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Dendrites and Axons

Edited by Gonzalo Emiliano Aranda Abreu and María Elena Hernández Aguilar

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Meet the editors

Professor Dr. Gonzalo Emiliano Aranda Abreu (Centro de Investigaciones Cerebrales, Universidad Veracruzana, Xalapa, Veracruz, Mexico) was born in 1968 in Ciudad del Carmen, Campeche, Mexico. He received his BSc (Experimental Biology) degree from the Universidad Autónoma Metropolitana, Iztapalapa, Mexico, in 1992, his MSc (Molecular Biology and Genetics) degree from the Centro de Investigación y Estudios Avanzados, IPN, Mexico, in 1996, and his PhD (Neurobiology) degree from the Weizmann Institute of Science, Rehovot, Israel, in 2001. He has more than 20 years of experience in Alzheimer´s disease research. He has written several articles about tau mRNA transport in the neuronal axon and Alzheimer´s disease, as well as book chapters where the main theme is brain rehabilitation. He is president and member of the Southeastern Mexican Chapter of the Society for Neuroscience, USA. He is involved in the training of doctoral students. He teaches molecular and cellular neurobiology and bioinformatics to PhD students.

Professor Dr. María Elena Hernández Aguilar (Centro de Investigaciones Cerebrales, Universidad Veracruzana, Xalapa, Veracruz, Mexico) was born in the city of Xalapa Veracruz, Mexico. She received her BSc (Biology) degree from Universidad Veracruzana, Mexico, in 1989, her MSc (Physiology) degree from the Universidad Nacional Autónoma de México, Mexico, in 1993, and her PhD (Physiology) degree from the Universidad Nacional Autónoma de México, Mexico, in 1997. Her area of research is the mechanisms that lead to prostate cancer. She has written a considerable number of articles and book chapters related to prostate cancer. She also investigates how major pelvic ganglia neurons may be involved in the generation of prostate cancer when they have degenerated. She coordinates PhDs in brain research studies and teaches neuroendocrinology to PhD students.

Contents

Preface

Neurons, Dendrites and Axons is an update of the information that exists about how axons develop and which signaling pathways are involved in the process. This book is divided into five chapters. The first chapter is about axon guidance through the action of the interaction between neurons, as well as the interaction with the extracellular matrix, leading to elucidate the complex connection that exists in the brain during its origin and development. The second chapter is about semaphorins and their relationship with neurodegenerative diseases such as Alzheimer's, Parkinson's, and sclerosis. It also describes the molecular mechanisms that could generate protection as a possible therapy for neurodegenerative diseases. Neurodegenerative diseases and their therapeutic approaches are described in third chapter. The fourth chapter focuses on the gap junction of the dorsal root of the ganglion where the morphology of neurons is described, as well as their neurobiology. Last chapter focuses on the development of bioelectrical nerve interfaces that could be implanted to regenerate damaged nerves.

The editors would like to express their deep gratitude to all the authors who contributed their knowledge to the development of this book. We also thank InTech Publishing for spreading the knowledge worldwide.

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Section 1

Axon Guidance

Chapter 1

Cell-Cell and Cell-Matrix Interactions during Axons Guidance

Vela-Alcántara Ana and Tamariz Elisa

Abstract

The establishment of neuronal connections during development is a critical process for the correct function of central nervous system and for their regeneration during adult stages. Axon extension and guidance toward their targets are a complex process involving several signals provided by extracellular milieu where secreted factors, other cells, axons, and extracellular matrix proteins are interacting to establish the wiring of the brain. The expression of those signals at specific time and space, and their mechanisms of action during axon projection are the subject of numerous studies. This knowledge had contributed to understand the complex panorama of brain wiring during development and the origin and possible cure of central nervous system diseases. In this chapter, we focus on cell-cell and cell-matrix interactions as two important signals during axon guidance, and how these interactions impact the response to diffusible guidance cues. We emphasize the need and the challenge to understand the complex relations among simultaneous signals to guide axons projections, and how this knowledge could influence approaches to deal with neural regeneration issues.

Keywords: growth cone, guidance cues, fasciculation, extracellular matrix, axonal regeneration

1. Introduction

After neural tube formation, multipotent stem cells migrate and generate precursor cells that will differentiate into neurons and glial cells. Neurons will extend cell projections to become integrated to the brain circuits by a finely regulated process. Through cell extensions, classified as dendrites and axons, neurons are responsible for the perception of the external world, the former are in charge of receiving electric impulses of other cells, and the last transmit the impulses far from the cell body. Axons projections are stereotyped, and the accuracy of reaching their target is fundamental for the correct central nervous system (CNS) functioning. Axons project by specialized and motile structures located at the end of the axons called growth cones. These specialized regions sense the external milieu, detecting signals that originate complex cellular mechanism involved in axon elongation; therefore, neuronal pathfinding is highly regulated by the availability of the external signals, by the expression of cell receptors, and by specific molecular mechanisms that stimulate or inhibit growth cone displacement. Extracellular matrix

components present in axons pathway can be signaling by forming soluble chemotropic protein gradients and/or by direct interaction with membrane receptors at the growth cones. Besides, other axons present in the pathway could be promoting axons-axons interactions or fasciculation, allowing the guidance of projections toward their final targets. In this chapter, we make a rough description of cellular mechanisms of growth cone motility that drives axon elongation, mainly focused in the cell adhesion and cytoskeleton regulation by guidance cues, followed by some of the evidences about cell-extracellular matrix and cell-cell interactions relevance during axons projection; finally, we address the importance of synergic interaction among the signals, and how they can modulate the response of axons during pathfinding toward their targets.

2. Cellular mechanisms of axon projection

The growth cone is a specialized structure located at the end of axons or dendrites, capable to detect extracellular guidance cues and to integrate them into a projection or retraction movement that guides the axon toward their innervation target [1]. During axonal elongation, changes in the growth cone morphology and in the direction of its projection depend on the cytoskeleton dynamics and on the regulation of cell adhesion, inducing the formation of filopodia and lamellipodia at the leading edge and exerting tensile and traction forces that will influence neuron elongation [2, 3]. According to the cytoskeleton distribution, the growth cone can be divided into three structural domains: the central domain (C domain), in which there are stable microtubule (MT) bundles entering the growth cone from the axon axis, organelles, vesicles and actin bundles; the peripheral domain (P domain), located in the distal part of the growth cone, containing actin filaments (F-actin bundles) that form the filopodia and lamellipodia, and dynamic MT that extends from the C domain and invades the P domain following the F-actin bundles. Finally, the intermediate zone between the C and P domain called the transition zone (T zone), which contains actomyosin contractile structures located perpendicular to the F-actin bundles [4, 5].

The lamellipodia and filopodia are dynamic structures, from which the elongation process starts. Lamellipodia are broader structures, rich in actin filament networks, while the filopodia are thin extensions, about 100–200 nm diameter and 10 μm in length, constituted by a 10–30 very close actin filaments arranged in parallel [5]. The rate of F-actin and MT polymerization and the retrograde flow of F-actin determine the extension and retraction of the growth cone. In a rough description, actin monomers assemble into F-actin filaments at the cell membrane boundary of the P zone, pushing the membrane during the elongation, thus generating tensile forces. By a retrograde flow driven by actomyosin contractility and by the cell-extracellular matrix interaction, filaments push themselves backward to the T region where filaments are severed and recycled [2, 5, 6]. Assembly and disassembly of the F-actin filaments by controlling the polymerization rate of globular monomeric actin (G-actin) are important for the advance or retraction and is influenced by guidance cues [7, 8]. At the same time, MT plus ends point toward axon tips, and their assembly and disassembly at growth cone are regulated by the F-actin bundles and by the traction force exerted by the actomyosin contractility, allowing the capture and guidance of MT extension through the T and P domain, stabilizing the filopodia [5]. MT polymerization and invasion of P domain, coupled to substrate anchor of growth cone projections by cell adhesion sites linked to the cytoskeleton, promotes growth cone, pulling forward using the actomyosin-mediated

force projection; therefore, MT advances while the C domain transforms in an axon segment, consolidating the axon elongation [9]. Cytoskeleton dynamics and cell adhesion regulation therefore are very important during the response to guidance cues, for example, at the side of the growth cone turning toward an attractant cue, a stabilization and a decrease of F-actin retrograde flow and anchoring through adhesion sites is present, while the inhibition of F-actin and MT polymerization occurs at the growth cone side retracted in response to a repulsive guidance cues [10–12].

Adhesion of growth cones to the substrate is finely regulated during axon elongation or retraction. Adhesion sites or "contact points" (CP) are constituted by protein complexes that allow the adhesion and generation of traction force on the substrate [13]; these complexes mediate the anchoring of cells by the transmembrane protein integrins that have a primordial role, coupling the cytoskeleton to the extracellular matrix and by recruiting adaptor and signaling proteins at the cytosolic side [14]. Adhesion complexes include several associated proteins that mediate the interactions with the cytoskeleton, regulate actin polymerization, and participate in the signaling exerted by cell adhesion [2, 14, 15]. During axon projection, assembly-disassembly of CP are involved in response to guidance cues; the inhibition of turnover of CP inhibits axons outgrowth, while localized assembly and turnover of CP promote axon extension in response to guidance cues [13, 16, 17], for example, activation of integrins and subsequent focal adhesion kinase (FAK) phosphorylation are involved in the attraction of dendrites mediated by the chemotrophic protein semaphorin 3A [18], while repellant factors as myelin-associated glycoprotein (MAG) induce growth cone turning by a rapid endocytosis of integrins and loss of cell adhesions [19]. In summary, the projection or retraction of growth cones responds to extracellular signals that guide them to specific targets and trigger a complex network of signal transduction mechanisms that includes the dynamic remodeling of cell adhesion sites and cytoskeleton that together translate into elongation or retraction movements for the redirection of the neuronal projections.

2.1 Cell-extracellular matrix interactions

As mentioned earlier, neurons project their neurites to specific targets, guided by extracellular signals integrated by the growth cone. The mechanisms to direct axons projection are triggered by secreted chemotropic proteins, by proteins anchored to the substrate, or by direct interactions between axons mediated by proteins anchored to the cell membrane, as cell adhesion proteins or even chemotropic protein receptors [20, 21]. Growth cones respond to gradients of diffusible molecules, or of proteins associated with the substrate, that guide them to the innervation target; these molecules can be chemoattractive or chemorepellant, and the extracellular matrix can stabilize the gradients from target cells or intermediate cells, extending them at a greater distance [21]. Although classical chemotropic proteins as ephrins, netrins, slits, and semaphorins are some of the more studied guidance cues, in this chapter, we focus our attention to ECM and cell-anchored proteins as axon guidance cues.

The interaction with extracellular matrix (ECM) components was one of the first proposed axon guidance cues that exert a "contact guidance" effect, improving axon projection [22]. ECM components surround cells and are distributed along the pathways of axon projection [23, 24]; therefore, they are not only part of the support in which neurons are divided and maintained but also has a relevant role in the signaling and the determination of the differentiation and migration of neurons, and in the elongation processes of neurites [25–27]. In addition, the physical

properties of ECM as topography and stiffness have now an increasing interest as factors that influence axon projection [28].

ECM comprises about 40% of extracellular space in developing brains as compared with the 20% in adult brains [29]. Some of the most relevant ECM proteins implicated in axonal projection are laminin, fibronectin, collagen, and tenascin. Laminins are a heterotrimeric glycoproteins family, formed of α, β, and γ subunits. During CNS development, laminins have an important role in promoting cell migration and axonal outgrowth [26], and the absence of laminins results in important axon-targeting alterations [30–32]. Fibronectin is a glycoprotein present in the early development at central and peripheral nervous system in the spinal cord and cortex [33, 34] and is involved in cell migration, cell adhesion, and in stimulation of neurite outgrowth during development and after peripheral nervous system injury [35–37]. Both laminins and fibronectin have an important role in modulating the response to chemotropic proteins [38, 39]. Collagen is a family of fibrillar glycoproteins that gives structure and support to cells as well as anchorage for other proteins [40, 41]. Collagens have an important role in neurite outgrowth, axon guidance, and axon targeting, and their absence impacts central and peripheral axons, targeting as a motor axon guidance and retinal ganglion cell projection [42–44]. Tenascin is another ECM glycoprotein family with several functional domains [45]. *In vitro* and *in vivo* experiments have shown an inhibitory effect of tenascin for several kinds of axons as hippocampal and cerebellar neurons [46, 47]; however, specific alternative spliced variants promote neurite outgrowth, as the fibronectin type-III domain of tenascin C that induce cerebellar neurons outgrowth [48]. Chondroitin sulfate proteoglycans (CSPGs) are ECM proteoglycans with both inhibitory and attractant effects on axonal outgrowth. The accumulation of CSPG in scar tissue, after injuries in adult CNS, inhibits axon outgrowth [49]; however, it is also a permissive signal along axonal pathways during the development of retinal projection, or in the cortex [50–53], and their inhibitory effects are attenuated by the presence of laminin-1 [54, 55].

Recent studies have shown that the ECM stiffness determines cellular processes such as differentiation, proliferation, and migration [56–58]. Particularly, the work of Engler et al. demonstrated for the first time that the stiffness of the substrate in which stem cells are grown in vitro can modulate their differentiation into cell types such as bone, muscle, or neurons [59]. Probably, one of the first studied aspects has been the role of stiffness in the elongation of neurites; Flanagan et al. reported that when primary neurons of the mouse spinal cord (E13.5) grew in matrices with less stiffness, close to that found in the brain, the elongation of the neurites was greater [60]. However, there are divergences in the data depending on the model, since it has been reported that on softer substrates, PC12 cells show few neurites, relatively short and unbranched, whereas on stiffer substrates, cortical neurons and astrocytes (E17-E19) turn out to have longer and branched projections [57, 60, 61].

In the case of developing nervous system, variations in stiffness during development stages, and at different regions as cerebral cortex and optic tectum have been reported [62, 63]. Guidance by chemotropic proteins as slits and semaphorins of retinal ganglion cell (RGC) axons projecting from the retina to the optic tectum (OT) has been extensively reported [64, 65]; interestingly, tissue stiffness also determines their projection, since RGC axons project toward softer OT and grow as fascicles while traversing stiffer regions. Once the axons arrive at the OT, the softer tissue slows down the projections and splays apart the fascicles to branch them and to form synapses with their stereotypic targets [63]. On the other hand, the prevention of axon regeneration after injury can be in part due to changes in ECM and tissue stiffness, as shown for glial scar after spinal cord injury, where components as

collagen IV and laminin, and changes in glial intermediate filaments as vimentin and GFAP, soften the tissue at the scar [66].

Besides stiffness, ECM topography is also a factor that determines the orientation and projection of neurites. Since early observation about the alignment and orientation of axons by "contact guidance," and the improving of axon elongation by aligned collagen fibrils [22, 67], advance in micro- and nanofabrication of biocompatible fibrous substrates, with specific topography and orientation, has shown to improve neurite elongation and orientation, promoting nerve regeneration [68]. Fibers alignment and dimensions are important to improve axonal guidance and elongation, for example, the micrometer versus nanometer dimensions of poly(lactic-glycolic acid) PLGA fibers improve the alignment of neurites [69], and aligned versus nonaligned gelatin and chitosan fibers induce a higher formation of filopodia in Schwann cells, improving the orientation of axon projections along the fibers [70].

2.2 Cell-cell interactions

During the first stages of CNS formation, neuron clusters projects pioneering axons to form longitudinal, transversal, and commissural tracts [71], functioning as scaffolds for latter or follower axons. Early axon scaffolds are well conserved in vertebrates, and common tracts had been described in zebrafish, chick, mouse, sea lamprey, and others. Among common tracts, the ventral longitudinal tract (VLT) formed by the medial longitudinal fascicle (MLF) and the tracts of the post-optic commissure (TPOC) are present in all the studied vertebrates. In amniotes, there are five early axon scaffolds: the MLF, the TPOC, the mammillo-tegmental tract (MTG), the tract of the posterior commissure (TPC), and the tract of the mesencephalic nucleus of the trigeminal nerve (DTmesV) [72]. Axons scaffolds are established as early as embryonic day (E) 8.5 for the DTmesV or E9.5 for MLF in mouse, soon after neural tube closure [73]. Axon-axon interactions are regulated during axon projection, and fasciculation and de-fasciculation could be present along the neural pathfinding, as reported early in insect embryos as grasshopper [74] or fruit fly *Drosophila* [75]. Fasciculation is a regulated process since growth cones can distinguish among different fascicles, and this behavior is driven by the recognition of cell adhesion molecules, as will be mentioned ahead, mediating a stereotyped targeting. Axons fasciculation can be a permissive or a repulsive cue, promoting or inhibiting axon projection by guiding axons through previously established "routes" by pioneering axons, or by limiting axon projections away of the previously established fascicles. Pioneering axons therefore become an important guidance cue that can determine the routes and the correct pattern of tracts [76]; moreover, their growth cones exhibit different morphology as compared with growth cones of follower axons, and a different speed while approaching the midline at the post-optic commissure in zebrafish embryos, indicating that the response to guidance cues as extracellular matrix or chemotropic proteins is different in pioneering and follower axons, probably by modifying their accessibility or sensibility to the guidance cues [77]; however, if the pioneering axons are eliminated, follower axons can convert to pioneering to establish normal tracts [77, 78].

Axons fasciculation is mediated by cell adhesion proteins (CAMs). CAMs are proteins linked to cell membrane as transmembrane proteins or as GPI-anchored proteins, with homophilic or heterophilic interactions [79]. Among the most relevant CAMs are the members of the calcium-independent cell adhesion immunoglobulin superfamily, like neural cell adhesion proteins (NCAM), several proteins of L1 family as L1, CHL1, neurofascin and NrCAM, and a member of the classic

calcium-dependent cadherins family, N-cadherin [20, 79]. The regulation of axons fasciculation could be exerted by modifying CAM expression or by modifying CAM interactions by post-translational modifications, as the addition of polysialic acid to NCAM (PSA-NCAM) [80]. Enhancement of axonal outgrowth has been previously shown by culturing neurons over transfected fibroblast expressing NCAM, N-cadherin, or L1 [81–83], allowing homophilic interactions of CAM expressed in fibroblasts and axon. CAMs also establish heterophilic interactions among proteins as integrin β1 [84], and receptors of chemotropic proteins as the ephrin receptors, EphA3, EphA4 [85], or semaphorins receptor, neuropilin-1 (Npn-1) with L1 [86], or EphA7 receptor with CHL1 [85], modulating the response to chemotropic proteins but also their adhesion. It has been shown that Npn-1, a receptor for class 3 semaphorins, is involved in the fasciculation of motor and sensory axons during limb innervation, and the selective depletion of Npn-1 in dorsal root ganglion neurons leads to defasciculation of motor projections, even when motor neurons still express Npn1, resulting in dorso-ventral incorrect targeting of motor neurons [87]. Npn-1 depletion also affects fasciculation and targeting of cranial nerves and Schwann cells migration [87]. Interestingly, altered projections of descending GAD65-positive fascicles from the MTG tract, present in double knockout mice for Slit chemotropic protein receptors Robo1/2, modify the nigrostriatal projection (NP) of dopaminergic neurons, impairing both tracts interactions, probably by the absence of homophilic Robo-Robo interactions and heterophilic interaction with NCAM proteins [88], explaining some of the Slit1/2 independent role of Robo-expressing axons during NP projection [89]. These results show that besides their role of mediating attraction or repulsive responses, some receptors for chemotropic proteins also mediate axons fasciculation by homo- and heterophilic interactions, and this role could be concealed or dismissed by the more characterized chemotropic response. Moreover, the absence of the expression of receptors Npn-1 and 2 in some of the DA axons projecting to the striatum and driven by semaphorins during NP formation suggests that fasciculation could be a relevant mechanism of guidance for these axons, and a complementary strategy for the projection of DA axons in addition to the chemotropic response [90].

3. Synergic effects of guidance cues

As mentioned before, chemotropic proteins, extracellular matrices, and axon fasciculation are the main guidance cues during axon projection, their effects and mechanisms of action had been mainly studied as a separated stimulus by in vitro assays in explants or cell cultures, or in knockout animals; however, the panorama of axon projection during CNS formation implies simultaneous guidance cues, and projecting neurons should be responding and adapting according to all of them. Interactions among ECM with secreted chemotropic factors can modify their effects on axon projection, for example, it has been shown that the attraction response of RGC to chemotropic protein netrin-1 can be modified to repulsion after neurons interact with laminin-1 or with a laminin-soluble peptide fragment [38]; a similar substrate-dependent response was observed for the membrane-bound chemotropic protein ephrin A-5 in RGC of *Xenopus*; a repulsive response was observed when cells were grown on fibronectin, while a response of attraction was exerted when cells grew over laminin [91]. The induction of neurite outgrowth in DRG neurons by nerve growth factor (NGF) and neurotrophin-3 (NT3) is inhibited when aggrecan or aggregates of aggrecan and hyaluronan are present [92], indicating that ECM component can also modify neurite response to secreted trophic factors. Cross-talk among ECM receptors, chemotropic proteins, and neurotrophin receptors has been well documented, for example, the proper innervation of sensory DRG seems to be

dependent on the integrin expression, since a differential expression of α7 integrin in the subpopulations of DRG determines their response to NGF and NT-3 neurotrophin [93]. Semaphorin 3D, a repulsive chemotropic protein, can regulate MLF axons fasciculation by regulating the expression of L1 CAM in zebrafish, suggesting that besides the reported repulsive effects of semaphorins, their action could be exerted by the regulating expression of cell adhesion molecules [32]. Moreover, a complex interrelation among ECM, response to neurotropic factors, and cell-cell interactions has been reported for chick embryo DRG axons from *in vitro* explants cultured over a bioactive substrate; the NGF-induced outgrowth of DRG axons and Schwann cells from tissue explants was dependent on the density of the Arg-Gly-Asp (RGD) integrin-binding domain of fibronectin, and this effect was mediated by the upregulation of L1 and NCAM proteins by NGF that allowed the interactions among DRG neurons and Schwann cells [94].

4. Guidance cues during regeneration

The generated knowledge about guidance cues as chemotropic proteins and ECM in axon guidance has led to multiple approaches to use them into the regeneration of CNS [95–97]. When the axonal continuity is interrupted by an injury or a disease, a correct axonal regeneration is required to effectively restore the nerve; in this process, cells and ECM interactions, chemotropic proteins, and factors as substrate stiffness are important. In vitro use of ECM as fibronectin has shown to support mouse cortical and hippocampal neurons axonal outgrowth mediated by α5β1 integrin [98]; in vivo application of fibrin/fibronectin gel at the rat spinal cord injury site is permissive to axonal outgrowth [99], and when fibrin glue is applied as a microsurgery suture at a sciatic nerve transplantation model in mouse, axons were more branched and travel longer distances reducing the regeneration time [100]. In the area of biomaterials engineering for axonal regeneration, several approaches promote neural outgrowth, combining ECM components and neurotrophic factors as laminin plus microspheres with neural growth factor and neurotrophin-3 for the repair of sciatic nerve in rats [101], or carbon-coated microfibers plus basic fibroblast growth factor and fibronectin for spinal cord injury [102]; moreover, the integration of stiffness, porosity, and adhesion promotion shows that an approach considering multiple factors can help to promote and orient axon outgrowth [103], and a soft and aligned fibrillary fibrin hydrogel promotes and directs axonal projection in a spinal cord injury in mouse [104]. The big challenge therefore is to integrate several cues to obtain a better and controlled growth cone response; the desired response could be obtained by developing biocompatible materials that allow an adequate scaffold containing both the chemical and physical cues, to allow an effective neural regeneration.

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Conflict of interest

The authors declare no conflict of interest.

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Section 2

Neurons and Neurodegenerative Disease

Chapter 2

Roles of Semaphorins in Neurodegenerative Diseases

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Abstract

Semaphorins are secreted and transmembrane proteins that bind plexin/neuropilin or integrin receptors, providing paracrine axonal guidance signals and ultimately leading to a functional and developed neuronal network. Following semaphorin's initial discovery, their relevance in the central nervous system (CNS) soon intrigued researchers about the possible links between semaphorins, their receptors and signaling mechanisms and different neurodegenerative diseases. Here, we explore the current knowledge of semaphorin's function and signaling in Alzheimer's disease (AD), Parkinson's disease (PD), Charcot-Marie-Tooth disease (CMT), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP). We focus on the effects of the most known semaphorin subclasses 3A and 4D, yet extending our discussion to other semaphorins that have been found involved in specific neuropathologies and the potential effect of semaphorins modulating the immune system in disorders with inflammatory components. Molecular, cellular, and genetic evidences are reviewed, highlighting the relevance of semaphorins on each disease etiology, pathophysiology, and progression. The newly discovered semaphorin functions in neurological disorders even suggest alternative therapies that may be highly valuable in diseases that have no current cure.

Keywords: semaphorin, neuropilin, plexin, neuroimmune cross talk, neurodegeneration

1. Introduction

Semaphorins (Sema) are a large family of proteins originally discovered as axon guidance signals during development as signals toward proper innervation of targets [1]. Semaphorin function is fundamental during embryonic development, yet they are also largely expressed in the adult brain. In the past decades, an increasing amount of evidence shows that semaphorins participate in refining synaptogenesis, dendritic and axonal exuberance, remodeling of the synaptic network, and even modulating neuronal response to reactive oxygen species and neuronal apoptosis. The association of semaphorins to neuronal function and cell death was soon explored in the context of neurological diseases [2–6]. Several reports linking alteration of semaphorin function or expression in neuropathologies opened an unexplored door to understand the mechanisms and look for treatment alternatives

of disorders with unknown or poorly understood pathological origins. Here, we aim to summarize the state of the art involving semaphorins on Alzheimer's disease (AD), Parkinson's disease (PD), Charcot-Marie-tooth disease (CMT), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP caused by HTLV-1 infection).

The role of semaphorins in neurological diseases depends on the different types of semaphorins, their receptors, different signaling pathways they activate, and the neuronal context [1]. For instance, some semaphorins are considered axon repellent in a particular neuronal context/disease, while chemoattractant in others. Similarly, some semaphorins are toxic to neurons and promote apoptosis, while others are neuroprotective. The bifunctionality of semaphorins is mirrored in a myriad of neurological affections and, therefore we, by no means, attempt to provide the reader with a complete list of diseases affected by Sema signaling or a thorough understanding of the Sema family members, their receptor, and their signaling pathways (refer to [2] instead), but rather provide an introductory reading for understanding semaphorin function in neurological pathologies.

1.1 Neurological disorders and the common factor of semaphorin

Neurological disorders of the central nervous system (CNS) are diseases with structural, biochemical, or electrical abnormalities in the brain and spinal cord caused by gene mutations, neuron damage, dysfunction of axon-dendrite connections, myelin loss, and/or damage of the surrounding vascular system. Neurological disorders include AD, PD, CMT, and ALS, sometimes showing an important immune component as MS and HAM/TSP [7, 8]. Currently, semaphorins are related with health and disease in the cardiovascular, immune, and central nervous systems. Although neurological disorders have different pathological origins, the participation of Sema signaling is a common factor [1, 9, 10]. Activation of semaphorin receptors in the neuronal growth cone promotes changes of cytoskeletal dynamics, resulting in an axon extension alteration and therefore possible neuronal dysfunctions in the context of neuropathologies [1, 11].

2. Semaphorin signaling

Semaphorins are a family of eight different subclasses with several members each, grouped based on amino acid sequence and structural similarities. Semaphorins include secreted and membrane-bound glycoproteins that bind mainly to plexin receptors (most relevant semaphorins related with neurological disorders are summarized in **Table 1**) [1, 2]. Plexins (PLXN) are a family of nine types of transmembrane semaphorin receptors (plexins A1, A2, A3, A4, B1, B2, B3, C1, and D1). Class 3 semaphorins bind to neuropilins (NRP1 and NRP2) that act as co-receptors forming an heterocomplex with type A plexins (the transducing unit), and in some cases, the Sema3-plexin-neuropilin complex may also associate with cell adhesion molecules of the IgCAM superfamily. Class 7 semaphorins bind to integrin receptors instead of plexins and neuropilins [1–3, 11–15]. While plexins seem to act as receptors for semaphorins only, the cell surface NRP receptors have pleiotropic functions, being also co-receptors for vascular endothelial growth factor (VEGF). NRP1 has high affinity for VEGF-A and is required for signal transduction after association to the VEGF receptor [16, 17]. Competition between VEGF and Sema3A for partially overlapping binding sites on NRP1 may produce a signaling unbalance potentially involved in neuropathologies.

Sema	Disease	Receptors/coreceptors	Cell expression	Functions
Sema2A	CMT	Plexin B Sema1A	Neurons	Neuronal connectivity, cell migration
Sema3A Sema3B Sema3C Sema3D Sema3E Sema3F	AD, PD, ALS, MS	Plexin A1-4/Nrp1/Nrp2, IgCAM, RTK, integrins, proteoglycans VEGFR2	Neurons, glia, immune cells	Cytoskeletal organization, neuronal connectivity, regeneration, synaptic transmission, regeneration, cell migration, angiogenesis, immune responses
Sema4A Sema4D	MS, HAM/TSP	Plexin B1/Met, ErbB2, Timp2, RTK	Neurons, glia, immune cells, endothelial cells, thymus	Cytoskeletal organization, neuronal connectivity, angiogenesis, cell migration, synaptic transmission, regeneration, immune responses
Sema5A	AD, PD,	Plexin B3/Nrp2, CSPGs, HSPGs, TK	Neurons, glia	Cytoskeletal organization, neuronal connectivity, synaptogenesis, vascular patterning
Sema7A	PD, MS	b1-integrins, α,b-integrins, plexin C1	Neurons, glia, immune cells, fibroblasts, thymus	Cytoskeletal organization, cell migration, cell adhesion, immunomodulation, stimulating cytokine production, proinflammatory responses

Receptors [1, 2, 10, 12, 15], cell expression [9, 10] functions [9], and diseases discussed here are indicated.

Table 1.
Semaphorins in neurological diseases.

Once semaphorins bind to their receptors, the transducing unit triggers signaling pathways linking several protein kinases and downstream substrates that overall change microtubule and actin dynamics, promoting growth cone collapse and axon repulsion in neurons. Nevertheless, changes in the neuronal environment or the type of semaphorin/receptor complex may shape a different transduction effect. Plexin receptors contain a cytoplasmic region acting as a GTPase-activating protein to bind and stimulate GTPase activity of Rho, Rac1, Rnd1, and R-Ras proteins. For instance, the G-protein R-Ras is involved in neuronal sprouting and cell adhesion via activation of integrins. Semaphorin signaling via plexin A1/B1 inactivates R-Ras. Sema3A- and Sema4D-mediated signaling, therefore, inhibit integrin β1 subunits through downregulation of R-Ras, leading to a reduction of growth cone adhesion and allowing collapse responses [1, 2, 10, 15]. Another central protein participating in semaphorin-induced growth cone collapse signaling is collapsin response mediator protein-2 (CRMP-2). CRMP-2 is a phosphoprotein mostly expressed in the CNS and involved in the cytoskeleton structure and function of neuronal cells through the induction of microtubule dynamics/assembly by binding to α- and β-tubulin heterodimers. The complex CRMP-2/kinesin-1 regulates soluble tubulin transport to the distal part of the growing axon and also neurite formation by modulating tubulin GTPase via intramolecular interaction with its N-terminal inhibitory region [18–20]. The affinity of CRMP-2 for tubulin is significantly diminished when specific residues are phosphorylated, leading to axon retraction and growth cone collapse [21, 22].

3. Semaphorin regulatory functions on neuronal and non-neuronal cells

3.1 Axonal degeneration associated to cytoskeleton organization

The roles of Sema3A and Sema4D are to produce alteration of both actin and microtubule dynamics in the cytoskeleton organization (ratio of the polymerization/depolymerization rates). The effects of Sema3A and Sema4D on actin dynamics include the downregulation of PI3K-Akt signaling pathway, inhibiting integrin-mediated adhesion as well as repulsive effects on axonal growing associated to actin-rich structure loss of lamellipodia and filopodia as part of the cofilin pathway triggered by Sema3A, or activation of myosin II (MyoII) and F-actin bundles promoted by Sema4D-ROCK signaling [2, 23, 24]. Sema3A additionally mediates an increase of the nonphosphorylated active form of myosin II (MyoII) and decreases the phosphorylation levels of Ezrin, Radixin, and Moesin (ERM) proteins. The nonphosphorylated active form of MyoII promotes retraction, while low ERM phosphorylation reduces the crosslinking between actin filaments and the plasma membrane [2, 25, 26]. Regarding microtubule dynamics, Sema4D signaling induces the inactivation of CRMP-2 by glycogen synthase kinase 3 beta (GSK3B)-mediated phosphorylation, leading to reduced binding of CRMP-2 to tubulin and consequently limiting its stabilizing function at the plus end of microtubules. Sema3A induces a similar CRMP-2-inactivating mechanism, yet requiring phosphorylation by cyclin-dependent kinase 5 (Cdk5) at Ser522 and GSK3B at Thr509/514 [2, 21, 22].

3.2 Axonal degeneration associated to synaptogenesis and synaptic plasticity

Synapsis formation of the neuronal network requires local participation of cell adhesion molecules, extracellular proteins, and axon guidance molecules at axodendritic sites [6]. Sema3A and VEGF signaling have been proposed to actively modulate the synaptogenesis and synaptic plasticity, since they are dysregulated in neurological diseases, such as AD [4, 16, 17]. Proper regulation of the synapse formation and dendritic branching contributes to a normal balance between excitatory and inhibitory synaptic transmission; dysregulation of this balance would interfere with the regeneration of damaged CNS axons [6, 11, 15, 27, 28].

3.3 Semaphorin function on axonal regeneration

Models of axonal regeneration have been studied after spinal cord injury, which can produce a permanent damage because axonal growth and regeneration are limited after injury [29]. In adult mammals, some myelin proteins produced by myelinating oligodendrocytes, such as Nogo-A, MAG and OMgp, inhibit spinal cord regeneration [4]. Additionally, glial cells release chondroitin sulfate proteoglycans (CSPGs) and lecticans, such as neurocan, brevican, phosphacan, and tenascin to form the neuronal extracellular matrix (perineuronal nets). The perineuronal nets, together with other extracellular proteins, such as, ephrins, slits, netrins, bone morphogenic proteins, Wnts, and semaphorins are among the molecules most likely involved in limiting axonal regeneration [4, 30–33]. However, the function of semaphorins is dependent on the cellular context and may also favor axon regeneration. For instance, nerve growth factor (NGF) co-injected with Sema3A in trigeminal neuronal cell culture induced neuron regeneration [34]. Sema3A has also been implicated in the restoration of functionally motor innervation required to regenerate fibers [35]. Sema4D has shown enhanced locomotor recovery and axon regeneration when expressed in motoneurons, attributed to regulation of microglia function following spinal cord injury in adult zebrafish [36].

3.4 Semaphorin function on revascularization

Revascularization (restoration of perfusion) is regulated by several growth factors secreted from endothelial cells, such as VEGFA, FGF, and PDGFs [4]. Class 3 semaphorins are considered anti-angiogenic semaphorins (e.g., Sema3A, Sema3E, and Sema3F), because they interfere with the effect of VEGF by competing for the same NRP receptor [37, 38]. Sema3A not only targets the actin cytoskeleton, but also the assembly/disassembly of focal adhesions, altering migration, proliferation, and adherence of endothelial cells [37]. Sema3A and Sema4D also produce alteration on the blood-brain barrier (BBB) by disrupting endothelial tight junctions and thus increasing its permeability. BBB damage has been related to higher infiltration of immune cells mediated by increasing levels of Sema4D in MS [4, 39]. As opposed to Sema3A, Sema4D is pro-angiogenic and promotes endothelial cell migration via plexin-B1-PI3K-Akt signaling pathway [37, 40].

3.5 Semaphorin function on remyelination

Axon myelination in the CNS is essential for regulating fast and slow axonal transport rates. Myelination requires interaction among axons, oligodendrocytes, and semaphorins. Semaphorins regulate the migration of oligodendrocyte precursor cells (OPC) during normal development and toward demyelinated lesions. Demyelinization, caused by loss of oligodendrocytes and myelin sheaths around axons, is a pathological condition that results in axonal dysfunction, degeneration and loss of sensory and motors neurons [2, 4, 41–43]. Oligodendrocyte death can be produced by genetic defects, infections, autoimmune reactions, and trauma, along with unknown causes. In some CNS pathologies related to myelination, astrocytes clear off myelin debris, modulating oligodendrocyte activity, myelin maintenance, and its synthesis [43]. Semaphorins (Sema3A, Sema4D, Sema5A, Sema6A, and Sema7A) inhibit OPC recruitment into demyelinated lesions and its differentiation to oligodendrocytes [9, 42, 44, 45]. Sema4D, Sema6, and Sema7A have been detected in myelin, and their expression found strongly upregulated in oligodendrocytes located near the injury site [9, 2, 45].

3.6 Semaphorin function on immune responses

Sema4D, formerly known as CD100, was called the "immune semaphorin" because it was originally found in lymphocytes [3]. The Sema4D receptor in neuronal cells is plexin B1, whereas in immune cells, besides binding to plexin B1, Sema4D also binds to a signaling surface receptor CD72. CD72 is considered a regulatory receptor, because it activates suppressive signals and prevents some forms of autoimmunity [46, 47]. Sema4D is a membrane-bound protein that can be proteolytically cleaved by MT1-MMP metalloprotease, releasing a 120-kDa soluble form of Sema4D, which can act paracrinally on other systems [40, 47]. Immune semaphorins also include Sema3A, Sema4A, Sema6D, and Sema7A expressed on T-cells, B-cells, natural killer cells, neutrophils, platelets, and mature dendritic cells (DC) [39, 47]. The neuronal system sense changes to maintain CNS homeostasis and communicates to the immune system by soluble factors to inhibit further inflammatory responses. For instance, neurons control T-cell and glia functions mediated by membrane-bound or secreted molecules such as semaphorins, neurotrophins, neurotransmitters, neuropeptides, and cytokines [48, 49]. Sema3A and Sema7A expression in neurons attenuate T-cell activation, proliferation, and function through T-cell receptor signaling [49–51]. Sema3A, additionally, downregulates autoimmunity by suppressing B- and T-cell-mediated autoimmune over-activity

responses [52]. In addition, cell adhesion molecules expressed by neurons, such as NCAM, cadherins, and integrins are active molecules in neurogenesis and synaptic plasticity that also can ameliorate inflammation and neurotoxic effects, while strengthening neuroprotection of immune components in pathology [48].

4. Semaphorins and their current association to neurological diseases

4.1 Alzheimer's disease

Alzheimer's disease (AD) is the most invalidating, common, and widespread elderly associated neuropathology. An estimated of over 30 million people are affected worldwide, increasing its incidence with age [53]. Patients with AD suffer progressive neuron lost, mostly from the prefrontal cortex and the hippocampus. As a consequence of neuronal death, patients experience memory, cognitive, and behavioral problems, leading within an average of less than 10 years to dementia and/or death [53]. Given its prevalence and the continuing aging of the population, AD threatens to generate an epidemic healthcare crisis in the next decades and yet its cause remains unknown. Current treatments target late symptoms, improving the patient's quality of life; however, they have a minute contribution on the disease impact and its inevitable progression. Two major features are distinct from Alzheimer's brains that have intrigued researchers since Alois Alzheimer described them in 1906: presenile plaques and neurofibrillary tangles (NFT). Plaques are extracellular insoluble aggregates, mostly composed of a misfolded amyloid beta (Aβ) peptide, whereas NFT are intraneuronal aggregates of hyperphosphorylated Tau (a microtubule-associated protein). It is yet controversial whether tangles, plaques, or both are causes or consequences of AD. Semaphorins have been long suggested to play a role in AD, because they initially were found largely expressed in adult brain tissues [54] and later found to have abnormal neurohistological patterns in affected cortical and hippocampal areas of AD brains [55], especially on vulnerable neurons [56, 57].

It has been hypothesized that Sema3A may be involved in the early stages of Alzheimer's degeneration. By analyzing the histological localization of Sema3A in vulnerable hippocampal areas that are first affected by neurodegeneration, a dysregulation in Sema3A expression and release has been proposed as a possible early sign in AD brains, observed even in neurons lacking NFT and therefore possible preceding Tau hyperphosphorylation and aggregation [57]. An intracellular form of Sema3A was also found associated to NFT, along with Plexins, hyperphosphorylated CRMP-2, and microtubule-associated protein 1B (MAP-1B) [57]. The association between Sema3A and a possible downstream Tau hyperphosphorylation may come from the kinases involved in Sema3A signaling. Yoshida et al. initially found a phosphorylated form of CRMP-2 associated to NFT and significantly higher in AD brains [58]. CRMP-2 was originally discovered form its chicken homolog, CRMP-62, as a downstream effector of semaphorin (formerly known as collapsin) signaling. Injection of blocking antibodies against CRMP-62 into dorsal root ganglion inhibited the growth cone collapse induced by Sema3A, suggesting that CRMP-62 is a downstream effector in Sema3A signaling [59]. Since then, CRMP-2 has been associated to NFT in AD [58] and found phosphorylated as a result of semaphorin signaling [60, 61]. The signaling mechanism involves phosphorylation of CRMP-2 by GSK3B after a priming phosphorylation at Ser522 [61, 62]. The phosphorylation at Ser522 is required for further phosphorylation of GSK3B. In rat models, Cdk5 has been found responsible of Ser522 phosphorylation, priming the phosphorylation site of GSK3B both *in vitro* and *in vivo* [63, 64]. The sequential

phosphorylation of CRMP-2 reduces its affinity to tubulin and triggers microtubule destabilization and Sema3A-mediated growth cone collapse associated to AD pathogenesis [63, 64]. Other kinases, such as Fyn, cyclin-dependent kinase 1, and dual specificity tyrosine-phosphorylation-regulated kinase 2 may also be involved in the priming phosphorylation at Ser522 [61, 64, 65]. The participating kinases and the Sema3A-mediated sequential phosphorylation mechanisms of CRMP-2 are strikingly similar to the pathway leading to Tau hyperphosphorylation, aggregation, and microtubule destabilization observed in AD [66].

A recent study also links Tau phosphorylation to Sema3A signaling by discovering several single-nucleotide polymorphisms (SNPs) associated to AD in the PLXNA4 gene, which codifies for plexin A4 [62]. Most of the top-ranked SNPs associated to AD were located in the region coding the Sema3A-binding site [62]. Cells expressing PLXNA4 and stimulated with Sema3A showed Sema3A-induced phosphorylation of Tau, enhanced by overexpression of the full-length PLXNA4 receptor, whereas expression of soluble forms of PLXNA4 inhibited Tau phosphorylation, presumably by binding Sema3A and competing with the endogenous Plexin receptor, both in a cell line model and in rat hippocampal neurons [62]. The full-length PLXNA4 expression was found higher in AD brains, and also significantly correlates with clinical and neuropathological disease severity measures, such as dementia. The Sema3A-induced kinase activities affecting CRMP-2 and Tau may ultimately lead to neurofibrillary tangle formation and neuronal dead in AD. If Sema3A signaling is effectively involved in the early stages of neurodegeneration, it would be worth to further study its association with AD toward the discovery of new biomarkers and drugs, such as specific kinase inhibitors (some of them already in clinical trial) [67]. An example of a different treatment approach involves modulating the interaction of semaphorins with the neuronal extracellular matrix or perineuronal nets. Differential expression of several proteins related to the extracellular matrix, among them Sema3C, has been found in AD-vulnerable brain areas [56]. Additionally, memory restoration in AD mice models has been achieved by digesting CSPs, a major component of perineuronal nets, using chondroitinase [68, 69]. Disruption of perineuronal nets presumably allows the formation of new synapsis sites and thus increases adult brain plasticity. An important effector of perineuronal nets is Sema3A by binding to chondroitin sulfate, a main component of CSPs [70]. A recent study showed restoration of object recognition memory in a tauopathy mice model via reducing Sema3A binding to perineuronal nets by perirhinal cortex injections of an inhibitory proteoglycan-neutralizing antibody against chondroitin 4-sulfate [69]. Therefore, blocking the binding of Sema3A to perineuronal nets can restore memory function in adult AD-mice model.

It is also interesting to note the bifunctional effect of semaphorins in AD. For instance, Sema3A has been shown to promote apoptosis and neurodegeneration [57, 61], whereas Sema3C has been related to neuroprotection [56, 71]. Such duality, along with several other genetic, environmental, and aging components, gives to AD its multifactorial category. Genetic factors are thought to account for over 50% of the disease, yet these risk factors are not determinants to causing AD. In rare cases of early-onset familial AD, the disease is linked to mutations on genes involving Aβ metabolism, such as the amyloid precursor protein (APP) and presenilin (PSEN1/2) genes. APP is a transmembrane protein from which Aβ peptide is generated by the cleavage of a gamma secretase complex. Presenilin-1 and presenilin-2 are part of the gamma secretase complex. However, in the most common sporadic (nonfamilial) late-onset AD, the genetic variants only explain part of the disease etiology. Several genes have been considered a risk factor, such as the APOE-ε4 polymorphic isoform of the apolipoprotein E gene (APOE), the main cholesterol carrier in the CNS. The role of apolipoprotein E in AD is, however, poorly understood and thought to be related to Aβ degradation [72]. Semaphorin polymorphs

have also been studied given their relevance in neuronal apoptosis and the findings of semaphorin polymorphisms related to other syndromes. The first attempts in relating semaphorin SNPs with AD were, however, unfruitful. Evaluation of two exonic SNPs of semaphorin 3A and 4D genes in patients with AD showed no correlation, even though modeling analysis predicted a damaged variant of the affected proteins [73]. Recently, however, a novel proposed method for detecting hidden SNPs that would otherwise appear undetected by commonly used tests found six SNPs on noncoding regions near the semaphorin 5A gene [74]. Schott et al. [75] also found in a noncodifying region of the SEMA3C gene a polymorphism associated to posterior cortical atrophy, a variant of AD. The SEMA3C SNP, therefore, suggests a role of Sema3C during development that may influence later neurodegeneration associated to specific brain regions that ultimately lead to differential degeneration phenotypes [75]. In light of these recent associations of semaphorins to AD [62, 74, 75], semaphorin gene variants and their receptors are expected to participate as a risk factor of AD and its associated neuropathologies, opening a relatively underexplored door toward new discoveries.

4.2 Parkinson's disease

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder after AD, affecting up to 1% of the worldwide population above 60 years old [76]. PD patients suffer a progressive and selective loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc), which innervates neurons in the dorsal striatum and regulates its effects on motor functions. The result of neurodegeneration manifests mostly with movement disorders or dyskinesia, where tremor at rest, rigidity, and bradykinesia are cardinal for diagnosis [76]. The reason why SNpc dopaminergic neurons die is not well understood. A composite interaction between genetics and environmental factors in the context of aging has been intensively studied to find the causes, preventing strategies and potential therapies of PD. Semaphorins in their role of apoptotic mediators in neurodegeneration have early on been suggested to be involved in the pathogenesis of PD [77–80].

Even though it is currently accepted that genetic plays a minor role in sporadic (nonfamilial) PD based on studies with relatives [81], several genes are known to be involved in the disease etiology and progression. For instance, mutations in the SNCA gene are known to be associated with familial PD and increased risk of sporadic PD. The SNCA gene encodes alpha-synuclein, which is the main protein found in Lewy bodies as insoluble aggregates inside SNpc neurons of patients with PD. Interestingly, population genetic studies have also found SNPs in the Sema5A gene that may be related to PD. In the pioneer work of Maraganore et al., 11 SNPs were associated to PD in Caucasian Americans by using a two-stage whole-genome association analysis including 198,345 SNPs. The SNP with the lowest p-value associated to increased risk of PD was located in the Sema5A gene (rs7702187 polymorphism, corresponding to a thymine substituted by adenine in an intronic sequence) [82]. Given the relevance of semaphorins in neuronal apoptosis, the polymorphism in Sema5A found by Maraganore et al. [82] was soon assessed by different research groups, though having conflicting results that still remain unclear. The controversies may come from the different populations evaluated in each analysis. Some studies have shown that the rs7702187 polymorphism is not associated to PD [83, 84] or even conflict to the extent of finding the polymorphic variant protective rather than a risk factor on a population-dependent basis [85]. Taiwanese Asians showed significant associations of the Sema5A rs7702187 polymorphism [85], whereas Finnish Caucasians, Polish Caucasians, Singapore Asians, and Chinese Han populations were not associated to PD [83–86]. In addition, a different SNP, the rs3798097

(C > T), located in the 5'UTR of the Sema5A gene, was also found associated with PD [85]. Ding et al. [86] showed that although the genotypes are not necessarily associated to PD in a Chinese Han population, the haplotypes involving these two SNPs in the context of a particular ethnicity may be implicated in the disease by finding the AC haplotype associated with an increased risk of PD compared to the common TC haplotype, whereas the AT haplotype was found protective against PD [86]. A more recent meta-analysis on the rs7702187 polymorphism concluded that the A allele frequency was associated to increased risk of PD only in Western population [87]. Both polymorphisms do not affect the sequence of the protein, but rather may be regulatory sequences affecting expression dynamics of Sema5A. These SNPs may be associated to PD; however, less studied polymorphic variants together with other cellular and molecular factors should be considered as well, such as the expression of other semaphorin classes and their receptors that may result as a confounding factor across different population genetic studies.

Besides the genetic highlights on PD, the mechanisms by which semaphorins participate in the disease etiology and progression are poorly understood. Although semaphorins had been suggested to promote neurodegeneration in PD, limited studies were known to link semaphorins in the context of the disease pathogenesis [77, 78, 88, 89]. After a decade of research since the study of Maraganore et al. [82], several lines of evidence indicate possible direct mechanisms of semaphorins (though, not Sema5A) in PD etiology. Recent evidence in a neurotoxin (MPTP)-induced PD mouse model directly involves Sema3A effects through Rho-ROCK signaling pathway. Rho kinase inhibition as well as heterozygous mice knockouts in both Rho and ROCK protect from MPTP-induced damage of dopaminergic neurons, increase dopamine and its metabolic products at the striatum, showed reduced protein expression of Sema3A and its receptors, plexin A and NRP-1, and overall alleviate the behavior damage compared to control PD mice [90–93]. The effects of Sema3A on Rho-ROCK signaling pathway could mediate several cytoskeleton effectors, contributing to growth cone collapse as well as regulation of neuronal apoptosis. Animal models and SNpc of human brains from PD-affected subjects show apparent neuronal apoptotic processes, probably triggered by oxidative stress [94]. Whether semaphorins are directly involved in causing neuronal apoptosis in PD is debatable and may be associated in part to their specific receptors and signaling pathways. For instance, although Sema3A through plexin A/NRP-1 and activation of Rho kinase was found to promote dopaminergic damage [92], Sema7A has been found protective against ROS-mediated neurodegeneration [95]. Sema7A binds to integrin receptors and could potentially regulate apoptosis through different mechanism. Sema7A reduces the large axonal arborization on dopaminergic neurons, which potentially decreases mitochondrial oxygen demand, ROS production, and neuronal vulnerability observed in PD [95]. However, even different ligands to the same receptor for Sema3A have been found protective against neuronal apoptosis. For instance, VEGF shows neuroprotection in PD models likely by binding to neuropilin receptors, though it is unclear whether the downstream VEGF signaling effectors differ from the semaphorin transduction pathway or VEGF indirectly promotes protection by competition to the same receptor. Alternatively, VEGF could mediate apoptosis by other processes such angiogenesis [89]. It would be interesting to evaluate in future research different ROS-mediated apoptotic pathways and their interplay with semaphorin-induced signaling, such as the phosphorylation targets of ROCK resulting in growth cone collapse (which could mediate CRMP-2 phosphorylation by a similar mechanism to what described previously for AD), to find out how these pathways are regulated in an effort to evaluate new drug candidates for PD prevention and treatment.

Paradoxically, the Sema3A found to promote neurodegeneration in PD pathogenesis, at the same time may be useful for steam cell transplantation therapy in PD patients [96–99]. Given that semaphorins participate in the formation of the nigrostriatal pathway during prenatal development, they have also been proposed to guide axons to their appropriate targets after possible cell replacement therapy with dopaminergic neurons [96, 100–105]. Embryonic stem cells differentiated to tyrosine hydroxylase-expressing neurons have been shown to have similar phenotype, expression of neuropilins, and response to Class 3 semaphorins than embryonic ventral mesencephalon neurons [96, 97, 106]. Via neuropilin-mediated signaling, Sema3A increases axonal length in collagen gel coculture experiments. Sema3C, besides increasing length, also attracts axons, whereas Sema3F produces either no effect or axon repulsion [96, 97]. Semaphorin axonal guidance results are promising toward the recovery of parkinsonian symptoms in transplanted PD animal models [98, 99]. Therefore, even though semaphorins may be directly involved in promoting PD neurodegeneration, they could also be a strategy to restore the dopaminergic function by providing axon guidance cues after embryonic stem cell intranigral transplantation.

4.3 Charcot-Marie-Tooth disease

Charcot-Marie-Tooth disease (CMT) is an inherited peripheral neuropathy associated with mutations in more than 90 different genes. CMT is divided into different forms based on the inheritance pattern and neurophysiological observations. The most common types are autosomal-dominant forms, and they are categorized into demyelinating with reduced nerve conduction velocities (CMT type 1) and axonal-loss type with relatively normal nerve conduction velocities (CMT type 2). Patients with CMT type 2 comprise about 20% of all cases [107–110].

Mutations in the gene *GARS*, encoding glycyl-tRNA synthetase (GlyRS), have been related to peripheral nerve degeneration and CMT type 2 [111]. In addition, mutated GlyRS has shown to bind neuropilin-1 in mice [112]. Besides its housekeeping intracellular function during protein synthesis, GlyRS can be secreted and produce different cellular effects from the extracellular space [113]. In *Drosophila*, the Cader lab showed that mutant GlyRS is secreted by muscles and interacts with the neuromuscular junction [114]. Recently, they showed that the P234KY mutant version of GlyRS (mutation associated to CMT type 2) colocalizes with plexin B in presynaptic neurons. Also, Sema2A overexpression, but not Sema1A overexpression, decreased the effect that mutant GlyRS produced on muscle contraction, suggesting that plexin B signaling could be affected by mutated GlyRS by competition with Sema2A [115]. Also, other ligands for neuropilin should be taken into account, such as VEGF. He et al. suggest that CMT type 2 mutations in GlyRS promote its abnormal binding to neuropilin-1, antagonizing the binding of VEGF and blocking the VEGF/neuropilin-1 signaling essential for survival and function of motor neurons [112]. Nevertheless, the neuropilin sequestration by mutant GlyRS has shown to be less detrimental in other tissues, given that this abnormal interaction is permissive to maturation and maintenance of the vasculature in CMT type 2 mice [116].

It is important to consider that in addition to the extracellular function, mutated GlyRS can have abnormal intracellular functions that could also contribute to the CMT pathogenesis, suggesting that multiple mechanisms could be participating. For example, human GlyRS mutations related to CMT (S581 L and G598A E71G, L129P, S211F, G240R, E279D, H418R, and G526R) have shown to have a gain-of-function effect binding to histone deacetylase 6 (HDAC6) and enhance its function, promoting α-tubulin de-acetylation and leading to axonal transport deficit. It is

relevant to highlight that G598A patients have more severe distal weakness and wasting in the lower limbs, and in that same article, this mutation showed one of the strongest affinities for HDAC6 [117]. Thus, the most severe mutations in GlyRS could eventually promote abnormal interaction with both NRP1 and HDAC6. A combination of intracellular and extracellular effects could eventually explain the severity and early-onset clinical symptoms of the patients carrying the G598A mutation, as the authors suggested. Future experiments will have to address in more detail the contributions that different plexin/neuropilin ligands may have in CMT, and also link the phenotypes with abnormal activation or deactivation of transduction pathways controlled by these receptors.

4.4 Amyotrophic lateral sclerosis

ALS is a neurological disorder with motor neuron degeneration. Neuron loss leads to paralysis in muscles and death mostly by respiratory failure. Most of the studies in animal models related to ALS use superoxide dismutase (SOD) mutations in mice (in particular, the SOD1G93A transgenic mouse), although the mechanism by which SOD mutations cause ALS is not clear. In these mice models, modifications in axons and nerve terminals are observed even before the clinical symptoms [118].

The first report linking semaphorins and ALS was published in 2006 by De Winter et al. showing increased Sema3A mRNA levels in the SOD1G93A transgenic mice model [119]. Nevertheless, a more recent report from the same lab showed that ALS mice expressing a mutant version of Sema3A (K108 N mutation that produces diminished signaling capacity) had no difference in ALS-induced decline in motor behavior, contrary to what was initially expected [120]. These last results point to a minor contribution of Sema3A to ALS pathology, although other articles are in clear contradiction with this claim. A clear example is the article published by Venkova et al. who hypothesized that Sema3A is able to trigger distal axonopathy and muscle denervation in the SOD1G93A mouse model of ALS [121]. They propose that Sema3A released from terminal Schwann cells activates plexin-A/neuropilin-1, promoting the regulation of kinases such as CDK5 and GSK3B that could alter CRMP-2 phosphorylation and leading to microtubule instability and actin cytoskeletal rearrangements. The Sema3A-mediated signaling could inhibit compensatory axon sprouting and coordination of neuromuscular junction remodeling after injury, contributing to distal axonopathy [121]. Anti-neuropilin-1 antibodies that block the Sema3A docking site in differentiated motor neuron-like cells (NSC-34) prevented Sema3A-induced growth cone collapse. Furthermore, injections of blocking antibodies delayed and even temporarily reversed the motor functional decline while prolonging the life span of SOD1G93A mice. Histologically, the antibody reduced neuromuscular junction denervation and attenuated pathologic alterations in ventral roots at late stages of the disease [121] [121].

In parallel, Miyazaki et al. focused on extracellular protein changes in SOD1G93A mice during the development of ALS to characterize changes in the cellular environment that could affect regeneration [122]. They found decreased Sema3A levels in the anterior half of the lumbar cord of ALS mice. Sema3A immunochemistry at ages 15 and 18 weeks showed a progressive decrease of staining in the neuropil of ALS mice compared to wild type, while Sema3A-positive astrocyte appeared [122]. In addition, it was found that *Sema3D* gene expression levels are decreased 2.5-fold with respect to wild type in another ALS mouse model (SOD1G37R mutation) [123].

Another piece of evidence for the role of semaphorins on ALS is related to microribonucleic acids (miRNAs). miRNAs are small single-stranded, noncoding RNAs that alter gene expression through post-transcriptional regulation by

binding to the 3′-untranslated region of target mRNAs [124]. The Perlson lab [125] analyzed miRNA profiles from axons and somas of two ALS mouse models, SOD1 with G93A mutation and TDP43 with A315T mutation. They showed that different miRNAs were significantly altered in the axons expressing ALS mutations, but not in the somas, indicating that miRNA could be regulating local functions in motor neuron axons [125]. Later, the same lab using qRT-PCR showed that one of these miRNAs, miR126-5p, downregulates Sema3A, Sema3B, neuropilin-1, and neuropilin-2 transcript levels in HeLa cells. Primary myoblasts with the SOD1G93A mutation were transfected with miR126-5p and cultured in a distal compartment of a microfluidic chamber together with a motor neuron explant placed in the proximal compartment. They showed that in the microfluidic chamber, the rate of axons that traversed the distal compartment was increased respect to the control condition of myoblasts transfected with an irrelevant miRNA. In addition, the injection of miR126-5p to ALS mice increased the amount of intact neuromuscular junctions revealing higher innervation in treated muscles compared to the mock condition. Three parameters: Mean Stand Index (measurement of the speed at which the paws detach from the walking surface), single-support parameter (the relative duration of all combined paws in contact with the glass floor), and base of support parameter (the average width of limb spreading between front or hind paws) were measured in ALS mice, and in all cases, the injection of miR126-5p improved all parameters respect to the control [126]. Based on these observations and previous reports, the authors suggested an attractive model of Sema3A/neuropilin-1 interaction that explains how the motor neuron degeneration in ALS could be regulated by miR126-5p. miR126-5p decrease in ALS could enhance Sema3A secretion in muscle and overexpression of neuropilin-1 in axons, increasing Sema3A signaling in the neuromuscular junction and leading to axon degeneration [126].

It is of consideration to test the results obtained with ALS mouse models in human samples. Motor cortex tissue samples showed increased Sema3A mRNA levels by quantitative RT-PCR in ALS patients (eight cases aged 44–72 years) compared to control samples (six subjects aged 45–84 years, with no neurological disease history). Likewise, by immunohistochemistry, the motor cortex showed stronger cytoplasmic and axonal Sema3A labeling in motor neurons of ALS patients compared to controls. Sema3A mRNA levels and immunohistological labeling showed, however, no difference between ALS patients and controls in spinal cord tissue samples [127]. Sema3A levels in human samples support the previous findings in ALS mouse models discussed above. However, other semaphorins and neurological factors not studied yet in the context of ALS may provide a better understanding of semaphorin function and mechanisms on ALS pathology.

4.5 Spastic paraparesis associated to HTLV-1

HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is produced by infection with the retrovirus HTLV-1 (Human T-cell lymphotropic virus type 1 (HTLV-1) [128, 129]. HTLV-1 is transmitted by breast-feeding, sexual intercourse, and parenterally [130]. Worldwide, around 15–20 million people are infected with HTLV-1; however, only 3–5% develop HAM/TSP. Another ~5% develop adult T-cell leukemia/lymphoma (ATL), whereas over 90% of infected people are asymptomatic carriers [131]. The most common HAM/TSP symptom is lower limb motor dysfunction, followed with bladder/bowel dysfunctions and sensory alterations [132]. The virus mainly infects CD4+-T-cells, while monocytes, B-cells, CD8+-T-cells, and DC are infected to a lesser extent and found in spinal cord lesions together with infected astrocytes and endothelial cells [7, 133]. HAM/TSP causes alteration of CNS axonal transport based on the presence of APP deposits

in the axons, a classical marker of defects in fast axonal transport [134, 135]. Immunological studies have shown a chronic infiltration of activated CD4+ and CD8+-T-cells into the CNS [136].

It is a consensus that the paraparesis axonopathy generates as a consequence of chronic extracellular action of viral proteins secreted by the infected lymphocytes present in the CNS [137, 138]. Among secreted proteins, Tax viral protein acts on several viral and cellular processes, modulates various cellular signaling pathways, and has also been detected in the CSF of HAM/TSP patients. In the cytoplasm of infected lymphocytes, Tax activates NF-kB pathway responsible for proliferation and differentiation of T-cells, whereas in the nucleus, Tax activates the ATP/CREB pathway. Tax can also be secreted via endoplasmic reticulum-Golgi apparatus and by exosomes [136–141]. Tax secreted from activated peripheral blood mononuclear cells (PBMCs) could explain the presence of Tax in the plasma and CSF of infected patients and carriers [141].

Using *in vitro* culture of PBMCs from HAM/TSP patients, it has been recently found that the levels of secreted Sema4D were increased compared to healthy subjects [142]. Elevated Sema4D could be explained as a result of increased levels of MT1-MMP—the enzyme responsible for generating soluble Sema4D (Sema4D) from the transmembrane Sema4D—found in PBMCs from HAM/TSP patients. It has been also found that Tax and SEMA4D co-immunoprecipitate from PBMC culture medium. To test the effect of Tax and Sema4D (or the Tax/Sema4D complex) in neuronal cells, culture media from infected lymphocytes were added to PC12 cells during their differentiation to neuronal type, finding decreased neurite length as a result. The effect of HTLV-1-infected PBMC culture media was blocked by both anti-Sema4D and anti-Tax antibodies, suggesting neurite length reduction by a Tax/Sema4D complex [142]. In the same report, it was shown that infected lymphocytes strongly migrate in response to Sema4D using a trans-well system. It was found that in the population of migrated lymphocytes, the levels of CRMP-2 phosphorylation at Ser522 were increased [142]. A change in Sema4D-mediated phosphorylation of CRMP-2 could be responsible for the increased motility. Authors proposed that infected lymphocytes have an increased migratory response toward Sema4D, making them able to cross the BBB [142]. Once in the CNS, infected lymphocytes secrete Tax and Sema4D, attracting more HTLV-1-infected lymphocytes at the same time that these proteins could mediate pathological disturbances on neuronal cells.

4.6 Multiple sclerosis

MS is a CNS disease mostly considered of autoimmune etiology. It shows demyelinated plaques that sometimes remyelinate spontaneously. Remyelination involves the recruitment of OPC, which differentiate into mature oligodendrocytes in damaged areas to promote remyelination. Nevertheless, the remyelination process is prone to fail, leading to progressive disability [41, 143]. Even though there are multiple reports linking semaphorins with lymphocyte signaling during MS; in this section, we will focus on discussing the reports that have linked semaphorin signaling in oligodendrocytes during MS.

Sema3 proteins are the main semaphorins related to MS, although there is an increasing evidence of Sema4 involvement as well. Using postmortem human samples, the Lubetzki lab [144] showed the presence of numerous cells positive for Sema3A or Sema3F transcripts around and within demyelinating white matter lesions in MS brains, whereas these transcripts were absent in control adult brain white matter. The differential expression of Sema3A and Sema3F was strictly restricted to active plaques. No expression was detected in normal white matter

distant to active lesions, around/within chronically demyelinated lesions or remyelinated plaques [144]. Later, also in human MS tissue samples, it was shown that although the chemoattractant Sema3F and chemorepellent Sema3A had similar protein expression patterns in some lesions, Sema3A was predominantly expressed in chronic active lesions, which mostly contain few OPCs [145].

The Lubetzki lab [42] also used a mouse model where demyelinated lesions are induced by lysophosphatidylcholine injection. They found that adult OPCs express Sema3 receptors (plexin A and neuropilin 1 and 2) and that the expression of these receptors, together with Sema3A and Sema3F, is increased after the induction of lesions. Interestingly, *in vivo* lentiviral expression of Sema3A decreased the OPC density in induced lesions, whereas Sema3F produced the opposite effect. When a transgenic mouse with a mutated NRP1 preventing Sema3A binding was used, an increase in OPC density was found after the induction of lesions compared to wild-type mice. The density of remyelinated axons increased in lesions of animals receiving the Sema3F, but not the Sema3A lentiviral vector [42]. Using a similar approach, in a more recent publication 145, the authors injected recombinant Sema3A and Sema3F to mice. Sema3A-treated mice had significantly fewer OPCs on the side of the lesion compared to the opposite side without lesion, whereas Sema3F-treated mice had increased number of OPCs in the lesion side [145]. Parallel studies in rats have shown that Sema3A inhibits CNS remyelination and the lineage progression of OPCs in demyelinated lesions, arresting OPCs at a premyelinating state [44]. Finally, a recent report using exome sequencing analysis found an association of a missense mutation in the *plexin A3* gene (receptor of Sema3A and Sema3F) with increased disability in MS males. Given the gender association, the authors debated whether the *plexin A3* mutation could alter the protein stability, interfering with its ligand binding and arguing the possibility of protective effects of estradiol in females [146]. Considering that in MS lesions, Sema3A and its receptors are also expressed in neurons, reactive astrocytes, and microglia/macrophages [147], the source of Sema3A can be multiple and simultaneously affect not only OPCs signaling, but also other cell types.

There are also some reports linking MS with Sema4. Ferritin uptake by oligodendrocytes is mediated by the Tim2 receptor and required for iron acquisition. In addition to ferritin, Tim2 binds Sema4A [148]. Recombinant Sema4A exposure to primary rat OPCs resulted in dose-dependent OPC cytotoxicity. Astrocytes and mature oligodendrocytes were, however, unaffected. The authors suggested that the observed cytotoxicity could be mediated by Tim2 receptor. Lymphocytes, macrophages, or microglia could be the source of Sema4A *in vivo* [149]. Later, the same group found that human oligodendrocytes undergo apoptosis when exposed to Sema4A and that the levels of this protein are increased in multiple sclerosis patients [150]. A different research group used recombinant Sema4D in an *in vitro* model of cultured OPC, resulting in actin filament rearrangement indicative of cytoskeletal collapse, along with an increase in apoptotic cells and fewer OPC differentiating into mature oligodendrocytes. All these effects were avoided by incubation with anti-Sema4D antibody [39]. The relative contribution of different semaphorins remains to be tested in future experiments in order to understand their role in the nervous system during MS.

4.7 Cross talk between the immune and the nervous systems

Even though the semaphorin signaling in lymphocytes is not the main subject of this chapter, it is impossible to completely dissociate the semaphorin signaling

in the nervous system from their roles in the immune system. The cross talk between these systems is extensive, and different neurological disorders are considered to have an important neuroinflammatory component. For example, the most commonly used animal model for MS is the experimental autoimmune encephalomyelitis (EAE) model, which resembles neuroinflammatory conditions. Different authors suggest that the effect of semaphorins in the nervous system is most likely an indirect effect through modulation of the neuroinflammation produced by immune cells in the nervous system, supported by using the EAE model. For instance, the EAE pathology is exacerbated in Sema7A-deficient mice, and T cells are hyperactive in response to activation in this model. Similarly, Sema4A is increased in MS patients and associated to nonresponsiveness to IFN-β therapy. Anti-Sema4D antibodies inhibited neuroinflammation during EAE [151–153].

Most of the patients who later will develop MS, usually, have an acute episode of neurological disturbance known as clinically isolated syndrome (CIS). The Montalban lab [154], using mass-spectrometry analysis, identified proteins associated with conversion to MS in CSF samples from CIS patients in a follow-up study. They found that Sema7A was downregulated in patients who later converted to MS [154, 155]. Using the EAE model, the same group found that Sema3A is increased in the CNS and decreased in the immune system, whereas Sema7A is increased in both systems [156]. The above results suggest an intricated system where different semaphorins can be participating at the same time. It is important to understand the relative contribution of different neuronal types and different immune cell types to the pathology and also the amount of soluble semaphorins available to interact with these cells.

5. Conclusion and future perspectives

Throughout this chapter, we have reviewed the currently known implications of different semaphorin classes to some relevant neurological disorders, highlighting their receptors and signaling pathways that could be affected in neuropathologies. Even though the diseases we discussed here represent just a fraction among several other semaphorin-affected neurodegenerative, psychiatric, and immunological disorders, they are also likely representative of the semaphorin function. The advances so far in this field are promising, yet the results obtained from murine systems require testing on human models and subsequently, approaching to eventual therapies and clinical trials. We have already mentioned the potential of semaphorins for cell replacement therapy, such as in the recent approaches on PD [105], or the alternative new drug developments to target specific semaphorin-induced kinases, such as in AD [67]. All these new treatment alternatives emerged in the recent years from the advances in understanding semaphorin-mediated mechanisms on human diseases. In addition, semaphorins have been recently pointed as the center for new therapeutic strategies using blocking antibodies. For example, the VX15/2503, an anti-Sema4D antibody has been characterized for clinical development on MS, Huntington's disease, and cancer [157]. LaGanke et al. carried out a phase 1 study providing evidence that the VX15/2503 anti-semaphorin 4D antibody administered at various doses was safe and tolerated in patients with MS [158]. It is expected in following years that new breakthroughs will further highlight semaphorin function in neurodegenerative conditions and contribute to future therapeutic strategies.

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Chapter 3

Neurodegenerative Diseases and Their Therapeutic Approaches

Farhin Patel and Palash Mandal

Abstract

Alzheimer's disease and Parkinson's disease are characterized as a chronic and progressive neurodegenerative disorder and are manifested by the loss of neurons within the brain and/or spinal cord. In the present chapter, we would like to summarize the molecular mechanism focusing on metabolic modification associated with neurodegenerative diseases or heritable genetic disorders. The identification of the exact molecular mechanisms involved in these diseases would facilitate the discovery of earlier pathophysiological markers along with substantial therapies, which may consist (of) mitochondria-targeted antioxidant therapy, mitochondrial dynamics modulators, epigenetic modulators, and neural stem cell therapy. Therefore, all these therapies may hold particular assurance as influential neuroprotective therapies in the treatment of neurodegenerative diseases.

Keywords: neurons, mitochondria-targeted antioxidants, mitochondrial dynamics, epigenetic regulations, stem cell, neurodegenerative diseases

1. Introduction

1.1 What are neurons?

Neurons or nerve cells are the functional unit of the brain and nervous system, and they produce electrical signals known as action potentials. Action potentials permit them to speedily pass on the details over long distances. Their connections define who we are as a person. The creation of new neurons in the brain is known as neurogenesis [1].

1.2 Anatomy of a neuron

Different types of neurons may differ in a number of ways, but they all include three distinct regions with differing functions, that is, the cell body (soma), followed by the dendrites, the axons, and the connected axon terminals (**Figure 1**).

a. Cell body: It is the place of biogenesis of almost all neuronal proteins and membranes. It contains a nucleus.

b. Dendrites: The extensions of neurons that receive signals and conduct them toward the cell body (soma) are known as dendrites.

c. Axon (nerve fiber): The extensions of neurons that conduct the signals away from the cell body to the other nerve cells or neuron are known as axons.

d. Axon terminal (end-plate): The end part or terminal part of axons that makes a synaptic contact with other nerve cells is known as an axon terminal. It is responsible for the initiation of transmission of nerve impulse to another nerve cell [2].

1.3 Functions of neurons

a. Conduction and transmission of nerve impulses

b. Initiation and conduction of action potential

c. Synaptic transmission [3]

1.4 How neurons transmit information throughout the body?

Neurons converse with other neurons through axons and dendrites. When a neuron receives information from another neuron, it transmits an electrical signal along the length of the respective axon, known as action potential. At the axon terminal, the electrical signal is changed into chemical signal. The axon releases chemical messengers called neurotransmitters. The neurotransmitters are released into the gap between the axon terminal and the tip of a dendrite (receptor site) of a further neuron. The space between the axon terminal and the tip of a dendrite is called a synapse. The neurotransmitters travel along the short distance through the synaptic gap to the dendrite. The dendrite receives the neurotransmitters and translates the chemical signal into electrical signal. This electrical signal travels all the way through the neuron, to be converted back into a chemical signal when it gets to adjoining neurons [4].

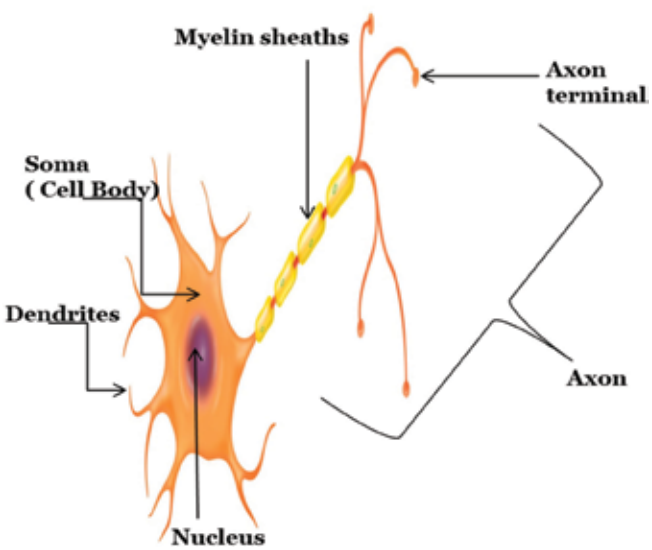

Figure 1.
Anatomy of neuron.

2. Neurodegenerative diseases

Etymologically, the word neurodegeneration comprises of "neuro," which refers to neurons, and "degeneration," which refers to the process of losing structure and/or function of either tissues or organs [5]. A neurodegenerative disease is considered as a slow, progressive failure of nerve cells within the central nervous system (CNS). This leads to deficits in particular brain functions like learning, movement, and cognition generally performed by the CNS (brain and spinal cord).

2.1 Factors associated with neurodegenerative diseases

a. Aberrant protein dynamics with aggregation and degradation of defective protein [6]

b. Oxidative stress and reactive oxygen species (ROS) formation

c. Impaired bioenergetics and mitochondrial dysfunction

d. Excessive exposure to metals and pesticides (**Figure 2**)

2.2 Classification based on molecular defects

a. Trinucleotide repeat diseases: HD, spinal cerebellar atrophy, and myotonic dystrophy [7].

b. Prion diseases: Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, and fetal familial insomnia [8].

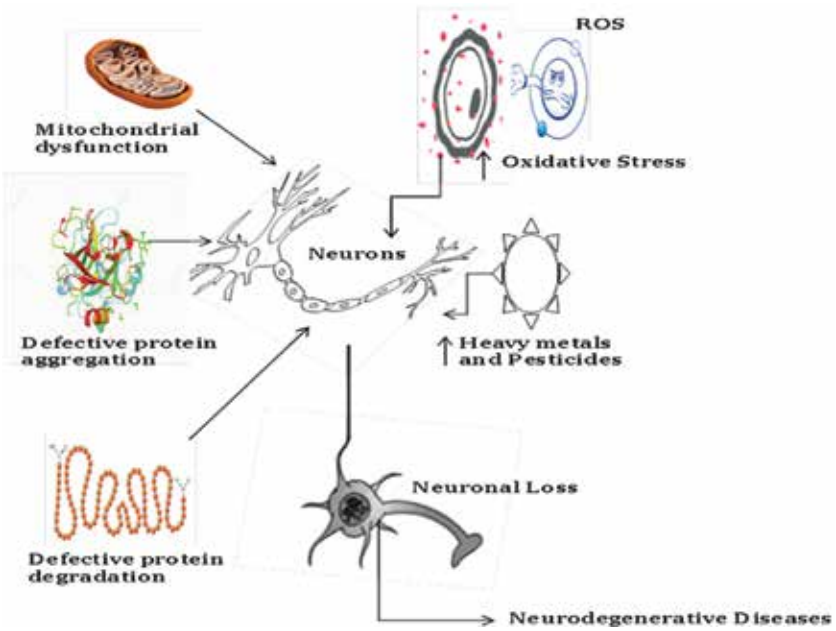

Figure 2.
Factors associated with neurodegenerative diseases.

c. Synucleinopathies: PD, progressive supranuclear palsy and diffuse Lewy body dementia [9].

d. Tauopathies: Corticobasal degeneration, frontotemporal dementia with parkinsonism linked to chromosome 1\(FTDP-17), and pick disease [10].

3. Alzheimer's disease

Alzheimer's disease (AD) is an irreparable, progressive neurodegenerative disease that affects normal brain functioning [11]. It is mainly the general cause of dementia [12]. Dementia is a syndrome associated with memory loss and loss of abilities like thinking, reasoning, and language skills along with other mental illness [12].

3.1 History

This disease is named after Dr. Alois Alzheimer. He observed some brain tissue abnormalities in an old woman who died due to some unusual mental illness. Later, he examined her brain and found many abnormal tangled bundles of fibers (called as tau tangles, neurofibrillary) and clumps (called as amyloid plaques). That is how he found the cause of AD [13].

3.2 Causes

The cause of AD is not clearly understood.

a. Genetic: Nearly, 70% of the cases are related to genetic factors with the involvement of many specific genes [14].

   1. Autosomal dominant inheritance: Also known as early-onset familial AD [15], it occurs due to the mutation in one of the three genes: Presenilin 1, presenilin 2, or amyloid precursor protein (APP) [16].

      Aβ42: A protein that is the main component of senile plaques, and the levels are increased due to mutation in APP and presenilin genes [17].

   2. Sporadic Alzheimer's disease: In this type of AD, genetic and environmental factors play a major role.

      Example: Inheritance of the epsilon 4 allele of the apolipoprotein E (APOE) [18, 19].

b. Cholinergic hypothesis: The cholinergic hypothesis states that AD is caused by the reduced synthesis of neurotransmitter acetylcholine [20].

c. Amyloid hypothesis: The amyloid hypothesis states that AD is caused by the deposits of extracellular amyloid beta (Aβ) [21].

d. Tau hypothesis: The tau hypothesis states that AD is caused due to abnormalities in tau protein, leading to the disintegration of microtubules in nerve cells [22, 23].

3.3 Molecular mechanism

(a) Proteopathy: AD has been recognized by plaque formation occurring due to abnormal folding of amyloid beta (Aβ) protein and tau protein in the nerve cells

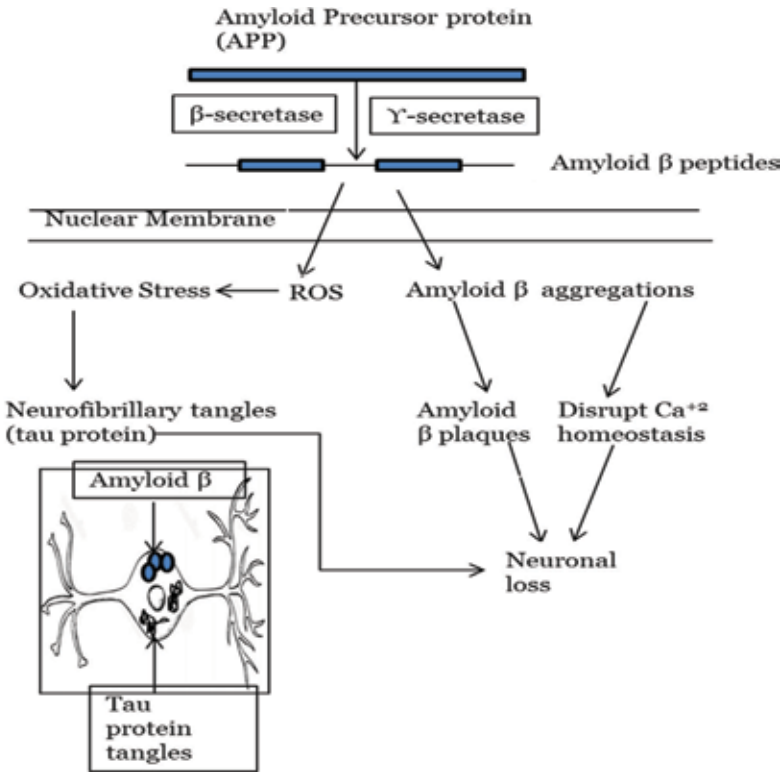

Figure 3.
Molecular mechanism of AD.

(brain) leading to the degeneration of nerve cells [24]. The amyloid precursor protein (APP) leads to the formation of Aβ. APP plays an important role in neuron-like developments and post-injury repair mechanism and survival [25, 26]. In AD, secreting enzymes like β-secretase and γ-secretase together will break down APP into small fragments that penetrate through the neuron membrane [27]. This leads to the formation of Aβ fibrils that later cluster together to form senile plaques and deposits in the outer side of neurons [28, 29]. Aggregated amyloid fibrils accumulation leads to the disruption of cell's calcium ion homeostasis, which results in apoptosis [30] (**Figure 3**).

(b) Tauopathy: In AD, there is an abnormal accumulation of tau protein. Upon phosphorylation, tau protein stabilizes the microtubules, and it is known as microtubule-associated protein. Tau protein undergoes certain chemical changes, and becomes hyperphosphorylated. This leads to the formation of neurofibrillary tangles upon aggregation with other threads, which results in decaying the neurotransport system [31].

3.4 Therapeutic approaches

3.4.1 Mitochondrial-directed therapies

Decline of N-acetyl aspartate and creatine is associated with dementia [32]. Supplementation of creatine was found to protect neurons in AD [33]. In hippocampal neurons, administration of creatine defends against glutamate and Aβ toxicity in rats [34].

In AD patients, administration of lipoic acid (600 mg/day) [LA - an antioxidant; coenzyme for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase]

stabilizes the cognitive measures [35, 36]. Decreased oxidative stress of mitochondria in fibroblasts was found in AD patients due to LA and/or N-acetyl cysteine (antioxidant and glutathione precursor) administration [37].

CoQ10 (an antioxidant and cofactor of the electron transport chain) blocks apoptosis by inhibiting the permeability transition pore (PTP) of mitochondria [38]. Treatment of CoQ10 neutralizes the brain mitochondrial alterations made by amyloid-β1–40 [39]. CoQ10 was shown to protect paraquat and rotenone-induced mitochondrial dysfunction and neuronal death in SHSY-5Y cells (human neuroblastoma cells) and primary rat mesencephalic neurons, [40, 41]. In R6/2 mice, combined treatment of CoQ10 and minocycline reduces HTT accumulation, brain atrophy, and striatal neuron atrophy [42].

MitoQ (mitochondrial coenzyme Q) reduces oxidative stress and prevents mitochondrial dysfunction [43]. Oral administration of MitoQ (1 mg/kg body weight) showed better pharmacokinetics behavior with plasma (Cmax = 33.15 ng/ml and Tmax = 1 hr.) in Phase I trial (Antipodean Pharmaceuticals Inc., San Francisco, CA).

3.4.2 Stem cell therapy

Neural stem cell therapy provides a potential to neurons derived from stem cells to integrate with existing neuronal network of the host brain [44]. In animal models, stem cell transplantation elevates the level of acetylcholine, resulting in an improved cognitive and memory function. Stem cells secrete neurotrophic factors, which modulate neuroplasticity and neurogenesis [45, 46].

Embryonic stem cells (ESCs)-derived neuron progenitor cells (NPCs) when transplanted into an amyloid-β injured in vitro model, after 2 weeks of amyloid-β injection, showed an increased escape latency when compared with phosphate-buffered saline-treated controls [47]. It has been reported that ESCs-derived NPCs improve memory impairment in AD models [48].

Human induced pluripotent stem cell (iPSC) therapy delivers a possible strategy for drug development against AD [49]. Neurons differentiated from iPSCs increase the secretion of amyloid-β42 as it is affected by γ-secretase inhibitors [50].

Bone marrow (BM)-derived mesenchymal stem cells (MSCs) play an important role in the removal of amyloid-β plaques from the hippocampus [51]. Human MSCs promoted amyloid-β clearance and enhanced autophagy and neuronal survival in an amyloid-β-treated mouse model [46]. Transplantation of adipose-derived MSCs (AMSCs) into AD brain improved the acetylcholine levels along with microglia activation and cognitive functions [52, 53]. In a transgenic mouse model, human umbilical cord-derived MSCs differentiated themselves into neuron-like cells, and these cells when transplanted into an amyloid-β precursor protein (AβPP) and PS1 (AβPP/PS1) resulted in improved cognitive function and decreased amyloid β deposition [54].

3.4.3 Epigenetic modulators

Histone deacetylases have been linked to AD. Treatment with HDACi (histone deacetylase inhibitors) induced dendrite growth, increased the number of synapses, and restored learning and memory deficits in mice with AD [55] (**Table 1**).

3.4.4 Mitochondrial dynamics modulators

Two recent studies have also shown the protective effects mediated by inhibition of mitochondrial fission via Drp1 deficiency on mitochondria and neurons in tau and APP transgenic animal models for AD [60, 61].

HDACi	Function	References
Sodium butyrate	In neuroblastoma cells, it induces phosphorylation of tau protein and programmed cell death resulting in restoring memory.	[56]
Phenylbutyrate (4-PBA)	InTg2576 mouse model, 4-PBA restores fear learning and rescues dendritic spine losses that are associated with memory shortage.	[57]
Suberoylanilide hydroxamic acid	In mutant mice model, systemic treatment restores contextual memory	[58]
Resveratrol (activator of class III HDAC)	In in vivo and in vitro studies, SIRT1 reduces the amyloidogenic processing of APP	[59]

Table 1.
Histone deacetylase inhibitors and their respective functions in AD.

4. Parkinson's disease

Parkinson's disease (PD) is a progressive, long-term neurodegenerative disorder that affects the motor neurons [62]. It is caused by a loss of neurons in the brain part known as substantia nigra leading to a reduction in a neurotransmitter called dopamine [62].

4.1 History

In 1817, James Parkinson (before known as Jean-Martin Charcot) published an essay named "Shaking Palsy" describing six cases of paralysis agitans showing certain characteristics of this disease [63, 64].

In 1865, William Sanders termed this disease as Parkinson's disease [65].

4.2 Causes

The following are the causes of PD:

(a) Environmental factors: Exposure to metals, solvents, and pesticides, or any head injuries are considered to be a factor for the onset of PD [66, 67].

(b) Genetics: Few percent of cases are developing this disease due to mutation in one specific gene out of several genes related to PD (**Table 2**).

4.3 Molecular mechanism

The mechanism involved in the development of PD includes various factors like the aggregations of misfolded proteins, activation of protein degradation

	Name	Gene	References
Autosomal-dominant PD	PARK1/PARK4	SNCA (α-synuclein)	[68, 69, 72]
	PARK2	Parkin	[68, 69, 72]
	PARK5	UCHL	[68, 69, 72]
	PARK8	LRRK2	[68, 69, 72]
Autosomal-recessive PD	PARK6	PINK1	[68, 70–72]
	PARK7	DJ-1	[68, 70–72]

Table 2.
Genes involved in PD.

pathways, mitochondrial damage, and oxidative stress, along with certain gene mutations [73–75].

4.3.1 Aggregation of misfolded proteins

- Accumulation of Lewy bodies in dopamine neurons of the substantia nigra pars compacta [75]
- Hyperphosphorylation of tau protein causes accumulation of neurofibrillary tangles [76]

4.3.2 Protein degradation pathways

- Ubiquitin-proteasome system (UPS): It is responsible for the degradation of misfolded or damaged proteins in the cytosol, nucleus, or endoplasmic reticulum (ER) [77]. Impairment in this system leads to aggregation of misfolded amyloid proteins (Lewy bodies) [78]. Other proteins like UCH-L1 and Parkin are involved in the degradation of misfolded α-synuclein [79]
- Chaperones (heat shock proteins/HSP): Chaperones undergo dysfunctioning in PD, as they play a vital role in cell-defense mechanism involved in protein degradation and folding of proteins. Major HSPs involved are HSP 26, HSP40, HSP 60, HSP 70, HSP 90, and HSP 100 [80]. HSPs aggregate with α-synuclein or tau protein and form insoluble structures resulting in reduced toxicity of α-synuclein or tau protein [81, 82]
- Autophagy-lysosomal pathway (ALP): It serves to clear Lewy bodies in PD acting as an alternative clearance mechanism for proteins [83, 84]. Chaperone-mediated autophagy (CMA) helps in the degradation of α-synuclein by selectively translocating into lysosomes [83]. Therefore, dysfunctioning of CMA decreases the efficiency of α-synuclein, leading to excessive accumulation of this protein. This results in impaired neuronal activity as observed in PD [73, 85]. Failure of formation of autophagosome, its inability to bind with lysosomes due to deficiency of lysozymes, or dysfunction of HSP70 results in dysfunction of ALP in PD [73, 85] (**Figure 4**)

4.3.3 Damage to mitochondria and oxidative stress

- Abnormality of complex-I in mitochondria directly interferes with ATP production in the cell, resulting in cell death [86]. Monoamines such as dopamine are cleaved by monoamine oxidase-B (MAO-B) and combined with oxygen-forming reactive oxygen species (ROS) [87]. Increased oxidative stress was observed in PD.

4.3.4 Genetic mutations

The most common genes related to PD are α-synuclein, DJ-1, PINK1, and Parkin [88] (**Table 3**).

4.4 Therapeutic approaches

4.4.1 Mitochondria-directed therapies

Administration of creatine increases tyrosine hydroxylase immunoreactive fiber density and soma size of dopaminergic neurons in mesencephalic cultures by protecting against neurotoxic insults induced by serum and glucose deprivation, MPP+, and 6-hydroxydopamine [33, 92]. It has been reported that dopamine loss was prevented by administration of creatine. In substantia nigra, creatine also

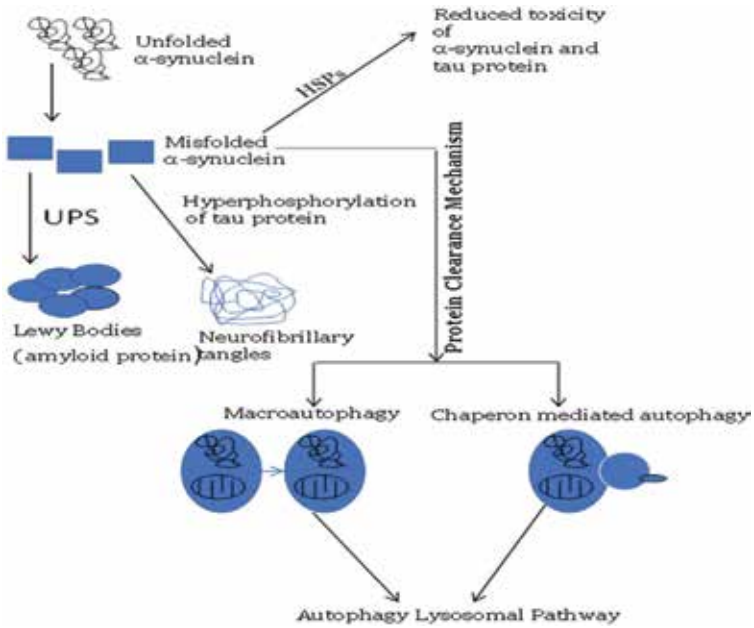

Figure 4.
Molecular mechanism of PD.

Genes	Dysfunction	References
α-synuclein	Aggregation of misfolded amyloid proteins	[89]
Parkin	Aggregation of misfolded amyloid proteins within SNpc	[89]
DJ-1 (PARK7)	Activities like transcriptional regulation, antioxidants, chaperone, and protease are dysregulated	[90]
PINK1 (PARK6)	Mitochondrial dysfunctioning Degeneration of substantia nigra neuron	[91]

Table 3.
Specific gene mutations and their dysfunction involved in the development of PD.

reduces loss of neuron in the mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [93].

CoQ10 protects against iron-induced apoptosis in dopaminergic neurons [94]. In vitro, CoQ10 exerts anti-amyloidogenic effects by disrupting preformed amyloid-β fibrils [95].

SS peptides (Szeto Schiller) act as antioxidants that target mitochondria in an independent manner. In mice, reports showed that SS-20 and SS-31 provide protection against MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) neurotoxicity. SS-31 provides protection against dopamine loss in the striatum. In substantia nigra, SS-31 also provides protection against the loss of tyrosine hydroxylase immunoreactive neurons. In MPTP-treated mice, SS-20 provides potential neuronal protection on dopaminergic neurons in PD [96].

4.4.2 Stem cell therapy

In the first trial of cell-based therapy, post-mitotic dopamine neuroblasts isolated from human embryonic mesencephalic tissue have been successfully grafted in PD patients [97]. It has been confirmed through increase in 18F-dopa intake,

detected through positron emission tomography (PET) [98, 99]. The grafts restore dopamine release. Disadvantages of this therapy are limited tissue availability and grafts standardization.

Recently, researchers have shed light on stem cell therapy. The production of dopamine neuroblasts from stem cells for transplantation in PD patients has been focused on. The aim was to release dopamine in a stable manner and exhibit the electrophysiological, molecular, and morphological properties of substantia nigra neurons [100, 101]. In clinical trials, it has been found that dopaminergic cells derived from embryonic stem cells can survive and reverse behavioral deficits after transplantation in PD animal models [102, 103].

4.4.3 Epigenetic modulators

In sporadic PD patients, there is an increased α-synuclein expression in dopaminergic neurons, which is linked with α-synuclein hypomethylation [104]. In familial PD patients, decreased histone acetylation is linked with increased α-synuclein levels [105]. In vitro model, mutation in α-synuclein leads to increased histone acetylation mediated through HDAC Sirt2. Treatment of Sirt2 siRNA resulted in decreased α-synuclein-mediated toxicity [106]. Administration of levodopa elevated the dopamine level, which partially showed decreased symptoms of PD.
It is correlated with deacetylation of H4K5, K12, and K16 [107].

4.4.4 Mitochondrial dynamics modulators

Recombinant adeno-associated virus expressing the dominant negative Drp1 (dynamin-related protein 1) mutant or Mdivi-1, a small molecular inhibitor of Drp1, has been reported to inhibit mitochondrial fragmentation, restore dopamine release, and prevent dopamine neuron loss in PD animal models [108].

Activation of DRP1-mediated mitochondrial fission is an important contributing factor in the progression of PD. Neurons lacking PINK or Parkin accumulate DRP1, resulting in excessive mitochondrial fission, increased oxidative stress, and reduced ATP production [108, 109]. These defects can be reversed by the inhibition of mitochondrial fission with the use of mdivi-1, an inhibitor of the DRP1 pathway, or by overexpression of MFN2 (Mitofusin 2) or OPA1 (Optic atrophy protein 1) [109, 110].

In vitro models of glutamate-toxicity or OGD (oxygen-glucose deprivation) in mouse hippocampal neurons or in vivo mouse models of transient focal ischemia can be protected from enhanced mitochondrial fission and apoptosis by DRP1 knockdown or mdivi-1 inhibition [111, 112].

5. Conclusion

The recent advancements in the field of neurodegenerative diseases like AD and PD are based on targeting the degenerative progressions that lead to the death of neurons. The death of neurons leads to irreversible neuropathological conditions, making it difficult to be functional in humans. Because of the intricacy involved in respective neurodegenerative diseases, researchers have identified few potential biomarkers. At present, many therapeutic approaches have been suggested to treat the symptoms of both neurodegenerative diseases. Yet there exists a lacuna for the effective therapies. Hence, few therapeutic approaches like mitochondria-targeted antioxidant therapy, mitochondrial dynamics modulators, epigenetic modulators, and neural stem cell therapy may prove to have a potential in treating AD and PD.

Conflict of interest

Authors have no conflict of interest to declare.

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Section 3

Gap Junction

Chapter 4

Gap Junctions in the Dorsal Root Ganglia

Vishwajit Ravindra Deshmukh

Abstract

Dorsal root ganglion (DRG) or spinal ganglia are present in relation to the dorsal ramus of the spinal nerves. The neurons in the dorsal root ganglion are pseudounipolar in type. The single process from the soma or body will divide into the central and peripheral processes. Dorsal root ganglion neurons constitute the first-order neurons for the pain pathways and can be categorized as small, medium and large varieties. Peripheral process collects the impulses from the peripheral receptors and the central process reaches out to the central nervous system. The neurons in the DRG were surrounded by the satellite glial cells (SGC). These cells ensheath the neurons from all the sides. Besides covering the neurons, they share features very much similar to the astrocytes such as expression of glutamine synthetase. Many quantitative studies have identified the different proportion of satellite glial cells for individual neurons. These cells have been identified to get activated when confronted by the noxious stimuli, injury or inflammation. Clinically, these cells were implied to be related to the many neurological disorders.

Keywords: neurons, satellite glial cells, communicating junctions, pain, connexin-43, glial fibrillary acidic protein, peripherin, Nissl stain, immunohistochemistry

1. Introduction

The human nervous system is an extremely efficient, compact, fast and reliable computing system, yet it weighs substantially less than most of the computers and performs at an incredibly greater capacity.

The nervous system is subdivided, morphologically into two components, the central nervous system (CNS) consisting of the brain and spinal cord and the peripheral nervous system (PNS) comprising of cranial and spinal nerves and ganglia.

Discrete collections of nerve cell bodies in the CNS are known as nuclei while in PNS, these are called ganglia. The nerve cell bodies are of varying sizes and shapes. Ganglia are present in the dorsal root of spinal nerves, the sensory root of the trigeminal nerve (Vth), Facial (VIIth), Glossopharyngeal (IXth), Vagus (Xth) nerves and in the autonomic nervous system [1]. Some of them have independent nomenclature like the "Gasserian ganglion" for the Vth nerve. Thus ganglia can be divided into two types somatic and autonomic (**Figure 1**). The nerve cell bodies in each of these differ in their size and shape. Somatic ganglia contain small to large pseudounipolar neurons while the autonomic ganglia contain small multipolar neurons.

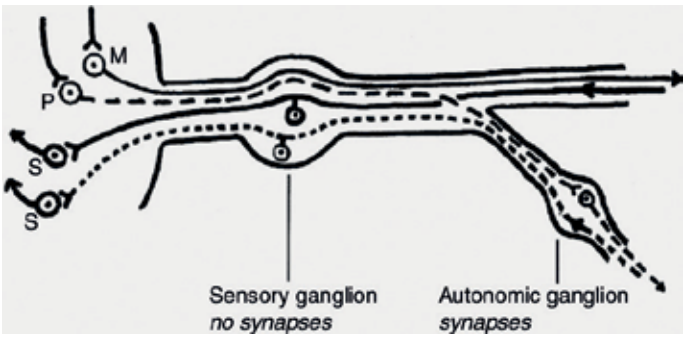

Figure 1.
Differences in sensory and autonomic ganglia (courtesy: Cranial Nerves and Functional Anatomy, 1st ed. p. 12).

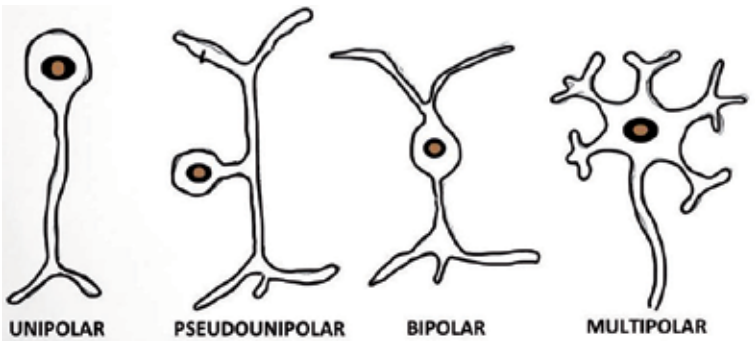

Figure 2.
Types of neurons in nervous system.

Depending on the number of processes, a neuron can be classified into various categories. Unipolar neurons (no dendrites only an axon) are rare in vertebrates, bipolar neurons (possesses an axon and a dendrite) present in olfactory mucosa and the retina and multipolar neurons (single axon and two or more dendrites) present in the central nervous system except the mesencephalic nucleus of the Vth cranial nerve. An additional type of neuron, the pseudounipolar neuron is present in sensory ganglia and the ganglia of Vth, VIIth, IXth and Xth cranial nerves. It divides into a central and peripheral process (**Figure 2**).

The neurons in sensory ganglia are at first bipolar, but the two neurites soon unite to form a single process during development. Structurally and electrophysiologically, both these processes show characteristic features of the axon [2]. Small satellite glial cells tightly wrap the cell bodies of the pseudounipolar neurons in the ganglion. The satellite cells that surround the pseudounipolar neuron are continuous with the Schwann cell sheath that surrounds the axon [3]. A distinctive feature of satellite glial cells by which they are distinguished from astrocytes is that they completely surround the individual sensory neuron. The neuron and its surrounding satellite glial cells form a distinct morphological and probably a functional unit [4]. The somatic ganglia of all the mammalian and avian species demonstrate this arrangement [5]. Satellite glial cells have been implicated in neuronal nutrition, homeostasis, and the process of apoptosis. It is known that astrocytes in the central nervous system perform 'spatial buffering' (regulation of K^+) and it is presumed

that SGCs also perform the same function [5]. Removing K^+ from the perineuronal environment would reduce neuronal excitation and therefore contribute to the lowering of pain.

2. Morphology of Dorsal root ganglia (DRG)

Dorsal root ganglia (sensory ganglia) contain the cell bodies of primary afferent neurons that transmit the sensory information from the periphery into the central nervous system (CNS) [6]. Sensory ganglia were located near the entrance of dorsal root into the spinal cord, and are not a part of CNS. Sensory (somatic) ganglia lie outside the blood-brain barrier and are densely vascularized by fenestrated capillaries, making the neurons and SGCs easily accessible to compounds in the circulation, including chemotherapeutic drugs [7]. Chemotherapeutic drugs show greater accumulation in sensory ganglia than in peripheral nerves [8]. Dorsal root ganglia are more sensitive to heat than other nervous tissues [9]. It is known that pulsed radiofrequency can selectively block sensory nerves while minimizing the destruction of motor nerves. Sluijter et al. reported that the placement of a cannula 1–2 cm peripheral to the dorsal root ganglia could result in maximum effect when pulsed radiofrequency was applied on dorsal root ganglia of the spinal cord [10]. Kikuchi et al. [9] classified anatomical positions and variations of dorsal root ganglia into intraspinal (IS), intraforaminal (IF), and extraforaminal (EF) (**Figure 3**).

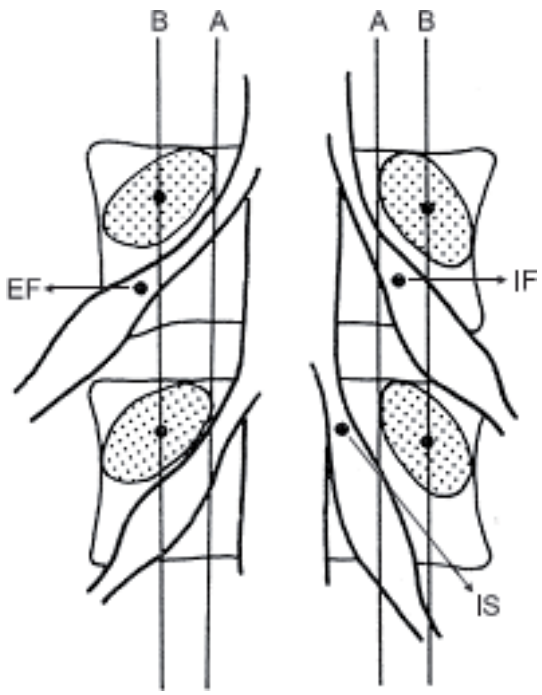

Figure 3.
Positions of dorsal root ganglia (DRG) were determined by two schematic lines and classified into three types. Line A: aligning the medial borders of L4 and L5 pedicles, Line B: aligning the centers of L4 and L5 pedicles, Intraspinal type (IS): DRG located proximal to line A, Intraforaminal type: DRG located between line A and B, Extraforaminal type: DRG located distal to line B [9].

3. Morphology and histology of sensory (somatic) ganglia

The segmental nature of the spinal cord is demonstrated by the presence of 31 pairs of spinal nerves, but there is little indication of segmentation in its internal structure. Each dorsal root is broken up into a series of rootlets that are attached to the spinal cord along the corresponding segment. The ventral root arises similarly as a series of rootlets. These rootlets join to form the ventral and dorsal roots. The dorsal and ventral roots traverse the subarachnoid space and pierce the arachnoid and dura mater. At this point, the dura mater becomes continuous with the epineurium. After passing through the epidural space, the roots reach the intervertebral foramina, where the dorsal root ganglia are located on the dorsal root.

Certain authors have put forward their views regarding the classification of the neurons in the dorsal root ganglia based upon their staining properties into two histological types called "large light" and "small dark", visible under the light microscope. This has been confirmed by recent electron microscopic analysis that indicates [11] the existence of two basic types of DRG neurons usually termed as type A and type B rather than large light and small dark [12]. The neurons in the dorsal root ganglion can also be divided into three types (small, medium and large neurons) based upon the size of their cell bodies. This classification seems to be more appropriate because the size of the neuronal cell bodies determine their function. The large neurons are mainly concerned with the transmission of proprioception and discriminative touch while the medium-sized neurons transmit nerve impulses associated with sensations like light touch, pressure, pain and temperature. However, the small-sized neurons exclusively transmit action potentials related to pain and temperature.

Glial cells are involved in various pathological processes affecting the central nervous system [13]. There is strong evidence that CNS glial cells are involved (microglia and astrocytes) in the induction and maintenance of neuropathic pain [14]. Following injury of a peripheral nerve, satellite glial cells (SGCs) in the dorsal root ganglia undergo changes in cell number, structure and function, similar to those in the CNS

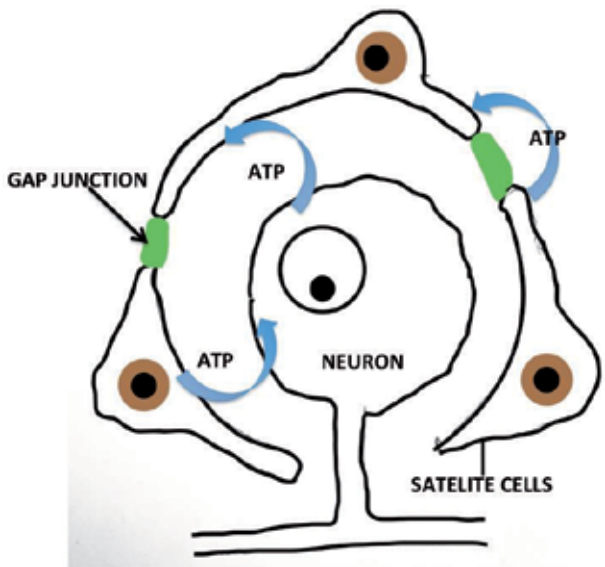

Figure 4.
Schematic diagram describing the structural and functional relations between SGCs and neurons in sensory ganglia, and the consequences of peripheral injury.

[15]. Peripheral nerve transection increases gap junctions and intercellular coupling of SGCs. SGCs also upregulated the production of proinflammatory cytokines such as tumor necrosis factor-α after lumbar facet joint injury [16].

Thus it is well established that glial cells play a critical role in the genesis and persistence of pain [17]. This is particularly true for the sensory ganglia. Though there are far fewer satellite glial cells than astrocytes or Schwann cells, yet because of their unique location in sensory ganglia, SGCs can strongly influence the afferent sensation. They also respond to the nerve injury by upregulating glial fibrillary acidic protein (GFAP) [18]. One of the ways glial cells in the sensory ganglia transmit signals is through intercellular calcium waves (ICWs) via gap junctions and adenosine-5′-triphosphate (ATP) acting on purinergic type 2 (P2) receptors [19]. This signaling has been shown to be bi-directional between SGCs and neurons (**Figure 4**).

4. Classification of pseudounipolar neurons of dorsal root ganglia into small, medium and large

Older literature suggests that neurons in dorsal root ganglia can be divided into two histological types called "large light (LL)" and "small dark (SD)" on the basis of staining properties under the light microscope [20]. This population overlaps, but still, they show the several physiological, biochemical and functional differences. Small dark neurons transmit the sensation particularly carried by C fibers (non-myelinated, slow conducting) [21]. Whereas Large light transmits the sensation carried via a fiber (myelinated and fast conducting). Many of the small dark neurons contain substance P or calcitonin gene-related peptide, and they are concerned with thermo- and mechanoreception, and many of them are nociceptive. The terminals of Large light neurons are low threshold mechanoreceptors [22]. Neurons in the sensory ganglia have no dendrites and also do not receive synapses but are still endowed with receptors for numerous neurotransmitters. More recently depending upon the electron microscopic appearance neurons in the dorsal root ganglia were divided into Type A and Type B for large light and small dark neurons respectively. Various other electrophysiological classification depending upon conduction velocity, modality and adaptation rate serves to distinguished large number of functional types of sensory neurons, but it is not clear how these are related to the two basic histological types.

There are contradictions among the researchers regarding the classification of dorsal root ganglia neurons into small, medium and large categories.

One of the studies involving chronic constriction injury model of Bennet and Xie [23] that retains the connection with the original receptive field so that hyperalgesia and allodynia can be demonstrated, classify the neurons in DRG into small (23–30 μm), medium (31–40 μm) and large (41–53 μm), based on the optical measurement of the average diameter [23]. These grouping roughly correspond to those giving rise to C, Aδ and Aβ fibers, respectively [21].

More recently sensory neurons in dorsal root ganglia were classified depending upon the immunohistochemical staining such as Nav1.8 expression in sensory neurons isolated from dorsal root ganglia into small (27–31 μm), medium (31–40 μm) and large (40–50 μm) [24]. There are two factors, namely DNA content and transcriptional activity, that are determinants of cell size [25]. Differences in neuronal body size seem to be primarily determined by the transcriptional activity. A positive correlation between the cell body and total RNA synthesis has been demonstrated in frog neurons, indicating that large neurons need higher transcriptional activities to maintain their large size [26]. The neurons transcription rate is, in turn, positively related to the magnitude of interactions between neurons and their targets, which contributes to the regulation of the soma size and metabolic activity [27].

Sensory neurons of the dorsal root ganglia express multiple voltage-gated sodium channels that substantially differ in gating kinetics and pharmacology. Small diameter (less than 25 μm) neurons isolated from the rat DRG express a combination of fast tetrodotoxin-sensitive (TTX-S) and slow TTX-resistant (TTX-R) sodium channels while large diameter neurons (more than 30 μm) predominantly expresses TTX-S Na current [28].

Viral study including adeno-associated viral vectors (AAV) are increasingly used to deliver therapeutic genes to the central nervous system where they promote transgene expression in postmitotic neurons for long periods with little or no toxicity. In adult rat dorsal root ganglia authors investigated the cellular tropism of AAV8 containing green fluorescent protein gene (GFP) after intra-lumbar DRG injection. And after injection, 2% of small DRG neurons (less than 30 μm) were GFP (+) as compared to 32% large (more than 60 μm) DRG neurons [29].

Electron microscopic features of dorsal root a ganglion divides the neurons depending upon their size and the distribution of their organelles (**Figure 5**). They were further subdivided into six subtypes according to the arrangement and three-dimensional organization of the Nissl bodies and Golgi apparatus in the perikarya. Type A1 cells were large, clear neurons in which Nissl bodies, separated from each other by pale narrow strands of cytoplasm containing small stacks of Golgi saccules and rod-like mitochondria, were evenly distributed throughout the perikaryon. In type A2, the Nissl bodies assumed a similar distribution but were separated by much wider strands of cytoplasm. Type A3, the smallest of the type A category, displayed densely packed Nissl bodies and long stacks of Golgi saccules which formed a perinuclear ring in the midportion of the perikaryon. Type B cells were smaller and showed a concentric zonation of their organelles. In type B1, large Nissl bodies located in an outer cytoplasmic zone were made of long piles of parallel cisternae interrupted by curved Golgi stacks. Type B2 was characterized by a ring-like Golgi apparatus separating the perikaryon in a cortical zone composed mainly of Nissl substance and a juxtanuclear zone containing mitochondria and smooth endoplasmic reticulum. Type C cells were the smallest of the ganglion cells

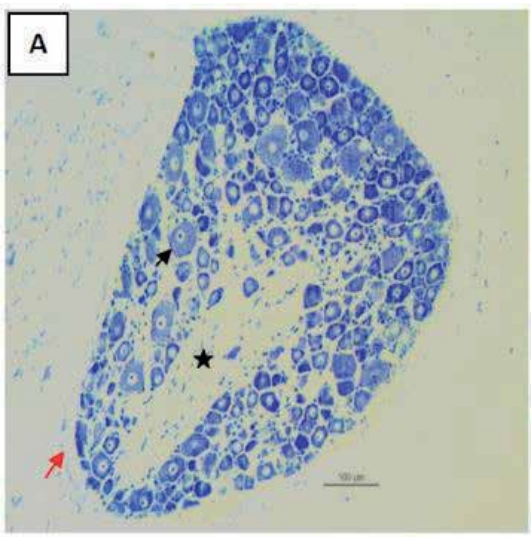

Figure 5.
Nissl's staining showing the variety of neurons in the dorsal root ganglion. Black arrow represents the large neurons, red arrow represents the surrounding capsule and the asterisk showed the location of centrally placed collection of nerve fibers [33].

and contained small, poorly demarcated Nissl bodies and a juxtanuclear Golgi apparatus [30].

Neurotransmitter study involving tachykinin like substance P (SP) and neurokinin A, which are released by the C-type primary afferent terminals of the small DRG neurons, plays important role in spinal nociception. By means of non-radioactive in situ hybridization and whole-cell recording, authors showed that the small rat DRG neurons also express the NK-1 tachykinin receptor. In situ hybridization demonstrated that the positive neurons in rat DRG sections were mainly small with a diameter of less than 25 μm. And the remaining positive neurons were cells with a medium diameter between 26 and 40 μm. No positive large neurons (more than 40 μm) were observed [31].

Depending upon the molecular weight of neurofilaments and their expression in various categories of neurons in dorsal root ganglia, three different neurofilament subunits have been identified, i.e. light (NF-L), middle (NF-M) and high (NF-H). Previous data showed that all the dorsal root ganglia neurons express NF-M and NF-H while only NF-L defines a distinct group of neurons and significantly large-light neurons [32].

5. Peripherin: marker to differentiate the neurons in the DRG

Peripherin, a protein formerly called Y, was first identified by two-dimensional gel electrophoresis in the insoluble fraction of cellular extracts from mouse neuroblastoma cell lines [34]. Its presence has been previously established in the rodent peripheral nervous system mostly by biochemical studies; moreover, biochemical characterization following nerve transection also supports its localization in neurons within the peripheral nervous system [35]. This observation leads to coining of the term "Peripherin" to designate this particular protein entity. Peripherin is a 57-kDa-type III neuronal intermediate filament protein, which is capable of either self-assembling or co-assembling with all of the individual neurofilament subunits [36]. In particular, the small cells of the dorsal root ganglia neurons selectively contain peripherin [35] and thus becoming a useful marker to define the small ganglion cell subpopulation. The exact function of the peripherin is still unknown though it has been suggested to be a determinant of the shape and architecture of the peripheral nerve axons and also provides structural integrity to the cells [37]. Peripherin immunolabeling has seen to be an important marker especially for the study of peripheral nerve development and regeneration since this intermediate filament protein is highly over-expressed during axon elongation [38]. Previously this neurofilament were thought to be inert but in fact these are highly dynamic structures with many diverse function such as relaying the signals from the plasma membrane to the nucleus [39], maintaining the position and function of cellular organelles, and also regulating the protein synthesis [40]. This neurofilament is clinically relevant because of their association with the pathogenesis of some major neuronal disorders. Mainly, accumulation of neurofilament protein and peripherin in proximal axons are associated with amyotrophic lateral sclerosis [41] and also seen in other diseases such as Alzheimer's disease [42]. Peripherin was used to identify the small to medium-sized neurons in the rat dorsal root ganglia in the present study as because these are associated with the transmission of pain from the periphery to the central nervous system. This would give an idea as to the actual number of neurons within the dorsal root ganglia involved in the transmission of pain (**Figure 6**).

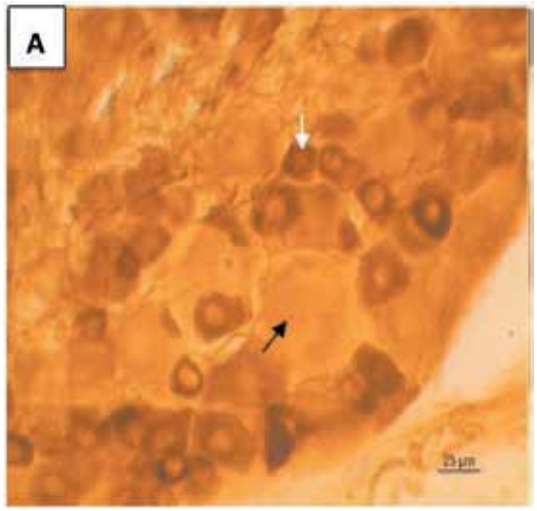

Figure 6.
Immunohistochemical stained section with peripherin antibody of dorsal root ganglion representing the specific staining in small to medium sized neurons (white arrows). Larger neurons (black arrows) [33].

6. Satellite glial cells

Sensory neurons in the dorsal root ganglia are ensheathed by specialized glial cells termed 'satellite glial cells' (SGCs). Recently, there has been considerable interest in these cells as they are profoundly altered by peripheral injuries used to study pain behavior and appear to contribute to chronic pain [43]. Satellite glial cells are the peripheral glial cells, but share many properties with astrocytes in the central nervous system (CNS), including the expression of glutamine synthetase and transporters of amino acids neurotransmitters. However, satellite glial cells differ in some respects from astrocytes, particularly by the tight sheath they make around the neuronal cell bodies [44]. In the dorsal root ganglion, Schwann cells and the satellite cells are activated in response to ischemia, traumatic injury and inflammation [45]. Application of various cytokines to the exposed Dorsal root ganglia resulted in an increase in the discharge rate as well as increased mechanosensitivity of DRG and peripheral receptive fields [46]. Satellite glial cells are the consistent component of the DRG in all the species, yet their contribution to the basic neuronal function remains unknown, although these satellite cells were implicated in neuronal nutrition, homeostasis and the process of apoptosis [5].

Recent studies have demonstrated that a specific glial cell population, the satellite glial cells, has the ability to regulate ion concentration [47] and possess mechanisms for the release of cytokines [48], ATP [19] and other chemical messengers like calcium. Satellite glial cells influence neuronal excitability via the gap junctions [49]. The satellite glial cells undergo major changes as a result of injury to peripheral nerves and appear to contribute to chronic pain [4]. Quantitative studies on several species showed that a number of satellite glial cells per neuron increases in proportion to the neuron's volume, consistent with the idea that these satellite glial cells support the neurons metabolically [50].

During pathological conditions, such as nerve injury or inflammation, SGCs demonstrate an altered phenotype similar to that seen in activated astrocytes, which includes increased expression of the glial fibrillary acidic protein (GFAP) and synthesis of cytokines [51]. SGCs are therefore said to undergo activation due

to injury. Increased coupling by gap junctions between SGCs has been observed in several inflammatory pain and axotomy models [52].

7. Satellite glial cells as a structural unit

Satellite glial cells (SGCs) in sensory ganglia wraps completely around the neuron. Several investigators claimed that SGCs bear processes and are therefore structurally similar to astrocytes but recent researches are that SGCs are laminar and have no true processes. In general, each sensory neuron has its own SGCs sheath, which usually consists of several SGCs, and thus the neuron and its surrounding satellite glial cells form a distinct morphological and probably functional unit. The region containing connective tissue separates these units. In some cases (5.6% in rat DRG) neurons from a small group containing two to three cells that are enclosed in common connective tissue space [44]. The neurons in the clusters are in most cases separated from each other by SGC sheath. The SGCs envelope usually consists of flat processes that lie close to the neuronal plasma membrane. The distance between the glial cell and neuronal plasma membrane is about 20 nm [44]. The neurons send numerous fine processes (microvilli), some of which fit into the invaginations of SGCs thus increasing the neuronal surface area and may allow an extensive exchange of chemicals between two cell types. A study on cultured SGCs of embryonic and neonatal rats showed that SGCs could transform into astrocytes, Schwann cells and oligodendrocytes [53].

Quantitative studies on several species showed that the number of SGCs per neuron increases in proportion to the neuron volume [50] consistent with the idea that SGCs support the neurons metabolically. It was also found that the mean volume of the nerve cell body corresponding to an SGC was lower for small neurons than for large neurons, which implies that the metabolic needs of small neurons are better satisfied than those of large ones. Therefore, smaller neurons have a higher resistance to insults, which seems to be the case for mercury poisoning. However, there is experimental evidence that smaller neurons are more likely to die following axonal damage [54]. As sensory ganglia are not protected from substances circulating in the blood, SGCs may be important in the context of exposure to toxic substances. In several studies, SGCs were examined after poisoning with heavy metals and it was found that these cells take up organic mercury compounds [55], and lead [56]. Mercury poisoning also caused SGCs proliferation [57]. Nineteen days after the administration of organic mercury to rats, SGCs in DRG were heavily labeled for mercury, and their ability to take up GABA was greatly diminished. Interestingly, small neurons were considerably less labeled for mercury than large neurons, which could be attributed to a more effective protection by SGCs. Prolonged (3–18 months) administration of lead acetate to rats resulted in prominent changes in SGCs in DRG, which included proliferation and hypertrophy of these cells. Although a certain degree of neuronal damage was observed, it can be proposed that the changes in SGCs provide a better protection to the neurons during lead poisoning.

8. Satellite glial cells maintain ionic concentration

The satellite glial cells neighboring the pseudounipolar neurons have a highly negative resting membrane potential and noticeable potassium permeability. The primary means of limiting extracellular levels of potassium in the sensory ganglia occurs through the process commonly called spatial buffering or syphoning which

is mediated by satellite glial cells. The maintenance of a low extracellular potassium concentration is crucial for controlling the neuronal resting membrane potential and neuronal excitability. In sensory ganglia increased neuronal excitability has been associated with the occurrence of altered sensation, including the development of the neuropathic pain [58]. In the CNS buffering of extracellular potassium ions is carried by astrocytes, which consist of uptake by inwardly rectifying potassium (Kir) channels and dissipation through other channels and gap junctions [59]. It is established that the Kir current and Kir4.1 expression occur in the satellite glial cells [60]. Voltage-gated potassium channels are one of the important physiological regulators of the membrane potentials in excitable cells including sensory ganglion neurons.

9. Neuron-glial interactions

Central nervous system glial cells are increasingly known to be important regulator of synaptic activity and the key functional unit of nervous system [61]. Even though many of the same voltage-sensitive ion channels and neurotransmitter receptors of neurons are found in glia; glial cells lack the membrane properties obligatory to fire action potentials. Nevertheless, these ion channels and electrogenic membrane transporters permit glia to sense indirectly the level of neuronal activity by monitoring activity-dependent changes in the chemical surroundings shared by these two cell types. Complex imaging methods, which allow observation of changes in intracellular and extracellular signaling molecules in real time, show that glia, communicate with one another and with neurons primarily through chemical signals rather than electrical signals. Many of these signaling systems overlap with the neurotransmitter signaling systems of neurons, but some are specialized for glial-glial and neuron-glial communication. Neuron-glia cell interaction through gap junctions and extracellular paracrine/autocrine processes are believed to be important in the development of peripheral sensitization within the trigeminal ganglia [62]. Peripheral sensitization, which is characterized by increased neuronal excitability and a lowered threshold for activation, may possibly trigger a migraine attack. Moreover, activation and sensitization of the trigeminovascular afferent fibers appear crucial for initiation of migraine pain and for subsequent central centralization, in which increased excitability of second-order neurons leads to pain and allodynia. Increased gap junction communication between neurons and satellite glial cells was observed in the trigeminal ganglion in response to chemical activation of sensory trigeminal nerves [62].

Increased neuronal-glial signaling by way of gap junctions is common in neuroinflammatory CNS disorders, such as cerebral ischemia and Alzheimer's disease and may have underlying pathological significance [63]. Tonabersat (SB-220453), a compound that binds selectively and with high affinity to a unique stereoselective site i.e. the gap junctions and inhibits it in rats and human brains [64]. After an injury, the numbers of gap junctions that connect satellite glial cells increases [43] in a probable adjust to the greater release of potassium ions with intense neuronal activity. Injury to a peripheral nerve does not directly impact satellite glial cells integrity. However, changes in injured neurons can influence the ability of the surrounding SGCs to regulate K^+ via neuromodulators such as adenosine triphosphate (ATP) and nitric oxide (NO) [65].

Satellite glial cells have unique proteins that include the inwardly rectifying K^+ channel Kir4.1 [43], the connexin-43 (Cx43) subunit of gap junctions the purinergic receptor P2Y4 [66] and soluble guanylate cyclase. There is also evidence of the presence of small-conductance Ca^{2+}–activated K^+ channel SK3 that is present only in satellite glial cells. All the above proteins are involved, either directly or indirectly,

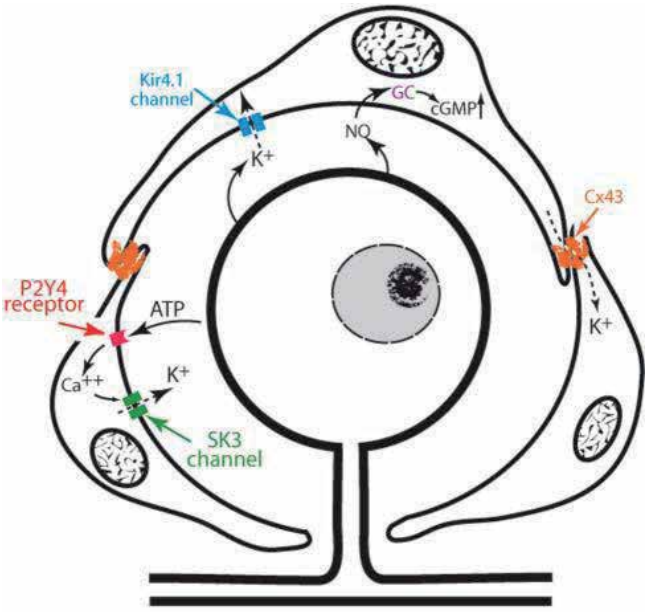

Figure 7.
Satellite glial cells involved in maintenance of potassium homeostasis [66].

in potassium ion (K^+) buffering and, thus, can influence the level of neuronal excitability, which, in turn, has been associated with neuropathic pain conditions (**Figure 7**). They also used in vivo RNA interference to reduce the expression of Cx43 (present only in SGCs) in the rat trigeminal ganglion and showed that this resulted in the development of spontaneous pain behavior. The pain behavior is present only when Cx43 is reduced and returns to normal when Cx43 concentrations are restored [66, 67].

10. Glial fibrillary acidic protein (GFAP): locator molecule for the satellite glial cells

Glial fibrillary acidic protein is principle intermediate filament in mature astrocytes of the central nervous system and satellite glial cells of sensory ganglia [4]. GFAP is strongly unregulated in response to CNS damage [68]. It is thought to be important in astrocyte neuronal interactions, astrocyte mobility and shape and for maintenance of homeostasis and vascular permeability at the blood-tissue interface [69]. GFAP is essential for normal white matter architecture and blood-brain barrier integrity and its absence leads to late-onset CNS dysmyelination [70]. Increased GFAP expression occurs in activated glial cells. Activated astrocytes are characterized by hypertrophy, the release of pro-inflammatory cytokines (IL-1, IL-6 and TNF-a), the release of nitric oxide and prostaglandins, and up-regulation of the intermediate filaments GFAP and vimentin [17]. Likewise, satellite glial cells (SGCs) display increased expression of GFAP after neuronal injury or inflammation and undergo a number of changes similar to those seen in astrocytes, such as synthesis of cytokines [71]. GFAP expression increases in the satellite glial cells of trigeminal ganglia after tooth pulp injury [72]. The present study also investigated the expression of GFAP in the satellite glial cells following acute pain (**Figure 8**).

GFAP is a marker of activated satellite glial cells and astrocytes [48]. These ropes like filaments are called intermediate filaments because their diameter of 8–10 nm is

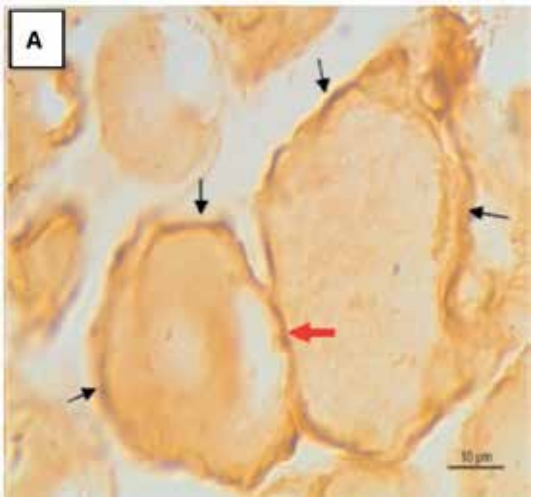

Figure 8.
Immunohistochemical staining for the section of DRG using GFAP antibody. Black arrows representing the location of satellite glial cells. Red arrow showing the communication between two neurons [33].

between those of actin filaments and microtubules. Nearly all-intermediate filaments consist of subunits with a molecular weight of about 50 kDa. Some evidence suggests that many of the stable structural proteins in intermediate filaments evolved from highly conserved enzymes, with only minor genetic modification. Intermediate filaments are formed from nonpolar and highly variable intermediate filament subunits. Unlike those of microfilaments and microtubules, the protein subunits of intermediate filaments show considerable diversity and tissue specificity. In addition, they do not possess enzymatic activity and form nonpolar filaments. Intermediate filaments also do not typically disappear and reform in the continuous manner characteristic of most microtubules and actin filaments. For these reasons, intermediate filaments are believed to play a primarily structural role within the cell and to compose the cytoplasmic link of a tissue-wide continuum of cytoplasmic, nuclear, and extracellular filaments. A highly variable central rod-shaped domain with strictly conserved globular domains at either end characterizes intermediate filament proteins. Although the various classes of intermediate filaments differ in the amino acid sequence of the rod-shaped domain and show some variation in molecular weight, they all share a homologous region that is important in filament self-assembly. Intermediate filaments are assembled from a pair of helical monomers that twist around each other to form coiled-coil dimers. Then, two coiled-coil dimers twist around each other in antiparallel fashion (parallel but pointing in opposite directions) to generate a staggered tetramer of two coiled-coil dimers, thus forming the nonpolarized unit of the intermediate filaments. Each tetramer, acting as an individual unit, is aligned along the axis of the filament. The ends of the tetramers are bound together to form the free ends of the filament. This assembly process provides a stable, staggered, helical array in which filaments are packed together and additionally stabilized by lateral binding interactions between adjacent tetramers [2].

Total six classes of intermediate filament are present in body, e.g., Class II and I include keratin and cytokeratin and class III include vimentin, glial acidic fibrillary protein (GFAP) and peripherin.

GFAP is the principal intermediate filament in mature astrocytes. GFAP is a soluble protein isolated from the multiple sclerosis plaques and presumably arising from the glial filaments [73]. The GFAP gene is located on the long (q) arm of chromosome 17 at position 21. Mutation in the GFAP results in Alexander disease

characterized by rare leukoencephalopathy affecting predominantly the brainstem and cervical cord with insidious onset of clinical features and unified by the presence in astrocytes of Rosenthal fibers (protein aggregates mainly contain glial fibrillary acidic protein (GFAP) and small stress proteins) in the astrocytes especially in the subpial and subependymal in location. It is strongly upregulated in response to the CNS damage [68]. It is thought to be important in astrocyte-neuronal communication and is believed to modulate astrocyte motility and shape. Satellite glial cells (SGCs) responsible for the maintenance of homeostasis and vascular permeability at the blood-tissue interface [69]. In the peripheral nervous system, neurons located in sensory ganglia are tightly surrounded by SGCs, following injury these cells undergo modification in structure and function [15]. According to Feng et al., after ligation of the L5 spinal nerve, mechanical allodynia developed in the ipsilateral hind paw and expression of GFAP in the ipsilateral DRG increased significantly as early as 4 hours after surgery, and gradually increases up to peak level at day 7 and then stayed at high level till day 56 [74]. Significant change seen among the sizes of neurons means small to medium size neurons shows maximum GFAP immunoreactivity at 12 hours and on day 7, a number of larger neurons was surrounded by GFAP stained satellite cells.

11. Gap junctions in the nervous system

Gap junctions, tight junctions, adherens junctions, desmosomes, hemidesmosomes, focal adhesions, chemical synapses, and immunological synapses are complex multiunit plasma membrane structures that assemble in a localized spatial and temporal organization to maintain structural tissue organization and to provide the cell signaling functions. At least nine connexins (Cx26, Cx32, Cx33, Cx36, Cx37, Cx40, Cx43, Cx45, Cx46) are expressed to various degrees in the nervous system. Functional studies in diverse cell types and in various exogenous expression systems have revealed that gap junction channels formed by different connexins are regulated differently, both at the single channel level (gating controls such as voltage sensitivity and variations in unitary conductance) and at the level of synthesis (expression, altered for example by hormones, extracellular matrix). Some gap junction channels are more sensitive to various gating stimuli than others, some display some degree of ionic selectivity, and some will pair promiscuously with other connexins (heterologous channels) while others are quite selective in their interaction (homologous channels). Such differences are important from the standpoint of the physiological roles of gap junctions in different cell types, as well as in the establishment of communication compartments within the nervous system [75]. Connexins are differentially expressed in the brain during ontogeny. Most recently, tissue culture preparations from embryonic neural tissue have allowed manipulation of individual cells and evaluation of changes in junctional distribution and expression during maturation. Such studies have clarified the relationships between sequential changes in phenotypes of neural cells, with the extent of coupling mediated by Cx43 (which is abundant in neural precursor populations) and the appearance of other gap junction proteins. Expression pattern of Cx32, Cx43 and Cx30 during the development in rat brain indicates the Connexin-43 appears first at embryonic days 12-18 [76] and that Cx32 protein and mRNA appear during first or second postnatal week and increases during development. Immunohistochemical analysis of postnatal rat brain has shown that Cx43 first appears along radial glial cells and is most intense along cerebellar Bergmann glial cells [77]. Glia represents the major cell population in the CNS coupled by gap junctions. Indeed, compared to neurons, the level of connexin expression is high in these cells and persists until the adult stage [75]. For the two main types

of macroglial cells, the astrocytes and the oligodendrocytes, several connexins have been detected [78]. Gap junctional communication is not limited to either astrocyte-to-astrocyte or oligodendrocyte-to-oligodendrocyte, but it also occurs in between both cell types. In the adult brains, the predominant connexin is Cx43, which is abundant in astrocytes and is also expressed in leptomeninges, endothelial cells and ependyma. The second type of microglia, the oligodendrocytes (and their peripheral counterparts, the Schwann cells), appear to express a different gap junction protein, Cx32, although to a lower extent in situ than the level of Cx43 expression exhibited by astrocytes. Astrocytes express Cx43 and are well coupled in vivo and under culture conditions. However, the strength of coupling and degree of Cx43 expression between astrocytes varies depending on brain regions being higher in the hypothalamus than in the striatum. Although glial gap junctions do not generate action potentials in normal conditions and are devoid of synaptic contacts, connexin channels provide a route that allows changes in membrane potential to be transmitted from one cell to its neighbors. Recently, the participation of astrocytic gap junction in neuroprotection has been investigated by comparing neuronal vulnerability in the presence of either communicating or non-communicating astrocytes [75].

12. Gap junctions and connexins

Gap junctions and their consistent connexin proteins have represented a new challenge in all tissues where they occur but no structure is more complex or more interconnected than the mammalian central and peripheral nervous systems (CNS and PNS). The term "Gap junctions" arose from the work of Revel and Karnovsky, who described the fine structure of the interconnections between mouse cardiomyocytes and between hepatocytes. Later development of specific antibodies to gap junction proteins and eventually the cloning of these connexin molecules have now led to the availability of a variety of techniques by which the distribution and expression patterns of specific types of gap junctions have been defined in a varied number of tissues, including the brain. Gap junctions are the clusters of intercellular channels that are composed of 12 subunits, 6 of which form a connexion or hemichannel contributed by each of the coupled cells [79]. Gap junctions are permeant to molecules up to 1 kDa and are found in virtually all cell types in mammals; few exceptions include circulating erythrocytes, spermatozoids and adult innervated skeletal muscle cells [80]. Gap junctional communication is essential for many physiological events, including cell synchronization, differentiation, cell growth, and metabolic coordination of avascular organ including epidermis and lens [81]. Connexin family members share a similar structural topology. Each connexin has four transmembrane domains that constitute the wall/pore of the channels. These domains are linked by two extracellular loops that play roles in the cell-cell recognition and docking processes. There are three unchanged cysteine residues in each loop, which solely form intraconnexin disulfide bonds [82]. The transmembrane domains and extracellular loops are highly conserved among the family members. Furthermore, connexin proteins have cytoplasmic N- and C-terminal and a cytoplasm loop linking the second and third transmembrane domains. Although the N-terminus is conserved, the cytoplasmic loop and C-terminus show great variation in terms of sequence and length. The cytoplasmic tail and loop are susceptible to various post-translational modifications (e.g., phosphorylation), which are believed to have regulatory roles [83]. Connexons (hemichannels) are then carried to the cell surface via vesicles transported through microtubules, which fuse to the plasma membrane. These hemichannels can either form nonjunctional channels in unopposed areas of the cell membrane or diffuse freely to regions of cell-to-cell contact to find a partner connexon from a neighboring cell to complete

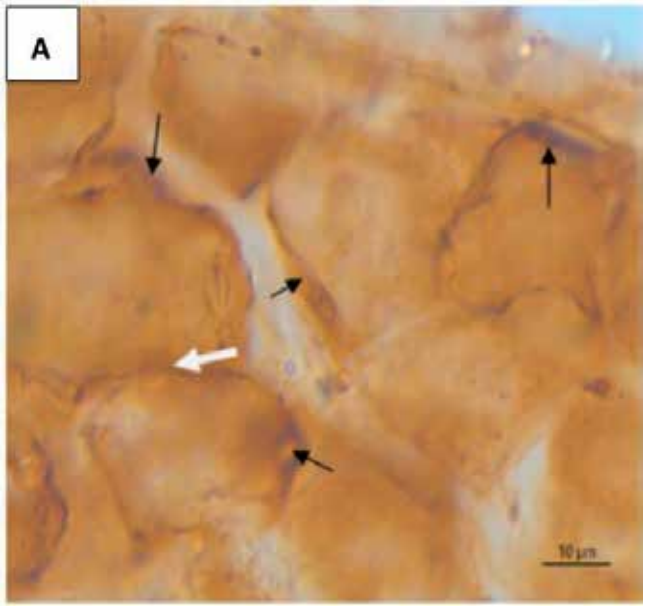

Figure 9.
Immunohistochemical staining using connexin-43 antibody. Black arrows represent the location of gap junctions between the satellite glial cells and the neuronal bodies [33].

the formation of intercellular channels. Intercellular channels then cluster into gap junction plaques, a highly dynamic event involving removal of old channels from the center of the plaque, while adding new gap junction subunits to the periphery [84]. The intercellular channels from the middle of the plaque are internalized into vesicular structures called "annular junctions" [85], which either fuse with the lysosome for degradation by lysosomal enzymes or are targeted to the proteasomal pathway [86]. The continuous synthesis and degradation of connexins through these mechanisms may provide for the quick adaptation of tissues to changing environmental conditions. Unopposed hemichannels can also be functional under certain conditions, including mechanical and ischemic stress. Under these circumstances, open hemichannels are thought to facilitate the release of a variety of factors such as ATP, glutamate, and NAD+ into the extracellular space, generating different physiological responses [87].

Up to date, there were 20 proposed members of the connexin family of proteins that form gap junctional intercellular communication channels in mammalian tissues, and over half are reported to be present in the nervous system. Identification of the several connexin proteins at gap junctions between each neuronal and glial cell type is necessary for the sensible design of investigations into the functions of gap junctions between glial cells and into the functional contributions of electrical and "mixed" (chemical plus electrical) synapses to communication between neurons in the mammalian nervous system (**Figure 9**).

13. Pathophysiology of connexins

Gap junction's role has been well evaluated concerning cell-to-cell interaction. There are two effects derived from gap junction's function that may determine life and death of the connected cells [89]. The bystander effect promotes the death of normal cells adjacent to an apoptotic cell by diffusing toxic metabolites through gap junctions. In the same way there is the Good Samaritan effect that allows

a condemned cell to live by draining the toxic metabolites to adjacent cells and maintaining cells integrity and thus tissue homeostasis. In this way gap junctions perform a dual function either saving or killing interconnected cells [88]. Some pathological conditions are directly related to gap junctions or to their altered function. Some human diseases are caused by mutated connexins [89]. Mutations on Cx32 induce a peripheral neuropathy named Charcot-Marie-Tooth disease. The many conductivity changes observed in this disease may be caused by altered protein traffic to the junctions, altered channel permeability and, sometimes, altered conformation of heterotypic channels [78]. Mutations of Cx36 may lead to the most common hereditary non-syndromic deafness. Cx43 structure may be altered in some forms of human epilepsy where Cx43 mRNA expression may or may not be altered. High Cx43 levels have been detected in β-4 positive amyloid plaques of Alzheimer's disease [77], indicating either astrocytes invasion of the plaques or increased Cx43 expression by astrocytes, as observed in PC12 cells (cells from a rat pheochromocytoma) with increased expression of carboxy-terminal portions of amyloid precursor protein [90]. However a higher Cx43 expression in that area may reflect the existence of many activated macrophages/microglia. The decrease of Cx43 within an inflammatory focus suggests that factors as IL-1 β are involved in astrocytic connectivity decrease as observed in autoimmune experimental encephalitis.

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Section 4

Nerve Interface

Chapter 5

Interface Nerve Tissue-Silicon Nanowire for Regeneration of Injured Nerve and Creation of Bio-Electronic Device

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Abstract

This overview presents the results of scientific and practical research into the development of the interface "neuron-electronic device" based on silicon nanowire. The work has been carried out for several years by a team of scientists specializing in various fields of science and technology: neuroscience, surface science, nanoelectronics, crystal growth, physics and chemistry of nanotechnology, and nanocomputing. The technology of formation of the interface "nerve fiber-silicon nanowire" was developed. The experiments were performed *in vivo* on Wistar rats. The developed technology was used in the manufacture of implants for the regeneration of the injured sciatic nerve. The results of the studies showed the effectiveness of using such implants not only for the regeneration of nerves with severe injuries but also for the creation of a *bioelectronic* interface for *neurocomputers* that can be used *in vivo* for a long time.

Keywords: interface, silicon nanowires, Wistar rats, sciatic nerve, experiment *in vivo*, laser heterodyne interferometric technique, application of SiNW-FET, physical model of the interface nervous tissue-silicon nanowires

1. Introduction

In the last decade, along with the solution of medical problems on the restoration of the human nervous system by traditional methods, a new direction of neuroscience arose related to the development of hybrid intellect that has to combine the best intellectual resources of human brain and the best achievements of nano- and quantum computing. By the computation speed, modern computers considerably exceed human capabilities, but they have two significant drawbacks. Providing their work with the intellectual capabilities of man using modern nanoelectronics requires considerable power consumption. This leads to an increase in the physical dimensions of the computer in order to provide a thermal regime acceptable for modern nanoelectronics. On the other hand, the human brain, accomplishing a huge amount of work on physical and intellectual interaction and to ensure the correlated work of the organism as a whole, is characterized by *extremely low energy costs* in comparison with quantum computers. Therefore, an idea arisen on creating

a hybrid intellect that physically combines the neural networks of the brain with modern, including quantum, computing devices. The development of such devices required detailed studies of the structure of neural networks of the brain and subsequent modeling of such networks on silicon nanostructures using living neurons or by implanting silicon nanostructures into a neural network of a living organism. The end product of this device will be a hybrid brain that is capable, unlike traditional computers, including quantum ones, to apply both logical and associative methods of solving problems with low energy consumption. The main task of the hybrid brain is to provide a constant two-way communication between the central and peripheral nervous system, which will allow, in extreme case, to constantly monitor the organism as a whole and, if necessary, already in the first stages of the disease, to correct the work of those organs that have deviations from the norm, using, first of all, the internal resources of the body.

By 2015, the strategy for creating hybrid brain has already been developed [1], and a call has been issued [2, 3] to the international community to concentrate scientific and financial resources on the solution of the problem that will make a revolution not only in the field of medicine but also in all spheres of human existence.

Detailed studies of neural networks were started about 10 years ago with the work on the study of the morphology of neural network [4]. Then, a large number of animal studies were made of the relationship between the structure of neural networks and the behavioral characteristics of animals. A detailed review of these studies was published recently [5]. In parallel with studies of the central nervous system and its connection with the peripheral nervous system, work was begun on the creation of a bioelectronic complex on silicon nanostructures with the artificial cultivation of neural networks in a biological environment [6–8]. The results obtained in experiments *in vitro* allowed the transition to animal experiments and then to begin clinical experiments for the treatment of diseases that could not be treated with application of traditional medicine. *Massachusetts General Hospital and Draper Labs* develop a tiny, implanted chip to place it between a patient's skull and scalp. A series of electrodes placed at varying depths in different regions of the brain would record neurological data. In the framework of the program *ElectRx, a closed-loop system* is developed to monitor and to regulate organ functions using the internal resources of the body. *Silent speech information generated directly from the activity of neurons* is involved in speech production via an intracortical microelectrode brain-computer interface [9]. It was shown that *Macaca nemestrina* monkeys can *directly control stimulation of muscles using the activity of neurons in the motor cortex*. Monkeys learned to use artificial connections from cortical cells to muscles to generate bidirectional wrist torques and controlled multiple neuron-muscle pairs simultaneously [10].

Despite encouraging results in the development and testing of bioelectronic complexes capable of recording neural impulses produced by a neuron and transferring them to subsequent processing into a nanocomputer, there are still many unresolved problems, the first of which is the development of a central link of the hybrid intelligence the "neuron-electronic device" interface [11–15]. To date, the greatest difficulty in creating such an interface is the problem of maintaining its working capacity in a living organism for a time comparable to the human lifespan. The most suitable material for creating such an interface is crystalline silicon. First, silicon is a biocompatible material, and, second, it is the main material of nano- and microelectronic technology, which makes it easy to integrate it into electronic circuits for subsequent signal processing. Taking into account the size of neurons (of the order of tens of micrometers), the silicon wires are the most suitable for creating an interface with a neuron. So, in the past decade, the "silicon crystal-nervous tissue" interface has been attracting huge interest. Various designs of electronic circuits of field-effect transistors [16] (SiNW-FET) have been developed. The main attention

in the development of these devices was given to obtaining a high sensitivity of the device for reliable registration of nerve impulses. For this purpose, the SiNW-FET design was developed, in which a dielectric layer between the neuron and the SiNW was created of the ultimate small thickness. The best results on the sensitivity of the SiNW-FET were obtained using the chemical compound poly-L-Lysine as a dielectric between neuron and FET. However, after long-term tests of this design, it was found that the lifetime of such an interface is estimated in a several days or a maximum of a few weeks.

So, a major hurdle in brain-machine interfaces (BMIs) is the lack of an implantable neural interface system that *remains viable for a substantial fraction of the user's lifetime and* the lack of a *high-density, chronic interface to enable recording and stimulation from thousands of sites* in a clinically relevant manner with little or no tissue response remains as one of the grand challenges of the twenty-first century.

The success of the research of our group in experiments on laboratory animals has shown the prospects for application of silicon nanowires in creation of bio-consistent and bioactive implants. The key problem of these works was the study of the biophysical state of the interface "neuron-silicon nanowire" and the development of methods for the purposeful management of its properties. At present, on the basis of this interface, we have developed and patented technology for the manufacture of implants [17], which provides auto-electronic stimulation of the regenerative processes of damaged nerve tissue. The most important feature of the developed technology that significantly distinguishes it from existing ones is to provide conditions for the continuous effective migration of biological cells to the implant site. This feature indicates the promise of its use for the development of neuro-electronic interfaces for neurocomputers, suitable for use over a long period of time, comparable to the years of human life.

In 2-d part of the overview, we present the research on formation of the interface "silicon wire-nerve tissue." Experiments were carried out *in vivo* by simulation of a sciatic nerve injury and following recovery of the injury using silicon nanowires.

In 3-d part, we present experimental techniques used to test how nerve fibers restore functional ability after implantation a conduit with silicon nanowires. In addition to the techniques traditionally used for this purpose, we apply a test to evaluate bidirectional communication between the brain and corresponding peripheral nerve by registration *in real-time in vivo* a nerve displacement initiated due to action potential propagation. We apply additionally SiNW-FET to measure charge state of the interface, when it forms. Furthermore, this experiment gives rise to direct definition of sign and surface charge densities both on silicon wire and nerve fiber in living organism.

In 4-d part we present experimental results on evolution of the restore functionality of the damaged nerve after implantation conduit with silicon nanowires. We analyze prospects to use the interface nervous tissue—silicon nanowire in the global problem brain-computer interface—particularly on possible application quantum HEM device [18] based on silicon nanowires as a nerve pulse binary adder [19, 20].

2. Formation of the interface silicon wire-nerve tissue

The research on formation of the interface "silicon wire-nerve tissue" was carried out *in vivo* on Wistar rats by simulation of a sciatic nerve injury and further replacement fault of the nerve trunk by implant with a set of silicon nanowires.

One of the procedures published in details elsewhere [21, 22] includes several stages: growing of silicon wires, handling the implants, surgical procedure, and various test experiments in vivo for evaluation of motor function recovery by "the method of walking track" [23] and by recording a bilateral interaction between neuronal nets of a brain

and actuators of peripheral nerve by real-time registration nerve displacements due to action potential propagation [24–27] and test experiments in vitro to examine morphological features of the interface by optical and electron transmission microscopy.

2.1 Growth of silicon wires

Silicon wires (see **Figure 1**) were grown by the technology developed by Sandulova et al. [28]. This technology is based on a method of gas-phase reaction in a sealed tube at a temperature gradient. In order to provide the chemical reactions and to stimulate rapid growth of the wires, we used bromine and gold.

For growing wires with a prespecified type and value of conductivity, we added doping impurities into hot part of the tube. Due to differences in the reaction-binding energies of gold and the doping impurities with bromine, the temperature gradient provides a different amount of precipitation of these materials along the tube. That is why grown silicon wires are distributed along the tube by size (diameter, length) and by the level of doping [29, 30]. The thinner the diameter of a wire is, the smaller is the concentration of dopants. The diameter of the grown wires ranges from 10 nm to several tens of microns. Their length varies in a range from tens of microns up to a few centimeters. Furthermore, the shape of wires depends on their diameter, too.

The wires, which diameter was of nanometers, were cylindrical, while the wires with much greater diameters were hexahedral.

2.2 Handling the implants

Wires for preparation of implants are shown in **Figure 1**. Making of implant started from dividing the wires by diameters. The prepared set of nanowires was treated for purification of a surface in different etchants. Thereupon, the wires were oxidized by storage under ambient atmosphere at room temperature. The thickness of silicon oxide does not exceed one to two nanometers. Just before surgical operation, an antispiking gel ("Mesogel," Linteks Ltd., Russian Federation) was introduced into the aorta extracted from another rat. In order to avoid a rejection of

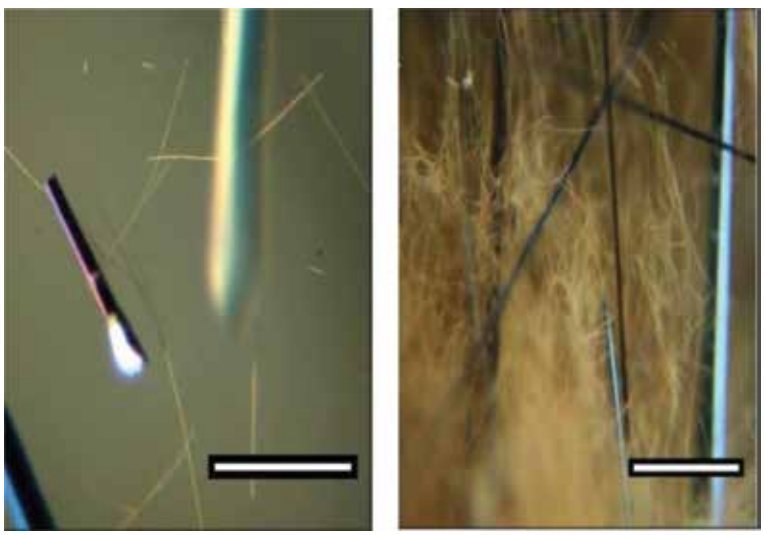

Figure 1.
Wires for preparation of implants. Scale bar is 80 μm on the left and 250 μm on the right side of the figure.

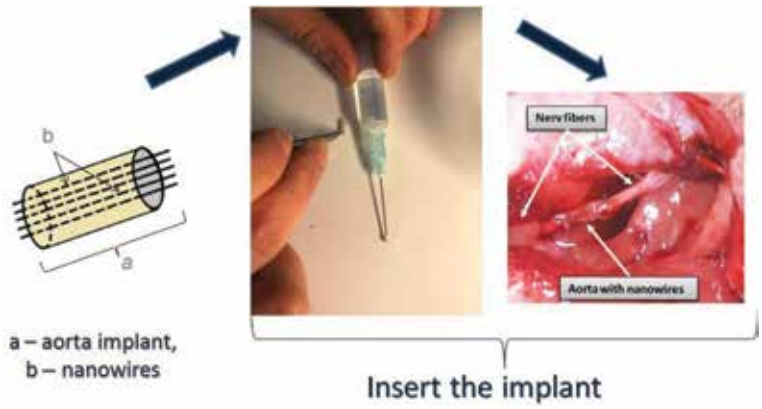

Figure 2.
Surgical procedure.

the transplant, the aorta has been prefrozen in liquid nitrogen. Then, the set of the wires was placed into the gel and oriented along an axis of the aorta.

2.3 Surgical procedure

Experiment was carried out on rats, weighing 180–250 g, that were housed in standard conditions with free access to food and water and natural light-dark cycle. The rats were randomly divided into several groups. Under thiopentone general anesthesia (40–60 mg/kg intraperitoneally), right sciatic nerve of animals were exposed in middle third, separated from surrounding tissues for approximately 10 mm in length, and isolated from underlying muscles. For the animals of one group, after dissection of sciatic nerve, we inserted the implant (**Figure 2**). Animals of the other groups were used for trauma simulating of the nerve and as sham-operated ones.

Animal care, housing, and all experiments were performed in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The research was approved by Bioethical Committee for human subjects or animal research at Bogomolets National Medical University, December 30, 2015.

3 Methods for evaluation of nerve recovery after implantation

Along with traditional methods of evaluation of a nerve recovery, we first used laser heterodyne interferometric techniques that give rise to record *in vivo* in real time an evolution of bilateral interaction of a brain and peripheral nerve [31] that reflect a quality of the nerve recovery.

3.1 Experiment *in vivo* for evaluation of motor function recovery

In the course up to 5 months, rats were tested on a degree of nerve regeneration. For evaluation of motor function recovery, we used "the method of walking tracks" [23]. The degree of motor function recovery was determined by the shape and size of prints of hind paws of animals when they pass through a narrow corridor. For quantitative assessment of sciatic function index (SFI), we used print length, toe spread, and intermediate toe spread on the prints of both post-operated and healthy hind limbs.

3.2 Experiment *in vivo* for evaluation of a bilateral interaction of a brain and peripheral nerve

To evaluate a recovery of bilateral interactions between neuronal net of a brain and actuators of peripheral nerve, we designed a setup on detection of nanometer displacement of nerve fibers [24–27] using laser heterodyne interferometric techniques with next specifications:

- Laser wavelength 0.63 μm
- Power of the probing radiation 1 mW
- Bandwidth of the receiver 1–30 KHz
- Noise level at frequency 1 KHz with bandwidth 3 KHz on distance 1 m about 0.1 nm
- Optical setup for detecting the surface displacement that is accompanying neuronal activity is presented in **Figure 3**.

The installation includes a laser heterodyne displacement meter, a computer for controlling the meter, and a computer for processing and displaying measurement results. The principle of operation of the displacement meter is based on detecting changes in the phase of scattered radiation from the object under study, relative to the phase of radiation of the laser heterodyne. The information signal is a phase-modulated variable component of the photodetector current, which is formed as a result of interference of laser radiation and scattered radiation from the object under study, in the current case from the nerve of rat.

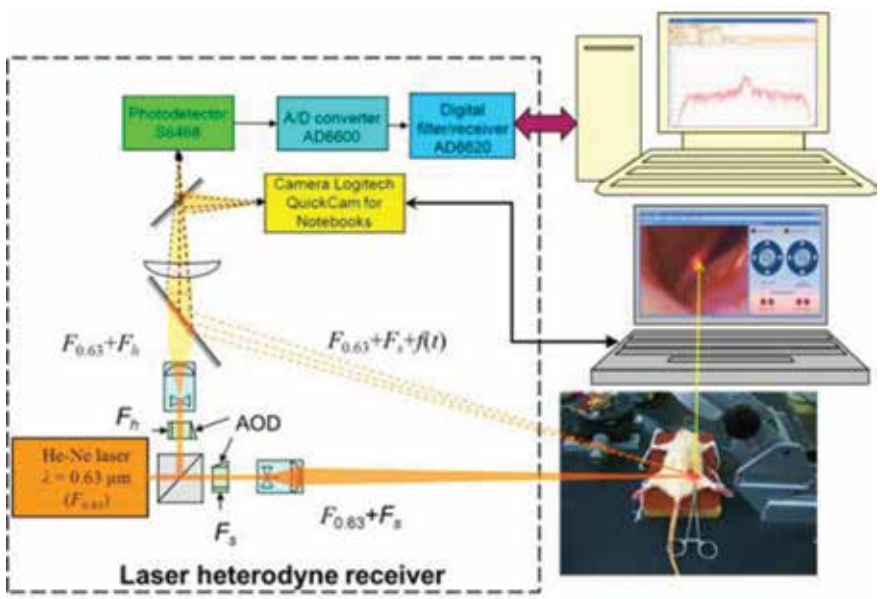

Figure 3.
Optical setup for detecting neuronal activity.

3.3 Examination of morphology of the interface

Around 6 weeks after the implantation, animals of the first group were taken out of the experiment by decapitation with the use of an overdose of thiopental anesthesia. Nerves with the implant were extracted, and slices were produced using a cryotome (MK-25, "Tekhnolog" Russia). Thereupon, the slices were stored during the day in 10% neutral formalin, next rinsed in distilled water, and fixed on a microscope slide. For the purposes of microscope investigation of the nerve fibers, samples were stained with silver nitrate [32]. Prepared slices of the interface "nerve fiber-silicon wire" were examined by light microscopes Carl Zeiss NU-2E and Olympus BX 51 equipped with a digital camera and transmission electron microscope TEM-125K (SELMI, Ukraine).

For light microscopy, material was prefixed by intracardiac perfusion with 10% formalin in 0.1 M phosphate buffer, postfixed in 10% formalin, dehydrated, and embedded in paraffin. Sections were cut and strained with hematoxylin-eosin, by the van Gieson method, impregnated with nitric silver. For TEM, material was prefixed by intracardiac perfusion with 1% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% glutaraldehyde, 1% osmium oxide, dehydrated, and embedded in epone-araldite. Semi-thin and ultrathin sections were cut, contrasted by lead citrate and acetate.

3.4 Experiment on ascertain energy state of the interface nerve tissue-silicon nanowire

To elucidate the energy state of both constituents of the interface, we carry out experiment with application of SiNW-FET biosensor based on SOI structure with two gates [33–35] as the sensor element to evaluate charge states of the surface of silicon nanowire and the nerve fiber during the interface formation. A schematic representation of this transistor is shown in **Figure 4**.

In this transistor, the substrate is used as a control gate (back-gate, BG), modulating their conductivity. An analyte, which adheres to the free surface of the transistor, plays the role of the second gate (virtual local gate). If the charge at the surface of the nanotransistor changes due to adsorption of the analyte, so will change the conductivity of the nanotransistor and will shift its current-voltage $I_{ds}(V_{bg})$ characteristic along voltage axis. A sign and value of the shifting allow determining both the sign and the density of the adsorbed charge.

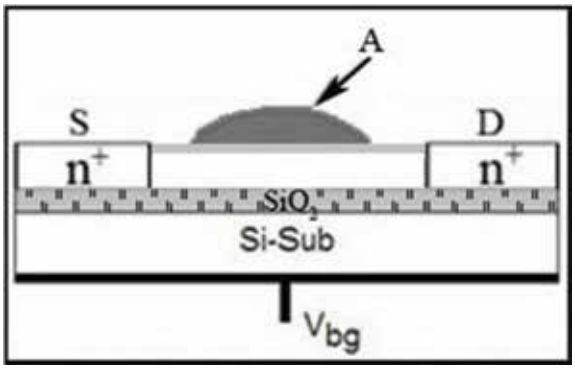

Figure 4.
Schematic presentation a dual-gated SiNW-FET biosensor based on SOI structure.

To elucidate how the charge state of the nanotransistor surface changes during the interface formation, we carried out experiment *in vitro* and studied the current-voltage characteristics $I_{ds}(V_{bg})$ in three cases: (1) initial state of the surface of the nanotransistor (without any analyte, i.e., a free surface covered with native oxide only), (2) the surface of the nanotransistor in contact with the physiological environment, and (3) the surface of the nanotransistor after adherence of a neuron when it is immersed into the physiological environment. The measured current-voltage characteristics for these three cases give rise to calculate the surface density of the initial charge on silicon nanowire (biosensor), after adherence of components of the physiological environment and after adherence of a neuron as well.

4. Results and discussion

4.1 Evaluation of motor function

Results on recovery motor function of the limb by "the method of walking tracks" [23] are presented in **Figure 5**. It is seen that the sciatic function index (SFI) related to motor function of the limb, being normal before simulation of a sciatic nerve injury, instantly after implantation sharply decreases to abnormal state. However, in postoperative period about several months, functionality of the limb, even if slowly, improves.

4.2 Evaluation of bidirectional communication between the brain and peripheral nerve

To evaluate regeneration of sciatic nerve *in vivo*, we used additionally the laser heterodyne interferometric technique that allows in real-time record of efferent and afferent nerve impulses that provide bidirectional communication between neuronal net of a brain and actuators of peripheral nerve, notably a limb.

Propagation of nerve electrical impulses along the axon is known, to be accompanying several other phenomena such as displacements of the axon, propagation of elastic and thermal waves, and magnetic oscillations as well [36, 37].

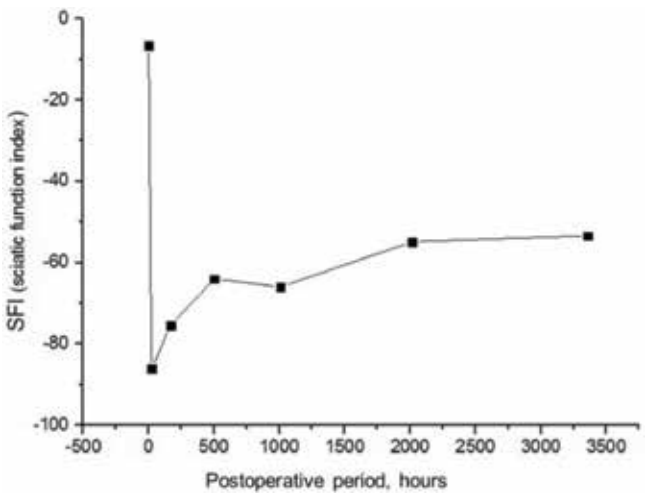

Figure 5.
Evolution of the motor function recovery in postoperative period.

In this work we study in real-time *in vivo* displacements of the nerve induced by nerve electrical impulses propagation. First of all, it should be noted that we measure the sum of the displacements of a bundle of the axons that forms the sciatic nerve. That is why the recorded displacements present the sum of independent cycles of a large number (about 1000 [38]) of the axon excitations. Consequently, we cannot observe a single excitation of regularly shaped spikelike to the observed *in vitro* on squid giant axon [39]. Furthermore, a magnitude of the spikes observed in the current experiment considerably exceeds the observed on a single axon.

The first step of our measurement was recording *in vivo* in real time the displacements of healthy nerve that is shown in **Figure 6** (top line). The next step is dissection of the healthy nerve and a measurement of activity in both dissected proximal and distal parts of the nerve presented in **Figure 6** (center and bottom lines). From a comparison of the intensities of the nerve impulse generation of a healthy nerve, **Figure 6** (top line), and the proximal and distal parts of the dissected nerve (central and bottom line accordingly), it is seen that intensity of nerve impulses propagation persists enough high, especially on the proximal part.

The second step is to carry out surgical operation on implantation of the aorta filled by the silicon nanowires. Then, we measure nerve displacements immediately after implantation (**Figure 7**) in three places of the post-operated nerve: proximal part, implant and distal part.

It is seen that immediately after implantation (**Figure 7**), the intensity of the nerve impulses generation on the proximal segment of the nerve slightly decreased,

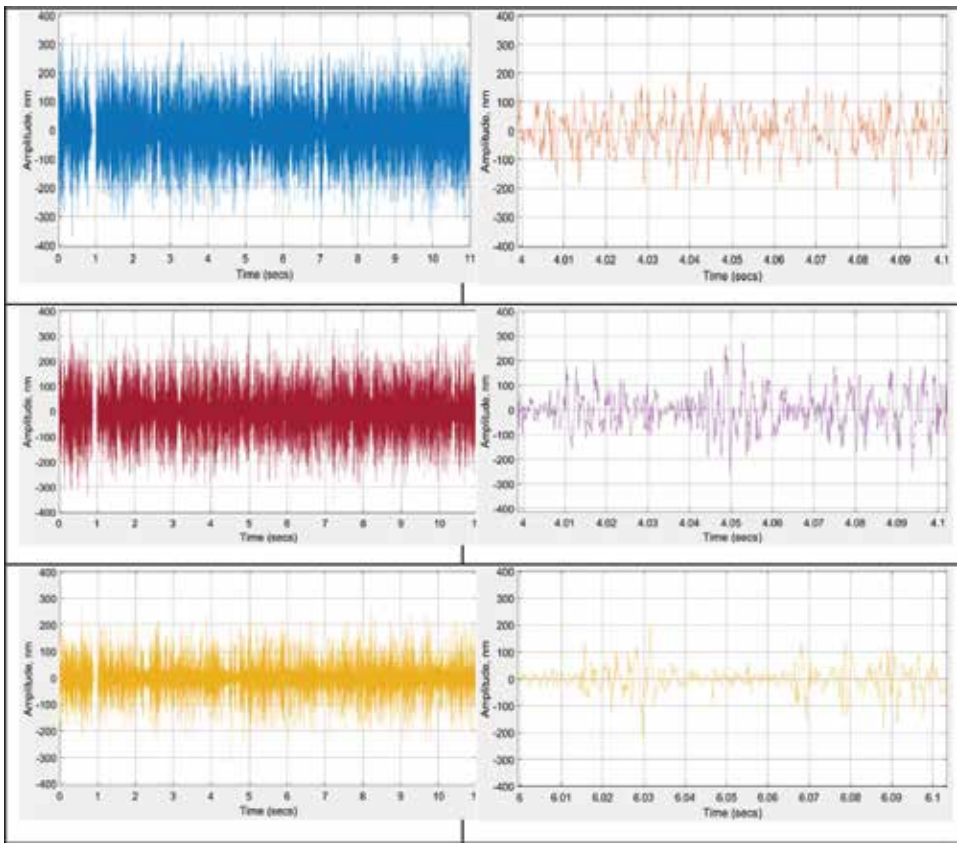

Figure 6.
Record in real-time in vivo displacements of the healthy nerve (the top line) and after dissection of the nerve (the center line, the proximal part; the bottom line, the distal part of the nerve).

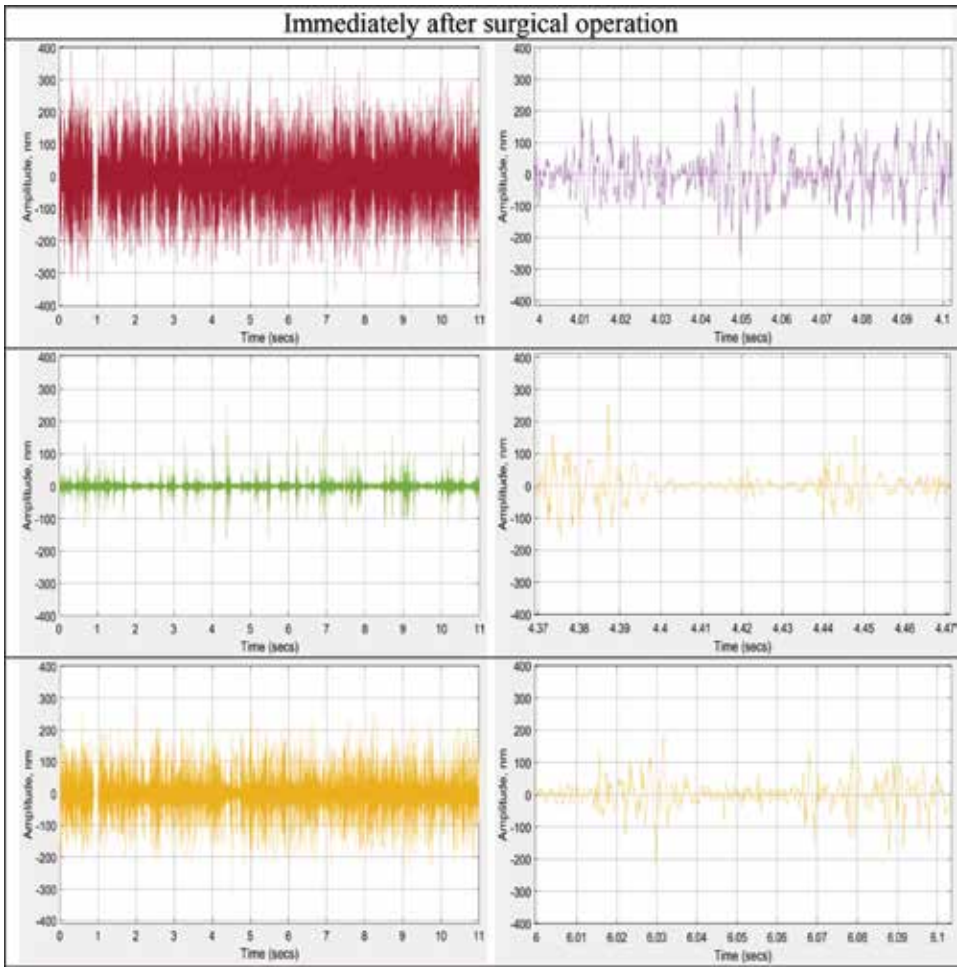

Figure 7.
Record in real-time in vivo displacements of the nerve immediately after implantation in three places of the post-operated nerve: the proximal part (the top line), the implant (the central line) and the distal part (the bottom line).

which could be expected, because this part of the nerve no longer receives signals generated in the paw. Generation of impulses produced in the paw (distal portion of the nerve) decreased more significantly. Nerve impulses in the area of the implant immediately after surgery are practically absent (**Figure 7**).

However, after 3 months the passage of nerve impulses through the regenerated nerve is already restored (**Figure 8**). Intensity propagations of nerve impulses through implant and distal parts are similar.

We conducted up to 20 experiments to study the passage of nerve impulses through the regenerating nerve. Summarizing results of all the test experiments, we can conclude that quality of restoration of the limb functionality depends on duration of postoperative period, number of silicon wires filling a gap, and physical properties of the wires.

4.3. Morphology of the interfaces nerve fiber-silicon nanowires

To understand the mechanism of neural tissue regeneration, a series of experiments were carried out to elucidate the morphological features of the interface "neuron-silicon nanowire." This research was published elsewhere [21, 22, 40],

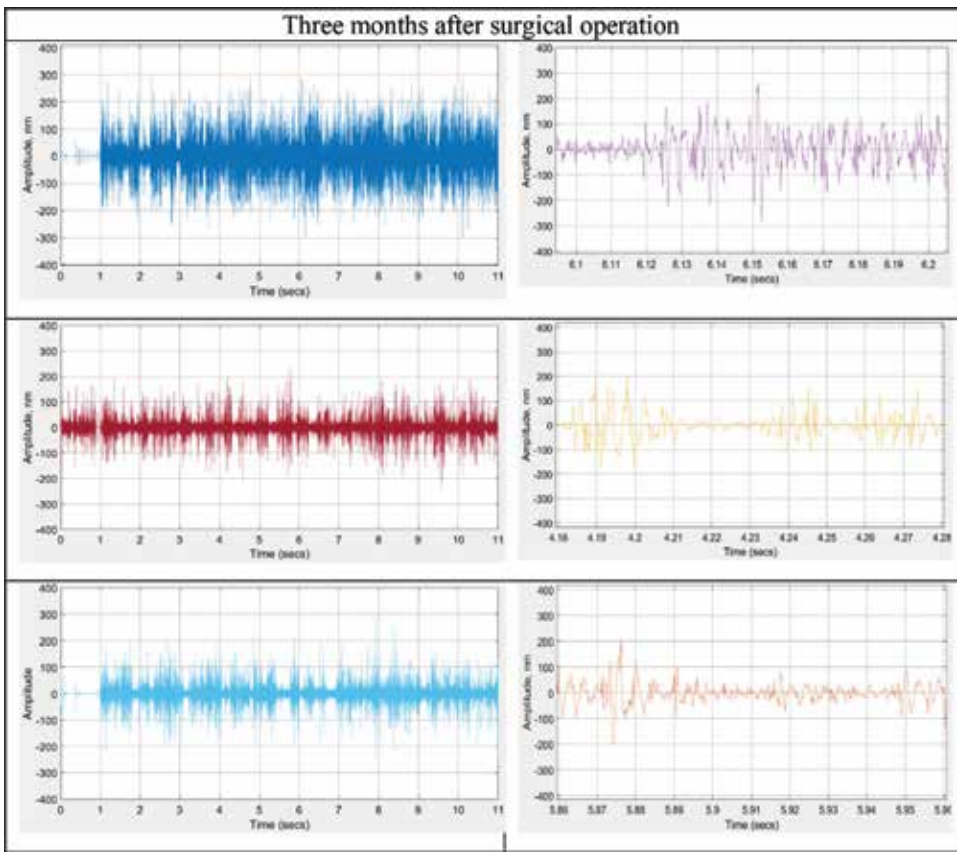

Figure 8.
Record in real-time in vivo displacements of the nerve after 3 months post-implantation in three places of the post-operated nerve: the proximal part (the top line), the implant (the central line), and the distal part (the bottom line) of the nerve.

while the main results and discussion are given below. The interfaces prepared *in vivo* were examined in various post-operation periods ranging from 3 weeks up to 12 months. The growing nerve fibers formed within the short period were unmyelinated, while the others had myelin sheath whose thickness depended on the length of the postoperative period. Micrographs of the interfaces "nerve fiber-silicon wire" studied in light microscope are presented in **Figures 9** and **10a**.

Before analyzing the micrographs, it is worthy to point out a specification of the preparation of slices that induced high difference in mechanical strength of nerve fiber and silicon wire. We attempted to prepare all the slices oriented primarily along the large axis of the wires. High deviation from this direction resulted in breaking off and falling out a piece of the crystal and in a persistence of a mark of the crystal-removed part as a residual of the biomaterial.

This may be seen in the micrographs of **Figure 9**(**a**, **b**). Slight deviation from this direction resulted in persistence of beveled cut of biomaterial placed on the crystal surface. In case, if the persistent layer of biomaterial is sufficiently thin, then one can see crystals, which accrete from every side by arrays of regenerating nerve fibers. In another case, interface "nerve fiber-silicon wire" is clearly seen along all lengths of the wire (see **Figure 9c**). High sensitivity of the nerve fibers to silicon wires is clearly seen from **Figure 9d**, which presents how the array of growing nerve fibers changes a direction of their growth, when it meets the silicon wire, adsorbs on a surface of the wire, and carries on further growth across the surface.

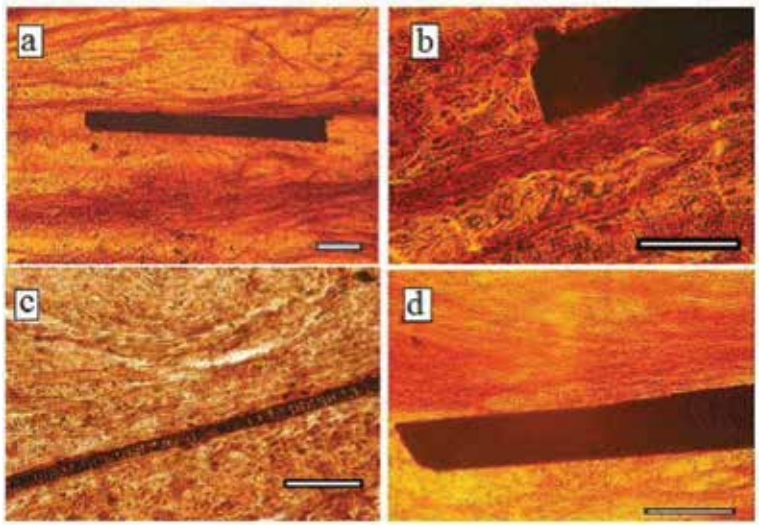

Figure 9.
Micrographs of the affected nerve with the implanted silicon wires. Scale bar is: (a) 150 μm, (b) 50 μm, (c) 60 μm, and (d) 80 μm.

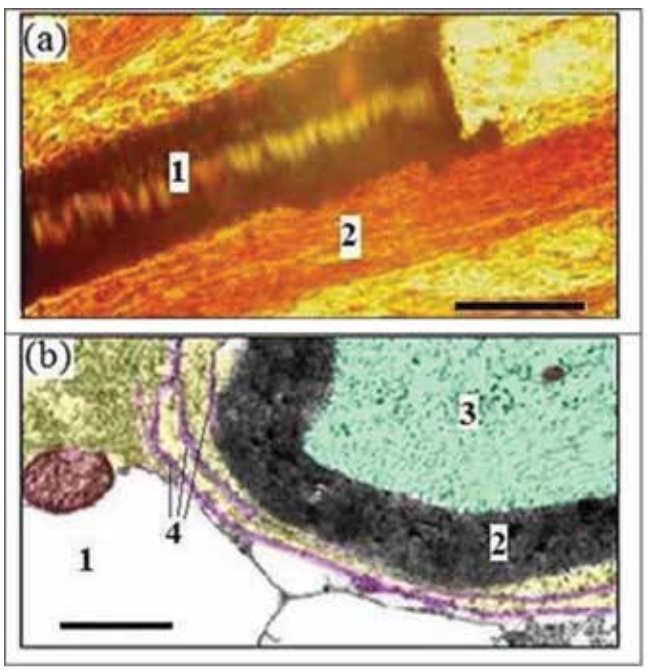

Figure 10.
Micrographs of the interfaces: (a) made with light microscope, the slice is impregnated with nitric silver; plane of the slice coincides with the long axis of the silicon wire; here 1 is the silicon wire, and 2 is a bundle of the newly formed nerve fibers; (b) made with transmission electron microscope, the slice treated with 1% water solution of osmic acid; plane of the slice was perpendicular to the long axis of the silicon wire; here 1 is the silicon wire, 2 is myelin sheath, 3 is axoplasm, and 4 is new layers of the myelin sheath formed of Schwann cells. Scale bar: (a) 40 μm and (b) 50 nm.

In all these cases, though their variety, we can conclude on high sensitivity of the growing nerve to the surface of silicon crystals. Typical micrographs of the "silicon wire-nerve fiber" interfaces made with the light and transmission electron microscopes are shown in **Figure 10**.

Examination of the interface with different magnifications allowed seeing general picture of the growing nerve fibers in the vicinity of the silicon wire and a set of various cells supporting growth of the nerve. In **Figure 10** a micrograph of the interface made with light microscope demonstrates how a bundle of the newly grown young nerve fibers tightly adhere to the silicon wire. The micrograph of the interface made with transmission electron microscope (**Figure 10b**) shows how the newly formed layers of cell membrane of regenerating nerve fiber adhere to silicon crystal. The distance between membrane and silicon wire is less than a few nanometers. Having analyzed a great number of micrographs, we can conclude that young regenerating nerve fibers adhere to the surface of silicon crystals.

To understand the affinity of the nerve fiber to the surface of the silicon nanowires found experimentally, we have to consider the composition and the energy state of both constituents of the interface, i.e., the nerve fiber and the silicon wire.

The energy state of the near-surface region of the silicon wire at room atmosphere is shown in **Figure 11a**. In our experiment, we used silicon wires doped by boron that means that position of the Fermi level in the bulk of the crystal E_f is placed nearby the top of the valence band E_v.

A specific lattice restructuring of a few external atomic layers proper to the silicon surface is known [41] to initiate two energy bands located immediately at the surface. Density of the states in each of these bands is very high and approaches density of atoms at the surface (~10^{14} cm^{-2}); therefore, the Fermi level at the surface is placed near the middle of the energy gap E_i, and its position slightly depends on doping [42, 43] and growth of a thin native oxide as well. However, in p-type of silicon, which is used in our experiment, a positive charge at the surficial bands exceeds the negative one.

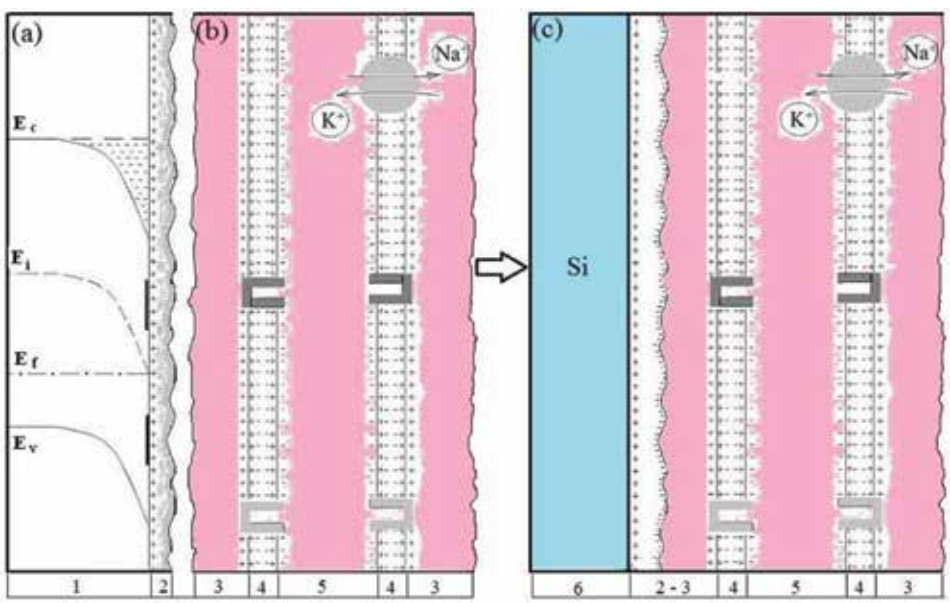

Figure 11.
(a) The near-surface region of silicon wire, where 1 is the energy structure of the near-surface region of the silicon wire and 2 is a native oxide layer on the nanowire surface. (b) A structure of the membrane of nerve fiber (axon) in the living organism, where 3 is the extracellular physiological environment, 4 is the axon membrane composed of phospholipids molecules, and 5 is the axoplasm. (c) A morphology of the "silicon wire-nervous tissue" interface generated in the living organism, where 6 is the silicon wire, 2–3 are the interface of a negatively charged native oxide and positively charged outer surface of the nerve fiber membrane, 4 is the axon membrane, and 5 is the axoplasm.

Thus, the silicon wire being at vacuum or covered by the thin native oxide is entirely neutral, though the external surface of the silicon wire is charged positively.

The structure of the nerve fiber membrane inside a living organism is shown in **Figure 11b**. In our case preparation of the interface from the sciatic nerve of rats, the axon membrane is composed of phospholipid molecules that are known [44] to consist of polar heads and nonpolar tails and form the membrane in a shape of bilayer. It is worthwhile to emphasize that the outer side of the polar heads is charged positively. Surface density of this charge, according to the Richardson structure model, equals about 2×10^{13} cm^{-2}. So, a large positive charge of about 2×10^{13} cm^{-2} is permanently located at the outer side of the membrane.

Summarizing the above consideration, we can draw the following conclusion. If the near-surface region of the silicon nanowire conserves its charge state inside the living organism, then the silicon wire and the nerve fiber are similarly charged and have to repulse each other. Nevertheless, we do observe a strong adherence of the nerve fiber to the silicon nanowire that allows supposing that the physiological environment (interstitial fluid, cell cytoplasm, etc.) contributes to the formation of the interface. Analyzing how the environment may influence the charge state of silicon nanowire, we paid attention to the main properties of the physiological environment. About 80% of the environment consists of water and its pH > 7. On the other hand, thin native oxide layer, that covers the wires, is known [45] to consist primarily of intermediate oxidation states of Si atoms, in particular, $Si^{1+}(Si_2O)$, $Si^{2+}(SiO)$, and $Si^{3+}(Si_2O_3)$. Thus, we can suppose that sub-oxidized Si atoms chemically react with OH^- radicals of the environment, charge the surface of the nanowire negatively, and, thereby, provide Coulomb attraction between silicon wire and nerve fiber. To validate this assumption, we used a model experiment on contact of the nerve cells with silicon nanowire in the electrolyte with pH > 7, close to the physiological environment.

4.4 Evaluation of the charge state of the interface nerve tissue-silicon nanowire

In this experiment SiNW-FET based on SOI structure with two gates [32–34] has been used as the sensor element to evaluate charge states of the silicon nanowire during the interface formation.

An optical image of the nerve cell after its adherence on SiNW-FET is shown in **Figure 12**. In this transistor, the substrate is used as a control gate (back-gate, BG), modulating their conductivity. An analyte which adheres to the free surface of the transistor plays the role of the second gate (virtual local gate). If the charge at the surface of the nanotransistor changes due to adsorption of the analyte, so

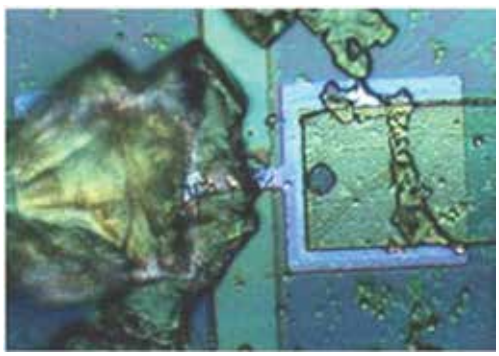

Figure 12.
Micrograph of a neuron adhering to the surface of biosensor.

will change the conductivity of the nanotransistor and will shift its current-voltage $I_{ds}(V_{bg})$ characteristic along voltage axis. A sign and value of the shifting allow determining both the sign and the density of the adsorbed charge.

To elucidate how the charge state of the nanotransistor surface changes in contact with the physiological environment and after adherence of a neuron, we studied current-voltage characteristics $I_{ds}(V_{bg})$ in three cases: (1) initial state of the surface of the nanotransistor (without any analyte, i.e., a free surface covered with native oxide only), (2) the surface of the nanotransistor in contact with the physiological environment, and (3) the surface of the nanotransistor after adherence of a neuron when it is immersed into the physiological environment. The current-voltage characteristics for these three cases are shown in **Figure 13**.

It is seen that, when we immerse the nanotransistor into the physiological environment, the current-voltage characteristics shift to the greater voltage V_{bg} that corresponds, by conditions of our experiment, to a negative charging of the surface of the nanotransistor. Then, we immerse a neuron into the physiological environment and observe its adherence to the surface of the nanotransistor (**Figure 12**). The adherence of the neuron is accompanied by shifting of the current-voltage characteristic in the opposite direction, in particular, to the smaller voltage V_{bg} that means an accumulation of a positive charge at the surface of the nanotransistor. Knowledge of the shifting of the current-voltage characteristics and geometric parameters of the nanotransistor allows calculating the surficial charge at the surface of the nanotransistor induced by the adherence of the analyte. We calculated the surface density of this charge after adherence of components of the physiological environment and after adherence of a neuron as well. We found that the charge accumulated in physiological environment on the surface of the silicon nanotransistor is negative and its density equals ~$1 \cdot 10^{14}$ cm^{-2}. On the other hand, the adsorption of a neuron initiates accumulation of a positive charge on the surface of nanotransistor. The density of this charge is equal to ~$2 \cdot 10^{13}$ cm^{-2}.

So, the experiment *in vitro* proved the above-made assumption about chemical reaction of native oxide with OH^- radicals and, hereby, negatively charging a

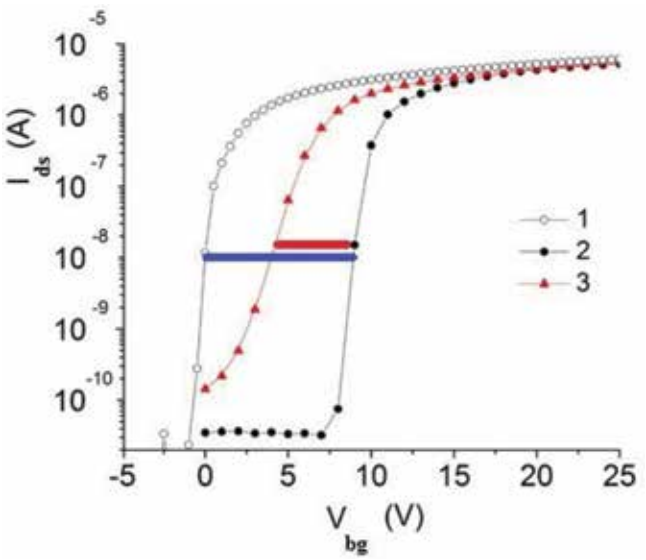

Figure 13.
Current-voltage characteristics $I_{ds}(V_{bg})$ for three cases of the surface of biosensor. (1) The surface covered by native oxide (without any analyte), (2) the surface in contact with the physiological environment, and (3) the surface in contact with adsorbed neuron that was immersed into the physiological environment.

surface of the native oxide of silicon wire. Furthermore, the density of the positive charge accumulated at the silicon nanotransistor after adsorption of the neuron coincides with the known value of the surface density of polar head of phospholipid molecules by the Richardson structure model [44]. So, from the *in vitro* experiment, we can draw a conclusion on the Coulomb origin of the interface formation and present morphology of the "silicon wire-nervous tissue" interface as it is shown in **Figure 11c**.

It is also evident that a propagation of the nerve impulse through the nerve fiber has to occur in a quite different way than the case when the nerve impulse passes through a free nerve fiber. A charge state of the formed interface during propagation of nerve impulse schematically is shown in **Figure 14**.

At a normal (resting) state of the nerve fiber, besides a permanent positive charge at the outer side of the membrane, there is an additional positive charge located inside the extracellular medium and the negative charge located inside the axoplasm. These charges produce potential difference across the axon membrane, the so called resting potential (V_{rest} ~ 70 mV) that acts throughout the entire length of the nerve fiber in a normal (resting) state of the nerve. However, when a nerve impulse passes along the nerve fiber, it reverses the potential difference across the axon membrane, the so called, "action potential" (V_{action} ~ 40 mV). So, propagation

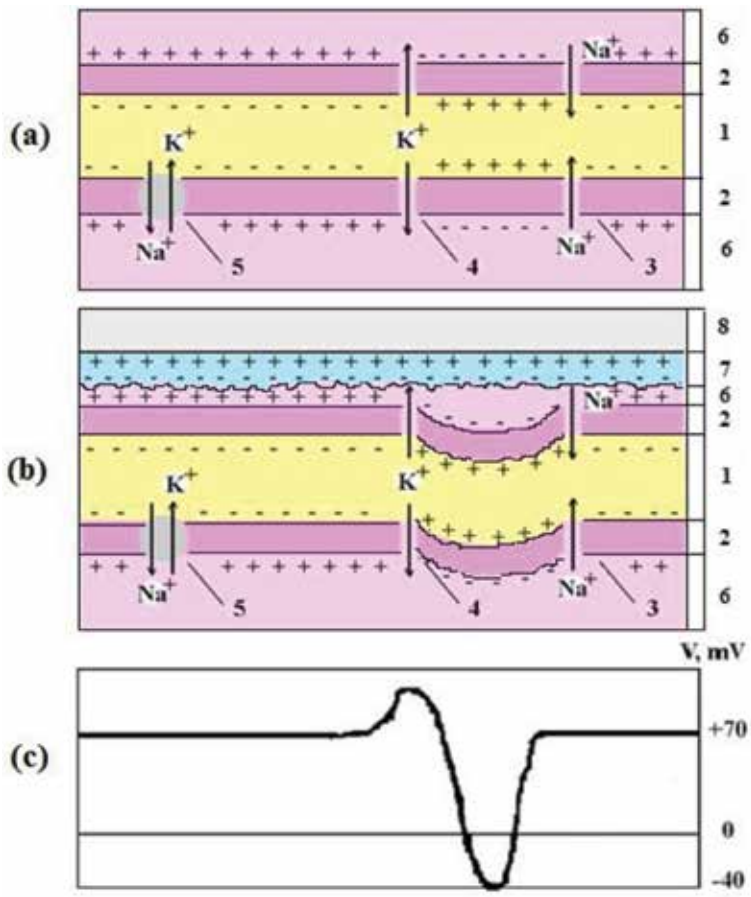

Figure 14.
Charge state of the nervous fiber in physiological environment (a) and charge state of the interface (b) during a nerve impulse propagation (c). Here 1 is the axoplasm; 2 is the axon membrane; 3, 4, and 5 are the ion channels; 6 is the extracellular physiological environment; 7 is the native oxide with negative charge on its surface; and 8 is the silicon wire.

of the nerve impulse along the nerve fiber has to be accompanied by a flexural wave in the nerve due to recharge of the external side of the membrane and subsequent changing of the Coulomb attraction of the nerve fiber to the silicon wire by the Coulomb repulsion. Additionally, propagation of the nerve impulse has to generate an electronic surficial wave in a space charge region of the silicon wire. The latter may be used for extracellular recording of neuronal signal. Details of this process have to depend strongly on properties of silicon wires and call for further research.

5. Conclusion

Here we presented the study of the "silicon wire-nerve tissue" interface formed both *in vivo* and *in vitro* experiments. We have shown experimentally that there is a very good adhesion, of a nerve tissue to silicon wire, covered by thin native oxide, in the living organism. We analyzed the morphology of the interface from a physical point of view taking into account the energy structure of silicon surface, morphology of the surface layer of nerve fiber, and the composition of nutrient medium as well. Result of the analysis is indicated on Coulomb interaction between the constituents of the interface. To verify this conclusion, we carried out experiment using doubly gated SOI-SiNW-FET that is given rise to measure the surface densities of the charge both on the surface of silicon wire and on the surface of nerve fiber. This experiment has shown that strong adhesion of silicon wire and nerve fiber is given rise to Coulomb mutual attraction of the oppositely charged surfaces of the nerve fiber and silicon wire. We analyzed Coulomb interactions at the interface during propagation of a nerve impulse and concluded that nerve impulse has to initiate a flexural wave in the nerve fiber and to generate an electronic surficial wave in a space charge region of silicon wire. Moreover, the flexural wave has to provide *metabolism in the nerve fiber and, hereby vital capacity of the interface*. On the other hand, the electronic wave in the space-charge region of silicon nanowire allows using it for extracellular recording of neuronal signal. So, it is evident that the proposed method of the interface "nervous tissue-silicon nanowire" preparation is promising for application in the global project brain-computer interface, particularly on possible application quantum HEM device based on silicon nanowires as a nerve pulse binary adder.

Conflict of interest

We have no conflicts of interest to disclose.

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The brain is the most complex structure that exists in the universe, consisting of neurons whose function is to receive information through dendrites and transmit information through the axon. In neurosciences one of the main problems that exists are neurodegenerative diseases for which until now there has been no cure. This book is mainly focused on updating the information on the signaling process carried out in the development of axons. Topics such as axon guidance and its interaction with the extracellular matrix are discussed. Other important topics are semaphorins and their relationship with neurodegenerative diseases, and the neurobiology of the gap junction in the dorsal root ganglion. Finally, the topic of bioelectrical interfaces destined to regenerate damaged nerves is covered. The information in this book will be very important both for researchers who work with these issues and doctoral students who are involved in neuroscience.

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