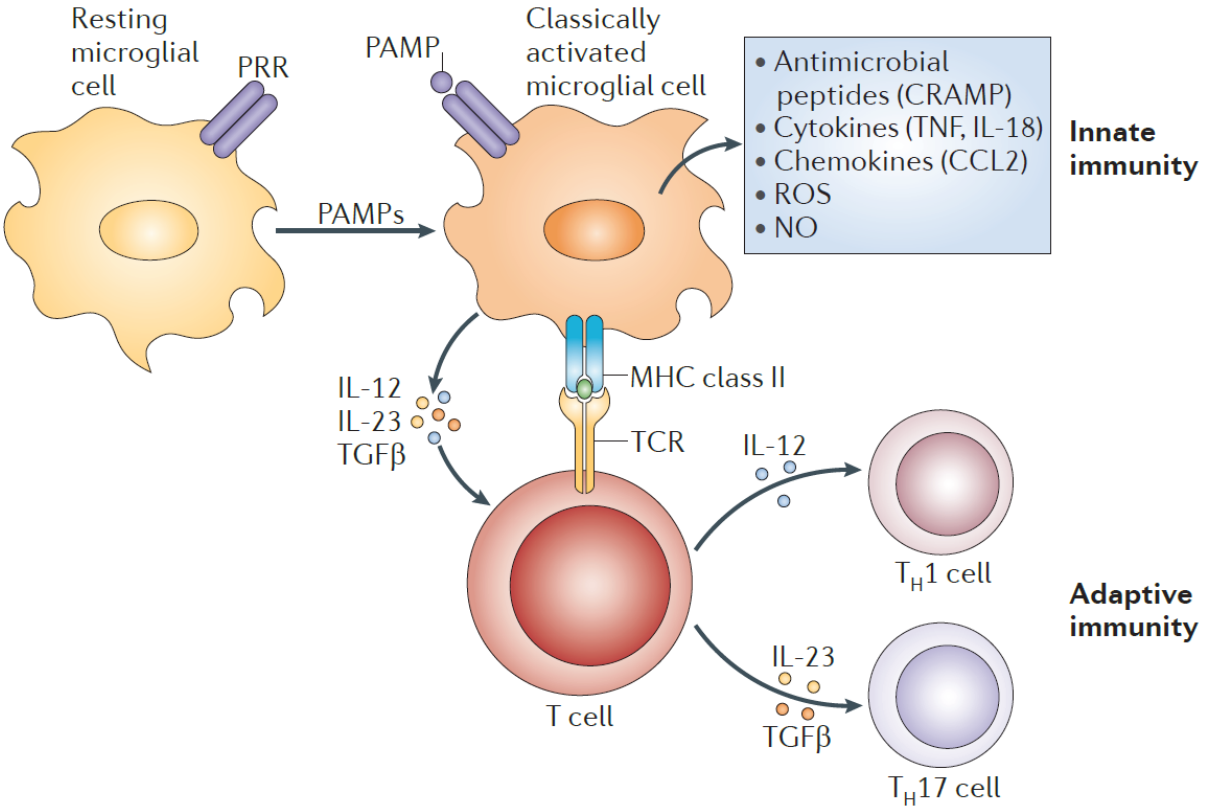


Figure 5. Activation pathway of classically activated (M1) microglia (6).

A.1. Pattern recognition receptors

Same as macrophages, microglia express receptors for recognition of pathogen-associated and

damage-associated molecular patterns (PAMPs and DAMPs, respectively). The most prominent pattern-recognition receptors (PRRs) which are mainly expressed by the cells of the innate immune system, are membrane-bound toll-like receptors (TLRs) and C-type lectin receptors (CLRs), and cytoplasmic NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and RLRs (6).

Upon PAMP recognition, TLRs activate multiple downstream signaling cascades dependent on myeloid differentiation primary response protein (MYD88) and TIR domain-containing adaptor protein inducing interferon-β (IFNβ) (TRIF). These multi-domain proteins are adaptor molecules which play a role in signal transduction (6). MYD88 is known as a universal adapter protein. It is used by all TLRs except TLR3 in order to activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen activated protein kinase (MAPK) pathways. These pathways, in turn, activate transcription of pro-inflammatory mediators crucial for inducing the M1 microglial phenotype (6). On the other hand, TRIF serves as an intermediary molecule in signal transduction from TLR3 and TLR4 and induces NF-κBand interferon-regulatory factor 3 (IRF3) pathways. On top of this, TRIF can also cause further transcription of pro-inflammatory mediators such as type I interferons (IFNs) (25,26).

Contrary to microglia which express TLR4 and CD14, astrocytes express only TLR4, and to a much lesser extent. Because of this, astrocytes are more sensitive to PAMP detection within the CNS, which is mainly mediated by TLR4 (27). In addition to recognizing PAMPs, microglia also recognize DAMPs, such as the oxidized low-density lipoprotein (LDL) (28–30). Under pathological conditions, microglial TLRs might become overactivated by surrounding harmful signals and contribute to initiation or further propagation of neurodegeneration as well as neuroinflammation.

Cytoplasmic NOD-like receptors are PRRs which often form multiprotein complexes called inflammasomes and initiate the immune response. These inflammasomes play a role in pathogen detection and activation of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and interleukin-18 (IL-18) (6,31). In cases of neurodegenerative diseases, the most commonly studied is the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) (6,31). The main structural components of the NLRP3 inflammasome are the sensor molecule NLRP3, adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase 1 (31). Its function greatly relies on sensing PAMPs and DAMPs, such as a variety of pathogen molecules and extracellular adenosine triphosphate (ATP), respectively (6,31–33).

A.2. Ion channels and neurotransmitter receptors

Besides PRRs, microglia also express many ion channels and neurotransmitter receptors (34,35) which assist in DAMP recognition. These also play a crucial role in debris clearance after cell death as well as CNS tissue repair. They mostly fall into two main categories: purinergic receptors (PR) and receptors for advanced glycation end-products (RAGE) (6,34–36).

A.2.1. Purinergic receptors

Purinergic receptors (purinoreceptors) are molecules on the cellular plasma membrane. They bind ATP, adenosine or other nucleoside diphosphates (NDPs) and triphosphates (NTPs) (37). As such, PRRs can, generally, be divided into two main categories: metabotropic and ionotropic receptors. They can further be subdivided into two distinct types based on their activation mechanism into: P1: adenosine receptors, and P2: ATP/nucleotide receptors (38) (Table 3).

Table 3. General types of purinergic receptors (6,38,39)

Category	Type	Activation	Class
Metabotropic receptors	P1	adenosine	G-protein coupled receptor
	P2y	NTP/NDP	G-protein coupled receptor
Ionotropic receptors	P2x	ATP	Ligand-gated ion channel

P2x ionotropic receptors fall into the class of ligand-gated ion channels. They constitute ion channels which open by ATP binding. Metabotropic receptors such as P1 and P2y bind purines and pyrimidines and use G-proteins to activate many downstream signaling cascades, deeming them G-protein coupled receptors (6,40).

P1 adenosine receptors are generally designated as A-type purinergic receptors which are further divided into four distinct categories: A1, A2a, A2b and A3. On the other hand, P2x, P2y, P2z, P2t and P2u belong into the category of P2 receptors (40). Because little is known about them, P2z, P2t and P2u receptors are generically all sorted into subtypes of P2Y receptors, even though P2z is not a G-protein coupled receptor but rather an ionotropic one, just as is the ligand-gated ion channel P2x (6,38,39).

Microglia express both P2x and P2y subtypes of purinergic receptors (40). Injured or dying cells

within the CNS release nucleoside triphosphates (NTPs), such as ATP, into the extracellular space which bind to microglial P2x or P2y receptors and serve as "danger signals" (41). This, in turn, activates the downstream extracellular signal-regulated kinase (ERK) pathway and, through activation of NF-κB and activator protein 1 (AP1) transcription factors, results in an increased expression of pro-inflammatory cytokines in microglia (6,36,42). Dying neurons also release uridine diphosphate (UDP) which triggers the activation of P2Y6 receptors on microglia and onsets phagocytosis of neurons (43). Because of the important role that purinergic receptors play in regulating microglial function, their deregulation has been implicated in many neurodegenerative or neuroimmune disorders, reactive astrogliosis and synaptic dysfunction (41).

A.2.2. Receptors for advanced glycation end-products

Far less is known about RAGE. It plays an important role in maintaining homeostasis within the CNS, whilst its overexpression in microglia promotes many pathological conditions (44) and exaggerates the process of neuroinflammation (45,46). Necrotic cell death in the CNS causes a release of nuclear protein high mobility group box 1 (HMGB1) which triggers TLR2, TLR4 and RAGE activation. This event promotes further transcription of pro-inflammatory genes (6,44,47) and causes mitochondrial injury, oxidative stress and M1 microglial polarization (46).

B. Alternatively activated microglia (M2)

The phenotype associated with alternatively activated microglia (M2-type) is most commonly observed in primary and metastatic tumors (48,49). This microglial phenotype is mainly distinguished from M1-microglia by an "upregulation of chitinase-like 3 (Chil3), frizzled class receptor 1 (Fzd1) and arginase 1 (Arg1)" (50,51). Within the CNS, the cells with that specific phenotype are considered to fall into the category of glioma-associated microglia/macrophages (GAMs).

Much research and clinical evidence has been gathered to support the hypothesis that GAMs, indeed, play a role in tumor progression and the subsequent impairment of the immune response upon their activation by tumor cells (48,52–54). These tumor cells induce a maladaptive phenotypic switch in resident microglia into GAMs through secretion of macrophage colony-stimulating factor (M-CSF), also known as colony stimulating factor 1 (CSF-1) (55). Once M-CSF causes a polarization of GAMs into their M2 phenotype, M2-GAMs then further contribute to tumor growth and local invasion as well as tumor cell migration (49,56).

Alternatively activated microglia and tumors

The most common primary brain and spinal cord tumors originating from glial cells are gliomas. Similar to an increased presence of overactive TAMs, much clinical evidence exists depicting microglial proximity to the tumor or within the glioma tissue itself (49,55,57,58). Even though it is still unclear whether these "tumor-associated microglia" are, indeed, derived from resident microglial cells, emerging research evidence suggests that these cells differ from classically activated microglia and are functionally closer to "alternatively activated macrophages" – earning them the name of "alternatively activated microglia" (Fig. 3.b) (6,59–61).

In order to induce alternatively activated microglia, on top of secreting M-CSF, glioma cells also secrete transforming growth factor β (TGF-β), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 10 (IL-10) and prostaglandin E2 (PGE2). In turn, these factors can suppress the M1-like and promote an M2-like microglial phenotype (6,49). Whilst TGF-β appears to inhibit the proliferation of microglia and microglia-mediated secretion of proinflammatory cytokines *in vitro* (6,62), interleukins 4, 6 and 10 induce M2-like, alternative, microglial phenotype (Fig. 2.b) (59). In this case, M2-like polarization of microglia stands for the anti-inflammatory phenotype which can be beneficial in many neurodegenerative and neuroimmune diseases, whilst also being very harmful in cases of brain tumors (49,59).

1.1.2.2. Astrocytes

Astrocytes are the most abundant cells in the CNS. They are involved in numerous activities such as maintaining extracellular ion homeostasis, supporting the endothelial cells of the blood brain barrier (BBB) and participating in the immune response (Fig. 6). They also assist in generating new blood vessels and controlling the flow of blood (63).

Through secretion of various factors, astrocytes protect neurons from degeneration and promote synapse formation (63). Moreover, through astrocyte-microglia crosstalk, they also play a role in modulation of microglial phenotypes and function (64).

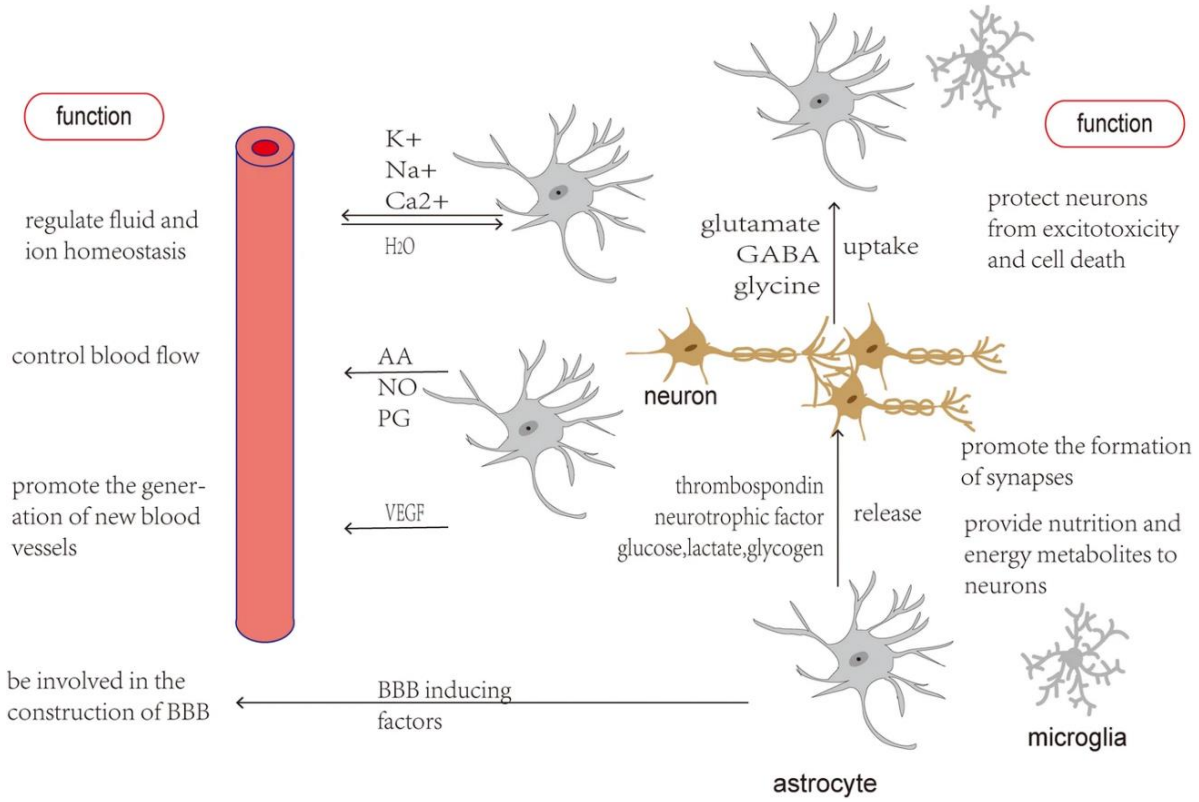


Figure 6. Multiple functions of astrocytes in the CNS (63).

Finally, astrocytes also play a role in initiating the development of a glial scar – formation of which precedes tissue regeneration (65,66). The formation of a glial scar occurs through a process of astrogliosis, during which the number of astrocytes in the CNS abnormally increases due to tissue damage or an overactive immune response (65,66). Astrogliosis, in turn, alters the gene expression, activity and morphology of astrocytes, leading to scar formation. Although crucial for tissue repair, scar formation can also inhibit axonal regeneration, furthering their damage and diminishing their function (67,68).

Reactive and scar-forming astrocytes

Upon noxious stimulation or nerve injury, naïve astrocytes undergo a process called reactive astrocytosis (63). It is characterized by extension of astrocyte's processes, their hypertrophy and increase in glial fibrillary acidic protein (GFAP) expression. These features are the main hallmarks of "reactive astrocytes" (63). After subsequent proliferation and migration to the injury site, reactive astrocytes convert to "scar-forming astrocytes" (63) (Fig. 7).

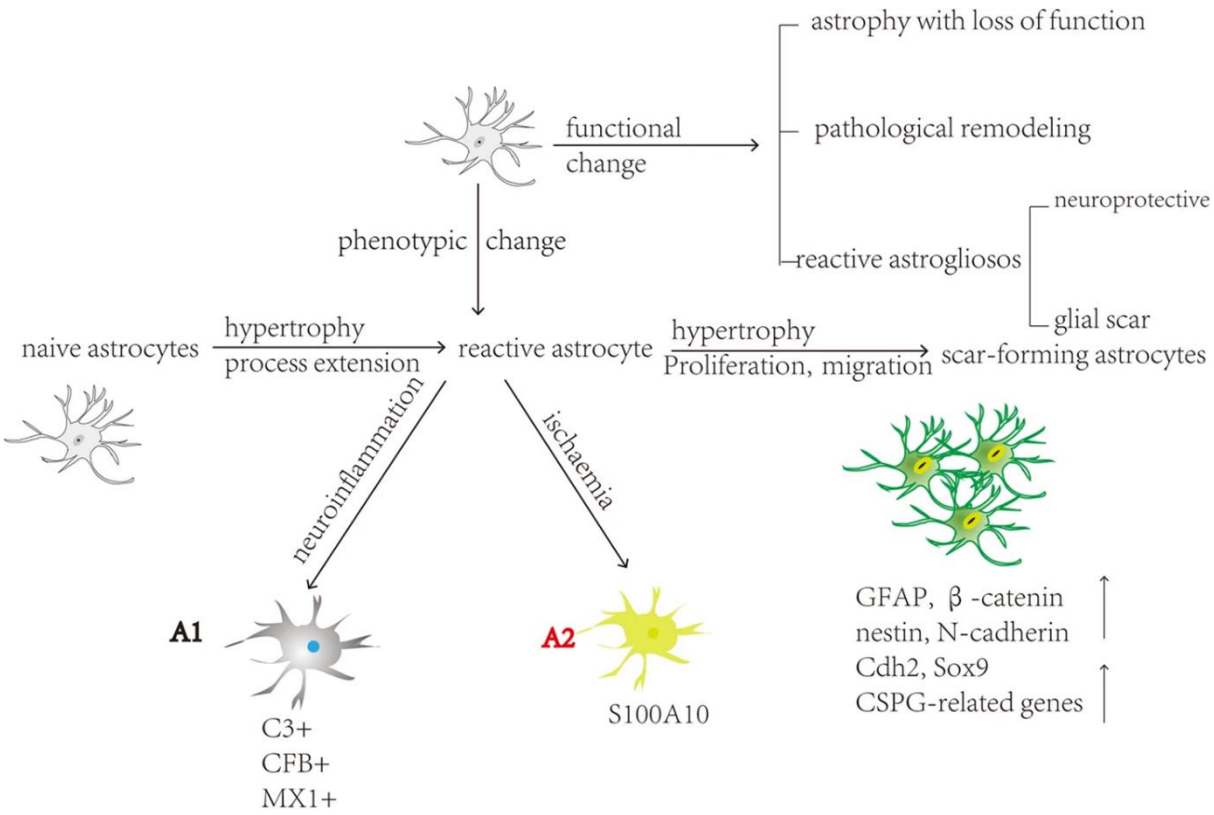


Figure 7. Graphical depiction of the variety of changes astrocytes undergo after being subjected to noxious stimulation and nerve injury (*adapted from* (63)).

Although both reactive and scar-forming astrocytes express GFAP, β-catenin, nestin and N-cadherin (63), they additionally express set of specific marker genes (Table 4) (63).

Table 4. Specific marker genes expressed by reactive and scar-forming astrocytes and their respective proteins (63).

Marker genes unique to reactive and scar-forming astrocyte			
Reactive astrocytes		**Scar-forming astrocytes**	
Protein	Gene	Protein	Gene
matrix metalloprotease 2	*MMP2*	N-cadherin (cadherin-2)	*CDH2*
plasminogen activator, urokinase receptor	*PLAUR*	SRY-box transcription factor 2	*SOX2*
matrix metallopeptidase 13	*MMP13*	chondroitin sulfate N-acetylgalactosaminyltransferase 1	*CSGALNACT1*
axis inhibition protein 2	*AXIN-2*	carbohydrate sulfotransferase 11	*CHST11*
		aggrecan core protein	*ACAN*

		slit homolog 2 protein	*SLIT2*

Thus, astrocyte activation and their transformation from resting to reactive state is a complex process involving many molecular regulators and activation of multitude signaling pathways involving neurons, other glial cells or cells of the immune system (Fig. 8) (63).

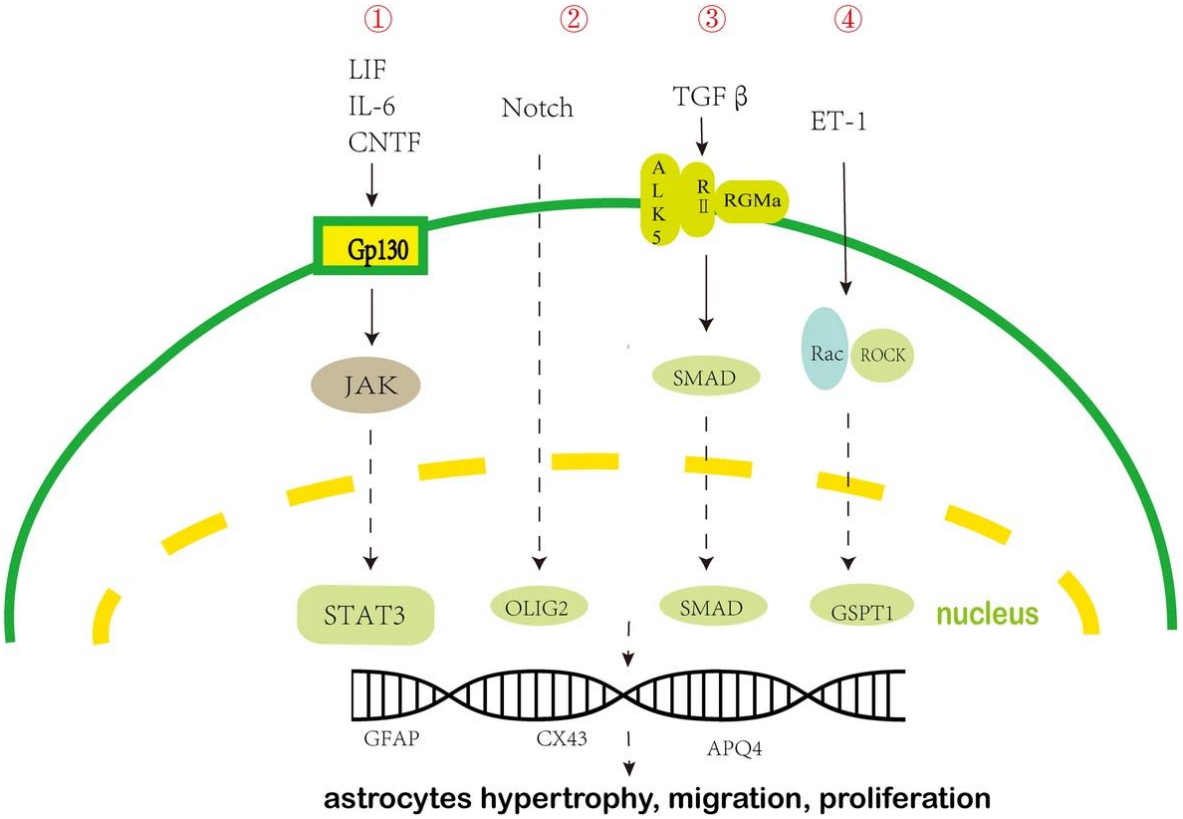


Figure 8. Intracellular and extracellular signaling pathways leading to astrocyte activation (63).

Depending on mediator molecules, the activation of astrocytes occur through four fundamental molecular signaling pathways (63):

1) Gp130-JAK-STAT3 signaling pathway;
2) Notch-OLIG2 signaling pathway;
3) TGFβ-RGMa-SMAD signaling pathway;
4) Rac-GSPT1 signaling pathway.

These all appear to play a role in initiating or propagating the process of reactive astrogliosis through upregulating genes coding for *GFAP*, connexin 43 (*CX43*), and aquaporin-4 (*AQP4*). The GFAP protein is involved in cellular communication and maintenance of proper functioning of the BBB. It also plays a crucial role in the formation of a glial scar (69). On the other hand, connexin 43 is a gap junction protein which can act as a hemichannel in order to control

glutamate and ATP release into the extracellular space. When present in excessive concentrations, both glutamate and ATP can be damaging to cells in their vicinity, triggering secondary damage and subsequent cellular death (70). The major controllers of opening of these hemichannels are intracellular Ca^{2+}, oxidative stress (OS) and inflammation (70). Finally, aquaporin-4 is a water channel membrane-bound protein that can also play roles associated with cellular adhesion and migration. It can also be involved in the processes relevant for the level of neuroexcitation, synaptic plasticity and the neuroimmune response (71).

The existence of at least two distinct types of activated astrocytes has been proposed recently. These are: classically activated (A1) astrocytes induced by neuroinflammation, and alternatively activated (A2) astrocytes induced by ischemia (63).

Distinct from their naïve state, A2 reactive astrocytes are characterized by an upregulation of various neurotrophic factors that support neuronal survival and growth: S100 calcium binding protein A10 (S100A10), leukemia inhibitory factor (LIF), cardiotrophin-like cytokine factor 1 (CLCF1) and BDNF. Thrombospondins, a class of proteins that are sufficient to promote synapse repair –implying a protective, or "helpful", function of the A2 phenotype – are also upregulated (72).

On the other hand, it was shown that A1 reactive astrocytes exhibit an upregulation in classical complement cascade (*C1r, C1s, C2, C3, C4*), complement factor B (*CFB*) and MX dynamin-like GTPase 1 (*MX1S*) genes (50,63,72) (Fig. 7). The complement cascade is a molecular pathway which is not involved only in microglial targeting of synapses for their destruction during normal brain development, but also in neurodegenerative diseases such as Alzheimer's disease (AD) (73). This suggests a "harmful" function of A1 astrocytes (74). Therefore, depending on the phenotype assumed, astrocytes' role can be either beneficial or a detrimental (Fig. 9) (63).

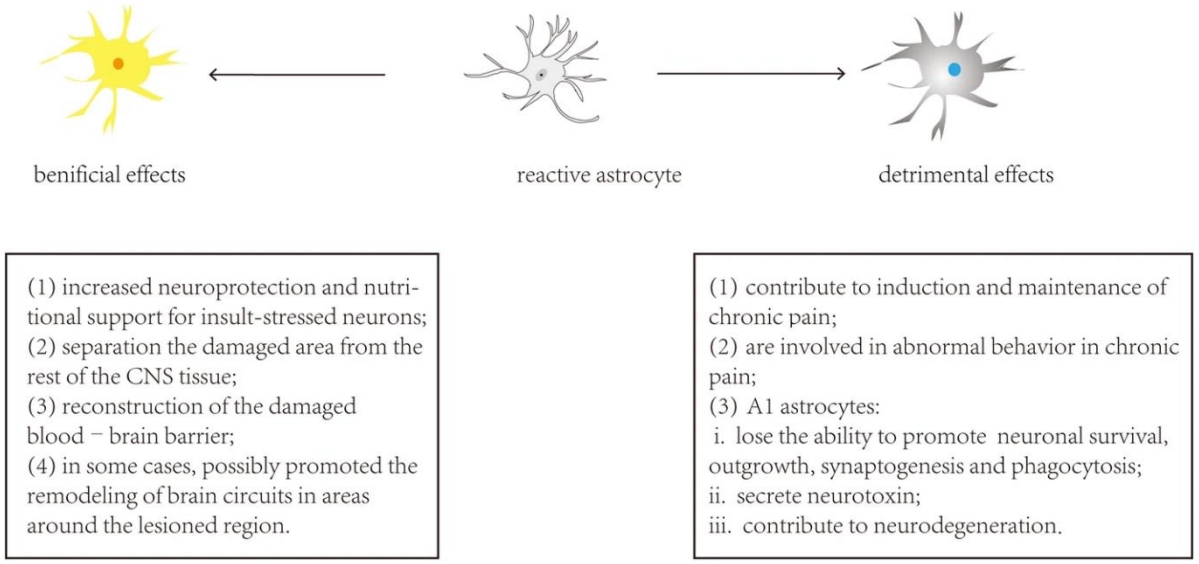


Figure 9. Beneficial and detrimental effects of astrogliosis (*adapted from* (63)).

The beneficial effects of reactive astrocytes, mostly associated with the A2 phenotype, include the rewiring of neuronal connections, promotion of tissue repair and increase in neuroprotection. On top of this, A2 reactive astrocytes also have the ability to reconstruct the damaged BBB through interaction with type I collagen and inducing the subsequent formation of an astrocytic scar (75) as well as to limit the infiltration of peripheral leukocytes (76). Contrastingly, the detrimental effects of the A1 phenotype include unknown neurotoxin release and contribution to further tissue degeneration (63,74).

A) Classically activated astrocytes (A1)

A candidate screen of possible A1-inducing-molecules has revealed only three cytokines. Each of them induced a subset of A1 genes - interleukin 1 α (*IL-1α*), tumor necrosis factor (*TNF*) and the initiating component of the classical complement cascade (*C1q*) genes (50,64,74) (Fig. 10). Interestingly enough, these three genes have one thing in common – they are all very highly expressed by microglial cells. This suggests that microglia play a very active and important role in inducing the A1 phenotype. Each of these cytokines, alone, is only able to induce a partial A1 phenotype but, when present together, they induce a complete A1 phenotype (74). This induction is highly dependent on the microglial state; only activated microglia can induce astrocyte reactivity (50).

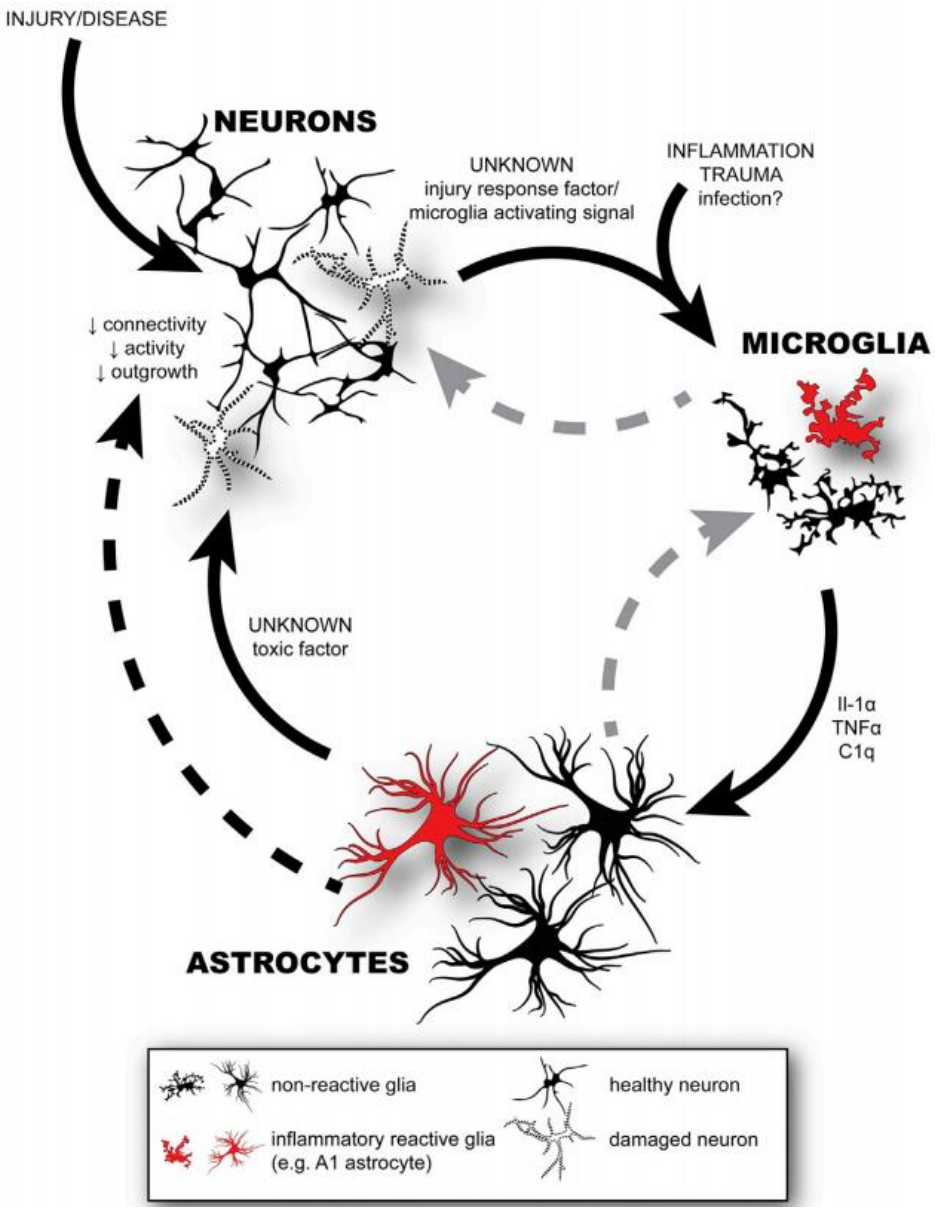


Figure 10. Model of microglia activation leading to induction of A1 phenotype in astrocytes and subsequent neurotoxicity (50).

Upon activation by an injury, neurodegeneration or inflammation, damaged neurons release unknown injury response factors that activate naïve microglia. Newly activated microglia then release IL-1α, TNF and C1q, and activate astrocytes in their vicinity towards A1 phenotype. The A1 astrocytes then release a yet-to-be-identified neurotoxin (50,74) which, in turn, decreases neuronal connectivity, activity and outgrowth. This is followed by further propagation of neurodegeneration or an increased pro-inflammatory response. Finally, in order to maintain their function, rewiring of neural circuits occurs (74).

When compared to naïve astrocytes, A1 astrocytes exhibit several notable, distinct properties:

loss of motility, increased inhibition or failure of axonal outgrowth and neuronal survival promotion, as well as loss of ability to phagocyte synapses and myelin debris. They are mostly associated with neuroinflammation and have been deemed "harmful" astrocytes (Table 5) (50).

Table 5. The main characteristics of A1 reactive astrocytes (50).

Properties of A1 reactive astrocytes
Loss of motility
Inhibition and/or failure to promote axon outgrowth
Inability to promote neuronal survival
Impaired phagocytosis of synapses and myelin debris
Loss of glutamate receptivity and altered frequency of calcium oscillations
Decreased gap junction coupling
Failure to promote synapse formation and function

A majority of these detrimental properties of A1 reactive astrocytes have already been studied in a variety of disease models in mice, such as Alzheimer's disease, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) (50,77–81). Most of these studies have reported some form of involvement of the NF-κB pathway which controls production of cytokines as well as cell survival (50). Because of this, the NF-κB pathway is considered to be the most crucial molecular pathways involved in neuroinflammation and neurodegeneration as well as the microglia-astrocyte crosstalk (79,82,83).

The crucial role of NF-κB pathway in induction of inflammatory reactive astrocytes and subsequent neuroinflammation has recently been confirmed in the study performed on human pluripotent stem cell (HPSC) model systems (84). This study has shown that IL-1B and TNF treatment of HPSCs induces a reactive astrocyte phenotype. As opposed to the control group where NF-κB was ubiquitously expressed in the cytoplasm, NF-κB was activated and translocated to the nucleus after cytokine stimulation, with over 50% of astrocytes showing NF-κB pathway activation (84). The activity of NF-κB complex was estimated through the strength of nuclear RELA staining. Here, even though the study defined NF-κB pathway activation as the percentage of nuclei with NF-κB translocation, i.e. the translocation of v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA or p65), in order for this statement to be properly quantified, additional tests were supposed to be carried out to assess the activity of the gene for COX2 which is the bona fide target of NF-κB. As such, no clear demonstration

whether NF-κB complex did, indeed, bind to a specific promoter has been done. Moreover, additional experimental mouse studies done on AD and HD, and clinical studies including patients with ALS, demonstrate a high NF-κB activity in astrocytes, associated with chronic inflammation and disease progression (77–81). Since most A1 reactive astrocytes present evidence of NF-κB activation (78,84), it is reasonable to assume that they play a role in promoting neurodegeneration and neuroinflammation as well exerting many other harmful effects impacting disease pathophysiology (50,84).

B) Alternatively activated astrocytes (A2)

Astrocytes can also assume an alternatively activated A2 phenotype, like the M2 type in macrophages and M2-like phenotype in microglia. This phenotype is usually induced by ischemic events within the CNS and seems to play a role in its repair and recovery (50,85–87). Many studies have been performed to probe the helpful function of A2 astrocytes mainly through modification of signaling pathway mediated by signal transducer and activator of transcription 3 (STAT3) (88,89).

STAT3 is a transcription factor which plays a role in cell proliferation, migration, apoptosis and regulation of inflammation (50). Studies have shown that a conditional knockout of STAT3 causes the removal of alternatively activated (A2) astrocytes and subsequent increase in monocyte, macrophage, lymphocyte and neutrophil infiltration into the CNS with consequential axonal death and tissue degeneration (88,89). The occurrence of tissue degeneration related to STAT3 knockout suggested that A2 astrocytes can also be classified as "scar-forming reactive astrocytes" with beneficial function (50).

1.2. Neurodegeneration

1.2.1. Classification

Once damaged by an internal or external event, neurons spend much energy to maintain proper functioning (90). As opposed to most other cells in the human body which can promptly regenerate to preserve tissue homeostasis, neurons lack the ability to self-repair, or this ability is kept to a minimum (90). Therefore, neuron loss which occurs with increasing age, leads to functional decline of the whole organism. This concept is the backbone of neurodegeneration, as well as neurodegenerative diseases (NDDs) themselves.

Etymologically, the word „neurodegeneration“ consists of the term „neuro-„ which stands for nerve cells or neurons, and „-degeneration“ which means loss of structure or function of something (91). Because of its name, neurodegeneration can be deemed to envelop any pathological condition which appears to be causing a loss of structure or function of neurons, be it primary or secondary through various molecular and genetic pathways (64).

Neurodegeneration is defined as the process of progressive decay and loss of neuronal function which, in most cases, leads to neuronal death. Most NDDs are characterized by alterations of physicochemical properties of proteins and their aberrant deposition in the brain. Diseases that fall into this category are thus defined as conformational diseases, getting their name from “misfolded proteins also called pathological conformers” (92). They are often also characterized by additional pathologies such as “disruption of normal axonal transport and synaptic functioning, dysfunction in mitochondrial and lysosomal mechanisms, oxidative stress and glial activation followed by neuroinflammation” (90).

These pathologies then affect many of subsets of neurons and contribute to a variety of heterogeneous clinical manifestations of symptoms (91). Nevertheless, not all diseases that result in loss of structure and function of neurons should be characterized as neurodegenerative. Excluded from the group of neurodegenerative diseases are those diseases which are not primary neuronal diseases such as edema, hemorrhage, ischemic stroke, traumatic brain injury and neoplasm (91,92). Even though multiple sclerosis (MS) does not fit the category of neurodegenerative diseases, because its etiology primarily includes the loss of myelin sheath, it involves neuronal death, apoptosis, necrosis and hypoxia – suggesting a common thread with neurodegeneration. Finally, the diseases in which neurons die as a result of a known and somewhat expected cause, such as “hypoxia, poison, metabolic defects” and various types of infections of the brain, do not fall into the category of neurodegenerative diseases (91). Based on the current classification, major neurodegenerative disorders are Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) (92,93).

Currently, there are estimated over hundreds of various types of neurodegenerative diseases. Many of them overlap clinically and pathologically so categorizing them within the broad spectrum of neurodegeneration becomes quite challenging (92,93). Moreover, when it comes to more complex diseases which can manifest through different combination of lesions throughout

the brain, like multisystem atrophy (MSA), they can also result in a variety of divergent clinical pictures. This additionally increases the problem of precise classification and hinders the improvement of potential treatment options. Depending on the array of genetic and environmental factors, even the same neurodegenerative disorders can be manifested through different regions of the brain at their onset. This usually leads to presentation of various clinical symptoms and further complicates the establishment of an accurate diagnosis.

Despite these inconsistencies in disease onset, presentation, and progression, the standard categorization of neurodegenerative disorders still can be achieved based on molecular pathologies (92) or the "predominant clinical feature, topography of the predominant lesion or a combination of both" (91). Thus, the most general classification of the neurodegenerative disorders of the CNS divides them into four main categories (91,92), based on the predominant lesion location in the brain. This gives rise to the anatomical classification of NDDs:

a) Cerebral cortex:

When looking at the neurodegenerative diseases impacting the cerebral cortex, they can be further subdivided into

I. Dementing conditions;
II. Nondementing conditions.

b) Basal ganglia

The next group of neurodegenerative diseases, primarily characterized by abnormal movements, is the one involving the basal ganglia. They can be divided into

I. Hypokinetic;
II. Hyperkinetic.

Hypokinetic neurodegenerative disorders are characterized by a decreased voluntary movement velocity and amplitude, or even its complete absence. The most represented hypokinetic disorder is PD. For that reason, the term "parkinsonism" is often used to unify all existing symptoms and assist in association of different diseases into their respective categories. In order for a group of symptoms to qualify as "parkinsonism", a patient has to present with more than two clinical signs amongst the following four: resting tremor, slow movements, stiffness and postural instability (91,92). If a person has parkinsonism-associated-symptoms, joined with some additional symptoms, the condition is called Parkinson-plus syndrome.

On the other hand, hyperkinetic neurodegenerative disorders are characterized by excessive abnormal movements which interfere with normal voluntary movements (91). The most representative examples of these disorders are Huntington's disease (HD) and essential tremor (ET). Although these hyperkinetic disorders also manifest themselves with a variety of different symptoms, the correct diagnosis can be established early due to the availability of specific tests covering existing gene mutations and other disease markers.

c) Brain stem & cerebellum

Due to a great overlap in pathological and clinical presentation of the diseases associated with brain stem and cerebellum, it is particularly challenging to come up with an accurate diagnosis. Only a few of the disorders of the cerebellum can be separated into three clear neuropathological types (91):

I. Cerebellar cortical atrophy (CCA);
II. Pontocerebellar atrophy (PCA);
III. Friedrich ataxia (FA).

The CCA affects Purkinje cells and inferior olives and PCA is characterized by degeneration of several cerebellar and brain regions. Lastly, Friedrich ataxia (FA) affects the posterior column of the spinal cord, peripheral nerves and the heart (91,92).

On the other hand, there are also diseases that do not fit in any of these categories. Good examples are dentatorubral-pallidoluysian atrophy (DRPLA) and the Machado-Joseph disease (MJD), which affect dentate and red nuclei or the dentate system, motor neurons and substantia nigra, respectively (91,93). Both diseases have complex pathologies which involve multiple brain structures. For that reason, the clinical presentation is colorful and establishment of correct diagnosis is problematic (91).

d) Spinal cord

Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are the best studied neurodegenerative disorders affecting the spinal cord. Friedrich ataxia (FA) could also fit into the category of the diseases of the spinal cord because it is the spinal cord which gets the most severely degenerated (91,92).

e) Diseases with no apparent structural abnormalities

Finally, there also exist diseases whose clinical presentation suggests existence of

neurodegeneration, but the structures of the patient's CNS do not show any structural abnormalities. This category encompasses torsion dystonia, Tourette syndrome, essential tremor and schizophrenia (91).

With an increasing development of novel diagnostic and research tools in the past decades, the characterization of neurodegenerative diseases has also increasingly started to rely on the diseases' molecular characteristics as opposed to their "neuropathological hallmarks" (92,93).

Therefore, many neurological neurodegenerative diseases that were previously thought to be unrelated, have now been grouped based on their similar molecular defects (92,93). This gave rise to a more contemporary clustering of NDDs based on their molecular features into (91,92):

1) Trinucleotide repeat diseases:
   - Huntington's disease (HD);
   - Spinal cerebellar atrophy (SCA);
   - Myotonic dystrophy (MD).
2) Prion diseases:
   - Creutfeldt-Jakob disease (CJD);
   - Gerstmann-Straussler-Scheinker syndrome (GSSS);
   - Fetal familial insomnia (FFI).
3) Synucleinopathies:
   - Parkinson's disease (PD);
   - Progressive supranuclear palsy (PSP);
   - Diffuse Lewy body dementia (DLB).
4) Tauopathies:
   - Corticobasal degeneration (CBD);
   - Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTD-17);
   - Pick disease or frontotemporal dementia (FTD).

One of the more debated topics in the research of neurodegenerative disease is the contribution of genetic and environmental factors in their initiation (92). Which factor plays the most important role and which one can cause the disease without the action of the other one?

1.2.2. Neuropathology and molecular mechanisms in neurodegenerative diseases

Pathological changes in the brain of patients with neurodegenerative diseases are mostly described as a "focal loss of neurons with reactive gliosis" (91). The remaining, not fully degenerated neurons exhibit distinct morphological features: from seemingly normal appearance to an acute distortion. The abnormal features most commonly seen in residual neurons are listed in Table 6 (91).

Table 6. Abnormal features commonly observed in residual neurons of patients with neurodegenerative diseases (91).

Abnormal features of neurons observed in neurodegenerative diseases
Hypertrophy of main cellular processes (94)
Changes in the cell body and nucleus (95)
Fragmentation of organelles (95)
Dispersion of Nissl bodies (96)
Cytoplasmic vacuolization (97)
Condensation of chromatin (98)

Neurons can undergo shape and size alteration of the cell body and nucleus. Organelle fragmentation and dispersion of Nissl bodies can also occur, leading to apoptosis. Finally, during some neurodegenerative processes cytoplasmic vacuolization and chromatin condensation can also be present.

In some cases, specific distortions observed in residual neurons, such as "proteinaceous inclusions", assist in delineating the particular type of disorder (92). The best example where such specific distortions of residual neurons can assist in reaching a correct diagnosis can be seen in patients with PD, where the "presence or absence of intraneuronal inclusions called Lewy bodies" allows to define the proper PD subtype (91).

1.2.2.1. Cell death in neurodegenerative diseases

There are at least four major types of cell death associated with neurodegenerative diseases: apoptotic, necrotic, autophagic and cytoplasmic (99–103). Since all are controlled by a distinct molecular mechanism, they are unique with respect to clinical meaningfulness (102–107).

I. Apoptosis and necrosis in neurodegenerative diseases

Only two decades ago, it was thought that only necrosis can induce neuroinflammation in neurodegenerative diseases. It was a false preconception as the inflammatory reaction occurs through processes of necrosis as well as apoptosis. During necrosis, exudative type of inflammation is the predominant one (108). It is characterized by infiltration of the circulating neutrophils and monocytes followed by an increase in necrotic cell volume. Morphological feature of apoptosis is the fragmentation of nucleus, plasma membrane blebbing and shrinkage of the apoptotic cell. As opposed to predominant infiltration of circulating immune cells in necrosis, apoptosis is characterized by mediation of the immune response by the resident innate immune cells – astrocytes and microglia.

II. Molecular pathways leading to neurodegeneration

Traditionally considered a consequence of inflammation and demyelination, neurodegeneration is, based on recent data, considered as a process that can also develop independent of these two processes. The complex occurrence of neurodegeneration depends on many interconnected molecular pathways (Fig. 11) (109).

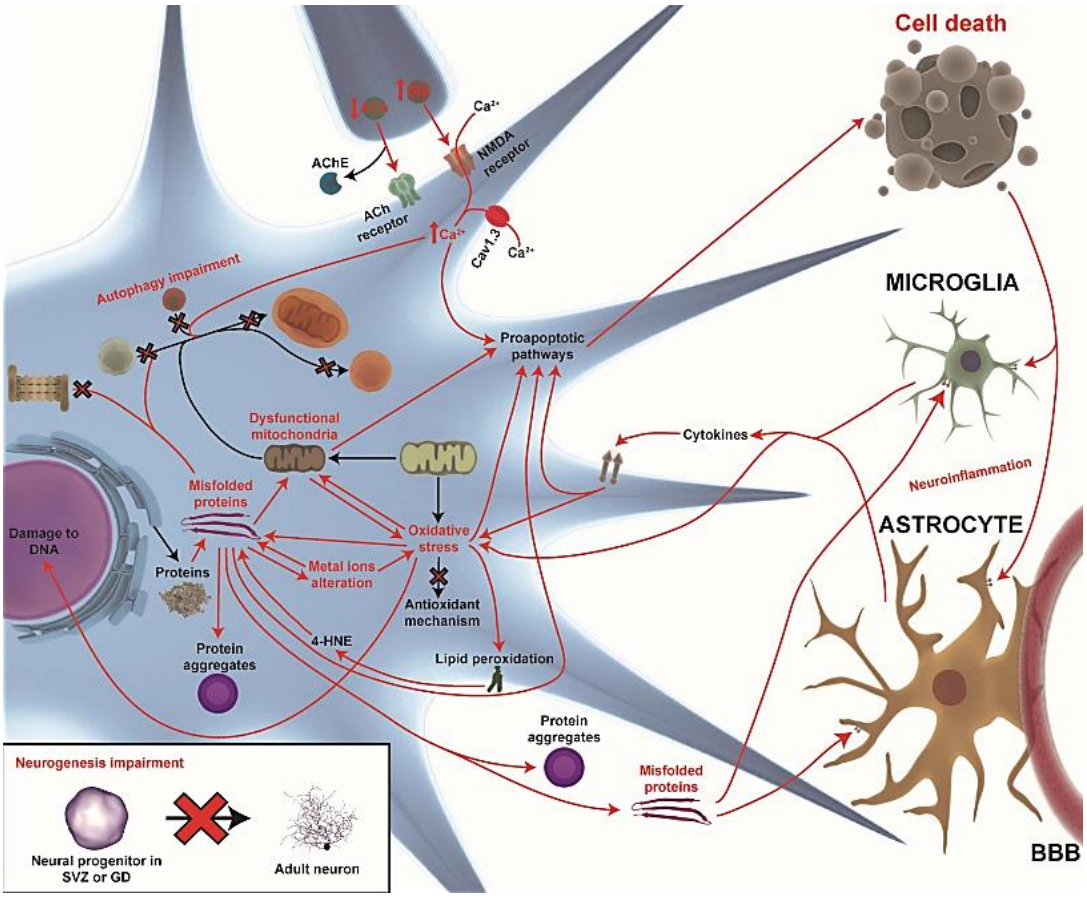


Figure 11. Pathological processes and their involvement neurodegenerative diseases (109).

Thus, the main pathways leading to neurodegeneration include (109,110):

a) Deregulation of autophagy;
b) Disturbance in apoptosis and necroptosis;
c) Lysosomal dysfunction;
d) Mitochondrial dysfunction;
e) Impaired protein homeostasis: accumulation, seeding and aggregation of misfolded proteins;
f) Oxidative stress;
g) Alteration of intracellular Ca^{2+} homeostasis;
h) Impairment of neurogenesis;
i) Alteration of metal ion homeostasis;
j) Disruption of RNA homeostasis;
k) Glia and neuroinflammatory responses.

a) Deregulation of autophagy

Autophagy is a form of cellular death which ensures proper degradation of different cellular components as well as misfolded proteins and their aggregates (109) in three different ways: chaperone dependent autophagy, microautophagy and macroautophagy (109,111). Whilst chaperone dependent autophagy depends on misfolded protein ubiquitination and subsequent chaperone activity, microautophagy occurs by invagination of cytosolic contents in lysosome membrane and their subsequent degradation (111). On the other hand, macroautophagy, commonly denoted just by the word "autophagy" refers to autophagy in its traditional sense. It takes place when a phagophore engulfs the cytoplasmic cargo, closes off its edges and becomes an autophagosome which then fuses with the lysosome, causing degradation of all of its contents (111).

A secondary function of autophagy is to regulate the oxidative stress, through degradation of dysfunctional mitochondria (109,112). As such, mutations in autophagy-regulating genes can contribute to neurodegenerative disease development and occurrence, such as Alzheimer's and Parkinson's disease as well as ALS. When it comes to overexpression of disease-related proteins, such as α-synuclein or superoxide dismutase 1 (SOD1), autophagy initiation appears to possess a protective function in attempted clearance of excessive mutant or damaged protein buildup in early stages of the disease (113). Nevertheless, as the disease progresses, deregulation in the

autophagy pathway can lead to improper mitochondrial disposal as well as an increased oxidative stress with consequential propagation of tissue degeneration.

b) Disturbance in apoptosis and necroptosis

Apoptosis is a type of programmed cell death which results in fragmentation of DNA, degradation of nuclear and cytoskeletal proteins and subsequent phagocytosis by cells of the immune system (90). The two main apoptosis-inducing mechanisms are the intrinsic, mitochondrial pathway and the extrinsic pathway (100,114).

Whilst the intrinsic pathway gets activated by intracellular signals of stressed cells and highly depends on protein release from the mitochondrial space (114), the extrinsic pathway's functioning depends on the proper binding of extracellular ligands to cell-surface-death receptors (90,114). As such, the intrinsic pathway of apoptosis is usually activated as a response to injury or other stressors through release of cytochrome *c* from mitochondria into the cytosol, where it binds to the apoptotic protease activating factor-1 (APAF1). APAF1 then oligomerizes into an apoptosome and recruits caspase-9. This is followed by an activation of downstream procaspases which, in turn, induce the process of apoptosis (114). On the other hand, the extrinsic pathway activation occurs after Fas ligands from the surface of a killer lymphocyte activate Fas on the target cell's surface. Next, intracellular adaptor proteins are recruited which, in turn, further recruit caspase-8 and/or caspase-10. Once formed, this complex is called a death-inducing signaling complex (DISC). Its formation is followed by an activation of downstream procaspases which, as in the intrinsic pathway, induce apoptosis (114).

Neuronal apoptosis occurs naturally to remove the excess of neurons during development, but it can also be seen in adulthood following acute injuries or chronic diseases, including neurodegenerative diseases (Fig. 12.A) (115,116). During neurodegeneration, apoptosis and necroptosis are prominent, while cell autolysis and autophagy have a far less prominent role. One of the most important contributors to neuronal apoptosis is mitochondrial permeabilization (115,116).

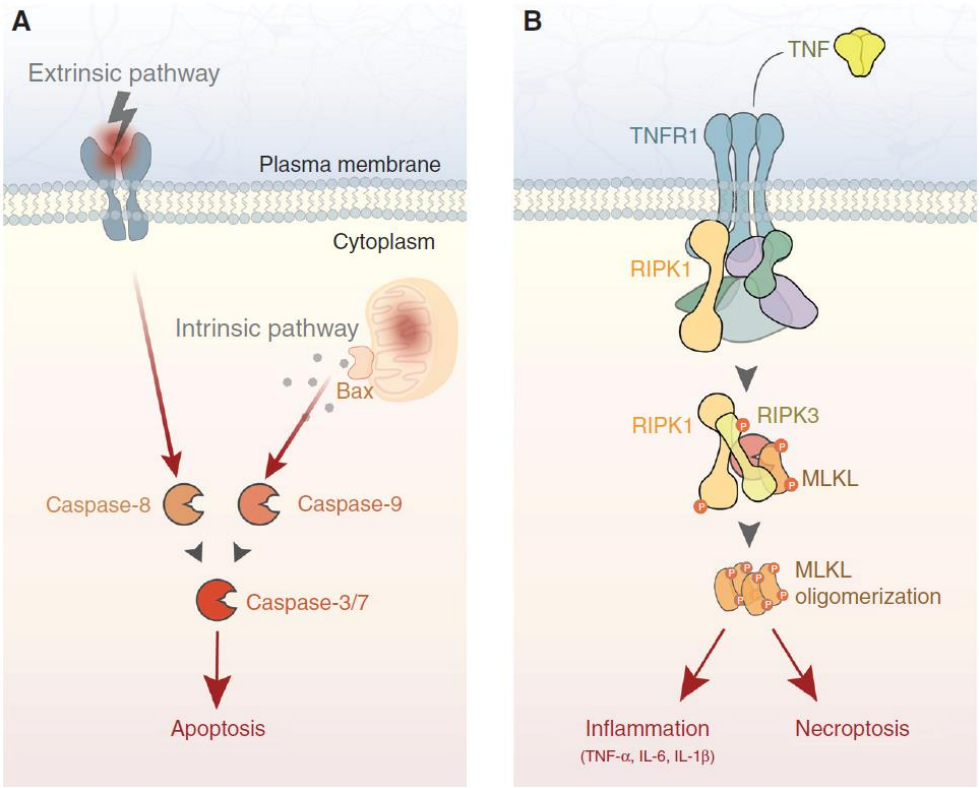


Figure 12. Pathways of (A) apoptosis and (B) necroptosis in neurons (90).

Whilst the extrinsic pathway of apoptosis occurs via caspase-8 or caspase-10, the mitochondrial damage following intrinsic pathway activation relies on caspase-9 which gets triggered through activation of proapoptotic BCL-2 family protein BAX and subsequent release of cytochrome *c* from the mitochondrion (90,117). Both intrinsic and extrinsic pathways of apoptosis are mediated by caspase-3 (118) (Fig. 12.A).

Neuronal cell death due to mitochondrial dysfunction can also take place through necroptosis, a programmed form of necrosis which most often occurs due to an inflammatory reaction (119). It is a caspase-independent type of cell death associated with cellular necrotic morphology (90) (Fig. 12.B). Necrosis results in disruption of cell membrane and the subsequent release of cellular components into the extracellular space, culminating in cell death by autolysis (90,120).

The most important molecular factors related to necroptosis are the tumor necrosis factor (TNF), interferon and Toll-like receptor (TLR) signaling pathways (Fig. 12.B) (90). Upon TNF binding, receptor-interacting serine/threonine-protein kinase 1 and 3 (RIPK1/3) phosphorylates mixed lineage kinase domain-like protein (MLKL) and instigates its oligomerization (90). This leads to necroptosis or further tissue inflammation mediated by release of pro-inflammatory

cytokines TNF, IL-6 and IL-1β (90) (Fig. 12.B). In microglia, RIPK1 plays a role in mediating the "cell-autonomous proinflammatory response" (90,121,122) and, by occurring in both neurons and glia, holds a crucial role in direct or indirect neurodegeneration.

One of the main characteristics of many neurodegenerative processes directly connected to apoptosis and necroptosis is axonal degeneration (90) – a programmed process which includes the disruption of axonal processes and loss of their structure and their connectivity (90,123). Axonal degeneration depends not only on "local axonal signaling and transcriptional regulation" within the neuron but also on other forms of programmed cell death – even though it can also occur in its absence (90,124). Here, a crucial role is played by the dual leucine zipper kinase (DLK)/c-Jun N-terminal kinase (JNK) signaling pathway which controls axon growth during development, axonal degeneration and subsequent neuronal apoptosis (Fig. 13). Neuronal stress leads to phosphorylation of DLK and downstream kinases MKK4/7 and JNK2/3 (90), a transcriptional response and consequential induction of the caspase signaling pathway. This event is associated with the activation of sterile alpha and toll/interleukin receptor motif containing 1 (SARM1), which leads to reduction of nicotinamide adenine dinucleotide (NAD^+) and energetic failure. Both events result in activation of calpains and subsequent apoptosis-induced axon degeneration.

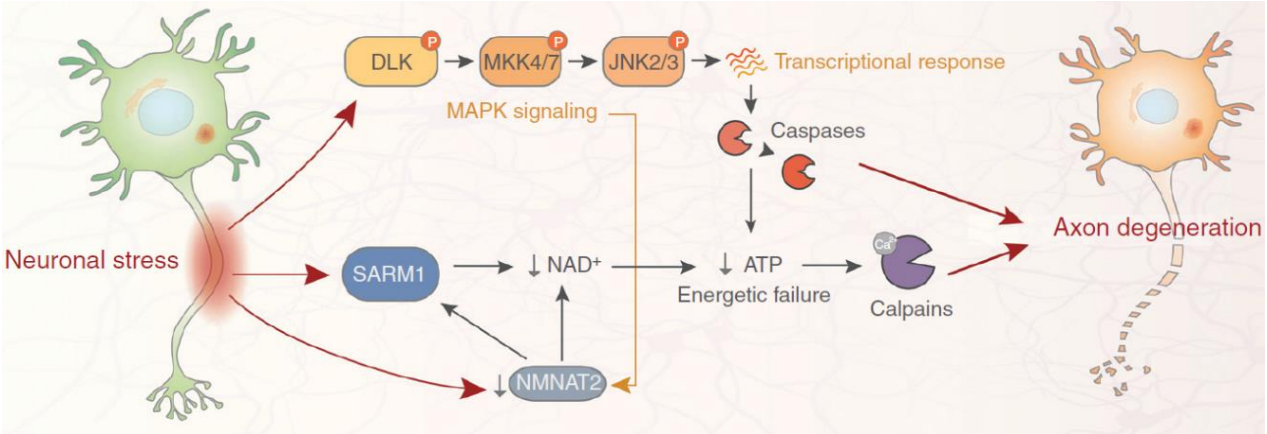


Figure 13. Molecular pathways leading to degeneration of axons (90).

c) Lysosomal dysfunction

Functional impairment of lysosomes also plays an important role in neurodegeneration. These membrane-bound cell organelles play a role in breakdown of many biomolecules. Disruption in lysosomal function is linked to over 50 lysosomal storage diseases. Many of them also exhibit various forms of neurodegeneration (108,110).

The main role of lysosomes is the removal of cellular debris. Accordingly, their dysfunction, (together with dysfunctional autophagy mechanisms), results in accumulation of misfolded proteins and their improper cellular clearance shown in many neurodegenerative diseases (AD, PD, HD) and other neurodegenerative pathologies.

Lysosomal dysfunction is not the only impaired cellular organelle observed in neurodegeneration. Mitochondrial dysfunction is also implicated in many NDDs. Previously thought to be a consequence of neurodegeneration, mitochondrial dysfunction is currently considered as an underlying reason for its onset (90,125,126).

d) Mitochondrial dysfunction

Mitochondria are the powerhouse of the cell. They are involved in generation of ATP, maintenance of Ca^{2+} homeostasis, apoptosis activation and regulation of oxidative stress (127). But, through alternating mitochondrial dynamics and DNA, the process of biogenesis, Ca^{2+} homeostasis, malfunction of apoptosis and alteration of cellular metabolism, mitochondria also play a large role in contributing to neuronal death and degeneration. Many research studies explore the connection between mitochondrial dysfunction and misfolded protein aggregation in major neurodegenerative diseases (128).

Numerous genes, directly, or indirectly associated with the onset of PD, have also been shown to play an important role in mitochondrial functioning. The one that has been best studied so far is PTEN-induced kinase 1 (*PINK1*). Associated with an early-onset PD, mutations in PINK1 disrupt electron transport chain as well as the mitochondrial membrane potential, ultimately resulting in impaired mitochondrial function (110,129).

Another protein, Parkin, which is also involved in early-onset PD, plays an opposing role with respect to mitochondrial function. When mutated in patients with PD, Parkin serves a protective role through amplifying PINK1 mitophagy signal (110).

Deregulated function of PINK1 and Parkin manifests as cellular inability to clear damaged mitochondria from axons, via mitophagy. Under stress conditions, PINK 1 and Parking also play a role in weakening the apoptosis pathway by diminishing BAX recruitment to the membrane of the mitochondrion (90,130).

e) Impaired protein homeostasis: accumulation, seeding and aggregation of misfolded proteins

Proteinopathies are diseases which are characterized by pathological occurrence of clumps of accumulated, misfolded proteins with disoriented domains and without specific secondary structure (109). Once proteins change their conformation they can act like "seeds" that alter the conformational state of other proteins in the brain, leading to their misfolding and subsequent aggregation (110,131).

The innate physiological mechanisms for preventing protein aggregation (macroautophagy, chaperone-mediated autophagy and the ubiquitin-proteasome system), are altered in NDDs, at least to a certain extent (109). Exhibiting properties of prion-like spreading, the misfolded proteins can be seeded and propagated throughout the brain in a "connectome-dependent fashion" (132,133). This is best seen in the cases of AD and PD and mostly occurs in proteins which are biased to misfolding due to their "higher cellular concentration relative to their intrinsic solubility" (134,135).

The most common proteinopathies include AD, PD, HD, frontotemporal dementia and spinocerebellar ataxia type I.

In AD, protein aggregates can occur in one of two states: "soluble intracellular (monomer to oligomer) aggregates" or "insoluble extracellular (proto-fibril to fibrils) aggregates", both of which consist mainly of beta amyloid (Aβ) and hyperphosphorylated tau protein (tau) neurofibrillary tangles (NFT) (109,136,137). In PD, on the other hand, intracellular aggregates are formed by α-synuclein (α-Syn) proteins and are known as Lewy Bodies (132). The precise underlying causes behind the formation of neurofibrillary tangles and Lewy Body formations is still unknown.

Since the main characteristics of NDDs is the death of neurons and two of the most widespread NDDs (AD, PD) occur due to an aggregation of toxic proteins, it is highly likely that this protein aggregation somehow plays a role in neurodegeneration associated cell death. The most widely accepted theory is that aggregate formation disables protein's physiological functions at their native location (90).

On the other hand, many studies claim that the "toxic-gain-of-function" rather than "loss-of-

function" of misfolded proteins is what actually contributes to neuronal cell death in NDDs (90). This toxic-gain-of-function is thought to be mediated by severe stress of the endoplasmic reticulum (ER) which coincides with the onset of neurodegeneration. The proposed mechanism shifts the unfolded protein response (UPR) pathway towards proapoptotic signaling (138,139). Such severe or sustained ER stress is a direct result of the action of misfolded proteins.

f) Oxidative stress

Oxidative stress is a phenomenon caused by an imbalance between reactive oxygen species (ROS) generation and cellular inability to remove them. ROS is normally produced during oxidative phosphorylation and removed with cellular antioxidative mechanisms: "glutathione, superoxide dismutase, catalase or peroxiredoxins" (109). This delicate intracellular balance between antioxidants and oxidants is highly dependent on mitochondria, ER, peroxisomes and different enzymes (e.g., NADPH oxidases) (109). Upon occurrence of mitochondrial dysfunction as well as upregulation of free radical production, associated with insufficient endogenous cellular antioxidative mechanisms, - oxidative stress occurs (109).

In neurodegenerative diseases such as AD, the aggregation of misfolded proteins can induce activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases which, in turn, increases the production of free radicals and increase the affinity of N-methyl-D-aspartase receptors (NMDAR) for N-methyl-D-aspartate (NMDA). This newly formed excitotoxicity, in the form of excess free radical production, promotes Ca^{2+} influx into the cell, which further increases production of ROS (109,140,141).

Sharing a great overlap in pathophysiology, a similar case could be made for oxidative stress in PD. In PD, dopamine gets auto-oxidized within the cytoplasm of dopaminergic (DA) neurons (109,142) and, through provoking a constant flow of Ca^{2+} ions, controls the basal firing rate of these neurons (109,143). This surged energy demand increases neuronal susceptibility to oxidative stress through overproduction of free radicals (109,142,144).

The generation of free radicals, which can be recorded in most neurodegenerative conditions, causes peroxidation of lipids and formation of 4-hydroxynonenal (4-HNE) (109). Newly formed 4-HNE reacts with proteins, altering their conformation and generating adducts in both the neurons containing neurofibrillary tangles as well as the normal pyramidal neurons in hippocampus, as shown in AD patients (145).

The accumulation of such events that lead to excessive generation of free radicals disrupts the balance between oxidants and antioxidants within the system. In turn, this activates various proapoptotic pathways leading to death of neurons (109).

g) Alteration of intracellular Ca^{2+} homeostasis

Intracellular Ca^{2+} is one of the crucial second messengers in many pathways throughout the CNS. It plays a role in "neurotransmitter release, synaptic plasticity, astrocyte Ca^{2+} waves, activation of proapoptotic enzymes and many other processes" (109,146). Therefore, the deregulation of calcium homeostasis is one of the main disruptive mechanisms present in neurodegenerative pathologies (147). An imbalance in intracellular Ca^{2+} concentration can give rise to an elevated number of protein aggregates which further alters lysosomal function (109,148), resulting in a disrupted autophagy pathway. Moreover, depending on the degree of deregulation in Ca^{2+} homeostasis, it can also lead to a decreased long-term potentiation (LTP) and increased long-term depression (LTD) (109).

It is hypothesized that the overactivation of glutamate NMDAR presents the main molecular mechanism behind the deregulation of Ca^{2+} homeostasis, characterized by cytotoxic influx of Ca^{2+} into the cell (109).

h) Alteration of metal ion homeostasis

The metal ion homeostasis in the CNS and its alteration are recognized as important factors in neurodegeneration. Because of the nature of charge on these ions, they can react with free radicals in the extracellular space, surging oxidative stress on the cell (109). They can also, indirectly, activate some pro-oxidative enzymes (xanthine oxidase or nitric oxide synthase). Consequently, cytotoxic aggregations of misfolded proteins occur. Iron ions, for example, induce a form of programmed cell death called ferroptosis (109,149).

i) Impairment of neurogenesis

Neurogenesis is the process of production of neurons by neural stem cells (NSC). The word "neurogenesis" exclusively describes the process in adults, as opposed to the term "prenatal neurogenesis", which is used for defining the same process during embryonal/fetal development. The most debating facts in this field relate to the adult neurogenesis existence itself. Many researchers in the field postulate that it is a crucial mechanism of cerebral plasticity

– playing a role in appropriate neural functioning and disease pathogenesis, if deregulated (109,150). One of the crucial regulators of neurogenesis is ongoing neuroinflammation which, in most cases, accompanies some form of neurodegeneration (109,151).

Cognitive deficits are commonly associated with neurodegenerative processes. In these cases, the impairment of neurogenesis is thought to be the main pathogenic molecular mechanism related to a specific set of symptoms. There is evidence of such a phenomenon occurring in rodent models – suggesting a connection between cognitive decline associated with neurodegeneration and decreased neurogenesis (109,152,153).

j) Disruption of RNA homeostasis

Even though many studies were done on genetic mechanisms behind major NDDs, more work remains to be done on demystifying the role of RNA regulation or deregulation (110). As is hypothesized, RNA regulation is at the basis of many neurodegenerative events and its processing is regulated by RNA-binding-proteins (RBP) (154).

The diseases in which disruption of RNA homeostasis makes a significant contribution to pathogenesis, such as ALS, frontotemporal dementia (FTD) and inclusion body myopathy (IBM), exhibit similarities in their clinical presentation, disease pathology and underlying genetic mechanisms. These genetic mechanisms mostly appear to be related with "mutations in low-complexity sequence domains of RBPs" (110). Ultimately, this causes "ubiquitin-dependent autophagy" and an increased functional damage to the membrane organelles such as RNA transport granules – leading to an alteration in RNA homeostasis and propagation of neurodegeneration (110).

k) Glia and neuroinflammatory responses

Glial cells, such as astrocytes and microglia, form a "positive feedback loop between neuronal death and neuroinflammation" (109,155).

Two representative diseases, AD and ALS, show the important role glial cells play in neurodegeneration. In AD, the two major risk factors are specific apolipoprotein E (*APOE*) alleles and aberrant expression of triggering receptor expressed on myeloid cells 2 (*TREM-2*) genes (156). Whilst the occurrence of *APOE* ε4 allele increases the risk of AD, ε2 allele has an opposing role (110,157,158). On the other hand, *TREM2* gene is expressed primarily in microglia

and it enables them to modulate protein aggregates. Therefore, when its expression is altered, *TREM2* gene possesses the ability of altering AD progression and associated level of neurodegeneration (110,159). Contrastingly, mutations in the *SOD1* gene are known to cause ALS (160). In this case, multiple studies have shown that astrocytes play a crucial role in ALS progression. Astrocytes isolated from patients with ALS release cytotoxic factors (such as misfolded wtSOD1) and can even, in human spinal motor neurons (MNs), evoke symptoms and pathological events characteristic for motor neuron diseases (161).

1.3. Neuroinflammation

Neuroinflammation is a process orchestrated and executed by glial cells and peripheral immune cells (162). Even though these cells play the most important role in initiating and sustaining the inflammatory response within the CNS, deeper understanding of the microglia-astrocyte crosstalk and its consequential reshaping of the immune response is still lacking.

Most imbalances which affect the innate immunity in the CNS are also the key factors involved in onset and progression of many, if not most, neurological diseases. Thus, for better understanding of the pathogenesis of the diseases of the CNS, the relationship between the CNS and the immune system, both the innate and adaptive, should be clearly elucidated.

Neuroinflammation is a process occurring due to onset of innate immune response within the neural tissue in order to A) confine potential, or ongoing, infections and B) eradicate pathogens, as well as cell debris and misfolded proteins (162). However, when it comes to chronic neurological disorders, the same process of neuroinflammation becomes permanent and destructive for neuronal cells (162).

Development of neuroinflammation relies heavily on glial crosstalk. Since these cells are present only in the nervous system and are directly, and to the greatest extent, involved in innate immune response, their involvement is crucial for differentiating neuroinflammation from inflammation of the peripheral tissue (163). Still, the exact nature and mechanism of glial cells interaction in neuroinflammatory processes is still not fully understood (162,164,165).

The main problem here relates to the fact that the process of neuroinflammation is extremely

variable and highly dependent on the nature of inflammatory cues, impacting the biological function of both adaptive immune cells and glia. Depending on the onset and course of inflammation, glial cells can either exacerbate or diminish the pro-inflammatory response. As the innate immune response in the CNS constitutes an intertwined crosstalk between glial cells, it is important to not only understand the function and activity of individual cells but rather their communication, specifically the microglia-astrocyte crosstalk.

1.3.1. Classification

Because etiology of many neuroinflammatory disorders and the occurrence of neuroinflammation in neurodegenerative diseases is not well studied, there is no standardized categorization of neuroinflammatory disorders – even though they are presented with distinct and varied clinical symptoms and pathologies. Most common neural disorders that have been classified as primary neuroinflammatory diseases include multiple sclerosis (MS), neuromyelitis optica (NMO) and acute disseminated encephalomyelitis (ADEM) – all of which are consequences of demyelination.

A) Multiple sclerosis (MS)

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disorder of the CNS. The name of the disease stems from the Greek word 'skleros', meaning hard. The main characteristic of MS is the appearance of scarring throughout multiple regions of the brain. Depending on its course and clinical outcome, MS is divided into four distinct types (Table 6).

Table 6. Classification of MS into four different types based on disease progression (166).

Types of MS	Description
Relapsing-remitting (RRMS)	The most common form of MS characterized by temporary flare-ups upon appearance of novel symptoms. This is followed by periods of remission where patients present with no new disease symptoms, even though the disease is still present, and demyelination can still occur.
Secondary-progressive (SPMS)	Characterized by worsening of symptoms over time. Patients with this form of MS can still present with both relapses and remissions but maintain to suffer nerve damage and

	demyelination.
Primary-progressive (PPMS)	PPMS presents itself with slow progression of the symptoms, with no periods or relapse or remission.
Progressive-relapsing (PRMS)	The rarest form of MS which displays acute relapses without remissions, followed by a steady worsening from the beginning of the processes.

The main cause of MS is thought to be an aberrant activation and proliferation of CD4+ T helper cells which penetrate the blood brain barrier and enter the CNS (166). This is possible because they express cell surface molecules P-selectin glycoprotein ligand-1 (PSGL-1), very late antigen-4 (VLA-4), and lymphocyte function-associated antigen 1 (LFA-1) that bind to the corresponding cell adhesion molecules, mucosal vascular addressin cell adhesion molecule 1 (MAdCAM), vascular cell adhesion protein 1 (VCAM), and intercellular adhesion molecule 1 (ICAM) expressed on the BBB cells (Fig. 14) (167). Shortly after passing the BBB, cell surface molecules expressed on CD4+ T helper cells activate surrounding microglia which, in turn, begin secreting cytokines that trigger additional activation of T cells and astrocytes. Consequently, an inflammatory cascade, which leads to neuronal injury and neuronal phagocytosis, develops. Because of these events, new classifications categorize MS as a neurodegenerative disorder (168).

On top of apparent neurodegeneration, these events also initiate an inflammatory cascade that is, to some extent, mediated by innate immune cells of the CNS – astrocytes and microglia. This results in myelin sheath damage followed by demyelination and, in many cases, death of oligodendrocytes and neurons. Because of the complexity of the immune response and the involvement of multiple cell types and molecular mediators (such as cytokines, chemokines and co-stimulatory molecules), the onset of MS is an extremely complex process, as documented with heterogenous clinical presentations and primary symptoms.

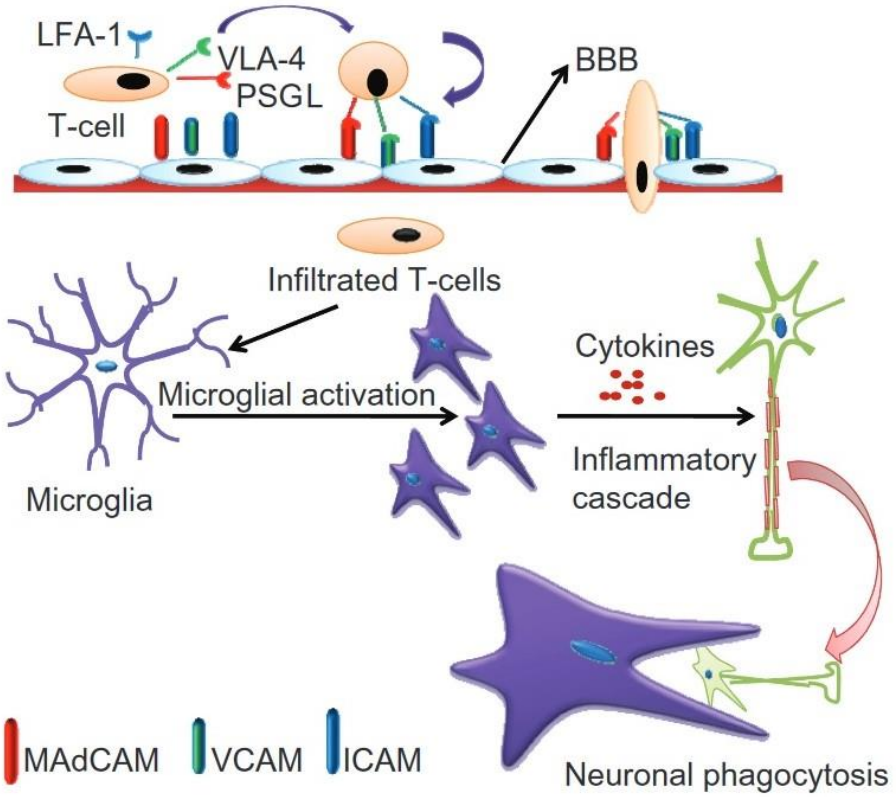


Figure 14. Proposed model of neuroinflammation in MS (*adapted from* (167)).

B) Neuromyelitis optica (NMO)

Neuromyelitis optica (NMO) is also a chronic inflammatory demyelinating disorder which has long thought to be just a form of MS (166). Nevertheless, as of 2006, NMO has been recognized as a distinct neural disorder which presents itself in the form of an optic neuritis and myelitis with a low recovery rate and common relapses (169). One of the most prominent features of NMO is pronounced necrosis and loss of neurons in diencephalon and hypothalamus (166).

Patients with NMO often present with a very high concentration of IL-6 in the CSF as well as aquaporin-4 autoantibodies (AQP4-Ab, also known as NMO-IgG) (166). On the other hand, patients which do not develop AQP4-Ab possess antibodies against myelin oligodendrocyte glycoprotein (MOG) (166).

C) Acute disseminated encephalomyelitis (ADEM)

Finally, as opposed to MS and NMO which are chronic diseases, acute disseminated encephalomyelitis (ADEM) is an acute inflammatory demyelinating disorder often categorized as a type of MS (166). Because it presents itself in the form of encephalopathy as well as

multifocal inflammation of the CNS, it is also known as a clinically isolated syndrome (CIS) (166). Unlike in patients with MS, patients with ADEM present with lymphocytic pleocytosis (an abnormal number of lymphocytes in the CSF) and less cerebrospinal fluid (CSF) oligoclonal bands (OCBs) than the patients suffering from MS (166).

Even though its clinical presentation will include visible lesions throughout the brain, ADEM is, as opposed to MS and NMO, a "monophasic illness" and it needs no disease modifying therapy (DMT) (166).

1.3.2. Neuropathology and molecular mechanisms of neuroinflammation

The innate immune system responds instantaneously to an injury or a pathogen in a stereotypical manner following a rule of a general response pattern. On the other hand, the cells of the acquired immune system adapt their response to individual threats through antibody- and antigen- recognition system. Within the CNS, a large portion of the immune response is mediated by astrocytes and microglia.

One of the most important structural components of microglial cells are their motile processes whose motion is regulated by release of ATP (162). This tonic increase in extracellular ATP occurs via connexin and pannexin hemichannels in astrocytes and through pannexin 1 hemichannels in neurons, both of which are triggered by activation of N-methyl-D-aspartate receptor (NMDAR) (162) (Fig. 15).

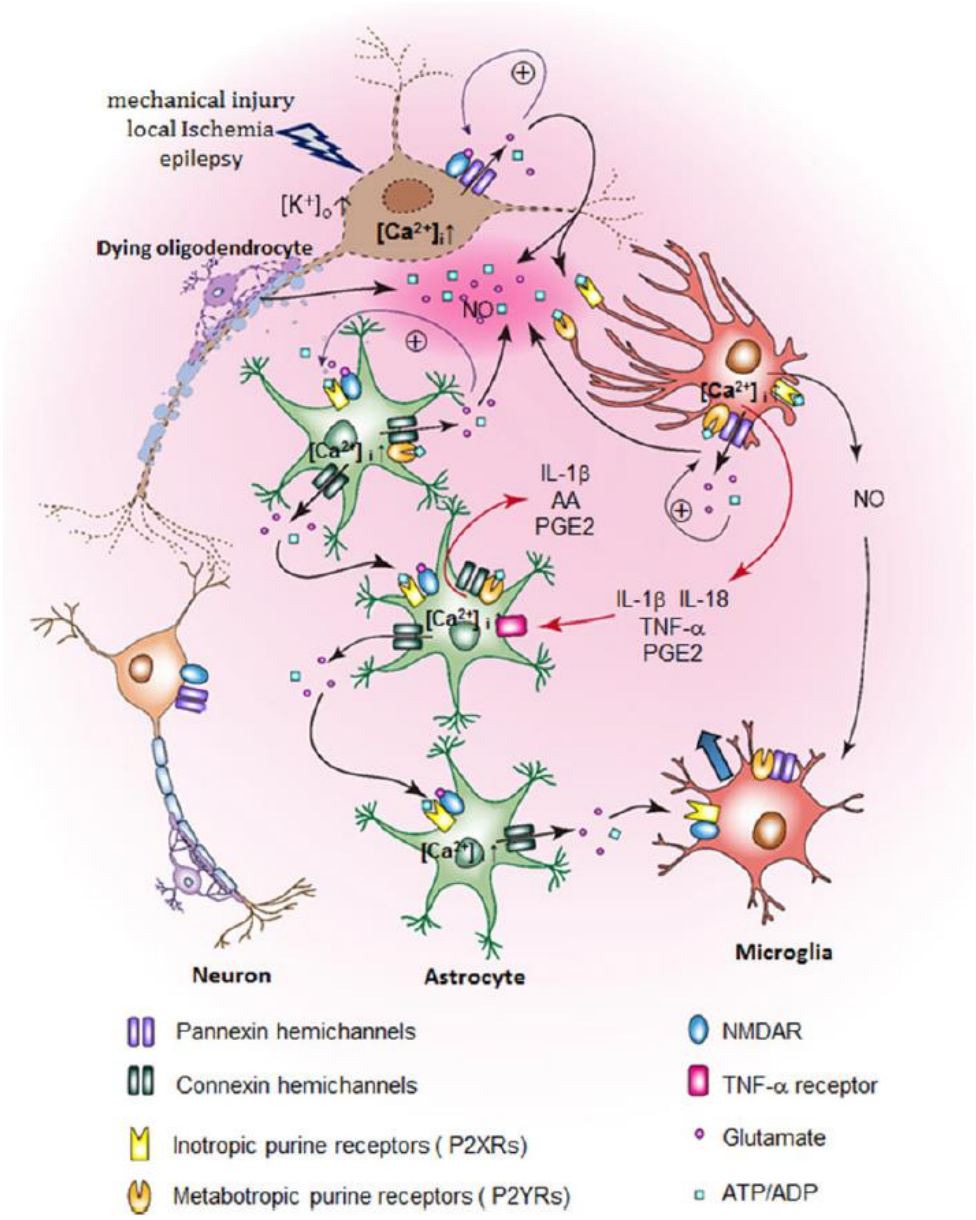


Figure 15. The role of glial cells in initiation and propagation of neuroinflammation (162).

Upon occurrence of a large energetic and metabolic stress in CNS induced by noxa, such as local ischemia or mechanical injury, dying neurons and oligodendrocytes initiate a release ATP in high concentrations. The upregulation of extracellular ATP recruits nearby glial cells, instigating the formation of bulbous termini by microglia and extending the injury site (170). This process is followed by induction of microglial chemotaxis by extracellular ATP/ADP through activation of metabotropic P2Y12 (P2Y12R) and ionotropic P2X4 receptors (P2X4R) expressed on microglial processes (162). Once activated, these receptors induce the activation of intracellular phosphoinositide 3-kinase (PI3K) signaling and cause an increase in intracellular Ca^{2+}. As a consequence, integrin activation occurs, resulting in "promotion of adhesion of microglial processes to the extracellular matrix" (162,171).

Further activation of P2C7R on microglia by increased extracellular ATP can also induce intracellular Ca^{2+} influx and K^{+} outflux (162). This is followed by P2X7R/NLRP3 association and onset of NLRP3 inflammasome and caspase-1 cascades, resulting with an overproduction of pro-inflammatory cytokines IL-1β and IL-18 (162,172). Since the increase of potassium in the extracellular space can open pannexin 1 channels on neurons and astrocytes, this process also results in further activation of other NLRP inflammasomes (NLRP1 and NLRP2), increasing the processing rate of caspase-1 and enhancing the production of pro-inflammatory cytokines (162,173).

Elevated levels of ATP in the extracellular space can also activate P2Y1R present on astrocytes, inducing production of arachidonic acid (AA) and PGE2 (162). This, in turn, causes an activation of P2Y6R on microglia, altering the release of NO and furthering the inflammatory response (162,174).

All of these establish a positive feedback loop between microglial and astrocytic activation, leading to further elevation of extracellular ATP associated with subsequent microglial release of pro-inflammatory factors (IL-1β, TNF and PGE2) as well as astrocytic activation and initiation of Ca^{2+} wave propagation (162,175). This induces further release of ATP and glutamate by astrocytes and sets off Ca^{2+} transients in surrounding cells within the tissue through activation of NMDA and purinergic receptors (162,176).

On top of extracellular ATP and intracellular calcium levels, PAMPs and DAMPs can also activate the innate immune response through PRRs. Primary PRR expressed by astrocytes and microglia are TLRs. As much as their main role is mediation of the innate immunity, they can also contribute to CNS tissue damage. Upon TLR activation, astrocytes and microglia initiate cytokine release (IFN-α, IFN-β, IL-1β, IL-6, IL-10, IL-12, IL-18 and TNF), production of ROS and chemokine secretion (162,177) which can, in acute phases of inflammation, amplify the immune response and contribute to further tissue damage.

1.4. Electromagnetic fields

Electromagnetic field (EMF) is a vector field created by the displacement of electrically charged

objects where energy interactions with other electric charges or electromagnetic fields occur. As the name suggests, it is the combination of both electric and magnetic fields.

Whilst electric field describes the space within which an electric charge exerts an electric force upon another charged body in its vicinity, a magnetic field is a field within which a charge will experience a magnetic influence (178).

According to their nature, strength and direction, electromagnetic fields are divided into homogeneous and inhomogeneous. While homogeneous fields are characterized by spatial consistency, inhomogeneous fields are variable in space. If there is a change in the nature of the field in time, the field is called a time-varying field. Not only do these fields then interact with charges in their vicinity, but they also have the ability to influence each other. Just as a changing magnetic field induces an electric field, a changing electric field induces a magnetic field (174). Detailed description and significance of charges and electromagnetic fields can be found in Appendix B: Electrostatics – charges and fields, Appendix C: Electric fields in matter, Appendix D: Magnetostatics and Appendix E: Magnetic fields in matter.

Generally, the actions of the electromagnetic field itself can be divided into four different parts of the loop (179):

1. the displacement of electric charges induces an electric and magnetic field;
2. the electric and magnetic fields interact;
3. electric and magnetic fields exert forces on electric charges in their vicinity;
4. electric charges move in space.

1.4.1. Innate inhomogeneous electromagnetic fields in the central nervous system

Within the CNS, the neurons propagate signals in the form of an action potential, generated by the flow of ionic currents through the axon. This movement of ions results in intracellular (or longitudinal – parallel to the cellular membrane) and extracellular (or transverse – along the normal to the cellular membrane) current flow; generating a difference in membrane potential together with axial , or "fractional", current flow propagating from the previous to the following axonal segment (180).

Right before the onset of AP propagation, all of the neuronal compartments and segments,

including those that are myelinated, as well as the nodes of Ranvier, carry positive intracellular and negative extracellular surface charge (180). Once the AP starts spreading, it causes a depletion of net charge in the intracellular and deposition of this charge in the extracellular space (180). This current flow, coupled with redistribution of charge along the axonal membrane, generates a time-varying magnetic field around the axon. Since this magnetic fields is changing in time it, in turn, induces an electric field – resulting in a time-varying electromagnetic field surrounding the axon that is of inhomogeneous nature or an inhomogeneous time-varying electromagnetic field (iIT-EMF) (180). The fact that this field is inhomogeneous means that, even though it can be symmetrical, it is not constant in space.

Innate electromagnetic fields are clear indicators of brain activity. Because of this, they are most commonly used to assess the functioning of the brain through a procedure called magnetoencephalography (MEG). MEG records those magnetic fields that result from synchronized, systemic activity of neurons throughout the CNS. As opposed to those uniform static magnetic fields that are measured by MEG, iIT-EMFs around axons occur on a microscopic level due to individual ion influx and outflux through ion channels as well as minute fractional, also called "axial", current flow (180). Because it is highly related to proper functioning of neurons on a microscopic level, this proposed existence of innate inhomogeneous time-varying electromagnetic fields around axons can open novel perspectives towards disease onset. Moreover, these iIT-EMFs could also be used to clarify some neurodegenerative or neuroinflammatory pathologies where neurons get flagged for destruction by immune cells.

The nature of innate electromagnetic fields around neurons greatly depends on the location along the neuron at which they occur or initiate their propagation. The main electrical characteristic of the myelin sheath is its dielectric property which averts charge propagation past its border – and serves as an electrical insulator for the axon (181). Because of this, it prevents dissipation of electrical current, potentiates faster AP propagation and enables AP to maintain its strength at large distances. Since such insulators prevent electric field penetration, only magnetic field can be observed at the regions of myelinated segments. On the other hand, this electromagnetic field presents itself with both the magnetic and electric components at the nodes of Ranvier. This creates apparent periodic breaks in the electromagnetic fields of neurons, potentially even sending aberrant signals to penetrating cells of the adaptive immune system.

All cells carry an innate electric charge, as a result of the presence of charged components on

the plasma membrane and the activity of ion channels (182). This means that they are not only susceptible to external electromagnetic field influence, but that electromagnetism did indeed, or still does, play a role in maintaining their structure or directing their function. Interestingly, some studies have been done aiming to prove the existence of electromagnetic field receptors on human cells. These studies have yielded interesting results which suggest the possibility of humans possessing the ability of magnetoreception (183,184).

1.4.1.1. Source

The main sources of inhomogeneous time-varying electromagnetic fields around neurons are point charge movements within the axon (Fig. 16) (180).

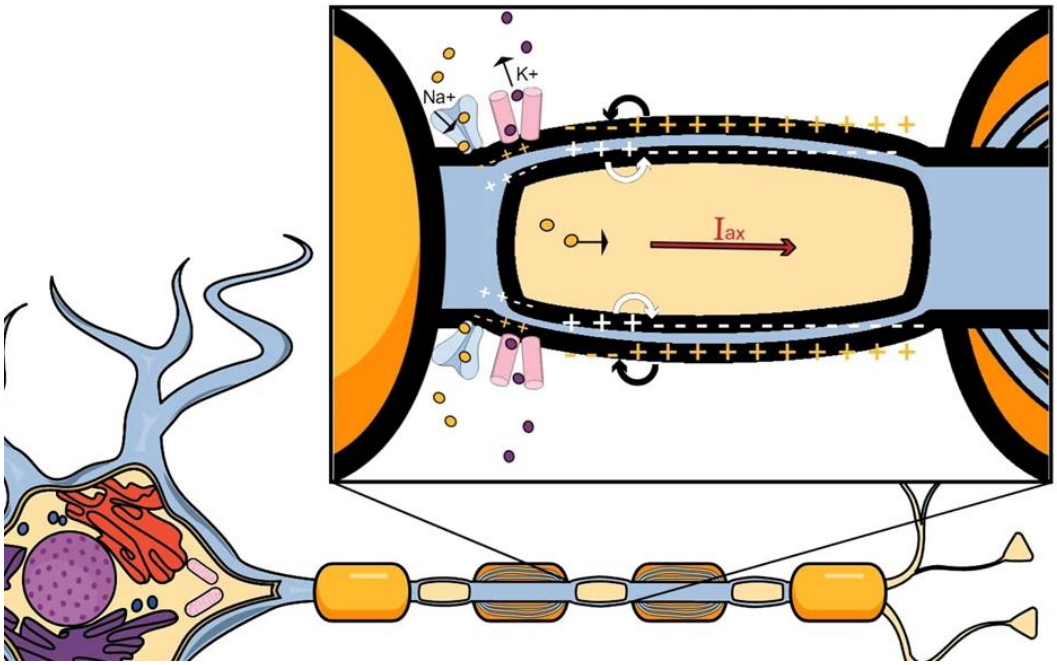


Figure 16. Sources of intracellular (axial/fractional current) and extracellular current (ionic current) within an axon and corresponding innate electromagnetic fields (*adapted from* (180)).

1.4.1.2. Function

Innate inhomogeneous electromagnetic fields around neurons can influence dipole movement within its range. Accordingly, they maintain neuronal signaling and direct cellular function, movement and activity.

Moreover, since some animals such as birds, bees and turtles have magnetoreceptors on most of their cells, enabling them to sense earth's magnetic fields and their changes, it is rational to conclude that electromagnetic fields could have a more important role in organism functioning

and operation than previously thought. The existence of magnetoreceptors could even be one of the evolutionary adaptations, ensuring better survival of the species – potentially even playing the role usually taken by signaling molecules and pathways.

1.4.2. Externally applied homogeneous electromagnetic fields

In recent years, external electromagnetic fields have focused on many organs and tissues in the human body to aid in pro- or anti-inflammatory effects and to trigger tissue regeneration (185,186). For treating disorders of the nervous system, the most common application of external electromagnetic fields includes homogeneous EMF (eH-EMF).

1.4.2.1. Source

As a result of ongoing degeneration, demyelination or inflammation, many ion channels undergo changes in their activation kinetics and their distribution along the cell membrane, resulting in an imbalance of ion flow and extra- or intra-cellular concentration. Since one of the main properties of electromagnetic fields is their ability to influence charged objects in their vicinity, innate and external EMFs can modify charge distribution along the cell membrane. They can also direct movement of ions and control the activity of voltage-gated ion channels (180).

Development of novel therapeutic approaches, and demystification of the etiology of the diseases of the nervous system, is an imperative. Currently, the most common methods of eH-EMF administration to the brain tissue consist of vagal nerve stimulation (VNS), (repetitive) transcranial magnetic stimulation (rTMS), deep brain stimulation (DBS), pulsed electromagnetic fields (PEMFs), low-frequency magnetic fields (LF-MF) and tumor treating fields (TTF) (Table 7).

Table 7. Types of electromagnetic field treatments for the central nervous system (187).

Technology	Physical characteristics	Indications
Vagal nerve stimulation (VNS)	Invasive (implanted neurostimulator) or non-invasive (external stimulator); delivers electric pulses to the left afferent nervus vagus. Uses 20–30 Hz frequencies.	refractory epilepsy (188), major depressive disorder (189) and chronic pain (190)

Repetitive transcranial magnetic stimulation (rTMS)	Non-invasive; delivery of static or time-varying magnetic fields by external coils. Uses both low (≤1 Hz) and high –frequency (>5 Hz) pulses.	treatment resistant depression (191), other anxiety and panic disorders (192)
Deep brain stimulation (DBS)	Invasive; surgically implanted electrodes serve to deliver electrical currents to various cells of the brain. Uses weak currents and frequencies up to hundreds of Hz.	essential tremor (193), Parkinson's disease (194), OCD (195), depression (196) and epilepsy (197)
Transcranial pulsed electromagnetic field (tPEMF)	Non-invasive; externally located coils that emit magnetic fields of 50 Hz in the form of square wave pulses.	therapy resistant depression (198)
Low frequency magnetic field (LF-MF)	Non-invasive; a sine wave delivered continuously, or as a pulse, with a frequency ≤ 300 Hz.	spinal cord injuries (199)
Tumor treating fields (TTF)	Non-invasive; extremely low-intensity electric fields with frequencies ranging from 100 to 300 kHz.	glioblastoma (200) and other tumor types

1.4.2.2. Function

The innate charge on all the cells within the central nervous system makes them susceptible to electromagnetic field influence. In order to take advantage of that, many novel therapies for neuroregeneration or neurorestoration are being designed with the sole purpose of influencing cellular migration, activation and activity.

Several published studies have, thus far, aimed to clarify the importance of EMF in immunomodulation. For example, continuous administration of 60 Hz, 1 mT electromagnetic fields for 13 weeks reduces or suppresses the activity of natural killer cells (NKCs) and causes a decrease in the activation and migration of T cell, neutrophils and macrophages (201). On the other hand, although extremely low-frequency electromagnetic fields (ELF-EMF), in the form of sinusoidal magnetic fields of 300 μT for 1, 2, 3, or 4 h, could not induce neutrophil extracellular traps (NETs), their application could increase the rate of NET formation in neutrophils

previously activated with phorbol 12-myristate 13-acetate (PMA) (202). Additionally, continuous administration of 60 Hz EMF and 0.3 mT has been shown to enhance TGF-β signaling and increase T cell formation (203). Finally, application of both ELF-MF (sinusoid; 50 Hz; 1.0 mT; 45 min) and PEMF (50 Hz; 2.25 mT) to human umbilical cord blood-derived macrophages (204) and macrophage-like cells (205) was shown to induce ROS release (204) and reduce expression of inflammatory cytokines (IL-1β and TNF) (205).

Based on these observations, new applications of electromagnetic fields have emerged, most notably in the form of low frequency electromagnetic fields (ELF-EMF), pulsed electromagnetic fields (PEMF), and tumor treatment fields (TTF). ELF-EMF has been shown to improve patients' functional and mental status. Administration of ELF-EMF was shown to significantly increase the levels of 3-nitrotyrosine and nitrate/nitrite, causing vasodilation and restoration of blood flow in the damaged tissue of post-stroke patients (206). Moreover, studies looking at bone healing and growth have also been performed showing that administration of PEMF increases osteogenesis (185). In addition, research has shown that administration of PEMF causes a great degree of pain relief as well as increased mobility in patients suffering from lower back pain due to degenerative disc disease (207).

1.5. Research aims and hypotheses

Electromagnetic fields are generated around projections of nerve cells (axon, dendrites) during AP propagation. These fields impact cell interactions. Thus, they could also play a role in altering the immune response of the system and impact the progression of some neurodegenerative and neuroimmune disorders. Due to the electromagnetic properties of cells, external electromagnetic fields can also affect the immune response and initiate regeneration of the nervous system.

Starting from the idea that the etiology and molecular mechanisms behind progression of many brain diseases are unclear, the main goal of this work is to describe and explain the influence of electromagnetic fields on inflammation and regeneration of the CNS through modulation of glial cell activity. This will be achieved by describing the nature of inhomogeneous electromagnetic fields around axons, followed by updating the Hodgkin-Huxley model to include transverse and longitudinal current flows. Finally, this work will outline the presumed

influence of electromagnetic fields on glial cells and neurons and predict their potential role in disease etiology and development, as well as nerve tissue regeneration.

2. MATERIALS AND METHODS

In order to propose the very existence of innate inhomogeneous time-varying electromagnetic fields (iIT-EMF) around axons, the time flow of transverse and longitudinal current through the axon was first modeled (180), as seen in the section Quantification of longitudinal and transverse current flow through axons based on the Hodgkin-Huxley (HH) model of a neuron. Then, the strength of the electromagnetic fields around the axon was calculated in a novel way that includes the temporal and spatial propagation of the action potential, obtained from the first step, which can be found in the section Modeling of inhomogeneous electromagnetic fields around neurons generated by the flow of longitudinal and transverse currents. This addition of both the temporal and spatial components of the AP propagation serves as an update to our previous model (180) with inhomogeneous forms of Maxwell's equations. Moreover, this part of the research provides information on the nature of inhomogeneous electromagnetic fields around axons and suggests that, after they become aberrant during degeneration or demyelination, they can positively or negatively affect the response of the cells within the adaptive or innate immune system and, consequently, neuroregeneration or further tissue degeneration.

Furthermore, to propose potential molecular mechanisms of astrocyte and microglia activation under the influence of electromagnetic field, either those around the axons themselves or the external fields, a systematic review of literature was performed, and the proposed molecular mechanisms were summarized (64). Detailed description of all-inclusive and exclusive criteria, as well as the protocol itself, is outlined in the section Systemic literature review: microglia-astrocyte crosstalk and the influence of externally applied homogeneous electromagnetic fields.

2.1. Quantification of longitudinal and transverse current flow through axons based on the Hodgkin-Huxley (HH) model of a neuron

2.1.1. Origin of the Hodgkin-Huxley model

In order to enable mathematical modeling of an axon, a complex electrical and biological

system, it was approximated that an axon behaves like a simple core conductor (wire). As such, it was depicted as a long cylindrical tube consisting of a membrane filled with, and surrounded by, electrically conducing media: the axoplasm and the extracellular fluid, respectively (Fig. 17) (180,208,209). In its modelling, the following basic assumptions were followed:

1. There is no radiation, so Ohm's Law is valid;
2. Radial and/or longitudinal currents are present;
3. Membrane separates two homogeneous conductors – neuron and the extracellular fluid;
4. There is no variation of current or voltage in angular direction;
5. Inner and outer conductors are equipotential;
6. The potential varies in radial direction.

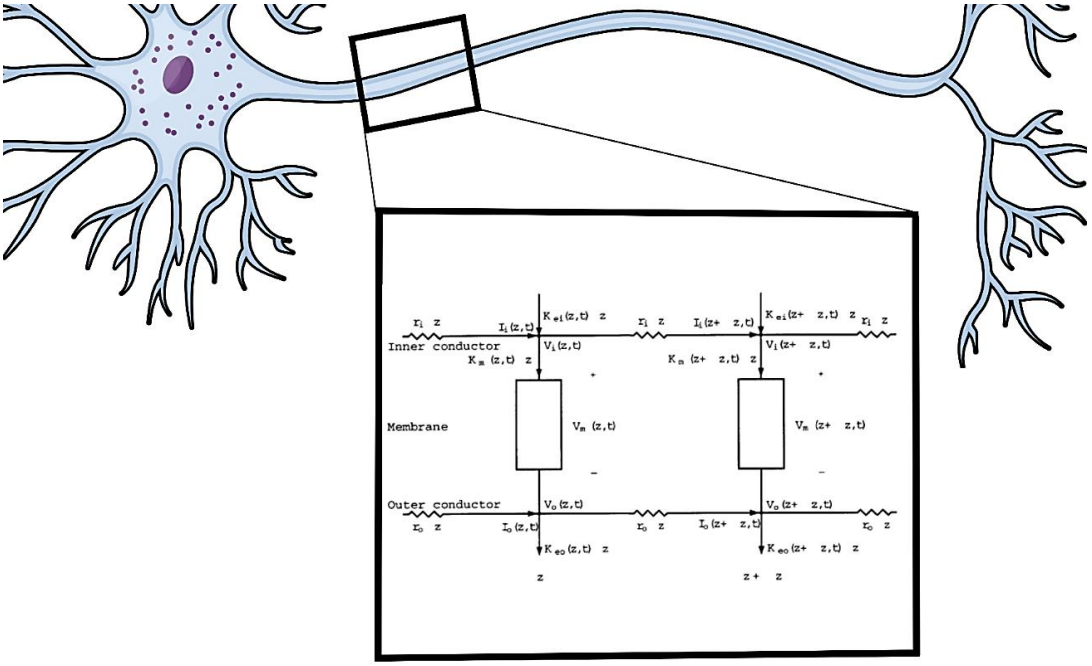


Figure 17. Equivalent circuit model depicting incremental nodes of an unmyelinated axon with denoted direction for both voltage and current variables (*adapted from* (180,209)).

Since the axonal membrane was now broken down into incremental nodes, its properties and activity can be depicted using Kirchhoff's Current Law, which states that a current coming into each of the nodes, or junctions between wires, must be equal to the current going out of the node. In the case of an axon (209,210) the results of these constitutive equations, upon taking the limit as its length approaches zero ($\Delta z \rightarrow 0$), is as follows:

$$\frac{\partial I_i(z,t)}{\partial z} = K_{ei}(z,t) - K_m(z,t) \quad (Eq.\,1.1)$$

$$\frac{\partial I_o(z,t)}{\partial z} = K_m(z,t) - K_{eo}(z,t) \quad (Eq\ 1.2)$$

$$\frac{\partial V_i(z,t)}{\partial z} = -r_i I_i(z,t) \quad (Eq.\ 1.3)$$

$$\frac{\partial V_o(z,t)}{\partial z} = -r_o I_o(z,t) \quad (Eq.\ 1.4)$$

$$V_m(z,t) = V_i(z,t) - V_o(z,t) \quad (Eq\ 1.5)$$

$$\frac{\partial V_m(z,t)}{\partial z} = -r_i I_i(z,t) + r_o I_o(z,t) \quad (Eq\ 1.6)$$

Definitions for each of the aforementioned variables can be found in Table 8 (209).

Table 8. Symbols used in the core-conductor model and their meaning

Symbol	Meaning
K_m	membrane current per unit length of axon
K_{eo}	extrinsic current applied externally
K_{ei}	extrinsic current applied internally
I_i	internal longitudinal current
I_e	external longitudinal current
V_m	potential across the membrane
V_i	internal potential
V_o	external potential

In order to obtain the relationship between the membrane potential (V_m) and the current density at the membrane (J_m), at time t and position z along its cylindrical unmyelinated segment, equations (Eq. 1.1 – 1.6) can be combined to form the core-conductor equation (209). This is done by replacing $K_m = 2\pi a J_m$, where a is the axonal radius.

$$\frac{1}{2\pi a(r_o + r_i)} \frac{\partial^2 V_m(z,t)}{\partial z^2} = J_m(z,t) - \frac{1}{2\pi a(r_o + r_i)} \left(r_o K_{eo}(z,t) + r_i K_{ei}(z,t)\right) \quad (Eq.\ 1.7)$$

This model now forms the basis of the HH model but with inclusion of additional terms which account for the electrical properties of the cell membrane in order to more realistically represent signal propagation through an unmyelinated axon of a giant squid.

2.1.2. The Hodgkin-Huxley model

The Hodgkin-Huxley (HH) model of an axon is a mathematical model describing initiation and

propagation of an action potential through neurons. Historical significance of the aforementioned model can be found in Appendix H: The history of the Hodgkin-Huxley model. It proposed that an axon can be described in terms of electrical circuitry where each ion channel can be represented through one branch of a parallel circuit with a capacitor cell (C) generating ionic and capacitive currents, respectively (Fig. 18). Their conductance (g) is introduced in a form of a variable resistor in each branch, enabling the circuit to account for the potential difference (V_m) across axonal membrane due to an "acquisition of net charge in the extracellular and its depletion in the intracellular space" (180).

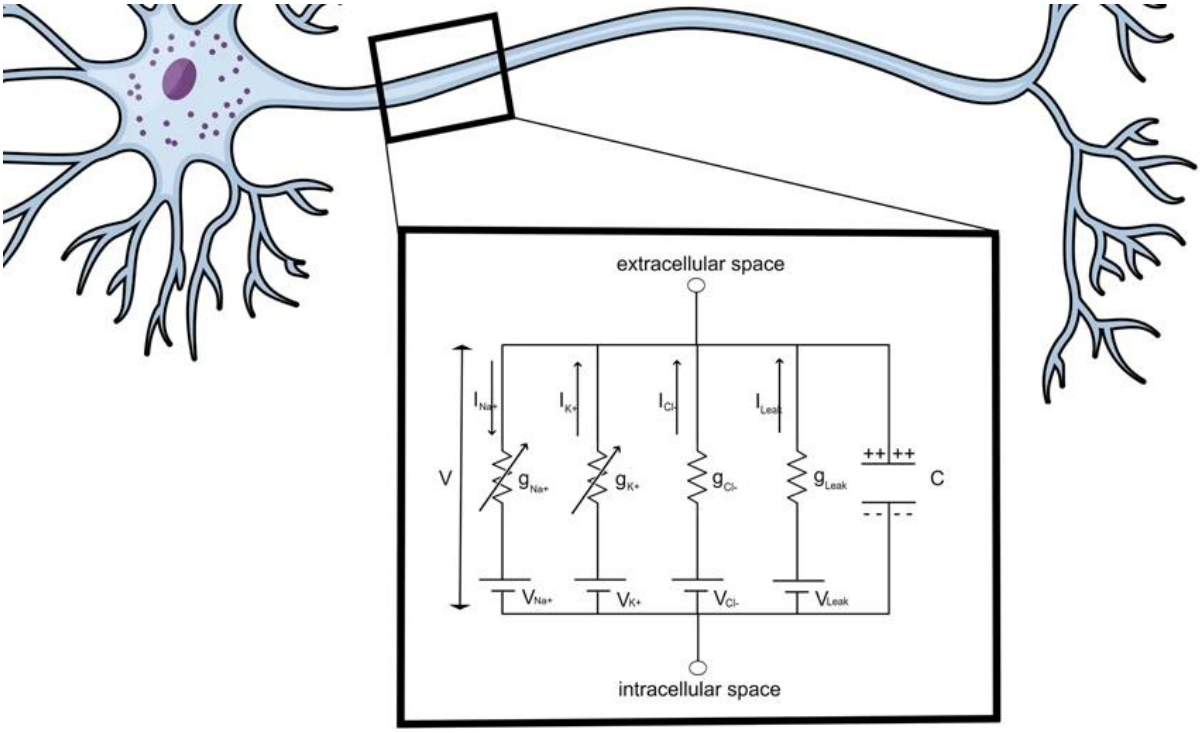


Figure 18. Hodgkin-Huxley model of a neuron (*adapted from* (180)).

Observing this simplified equivalent circuit for an axon, there are two main current sources that can be recognized: the capacitor cell and the ion channels. Whilst the ionic current is the sum of the individual ion currents through each of the channels (Eq 1.8), the capacitive current represents the "change in membrane voltage in time multiplied by cell capacitance" (Eq. 1.9) (180).

$$J_{ion}(z,t) = \sum J_i \quad (Eq.1.8)$$

$$J_C(z,t) = C_m \frac{\partial V_m(z,t)}{\partial t} \quad (Eq.1.9)$$

As Hodgkin and Huxley were working on the giant squid axon using an electrophysiological

setup, their equation also included an additional term in the form of J_{app}, the externally applied current density. Since Kirchhoff's Current Law states that a current coming into a node is exactly equal to the current coming out of the node, as no charge can be lost within the node, then the total membrane current density defined in the core-conductor equation (Eq. 1.7) has to be equal to capacitive and ionic current density within an axon (180).

$$J_m(z,t) = J_C(z,t) + J_{ion}(z,t) \quad (Eq.\,1.10)$$

In order to account for the application of external currents, ionic current density is combined with the term for external current density term (Eq. 1.7) to form,

$$J(z,t) = J_{ion}(z,t) - \frac{1}{2\pi a(r_o + r_i)}\left(r_o K_{eo}(z,t) + r_i K_{ei}(z,t)\right) \quad (Eq.\,1.11)$$

remodeling the core-conductor equation to

$$\frac{1}{2\pi a(r_o + r_i)}\frac{\partial^2 V_m(z,t)}{\partial z^2} = C_m\frac{\partial V_m(z,t)}{\partial t} + J(z,t) \quad (Eq.\,1.12)$$

Delving deeper into each individual segment of the HH mode, the ionic current is equal to the "conductance of the ion channel multiplied by the driving force across the membrane" and it includes the current through sodium, potassium and leak channels (Eq. 1.13) (180).

$$J_{ion}(z,t) = \sum J_i = \sum g_i(V_m,t)(V_m - V_i)$$
$$J_{ion}(z,t) = g_K(V_m,t)(V_m - V_K) + g_{Na}(V_m,t)(V_m - V_{Na}) + g_L(V_m,t)(V_m - V_L) \quad (Eq.\,1.13)$$

Whilst V_m is the membrane potential, V_w is the Nernst equilibrium potential of each ion w, where $w = (K, Na, L)$.

$$V_w = \frac{RT}{z_n F}\ln\left(\frac{c_w^o}{c_w^i}\right)$$

If an ion in question is univalent, such as sodium and potassium, then the Nernst equilibrium potential reads

$$V_w = 0.08616(T_c + 273.16)\ln(\frac{c_w^o}{c_w^i})$$

Corresponding symbols and their meaning can be found in Table 9.

Table 9. Symbols used in the equation for Nernst equilibrium potential and their meaning (209).

Symbol	Meaning
R	molar gas constant
T	absolute temperature
F	Faraday's constant
z_n	valence of the ions
c_w^o	outside concentration of ion w
c_w^i	inside concentration of ion w
T_c	temperature in degrees Celsius

Because sodium and potassium are the main ion channels contributing to charge accumulation and subsequent action potential propagation, they are each associated with an individual variable resistor (Fig. 18). Other ion channels, including those of chloride and calcium, which play a major role in many neurodegenerative and neuroimmune disorders, are accounted for by 'leak' channels and corresponding leak current and conductance distribution (Eq. 1.13) (180).

According to the HH model, this specific ion channel conductance, g_w, depends on activation and inactivation gates of each ion channel (180). At resting state, only the inactivation gate is open, and the activation gate is closed. Depending on the properties of each ion channel, the potential differences across the membrane will trigger opening or closing of different gates. Whilst the potassium ion channels depend on four activation gates, sodium channels depend on three activation and one inactivation gate (180). In HH model, this is taken into account in the term for specific conductance of each ion channel and is denoted by a superscript above specific probabilities - n, m or h. Here, n and m stand for the probability of the potassium and sodium activation gates being open, respectively, while h is the "probability of the sodium inactivation gate being open" (180). This means that, in simplified terms, the conductance of each ion channel at a specific time point depends on average conductance, g_w, and probabilities of activation or inactivation gates being open (180).

$$g_K = g_K(V_m, t) = \overline{g_K} n^4(V_m, t), \quad (Eq.\ 1.14)$$

$$g_{Na} = g_{Na}(V_m, t) = \overline{g_{Na}} m^3(V_m, t) h(V_m, t) \quad (Eq. 1.15)$$

These probabilities are functions of time and membrane potential.

$$\frac{dm}{dt} = \frac{m_\infty(V_m) - m(V_m)}{\tau_m(V_m)}$$
$$\frac{dn}{dt} = \frac{n_\infty(V_m) - n(VV_m)}{\tau_n(V_m)}$$
$$\frac{dh}{dt} = \frac{h_\infty(V_m) - h(VV_m)}{\tau_h(V_m)}$$

Once the membrane voltage is clamped at a fixed value V, the activation and inactivation gates which are in permissive state will, eventually, settle at a steady state value $\frac{dp}{dt} = 0$ as $t \to \infty$, where $p = (n, m, h)$. In order to define the time course needed to approach this equilibrium, the HH model defines a time constant $\tau_p\ (V_m)$, given by

$$\tau_p(V_m) = \frac{1}{\alpha_p(V_m) + \beta_p(V_m)}$$

The allowed rates at which gates transition between non-permissive and permissive state is designated by $\alpha_p(V_m)$ and $\beta_p(V_m)$ in sec^{-1}. As much as these terms are denoted as "rate constants" they are not an actual constant but rather dependent on membrane voltage V_m (209). Whilst $\beta_p(V_m)$ describes the gate transition from permissive to non-permissive state, $\alpha_p(V_m)$ describes the opposite. The rate constants are specific for each ion channel and have been experimentally measured by Hodgkin and Huxley in order to derive a general term describing their dependence on membrane potential (Eq. 1.19) (180,209).

$$\alpha_m(V_m) = -\frac{0.1(35 + V_m + \Delta V_{Ca} + V_{\alpha m})}{e^{-0.1(35+V_m+\Delta V_{Ca}+V_{\alpha m})} - 1} K_T K_m, \qquad \beta_m(V_m) = 4.0e^{\frac{-(V_m+\Delta V_{Ca}+V_{\beta m}+60)}{18}} K_T K_m \quad (Eq. 1.16)$$

$$\alpha_n(V_m) = -\frac{0.01(V_m + \Delta V_{Ca} + V_{\alpha n} + 50)}{e^{-0.1(V_m+\Delta V_{Ca}+V_{\alpha n}+50)} - 1} K_T K_n,$$

$$\beta_n(V_m) = 0.125e^{-0.0125(V_m+\Delta V_{Ca}+V_{\beta n}+60)} K_T K_n \quad (Eq. 1.17)$$

$$\alpha_h(V_m) = 0.07e^{-0.05(V_m+\Delta V_{Ca}+V_{\alpha h}+60)} K_T K_h, \qquad \beta_h(V_m) = \frac{1}{1 + e^{-0.1(V_m+\Delta V_{Ca}+V_{\beta h}+30)}} K_T K_h \quad (Eq. 1.18)$$

As opposed to the original HH model, an additional temperature factor was added, K_T, in order to account for the temperature effect on membrane's electrical properties (209,211).

$$K_T = 3^{\frac{T_c - 6.3}{10}}$$

Additionally, K_m, K_n and K_h (209) are also appended to the original HH equations in order to describe the relationship between rate constants and the membrane potential (V_m) to allow for individual modifications in rate constants of m, h and n, in cases of pathological changes. In order to further approximate the influence of calcium channels which have originally been disregarded in the HH model, a factor of ΔV_{Ca} has been added (209).

$$\Delta V_{Ca} = 0.02225(T_c + 273.16)[\ln(\frac{c_{Ca}^{o}}{c_{Ca}^{i}}) - 12.995] \quad (Eq. 1.19)$$

When the calcium concentrations are normal, $\Delta V_{Ca} = 0$. On the other hand, if a pathological event occurs or the signaling pathway gets disrupted by an ongoing immune response or tissue degeneration, $\Delta V_{Ca} \neq 0$. This additional term estimates the effect that changing levels of calcium have on action potential propagation and axonal properties (209,211). Besides that, terms such as V_{qp} (209), where $q = (\alpha, \beta)$ and $p = (n, m, h)$, which have a zero value in the original HH model, can also be useful in modelling the subsequent shift of rate constant values after alteration of the membrane potential.

Contribution of other ion channels is taken into account through leak channel conductance, denoted by a subscript L (180).

$$g_L = g_L(V)$$

An important simplification that is introduced here is the fact that ion channel conductance depends solely on the potential difference across the membrane - even though we now know that that is not always the case (180). Conductance of each individual ion channel not only depends on the membrane potential but also on the activity of the surrounding channels.

Knowing that ionic currents are not contained to merely their segments (180), the next step is to remodel the extrinsic current applied to the axon in the HH model into an additional longitudinal current term that contributes to AP propagation.

To account for the spatio-temporal propagation of an action potential not only under the

influence of externally applied impulses but also intracellular charge movement, the term "fractional current" is introduced (180). Fractional current is an update on the "axial current" term (180) which was a result of continuous flow of ions through the cell's membrane and through the axon. With that, the new fractional current term describes the fraction of the total current propagating from the previous to the subsequent axonal segment (Fig. 19).

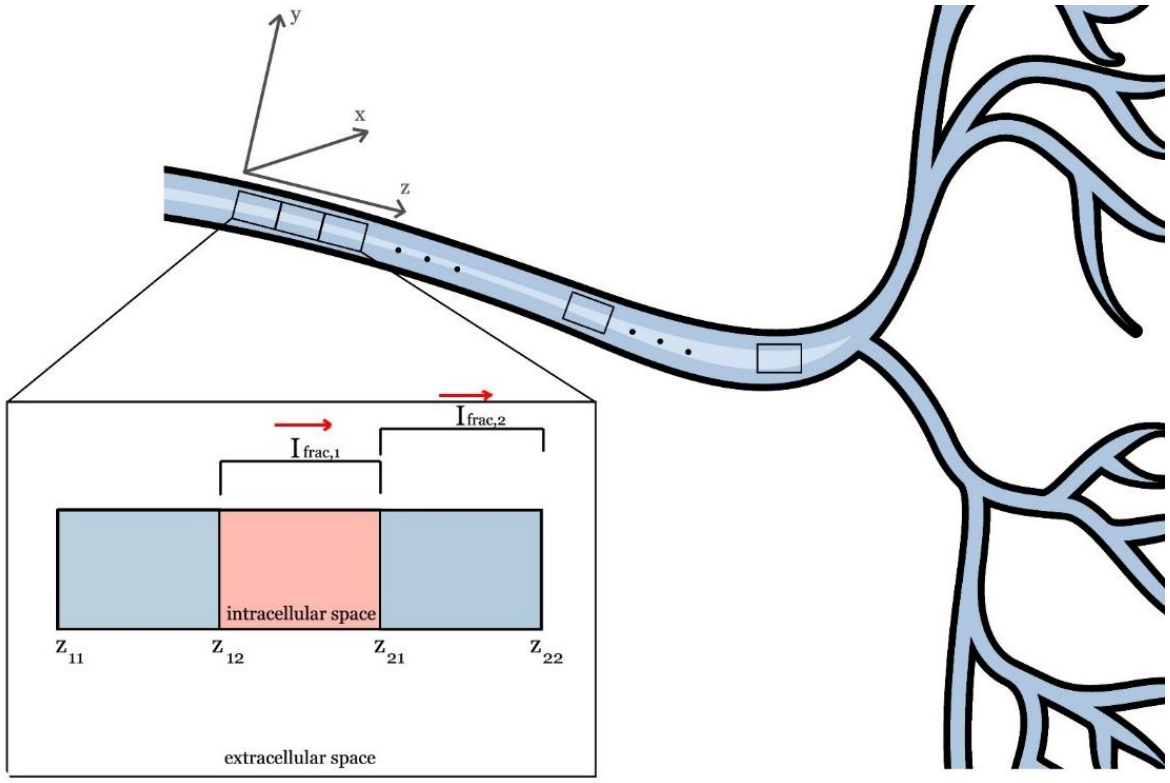


Figure 19. The relation of fractional currents in an axon with respect to z coordinates (*Created with MindTheGraph.com*).

As Kirchhoff's Current Law must hold true here as well, at any point along the axon the sum of the currents has to be equal to zero.

$$I_i(z,t) + I_o(z,t) + I_{frac}(z,t) = 0 \quad (Eq.\,1.20)$$

Whilst $I_i(z,t)$ is the intracellular and $I_o(z,t)$ the extracellular current, $I_{frac}(z,t)$ is the current propagating in $+z$ direction due to impulse propagation in the previous segment.

$$I_{frac}(z,t) = I_{frac,1}(t)(u(z-z_{12}) - u(z-z_{21})) + I_{frac,2}(t)(u(z-z_{21}) - u(z-z_{22})) \quad (Eq.\,1.21)$$

$u(z)$ is defined as a unit step function which follows (209)

$$u(z) = \begin{cases} 1 & if\ z > 0 \\ 0 & if\ z < 0 \end{cases} \quad (Eq.\ 1.22)$$

Considering that these fractional currents occur intracellularly, the current per unit length from the core-conductor equation (Eq. 1.7) can now be defined as

$$K_{eo}(z,t) = 0$$
$$K_{ei}(z,t) = -I_{frac,1}(t)(\delta(z - z_{12}) - \delta(z - z_{21})) + I_{frac,2}(t)(\delta(z - z_{21}) - \delta(z - z_{22})) \quad (Eq.\ 1.23)$$

Since the innate electromagnetic fields around axons are a result of ion movement in longitudinal and transverse direction, as well as subsequent action potential propagation, they are non-uniform and time-varying in nature.

The longitudinal current accounts for the current generated with propagation of the action potential as well as ion movement from one segment to another in the form of fractional current.

On the other hand, transverse current accounts for influx and outflux of ions between and from the intracellular and extracellular space that is not mediated by sodium, potassium and leak channels. Since the assumption is now made that the current flow through an axon occurs in two directions, those two directions must be defined.

2.1.2.1. Longitudinal current

Longitudinal currents are those currents whose direction of propagation is parallel to the axon and include the capacitive, ionic and fractional currents.

$$I_{lng,i}(z,t) = -\frac{1}{r_o + r_i}\frac{\partial V_m(z,t)}{\partial z} - \frac{r_o}{r_o + r_i} I_{frac}(z,t) \quad (Eq.\ 1.24)$$
$$I_{lng,o}(z,t) = \frac{1}{r_o + r_i}\frac{\partial V_m(z,t)}{\partial z} - \frac{r_i}{r_o + r_i} I_{frac}(z,t) \quad (Eq.\ 1.25)$$

2.1.2.2. Transverse current

Contrastingly, transverse currents are those which occur perpendicular to the axon. They mostly consist of ion flow through calcium and other channels that are not flowing in the z direction, since they do not directly contribute to AP propagation.

$$I_{tr}(y,t) = g_{Ca}(V_m,t)(V_m - V_{Ca}) \quad (Eq.1.26)$$

2.1.2.3. *Potential difference*

Defining the potential difference in terms of membrane potential as well as fractional and longitudinal currents results in rewriting of the basic terms in the form of

$$V_i(z_{f2},t) - V_i(z_{f1},t) = \frac{r_i}{r_i + r_o}(V_m(z_{f2},t) - V_m(z_{f1},t)) + \frac{r_i r_o}{r_i + r_o}\int_{z_{f1}}^{z_{f2}}(I_{frac}(z,t) + I_{tr,i}(z,t))dz \quad (Eq.1.27)$$

$$V_o(z_{f2},t) - V_o(z_{f1},t) = -\frac{r_o}{r_i + r_o}(V_m(z_{f2},t) - V_m(z_{f1},t)) + \frac{r_i r_o}{r_i + r_o}\int_{z_{f1}}^{z_{f2}}(I_{frac}(z,t) + I_{tr,o}(z,t))dz \quad (Eq.1.28)$$

Since I_{frac} is defined as in equation (Eq. 1.21), then the definite spatial integral for the longitudinal and transverse current can be rewritten as

$$\int_{z_{f1}}^{z_{f2}}(I_{frac}(z,t) + I_{tr,o}(z,t))dz = \int_{z_{f1}}^{z_{f2}} I_{frac}(z,t)dz + \int_{z_{f1}}^{z_{f2}} I_{tr,i}(z,t)dz$$

$$\int_{z_{f1}}^{z_{f2}}(I_{frac}(z,t) + I_{tr,o}(z,t))dz = I_{frac,1}(t)((z - z_{12})u(z - z_{12}) - (z - z_{21})u(z - z_{21})) + I_{frac,2}(t)((z - z_{21})u(z - z_{21}) - (z - z_{22})u(z - z_{22})) + X\int_{z_{f1}}^{z_{f2}} I_{tr,i}(z,t)dz \quad (Eq.1.29)$$

More extensive details on the electric potential can be found in Appendix C: Electric potential.

2.1.2.4. *The Crank Nicholson method for solving the Hodgkin-Huxley equation*

To account for the temporal dependence of the potential difference along the neuron, the HH model is solved in a compartmental model using the Crank-Nicholson method (180). For performing this, membrane potential and current density components of the remodeled core-conductor equation (Eq. 1.12) first need to be discretized by introducing increments in z and t, Δz and Δt, respectivelly (209).

$$V_i^j = V_m(i\Delta z, j\Delta t) = V_m(z,t)|_{z=i\Delta z, t=j\Delta t} \quad (Eq.1.30)$$

$$J_i^j = J(i\Delta z, j\Delta t) = J(z,t)|_{z=i\Delta z, t=j\Delta t} \quad (Eq.1.31)$$

Using the centered difference method, the second derivative can now be approximated as

$$\frac{\partial^2 V_m(z,t)}{\partial z^2} \approx \frac{V_{i+1}^{j} - 2V_i^{j} + V_{i-1}^{j}}{(\Delta z)^2} \quad (Eq.\,1.32)$$

whilst the temporal derivative at time t is

$$\frac{\partial V_m(z,t)}{\partial t} \approx \frac{V_i^{j+1} - V_i^{j}}{\Delta t} \quad (Eq.\,1.33)$$

In this way, the model takes into consideration both the spatial and temporal propagation of the action potential, increasing its fidelity and dispelling any numerical instabilities (180,209).

Once the spatial (Eq. 1.32) and temporal (Eq. 1.33) derivatives of the membrane potential are obtained, they can be introduced into the core-conductor equation (Eq. 1.12) to form

$$\frac{1}{4\pi a(r_o + r_i)}\left(\frac{V_{i+1}^{j+1} - 2V_i^{j+1} + V_{i-1}^{j+1}}{(\Delta z)^2} + \frac{V_{i+1}^{j} - 2V_i^{j} + V_{i-1}^{j}}{(\Delta z)^2}\right) = C_m \frac{V_i^{j+1} - V_i^{j}}{\Delta t} + \frac{1}{2}\left(J_i^{j} + J_i^{j+1}\right) \quad (Eq.\,1.34)$$

Next, in order to obtain the current density at J_i^{j+1}, where p is again set to stand for $p = (n, m, h)$, the probabilities of ion channel activation/inactivation gates opening need to be defined.

$$\frac{p_i^{j+1} - p_i^{j}}{\Delta t} = \frac{\alpha_p(V_i^{j+\frac{1}{2}}) - p_i^{j+1}(\alpha_p(V_i^{j+\frac{1}{2}}) + \beta_p(V_i^{j+\frac{1}{2}}))}{2} + \frac{\alpha_p(V_i^{j+\frac{1}{2}}) - p_i^{j}(\alpha_p(V_i^{j+\frac{1}{2}}) + \beta_p(V_i^{j+\frac{1}{2}}))}{2} \quad (Eq.\,1.35)$$

Solving for p_i^{j+1}

$$p_i^{j+1} = \frac{\frac{\Delta t}{2}\alpha_p(V_i^{j+\frac{1}{2}})}{1 + \frac{\Delta t}{2}(\alpha_p(V_i^{j+\frac{1}{2}}) + \beta_p(V_i^{j+\frac{1}{2}}))} + p_i^{j}\frac{1 - \frac{\Delta t}{2}(\alpha_p(V_i^{j+\frac{1}{2}}) + \beta_p(V_i^{j+\frac{1}{2}}))}{1 + \frac{\Delta t}{2}(\alpha_p(V_i^{j+\frac{1}{2}}) + \beta_p(V_i^{j+\frac{1}{2}}))} \quad (Eq.\,1.36)$$

Since p stands for n, m and h, the general form of equation (Eq. 1.36) can be introduced to the ionic current density equation (Eq. 1.13) combined with (Eq. 1.14) and (Eq. 1.15) to obtain ionic current density at time $j + 1$, after proper variable substitution.

$$(J_{ion})_i^{j+1} = \overline{g_K}(n_i^{j+1})^4\left(V_i^j - V_K\right) + \overline{g_{Na}}(m_i^{j+1})^3 h_i^{j+1}\left(V_i^j - V_{Na}\right) + g_L\left(V_i^j - V_L\right) \quad (Eq.1.37)$$

Now, according to equation (Eq.1.11) the total current density within a neuron is

$$J_i^{j+1} = (J_{ion})_i^{j+1} - \frac{1}{2\pi a(r_o + r_i)}\left(r_o(K_{eo})_i^{j+1} + r_i(K_{ei})_i^{j+1}\right) \quad (Eq.1.38)$$

2.2. Modeling of inhomogeneous electromagnetic fields around neurons generated by the flow of longitudinal and transverse currents

Since, according to Gauss' law for magnetism (Eq. 2.1), a magnetic field has no sources nor sinks - meaning that its field lines can only form closed loops - it is physically impossible for a homogeneous magnetic field to exist.

The general consensus within the field is that the properties of electromagnetic fields around neurons are the same at any point in space. Nevertheless, current dissipation, distribution of ion channels along the membrane as well as other infinitesimal charge movements cause the fields to vary in space. This means that the fields generated by impulse propagation along neurons can be symmetric but are not homogeneous (180).

In order to model those fields, Maxwell's equations must be modified to give rise to inhomogeneous wave equations for the electric and magnetic field. Maxwell's equations (Eq. 2.1, 2.2, 2.3 and 2.4) represent a set of coupled partial differential equations (PDEs) that provide a mathematical model for describing electromagnetic fields in SI units. Detailed description and origins of Maxwell's equations can be found in Appendix G.5: Maxwell's equations.

$$\vec{\nabla} \cdot \vec{B} = 0 \quad (Eq.2.1)$$

$$\vec{\nabla} \cdot \vec{E} = \frac{\rho}{\epsilon_0} \quad (Eq.2.2)$$

$$\vec{\nabla} \times \vec{E} = -\frac{\partial \vec{B}}{\partial t} \quad (Eq.2.3)$$

$$\vec{\nabla} \times \vec{B} = \mu_0(\vec{J} + \epsilon_0 \frac{\partial \vec{E}}{\partial t}) \quad (Eq.2.4),$$

where $\vec{E}$ represents the electric and $\vec{B}$ the magnetic field. $\vec{J}$ is the total current density and ϵ_0 stands for vacuum permittivity and μ_0 for vacuum permeability such that

$$\mu_0 \epsilon_0 = \frac{1}{c^2}$$

Gauss law for electricity (Eq. 2.2, Appendix B.4: Gauss's law for electricity) outlines the relationship between the electric displacement field and the free electric charge density, whilst Gauss's law for magnetism (Eq. 2.1) states that magnetic monopoles cannot exist. Faraday's law (Eq. 2.3, Appendix G.3: Faraday's law) defines that a time-varying magnetic field induces a non-conservative electric field. The Ampere-Maxwell law (Eq. 2.4) states existence of a direct relationship between electric currents, electric field and magnetic flux.

2.2.1. Maxwell's equations for the inhomogeneous time-varying electromagnetic field around an axon

As much as the current flowing through a neuron could be approximated as constant in time over an infinitesimal distance Δz, this would signify that the induced magnetic field around an axon due to action potential propagation is constant in time (180). This is not the case; the action potential has sinusoidal properties that greatly depend on infinitesimal charge movement. This means that the field varies in time and in space (180). Since Biot-Savart law for a magnetic field is used for fields with no temporal component, therefore it cannot be used to describe the inhomogeneous time-varying magnetic field around an axon. More details on the Biot-Savart law can be found in Appendix E.2: Biot-Savart law.

In order to update the previous model (180) and define the inhomogeneous time-varying electric field around an axon which is induced by the inhomogeneous time-varying magnetic field, Faraday's law (Eq. 2.3) has to be modified and combined with Gauss' law for electricity (Eq. 2.2). This gives rise to the first inhomogeneous wave equation describing neuron's electric field, $\boldsymbol{E}$.

$$\frac{1}{c^2}\frac{\partial^2 \boldsymbol{E}}{\partial t^2} - \boldsymbol{\nabla^2 E} = -\left(\frac{1}{\epsilon_0}\nabla\rho + \mu_0 \frac{\partial \boldsymbol{J}}{\partial t}\right) \quad (Eq.\ 2.5)$$

For the purpose of simplifying vector notations, the following substitutions have been made: $\vec{E} = \boldsymbol{E}$, $\vec{B} = \boldsymbol{B}$ and $\vec{J} = \boldsymbol{J}$, where all of these variables are now time-varying.

A neuron is most viewed as a static, perfect conductor in which no charge is considered to be contained and no change of electric field occurs with time. Nevertheless, since we modeled the axon with a presence of a fractional current, $I_{frac}(z,t)$, which is a function of time, this suggests that a time-varying electric field is also induced around a neuron (180).

By substituting Gauss' law for magnetism (Eq. 2.1) into Ampere-Maxwell law (Eq. 2.4) the new wave equation for neuron's magnetic field, $\boldsymbol{B}$, can be obtained.

$$\frac{1}{c^2}\frac{\partial^2 \boldsymbol{B}}{\partial t^2} - \boldsymbol{\nabla^2 B} = \mu_0 \nabla \times \boldsymbol{J} \quad (Eq.\,2.6)$$

Solving these wave equations (Eq.2.5 and Eq.2.6) gives rise to expressions quantifying the strength and direction of the inhomogeneous electromagnetic fields around axons (Eq.2.7).

$$\begin{cases} \boldsymbol{E}(z,t) = E_0 e^{-kz}\cos(kz - \omega t + \delta_E)\hat{\boldsymbol{x}} \\ \boldsymbol{B}(z,t) = B_0 e^{-kz}\cos(kz - \omega t + \delta_E + \phi)\hat{\boldsymbol{y}} \end{cases} \quad (Eq.\,2.7)$$

2.3. Systemic literature review: microglia-astrocyte crosstalk and the influence of externally applied homogeneous electromagnetic fields

In order to evaluate the molecular effects of eH-EMF on neuroregeneration mediated by the microglia-astrocyte crosstalk, a systemic review of literature was performed. Studies that were included in this work are those which are quantifying the effects of the application of magnetic or electromagnetic fields *in vivo* or *in vitro* on microglia and astrocytes, cells that play a role in tissue regeneration within the CNS (64). Any studies that were performed on other cell types were excluded from this search.

2.3.1. Information sources

In accordance with the Peer Review of Electronic Search Strategies (PRESS) Checklist (212), the search was designed using keywords and Boolean operators as follows: "(electromagnetic fields) AND (astrocyte OR microglia OR microglial OR astrocytic OR regeneration OR restoration)

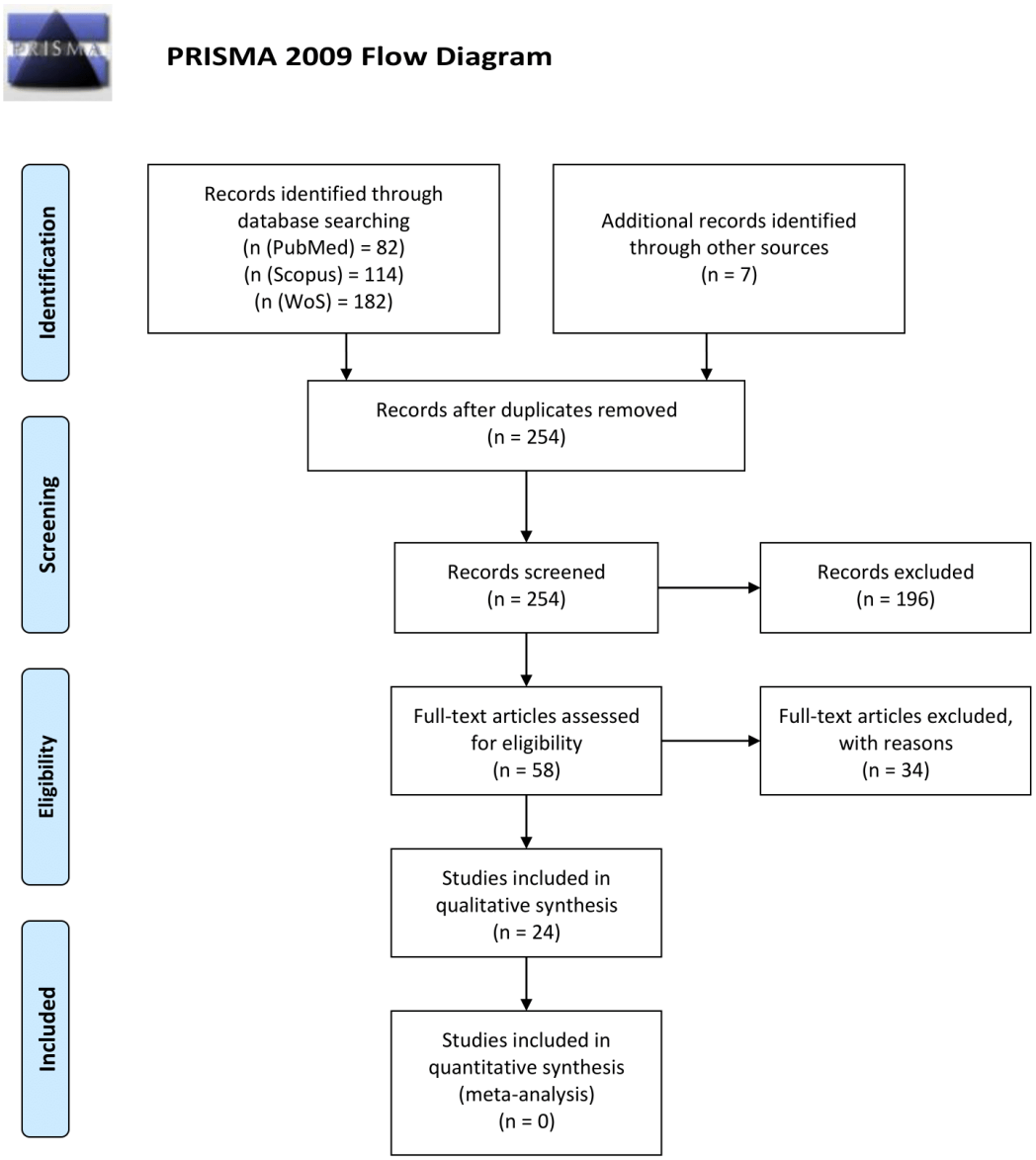
AND (brain)" (64).

2.3.2. Search

While searching through databases (PubMed, Scopus and Web of Science), a preliminary screening was performed. Duplicates, appearing in more than one database, were removed. Next, a primary screening was performed in order to classify the studies based on their status: “relevant”, “uncertain” and “irrelevant”. Studies classified as “irrelevant” were those which included literature reviews, theoretical or computational studies or pilot studies (64). After accomplishing this goal, a secondary screening was performed by accessing the abstracts of selected studies in order to confirm their relevance with respect to their previously determined status. All literature that was assigned as “relevant” was thoroughly searched to obtain the results and answer the question posed in this research (64).

2.3.3. Study selection

After the performed search, 82 studies were found in PubMed, 114 in Scopus and 182 in Web of Science databases (Figure 20) (64).



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Figure 20. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 flow diagram outlining the systematic review study selection process (64).

After successful selection and analyses of eligible studies, the results were synthesized in three major categories based on the molecular target for electromagnetic field application (64):

1. heat shock proteins (HSP);
2. adenosine triphosphate (ATP) and calcium ions (Ca^{2+});
3. hypoxia-inducible factor 1α (HIF1α).

3. RESULTS

3.1. Inhomogeneous electromagnetic fields in the CNS

The innate inhomogeneous time-varying electromagnetic fields around neurons can be described with a set of constitutive equations consisting of the time-varying current density, $\boldsymbol{J}$, obtained from modelling longitudinal and transverse current flow within an axon (Eq.1.11), as well as the solutions of the inhomogeneous wave equations representing electric, $\boldsymbol{E}$, and magnetic, $\boldsymbol{B}$, fields (Eq.2.7).

$$\boldsymbol{J} = \boldsymbol{J_{ion}} - \frac{1}{2\pi a(r_o + r_i)}(r_o \boldsymbol{K_{eo}} + r_i \boldsymbol{K_{ei}})$$

$$\begin{cases} \boldsymbol{E}(z,t) = E_0 e^{-kz}\cos(kz - \omega t + \delta_E)\hat{\boldsymbol{x}} \\ \boldsymbol{B}(z,t) = B_0 e^{-kz}\cos(kz - \omega t + \delta_E + \phi)\hat{\boldsymbol{y}} \end{cases}$$

where

$$J_{ion}(z,t) = g_K(V_m,t)(V_m - V_K) + g_{Na}(V_m,t)(V_m - V_{Na}) + g_L(V_m,t)(V_m - V_L)$$
$$K_{ei}(z,t) = -I_{frac,1}(t)(\delta(z - z_{12}) - \delta(z - z_{21})) + I_{frac,2}(t)(\delta(z - z_{21}) - \delta(z - z_{22}))$$
$$I_{frac}(z,t) = I_{frac,1}(t)(u(z - z_{12}) - u(z - z_{21})) + I_{frac,2}(t)(u(z - z_{21}) - u(z - z_{22}))$$

J_{ion} stands for the ionic current density (Eq.1.13), K_{ei} for extrinsic current per unit length (Eq.1.23), I_{frac} for the fraction of the total current propagating from the previous to the subsequent axonal segment (Eq.1.21) and g_K, g_{Na} and g_L are the specific conductance of potassium, sodium and leak ion channels, respectively.

Upon solving, these equations should provide the strength of the innate inhomogeneous time-varying electromagnetic field around axons and, building upon the Hodgkin-Huxley model, serve as a more optimal mathematical representation of the neuronal function and action as opposed to other currently used models (213–215).

At this point, the proposed model only provides a general theoretical framework. Still, one can appropriate it in order to quantify the innate inhomogeneous electromagnetic fields around neurons. The preliminary version of this model described in our publication (180) demonstrated that, at its strongest, the magnetic field strength around the axon is $B = 3.0 \times 10^{-12}\ T$. This

value is within the same order of magnitude ($10^{-12}\ T$) as the strength of those magnetic fields measured by MEG.

Nevertheless, since the model proposed here represents a major update to the previously used model (180), further work is needed in order to solve these equations and compare them to the results obtained by our previous model, as well as other models currently used in the field such as the FitzHugh-Nagumo (213), Hindmarsh–Rose (214) and Morris–Lecar model (215).

Ultimately, the importance of this model is reflected in the fact that it is an attempt towards initiating a discussion about clarifying disease etiologies in the setting of innate inhomogeneous electromagnetic fields around axons.

3.2. Mechanism of action of electromagnetic fields on glia and surrounding cells

In addition to iIT-EMFs which are generated within the CNS and play a role in modifying the microglia-astrocyte crosstalk during the process of neuroinflammation and neurodegeneration, electromagnetic fields can also occur in the form of external ones (eH-EMFs) – which can be applied to specific regions of the organism in order to initiate tissue repair. In the CNS, these fields then affect the behavior of innate immune cells and enhance the pathological process or initiate neuroregeneration.

3.2.1. Molecular mechanisms of the microglia-astrocyte crosstalk

3.2.1.1. Microglia-astrocyte crosstalk in normal physiological conditions

Because of their intimate molecular conversation, an important role in the innate immune response is taken up by the microglia-astrocyte crosstalk (Fig. 21).

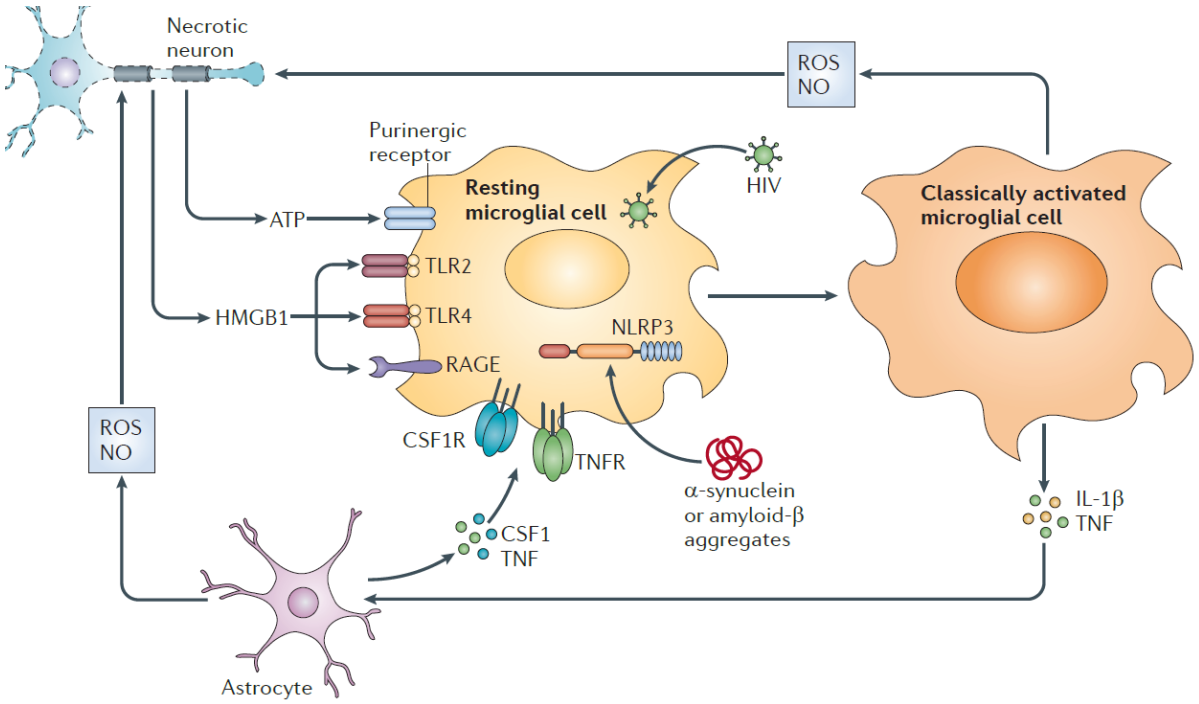


Figure 21. Role of classically activated and resting microglia in response to necrosis of neurons (6).

When activated through pattern recognition or purinergic receptors by detection of PAMPs or DAMPs, such as ATP or HMGB1, microglia start secreting pro-inflammatory factors IL-1β and TNF (6). Excess of pro-inflammatory signals in the extracellular space leads to their transition from a naïve to a M1-like phenotype, associated with colony-stimulating factor 1 (CSF1) and TNF production by astrocytes.

Newer studies have suggested that astrocytes play a crucial role in determining microglial functioning and the direction of innate immune response (59,216) through tight regulation of one of the main signaling molecules within the astrocyte activation cascade - orosomucoid 2 (ORM 2). ORM2 is a protein whose concentration increases during acute inflammation and its specific function has not yet been determined. Nevertheless, it appears to play a role in various aspects of immunosuppression, making astrocytes active players in neuroinflammation (59,216).

In the CNS, ORM2 is secreted by astrocytes and it appears to work through blocking CCL4-CCR5 interaction (217), inhibiting microglial activation and subsequent release of proinflammatory cytokines, or through amplifying the activity of microglia through upregulation of LCN2 (218,219), MCP-1/CCL2 (220), IP-10/CDCL10 (220) or TGF-β (64,219). This establishes a positive feedback loop between astrocytes and microglia associated with

expression of pro-inflammatory cytokines by microglia. Consequently, additional activation of astrocytes and subsequent amplification of the microglial response occur - leading to oxidative stress, inflammation and, ultimately, neuronal death.

3.2.1.2. Microglia-astrocyte crosstalk in neurodegenerative and neuroimmune disorders

As simply presented (Fig. 22), neurodegenerative mechanisms in demyelinated axons cause redistribution of ion channels which are physiologically distributed at nodes of Ranvier. This, in turn, activates the microglia-astrocyte crosstalk and causes further demyelination and tissue degeneration.

Upon noxious stimulation or PAMP/DAMP detection, microglia switch from their naïve to M1 reactive microglia which initiate the expression of MHC class II and release of pro-inflammatory cytokines (TNF, IL-1β, Il-1α) and ROS (221). The abundant pro-inflammatory mediators, in turn, activate not only the adaptive immune cells that have penetrated into the CNS such as T and B cells and macrophages, but also astrocytes.

Upon stimulation by Il-1α, TNF and C1q, astrocytes switch into their A1 reactive phenotype and start releasing neurotoxins, CSF 1 and TNF (6,50). These neurotoxins then decrease the connectivity, activity and outgrowth of neurons. At the same time, CSF1 and TNF establish a positive feedback loop between astrocytes and microglia, leading to further release of pro-inflammatory cytokines and microglial transition from naïve to M1 phenotype. The increase in pro-inflammatory cytokines in the extracellular space induces a stronger immune reaction, recruiting other cells from the adaptive immune system and initiating the destruction of the myelin sheath (222).

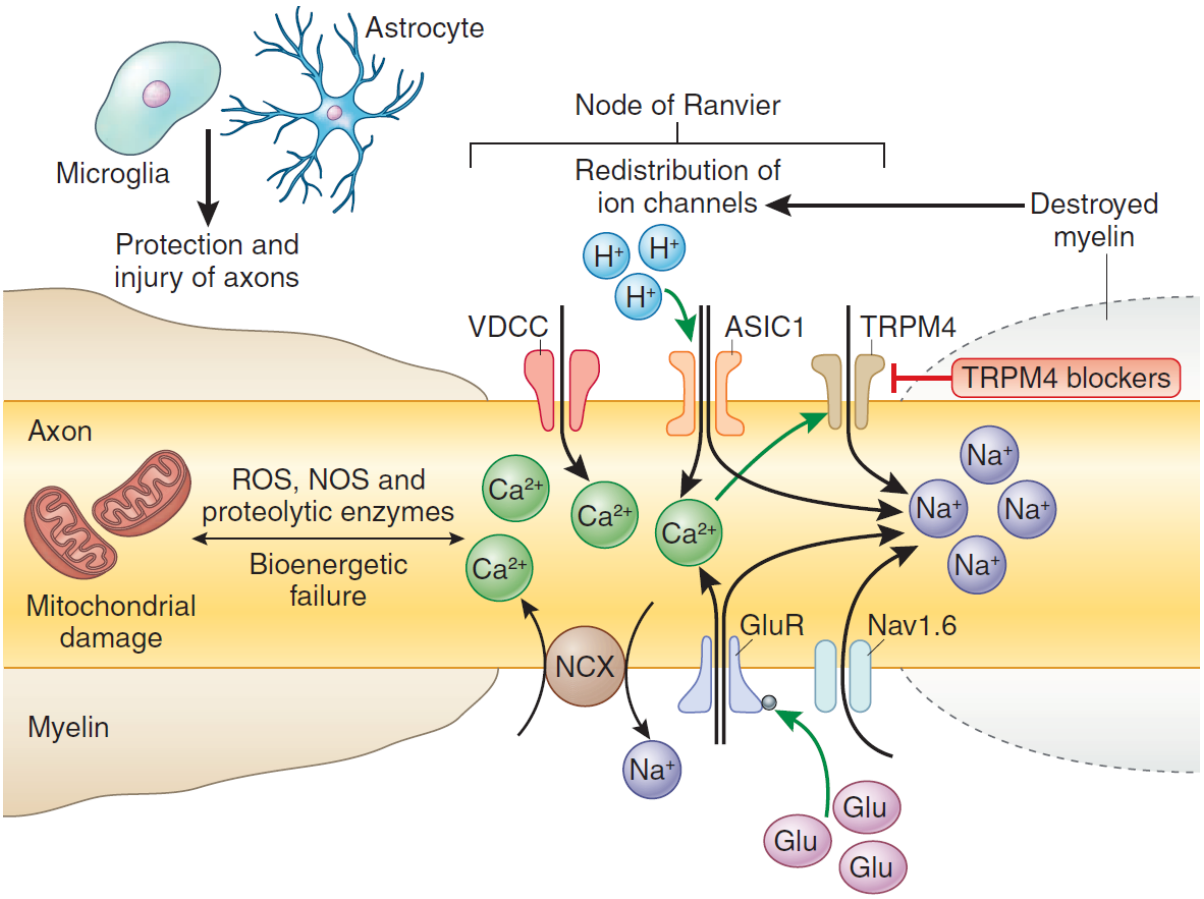


Figure 22. Mechanisms of neurodegeneration in demyelinated axons following neuroinflammation: the case of multiple sclerosis (222).

Demyelination then induces the redistribution of ion channels along the nodes of Ranvier, resulting in accumulation of sodium and calcium in the intracellular space of an axon (222). This is followed by a production of RONS as well as synthesis of various proteolytic enzymes, leading to bioenergetic failure and subsequent mitochondrial damage (222).

The increase in sodium and calcium influx after demyelination leads to further neurodegeneration. It occurs through "non-inactivating sodium channels (Nav1.6), glutamate receptors (GluR) and the sodium calcium exchanger (NCX)" (222). Such an accumulation of ions within the intracellular space is also further amplified by the activation of ASIC1, TRPM4 and voltage-dependent calcium channels (VDCC) (222). Whilst ASIC1 channels are activated by acidic pH, the opening of TRPM4 channels depend on elevated intracellular calcium, downstream of glutamatergic calcium influx. The VDCC channels become active following cellular depolarization (222).

During the progression of these harmful events, a crucial role is being played by the microglia-

astrocyte crosstalk. Through their intercellular communication they can amplify the inflammatory response and ATP release by necrotic neurons. These events lead to sustained tissue injury.

It is known that classically activated macrophages are crucial players in onset and progression in majority of many chronic inflammatory diseases. Nevertheless, the focus of recent research has shifted to the role of microglia in neurodegenerative and autoimmune diseases of the CNS - particularly Alzheimer's and Parkinson's disease as well as multiple sclerosis (8,23,223). The main molecular mechanism proposed to be behind the observed erroneous microglial activity in the CNS is the potential deregulation of M1-like and A1-like microglial and astrocytic cell activation, respectively.

A) Parkinson's' disease

The main pathological features of PD are the loss of dopaminergic neurons and α-synuclein aggregation which manifest with motor dysfunction: stiffness and bradykinesia (224). Many patients also present with clear signs of inflammation in the form of microglia activation and reactive astrogliosis. Microglial activation occurs due to increased death rate of dopaminergic neurons which activates PRRs and purinergic receptors, contributing to further damage to the neural tissue. Besides neuronal death, α-synuclein can also trigger microglial activation through PRRs (6,225). Since this protein exists in abundance in patients with PD, and its amount only increases in time, continuous aggregation or overproduction of α-synuclein can potentially result in chronic overstimulation of microglia towards their M1 phenotype, leading to pathological inflammation. This causes transition of naïve astrocytes to their A1 reactive phenotype and further exacerbation of the disease.

B) Alzheimer's' disease

Prominent clinical features of AD are memory loss and impairment of cognitive functions as consequences of the accumulation of amyloid-β plaques and neurofibrillary tangles (226,227). Formation of amyloid-β plaques correlates with increased activity of TLRs, NLRP3 and RAGE (228–230), contributing to onset of M1-like phenotype. Upon amyloid-β recognition, neurotoxic pro-inflammatory cytokines are released from surrounding cells, causing further damage (6) and driving astrocytes towards their A1 reactive astrocytosis. On the other hand, microglia can play neuroprotective roles in AD through phagocytosis of amyloid-β (231,232) and induction of astrocytes to A2 phenotype, also deemed the "scar-forming reactive astrocytes" (50). In turn, this could potentially cause glial scar formation and decrease the inflammatory response (89).

C) Multiple sclerosis

Finally, multiple sclerosis is a type-IV hypersensitivity reaction characterized by demyelination and degeneration consequential to aberrant autoimmune response. It involves activation of Th17, Th1 and B cells and their penetration into the CNS through BBB. Although these cells are known to be autoantigen-specific and highly relevant for the onset of MS, the precise etiology of this disease is still unknown. In addition to these cells, microglia also appear to have an important role in MS progression by contributing autoantigens to naïve T cells and secreting many pro-inflammatory cytokines (IL-6, IL-23, IL-1β and TGF-β) involved in establishment of a positive feedback loop with astrocytes (6,233). The aberrant activation of microglia, potentially towards their M1 phenotype, and recruitment of astrocytes and circulating monocytes (234) contribute to further progression of the disease and subsequent neurodegeneration.

Pathogenesis of most neurodegenerative diseases includes some form of microglial cell activation through TLRs and other PRRs (6). Since these receptors serve as activators of many pro-inflammatory pathways, a common mechanism might underline the subsequent neurotoxic inflammatory response shared by many neurological disorders. Nevertheless, this suggests that microglia, together with astrocytes, are crucial targets for novel therapeutic approaches aiming to diminish or reverse the polarity of the immune reaction within the CNS.

Since ion channel dysfunction occurs either prior or as a consequence of many neurodegenerative events, it is hypothesized to play several important roles in: demyelination, rising ion imbalance, mitochondrial injury, excitotoxicity and energy failure seen in many neurodegenerative and neuroimmune disorders (222).

With that being said, this work hypothesizes that the importance of ion channels and charged components on the plasma membrane can be recognized in the following:

1) External electromagnetic fields can influence movement of charges in their vicinity and, in this way, assist in decreasing the elements of disease;
2) Innate electromagnetic fields can contribute to disease development and further serve to deregulate the immune response within the CNS and cause more injury.

3.2.2. Molecular mechanisms behind the influence of externally-applied electromagnetic fields on glial cells

The main molecular targets for eH-EMF application on cells of the innate immune system are extracellular ATP and intracellular Ca^{2+}, hypoxia inducible factor 1α (HIF1α) and heat shock proteins (HSPs) (64,235–237).

3.2.2.1. ATP signaling, intra- and extracellular Ca^{2+} concentration

Elevated extracellular adenosine triphosphate (ATP) released by dying or necrotic neurons may be considered as a damage signal that induces inflammatory cytokine release from surrounding cells of the innate immune system (64). Although released by both astrocytes and neurons, the most significant source of ATP in pathological conditions are astrocytes. Its subsequent uptake takes place through G-protein-coupled adenosine receptors (AR) which are expressed in both astrocytes and microglia (238,239). Whilst A1 and A3 ARs inhibit the activity of adenyl cyclase resulting in decreased cAMP accumulation, A2A and A2B ARs cause an upsurge in cAMP accumulation (64). In addition to occurring through AR receptors, microglial recognition of ATP also occurs through P2X purinoreceptors 7 (P2RX7), a subgroup of PRRs involved in extracellular ATP-mediated apoptotic cell death and inflammation (64,240–243).

Studies evaluating the role of EMF in mediating extracellular ATP and intracellular Ca^{2+} signaling in astrocytes and microglia are scarce. Existing studies have mostly investigated application of ELF-MF or ELF-EMF on astrocyte cell line cultures (244,245). Nonetheless, there have been attempts to computationally model the influence of fields generated by an electroencephalogram (EEG) on calcium transients. One of such studies has shown that EEG waves can induce an increased frequency and occurrence of Ca^{2+} momentum waves in Purkinje cells (246). Within the CNS, the calcium momentum waves are a form of Ca^{2+} signal propagation between neighboring glial cells.

Whilst ELF-MFs increase the cytoplasmic Ca^{2+} concentration in astrocytes (244), ELF-EMFs appear to possess the ability to impact the rate of calcium influx, therefore changing the total cellular level of Ca^{2+}. The increase of intracellular Ca^{2+} concentration is usually a consequence of "astrocytic induction of diffusion of IP_3 through gap junctions and extracellular ATP signaling", that activates microglial cells within the surrounding area and changes both astrocyte morphology and function (64).

3.2.2.1. Hypoxia-inducible factor 1-α

Hypoxia-inducible factor 1-α (HIF1α) is a subunit of the HIF-1 transcription factor. Within the CNS, HIF1α is expressed mainly by glial cells (64). Its overexpression in microglia under ischemic conditions leads to induction of A1 reactive astrocytes (Fig. 10), recruitment of peripheral T cells and subsequent tissue damage. Numerous studies have demonstrated that application of EMF cases a decreased release of IL-1β, TNF, IL-6, IL-8 and human monocyte chemoattractant protein-1 (MCP-1/CCL2) (235,247,248). All these cytokines act as HIF1α inhibitors. Many of these studies have also shown that cytokine downregulation decreases neuroinflammation and subsequent death of neurons (235,247,248). Out of those, the most prominent results were obtained in a study investigating the influence of PEMF on microglia (248), showing that PEMF has the ability to inhibit HIF1α activation. This inhibition of HIF1α activation, in turn, impairs the microglial induction of A1 reactive phenotype in astrocytes, resulting in a decreased pro-inflammatory response and enabling the initiation of neurorestoration (248).

3.2.2.1. Heat shock proteins

Finally, heat shock proteins (HSPs) belong to a protein family that is produced by cells under specific stressful conditions (249). They are involved in protein folding and antigen-presenting cell (APC) activation. By upregulating pro-inflammatory cytokine release in APCs, HSPs play a role in inducing the adaptive immune response (249).

Even though the studies on HSPs expression in astrocytes or microglia and the influence on their crosstalk are rare, two studies exploring EMF and MF applications on astrocytes have been published (250,251). Interestingly, both of these studies concluded that EMF does not change the levels of HSP25, HSP60 and HSP70 in astrocytes, *in vitro*. When explored *in vivo* (brain of developing rats), the expression or abundance of HSP60, HSP70 and HSP90, was also shown to be stable (250,251). On the other hand, ELF-MF application on other cell types such as mouse macrophages and human leukemia cells has induced a steady generation of ROS and HSP70 expression, inactivating the NF-κB signaling pathway through matrix metallopeptidase (MMP) expression and decreasing the release of pro-inflammatory cytokines (235). Since EMFs play an important role in inducing HSP expression in other cell types, and are thought to have immunomodulatory and restorative functions, additional studies are required to elucidate the role EMFs play on HSP expression in astrocytes (250,251). Similarly designed studies in microglia are still lacking.

Therefore, it can be postulated that, based on downregulation of HIF1α, ATP release and intracellular Ca^{2+} concentration, external electromagnetic fields cause a decrease in pro-inflammatory factor expression by microglia and their potential transformation to the M2 phenotype. This, in turn, results in induction of A2 reactive astrocytosis and neurorestoration (Fig. 23).

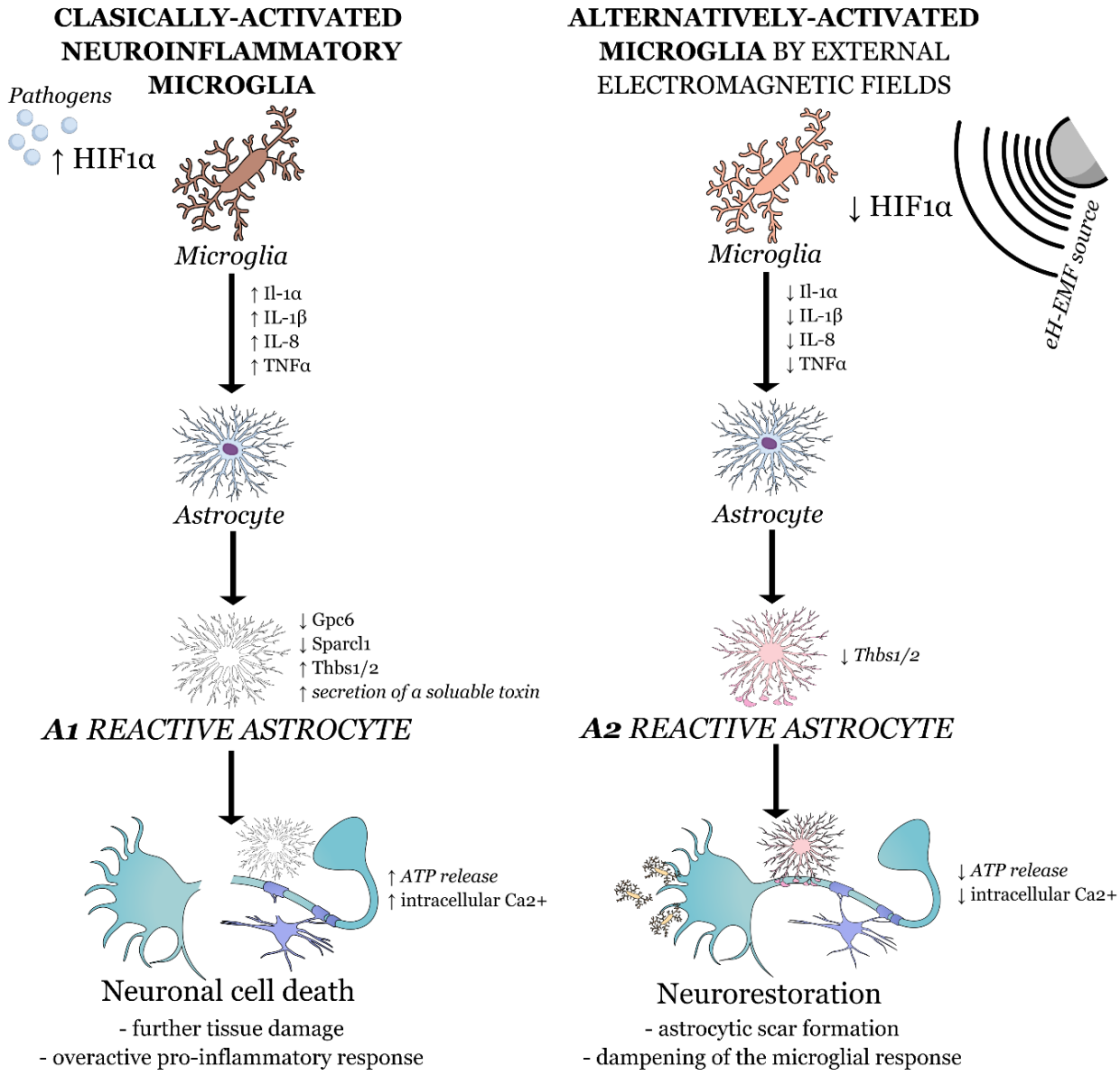


Figure 23. The influence of external homogeneous electromagnetic field (eH-EMF) application on the microglia-astrocyte crosstalk and their role in tissue regeneration (*adapted from* (64)).

This A2 phenotype then plays a role in glial scar formation as well as in further dampening of the immune response. These events induce an additional phenotype switch in microglia; from M1 to M2 microglia through the microglia-astrocyte crosstalk. This causes a decrease in the immune response and matches the benefits of eH-EMF application observed in many different

research studies (201–207).

4. DISCUSSION

This work reviewed up to date knowledge about select mechanisms involved in neuroinflammation and neurodegeneration and the basis for their modulation with application of electromagnetic fields. The scene was set by defining external as well as innate electromagnetic fields around axons and by combining relevant up to date knowledge in pathology of neural diseases and the influence of EMF. This opens a new category of possible therapeutic approaches and implies novel outlook on pathophysiology and treatment of neural diseases.

4.1. Neuroinflammation and neurodegeneration: the common pathways

Neurodegenerative diseases are primarily characterized by loss of structure and function of neurons, ultimately leading to cell death. The most important molecular signatures of many neurodegenerative diseases include misfolded protein aggregation and accumulation, dysfunction of mitochondria, oxidative stress and neuroinflammation (90,126,139). In these pathological events, the most important role is being played by glial cells. They not only regulate the process of neuroinflammation but can also rescue malfunctioning cells and diminish oxidative stress – preventing the onset or further propagation of neurodegenerative processes.

Since a decade ago, the main paradigm was that neurodegenerative diseases, as well as neurodegeneration itself, were purely neurocentric events. Nowadays we recognize that neurodegeneration is a multilevel process which also includes glial cells. Some newer studies even show the expression of major histocompatibility (MHC) class I and II molecules in the human brain tissue – suggesting the possibility that some neurons also express MHC class I molecules and, through those, become targets for the cells of the innate immune system which initiate their destruction (110,137). The expression of MHC class I molecules is highly regulated by microglial activity. This suggests that neurodegeneration and neuroinflammation share many common elements with respect to molecular pathways involved (137).

After the onset of neurodegeneration, an increasing number of dead neurons causes a surge in microglial activation, resulting in upregulation of MHC I expression and antigen display – activating the innate immune response (110). This is followed by an additional level of inflammation. On top of this, if there exists a peripheral injury or an underlying genetic mutation (such as the mutation in NLRP12 in patients with MS), a breach of the BBB by T lymphocytes occurs. In this case, the antigen presented on MHC I molecules could then cause the invading immune cells to target neurons and the myelin sheath, initiating their subsequent destruction. This mechanism was primarily known to be the basis for many neuroinflammatory and neuroimmune disorders, such as MS, but is also now increasingly being studied within the context of neurodegeneration and NDDs.

Similar to the large diversity of molecular pathways leading to neurodegeneration, there are many molecular mechanisms involved in neuroinflammation. Most of them include some form of TLR binding of misfolded and aggregated proteins as well as subsequent aberrant activation of damage-associated molecular pattern (DAMP) receptors. Misfolded proteins, such as those in AD and PD, as well as aberrant cell damage signals, lead to an overactive immune reaction mediated by glial cells. This activation of astrocytes and microglia causes an increased generation of free radicals as well as pro-inflammatory cytokines (109) and leads to neuronal death (252).

4.2. Application of electromagnetic fields for neurological disorders

Modern era is faced with an increase of old population, a population group with a high incidence of neurodegenerative diseases (253). This problem represents a large healthcare and financial burden on the society (110). Because of this, there is a strong need for novel therapies for neurodegeneration and not just NDDs. However, only a few novel ways of treatment have reached the market since 2003 (254). As science begins delving deeper into the molecular mechanisms underlying these pathologies, the need for the interdisciplinary approach is becoming obvious – certainly the one that considers the application of electromagnetic fields.

Numerous studies have demonstrated the effectiveness of electromagnetic fields in reducing pain (192,207,255). Accordingly, it has been hypothesized that administration of external

homogeneous electromagnetic fields may slow or reduce inflammation in the central and peripheral nervous systems through modulation of the microglia-astrocyte crosstalk.

Nonetheless, external electromagnetic fields are not the only form of electromagnetic fields existing in nature. As shown by our previous work, there also exist innate inhomogeneous time-varying electromagnetic fields around axons generated by action potential propagation (180). They play a crucial role in directing migration and activation of surrounding cells such as astrocytes and microglia.

The electromagnetic fields generated around the axons are inhomogeneous. They occur exclusively around axons and around some microorganisms. For that reason they can be misidentified by the host's immune system. As a consequence, an initiation of an autoimmune cascade occurs. This is especially important within the context of neuroimmune, neuroinflammatory and neurodegenerative diseases.

The main property of electromagnetic fields is their ability to influence charged objects in their close vicinity. When applied for neural disorders, the action of electromagnetic fields can be described within five steps:

1. *Reorientation of molecules and deformation of embedded ion channels* with consequential alteration of their activation kinetics and flow of ions (256);
2. *Modification of ion distribution* along the membrane (257) and within the intra- and extra-cellular space in general;
3. *Controlling the opening and closing of voltage-gated ion channels associated with* movement of calcium ions (64,258);
4. *Changed rate of ion and ligand binding* resulting in an amplification or diminishing of the propagating signal, as well as alteration of ion and ligand channel activation kinetics (259);
5. *Electromagnetic induction* at specific areas of the brain they are acting upon (260).

Since electromagnetic fields can either be homogeneous or inhomogeneous in nature, they have distinct methods of action and function within the CNS. Thus, depending on the nature of initiating stimuli, as well as the homogeneity of the fields themselves, EMFs could either cause disease onset, further tissue degeneration or its regeneration.

4.2.1. The role of innate inhomogeneous electromagnetic fields

This peculiar nature of innate inhomogeneous electromagnetic fields around axons, asks for a more appropriate approach towards their quantification and, at the same time, demystification of their role in physiological as well as pathological conditions. In order to tackle this issue, this thesis proposed a spatio-temporal modification to the existing Hodgkin-Huxley model of an axon to include the evolution of longitudinal and transverse currents induced by action potential propagation. A new term in the form of fractional current was introduced (180). With all these parameters included, this model allows for modelling of the strength, direction and force exerted by iIT-EMFs in order to explore their influence within the CNS.

As opposed to using the Biot-Savart Law for a constant magnetic field, this work proposed the use of inhomogeneous wave equation for electric (Eq. 2.5) and magnetic fields (Eq. 2.6) around an axon, stemming from inhomogeneous forms of Maxwell's equations (180). This inhomogeneity, reflected in the nature of innate fields, is a "direct consequence of the time-dependent current generated by action potential propagation" (180). Differentiating this model from others is very important because it accounts for current propagation between different axonal segments in the form of fractional current, I_{frac} (Eq. 1.21) – a modified version of the "axial current" proposed in our previous model (180). This fractional current stands in place of the applied current term usually disregarded in the Hodgkin-Huxley model when modelling the axonal dynamics. It also implies both temporal and spatial propagation of current flow within an axon.

Since the backbone of this model is the mutual interaction between electromagnetic fields and cells of the nervous tissue, it also includes assumptions about the existence of electro- or magneto-receptors on the cell surface. This is a potential evolutionary adaption needed for efficient regulation of cellular activity. If cells of the innate or adaptive immune system would, indeed, possess these receptors, they would actively respond to EMFs in their vicinity. This type of interaction would prove to be crucial in directing their response under pathological conditions.

4.2.1.1. Negative function of innate electromagnetic fields

As a result of ongoing degeneration, demyelination or inflammation, many ion channels undergo changes. These changes affect their activation kinetics and their distribution along the

cell membrane. This results in an imbalance of ion flow and extra- or intra-cellular ion concentration. Since electromagnetic fields are able to influence charged objects in their vicinity, innate and external EMFs can also modify charge distribution along the cell membrane, direct movement of ions and control the dynamics of voltage-gated ion channels (180).

Residing cells of the CNS are accustomed to the presence of iIT-EMF and might even be influenced by it. On the other hand, cells of the adaptive immune system coming from the periphery have not yet encountered inhomogeneous electromagnetic fields and can, therefore, misreact when in their presence. This concept can be best explained in the example of multiple sclerosis.

As much as the exact etiology of MS is still unknown, because of the occurrence of neurodegeneration following inflammation, it is nowadays not only classified as a neuroimmune but also potentially a neurodegenerative disorder. Knowing that cells, potentially, possess magnetoreceptors (184), and maybe even electroreceptors, it is reasonable to assume that they are able to recognize changes in electromagnetic fields and, possibly, even detect pathogens using those receptors.

Generally, it is thought, although not experimentally confirmed, that MS occurs when an autoantigen for myelin is presented to a macrophage in the periphery. It then gets devoured and presented to a T cell. Once activated, T cell starts releasing pro-inflammatory cytokines which multiplicate the inflammation cascade. These cytokines then induce an expression of surface adhesion molecules such as VCAM-1, which binds to VLA4 molecules expressed on the T cell surface. This enables T cells to penetrate the BBB and enter the CNS where they start releasing signaling molecules and recruiting resident immune cells: astrocytes, microglia, and B cells from the periphery. This chain of events leads to increase in the inflammatory response and cause demyelination of axons and their subsequent death. Although this cascade of events seems to lead to MS onset, it is still unclear whether an autoantigen for myelin exists, or this autoimmune response has a different cause.

Many probable causes for MS have been studied, from bacteria to viruses, but the precise driving factor that causes the T cells to initiate this autoimmune cascade is still unknown. Thus, being aware of the inhomogeneous nature of electromagnetic fields around neurons and their ability to influence charges in their vicinity, it is plausible to hypothesize that they also play a role in

MS onset. Even more than that, they may be involved in progression of many other neurological disorders which include penetration of adaptive immune cells into the CNS or deregulation of the innate immune response.

After reaching the CNS, T cells become influenced by iIT-EMFs. These iIT-EMFs can, in turn, exert an electromagnetic force on these cells, impacting their movement and charge distribution along their membrane which, in turn, may impact their function. The magnitude and direction of the electromagnetic force depend on the longitudinal and transverse current flow between axonal segments and are a direct result of the iIT-EMF action which we quantified by the inhomogeneous wave equation describing the electric (Eq. 2.5) and magnetic field (Eq. 2.6) of an axon. Moreover, by polarizing their charged elements, iIT-EMFs can, through magneto- or electro-receptors on the cell's surface induce a potentially unknown, novel T cell phenotype. Hypothetically, these T cells may be able to interact with axons, which are the source of these fields, and mistakenly recognize the myelin sheath as a pathogen because of its dielectric properties.

The myelin sheath is a dielectric material that prevents charge propagation past its borders and serves as an electrical insulator. Because of this, the already peculiar nature of iIT-EMF takes upon a new shape in myelinated axons. The myelinated regions display only the magnetic, whilst the nodes of Ranvier display both the electric and magnetic field component. This periodic nature of iIT-EMF around myelinated axons (Fig. 24), displaying only magnetic field component, can then be wrongfully recognized by invading cells as a region with an impaired electromagnetic colocalization. Lack of the electric field component can then trigger the autoimmune reaction through activation of only magneto- and not electro-receptors – which is an action usually associated with pathogens.

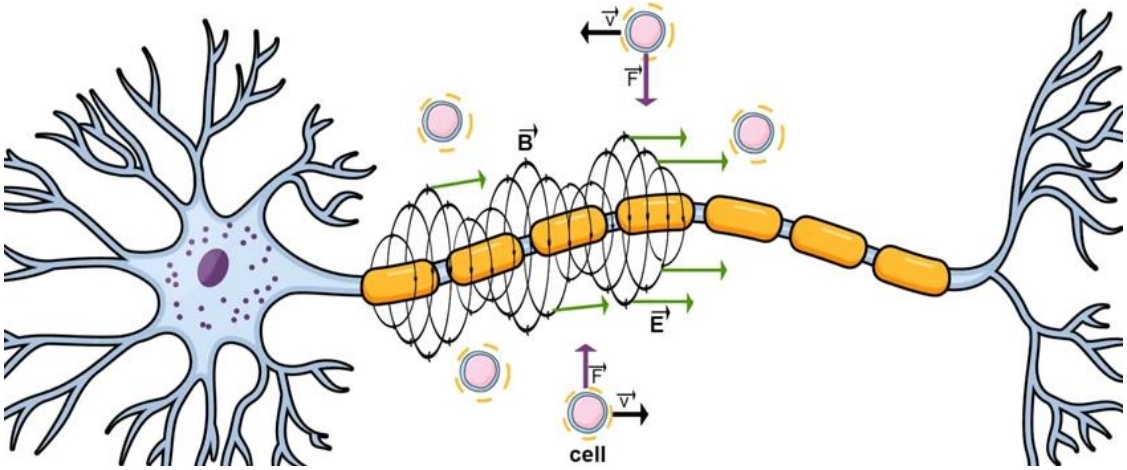


Figure 24. The nature of innate inhomogeneous time-varying electromagnetic fields around

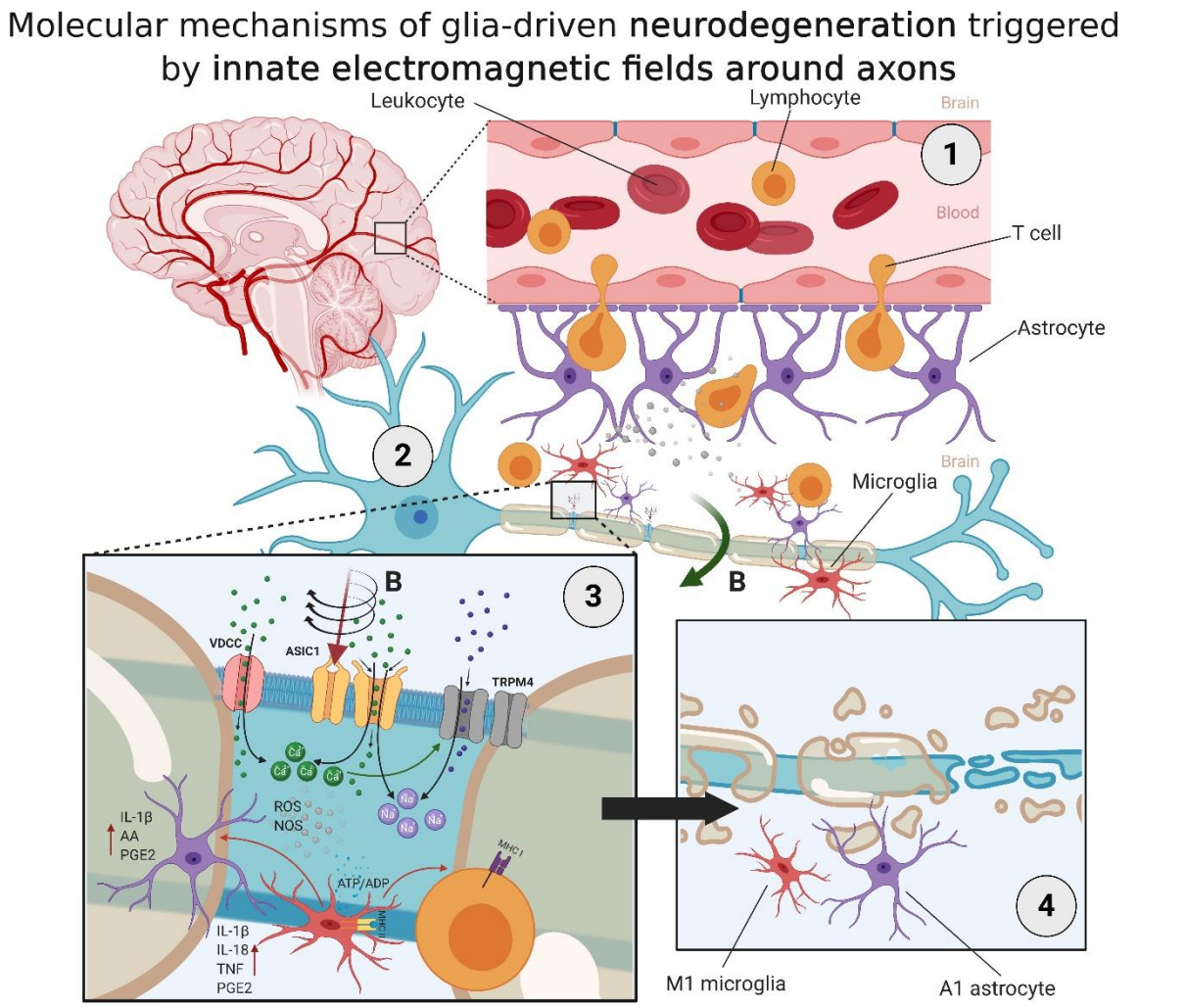
myelinated axons and corresponding forces acting upon cells in their vicinity (*adapted from* (180)).

Nonetheless, iIT-EMF does not only impact the function and activity of adaptive immune cells that have penetrated the BBB, but also the cells of the innate immune system. That concept that can be best explained on the example of multiple sclerosis (Fig. 25).

The pathogenic event most associated with MS is the penetration of the blood brain barrier by circulating lymphocytes. Once within the CNS, these lymphocytes encounter inhomogeneous electromagnetic fields around axon. This can result in erroneous activation of lymphocytes through still unknown molecular mechanisms, but possibly related with magnetoreceptors that are, potentially, present on these cells (184). Their activation leads to pro-inflammatory cytokine release and glial cell recruitment.

Resident glial cells then initiate further cytokine release such as IL-18 and IL-1β, amplifying the autoimmune reaction. This leads to an upregulation of extracellular RONS and ATP/ADP signaling, inducing the production of AA and PGE2. Following this is the polarization of microglia and astrocytes towards their M1 and A1 phenotypes, respectively. This overactive inflammatory response mediated by the innate immune cells of the CNS is only enhanced by the by ongoing demyelination which creates an electromagnetic field imbalance in the surrounding tissue.

Such an apparent imbalance in the nature of electromagnetic fields then manifests itself as a sudden appearance of electric field component at the previously myelinated segments due to their degradation – resulting in apparent changes in the nature of electromagnetic fields around axons and furthering the pro-inflammatory cascade. This can, together with potential activation of cells within the adaptive immune system, enhance some of the pathological events seen during onset of MS and many other neurodegenerative, neuroinflammatory or neuroimmune disorders.



1. Circulating lymphocytes penetrate the blood brain barrier in patients with multiple sclerosis.
2. Once within the CNS, they encounter inhomogeneous electromagnetic fields around axons which are not present elsewhere in the human body.
3. These lymphocytes mistakenly recognize the myelinated segments as pathogens - leading to pro-inflammatory cytokine release and glial cell recruitment. Resident glial cells initiate further cytokine release (IL-18 and IL-1β), amplifying the immune reaction. This leads to an upregulation of extracellular RONS and ATP/ADP signalling, inducing production of arachidonic acid (AA) and PGE2 as well as initiating a negative feedback loop between astrocyte and microglia overreactivity.
4. Subsequent microglia and astrocyte polarisation occurs, resulting in M1 and A2 phenotypes, furthering the pro-inflammatory immune response and initiating demyelination, as well as subsequent neurodegeneration.

Figure 25. Proposed molecular mechanisms of glia-driven neurodegeneration and demyelination triggered by innate electromagnetic fields around axons on the example of multiple sclerosis (*Created with BioRender.com*).

4.2.1.1. Positive function of innate electromagnetic fields

The innate electromagnetic fields around neurons are not necessarily associated only with negative effects in etiopathogenesis of neural diseases. They can also, potentially, exhibit a positive effect on glial cell functioning, through yet unknown mechanisms. These mechanisms may be similar to those behind the action of external electromagnetic fields – in order to potentially assist in inducing alternative microglial (M2) and astrocytic (A2) phenotypes and

initiate the formation of a glial scar and, with that, tissue regeneration.

If this was the case, it would suggest that such neural disorders, which include a pathological invasion of peripheral cells, could potentially be treated through regulation of blood brain barrier permeability. Another treatment option would be the application of external electromagnetic fields. They could hinder the immune response through upregulation of anti-inflammatory factors and even initiate neurorestoration through glial scar formation mediated by M2 and A2 microglia and astrocytes, respectively.

4.2.2. The role of externally applied homogeneous electromagnetic fields

As opposed to iIT-EMFs, external electromagnetic fields are homogeneous (eH-EMFs) and are used as therapy for many neural disorders (191-200). Because of their ability to induce deformations of embedded ion channels, control their opening/closing and change the rate of ion flow and ligand binding, eH-EMFs impact signal transduction within and between cells, regulating the release and concentration or many pro- and anti-inflammatory factors. Moreover, due to their reported beneficial function (74), they are thought to induce M2 and A2 phenotypes in microglia and astrocytes, respectively, leading to glial scar formation and tissue repair (Fig. 23). Since the effects of eH-EMF application depend on many factors such as field type, nature, strength, frequency and duration of application, each of the devices used for therapeutic application of EMF (Table 7) has its own specificities.

Vagal nerve stimulation (VNS) is an approach based on the activation of the cholinergic anti-inflammatory pathway for suppressing the innate immune response. A study done on the rat model of endotoxemia, a condition associated with a persistent inflammation due to presence of circulating endotoxins, has shown that VNS caused a decrease of TNF production in the spleen (261). This downregulation then inhibits the release of other proinflammatory cytokines such as IL-1 and HMGB1 from macrophages (262). This disables the immune response from spreading beyond the local tissue and prevents further damage. A similar mechanism could also hold true for astrocytes and microglia.

Furthermore, whilst the low frequency rTMS decreases, high frequency rTMS increases neuronal excitability and exerts long-term effects on synaptic plasticity as well as neurotransmitter function and release (187). Generally, TMS can evoke action potentials in a local population of neurons and impact neuronal excitability. Specific details on its action in a

variety of neurological disorders are still unknown.

As opposed to other eH-EMFs that are being used in treating neurodegenerative and neuroimmune disorders, the mechanism of DBS action is the most debated one (263). Generally, DBS is thought to normalize the processes involved in the pathophysiology of movement disorders through inhibition, excitation or disruption of neuronal function. This gave rise to three contrasting theories on DBS action: the "inhibition", "excitation" and "disruption hypothesis". The “inhibition” and “excitation” hypothesis postulate that DBS inhibits or activates local neuronal elements, respectively. (187,263). One of the newer theories about DBS action is the "disruption hypothesis" which proposes that DBS completely blocks abnormal flow of information through "dissociating input and output signals" in the target region (264,265).

Contrastingly, the main mechanism of action of tPEMF is its ability to induce electric fields in the brain tissue. This, in turn, potentially activates receptor tyrosine kinase signal transduction pathway and induces a gradual depolarization of membrane potentials (187).

Although studies show its beneficial effect on neural transmission and neurotrophic action, the exact functioning of low-frequency magnetic field is still unknown. Because of its ability to upregulate anti-inflammatory A2A and A3 adenosine receptors, decrease the expression of pro-inflammatory cytokines PGE2, IL-6 and IL-8 and inhibit transcription of NF-κB, LF-MFs can not only decrease chronic pain but can also, through promotion of glial scar formation, induce nerve regeneration (187,235,248).

Finally, as one of the newer forms of eH-EMF therapy, TTFs work by disrupting cell division through impeding mitotic spindle assembly and compromising normal cytokinesis. Accordingly, TTF may prevent cancer cell division (266), reduce the clonogenicity of the progeny and decrease metastatic potential (267–269).

All of the aforementioned therapeutic applications of electromagnetic fields exert effects through different molecular mechanisms of action. Still, they can be generally attributed to subsequent polarization of astrocytes into their A2 phenotype (Fig. 26).

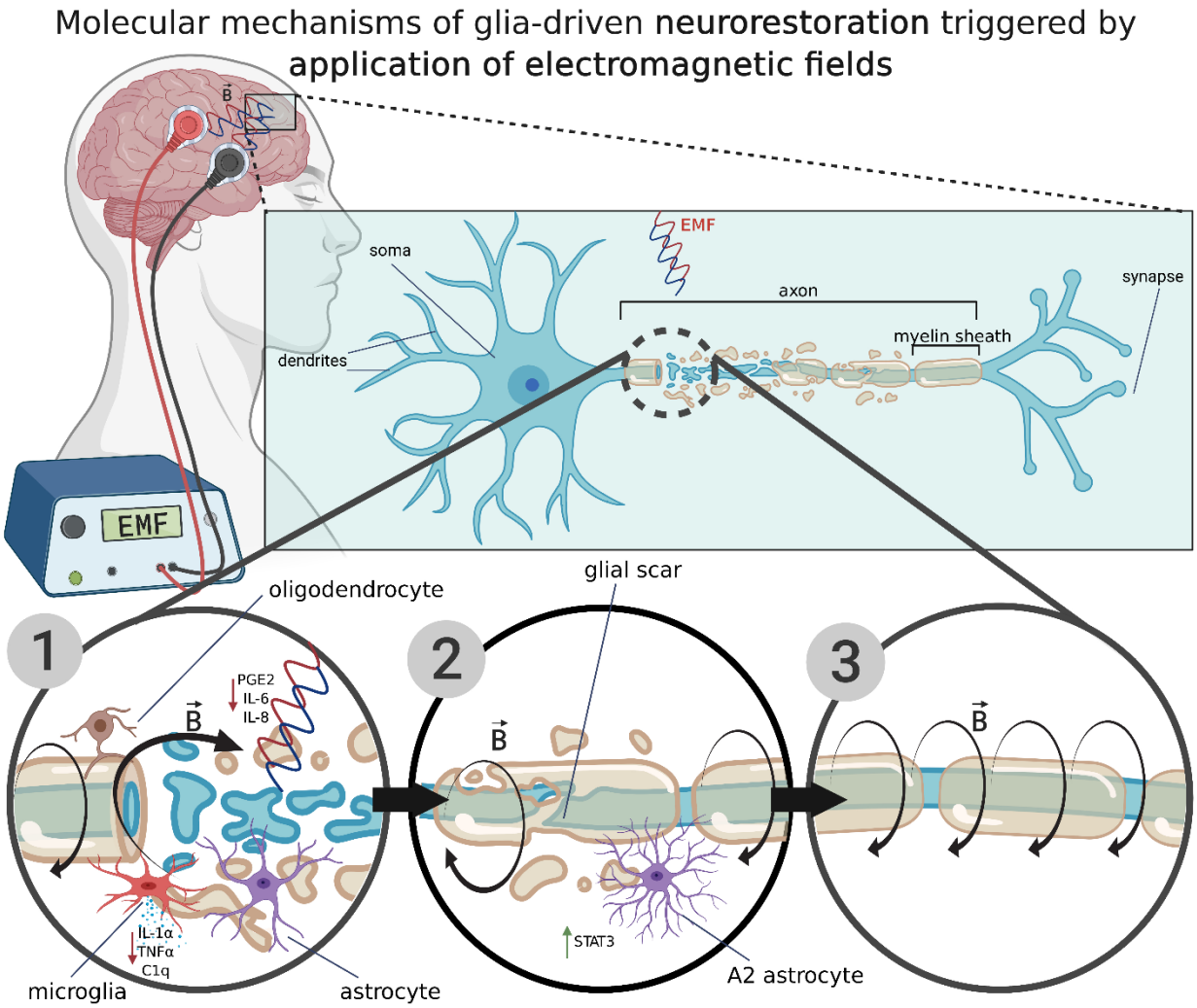


Figure 26. Proposed general molecular mechanisms of glia-driven neurorestoration triggered by application of external electromagnetic fields (*Created with BioRender.com*).

Occurrence of neurodegeneration or demyelination causes a change in the nature of the fields around demyelinated axons – completely abolishing them in some regions and inducing the occurrence of both electric and magnetic fields components at the myelinated segments. Upon application of external electromagnetic fields, a decrease in PGE2, IL-6 and IL-8 signaling occurs (187,235,248). This causes a reduction in IL-1α, TNF and C1q release by microglia, leading to polarization of astrocytes towards their A2 phenotype. Once in their A2 phenotype, astrocytes upregulate the STAT3 pathway, leading to a controlled inflammatory response and initiating the formation of a glial scar. Its formation enables tissue restoration and return of function to

astrocytes. Ultimately, as signal is now able to propagate through the repaired axon, normalization of the innate fields around axons occurs, while the pro-inflammatory response stops.

4.3. Molecular mechanisms behind the influence of externally-applied homogeneous electromagnetic fields on glial cells

Because of the tightly regulated microglia-astrocyte crosstalk, these two glial cell types represent two major molecular targets for EMF. In addition to working with astrocytes to initiate an immune response upon occurrence of an insult or an injury, microglia are also responsible for determining the fate of naïve astrocytes (64). Once they are activated, microglia start releasing many pro-inflammatory mediators and, depending on the nature of initial signal, can either conform to a classical (M1) or alternative (M2) activation pathway (248,270,271).

Classically activated microglia express MHC class II and release pro-inflammatory cytokines (TNF, IL-1β, IL-18) as well as reactive oxygen species. Upon PAMP or DAMP recognition, TLRs on microglia activate downstream signaling cascades contingent on MYD88 and TRIF adaptor molecules which play a role in signal transduction (6). This causes further activation of NF-κB and MAPK pathways, leading to an increased transcription of pro-inflammatory mediators (6).

On the other hand, alternatively activated microglia are contingent upon TGF-β, IL-4, IL-6, IL-10 and PGE2. These molecular factors suppress the M1 and induce the M2 microglial phenotype, decreasing the inflammatory response (6).

As opposed to microglial phenotypes which appear to be activated by the presence of an injury or a pathogen, pathways of astrocytic activation depend on the nature of microglial input. The main characteristics of A1 astrocytes, and what sets them apart from other phenotypes, are loss of motility, inhibition of axonal outgrowth and neuronal survival as well as loss of ability to phagocytose synapses and myelin debris (50). These properties promote further inflammation and tissue injury, earning them the name of "harmful" astrocytes.

Contrastingly, polarization of astrocytes into their A2 phenotype leads to controlling of the inflammatory response, decrease in pro-inflammatory mediators and formation of the glial scar

- classifying A2 astrocytes as "helpful" or "scar-forming reactive astrocytes" (50).

When it comes to EMF application and its role in directing the microglia-astrocyte crosstalk, three main molecular targets can be recognized: extracellular ATP and intracellular Ca^{2+}, hypoxia inducible factor 1α (HIF1α) and heat shock proteins (HSPs) (64). eH-EMF application has been shown to upregulate cytoplasmic Ca^{2+} levels in astrocytes (272) through increase of calcium influx (244). This then further regulates calcium waves and activates surrounding microglial cells - impacting the extracellular ATP concentration and changing astrocyte's morphology (64).

With respect to HIF1α, research (248,273) has shown that application of eH-EMF downregulates or, in some cases even inhibits, HIF1α expression through reduced release of pro-inflammatory mediators (IL-1β, TNF-α, IL-6, IL-8 and MCP-1/CCL2). As a consequence, an induction of M2 and A2 phenotypes of microglia and astrocytes occurs, respectively (64,248,273).

Finally, even though HSPs are major inducers of the adaptive immune response, only a few studies have been performed with respect to HSP expression after eH-EMF application (250,251,274). None of these studies have shown any change in the level of HSPs in astrocytes (250,274). Contrastingly, EMF application on mouse macrophages (275) and human leukemia cells line K562 (276), demonstrated an increase in ROS and HSP70 expression and an inactivation of the NF-κB signaling pathway. This is associated with decrease of the pro-inflammatory cellular response. However, since these studies on astrocytes were performed only in one set of experiments, it seems too early to conclude that this response is cell-specific. Further research is needed (64).

Nevertheless, all quoted studies have been performed with application of homogeneous EMF (eH-EMF). As such, there is a lack of knowledge in how inhomogeneous time-varying EMFs (iIT-EMF) impact the microglia-astrocyte crosstalk. Because the innate electromagnetic fields around neurons are inhomogeneous in nature, and exert a force on cells in surrounding tissue, it is worth to investigate inhomogeneous EMFs in more details. Due to their hypothesized link with both physiological and pathological events, iIT-EMFs can help us to better understand the pathophysiology of neural diseases. In addition, designing instruments which influence these fields can yield improved treatments of neurodegenerative and neuroinflammatory diseases.

5. CONCLUSIONS

Diseases of the nervous system place a large burden on the contemporary world. Thus, in order to combat the rising toll on the modern society, it is imperative to demystify disease etiologies and develop novel therapeutic approaches. This would not only ease the functioning of the healthcare system but would also improve the lives of those suffering from these diseases. One of the most promising approaches to this rising problem involves the study of electromagnetic fields – be it in the form of externally applied fields for the purpose of tissue regeneration or innate fields for the purpose of understanding their role in etiology of neurodegenerative, neuroimmune and neuroinflammatory diseases.

By presenting the idea of innate inhomogeneous time-varying electromagnetic fields around axons, caused by the temporal and spatial propagation of the action potential, this work has put forth a novel hypothesis: some neurological disorders might be caused, or further propagated, through interaction between these fields and surrounding glial cells. This idea has a potential to open novel approaches not only to neurodegenerative and neuroinflammatory disease's onset but also suggests the option to devise therapies that would target this electromagnetic imbalance within the central nervous system and assist in neuroregeneration.

The behavior of astrocytes and microglia is usually determined by the invading pathogen or penetration of lymphocytes and macrophages through the blood brain barrier. Since innate inhomogeneous time-varying electromagnetic fields act within the CNS, we can hypothesize that these fields are what actually causes astrocytic and microglial activation towards their A1 and M1 phenotypes. These events finally lead to exacerbation or even onset of some neurodegenerative or neuroimmune diseases or disorders. On the other hand, because these fields can impact the behavior of resident cells and further propagate various disease pathologies, they can also be used in order to regenerate the resident tissue and cause neurorestoration. Many studies tried to explain the influence of externally-applied homogeneous electromagnetic fields on glial cells as well as other cells of the immune system. Novel therapeutic devices have reached the market. Still, very little is known with respect to precise molecular mechanisms behind the influence of such fields on innate immunity and repair of the nervous tissue.

Nonetheless, the current studies investigating the influence of externally-applied homogeneous

electromagnetic fields on glial cells have shown that they may influence all three main molecular targets - ATP, HSP and HIF1α. Since these molecules represent crucial players in glial cell functioning and activity, modulation of their abundance could, potentially, lead to central nervous system tissue regeneration and partial restoration of its function through activation of A2 astrocytic and M2 microglial pathways. Because of their importance in the microglia-astrocyte crosstalk, ATP, HSP and HIF1α could also be the main molecular targets behind the action of innate inhomogeneous electromagnetic fields around axons.

In conclusion, this work has modeled the innate fields around axons and proposed potential mechanisms behind their action as opposed to externally applied fields. Nevertheless, more comprehensive studies that would be focused on the impact of both external and innate electromagnetic fields on the microglia-astrocyte crosstalk and their role in neurodegenerative, neuroinflammatory and neuroimmune disorders as well as neuroregeneration are clearly needed. Through careful studying of the impact electromagnetic field homogeneity has on the function of cells within the central nervous system, we can potentially devise novel therapies and demystify disease onset – getting us a step closer to our goal of healthy living and ageing.

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Summary

Neurological diseases are a great burden for patients. They also represent a financial problem for the society. Since the cost of annual medical care for people with neurological diseases in the European Union alone has reached 800 billion EUR, there is a great need for new therapeutic approaches. Although there are available drugs on the market which alleviate the symptoms of neurodegenerative, neuroimmune and neurovascular diseases, they are not selective. At the same time, they represent a great cost to public health institutions. Therefore, it is very important to discover the molecular mechanisms that form the basis of the origin and progression of these diseases. Thus, novel treatment strategies should be discovered and proposed. For that purpose, this work is focused on elucidating the significance of electromagnetic fields, both innate and external, on neural disease etiology and therapy, respectively.

Since electromagnetic fields can affect electrically charged cells located in their vicinity, they can influence the activity of neurons and glial cells and, with that, play a role in furthering tissue damage or initiate neuroregeneration. Using advanced mathematical modeling, the nature of innate inhomogeneous time-varying electromagnetic fields around axons was described first. Secondly, an update to the Hodgkin-Huxley model to include transverse and longitudinal current flow and qualification of the induced fields around neurons through modification of inhomogeneous forms of Maxwell's equations was made. The nature of the field is conditioned by the proper function of neurons. If there is a degeneration or demyelination of the tissue, the nature of the fields that arise during the expansion of the action potential changes. This change is reflected in the function of the surrounding cells. Furthermore, during the neuroinflammation that accompanies neurodegeneration, cells of the adaptive immune system infiltrate the central nervous system. These cells can mistakenly "recognize" the altered field around the axon as a microorganism and initiate an immune response. This could be the backbone for occurrence of many diseases s with unknown or not well explored etiology.

Thirdly, the presumed influence of electromagnetic fields on glial cells and neurons was described, using extensive review of the existing literature. This was followed by predicting their potential role in disease etiology and development as well as nerve tissue regeneration. Starting from the idea that the main molecular targets for electromagnetic field application are heat shock proteins (HSP), adenosine triphosphate (ATP), calcium ions (Ca^{2+}) and hypoxia inducible factor 1α (HIF1α), it was postulated that, through acting on these molecular mediators, external

electromagnetic fields induce the alternative activation pathways in microglia (M2) and astrocytes (A2). A protective role and initiation of glial scar formation and subsequent neuroregeneration may be beneficial consequences of these processes. On the other hand, because of their inhomogeneous nature, innate electromagnetic fields around neurons are thought to induce the classically activated microglia (M1) and astrocyte (A1) pathway, leading to further worsening of the injury, on overactive immune response and further tissue degeneration.

This research is a pioneering work trying to define the presence of innate inhomogeneous electromagnetic fields around axons and explain their importance in physiological and pathological processes within the central nervous system. Based on the model here, it is possible to design an instrument that could influence the innate fields within the nervous system and, consequentially, the process of neuroinflammation. This innovative concept could lead to a new approach to the treatment of diseases with an inflammatory or degenerative component and suggest alternative methods of glial cell activation in neurological diseases.

Sažetak

Neurološke bolesti ne predstavljaju samo veliko opterećenje za pacijenta, već i financijski problem za društvo u cjelini. Budući da je trošak medicinske skrbi za osobe oboljele od bolesti mozga samo u Europskoj Uniji dostigao 800 milijardi EUR godišnje, postoji velika potreba za novim terapijskim pristupima. Iako na tržištu postoje lijekovi koji ublažavaju simptome neurodegenerativnih, neuroimunoloških i neurovaskularnih bolesti, oni sustavno djeluju na cijeli organizam te predstavljaju velik trošak javnim zdravstvenim ustanovama. Stoga je vrlo važno otkriti molekularne mehanizme koji čine osnovu nastanka i napredovanja različitih neurodegenerativnih i neuroimunoloških bolesti te predložiti nove strategije liječenja. U tu se svrhu ovaj rad bavi opisom utjecaja elektromagnetskih polja, kako urođenih, tako i vanjskih, na etiologiju i terapiju za bolesti mozga.

Izraz "neurodegeneracija" opisuje progresivni gubitak strukture i funkcije neurona, što često dovodi do njihove smrti. S druge strane, „neuroinflamacija“ je proces koji uključuje odgovor središnjeg živčanog sustava (SŽS) na razne patogene i štetne podražaje, a posredovan je djelovanjem imunoloških stanica, krvnih žila i brojnih molekularnih posrednika. Neuroinflamacija se, u velikoj mjeri, sastoji od privlačenja i aktiviranja glija stanica te perifernih imunoloških stanica koje tada započinju kaskadu događaja koja dovodi do oštećenja ili regeneracije tkiva.

Mikroglija su imunološke stanice SŽS-a koje započinju imunološki odgovor. Osim toga, mikroglija može, putem oslobađanja proupalnih citokina putem STAT i MAPK puteva te fagocitoze oštećenih neurona i sinapsi, također igrati ulogu u neurodegenerativnim zbivanjima. Neki od najpoznatijih molekularnih elemenata koji sudjeluju u aktiviranju mikroglije su povećana ekspresija nuklearnog faktora-κB (NF-κB), interleukina 1α (IL-1α), faktora nekroze tumora (TNF) i komponente komplementa 1q (C1q). Ti citokini i proteinski kompleksi tada igraju ulogu u aktivaciji procesa reaktivne astrocitoze - puta koji vodi do daljnje upale i oštećenja tkiva. Tako, ovisno u vanjskim podražajima, mikroglije slijede jedan od dva moguća puta aktivacije – M1 ili klasično aktivirane mikroglije te M2 ili alternativno aktivirane mikroglije. Istovremeno, danas znamo kako astrociti igraju presudnu ulogu u usmjeravanju rada mikroglije i urođenog imunološkog odgovora tkiva uskom regulacijom jedne od glavnih signalnih molekula unutar aktivacijske kaskade astrocita - ORM 2.

Slično različitim putevima aktivacije makrofaga i mikroglije, astrociti se također mogu, ovisno

o prirodi upalnih znakova i mikrookruženju, polarizirati u dvije vrste: A1 ili klasično aktivirani astrociti te A2 ili alternativno aktivirani astrociti. Dok su A1 astrociti inducirani izlučivanjem IL-1α, TNF, C1q od strane mikroglije te imaju proupalnu funkciju, A2 astrociti imaju neuroprotektivnu ulogu te izlučuju proteine koji promiču sinaptogenezu.

Iako je ponašanje astrocita i mikroglije obično određeno pomoću prisutnosti patogena ili prodorom limfocita i makrofaga kroz krvno-moždanu barijeru, budući da unutar SŽS-a postoje urođena nehomogena elektromagnetska polja koja su promjenjiva u vremenu, postoji i mogućnost da su ta polja ono što zapravo uzrokuje aktivaciju astrocita i mikroglije. To bi zatim moglo dovesti do prekomjerne upalne reakcije što dovodi do pogoršanja ili čak nastanka nekih neurodegenerativnih ili neuroimunoloških bolesti.

Budući da elektromagnetska polja mogu utjecati na električno nabijene stanice koje se nalaze u njihovoj blizini, cilj ovog rada je objasniti utjecaj elektromagnetskih polja na ključne funkcije neurona i glija stanica te predložiti njihovu ulogu u daljnjem oštećenju tkiva i potencijalnoj neuroregeneraciji. Korištenjem naprednog matematičkog modeliranja, ovaj rad opisuje prirodu urođenih nehomogenih vremenski-promjenjivih elektromagnetskih polja oko aksona, ažurira Hodgkin-Huxley-ev model kako bi se obuhvatili longitudinalni i transverzalni protok struje te kvantificira polja oko neurona kroz korištenje nehomogenih oblika Maxwell-ovih jednadžbi. Međutim, budući da je priroda polja uvjetovana pravilnom funkcijom neurona, ako dolazi do degeneracije ili demijelinizacije tkiva, priroda polja koja nastaju tijekom širenja akcijskog potencijala se mijenja. Ova promjena ogleda se u funkciji okolnih stanica. Nadalje, tijekom upale koja prati neurodegeneraciju, stanice imunološkog sustava infiltriraju u središnji živčani sustav. Te stanice mogu pogrešno „prepoznati“ izmijenjeno polje oko aksona kao mikroorganizam te pokrenuti imunološki odgovor - ta bi ideja mogla biti okosnica mnogih bolesti mozga s nepoznatom ili nedovoljno istraženom etiologijom.

Slijedom toga, u ovom je radu opisan pretpostavljeni utjecaj elektromagnetskih polja na glija stanice i neurone korištenjem opsežnog pregleda postojeće literature te je predviđena njihova potencijalna uloga u razvoju bolesti i njenoj etiologiji, kao i obnovi živčanog tkiva. Polazeći od ideje da su glavne molekularne mete za primjenu elektromagnetskog polja proteini toplinskog šoka (HSP), adenozin trifosfat (ATP), kalcijevi ioni (Ca^{2+}) i hipoksijom inducirani faktor 1α (HIF1α), pretpostavljeno je da djelovanjem na ove molekularne posrednike, vanjska elektromagnetska polja induciraju alternativne puteve aktivacije mikroglija (M2) i astrocita (A2)

koji imaju zaštitnu ulogu i pokreću stvaranje glijalnih ožiljaka te naknadnu neuroregeneraciju. S druge strane, zbog svoje nehomogene prirode, urođena elektromagnetska polja oko aksona potiču put klasično aktiviranih mikroglija (M1) i astrocita (A1), što dovodi do pogoršanja ozljede, prekomjernog imunološkog odgovora i daljnje degeneracije tkiva.

Ovo je istraživanje po prvi puta definiralo prisutnost urođenih nehomogenih vremenski-promjenjivih elektromagnetskih polja oko aksona te objasnilo njihovu važnost u fiziološkim i patološkim procesima u središnjem živčanom sustavu. Na temelju predloženog novog modela, moguće je dizajnirati instrument koji bi utjecao na urođena polja unutar živčanog sustava i sam proces upale. S time, ovaj bi inovativni koncept mogao dovesti do novog pristupa liječenju bolesti s upalnom ili degenerativnom komponentom te predložiti alternativne metode aktivacije glija stanica u bolestima mozga.

APPENDICES

A. Vector analysis

A.1. Vectors and notation

Vectors are physical objects which have both a magnitude and a direction. In such, a vector can be written in the form of an ordered set $\vec{v} = (v_1, v_2, \dots v_n)$ or in matrix notation

$$\vec{v} = \begin{pmatrix} v_1 \\ v_2 \\ v_3 \end{pmatrix}$$

They are, most commonly, denoted as a non-bold italic serif letter accented by a right arrow ($\vec{v}$ or V) or, as often seen in typographic convention, a boldface letter ($\boldsymbol{v}$ or $\boldsymbol{V}$). Both of these notations are well accepted and widely used. Even though the arrow notation is most commonly used in handwriting, as boldface is impractical, both of these will be presented within this text and shall be used interchangeably.

A.2. The del operator

The del operator, denoted by the *nabla* symbol ($\boldsymbol{\nabla}$), resembling the inverted Greek letter *delta*, is a vector operator. This operator, essentially, serves as a shorthand instruction to differentiate what follows.

$$\boldsymbol{\nabla} = \hat{\boldsymbol{x}}\frac{\partial}{\partial x} + \hat{\boldsymbol{y}}\frac{\partial}{\partial y} + \hat{\boldsymbol{z}}\frac{\partial}{\partial z} \quad (Eq.\, A.\, 2.1)$$

Thus, if the del operator is acting on a scalar function T, we will obtain the gradient of such a function ($\boldsymbol{\nabla}$T). On the other hand, del acting upon a vector function via a dot product will yield the divergence of such a vector ($\boldsymbol{\nabla} \cdot \mathbf{v}$), whilst del acting on a vector via a cross product will result in the curl of the vector ($\boldsymbol{\nabla} \times \mathbf{v}$) in question – each of which has a distinct purpose (178).

A.3. The divergence of a vector

Starting from the divergence of a vector ($\nabla \cdot \mathbf{v}$), this dot product of the vector operator and the vector itself is just a measure of the spread (*divergence*) of the vector **v** from the point in question (Fig. A.1).

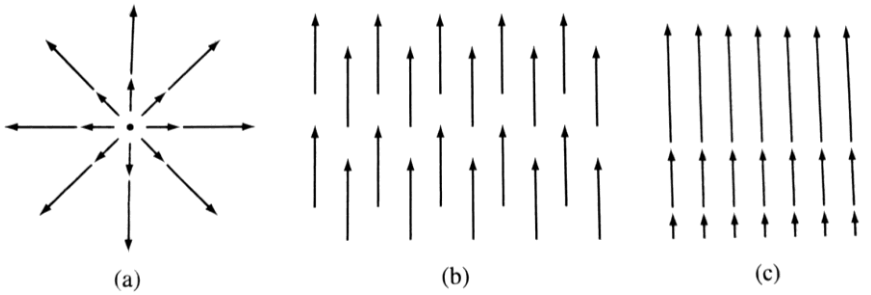

Figure A.1. Divergence of a vector function (a) large positive divergence, (b) zero divergence and (c) positive divergence (178).

In mathematical terms, the divergence of a vector function is a scalar (Eq.A.3.1).

$$\nabla \cdot \mathbf{v} = \left(\hat{\boldsymbol{x}}\frac{\partial}{\partial x} + \hat{\boldsymbol{y}}\frac{\partial}{\partial y} + \hat{\boldsymbol{z}}\frac{\partial}{\partial z}\right) \cdot (v_x\hat{\boldsymbol{x}} + v_y\hat{\boldsymbol{y}} + v_z\hat{\boldsymbol{z}}) \quad (Eq.A.3.1)$$

A.4. The curl of a vector

As opposed to the divergence of a vector which quantifies how much the vector spreads from the initial point, the curl ($\nabla \times \mathbf{v}$) is a measure of how much the vector swirls in space around the point in question (Fig. A.2).

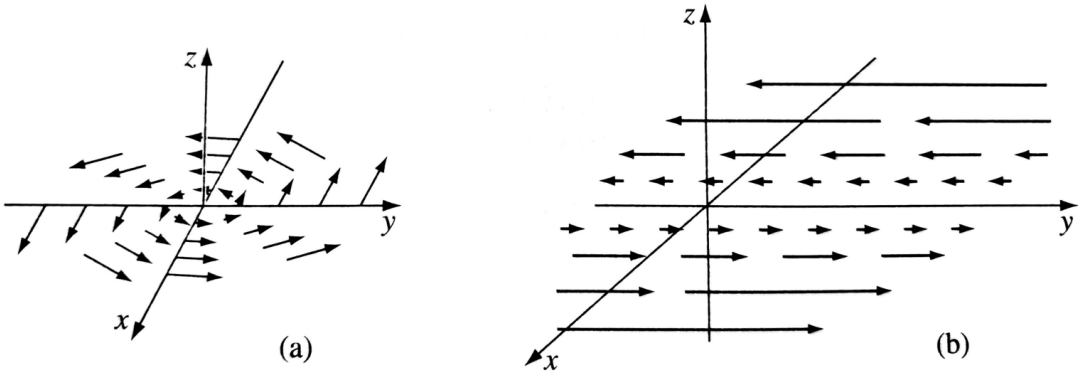

Figure A.2. Curl of vectors (a) and (b) pointing in the z-direction (178).

B. Electrostatics – charges and fields

B.1. Electric charge

The basic premise of classical electromagnetism is that one can deal with interactions between electric charges and currents in such a way that each variable could be measured independently. This depiction of the classical theory of electromagnetism means nothing more but the fact that it is "nonquantum". As opposed to classical theories, the quantum theories deal with properties and interaction of particles on a smaller scale of atoms and subatomic particles and, in itself, are an expansion, an upgrade of sorts, of the classical theories (178).

Electric charge is the physical property of matter that causes it to experience a force when placed in an electromagnetic field. There are two types of electric charge: *positive* and *negative* (commonly carried by protons and electrons respectively). Even though those terms do not necessarily denote the sign of the particle, these signs serve to describe the relationship between two different entities – where all within one variety repel each other whilst being attracted to the other variety. This duality of particles observed in nature is what is deemed as the idea of *antiparticles* – the fundamental concept within particle physics which deals with particles and their counter particles. In such, an electron, carrying a negative charge, has an antiparticle called the *positron* or an *antielectron*, with a positive charge and of the same magnitude. Same holds true for protons, neutrons etc. whose antiparticles are *antiproton* and *antineutron*, both of which have the same magnitude as their counterparts.

On top of existing in two distinct types, electric charges have two other essential properties defining the structure of matter – the ideas that charge is *conserved* and *quantized*. The conservation of charge means that the total amount of an electric charge within a closed system, meaning the algebraic sum of the positive and negative charges present, remains the same. On the other hand, the idea of charge quantization lies outside the scope of classical electromagnetism and essentially states that almost all electric charge found in nature comes in integer multipliers of the elementary charge of an elementary particle – the electron, e. The

exceptions here are *quarks* whose charge is quantized in multiples of $\frac{1}{3}e$ and *quasiparticles*, material systems that behave like particles but are not a part of the elementary particles and, as such, do not violate the charge quantization. As opposed to protons which are seen as composite subatomic particles that can be made up of more than one quark, elementary particles are subatomic particles with no substructure – such as electrons, muons and photons, etc.

B.2. Coulomb's law

In order to describe the interaction between such charges present in nature, Coulomb's law was formed based on experimental data. Coulomb's law describes the amount of force between two stationary electric charges, the source charge (q) and the test charge (Q) (Fig.B.1a) (178).

$$\boldsymbol{F} = \frac{1}{4\pi\epsilon_0}\frac{qQ}{r^2}\hat{\boldsymbol{r}} \quad (Eq.B.1.1)$$

Here, ϵ_0 is a constant describing the permittivity of free space, $\boldsymbol{r}$ is the separation vector from $\boldsymbol{r}'$ (location of the source charge, q) to $\boldsymbol{r}$ (location of the test charge, Q), with a magnitude r and direction $\hat{\boldsymbol{r}}$ (Fig.B.1b) (178).

$$\boldsymbol{r} = \boldsymbol{r} - \boldsymbol{r}' \quad (Eq.B.1.2)$$

This force is acting along the line joining these two charges and points from q to Q. It is repulsive in nature if the signs of these two charges are equal, and attractive if the signs are opposite.

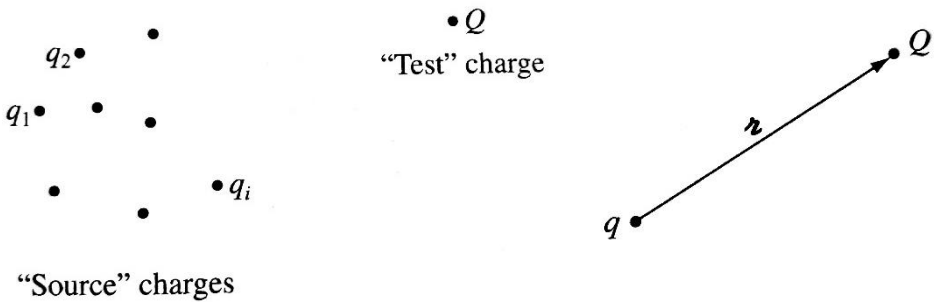

Figure B.1. Depiction of the (a) location of the source charges and the test charge and (b) the separation vector (178).

Coulomb's law then directly facilitates the *principle of superposition* (178). This principle states that an interaction between any two point charges is unaffected by the presence of other charges

in its surroundings, enabling us to compute forces between two point charges separately (q_1 and Q) and then add it up with the force between neighboring charges (q_2, q_3 …) and the charge of interest (Q). This yields the vector sum of all of the individual forces ($\boldsymbol{F_1}, \boldsymbol{F_2}, \boldsymbol{F_3}$ …) between point charges, yielding the definition of the total force ($\boldsymbol{F}$) on the test charge Q.

$$\boldsymbol{F} = \boldsymbol{F_1} + \boldsymbol{F_2} + \boldsymbol{F_3} + \cdots \quad (Eq.B.1.3)$$

B.3. Electric field

Now suppose we have an array of charges (q_1, q_2, q_3 …) arranged randomly and fixed in space. In their vicinity is another charge (q_0). We are not necessarily interested in the strength of the force they exert on one another but rather just the effect they have on this charge in their surroundings – bringing us to the idea of an electric field.

An *electric field* ($\boldsymbol{E}$) is a term describing the space within which an electric charge exerts a force upon another charged body in its vicinity (178). The derived SI units for the strength of an electric field are *volts per meter* (V/m). It can either be created by electric charges themselves or by time-varying magnetic fields. Whilst the former one is deemed to be an electrostatic field, the latter one is an electrodynamic field.

An electrostatic field is the one that remains constant in time and results from stationary charges and currents (178). Because it is a result of stationary charges, type of a field can then be fully described using Coulomb's law and the principle of superposition.

$$\boldsymbol{F} = \boldsymbol{F_1} + \boldsymbol{F_2} = \frac{1}{4\pi\epsilon_0}\left(\frac{q_1 Q}{r_1{}^2}\hat{\boldsymbol{r}}_{\boldsymbol{1}} + \frac{q_2 Q}{r_2{}^2}\hat{\boldsymbol{r}}_{\boldsymbol{2}} + \cdots\right) \quad (Eq.B.2.1)$$

$$\boldsymbol{F} = \frac{Q}{4\pi\epsilon_0}\left(\frac{q_1}{r_1{}^2}\hat{\boldsymbol{r}}_{\boldsymbol{1}} + \frac{q_2}{r_2{}^2}\hat{\boldsymbol{r}}_{\boldsymbol{2}} + \cdots\right) \quad (Eq.B.2.2)$$

This suggests that the force of another charge located in the vicinity of the test charge (Q) can be purely defined as

$$\boldsymbol{F} = Q\boldsymbol{E} \quad (Eq.B.2.3)$$

where

$$\boldsymbol{E}(\boldsymbol{r}) = \frac{1}{4\pi\epsilon_0}\sum_{i=1}^{n}\frac{q_i}{r_i^2}\hat{\boldsymbol{r}}_{\boldsymbol{i}} \quad (Eq.B.2.4)$$

This gives rise to the basic definition of an electric field ($\boldsymbol{E}$) of stationary charges.

This field is a function of position ($\boldsymbol{E}(\boldsymbol{r})$) since the aforementioned separation vectors ($\boldsymbol{r_i}$) depend on the location of the field point (P) upon which the electric field is acting (Fig.B.2). Technically speaking, one could view this electrostatic electric field as the force per unit charge that a point charge would experience if it was placed on the field point P (178).

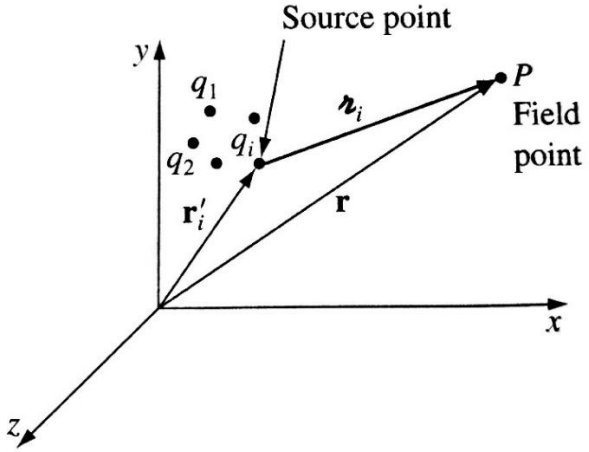

Figure B.2. Location of the field point with respect to the source point within a coordinate system (178).

The shape and direction of an electric field of a point charge depends on the nature of the charge itself. If there are two equal charges in their vicinity, the direction of the field points from the positive towards the negative charge (Fig. B.3a). On the other hand, if the sources are of the same charge, the field lines are divergent (Fig. B.3b) (178).

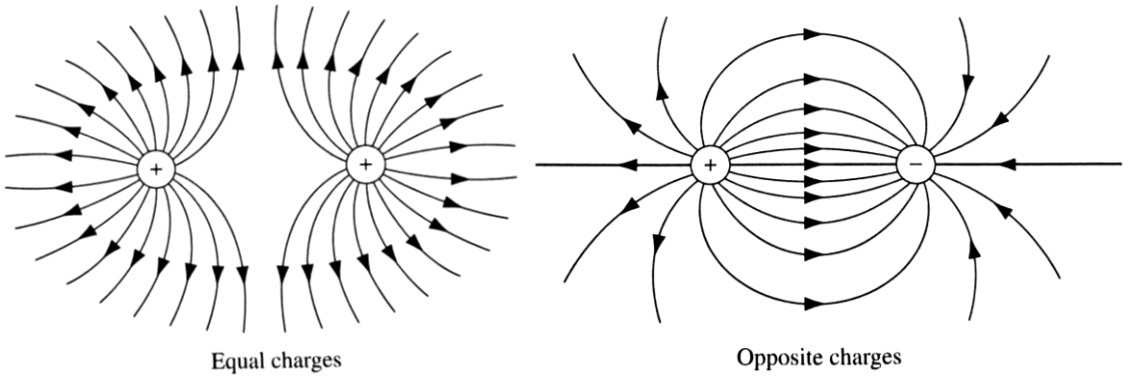

Figure B.3. Direction of the electric field of (a) equal charges and (b) opposite charges (178).

In general, electric fields can, together with later-mentioned magnetic fields, be classified into two main categories based on their magnitude and direction: homogeneous and inhomogeneous fields. Whilst homogeneous fields are those whose magnitude and direction are constant; the magnitude and direction of inhomogeneous fields varies throughout space. If these fields are also changing in time, they are called time-varying fields.

B.4. Gauss's law for electricity

In accordance with these field lines is the measure associated with electric fields is the number of field lines passing through a surface, S, also called the flux of the electric field. In such, the flux essentially stands for the number of the total charges inside a closed surface. The charge outside the surface itself can be disregarded as it will not contribute to the flux. In general, all of these statements describe Gauss's law – a principle that relates the total electric flux within a closed surface ($\boldsymbol{a}$) and the enclosed charge (Q_{enc}) (178).

$$\oint \boldsymbol{E} \cdot d\boldsymbol{a} = \frac{1}{\epsilon_0} Q_{enc} \quad (Eq. B. 3.1)$$

This is the integral form of Gauss's law, also called *Gauss's law for electricity*. In differential form, Gauss' law states

$$\boldsymbol{\nabla} \cdot \boldsymbol{E} = \frac{1}{\epsilon_0} \rho \quad (Eq. B. 3.2)$$

which stands for the divergence of the electric field where ρ stands for charge density. This vector operator produces a scalar field, quantifying the electric field at each point. On the other hand, the curl of an electric field is another vector operator but which, in turn, describes the rotation of the electric field in space (178). Since in order to move a charge within a closed loop in an electric field requires no work to be done, the line integral of the electric field must be zero – hence, the curl is also zero.

$$\oint \boldsymbol{E} \cdot d\boldsymbol{l} = 0 \quad (Eq. B. 3.3)$$

$$\boldsymbol{\nabla} \times \boldsymbol{E} = 0 \quad (Eq. B. 3.4)$$

Since they describe the fundamentals of electrostatic fields, the curl and the divergence of the electric field are the two most basic equations describing the field properties that hold true for any distribution of static charge (178).

C. Electric potential

Another quantity associated with electric fields is the electric potential. Electric potential quantifies the amount of work that is needed to move a stationary charge from one to another point within an electric field without causing any acceleration (178). As opposed to the electric field, which is a vector, the electric potential is a scalar. In such, the electric potential is a line integral of an electric field from a standard reference point $\mathcal{O}$ to point $\boldsymbol{r}$ around any closed loop. The derived SI units for electric potential are *volts* (V).

$$V(\boldsymbol{r}) = -\int_{\mathcal{O}}^{\boldsymbol{r}} \boldsymbol{E} \cdot d\boldsymbol{l} \quad (Eq.C.1)$$

Since this line integral is independent of the path, the potential difference between any two points $\boldsymbol{a}$ and $\boldsymbol{b}$ around a closed loop is

$$V(\boldsymbol{b}) - V(\boldsymbol{a}) = -\int_{\mathcal{O}}^{\boldsymbol{b}} \boldsymbol{E} \cdot d\boldsymbol{l} + \int_{\mathcal{O}}^{\boldsymbol{a}} \boldsymbol{E} \cdot d\boldsymbol{l} = -\int_{\mathcal{O}}^{\boldsymbol{b}} \boldsymbol{E} \cdot d\boldsymbol{l} - \int_{\boldsymbol{a}}^{\mathcal{O}} \boldsymbol{E} \cdot d\boldsymbol{l} \quad (Eq.C.2)$$

$$V(\boldsymbol{b}) - V(\boldsymbol{a}) = -\int_{\boldsymbol{a}}^{\boldsymbol{b}} \boldsymbol{E} \cdot d\boldsymbol{l} \quad (Eq.C.3)$$

Now, according to the fundamental theorem of gradients

$$V(\boldsymbol{b}) - V(\boldsymbol{a}) = \int_{\boldsymbol{a}}^{\boldsymbol{b}} (\boldsymbol{\nabla} V) \cdot d\boldsymbol{l} \quad (Eq.C.4)$$

Equating Eq.C.3. with Eq.C.4

$$\int_{\boldsymbol{a}}^{\boldsymbol{b}} (\nabla V) \cdot d\boldsymbol{l} = -\int_{\boldsymbol{a}}^{\boldsymbol{b}} \boldsymbol{E} \cdot d\boldsymbol{l} \quad (Eq.C.5)$$

Since this statement holds true for any two points $\boldsymbol{a}$ and $\boldsymbol{b}$ around a closed loop, the integrands must be equal.

This gives rise to the equation describing the electric field as the gradient of a scalar electric potential.

$$\boldsymbol{E} = -\boldsymbol{\nabla} V \quad (Eq.C.6)$$

D. Electric fields in matter

D.1. Polarization

When discussing electric fields in matter – be it in solid, liquid, gas, metal or any other form – most everyday objects can be generally assigned to one of two categories: *conductors* and *insulators* (dielectrics) (178). Whilst conductors contain moving charges within the material, insulators are made out of stationary charges which can move only slightly in the form of microscopic displacements. In such, charge distribution of these bound charges in dielectrics can be modified by electric fields trough two principal mechanisms: stretching and rotating (178).

But on top of influencing charged objects, electric fields can also impact neutral objects, as well as neutral atoms. As much as atoms, as a whole, are neutral, they are essentially made out of a positive core (nucleus) and a negative electron cloud surrounding it (178). This means that, when inside an electric field, the atom's nucleus will move with the direction of the field whilst the cloud of electrons will move the opposite way. Once these two opposing forces reach a balance, the atom will remain *polarized* – with its positive charge shifting in one, and negative charge in another direction (178). Because of this apparent occurrence of two poles within an atom that was previously seen as neutral, this is now called an *induced dipole*. This induced dipole has a small dipole moment ($\boldsymbol{p}$) which points in the direction of the electric field ($\boldsymbol{E}$).

$$\boldsymbol{p} = \alpha \boldsymbol{E} \quad (Eq.D.1.1),$$

where α is some proportionality constant. When this effect occurs in a substance, the material becomes polarized. Essentially, this polarization ($\boldsymbol{P}$) stands for the dipole moment per unit volume and it, in itself, also causes an electric field (178).

D.2. Field of a polarized object

Once a material gets polarized, it also generates an electric field. Since Eq.B.6. has established

a clear relationship between electric field and potential, the electric field of a polarized object can be obtained upon defining the vector potential for a single dipole $\boldsymbol{p}$ (Eq.D.2.1.) (178)

$$V(\boldsymbol{r}) = \frac{1}{4\pi\epsilon_0}\frac{\boldsymbol{p}\cdot\hat{\boldsymbol{r}}}{r^2} \quad (Eq.D.2.1)$$

Now, when it comes to a polarized object itself, its total scalar potential is

$$V(\boldsymbol{r}) = \frac{1}{4\pi\epsilon_0}\int_{\mathcal{V}}\frac{\boldsymbol{P}(\boldsymbol{r}')\cdot\hat{\boldsymbol{r}}}{r^2}d\tau' \quad (Eq.D.2.2)$$

where $d\tau'$ stands for each volume element associated with a dipole moment ($\boldsymbol{p} = \boldsymbol{P}d\tau'$) and ϵ_0 stands for vacuum permittivity.

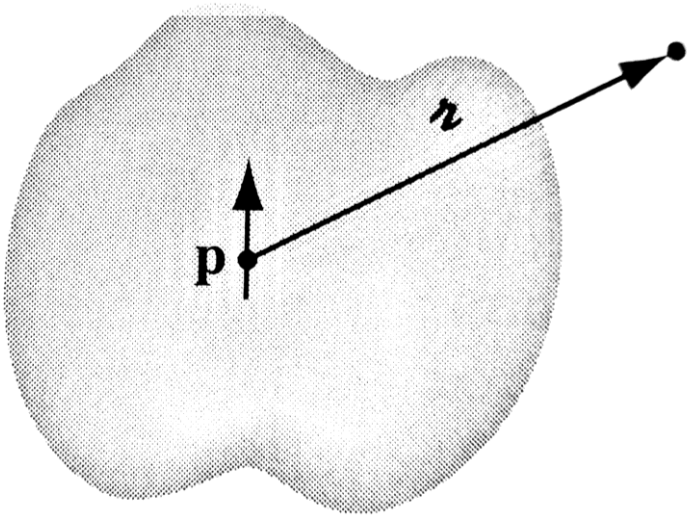

Figure D.1. Setup for determining the total vector potential of a material with a volume $d\tau'$ (178).

In order to rewrite the total vector potential in terms of potentials due to volume (σ_b) and surface (ρ_b) charges, the identity $\nabla'\frac{1}{r} = \frac{\hat{\boldsymbol{r}}}{r^2}$ has to be utilized (178). This yields the final form for the equation quantifying vector potential of an electric field (Eq. D.2.3.).

$$V = \frac{1}{4\pi\epsilon_0}\oint_{\mathcal{S}}\frac{1}{r}\boldsymbol{P}\cdot d\boldsymbol{a}' - \frac{1}{4\pi\epsilon_0}\int_{\mathcal{V}}\frac{1}{r}(\boldsymbol{\nabla}'\cdot\boldsymbol{P})d\tau' \quad (Eq.D.2.3)$$

Since

$$\sigma_b = \mathbf{P}\cdot\hat{\boldsymbol{n}} \quad (Eq.D.2.3)$$
$$\rho_b = -\boldsymbol{\nabla}\cdot\mathbf{P} \quad (Eq.D.2.4),$$

the vector potential of a field produced by a polarized object is

$$V = \frac{1}{4\pi\epsilon_0}\oint_S \frac{\sigma_b}{r}da' - \frac{1}{4\pi\epsilon_0}\int_V \frac{\rho_b}{r}d\tau' \quad (Eq.D.2.5).$$

D.3. Electric displacement

On the other hand, electric displacement ($\boldsymbol{D}$) is a vector field that results from movement of bound charges in the presence of an electric field (178).

$$\boldsymbol{D} = \varepsilon_0\boldsymbol{E} + \boldsymbol{P} \quad (Eq.D.3.1)$$

The aforementioned differential form of Gauss's law can now be rewritten in terms of displacement to read

$$\boldsymbol{\nabla}\cdot\boldsymbol{D} = \rho_f, \quad (Eq.D.3.2)$$

Consequently, the integral form is

$$\oint \boldsymbol{D}\cdot d\boldsymbol{a} = Q_{f_{enc}} \quad (Eq.D.3.3)$$

Here, ρ_f denotes the density of free charges whilst $Q_{f_{enc}}$ stands for the total free charge enclosed in the volume in question. Whilst free charge describes all the charge that is not a result of polarization of the medium, bound charge, on the other hand, is the charge that is a direct product of polarization within the dielectric (178).

Thus, the total free charge within a dielectric, or an insulator, can be written as

$$\rho = \rho_b + \rho_f \quad (Eq.D.3.4)$$

E. Magnetostatics

E.1. Magnetic field

Magnetic field (***B***) is a vector field within which a charge will experience a magnetic influence. As opposed to a stationary charge which generates only an electric field, a moving charge will also induce a magnetic field (178).

For example, suppose we have a wire carrying some current (***I***), characterized by a line charge (λ) (Fig. E.1) travelling at a certain speed ($\boldsymbol{v}$), the current through that wire can be described as

$$\boldsymbol{I} = \lambda \boldsymbol{v} \quad (Eq. D. 1.1)$$

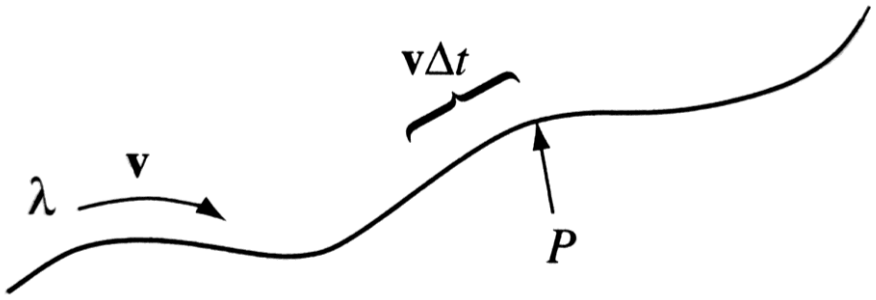

Figure E.1. Line charge travelling at a certain speed within a current-carrying wire (178). This line charge λ travelling at a speed v in some time Δt causes a current flow through the wire which can be observed at the point P.

Since current is just a directed movement of charges, these moving charges, in turn, generate a magnetic field consisting of closed circular field lines surrounding the wire (Fig. E.2) (178). The direction of these field lines is determined by the right-hand rule 2 (RHR-2) where the thumb points in the direction of the current flow and the fingers curl in the direction of the field (Fig. E.2).

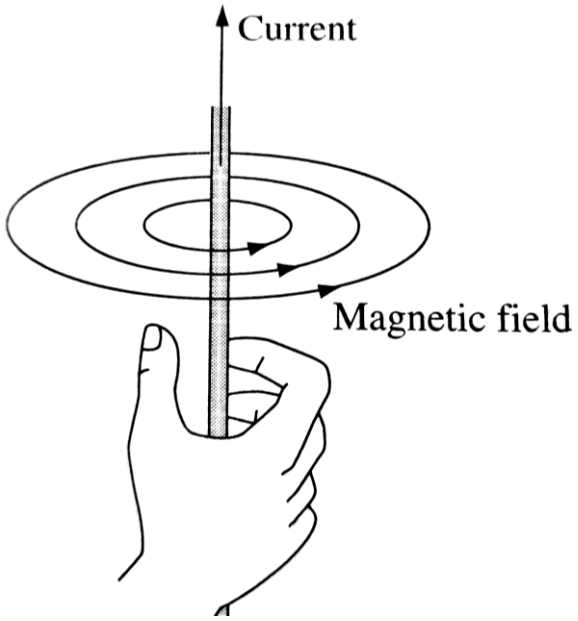

Figure E.2. Right-hand rule for the direction of a magnetic field around a wire (178).

When within such a field a charged particle will experience a magnetic force ($\boldsymbol{F_{mag}}$) (178). In such, the magnetic force on a charge (Q) that is moving with a certain velocity ($\boldsymbol{v}$) within some magnetic field ($\boldsymbol{B}$), also known as *magnetic induction*, is determined by the expression also called the Lorentz force law:

$$\boldsymbol{F_{mag}} = Q(\boldsymbol{v} \times \boldsymbol{B}) \quad (Eq.E.1.2)$$

This generalized expression for Lorentz force law is appropriate for the case of a current carrying wire to be

$$\boldsymbol{F_{mag}} = \int (\boldsymbol{v} \times \boldsymbol{B})\, dq = \int (\boldsymbol{v} \times \boldsymbol{B})\lambda\, dl \quad (Eq.E.1.3)$$

With appropriate substitution of equation Eq.E.1.1. into equation Eq.E.1.3.

$$\boldsymbol{F_{mag}} = \int (\boldsymbol{I} \times \boldsymbol{B})\, dl \quad (Eq.E.1.4)$$

or, if the magnitude of the current is constant along the length of the wire

$$\boldsymbol{F_{mag}} = I \int (d\boldsymbol{l} \times \boldsymbol{B}) \quad (Eq.E.1.5)$$

On top of line current density, one can also define surface ($\boldsymbol{K}$) (Fig. E.3a.) and volume current ($\boldsymbol{J}$) (Fig. E.3b.) densities.

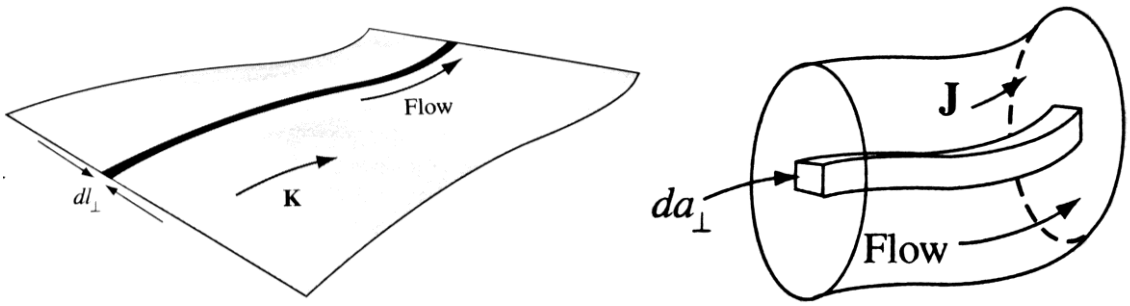

Figure E.3. Geometrical setup of (a) surface and (b) volume current density (178).

The surface current density ($\boldsymbol{K}$) (Fig. E.3a) is a measure of current per unit width and considers a slab of infinitesimal width ($dl_\perp$) that is parallel to the flow of the current ($d\boldsymbol{I}$) (178). It can be written as:

$$\boldsymbol{K} = \frac{d\boldsymbol{I}}{dl_\perp} \quad (Eq.E.1.6)$$

or

$$\boldsymbol{K} = \sigma \boldsymbol{v} \quad (Eq.E.1.7),$$

where σ is the surface charge density and $\boldsymbol{v}$ is the charge velocity.

On the other hand, volume current density ($\boldsymbol{J}$) (Fig. E.3b) describes the current per unit area within a tube volume of infinitesimal cross section ($da_\perp$) that is parallel to the flow of the current through the tube ($d\boldsymbol{I}$) (178), as described by

$$\boldsymbol{J} = \frac{d\boldsymbol{I}}{da_\perp} \quad (Eq.E.1.8)$$

or

$$\boldsymbol{J} = \rho \boldsymbol{v} \quad (Eq.E.1.9),$$

where ρ is the charge density enclosed within the volume travelling with a certain velocity ($\boldsymbol{v}$).

Rewriting Lorentz force law in terms of surface (Eq.E.1.7) and volume current (Eq.E.1.9) densities

yields:

$$\boldsymbol{F_{mag}} = \int (\boldsymbol{K} \times \boldsymbol{B})\, da \quad (Eq.E.1.10)$$

$$\boldsymbol{F_{mag}} = \int (\boldsymbol{J} \times \boldsymbol{B})\, d\tau \quad (Eq.E.1.11)$$

Since charge has to be conserved within any such system, a mathematical statement quantifying such conservation is called a *continuity equation* (Eq.E.1.12.) (178).

$$\boldsymbol{\nabla} \cdot \boldsymbol{J} = -\frac{\partial \rho}{\partial t} \quad (Eq.E.1.12)$$

E.2. Biot-Savart law

Whilst stationary charges produce constant electric fields, deeming this field of research *electrostatics*, steady currents generate constant magnetic fields. Thus, the study of constant magnetic fields is termed *magnetostatics* (178). As much as this term "steady current" or "stationary current" is contradictory term as the word "current" describes directed movement of charges, as discussed previously, it is often used within the study of magnetostatics to denote those currents that exhibit an infinite continuous flow (178). Even though both electro- and magnetostatics are impossible in practice and describe occurrences in artificial worlds, they are suitable approximations for those cases where fluctuations in movement of charges or currents are minute in time ($-\frac{\partial \rho}{\partial t} = 0$) (178). In such, the continuity equation (Eq.E.1.12.) becomes

$$\boldsymbol{\nabla} \cdot \boldsymbol{J} = 0 \quad (Eq.E.2.1)$$

The most common equation used to quantify the strength of magnetic fields generated by constant electric currents is the Biot-Savart law (Eq.E.2.2.). It relates the current flow through a wire ($\boldsymbol{I}$) with the element of length along this wire (dl') and the separation vector ($\boldsymbol{r}$) (Fig. E.4).

$$\boldsymbol{B}(\boldsymbol{r}) = \frac{\mu_0}{4\pi} \int \frac{\boldsymbol{I} \times \hat{\boldsymbol{r}}}{r^2} dl' = \frac{\mu_0}{4\pi} I \int \frac{d\boldsymbol{l}' \times \hat{\boldsymbol{r}}}{r^2} \quad (Eq.E.2.2)$$

where μ_0 is a constant denoting the permeability of free space and $\boldsymbol{r} = (x, y, z)$ is the point at

which the magnetic field is calculated. The derived SI unit for magnetic field is *Tesla* (T).

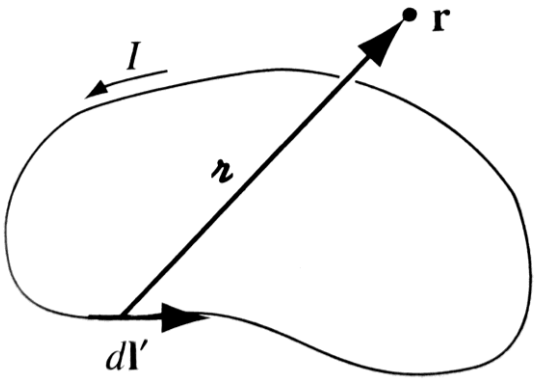

Figure E.4. Current flow through a loop of wire and the associated separation vector (178).

Analogous with the divergence of electric fields, the divergence of a magnetic field can also be obtained, this time starting from Biot-Savart law for the case of volume currents (Eq.E.2.3) (178).

$$\boldsymbol{B}(\boldsymbol{r}) = \frac{\mu_0}{4\pi}\int \frac{\boldsymbol{J}(\boldsymbol{r}') \times \hat{\boldsymbol{r}}}{r^2} d\tau' \quad (Eq.E.2.3)$$

where

$$\boldsymbol{B} = f(x, y, z),$$
$$\boldsymbol{J}' = f(x', y', z'),$$
$$\boldsymbol{r} = (x - x')\hat{\boldsymbol{x}} + (y - y')\hat{\boldsymbol{y}} + (z - z')\hat{\boldsymbol{z}},$$
$$d\tau' = dx'\, dy'\, dz',$$

as seen in Fig. E.5.

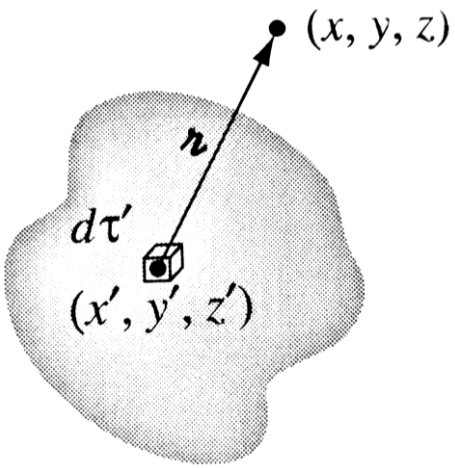

Figure E.5. Setup for determining the divergence of a magnetic field within a volume $d\tau'$ and the associated separation vector (178).

Taking the divergence to Eq.E.2.3, yields

$$\boldsymbol{\nabla} \cdot \boldsymbol{B} = \frac{\mu_0}{4\pi} \int \boldsymbol{\nabla} \cdot (\mathbf{J} \times \frac{\widehat{\boldsymbol{r}}}{r^2}) d\tau' \quad (Eq.E.2.4)$$

Rewriting the right-hand side (RHS) of this equation in terms of a product rule (Eq.E.2.5)

$$\boldsymbol{\nabla} \cdot (\mathbf{A} \times \boldsymbol{B}) = \boldsymbol{B} \cdot (\boldsymbol{\nabla} \times \boldsymbol{A}) - \boldsymbol{A} \cdot (\boldsymbol{\nabla} \times \boldsymbol{B}) \quad (Eq.E.2.5)$$

gives

$$\boldsymbol{\nabla} \cdot \boldsymbol{B} = \frac{\mu_0}{4\pi} \int \left(\frac{\widehat{\boldsymbol{r}}}{r^2} \cdot (\boldsymbol{\nabla} \times \boldsymbol{J}) - \boldsymbol{J} \cdot \left(\boldsymbol{\nabla} \times \frac{\widehat{\boldsymbol{r}}}{r^2} \right) \right) d\tau' \quad (Eq.E.2.6)$$

Since $\boldsymbol{\nabla} \cdot \boldsymbol{J} = 0$ and $\boldsymbol{\nabla} \times \frac{\widehat{\boldsymbol{r}}}{r^2} = 0$, the divergence of the magnetic field is zero as well. This is also known as *Gauss's law for magnetism* (178).

$$\boldsymbol{\nabla} \cdot \boldsymbol{B} = 0 \quad (Eq.E.2.7)$$

E.3. Ampère's law

On the other hand, upon applying the curl to Eq.E.3.3, the equation becomes

$$\boldsymbol{\nabla} \times \boldsymbol{B} = \frac{\mu_0}{4\pi} \int \boldsymbol{\nabla} \times (\mathbf{J} \times \frac{\widehat{\boldsymbol{r}}}{r^2}) d\tau' \quad (Eq.E.3.8)$$

Again, rewriting the RHS of the equation in terms of a product rule (Eq.E.3.9)

$$\boldsymbol{\nabla} \times (\mathbf{A} \times \boldsymbol{B}) = (\boldsymbol{B} \cdot \boldsymbol{\nabla})\boldsymbol{A} - (\boldsymbol{A} \cdot \boldsymbol{\nabla})\boldsymbol{B} + \boldsymbol{A}(\boldsymbol{\nabla} \cdot \boldsymbol{B}) - \boldsymbol{B}(\boldsymbol{\nabla} \cdot \boldsymbol{A}) \quad (Eq.E.3.9),$$

and dropping the terms that involve any derivative of $\boldsymbol{J}$, since $\boldsymbol{J} \neq f(x, y, z)$, yields

$$\boldsymbol{\nabla} \times \boldsymbol{B} = \frac{\mu_0}{4\pi} \int \left(\mathbf{J} \left(\boldsymbol{\nabla} \cdot \frac{\widehat{\boldsymbol{r}}}{r^2} \right) - (\boldsymbol{J} \cdot \boldsymbol{\nabla}) \frac{\widehat{\boldsymbol{r}}}{r^2} \right) d\tau' \quad (Eq.E.3.8)$$

$$\nabla \times \boldsymbol{B} = \frac{\mu_0}{4\pi} \int \boldsymbol{J}(\boldsymbol{r}') 4\pi \delta^3(\boldsymbol{r} - \boldsymbol{r}') d\tau' \quad (Eq.E.3.9)$$

Thus, the final equation for the curl of a magnetic field can be seen in Eq.E.3.10.

$$\nabla \times \boldsymbol{B} = \mu_0 \boldsymbol{J} \quad (Eq.E.3.10)$$

This equation for the curl of a magnetic field (Eq.E.3.10) is also known as the differential form of Ampere's law (178). The integral form of the same law can be seen in Eq.E.3.11.

$$\oint \boldsymbol{B} \cdot d\boldsymbol{l} = \mu_0 I_{enc} \quad (Eq.E.3.11)$$

where I_{enc} is the total current enclosed by the Amperian loop (Fig. E.6).

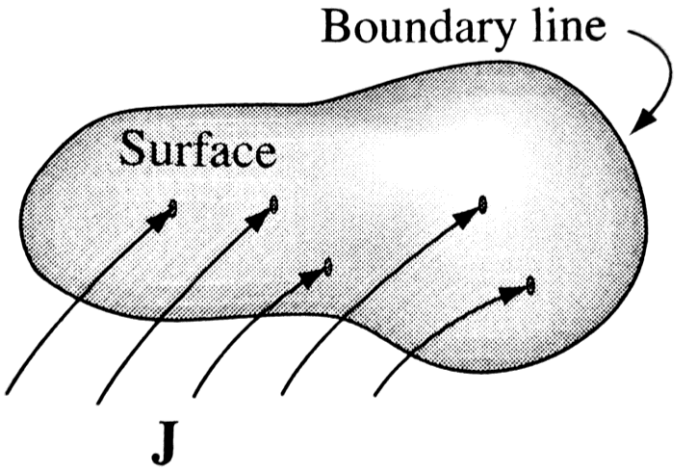

Figure E.6. Amperian loop with the total current density ($\boldsymbol{J}$) passing through the surface (178).

In essence, since the Biot Savart law plays the same role in magnetostatics as the Coulomb's law plays in electrostatics, Ampere's law plays the role of Gauss's law in magnetostatics (178).

$$\begin{cases} Electrostatics & : & Coulomb's\ law & \rightarrow & Gauss's\ law \\ Magnetostatics & : & Biot-Savart's\ law & \rightarrow & Ampere's law \end{cases}$$

F. Magnetic fields in matter

F.1. Magnetization

Even though not much thought is given to what gives rise to magnetism on an everyday scale, considering decorations for our fridges or compass needles, all of their properties are a direct result of microscopic movement of charges on an atomic scale (178). These charges will, similar to their movement in an electric field which results in *polarization*, realign in the presence of external magnetic fields – thus becoming magnetically polarized or *magnetized* (178). As similar as this phenomenon is to polarization, it is also extremely dissimilar to an extent where all the charges within an electric field will align with the direction of such a filed whilst those experiencing a magnetic force will either align parallel to the magnetic field or completely opposite to it. Whilst the former materials are known as *paramagnets*, the latter are called *diamagnets*. Even though these paramagnets and diamagnets experience magnetization whilst within a magnetic field, it vanishes once these fields are removed. The same cannot be said for *ferromagnets* – materials which maintain their magnetization even after they are removed from a magnetic field (178). In their case, it is not the presence of the field within a certain time period that makes them magnetized, but rather the presence of a magnetic field in general.

This notion of magnetization ($\boldsymbol{M}$), brings forth the idea of magnetic dipoles, similar to the electric dipoles that arise as a result of polarization in electric fields (178). In essence, magnetization is just a vector quantity that denotes the magnetic dipole moment ($\boldsymbol{m}$) per unit volume of the material and plays a role analogous to polarization ($\boldsymbol{P}$) in electrostatics (178).

F.2. Field of a magnetized object

Just as electric potential ($\boldsymbol{V}$) is associated with electric fields, magnetic field also has a vector potential ($\boldsymbol{A}$) associated with single magnetic dipoles ($\boldsymbol{m}$) as well as a total vector potential (178).

When it comes to a single magnetic dipole, its vector potential is given by Eq.F.2.1.

$$\mathbf{A}(\mathbf{r}) = \frac{\mu_0}{4\pi}\frac{\boldsymbol{m} \times \hat{\boldsymbol{r}}}{r^2} \quad (Eq.F.2.1)$$

Now, when it comes to a magnetized object itself, its total vector potential is

$$\mathbf{A}(\mathbf{r}) = \frac{\mu_0}{4\pi}\int \frac{\boldsymbol{M}(\boldsymbol{r}') \times \hat{\boldsymbol{r}}}{r^2} d\tau' \quad (Eq.F.2.2)$$

where $d\tau'$ stands for each volume element associated with a dipole moment ($\boldsymbol{m} = \boldsymbol{M}d\tau'$).

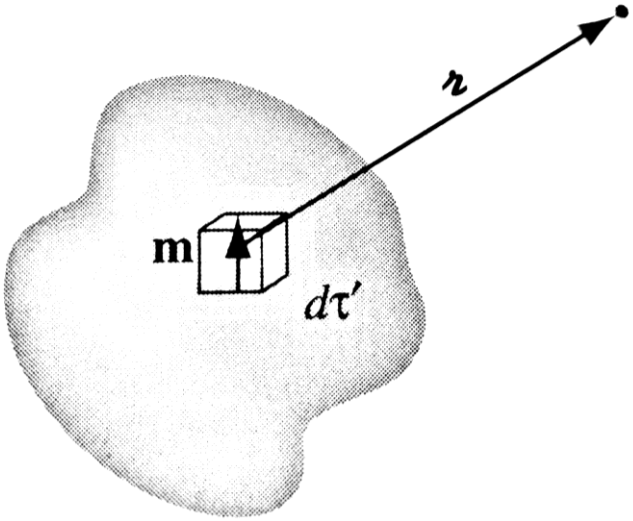

Figure F.1. Setup for determining the field of a magnetized object within a volume $d\tau'$ (178).

In order to rewrite the total vector potential in terms of magnetic potentials due to volume ($\boldsymbol{J_b}$) and surface ($\boldsymbol{K_b}$) currents, the identity $\nabla' \frac{1}{r} = \frac{\hat{r}}{r^2}$ has to be utilized (178). This yields the final form for the equation quantifying vector potential of a magnetic field (Eq. F.2.3.).

$$\mathbf{A}(\mathbf{r}) = \frac{\mu_0}{4\pi}\int_{\mathcal{V}} \frac{\boldsymbol{J_b}(\boldsymbol{r}')}{r} d\tau' + \frac{\mu_0}{4\pi}\oint_{\mathcal{S}} \frac{\boldsymbol{K_b}(\boldsymbol{r}')}{r} da' \quad (Eq.F.2.3)$$

where

$$\boldsymbol{J_b} = \boldsymbol{\nabla} \times \boldsymbol{M} \quad (Eq.F.2.4)$$
$$\boldsymbol{K_b} = \mathbf{M} \times \hat{\boldsymbol{n}} \quad (Eq.F.2.5)$$

which bear a striking similarity to the electric potential of a bound volume (Eq. D.2.4.) and surface charge (Eq. D.2.3).

F.3. Auxiliary field H

On top of bound currents that produce the magnetic field in a magnetized material, designated by Eq.F.2.4. and Eq.F.2.5., if such a material is plugged into an electrical network further generation of *free currents* within the material will occur (178). This means that the total current within such an object is

$$\boldsymbol{J} = \boldsymbol{J_b} + \boldsymbol{J_f} \quad (Eq.F.3.1)$$

Whilst the bound currents ($\boldsymbol{J_b}$) appear as a result of dipole realignment due to the presence of an external magnetic field, the free currents ($\boldsymbol{J_f}$) consist of charge transport through the material (178).

Rewriting the differential form of Ampere's law (Eq.F.2.10) in terms of bound currents (Eq.F.2.4.) and the total current within the material (Eq. F.3.1.) and multiplying each side of the equation by $\frac{1}{\mu_0}$ yields

$$\frac{1}{\mu_0}(\boldsymbol{\nabla} \times \boldsymbol{B}) = \boldsymbol{J_b} + \boldsymbol{J_f} \quad (Eq.F.3.2)$$

$$\frac{1}{\mu_0}(\boldsymbol{\nabla} \times \boldsymbol{B}) = (\boldsymbol{\nabla} \times \boldsymbol{M}) + \boldsymbol{J_f} \quad (Eq.F.3.3)$$

After rewriting the two terms involving the curl of the magnetic field ($\boldsymbol{\nabla} \times \boldsymbol{B}$) and the curl of magnetization ($\boldsymbol{\nabla} \times \boldsymbol{M}$) gives

$$\boldsymbol{\nabla} \times \left(\frac{1}{\mu_0}\boldsymbol{B} - \boldsymbol{M}\right) = \boldsymbol{J_f} \quad (Eq.F.3.4),$$

where

$$\boldsymbol{H} = \frac{1}{\mu_0}\boldsymbol{B} - \boldsymbol{M} \quad (Eq.F.3.5)$$

This newly introduced variable $\boldsymbol{H}$ is also known as the *auxiliary field* (or *magnetic field within a material*) which plays the same role in magnetostatics that electric displacement ($\boldsymbol{D}$) plays in electrostatics (178). It allows the expression of Ampere's law just in terms of free currents, just

as D allows the expression of Gauss's law in terms of free charge alone (Eq.D.3.2 and Eq.D.3.3) (178). The derived SI unit for the auxiliary field $\boldsymbol{H}$ is *Ampere per metre* (A/m).

Ampere's law in differential form now reads

$$\boldsymbol{\nabla} \times \mathbf{H} = \boldsymbol{J_f} \quad (Eq.F.3.6),$$

whilst the integral form is

$$\oint \boldsymbol{H} \cdot d\boldsymbol{l} = I_{enc} \quad (Eq.F.3.7.).$$

What is to be noted here, but with caution, is the fact that Eq. F.3.6. looks eerily similar to the original differential form of Ampere's law (Eq.E.2.10), but with a small caveat whereas the total current on the RHS of the equation is replaced by the free current and $\boldsymbol{B}$ is replaced by $\mu_0\boldsymbol{H}$ (178).

As much as this seems to indicate that $\mu_0\boldsymbol{H}$ is the same as $\boldsymbol{B}$, but just with a free current $(\boldsymbol{J_f})$ and not total current $(\boldsymbol{J})$ source – that parallel cannot be made in all cases (178). Even though $\boldsymbol{\nabla} \cdot \boldsymbol{B} = 0$, the divergence of $\boldsymbol{H}$ is, in general, different from zero (Eq. F.3.8.).

$$\boldsymbol{\nabla} \cdot \boldsymbol{H} = -\boldsymbol{\nabla} \cdot \boldsymbol{M} \quad (Eq.F.3.8)$$

In such, the above made parallel holds true only for specific situations where the divergence of $\boldsymbol{M}$ vanishes.

F.4. Linear and nonlinear media

As opposed to ferromagnetic materials, which maintain their magnetization $(\boldsymbol{M})$ even after they are removed from the external magnetic field $(\boldsymbol{B})$, the magnetization of paramagnetic and diamagnetic materials is contingent upon the presence of a magnetic field (178). Since thus magnetization is proportional to the magnetic field, and magnetized materials are, conventionally, primarily associated with their auxiliary field $\boldsymbol{H}$, it can be written that

$$\boldsymbol{M} = \chi_m \boldsymbol{H} \quad (Eq.F.4.1),$$

where the constant χ_m denotes magnetic susceptibility. It is reliant on the magnetic properties of an object in question and, as such, has different values for specific materials. Materials which exhibit such proportionality described by Eq.F.4.1. are also called *linear media* (178). For such materials

$$\boldsymbol{B} = \mu_0(\boldsymbol{H} + \boldsymbol{M}) \quad (Eq.F.4.2),$$

After substitution of Eq.F.4.1. into Eq.F.4.2., the relationship between $\boldsymbol{B}$ and $\boldsymbol{H}$ becomes

$$\boldsymbol{B} = \mu_0(1 + \chi_m)\boldsymbol{H} \quad (Eq.F.4.3).$$

In such a case, $\boldsymbol{B}$ is, indeed, proportional to $\boldsymbol{M}$, as discussed previously, with a proportionality constant of $\mu = \mu_0(1 + \chi_m)$ (178). This proportionality constant is also called the *magnetic permeability* of a material, almost analogous to electric permittivity (ϵ_0) in electrostatics. Whilst permeability depends on magnetization of a material and is a measure of its ability to conduct magnetic fields, permittivity depends on polarization and it quantifies the degree of obstruction a material provides during the formation of an electric field (178).

G. Electrodynamics and Maxwell's equations

G.1. Ohm's law

Even though the presumption that *a force is needed to push on the charges for a current to flow* is now a well-accepted, and a somewhat obvious statement, it was a very controversial topic when Ohm's law was proposed. In general terms, Ohm's law states that the density of a current flowing through a conductor, $\boldsymbol{J}$, is proportional to force per unit charge, $\boldsymbol{f}$ (178).

$$\boldsymbol{J} = \sigma \boldsymbol{f} \quad (Eq.G.1.1),$$

where *sigma* (σ) is a proportionality factor, different from surface charge density. This is an empirical constant that depends on the properties of the material – deeming it the *conductivity* of the medium. In some cases, a term ρ is also sometimes used, which denotes the *resistivity* of the material and is defined as $\rho = \frac{1}{\sigma}$, not to be confused with volume charge density (178).

Generally, the force that drives charge movement within a conductor can be of any nature, such as chemical or gravitational but, in most cases, it is seen to be the electromagnetic force (178). In such a case, Eq.G.1.1. becomes

$$\boldsymbol{J} = \sigma(\boldsymbol{E} + \boldsymbol{v} \times \boldsymbol{B}) \quad (Eq.G.1.2)$$

Since $\boldsymbol{v} \times \boldsymbol{B}$ is the vector product of velocity and the magnetic field, and the velocity of the charges themselves is usually sufficiently small, the second term is usually ignored yielding:

$$\boldsymbol{J} = \sigma \boldsymbol{E} \quad (Eq.G.1.3)$$

Such a simplification results in a general form of Ohm's law, as it relates to electric field and conductivity of the medium (Eq.G.1.3) (178).

Even though it is usually said that $E = 0$ inside perfect conductors, that holds true only for stationary charges in which $J = 0$. With that, in the case of a perfectly conducting medium, $\boldsymbol{E} = \frac{J}{\sigma} = 0$, even with current flowing through the conductor.

Since metals are good conductors, meaning that the electric fields needed to drive the movement of charges and current propagation are negligible, connecting wires in electrical circuits are usually viewed as *equipotential*, i.e. as perfect conductors (178). On the other hand, resistors are considered to be poorly conducting materials and, thus, when modelling current propagation, cannot be approximated as perfect conductors.

Nevertheless, one of the most common ways of writing Ohm's law, different from what is stated in Eq.G.1.3., is that the potential difference between two electrodes is equal to the multiplicative of current ($\boldsymbol{I}$) and resistance ($\boldsymbol{R}$) within the electrical circuit (Eq. G.1.4)

$$\boldsymbol{V} = \boldsymbol{IR} \quad (Eq.G.1.4),$$

where $\boldsymbol{R}$ is the *resistance* of the material. The derived SI unit for resistance is *ohm* (Ω).

G.2. Electromotive force

When observing current flow through a typical electrical circuit, such as a light bulb connected to a source of electricity (e.g. battery) (Fig. G.1.), it can be said that there are only two main forces involved in driving of the current through the loop – the force associated with the source ($\boldsymbol{f}_s$), such as the battery, and the electrostatic force ($\boldsymbol{E}$) (178). This means that the total force ($\boldsymbol{f}$) driving the current flow can be summarized as

$$\boldsymbol{f} = \boldsymbol{f}_s + \boldsymbol{E} \quad (Eq.G.2.1)$$

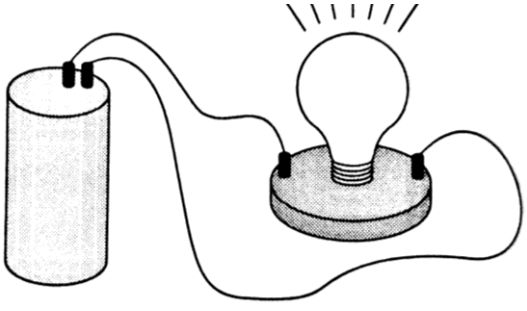

Figure G.1. Sketch of a typical electrical circuit – a light bulb connected to a battery (178).

The net effect of such a force within the electrical circuit is usually deemed as the electromotive force (ε), an integral of the force per unit charge (Eq.G.2.2) (178). In the case of an electrostatic field, the electromotive force (emf) is

$$\varepsilon = \oint \boldsymbol{f} \cdot \mathrm{d}\mathbf{l} = \oint \boldsymbol{f}_s \cdot \mathrm{d}\mathbf{l} \quad (Eq.G.2.2)$$

As it can be noted in Eq. G.2.2., $\boldsymbol{f}$ and $\boldsymbol{f}_s$ have been used interchangeably since $\oint \boldsymbol{E} \cdot \mathrm{d}\mathbf{l} = 0$ in an electrostatic field.

As opposed to the electromotive force associated with electrostatic fields, such as those produced by batteries, there also exists electromotive force associated with electrodynamic fields, such as those of generators (Fig. G.2) (178).

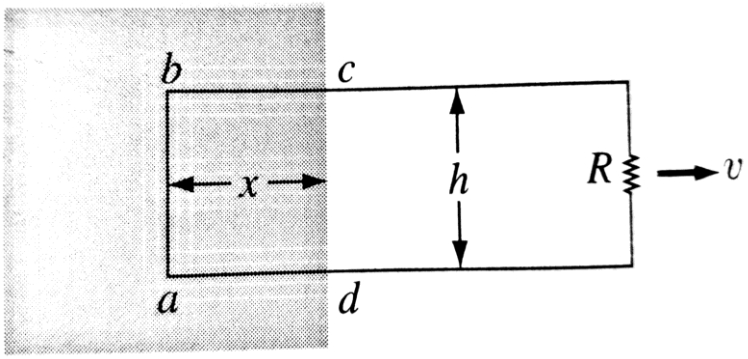

Figure G.2. Primitive model of a generator. The shaded region represents the region of the magnetic field whilst the resistor represents the electric appliance the generator is driving the current through (178).

In such a case, the emf can be written as a function of the speed (v) the current loop is being pulled with and the width of the loop (h) (178).

$$\varepsilon = \oint \boldsymbol{f}_{mag} \cdot \mathrm{d}\mathbf{l} = vBh \quad (Eq.G.2.3)$$

In order to obtain the rate of flow of emf through a loop of wire, ϕ is defined as the flux of $\boldsymbol{B}$ through the loop.

$$\phi = \int \boldsymbol{B} \cdot d\boldsymbol{a} \quad (Eq.G.2.4)$$

For a simple rectangular loop in Fig. G.2, the flux is

$$\phi = Bhx \quad (Eq.G.2.5)$$

When the loop is being moved through and from the region with the magnetic field, the flux decreases.

$$\frac{d\phi}{dt} = Bh\frac{dx}{dt} = -Bhv \quad (Eq.G.2.6)$$

Combining Eq.G.2.3. with Eq.G.2.6. the flux rule of motional emf can be obtained (Eq. G.2.7).

$$\varepsilon = -\frac{d\phi}{dt} \quad (Eq.G.2.7)$$

G.3. Faraday's law

In 1831, Michael Faraday coined what will turn out to be one of the most fundamental laws in classical electromagnetism – the fact that *a changing magnetic field induces an electric field*. He did this by performing a series of three experiments with a loop of wire and a magnet (Fig. G.3) (178).

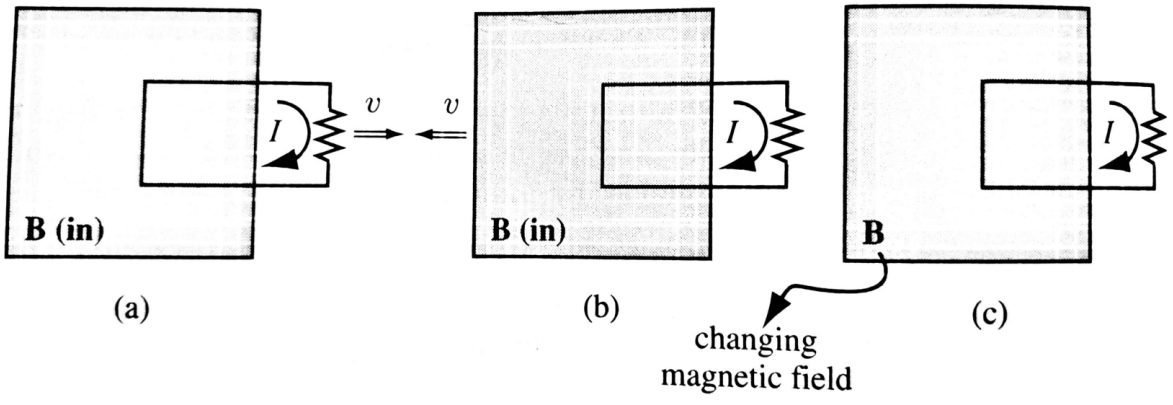

Figure G.3. Setup of Faraday's (a) first, (b) second and (c) third experiments (178).

The first experiment consisted of a static magnet through whose magnetic field he pulled a loop of wire (Fig. G.3a) – resulting in a current flow through the wire (178). On the other hand, the second experiment required for the loop of wire to be stationary while the magnet would be

moved (Fig. G.3b) – again, resulting in a current flow through the wire (178). Finally, the third experiment included changing the strength of the field with both the magnet and the wire loop being at rest (Fig. G.3c) (178). Not much to his surprise after the first two experiments, the third experiment also resulted in a current flow through the wire.

Unsurprisingly, the first experiment Faraday performed follows the flux rule (Eq.G.2.7), thus representing a classical case of motional emf (178). The same holds true for his second experiment where it is the magnet that is being moved, as it is only the relative motion between the magnet and wire loop that counts – not necessarily the movement of each individual component in itself (178).

Since

$$\varepsilon = \oint \boldsymbol{E} \cdot d\boldsymbol{l} = -\frac{\mathrm{d}\phi}{dt} \quad (Eq.G.3.1),$$

upon substitution of the flux of $\boldsymbol{B}$ (Eq.G.2.4) into the aforementioned expression, one can obtain Faraday's law in integral (Eq.G.3.2)

$$\oint \boldsymbol{E} \cdot d\boldsymbol{l} = -\int \frac{\partial \boldsymbol{B}}{\partial t} \cdot d\boldsymbol{a} \quad (Eq.G.3.2),$$

as well as the differential form (Eq.G.3.3).

$$\boldsymbol{\nabla} \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t} \quad (Eq.G.3.3)$$

Even though the change in the magnetic field in Faraday's third experiment occurred due to an entirely different reason, an electric field is, nevertheless, generated and the same equation for emf within the loop will hold true (178).

Getting back to the idea of Faraday's experiments, even though all three of his experiments appear to be demonstrations of Faraday's law, in such extent that 'changing magnetic fields induce electric fields', the situation is not so straightforward (178). In general, the first experiment Faraday performed seems to be a more clear-cut demonstration of the Lorentz's force law (Eq.E.1.2), in as much that Lorentz force law describes the force a charge would feel once under the influence of an external magnetic field. The main problem here is that,

technically, the emf generated in the first experiment is not *electric*, as is in other two experiments, but rather *magnetic* in nature (178). Essentially, in the first experiment, the work is being performed by the *magnetic field*, whilst in the second two experiments the work is being performed by the *electric field*, which has been induced by a *changing magnetic field* – which is, ultimately, the definition of Faraday's law (178).

G.4. Induced electric field

Speaking generally, the electric field ($\boldsymbol{E}$) induced by a changing magnetic field as described by Faraday's law, then the curl of $\boldsymbol{E}$ follows Faraday's law (Eq.G.3.3), whilst its divergence follows Gauss's law (Eq.B.3.2) (178). Since $\rho = 0$ then

$$\boldsymbol{\nabla} \times \boldsymbol{E} = 0 \quad and \quad \boldsymbol{\nabla} \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t} \quad (Eq.G.4.1)$$

which appear to be mathematically identical to curl and divergence of magnetic fields, as discussed in magnetostatics (Eq.G.4.2).

$$\boldsymbol{\nabla} \times \boldsymbol{B} = 0 \quad and \quad \boldsymbol{\nabla} \times \boldsymbol{B} = \mu_0 \boldsymbol{J} \quad (Eq.G.4.2)$$

As it is visible between (Eq.G.4.1) and (Eq.G.4.2), electric fields induced by changing magnetic fields (as described by Faraday's law) are governed by $-\frac{\partial \boldsymbol{B}}{\partial t}$ in the same way that magnetic fields are governed by $\mu_0 \boldsymbol{J}$ (178). This means that an analog to Biot-Savart law for volume currents (Eq.E.2.3) can be written for these Faraday-induced electric fields (Eq. G.4.3 and Eq. G.4.4.).

$$\boldsymbol{E} = -\frac{1}{4\pi}\int \frac{\frac{\partial \boldsymbol{B}}{\partial t} \times \hat{\boldsymbol{r}}}{r^2} d\tau \quad (Eq.G.4.3)$$

$$\boldsymbol{E} = -\frac{1}{4\pi}\frac{\partial}{\partial t}\int \frac{\boldsymbol{B} \times \hat{\boldsymbol{r}}}{r^2} d\tau \quad (Eq.G.4.4)$$

In accordance with aforementioned similarities, such analogy could also be made for the role $-\frac{\mathrm{d}\phi}{dt}$ plays in Faraday-induced electric fields with the role $\mu_0 I_{enc}$ plays in governing magnetic fields as described by Ampere's law (Eq. E.2.11.) (178). This yields another form of Faraday's law in integral form (Eq. G.4.5).

$$\oint \boldsymbol{E} \cdot d\boldsymbol{l} = -\frac{\mathrm{d}\phi}{dt} \quad (Eq.G.4.5)$$

G.5. Maxwell's equations

Combining all the equations encountered thus far, and that are related with the divergence and curl of electric and magnetic fields, yields a set of equations that described the fundamental laws of electrodynamics before Maxwell (178).

$$\boldsymbol{\nabla} \cdot \boldsymbol{E} = \frac{\rho}{\epsilon_0} \quad - \text{Gauss's law for electricity } (Eq.B.3.2)$$

$$\boldsymbol{\nabla} \cdot \boldsymbol{B} = 0 \quad - \text{ Gauss's law for magnetism } (Eq.E.2.7)$$

$$\boldsymbol{\nabla} \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t} \quad - \text{ Faraday's law } (Eq.G.3.3)$$

$$\boldsymbol{\nabla} \times \boldsymbol{B} = \mu_0 \boldsymbol{J} \quad - \text{ Ampere's law } (Eq.E.3.10)$$

In the mid-nineteenth century, when James Clerk Maxwell began his work, these laws were already starting to circle the scientific community. But one of them had one major flaw – Ampere's law was invalid in the case of *nonsteady* currents (178). Of course, this is something that makes perfect sense now that we can experimentally prove it – what else could one expect when the law itself is derived from the Biot-Savart law (178). Nevertheless, driven by a notion that the divergence of a curl is always 0, it is not difficult to prove the inaccuracies in of Ampere's law in cases of nonsteady currents.

Taking Faraday's law (Eq.G.3.3), for it to hold true in steady and nonsteady conditions, the divergence of a curl of the electric field has to be zero ($\boldsymbol{\nabla} \cdot (\boldsymbol{\nabla} \times \boldsymbol{E}) = 0$) (178). What this means is that the partial derivative of the magnetic field with respect to time on the RHS must be zero too (Eq.G.5.1).

$$\boldsymbol{\nabla} \cdot (\boldsymbol{\nabla} \times \boldsymbol{E}) = \boldsymbol{\nabla} \cdot \left(-\frac{\partial \boldsymbol{B}}{\partial t}\right) \quad (Eq.G.5.1)$$

$$0 = \frac{\partial}{\partial t}(\boldsymbol{\nabla} \cdot \boldsymbol{B}) \quad (Eq.G.5.2)$$

$$0 = 0 \quad (Eq.G.5.3)$$

Now, since we know, from Gauss's law for magnetism that a curl of the magnetic field is zero (Eq.E.2.7), the RHS of the equation (Eq.G.5.2) becomes zero too (Eq.G.5.3).

On the other hand, when taking the divergence of a curl of the magnetic field from Ampere's law (Eq.E.3.10)

$$\boldsymbol{\nabla} \cdot (\boldsymbol{\nabla} \times \boldsymbol{B}) = \mu_0 (\boldsymbol{\nabla} \cdot \boldsymbol{J}) \quad (Eq.G.5.4),$$

the left-hand side (LHS) of the expression indeed is zero, but a problem arises on the RHS. The divergence of $\boldsymbol{J}$ is zero for *steady* currents, making the RHS zero in that case but, for *nonsteady* currents, RHS is different from zero (Eq.G.5.4.). This suggests an innate fallacy in Ampere's law that Maxwell aimed to fix.

By applying the continuity equation (Eq.E.1.12) to Gauss's law for electricity, we can rewrite the divergence of $\boldsymbol{J}$ (Eq.G.5.5.).

$$\boldsymbol{\nabla} \cdot \boldsymbol{J} = -\frac{\partial \rho}{\partial t} = -\frac{\partial}{\partial t}(\epsilon_0 \boldsymbol{\nabla} \cdot \boldsymbol{E}) = -\boldsymbol{\nabla} \cdot \left(\epsilon_0 \frac{\partial \boldsymbol{E}}{\partial t}\right) \quad (Eq.G.5.5)$$

Combining the RHS term $\epsilon_0 \frac{\partial \boldsymbol{E}}{\partial t}$ with $\boldsymbol{J}$ in Ampere's law yields the *Ampere-Maxwell law* as we know it today (Eq.G.5.6.) (178).

$$\boldsymbol{\nabla} \times \boldsymbol{B} = \mu_0 \left(\boldsymbol{J} + \epsilon_0 \frac{\partial \boldsymbol{E}}{\partial t}\right) \quad (Eq.G.5.6)$$

With this alteration, Maxwell managed to successfully solve the inconsistencies in Ampere's law for nonsteady currents whilst still keeping it valid in magnetostatic cases of steady currents (178). Generally, this additional term Maxwell introduced suggested another interesting component to an already interesting law – just as a changing magnetic field induces an electric field (Faraday's law, Eq.G.5.1c), *a changing electric field induces a magnetic field* (178).

In order to give it additional appeal or try to reason the role it plays in magnetodynamics, Maxwell named it the *displacement current* ($\boldsymbol{J}_d$) (Eq.G.5.7).

$$\boldsymbol{J}_d = \epsilon_0 \frac{\partial \boldsymbol{E}}{\partial t} \quad (Eq.G.5.7)$$

but, this newly-introduced term has nothing to do with currents. Maxwell gave it that name to enable easier association with its function – adding onto $\boldsymbol{J}$ in Ampere's law to make it valid in cases of nonsteady currents (178).

All of this gave rise to what we now know under the name of *Maxwell's equations* (178).

$$\boldsymbol{\nabla} \cdot \boldsymbol{E} = \frac{\rho}{\epsilon_0} \quad - \text{Gauss's law for electricity}$$

$$\boldsymbol{\nabla} \cdot \boldsymbol{B} = 0 \quad - \text{Gauss's law for magnetism}$$

$$\boldsymbol{\nabla} \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t} \quad - \text{Faraday's law}$$

$$\boldsymbol{\nabla} \times \boldsymbol{B} = \mu_0 \left(\boldsymbol{J} + \epsilon_0 \frac{\partial \boldsymbol{E}}{\partial t} \right) \quad - \text{Ampere– Maxwell law}$$

G.6. Electromagnetic waves

When talking about propagation of electromagnetic fields, it is impossible to avoid the term *electromagnetic waves* (178). Electromagnetic waves are the oscillation of an electric and magnetic field perpendicular to the direction of wave propagation.

Depending on the medium electromagnetic waves are propagating within, Maxwell's equations will greatly differ between conducting and nonconducting media. Whilst Maxwell's equations take on a simpler form in linear nonconducting media, or in vacuum, since the free charge density (ρ_f) and the free current density ($\boldsymbol{J}_f$) are zero, this is not the case for electromagnetic wave propagation in conductors (178).

According to Ohm's law (Eq.G.1.3), the density of the current within a conductor is proportional to the electric field

$$\boldsymbol{J}_f = \sigma \boldsymbol{E} \quad (Eq.G.5.8)$$

After substituting this relationship into Maxwell's equations, they take the shape of

$$(i)\ \boldsymbol{\nabla}\cdot\boldsymbol{E}=\frac{\rho}{\epsilon_0}\quad (Eq.G.5.9)$$

$$(ii)\ \boldsymbol{\nabla}\cdot\boldsymbol{B}=0\quad (Eq.G.5.10)$$

$$(iii)\ \boldsymbol{\nabla}\times\boldsymbol{E}=-\frac{\partial\boldsymbol{B}}{\partial t}\quad (Eq.G.5.11)$$

$$(iv)\ \boldsymbol{\nabla}\times\boldsymbol{B}=\mu\left(\epsilon\frac{\partial\boldsymbol{E}}{\partial t}+\sigma\boldsymbol{E}\right)\quad (Eq.G.5.12)$$

Combining the continuity equation (Eq.E.1.12) for free charge with Ohm's (Eq.G.1.3) and Gauss's law for electricity (Eq.B.3.2) yields

$$\frac{\partial\rho_f}{\partial t}=-\sigma(\boldsymbol{\nabla}\cdot\boldsymbol{E})=-\frac{\sigma}{\epsilon}\rho\quad (Eq.G.5.13),$$

an expression that describes the change in free charge within a homogeneous linear medium through time

$$\rho_f(t)=e^{-\left(\frac{\sigma}{\epsilon}\right)t}\rho_f(0)\quad (Eq.G.5.14),$$

where $\tau=\frac{\sigma}{\epsilon}$ is the characteristic time needed for the initial free charge $\rho_f(0)$ to dissipate (178).

G.7. Maxwell's equations for inhomogeneous fields

On the other hand, if the aim is to describe inhomogeneous electromagnetic fields, slight modification of Maxwell's equations is needed.

As mentioned previously, inhomogeneous fields are a type of electromagnetic fields whose magnitude and direction varies in space. Because of this, a specific subset of expressions has to be defined that govern their behavior. In order to obtain this modified wave equation for $\boldsymbol{E}$ and $\boldsymbol{B}$, the curl has to be applied to equations Eq.G.5.11 (iii) and Eq.G.5.12 (iv) (178). This results in

$$\nabla^2\boldsymbol{E}=\mu\epsilon\frac{\partial^2\boldsymbol{E}}{\partial t^2}+\mu\sigma\frac{\partial\boldsymbol{E}}{\partial t}\quad (Eq.G.5.15),$$

$$\nabla^2\boldsymbol{B}=\mu\epsilon\frac{\partial^2\boldsymbol{B}}{\partial t^2}+\mu\sigma\frac{\partial\boldsymbol{B}}{\partial t}\quad (Eq.G.5.16)$$

After appropriate substitutions, the equations can take the form of

$$\frac{1}{c^2}\frac{\partial^2 \boldsymbol{E}}{\partial t^2} - \boldsymbol{\nabla^2 E} = -\left(\frac{1}{\epsilon_0}\nabla\rho + \mu_0\frac{\partial \boldsymbol{J}}{\partial t}\right) \quad (Eq.\,G.5.17)$$

$$\frac{1}{c^2}\frac{\partial^2 \boldsymbol{B}}{\partial t^2} - \boldsymbol{\nabla^2 B} = \mu_0 \nabla \times \boldsymbol{J} \quad (Eq.\,G.5.18)$$

Ultimately, the final solutions to these equations serve to describe the real electric and magnetic fields in conductors as seen in Fig. G.6 and are

$$\begin{cases} \boldsymbol{E}(z,t) = E_0 e^{-kz}\cos(kz - \omega t + \delta_E)\hat{\boldsymbol{x}} \\ \boldsymbol{B}(z,t) = B_0 e^{-kz}\cos(kz - \omega t + \delta_E + \phi)\hat{\boldsymbol{y}} \end{cases} \quad (Eq.\,G.5.19)$$

where k is the wave number, ω is the angular frequency such that $\omega = 2\pi\upsilon = k\upsilon$, δ_E is the phase of the electric field and ϕ is the angle such that $\phi = \tan^{-1}({}^{\kappa}/_{k})$ (178).

$$k = \omega\sqrt{\frac{\epsilon\mu}{2}}\left[\sqrt{1+\left(\frac{\sigma}{\epsilon\omega}\right)^2}+1\right]^{1/2} \quad (Eq.\,G.5.20)$$

$$\kappa = \omega\sqrt{\frac{\epsilon\mu}{2}}\left[\sqrt{1+\left(\frac{\sigma}{\epsilon\omega}\right)^2}-1\right]^{1/2} \quad (Eq.\,G.5.21)$$

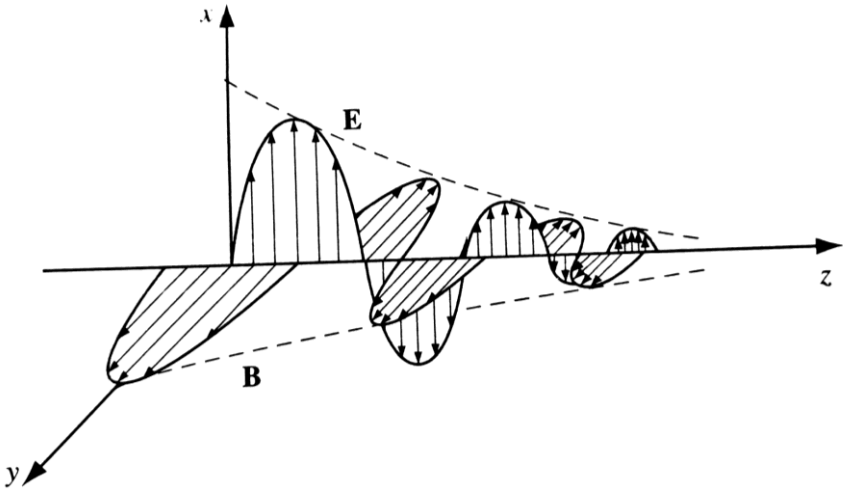

Figure G.4. Direction and shape of an electromagnetic wave within a coordinate system (178).

H. The history of the Hodgkin-Huxley model

Alan Lloyd Hodgkin and Andrew Huxley were researchers at Trinity College, University of Cambridge, in the 20th century and they are some of the most influential scientists in the history of physiology. Their work on nerve cell excitability not only shaped our current understanding of action potential initiation and propagation through opening and closing of voltage-gated ion channels but also enabled us to form fundamentals of computational modelling of nerve cell workings and dynamics (277).

They first began their work together in 1935, performing experiments on squid giant axons. Initially, their goal was to measure the viscosity of the axoplasm by observing the behavior mercury droplets make when passing through it (277). But this experiment was not a success and, most of the time, the droplets would just sit on top of the axon, passing through only if the axoplasm was previously damaged (277). Nevertheless, this did not pose a big derailment in their collaboration as they, soon after, decided to insert a fine capillary electrode inside the nerve fiber in order to measure the potential difference that exists across the membrane – an experiment that would end up being one of the biggest leaps in the history of physiology.

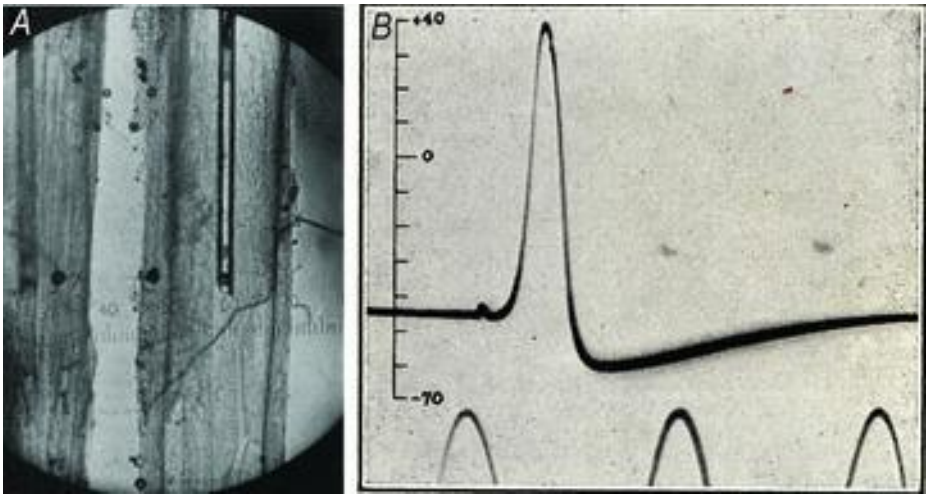

Figure H.1. (a) Photomicrograph of the electrode setup inside a squid giant axon (278) and (b) the recording of the action potential propagation through the squid giant axon (279).

In 1947, after returning from World War II, they resumed their work and started developing the

voltage-clamp technique – an experimental method which has become the fundamental research method in electrophysiology. The voltage-clamp method enables researchers to measure ionic currents flowing through membranes of excitable cells whilst setting the membrane voltage at a resting level. Essentially, the voltage clamp works by first measuring the electric potential of a membrane which is then followed by changing of this voltage to a goal value through addition of additional currents. As much as K. S. Cole and G. Marmont, researchers working at Woods Hole, were also working on the voltage clamp technique in 1947, Hodgkin and Huxley used a dual electrode approach, completely avoiding the problem that often occurred in such recordings and which is voltage polarization (277). In such, the method they used presented clear advantages over the Cole and Marmont technique and are, thus, most often considered as the inventors of the voltage-clamp method (277).

On top of the voltage-clamp method, the most innovative and revolutionary work Hodgkin and Huxley did, occurred in 1946 – when they first started working on their mathematical model of the action potential. Not only did their model present a first attempt at quantifying the degree and nature of electrical excitability in nerve cells, but they also included valuable constants which enabled faithful representation of experimental measurements in a mathematical model (277). What makes the Hodgkin-Huxley model even more interesting is the fact that it describes the electrical activity of neurons through both passive and active current flow – this is also one of the reasons why their model is still in wide use today.

Ultimately, their work on nerve cell excitability culminated in a seminal paper published in 1952 which earned them the Nobel prize in medicine in 1963.

Curriculum Vitae

Jasmina Isaković was born on 14th of January 1995 in Varaždin, where she finished the International Baccalaureate Diploma Programme at the First Gymnasium Varaždin. She obtained her BSc in Physics, with a minor in Neural Science and a disciplinary concentration in Art History, from New York University Abu Dhabi in 2017, with a full academic scholarship. Throughout these studies she was also supported by the Zois scholarship for gifted students, awarded by The Slovene Human Resources Development and Scholarship fund of the Republic of Slovenia. During her undergraduate studies, Jasmina has obtained research experience in the field of dark matter detectors at the Gran Sasso National Laboratory and NYU Abu Dhabi, as well as in neuroscience and molecular biology at the NYU New York, SUNY Downstate Medical Center and the Croatian Institute for Brain Research in Zagreb throughout 2015 and 2016. This interdisciplinary approach to neuroscience gave rise to her undergraduate thesis titled: "Theoretical modeling of the interaction between the T-cell surface charge and proposed electromagnetic fields around neurons: its role in disease etiology and therapy". As of 2018, she has been a part of Omnion Research International Ltd. where she works on modeling the electromagnetomechanics of the human body, with a specific emphasis on bridging the gap between the physics of electromagnetism, particle physics and biology. She is currently developing novel electromagnetic field application devices to utilize electric properties of cells in order to initiate tissue regeneration and modify the immune response. For this project she obtained one-year research funding as a part of the call "Program provjere inovativnog koncepta (PoC8)" by HAMAG BICRO in 2019, and has been serving as the Project Leader since. Up to date, Jasmina has participated in 6 research conferences, being an invited speaker on two of them. Jasmina has co-authored 5 scientific publications which have 10 citations and has an h-index of 2 (Google Scholar). In 2019, Jasmina has also started pursuing a PhD in Elementary Particle Physics at the University of Rijeka, whilst also working at the Department of Histology at the University of Zagreb School of Medicine as an External Associate in Education for the subject "Histology and Embryology".

LIST OF PUBLICATIONS

I. **Isakovic, J.**, Dobbs-Dixon, I., Chaudhury, D., & Mitrecic, D. (2018). Modeling of inhomogeneous electromagnetic fields in the nervous system: a novel paradigm in understanding cell interactions, disease etiology and therapy. Scientific reports, 8(1), 12909. https://doi.org/10.1038/s41598-018-31054-9

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IV. Mitrečić, D., Petrović, D. J., Stančin, P., **Isaković, J.**, Zavan, B., Tricarico, G., Kujundžić Tiljak, M., & Di Luca, M. (2020). How to face the aging world - lessons from dementia research. Croatian medical journal, 61(2), 139–146. https://doi.org/10.3325/cmj.2020.61.139

V. Tricarico, G., **Isakovic, J**., Song, M. S., Rustichelli, F., Travagli, V., & Mitrecic, D. (2020). Ozone influences migration and proliferation of neural stem cells in vitro. Neuroscience letters, 739, 135390. https://doi.org/10.1016/j.neulet.2020.135390